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PART II

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

■

REORGANIZATION AND REPUBLICATION

Title 21—Food and Drugs

CHAPTER I—FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

[Recodification Docket No. 2]

SUBCHAPTER F—BIOLOGICS

REORGANIZATION AND REPUBLICATION

The Commissioner of Food and Drugs, for the purposes of establishing an orderly development of informative regulations for the Food and Drug Administration, furnishing ample room for expansion of such regulations in years ahead, and providing the public and affected industries with regulations that are easy to find, read, and understand, has initiated a recodification program for Chapter I of Title 21 of the Code of Federal Regulations. This is the second document in a series of recodification documents that will eventually include all regulations administered by the Food and Drug Administration.

The regulations formerly under Part 273—Biological Products have been reorganized into nine parts in an effort to provide greater clarity and adequate space for the development of future regulations pertaining to biologics.

The new heading of Subchapter F reflects the transfer of the three other parts that were formerly in this subchapter to a new Subchapter L elsewhere in this issue of the FEDERAL REGISTER.

The changes being made are nonsubstantive in nature and for this reason notice and public procedure are not prerequisites to this promulgation. For the convenience of the user the entire text of the revised Subchapter F—Biologics is set forth below.

Dated: November 13, 1973.

WILLIAM F. RANDOLPH,
Acting Associate Commissioner for Compliance.

Therefore, Part 273 of Chapter I of Title 21 of the Code of Federal Regulations is redesignated as Subchapter F, Parts 600, 601, 610, 620, 630, 640, 650, 660, and 680 and republished to read as follows:

SUBCHAPTER F—BIOLOGICS

PART 600—BIOLOGICAL PRODUCTS: GENERAL

Subpart A—General Provisions

Subpart B—Establishment Standards

Subpart C—Establishment Inspection

AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—General Provisions

§ 600.3 Definitions.

As used in this subchapter:

(a) "Act" means the Public Health Service Act (58 Stat. 682), approved July 1, 1944.

(b) "Secretary" means the Secretary of Health, Education, and Welfare and any other officer or employee of the Department of Health, Education, and Welfare to whom the authority involved has been delegated.

(c) "Commissioner of Food and Drugs" means the Commissioner of the Food and Drug Administration.

(d) "Bureau of Biologics" means the Bureau of Biologics of the Food and Drug Administration.

(e) "State" means a State or the District of Columbia, Puerto Rico, or the Virgin Islands.

(f) "Possession" includes among other possessions, Puerto Rico and the Virgin Islands.

(g) "Products" includes biological products and trivalent organic arsenicals.

(h) "Biological product" means any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man:

(1) A virus is interpreted to be a product containing the minute living cause of an infectious disease and includes but is not limited to filterable viruses, bacteria, rickettsia, fungi, and protozoa.

(2) A therapeutic serum is a product obtained from blood by removing the clot or clot components and the blood cells.

(3) A toxin is a product containing a soluble substance poisonous to laboratory animals or to man in doses of 1 milliliter or less (or equivalent in weight) of the product, and having the property, following the injection of non-fatal doses into an animal, of causing to be produced therein another soluble substance which specifically neutralizes the poisonous substance and which is demonstrable in the serum of the animal thus immunized.

(4) An antitoxin is a product containing the soluble substance in serum or other body fluid of an immunized animal which specifically neutralizes the toxin against which the animal is immune.

(5) A product is analogous:

(i) To a virus if prepared from or with a virus or agent actually or potentially infectious, without regard to the degree of virulence or toxicogenicity of the specific strain used.

(ii) To a therapeutic serum, if composed of whole blood or plasma or containing some organic constituent or product other than a hormone or an amino acid, derived from whole blood, plasma, or serum.

(iii) To a toxin or antitoxin, if intended, irrespective of its source of origin, to be applicable to the prevention, treatment, or cure of disease or injuries of man through a specific immune process.

(i) "Trivalent organic arsenicals" means arsphenamine and its derivatives (or any other trivalent organic arsenic compound) applicable to the prevention, treatment, or cure of diseases or injuries of man.

(j) A product is deemed "applicable to the prevention, treatment, or cure of diseases or injuries of man" irrespective of the mode of administration or application recommended, including use when intended through administration or application to a person as an aid in diagnosis, or in evaluating the degree of susceptibility or immunity possessed by a person, and including also any other use for purposes of diagnosis if the diagnostic substance so used is prepared from or with the aid of a biological product.

(k) "Proper name", as applied to a product, means the name designated in the license for use upon each package of the product.

(l) "Dating period" means the period beyond which the product cannot be expected beyond reasonable doubt to yield its specific results.

(m) "Expiration date" means the calendar month and year, and where applicable, the day and hour, that the dating period ends.

(n) The word "standards" means specifications and procedures applicable to an establishment or to the manufacture or release of products, which are prescribed in this subchapter and which are designed to insure the continued safety, purity and potency of such products.

(o) The word "continued" as applied to the safety, purity and potency of products is interpreted to apply to the dating period.

(p) The word "safety" means the relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.

(q) The word "sterility" is interpreted to mean freedom from viable contaminating microorganisms, as determined by

the tests prescribed in § 610.12 of this chapter.

(r) "Purity" means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. "Purity" includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances.

(s) The word "potency" is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

(t) "Manufacturer" means any legal person or entity engaged in the manufacture of a product subject to license under the act.

(u) "Manufacture" means all steps in propagation or manufacture and preparation of products and includes but is not limited to filling, testing, labeling, packaging, and storage by the manufacturer.

(v) "Location" includes all buildings, appurtenances, equipment and animals used, and personnel engaged by a manufacturer within a particular area designated by an address adequate for identification.

(w) "Establishment" includes all locations.

(x) "Lot" means that quantity of uniform material identified by the manufacturer as having been thoroughly mixed in a single vessel.

(y) A "filling" refers to a group of final containers identical in all respects, which have been filled with the same product from the same bulk lot without any change that will affect the integrity of the filling assembly.

(z) "Process" refers to a manufacturing step that is performed on the product itself which may affect its safety, purity or potency, in contrast to such manufacturing steps which do not affect intrinsically the safety, purity or potency of the product.

(aa) "Selling agent" or "distributor" means any person engaged in the unrestricted distribution, other than by sale at retail, of products subject to license.

(bb) "Container" (referred to also as "final container") is the immediate unit, bottle, vial, ampule, tube, or other receptacle containing the product as distributed for sale, barter, or exchange.

(cc) "Package" means the immediate carton, receptacle, or wrapper, including all labeling matter therein and thereon, and the contents of the one or more enclosed containers. If no package, as defined in the preceding sentence, is used, the container shall be deemed to be the package.

(dd) "Label" means any written, printed, or graphic matter on the container or package or any such matter clearly visible through the immediate carton, receptacle, or wrapper.

Subpart B—Establishment Standards

§ 600.10 Personnel.

(a) *Responsible head.* A person shall be designated as the responsible head who shall exercise control of the establishment in all matters relating to compliance with the provisions of this subchapter, with authority to represent the manufacturer in all pertinent matters with the Bureau of Biologics, and with authority to enforce or to direct the enforcement of discipline and the performance of assigned functions by employees engaged in the manufacture of products. The responsible head shall have an understanding of the scientific principles and the techniques involved in the manufacture of products. The responsible head shall have the responsibility for the training of employees in manufacturing methods and for their being informed concerning the application of the pertinent provisions of this subchapter to their respective functions.

(b) *Other personnel.* Personnel shall have capabilities commensurate with their assigned functions, a thorough understanding of the manufacturing operations which they perform, the necessary training and experience relating to individual products, and adequate information concerning the application of the pertinent provisions of this subchapter to their respective functions. Personnel shall include such professionally trained persons as are necessary to insure the competent performance of all manufacturing processes.

(c) *Restrictions on personnel*—(1) *Specific duties.* Persons whose presence can affect adversely the safety and purity of a product shall be excluded from the room where the manufacture of a product is in progress.

(2) *Sterile operations.* Personnel performing sterile operations shall wear clean or sterilized protective clothing and devices to the extent necessary to protect the product from contamination.

(3) *Pathogenic viruses and spore-bearing organisms.* Persons working with viruses pathogenic for man or with spore-bearing microorganisms, and persons engaged in the care of animals or animal quarters, shall be excluded from areas where other products are manufactured, or such persons shall change outer clothing, including shoes, or wear protective covering prior to entering such areas.

(4) *Live vaccine work areas.* Persons may not enter a live vaccine processing area after having worked with other infectious agents in any other laboratory during the same working day. Only persons actually concerned with propagation of the culture, production of the vaccine, and unit maintenance, shall be allowed in live vaccine processing areas when active work is in progress. Casual visitors shall be excluded from such units at all times and all others having business in such areas shall be admitted only under supervision. Street clothing, including shoes, shall be replaced or covered by suitable laboratory clothing before entering a live vaccine processing unit. Persons caring for animals used in the manufacture of live vaccines shall be excluded from other animal quarters and from contact with other animals during the same working day.

§ 600.11 Physical establishment, equipment, animals, and care.

(a) *Work areas.* All rooms and work areas where products are manufactured or stored shall be kept orderly, clean, and free of dirt, dust, vermin and objects not required for manufacturing. Precautions shall be taken to avoid clogging and back-siphonage of drainage systems. Precautions shall be taken to exclude extraneous infectious agents from manufacturing areas. Work rooms shall be well lighted and ventilated. The ventilation system shall be arranged so as to prevent the dissemination of microorganisms from one manufacturing area to another and to avoid other conditions unfavorable to the safety of the product. Filling rooms, and other rooms where open, sterile operations are conducted, shall be adequate to meet manufacturing needs and such rooms shall be constructed and equipped to permit thorough cleaning and to keep air-borne contaminants at a minimum. If such rooms are used for other purposes, they shall be cleaned and prepared prior to use for sterile operations. Refrigerators, incubators and warm rooms shall be maintained at temperatures within applicable ranges and shall be free of extraneous material which might affect the safety of the product.

(b) *Equipment.* Apparatus for sterilizing equipment and the method of operation shall be such as to insure the destruction of contaminating microorganisms. The effectiveness of the sterilization procedure shall be no less than that achieved by an attained temperature of 121.5°C. maintained for twenty minutes by saturated steam or by an attained temperature of 170°C. maintained for two hours with dry heat. Processing and storage containers, filters, filling apparatus and other pieces of apparatus and accessory equipment, including pipes and tubing, shall be designed and constructed to permit thorough cleaning and, where possible, inspection for cleanliness. All surfaces that come in contact with products shall be clean and free of extraneous material. For products for which sterility is a factor, equipment shall be sterile unless sterility of the product is assured by subsequent procedures.

(c) *Laboratory and bleeding rooms.* Rooms used for the processing of products, including bleeding rooms, shall be effectively fly-proofed and kept free of flies and vermin. Such rooms shall be so constructed as to insure freedom from dust, smoke and other deleterious substances and to permit thorough cleaning and disinfection. Rooms for animal injection and bleeding, and rooms for smallpox vaccine animals, shall be disinfected and be provided with the necessary water, electrical and other services.

(d) *Animal quarters and stables.* Animal quarters, stables and food storage areas shall be of appropriate construction, fly-proofed, adequately lighted and ventilated, and maintained in a clean, vermin-free and sanitary condition. No manure or refuse shall be stored as to permit the breeding of flies on the premises, nor shall the establishment be lo-

cated in close proximity to off-property manure or refuse storage capable of engendering fly breeding.

(e) *Restrictions on building and equipment use*—(1) *Work of a diagnostic nature.* Laboratory procedures of a clinical diagnostic nature involving materials that may be contaminated, shall not be performed in space used for the manufacture of products except that manufacturing space which is used only occasionally may be used for diagnostic work provided spore-bearing pathogenic microorganisms are not involved and provided the space is thoroughly cleaned and disinfected before the manufacture of products is resumed.

(2) *Spore-bearing organisms for supplemental sterilization procedure control test.* Spore-bearing organisms used as an additional control in sterilization procedures may be introduced into areas used for the manufacture of products, only for the purposes of the test and only immediately before use for such purposes: *Provided,* That (i) the organism is not pathogenic for man or animals and does not produce pyrogens or toxins, (ii) the culture is demonstrated to be pure, (iii) transfer of test cultures to culture media shall be limited to the sterility test area or areas designated for work with spore-bearing organisms, (iv) each culture be labeled with the name of the microorganism and the statement "Caution: microbial spores. See directions for storage, use and disposition.", and (v) the container of each culture is designed to withstand handling without breaking.

(3) *Work with spore-bearing organisms.* Except as provided in the previous paragraph, all work with spore-bearing microorganisms shall be done in an entirely separate building: *Provided,* That such work may be done in a portion of a building used in the manufacture of products not containing spore-bearing microorganisms if such portion is completely walled-off and is constructed so as to prevent contamination of other areas and if entrances to such portion are independent of the remainder of the building. All vessels, apparatus and equipment used for spore-bearing microorganisms shall be permanently identified and reserved exclusively for use with those organisms. Materials destined for further manufacturing may be removed from such an area only under conditions which will prevent the introduction of spores into other manufacturing areas.

(4) *Live vaccine processing.* Space used for processing a live vaccine shall not be used for any other purpose during the processing period for that vaccine and such space shall be decontaminated prior to initiation of the processing. Live vaccine processing areas shall be isolated from and independent of any space used for any other purpose by being either in a separate building, in a separate wing of a building, or in quarters at the blind end of a corridor and shall include adequate space and equipment for all processing steps up to filling into final containers. Test procedures which potentially involve the presence of microorganisms other than the vaccine strains, or the use of tissue culture cell lines other than primary cultures, shall not be conducted in space used for processing live vaccine.

(5) *Equipment and supplies—contamination.* Equipment and supplies used in work on or otherwise exposed to any pathogenic or potentially pathogenic agent shall be kept separated from equipment and supplies used in the manufacture of products to the extent necessary to prevent cross-contamination.

(f) *Animals used in manufacture*—(1) *Care of animals used in manufacturing.* Caretakers and attendants for animals used for the manufacture of products shall be sufficient in number and have adequate experience to insure adequate care. Animal quarters and cages shall be kept in sanitary condition. Animals on production shall be inspected daily to observe response to production procedures. Animals that become ill for reasons not related to production shall be isolated from other animals and shall not be used for production until recovery is complete. Competent veterinary care shall be provided as needed.

(2) *Quarantine of animals*—(i) *General.* No animal shall be used in processing unless kept under competent daily inspection and preliminary quarantine for a period of at least 7 days before use, or as otherwise provided in this subchapter. Only healthy animals free from detectable communicable diseases shall be used. Animals must remain in overt good health throughout the quarantine periods and particular care shall be taken during the quarantine periods to reject animals of the equine genus which may be infected with glanders and animals which may be infected with tuberculosis.

(ii) *Quarantine of monkeys.* In addition to observing the pertinent general quarantine requirements, monkeys used as a source of tissue in the manufacture of vaccine shall be maintained in quarantine for at least 6 weeks prior to use, except when otherwise provided in this part. Only monkeys that have reacted negatively to tuberculin at the start of the quarantine period and again within 2 weeks prior to use shall be used in the manufacture of vaccine. Due precaution shall be taken to prevent cross-infection from any infected or potentially infected monkeys on the premises. Monkeys to be used in the manufacture of a live vaccine shall be maintained throughout the quarantine period in cages closed on all sides with solid materials except the front which shall be screened, with no more than two monkeys housed in one cage. Cage mates shall not be interchanged.

(3) *Immunization against tetanus.* Horses and other animals susceptible to tetanus, that are used in the processing steps of the manufacture of biological products, shall be treated adequately to maintain immunity to tetanus.

(4) *Immunization and bleeding of animals used as a source of products.* Toxins or other nonviable antigens administered in the immunization of animals used in the manufacture of products shall be sterile. Viable antigens, when so used, shall be free of contaminants, as determined by appropriate tests prior to use. Injections shall not be made into horses within 6 inches of bleeding site. Horses shall not be bled for manufacturing purposes while showing persistent general reaction or local reaction near the site of bleeding. Blood shall not be used if it was drawn within 5 days of injecting the animals with viable microorganisms. Animals shall not be bled for manufacturing purposes when they have an intercurrent disease. Blood intended for use as a source of a biological product shall be collected in clean, sterile vessels. When the product is intended for use by injection, such vessels shall also be pyrogen-free.

(5) [Reserved]

(6) *Reporting of certain diseases.* In cases of actual or suspected infection with foot and mouth disease, glanders, tetanus, anthrax, gas gangrene, equine infectious anemia; equine encephalomyelitis, or any of the pock diseases among animals intended for use or used in the manufacture of products, the manufacturer shall immediately notify the Director, Bureau of Biologics.

(7) *Monkeys used previously for experimental or test purposes.* Monkeys that have been used previously for experimental or test purposes with live microbiological agents shall not be used as a source of kidney tissue for the manufacture of vaccine. Except as provided otherwise in this subchapter, monkeys that have been used previously for other experimental or test purposes may be used as a source of kidney tissue upon their return to a normal condition, provided all quarantine requirements have been met.

(8) *Necropsy examination of monkeys.* Each monkey used in the manufacture of vaccine shall be examined at necropsy under the direction of a qualified pathologist, physician, or veterinarian having experience with diseases of monkeys, for evidence of ill health, particularly for (i) evidence of tuberculosis, (ii) presence of herpes-like lesions, including eruptions or plaques on or around the lips, in the buccal cavity or on the gums, and (iii) signs of conjunctivitis. If there are any such signs or other significant gross pathological lesions, the tissue shall not be used in the manufacture of vaccine.

(g) *Filling procedures.* Filling procedures shall be such as will not affect adversely the safety, purity or potency of the product.

(h) *Containers and closures.* All final containers and closures shall be made of material that will not hasten the deterioration of the product or otherwise render it less suitable for the intended use. All final containers and closures shall be clean and free of surface solids, leachable contaminants and other materials that will hasten the deterioration of the product or otherwise render it less suitable for the intended use. After filling, sealing shall be performed in a manner that will maintain the integrity of the product during the dating period. In addition, final containers and closures for products intended for use by injection shall be sterile and free from pyrogens. Except as otherwise provided in the regulations of this subchapter, final con-

tainers for products intended for use by injection shall be colorless and sufficiently transparent to permit visual examination of the contents under normal light. As soon as possible after filling final containers shall be labeled as prescribed in § 610.60 et seq. of this chapter, except that final containers may be stored without such prescribed labeling provided they are stored in a sealed receptacle labeled both inside and outside with at least the name of the product, the lot number, and the filling identification.

§ 600.12 Records.

(a) *Maintenance of records.* Records shall be made, concurrently with the performance, of each step in the manufacture and distribution of products, in such a manner that at any time successive steps in the manufacture and distribution of any lot may be traced by an inspector. Such records shall be legible and indelible, shall identify the person immediately responsible, shall include dates of the various steps, and be as detailed as necessary for clear understanding of each step by one experienced in the manufacture of products.

(b) *Records retention*—(1) *General.* Records shall be retained for such interval beyond the expiration date as is necessary for the individual product, to permit the return of any clinical report of unfavorable reactions. The retention period shall be no less than five years after the records of manufacture have been completed or six months after the latest expiration date for the individual product, whichever represents a later date.

(2) *Records of recall.* Complete records shall be maintained pertaining to the recall from distribution of any product upon notification by the Director, Bureau of Biologics, to recall for failure to conform with the standards prescribed in the regulations of this subchapter, because of deterioration of the product or for any other factor by reason of which the distribution of the product would constitute a danger to health.

(3) *Suspension of requirement for retention.* The Director, Bureau of Biologics, may authorize the suspension of the requirement to retain records of a specific manufacturing step upon a showing that such records no longer have significance for the purposes for which they were made: *Provided,* That a summary of such records shall be retained.

(c) *Records of sterilization of equipment and supplies.* Records relating to the mode of sterilization, date, duration, temperature and other conditions relating to each sterilization of equipment and supplies used in the processing of products shall be made by means of automatic recording devices or by means of a system of recording which gives equivalent assurance of the accuracy and reliability of the record. Such records shall be maintained in a manner that permits an identification of the product with the particular manufacturing process to which the sterilization relates.

(d) *Animal necropsy records.* A necropsy record shall be kept on each animal from which a biological product has been obtained and which dies or is sacrificed while being so used.

(e) *Records in case of divided manufacturing responsibility.* If two or more establishments participate in the manufacture of a product, the records of each such establishment must show plainly the degree of its responsibility. In addition, each participating manufacturer shall furnish to the manufacturer who prepares the product in final form for sale, barter or exchange, a copy of all records relating to the manufacturing operations performed by such participating manufacturer insofar as they concern the safety, purity and potency of the lots of the product involved, and the manufacturer who prepares the product in final form shall retain a complete record of all the manufacturing operations relating to the product.

§ 600.13 Retention samples.

Manufacturers shall retain for a period of at least 6 months after the expiration date, unless a different time period is specified in additional standards, a quantity of representative material of each lot of each product, sufficient for examination and testing for safety and potency, except Whole Blood (Human), Antihemophilic Plasma (Human), Cryoprecipitated Antihemophilic Factor (Human), Red Blood Cells (Human), Single Donor Plasma (Human), Source Plasma (Human), Normal Human Plasma and Allergenic Products prepared to physician's prescription. Samples so retained shall be selected at random from either final container material, or from bulk and final containers, provided they include at least one final container as a final package, or package-equivalent of such filling of each lot of the product as intended for distribution. Such sample material shall be stored at temperatures and under conditions which will maintain the identity and integrity of the product. Samples retained as required in this section shall be in addition to samples of specific products required to be submitted to the Bureau of Biologics. Exceptions may be authorized by the Director, Bureau of Biologics, when the lot yields relatively few final containers and when such lots are prepared by the same method in large number and in close succession.

§ 600.14 Reporting of errors.

The Director, Bureau of Biologics, shall be notified promptly of errors or accidents in the manufacture of products that may affect the safety, purity, or potency of any product.

§ 600.15 Temperatures during shipment.

The following products shall be maintained during shipment at the specified temperatures:

Product	*Temperature*
Cryoprecipitated Antihemophilic Factor (Human)	—18° or colder.
Poliovirus Vaccine, Live, Oral, Type 1	A temperature which will maintain ice continuously in a solid state.
Poliovirus Vaccine, Live, Oral, Type 2	
Poliovirus Vaccine, Live, Oral, Type 3	
Poliovirus Vaccine, Live, Oral, Trivalent	
Red Blood Cells (Human), Frozen.	—65° C. or colder.
Red Blood Cells (Human), Liquid.	Between 1° and 10° C.
Single Donor Plasma (Human), Frozen.	—18° C. or colder.
Smallpox Vaccine, Liquid.	A temperature which will maintain ice continuously in a solid state.
Source Plasma (Human).	—5° C. or colder.
Whole Blood (Human).	Between 1° and 10° C.
Yellow Fever Vaccine.	A temperature which will maintain ice continuously in a solid state.

Subpart C—Establishment Inspection

§ 600.20 Inspectors.

Inspections shall be made by an officer of the Food and Drug Administration having special knowledge of the methods used in the manufacture and control of products and designated for such purposes by the Commissioner of Food and Drugs, or by any officer, agent, or employee of the Department of Health, Education, and Welfare specifically designated for such purpose by the Secretary.

§ 600.21 Time of inspection.

The inspection of an establishment for which a license is pending need not be made until the establishment is in operation and is manufacturing the complete product for which a product license is desired. In case the license is denied following inspection for the original license, no reinspection need be made until assurance has been received that the faulty conditions which were the basis of the denial have been corrected. An inspection of each licensed establishment shall be made at least once each year. Inspections may be made with or without notice, and shall be made during regular business hours unless otherwise directed.

§ 600.22 Duties of inspector.

The inspector shall:

(a) Call upon the active head of the establishment, stating the object of his visit,

(b) Interrogate the proprietor or other personnel of the establishment as he may deem necessary,

(c) Examine the details of location, construction, equipment and maintenance, including stables, barns, warehouses, manufacturing laboratories, bleeding clinics maintained for the collection of human blood, shipping rooms, record rooms, and any other structure or appliance used in any part of the manufacture of a product,

(d) Investigate as fully as he deems necessary the methods of propagation, processing, testing, storing, dispensing, recording, or other details of manufacture and distribution of each licensed product, or product for which a license has been requested, including observation of these procedures in actual operation.

(e) Obtain and cause to be sent to the Director, Bureau of Biologics, adequate samples for the examination of any product or ingredient used in its manufacture.

(f) Bring to the attention of the manufacturer any fault observed in the course of inspection in location, construction, manufacturing methods, or administration of a licensed establishment which might lead to impairment of a product.

(g) Inspect and copy, as circumstances may require, any records required to be kept pursuant to § 600.12.

(h) Certify as to the condition of the establishment and of the manufacturing methods followed and make recommendations as to action deemed appropriate with respect to any application for license or any license previously issued.

PART 601—LICENSING

Subpart A—General Provisions

Sec.

Subpart B—Establishment Licensing

Subpart C—Product Licensing

Subpart D—Licensing of Foreign Establishments and Products

Subpart E—Suspension of Licenses and Appeals Procedure

AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES.—For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—General Provisions

§ 601.1 Two forms of licenses.

There shall be two forms of licenses: establishment and product.

§ 601.2 Application for establishment and product licenses; procedure for filing.

To obtain a license for any establishment or product, the manufacturer shall make application to the Director, Bureau of Biologics, on forms prescribed for such purpose, and in the case of an application for a product license, shall submit data derived from laboratory and clinical studies which demonstrate that the manufactured product meets prescribed standards of safety, purity and potency, a full description of manufacturing methods, data establishing stability of the product through the dating period, sample(s) representative of the product to be sold, bartered or exchanged or offered, sent, carried or brought for sale, barter or exchange, summaries of results of tests performed on the lot(s) represented by the submitted sample(s), and specimens of the labels enclosures and containers proposed to be used for the product. An application for license shall not be considered as filed until all pertinent information and data shall have been received from the manufacturer by the Bureau of Biologics.

§ 601.3 License forms.

(a) *Establishment license.* The establishment license form shall be prescribed by the Commissioner of Food and Drugs and shall include:

(1) The name and address of the manufacturer.

(2) The name and address of the establishment.

(3) The names and addresses of all locations of the establishment.

(4) The license number.

(5) The date of issuance.

(b) *Product license.* The product license form shall be prescribed by the Commissioner of Food and Drugs and shall include:

(1) The name and address of the manufacturer.

(2) The name and address of the establishment.

(3) The name and address of each location at which the product is manufactured.

(4) The license number of the establishment.

(5) The proper name of the product, with additional specifications, if any, which may be approved or required for additional labeling purposes.

§ 601.4 Issuance, revocation or suspension.

A license shall be issued by the Secretary upon the recommendation of the Commissioner of Food and Drugs and upon the determination by the Commissioner of Food and Drugs that the establishment or the product, as the case may be, meets the standards established by the regulations in this subchapter as herein prescribed or hereafter amended. Licenses shall be valid until suspended or revoked. An establishment or product license shall be revoked upon application of the manufacturer giving notice of intention to discontinue the manufacture of all products or of intention to discontinue the manufacture of a particular product for which a license is held. The Commissioner of Food and Drugs shall recommend to the Secretary that a license be suspended or revoked whenever he finds, after notice and opportunity for hearing, that (a) Food and Drug Administration inspectors after reasonable efforts have been unable to gain access to an establishment or a location for the purpose of carrying out the inspection required under § 600.21 of this chapter, or that (b) manufacturing of products or of a product has been discontinued to an extent that a meaningful inspection cannot be made, or (c) the establishment or any location thereof, or the product for which the license has been issued, fails to conform to the standards in the regulations in this subchapter, as herein prescribed or as hereafter amended, designed to insure the continued safety, purity, and potency of the manufactured product. In case of suspension, unless assurances satisfactory to the Commissioner of Food and Drugs (a) that access will be permitted or (b) that manufacturing will be resumed, have been provided or (c) if the faulty condition is not corrected within 60 days or within such other period as may be specified in the notice of suspension, whichever is applicable, he shall recommend that the license be revoked. Except as provided in § 601.40 prior to the institution of proceedings looking to the suspension or revocation of a license the licensee shall be advised in writing of the facts or conduct which may warrant such action and shall be accorded opportunity within a reasonable period prescribed by the Commissioner of Food and Drugs to demonstrate or achieve compliance with the regulations in this subchapter.

§ 601.5 Licenses heretofore issued.

Any license heretofore issued and in effect upon the effective date of the regulations in this subchapter shall remain in effect unless and until superseded by a new license, or suspended or revoked, pursuant to the regulations in this subchapter.

§ 601.6 Changes to be reported.

(a) *General.* Important proposed changes in location, equipment, management and responsible personnel, or in manufacturing methods and labeling, of any product for which a license is in effect or for which an application for license is pending, shall be reported to the Director, Bureau of Biologics, by the manufacturer, and unless in case of an emergency, not less than 30 days in advance of the time such changes are intended to be made.

(b) *Manufacturing methods and labeling.* Proposed changes in manufacturing methods and labeling may not become effective until notification of acceptance is received from the Director, Bureau of Biologics.

(c) *Failure to report.* Failure to report a change as required shall constitute a ground for summary suspension of a license.

Subpart B—Establishment Licensing

§ 601.10 Establishment licenses; issuance and conditions.

(a) *Inspection—compliance with standards.* An establishment license shall be issued only after inspection of the establishment and upon a determination that the establishment complies with the applicable standards prescribed in the regulations in this subchapter.

(b) *Availability of product; simultaneous request for and issuance of product license.* No establishment license shall be issued unless (1) a product intended for sale, barter or exchange or intended to be offered, sent, carried or brought for sale, barter or exchange is available for examination, (2) such product is available for inspection during all phases of manufacture and (3) a product license is requested and issued simultaneously with the establishment license.

(c) *One establishment license to cover all locations.* One establishment license shall be issued to cover all locations meeting the establishment standards.

§ 601.11 Registration of blood banks and other firms collecting, manufacturing, preparing, or processing human blood or blood products.

(a) All owners or operators of establishments that engage in the collection, manufacturing, preparation, propagation, compounding, or processing of human blood or blood products are required to register, pursuant to section 510 of the Federal Food, Drug, and Cosmetic Act. Registration and listing of products shall comply with Part 132 of this chapter. Registration does not permit any blood bank or similar establishment to ship blood or blood products in interstate commerce.

(b) Forms for registration of an establishment are obtainable on request from the Bureau of Drugs (HFD–315), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20852, or at any of the Food and Drug Administration district offices.

(c) The completed form should be mailed to Drug Registration Section, Bureau of Drugs (HFD–315), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20852.

Subpart C—Product Licensing

§ 601.20 Product licenses; issuance and conditions.

(a) *Examination—compliance with standards.* A product license shall be issued only upon examination of the product and upon a determination that the product complies with the standards prescribed in the regulations in this subchapter: *Provided*, That no product license shall be issued except upon a determination that the establishment complies with the establishment standards prescribed in the regulations contained in this subchapter, applicable to the manufacture of such product.

(b) *Manufacturing process—impairment of assurances.* No product shall be licensed if any part of the process of or relating to the manufacture of such product, in the judgment of the Commissioner of Food and Drugs, would impair the assurances of continued safety, purity and potency as provided by the regulations contained in this subchapter.

§ 601.21 Products under development.

A biological product or trivalent organic arsenical undergoing development, but not yet ready for a product license, may be shipped or otherwise delivered from one State or possession into another State or possession provided such shipment or delivery is not for sale, barter or exchange and is in accordance with section 505 of the Federal Food, Drug, and Cosmetic Act, as amended, and the regulations thereunder.

§ 601.22 Products in short supply; initial manufacturing at other than licensed establishment.

Licenses issued to a manufacturer for an establishment shall authorize persons other than such manufacturer to conduct at places other than such establishment the initial, and partial manufacturing of a product for shipment solely to such manufacturer only to the extent that the names of such persons and places are registered with the Commissioner of Food and Drugs and he finds, upon application of such manufacturer, that (a) the product is in short supply due either to the peculiar growth requirements of the organism involved or to the scarcity of the animal required for manufacturing purposes, and (b) such manufacturer has established with respect to such persons and places such procedures, inspections, tests or other arrangements as will assure full compliance with the applicable regulations of this subchapter related to continued safety, purity, and potency. Such persons and places shall be subject to all regulations of this subchapter except §§ 601.1 to 601.6, 601.10, 601.20, 601.21, 601.30 to 601.33, 601.40 to 601.44, and 610.60 to 610.65 of this chapter. Failure of such manufacturer to maintain such procedures, inspections, tests, or other arrangements, or failure of any person conducting such partial manufacturing to comply with applicable regulations shall constitute a ground for summary suspension or revocation of the authority conferred pursuant to this section on the same basis as provided in §§ 601.40, 601.42, and 601.43 with respect to the summary suspension and the revocation of licenses.

§ 601.25 Review procedures to determine that licensed biological products are safe, effective, and not misbranded under prescribed, recommended, or suggested conditions of use.

For purposes of reviewing biological products that have been licensed prior to July 1, 1972, to determine that they are safe and effective and not misbranded, the following regulations shall apply. Prior administrative action exempting biological products from the provisions of the Federal Food, Drug, and Cosmetic Act is superseded to the extent that these regulations result in imposing requirements pursuant to provisions therein for a designated biological product or category of products.

(a) *Advisory review panels.* The Commissioner of Food and Drugs shall appoint advisory review panels (1) to evaluate the safety and effectiveness of biological products for which a license has been issued pursuant to section 351 of the Public Health Service Act, (2) to review the labeling of such biological products, and (3) to advise him on which of the biological products under review are safe, effective, and not misbranded. An advisory review panel shall be established for each designated category of biological product. The members of a panel shall be qualified experts, appointed by the Commissioner, and shall include persons from lists submitted by organizations representing professional, consumer, and industry interests. Such persons shall represent a wide divergence of responsible medical and scientific opinion. The Commissioner shall designate the chairman of each panel, and summary minutes of all meetings shall be made.

(b) *Request for data and views.* (1) The Commissioner of Food and Drugs will publish a notice in the FEDERAL REGISTER requesting interested persons to submit, for review and evaluation by an advisory review panel, published and unpublished data and information pertinent to a designated category of biological products.

(2) Data and information submitted pursuant to a published notice, and falling within the confidentiality provisions of 18 U.S.C. 1905, 5 U.S.C. 552(b), or 21 U.S.C. 331(j), shall be handled by the advisory review panel and the Food and Drug Administration as confidential until publication of a proposed evaluation of the biologics under review and the full report or reports of the panel. Thirty days thereafter such data and information shall be made publicly available and may be viewed at the office of the Hearing Clerk of the Food and Drug Administration, except to the extent that the person submitting it demonstrates that it still falls within the confidentiality provisions of one or more of those statutes.

(3) To be considered, 12 copies of the submission on any marketed biological product within the class shall be submitted, preferably bound, indexed, and on standard sized paper, approximately 8½ x 11 inches. The time allotted for submissions will be 60 days, unless otherwise indicated in the specific notice requesting data and views for a particular category of biological products. When requested, abbreviated submissions should be sent. All submissions shall be in the following format, indicating "none" or "not applicable" where appropriate, unless changed in the FEDERAL REGISTER notice:

BIOLOGICAL PRODUCTS REVIEW INFORMATION

I. Label or labels and all other labeling (preferably mounted. Facsimile labeling is acceptable in lieu of actual container labeling), including labeling for export.

II. Representative advertising used during the past 5 years.

III. The complete quantitative composition of the biological product.

IV. Animal safety data.

A. Individual active components.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

B. Combinations of the individual active components.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

C. Finished biological product.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

V. Human safety data.

A. Individual active components.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

3. Documented case reports.

4. Pertinent marketing experiences that may influence a determination as to the safety of each individual active component.

5. Pertinent medical and scientific literature.

B. Combinations of the individual active components.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

3. Documented case reports.

4. Pertinent marketing experiences that may influence a determination as to the safety of combinations of the individual active components.

5. Pertinent medical and scientific literature.

C. Finished biological product.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

3. Documented case reports.

4. Pertinent marketing experiences that may influence a determination as to the safety of the finished biological product.

5. Pertinent medical and scientific literature.

VI. Efficacy data.

A. Individual active components.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

3. Documented case reports.

4. Pertinent marketing experiences that may influence a determination on the efficacy of each individual active component.

5. Pertinent medical and scientific literature.

B. Combinations of the individual active components.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

3. Documented case reports.

4. Pertinent marketing experiences that may influence a determination as to the effectiveness of combinations of the individual active components.

5. Pertinent medical and scientific literature.

C. Finished biological product.

1. Controlled studies.

2. Partially controlled or uncontrolled studies.

3. Documented case reports.

4. Pertinent marketing experiences that may influence a determination as to the effectiveness of the finished biological product.

5. Pertinent medical and scientific literature.

VII. A summary of the data and views setting forth the medical rational and purpose (or lack thereof) for the biological product and its components and the scientific basis (or lack thereof) for the conclusion that the biological product, including its components, has been proven safe and effective and is properly labeled for the intended use or uses. If there is an absence of controlled studies in the materials submitted, an explanation as to why such studies are not considered necessary or feasible shall be included.

VIII. If the submission is by a licensee, a statement signed by the responsible head (as defined in § 600.10 of this chapter) of the licensee shall be included, stating that to the best of his knowledge and belief, it includes all information, favorable and unfavorable, pertinent to an evaluation of the safety, effectiveness, and labeling of the product, including information derived from investigation, commercial marketing, or published literature. If the submission is by an interested person other than a licensee, a statement signed by the person responsible for such submission shall be included, stating that to the best of his knowledge and belief, it fairly reflects a balance of all the information, favorable and unfavorable, available to him pertinent to an evaluation of the safety, effectiveness, and labeling of the product.

(c) *Deliberations of an advisory review panel.* An advisory review panel will meet as often and for as long as is appropriate to review the data submitted to it and to prepare a report containing its conclusions and recommendations to the Commissioner of Food and Drugs with respect to the safety, effectiveness, and labeling of the biological products in the designated category under review.

(1) A panel may also consult any individual or group.

(2) Any interested person may request in writing an opportunity to present oral views to the panel. Such written requests for oral presentations should include a summarization of the data to be presented to the panel. Such request may be granted or denied by the panel.

(3) Any interested person may present written data and views which shall be considered by the panel. This information shall be presented to the panel in the format set forth in paragraph (b) (3) of this section and within the time period established for the biological product category in the notice for review by a panel.

(d) *Standards for safety, effectiveness, and labeling.* The advisory review panel, in reviewing the submitted data and preparing the panel's conclusions and recommendations, and the Commissioner of Food and Drugs, in reviewing and implementing the conclusions and recommendations of the panel, shall apply the following standards to determine that a biological product is safe and effective and not misbranded.

(1) Safety means the relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time. Proof of safety shall consist of adequate tests by methods reasonably applicable to show the biological product is safe under the prescribed conditions of use, including results of significant human experience during use.

(2) Effectiveness means a reasonable expectation that, in a significant proportion of the target population, the pharmacological or other effect of the biological product, when used under adequate directions, for use and warnings against unsafe use, will serve a clinically significant function in the diagnosis, cure, mitigation, treatment, or prevention of disease in man. Proof of effectiveness shall consist of controlled clinical investigations as defined in § 130.12(a) (5) (ii) of this chapter, unless this requirement is waived on the basis of a showing that it is not reasonably applicable to the biological product or essential to the validity of the investigation, and that an alternative method of investigation is adequate to substantiate effectiveness. Alternate methods, such as serological response evaluation in clinical studies and appropriate animal and other laboratory assay evaluations may be adequate to substantiate effectiveness where a previously accepted correlation between data generated in this way and clinical effectiveness already exists. Investigations may be corroborated by partially controlled or uncontrolled studies, documented clinical studies by qualified experts, and reports of significant human experience during marketing. Isolated case reports, random experience, and reports lacking the details which permit scientific evaluation will not be considered.

(3) The benefit-to-risk ratio of a biological product shall be considered in determining safety and effectiveness.

(4) A biological product may combine two or more safe and effective active components: (i) When each active component makes a contribution to the claimed effect or effects; (ii) when combining of the active ingredients does not decrease the purity, potency, safety, or effectiveness of any of the individual active components; and (iii) if the combination, when used under adequate directions for use and warnings against unsafe use, provides rational concurrent preventive therapy or treatment for a significant proportion of the target population.

(5) Labeling shall be clear and truthful in all respects and may not be false or misleading in any particular. It shall comply with section 351 of the Public Health Service Act and sections 502 and 503 of the Federal Food, Drug, and Cosmetic Act, and in particular with the applicable requirements of §§ 610.60 through 610.65 and 1.106 of this chapter.

(e) *Advisory review panel report to the Commissioner.* An advisory review panel shall submit to the Commissioner of Food and Drugs a report containing the panel's conclusions and recommendations with respect to the biological products falling within the category covered by the panel. Included within this report shall be:

(1) A statement which designates those biological products determined by the

panel to be safe and effective and not misbranded. This statement may include any condition relating to active components, labeling, tests required prior to release of lots, product standards, or other conditions necessary or appropriate for their safety and effectiveness.

(2) A statement which designates those biological products determined by the panel to be unsafe or ineffective, or to be misbranded. The statement shall include the panel's reasons for each such determination.

(3) A statement which designates those biological products determined by the panel not to fall within either subparagraph (1) or (2) of this paragraph on the basis of the panel's conclusion that the available data are insufficient to classify such biological products, and for which further testing is therefore required. The report shall recommend with as much specificity as possible the type of further testing required and the time period within which it might reasonably be concluded. The report shall also recommend whether the product license should or should not be revoked, thus permitting or denying continued manufacturing and marketing of the biological product pending completion of the testing. This recommendation will be based on an assessment of the present evidence of the safety and effectiveness of the product and the potential benefits and risks likely to result from the continued use of the product for a limited period of time while the questions raised concerning the product are being resolved by further study.

(f) *Proposed order.* After reviewing the conclusions and recommendations of the advisory review panel, the Commissioner of Food and Drugs shall publish in the FEDERAL REGISTER a proposed order containing:

(1) A statement designating the biological products in the category under review that are determined by the Commissioner of Food and Drugs to be safe and effective and not misbranded. This statement may include any condition relating to active components, labeling, tests required prior to release of lots, product standards, or other conditions necessary or appropriate for their safety and effectiveness, and may propose corresponding amendments in other regulations under this Subchapter F.

(2) A statement designating the biological products in the category under review that are determined by the Commissioner of Food and Drugs to be unsafe or ineffective, or to be misbranded, together with the reasons therefor. All licenses for such products shall be proposed to be revoked.

(3) A statement designating the biological products not included in either of the above two statements on the basis of the Commissioner of Food and Drugs determination that the available data are insufficient to classify such biological products under either subparagraphs (1) or (2) of this paragraph. Licenses for such products may be proposed to be revoked or to remain in effect on an interim basis. Where the Commissioner determines that the potential benefits outweigh the potential risks, the proposed order shall provide that the product license for any biological product, falling within this paragraph will not be revoked but will remain in effect on an interim basis while the data necessary to support its continued marketing are being obtained for evaluation by the Food and Drug Administration. The tests necessary to resolve whatever safety or effectiveness questions exist shall be described.

(4) The full report or reports of the panel to the Commissioner of Food and Drugs.

The summary minutes of the panel meeting or meetings shall be made available to interested persons upon request. Any interested person may, within 60 days after publication of the proposed order in the FEDERAL REGISTER, file with the Hearing Clerk of the Food and Drug Administration written comments in quintuplicate. Comments may be accompanied by a memorandum or brief in support thereof. All comments may be reviewed at the office of the Hearing Clerk during regular working hours, Monday through Friday.

(g) *Final order.* After reviewing the comments, the Commissioner of Food and Drugs shall publish in the FEDERAL REGISTER a final order on the matters covered in the proposed order. The final order shall become effective as specified in the order.

(h) *Additional studies.* (1) Within 30 days following publication of the final order, each licensee for a biological product designated as requiring further study to justify continued marketing on an interim basis, pursuant to paragraph (f) (3) of this section, shall satisfy the Commissioner of Food and Drugs in writing that studies adequate and appropriate to resolve the questions raised about the product have been undertaken, or the Federal Government may undertake the studies. The Commissioner may extend this 30-day period if necessary, either to review and act on proposed protocols or upon indication from the licensee that the studies will commence at a specified reasonable time. If no such commitment is made, or adequate and appropriate studies are not undertaken, the product license or licenses shall be revoked.

(2) A progress report shall be filed on the studies every January 1 and July 1 until completion. If the progress report is inadequate or if the Commissioner of Food and Drugs concludes that the studies are not being pursued promptly and diligently, or if interim results indicate the potential benefits do not outweigh the potential risks, the product license or licenses shall be revoked.

(3) Promptly upon completion of the studies undertaken on the product, the Commissioner of Food and Drugs will review all available data and will either retain or revoke the product license or licenses involved. In making this review and evaluation the Commissioner may again consult the advisory review panel which prepared the report on the product, or other advisory committees, professional organizations, or experts. The Commissioner shall take such action by notice published in the FEDERAL REGISTER.

(i) *Court Appeal.* The final order(s) published pursuant to paragraph (g) of this section, and any notice published pursuant to paragraph (h) of this section, constitute final agency action from which appeal lies to the courts. The Food and Drug Administration will request consolidation of all appeals in a single court. Upon court appeal, the Commissioner of Food and Drugs may, at his discretion, stay the effective date for part or all of the final order or notice, pending appeal and final court adjudication.

Subpart D—Licensing of Foreign Establishments and Products

§ 601.30 Licenses required; products for controlled investigation only.

Any biological or trivalent organic arsenical manufactured in any foreign country and intended for sale, barter or exchange shall be refused entry by collectors of customs unless manufactured in an establishment holding an unsuspended and unrevoked establishment license and license for the product. Unlicensed products which are not imported for sale, barter or exchange and which are intended solely for purposes of controlled investigation are admissible only if in accord with section 505 of the Federal Food, Drug, and Cosmetic Act, as amended, and the regulations thereunder.

§ 601.31 Procedure.

Except as otherwise provided in this subchapter, licenses for foreign establishments and products shall be issued, suspended, and revoked in the same manner as licenses for domestic establishments and products. Each foreign establishment holding a license and sending, carrying, or bringing any licensed product into any State or possession for sale, barter, or exchange shall file with the Director, Bureau of Biologics, the name and address of each person to whom such a product is thus sent, carried, or brought. Foreign licensees shall notify each person in the United States to whom such a product is thus sent, carried, or brought, to keep such records of distribution as are required of domestic licensed establishments. Failure to give such notice to maintain records shall constitute ground for revocation of license.

§ 601.32 Form of license.

Licenses for establishments located in foreign countries shall be in form similar to that for domestic establishments except that they shall authorize manufacture for sending, carrying, or bringing for sale, barter or exchange from the foreign country designated in the license into any State or possession of the United States and shall specify that it is issued upon the condition that the licensee will permit the inspection during all reasonable hours of the establishment by any officer, agent, or employee of the Department of Health, Education, and Welfare authorized by the Secretary for such purpose.

§ 601.33 Samples for each importation.

Random samples of each importation, obtained by the District Director of Customs and forwarded to the Director, Bureau of Biologics, shall be at least two final containers of each lot of product. A copy of the associated documents which describe and identify the shipment shall accompany the shipment for forwarding with the samples to the Director, Bureau of Biologics. For shipments of 20 or less final containers, samples need not be forwarded, provided a copy of an official release from the Bureau of Biologics accompanies each shipment.

Subpart E—Suspension of Licenses and Appeals Procedure

§ 601.40 Summary suspension.

Whenever the Commissioner of Food and Drugs has reasonable ground to believe that an establishment or product for which a license has been issued fails to conform to the standards prescribed in the regulations in this subchapter, and that by reason of such failure and of failure of the manufacturer to take prompt corrective measures on notice thereof, the distribution or sale of a licensed product would constitute a danger to health, or that the establishment and manufacturing methods have been so changed as to require in order to protect the public health a new showing that the establishment or product meets the standards prescribed in the regulations in this subchapter, he may recommend to the Secretary that the license for the establishment or the product be summarily suspended and the manufacturer be required (a) to notify the selling agents and distributors to whom such product or products have been delivered of such suspension, (b) to furnish complete records of such deliveries and notice of suspension, and (c) to show cause within 60 days or such other period as may be specified in the order why the license should not be revoked.

§ 601.41 Review Board.

When deemed advisable by the Commissioner of Food and Drugs, in matters involving the safety, purity, and potency of licensed products or products for which an application for license is pending, the reports of inspection and laboratory examinations, together with any pertinent data the establishment may submit, shall be passed upon by a special board of three officers appointed by the Commissioner of Food and Drugs for that purpose. The board shall report its findings to the Commissioner of Food and Drugs who will forward its report, together with his findings and recommendations, to the Secretary.

§ 601.42 Opportunity for hearing.

Any manufacturer whose application for a license has been denied, or whose establishment or product license has been summarily suspended, without prior opportunity for hearing, may appeal from such denial or suspension and shall be entitled to a hearing thereon before a review body constituted as provided in § 601.41. The Commissioner of Food and Drugs, upon review of the record, may affirm, reverse, or modify the findings of the review board, or may direct the taking of further testimony, and shall forward his determinations and recommendations to the Secretary.

§ 601.43 Suspension and revocation; publication.

Notice of suspension or revocation of license, with statement of cause therefor, may be published by the Secretary.

§ 601.44 Licenses; reissuance.

(a) *Compliance with standards.* An establishment or product license, previously suspended or revoked, whether upon application, or for failure to comply with standards or changes in standards prescribed in the regulations in this subchapter, may be reissued or reinstated upon a showing of compliance with required standards and upon such inspection and examination as may be considered necessary by the Director of the Bureau of Biologics.

(b) *Exclusion of noncomplying location.* An establishment or product license, excluding a location or locations that fail to comply with prescribed standards, may be issued without further application and concurrently with the suspension or revocation of the license for noncompliance at the excluded location or locations.

PART 610—GENERAL BIOLOGICAL PRODUCTS STANDARDS

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AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES. For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Release Requirements

§ 610.1 Tests prior to release required for each lot.

No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product. Each applicable test shall be made on each lot after completion of all processes of manufacture which may affect compliance with the standard to which the test applies. The results of all tests performed shall be considered in determining whether or not the test results meet the test objective, except that a test result may be disregarded when it is established that the test is invalid due to causes unrelated to the product.

§ 610.2 Requests for samples and protocols; official release.

Samples of any lot of any licensed product, together with the protocols showing results of applicable tests, may at any time be required to be sent to the Director, Bureau of Biologics. Upon notification by the Director, Bureau of Biologics, a manufacturer shall not distribute a lot of a product until the lot is released by the Director, Bureau of Biologics: *Provided*, That the Director shall not issue such notification except when deemed necessary for the safety, purity or potency of the product.

Subpart B—General Provisions

§ 610.10 Potency.

Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 600.3(s) of this chapter.

§ 610.11 General safety.

In addition to specified safety tests prescribed in this subchapter for individual products, a general safety test shall be performed in final container material, from each filling of each lot of all products intended for administration to man, either after the labels have been affixed to the final container, or affixed, both outside and inside, to the multiple container storage receptacle just prior to its sealing for storage purposes. Exceptions to this procedure may be authorized by the Director, Bureau of Biologics, when more than one lot is processed each day. The general safety test shall consist of the parenteral injection

of the maximum volume tolerated into each of two mice weighing approximately 20 gms. each and into each of two guinea pigs weighing approximately 350 gms. each but no more than 0.5 ml. need be inoculated into each mouse and no more than 5.0 ml. need be inoculated into each guinea pig. After injection the animals shall be observed for a period of no less than seven days and if neither significant symptoms nor death results during the observation period, the product meets the requirements for general safety. Variations of this test, either in the volume injected or in the species of test animal used shall be made whenever required because of the human dose level demanded of the product or because of any individual demands of the product itself.

§ 610.12 Sterility.

Except as provided in paragraphs (f) and (g) of this section, the sterility of each lot of each product shall be demonstrated by the performance of the tests prescribed in paragraphs (a) and (b) of this section for both bulk and final container material.

(a) *The test.* Bulk material shall be tested separately from final container material and material from each final container shall be tested in individual test vessels as follows:

(1) *Using Fluid Thioglycollate Medium*—(i) *Bulk and final container material.* The volume of product, as required by paragraph (d) of this section (hereinafter referred to also as the "inoculum"), from samples of both bulk and final container material, shall be inoculated into test vessels of Fluid Thioglycollate Medium. The inoculum and medium shall be mixed thoroughly and incubated at a temperature of 30° to 32° C. for a test period of no less than 14 days and examined visually for evidence of growth on the third, fourth, or fifth day and on the seventh or eighth day and on the last day of the test period. Results of each examination shall be recorded. If the inoculum renders the medium turbid so that the absence of growth cannot be determined reliably by visual examination, portions of this turbid medium in amounts of no less than 1.0 ml. shall be transferred on the third, fourth, or fifth day of incubation, from each of the test vessels and inoculated into additional vessels of medium. The material in the additional vessels shall be incubated at a temperature of 30° to 32° C. for no less than 14 days. Notwithstanding such transfer of material, examination of the original vessels shall be continued as prescribed above. The additional test vessels shall be examined visually for evidence of growth on the third, fourth, or fifth day of incubation and on the seventh or eighth day and on the last day of the incubation period. If growth appears, repeat tests may be performed as prescribed in paragraph (b) of this section and interpreted as specified in paragraph (c) of this section.

(ii) *Final container material containing a mercurial preservative.* In addition to the test prescribed in subparagraph (1) (i) of this paragraph, final container material containing a mercurial preservative shall be tested using Fluid Thioglycollate Medium following the procedures prescribed in such subparagraph, except that the incubation shall be at a temperature of 20° to 25° C.

(2) *Using Soybean-Casein Digest Medium.* Except for products containing a mercurial preservative, a test shall be made on final container material, following the procedures prescribed in subparagraph (1) (i) of this paragraph, except that the medium shall be Soybean-Casein Digest Medium and the incubation shall be at a temperature of 20° to 25° C.

(b) *Repeat tests*—(1) *Repeat bulk test.* If growth appears in the test of the bulk material, the test may be repeated to rule out faulty test procedures by testing at least the same volume of material.

(2) *First repeat final container test.* If growth appears in any test (Fluid Thioglycollate Medium or Soybean-Casein Digest Medium) of final container material, the test may be repeated to rule out faulty test procedures by testing material from a sample of at least the same number of final containers.

(3) *Second repeat final container test.* If growth appears in any first repeat final container test (Fluid Thioglycollate Medium or Soybean-Casein Digest Medium), that test may be repeated provided there was no evidence of growth in any test of the bulk material and material from a sample of twice the number of final containers used in the first test is tested by the same method used in the first test.

(c) *Interpretation of test results.* The results of all tests performed on a lot shall be considered in determining whether or not the lot meets the requirements for sterility, except that tests may be excluded when demonstrated by adequate controls to be invalid. The lot meets the test requirements if no growth appears in the tests prescribed in paragraph (a) of this section. If repeat tests are performed, the lot meets the test requirements if no growth appears in the tests prescribed in paragraph (b) (2) or (3) of this section, whichever is applicable.

(d) *Test samples and volumes*—(1) *Bulk.* Each sample for the bulk sterility test shall be representative of the bulk material and the volume tested shall be no less than 10 ml. (Note exceptions in paragraph (g) of this section.)

(2) *Final containers.* The sample for the final container and first repeat final container test shall be no less than 20 final containers from each filling of each lot, selected to represent all stages of filling from the bulk vessel. If the amount of material in the final container is 1.0 ml. or less, the entire contents shall be tested. If the amount of material in the final container is more than 1.0 ml., the volume tested shall be the largest single dose recommended by the manufacturer or 1.0 ml., whichever is larger, but no more than 10 ml. of material or the entire contents from a single final container need be tested. If more than two filling machines, each with either single or multiple filling stations, are used for filling one lot, no less than 10 filled containers shall be tested from each filling machine, but no more than 100 containers of each lot need be tested. The items tested shall be representative of each filling assembly and shall be selected to represent all stages of the filling operation. (Note exceptions in paragraph (g) of this section.)

(e) *Culture medium*—(1) *Formulae.* (i) The formula for Fluid Thioglycollate Medium is as follows:

FLUID THIOGLYCOLLATE MEDIUM

l-cystine	0.5 Gm.
Sodium chloride	2.5 Gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 Gm.
Granular agar (less than 15% moisture by weight).	0.75 Gm.
Yeast extract (water-soluble)	5.0 Gm.
Pancreatic digest of casein	15.0 Gm.
Purified water	1,000.0 ml.
Sodium thioglycollate (or thioglycolic acid—0.3 ml.).	0.5 Gm.
Resazurin (0.10% solution, freshly prepared).	1.0 ml.
pH after sterilization 7.1±0.2	

(ii) The formula for Soybean-Casein Digest Medium is as follows:

SOYBEAN-CASEIN DIGEST MEDIUM

Pancreatic Digest of Casein	17.0 Gm.
Papaic Digest of Soybean Meal.	3.0 Gm.
Sodium Chloride	5.0 Gm.
Dibasic Potassium Phosphate	2.5 Gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	2.5 Gm.
Purified water	1,000.0 ml.
pH after sterilization 7.3±0.2	

(2) *Culture media requirements*—(i) *Growth promoting qualities.* Each lot of dehydrated medium bearing the manufacturer's identifying number, or each lot of medium prepared from basic ingredients, shall be tested for its growth-promoting qualities using not more than 100 organisms of two or more strains of microorganisms that are exacting in their nutritive and aerobic-anaerobic requirements.

(ii) *Conditions of medium and design of test vessels.* A medium shall not be used if the extent of evaporation affects its fluidity, nor shall it be reused in a sterility test. Fluid Thioglycollate Medium shall not be used if more than the upper one-third has acquired a pink color. The medium may be restored once by heating on a steam bath or in free-flowing steam until the pink color disappears. The design of the test vessel for Fluid Thioglycollate Medium shall be such as is shown to provide favorable aerobic and anaerobic growth of microorganisms throughout the test period.

(iii) *Ratio of the inoculum to culture medium.* The ratio of the inoculum to the volume of the culture medium resulting in a dilution of the product that is not bacteriostatic or fungistatic shall be determined for each product, except for those tested by membrane filtration. Vessels of the product-medium mixture(s) and control vessels of the medium shall be inoculated with dilutions of cultures of bacteria or fungi which are sensitive to the product being tested, and incubated at the appropriate temperature for no

less than 7 days. Inhibitors or neutralizers of preservatives may be considered in determining the proper ratio.

(f) *Membrane filtration.* Bulk and final container material of products containing oil or products in water insoluble ointments shall be tested for sterility using the membrane filtration procedure set forth in The United States Pharmacopeia [1] (18th Revision, 1970), section entitled "Membrane Filtration," pages 853–854, except that (1) the test samples shall conform with paragraph (d) of this section, (2) the temperature of incubation for the test using Fluid Thioglycollate Medium shall be 30° to 32° C. and (3) in addition, for products containing a mercurial preservative, the product shall be tested in a second test using Fluid Thioglycollate Medium incubated at 20° to 25° C. in lieu of the test in Soybean-Casein Digest Medium. Such Membrane Filtration section is hereby incorporated by reference and deemed published herein. The United States Pharmacopeia is available at most medical and public libraries and copies of the pertinent section will be provided to any manufacturer affected by the provisions of this subchapter upon request to the Director, Bureau of Biologics or the appropriate Information Center Offices listed in 45 CFR Part 5. In addition, an official historic file of the material incorporated by reference is maintained in the office of the Director, Bureau of Biologics.

(g) *Exceptions.* Bulk and final container material shall be tested for sterility as described above in this section, except as follows:

(1) *Different sterility tests prescribed.* When different sterility tests are prescribed for a product in this subchapter.

(2) *Alternate incubation temperatures.* Two tests may be performed, in all respects as prescribed in paragraph (a) (1) (i) of this section, one test using an incubation temperature of 18° to 22° C., the other test using an incubation temperature of 35° to 37° C., in lieu of performing one test using an incubation temperature of 30° to 32° C.

(3) *Different tests equal or superior.* A different test (such as membrane filtration as set forth in paragraph (f) of this section) may be performed provided that prior to the performance of such test a manufacturer submits data which the Commissioner of Food and Drugs, finds adequate to establish that the different test is equal or superior to the tests described in paragraphs (a) and (b) of this section in detecting contamination and makes the finding a matter of official record.

(4) *Test precluded or not required.* The tests prescribed in this section need not be performed for Whole Blood (Human), Cryoprecipitated Antihemophilic Factor (Human), Leukocyte Typing Serum, Red Blood Cells (Human), Single Donor Plasma (Human), Source Plasma (Human), Smallpox Vaccine and other similar products concerning which the Commissioner of Food and Drugs, finds that the mode of administration, the method of preparation or the special nature of the product precludes or does not require a sterility test.

(5) *Viscid or turbid products.* Alternative Thioglycollate Medium may be used in place of Fluid Thioglycollate Medium for the testing of products that are viscid or turbid or otherwise do not lend themselves to culturing in Fluid Thioglycollate Medium, provided it has been freshly prepared or has been heated on a steam bath or in free-flowing steam and cooled just prior to use and is used in a suitable vessel that will maintain aerobic and anerobic conditions throughout the incubation period. The formula for the Alternative Thioglycollate Medium follows:

ALTERNATIVE THIOGLYCOLLATE MEDIUM

l-cystine	0.5 Gm.
Sodium chloride	2.5 Gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 Gm.
Yeast extract (water soluble)	5.0 Gm.
Pancreatic digest of casein	15.0 Gm.
Purified water	1,000.0 ml.
Sodium thioglycollate (or thioglycollic acid—0.3 ml.)	0.5 Gm.

pH after sterilization 7.1±0.2.

(6) *Number of final containers more than 20, less than 200.* If the number of final containers in the filling is more than 20 or less than 200, the sample shall be no less than 10 percent of the containers.

(7) *Number of final containers—20 or less.* If the number of final containers in a filling is 20 or less, the sample shall be two final containers, or the sample need be no more than one final container, provided (i) the bulk material met the sterility test requirements and (ii) after filling, it is demonstrated by testing a simulated sample that all surfaces to which the product was exposed were free of contaminating microorganisms. The simulated sample shall be prepared by rinsing the filling equipment with sterile 1.0 percent peptone solution, pH 7.1±0.1, which shall be discharged into a final container by the same method used for filling the final containers with the product.

(8) *Samples—large volume of product in final containers.* For Normal Serum Albumin (Human), Normal Human Plasma, Antihemophilic Plasma (Human), Plasma Protein Fraction (Human) and Fibrinogen (Human), when the volume of product in the final container is 50 ml. or more, the final containers selected as the test sample may contain less than the full volume of product in the final containers of the filling from which the sample is taken: *Provided,* That the containers and closures of the sample are identical with those used for the filling to which the test applies and the sample represents all stages of that filling.

(9) *Diagnostic products not intended for injection.* For diagnostic products not intended for injection, (i) only the Thioglycollate Medium test incubated at 30° to 32° C. is required, (ii) the volume of material for the bulk test shall be no less than 2.0 ml., and (iii) the sample for the final container test shall be no less than three final containers if the total number filled is 100 or less, and, if greater, one additional container for each additional 50 containers or fraction thereof, but the sample need be no more than 10 containers.

(10) *Immune globulin preparations.* For immune globulin preparations, the test samples from the bulk material and from each final container need be no more than 2.0 ml.

§ 610.13 Purity.

Products shall be free from extraneous material except for unavoidable bacteriophage. In addition, products shall be tested as provided in paragraphs (a) and (b) of this section.

(a) *Test for residual moisture.* Each lot of dried product shall be tested for residual moisture and other volatile substances.

(1) *Procedure.* The test for dried products shall consist of measuring the maximum loss of weight in a weighed sample equilibrated over anhydrous P_2O_5 at a pressure of not more than one mm. of mercury, and at a temperature of 20° to 30° C. for as long as it has been established is sufficient to result in a constant weight.

(2) *Test results; standard to be met.* The residual moisture and other volatile substances shall not exceed 1 percent except that for BCG Vaccine they shall not exceed 1½ percent, for Measles Virus Vaccine, Live, Attenuated, Measles-Smallpox Vaccine, Live; Rubella Virus Vaccine, Live and Antihemophilic Factor (Human), they shall not exceed 2 percent, and for Modified Plasma (Bovine); Thrombin; Fibrinogen; Streptokinase; Streptokinase - Streptodornase; and Anti-Influenza Virus Serum for the Hemagglutination Inhibition Test, they shall not exceed 3 percent.

(b) *Test for pyrogenic substances.* Each lot of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in subparagraphs (1) and (2) of this paragraph: *Provided,* That notwithstanding any other provision of this subchapter, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Cryoprecipitated Antihemophilic Factor (Human); Single Donor Plasma (Human); Source Plasma (Human); Normal Horse Serum; Normal Rabbit Serum; bacterial viral and rickettsial vaccines and antigens; toxoids; toxins, allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

(1) *Test dose.* The test dose for each rabbit shall be at least 3 milliliters per kilogram of body weight of the rabbit and also shall be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended, but need not exceed 10 ml. per kilogram of body weight of the rabbit, except that: (i) Regardless of the human dose recommended, the test dose per kilogram of body weight of each rabbit shall be, at least 1 milliliter for immune globulins derived from human

[1] Copies may be obtained from: United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852.

blood, at least 3 milliliters for Normal Human Plasma, and at least 30 milligrams for Fibrinogen (Human); (ii) for Streptokinase, Streptokinase-Streptodornase, Aggregated Radio-Iodinated (I^{131}) Albumin (Human), Radio-Chromater (Cr^{51}) Serum Albumin (Human), Radio-Iodinated (I^{125}) Serum Albumin (Human) and Radio-Iodinated (I^{131}) Serum Albumin (Human), the test dose shall be at least equivalent proportionately on a body weight basis to the maximum single human dose recommended.

(2) *Procedure.* Products shall be tested for freedom from pyrogenic substances by intraveneous injection of the test dose into three or more rabbits in overt good health and by recording for each rabbit a control temperature taken within one hour prior to injection, and three additional temperatures taken one, two, and three hours after injection. For purposes of subparagraph (3) of this paragraph, if there is no temperature increase over the control temperature (i.e. where the temperature remains unchanged or falls), the temperature rise shall be considered as zero. If there is an increase in temperature over the control temperature, the temperature rise shall be the difference between the highest of the three hourly readings and the control temperature reading.

(3) *Test results; standards to be met.* The results recorded for all rabbits used in all tests of a lot of a product shall be included in determining whether the standard for purity is met. The product fails to meet test requirements if one-half or more of all rabbits show a temperature rise of 0.6° C. or more or if the average temperature rise of all rabbits is 0.5° C. or more.

(c) *Different tests equal or superior.* A different test for residual moisture may be performed provided that prior to its performance the manufacturer submits data which the Commissioner of Food and Drugs finds adequate to establish that the different test is equal or superior to the test described in paragraph (a) of this section and makes the finding a matter of official record.

§ 610.14 Identity.

The contents of a final container of each filling of each lot shall be tested for identity after all labeling operations shall have been completed. The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory. Identity may be established either through the physical or chemical characteristics of the product, inspection by macroscopic or microscopic methods, specific cultural tests, or in vitro or in vivo immunological tests.

§ 610.15 Constituent materials.

(a) *Ingredients, preservative, diluents, adjuvants.* All ingredients used in a licensed product, and any diluent provided as an aid in the administration of the product, shall meet generally accepted standards of purity and quality. Any preservative used shall be sufficiently nontoxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in the combination used shall not denature the specific substances in the product below the minimum acceptable potency within the dating period when stored at the recommended temperature. Products in multiple dose containers shall contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine, Poliovirus Vaccine, Live, Oral, or to viral vaccines labeled for use with the jet injector, or to dried vaccines when the accompanying diluent contains a preservative. An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product. In no event shall the recommended individual dose of a biological product contain more than 0.85 milligram of aluminum, determined by assay, or more than 1.14 milligrams of aluminum, determined by calculation on the basis of the amount of aluminum compound added.

(b) *Extraneous protein; cell culture produced vaccines.* Extraneous protein known to be capable of producing allergenic effects in human subjects shall not be added to a final virus medium of cell culture produced vaccines intended for injection. If serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(c) *Antibiotics.* A minimum concentration of antibiotics, other than penicillin, may be added to the production substrate of viral vaccines.

§ 610.16 Total solids in serums.

Except as otherwise provided by regulation, no liquid serum or antitoxin shall contain more than 20 percent total solids.

§ 610.17 Permissible combinations.

Licensed products may not be combined with other licensed products, either therapeutic, prophylactic or diagnostic, except as a license is obtained for the combined product. Licensed products may not be combined with nonlicensable therapeutic, prophylactic, or diagnostic substances except as a license is obtained for such combination.

§ 610.18 Cultures.

(a) *Storage and maintenance.* Cultures used in the manufacture of products shall be stored in a secure and orderly manner, at a temperature and by a method that will retain the initial characteristics of the organisms and insure freedom from contamination and deterioration.

(b) *Identity and verification.* Each culture shall be clearly identified as to source strain. A complete identification of the strain shall be made for each new stock culture preparation. Primary and subsequent seed lots shall be identified by lot number and date of preparation. Periodic tests shall be performed as often as necessary to verify the integrity of the strain characteristics and freedom from extraneous organisms. Results of all periodic tests for verification of cultures and determination of freedom from extraneous organisms shall be recorded and retained.

Subpart C—Standard Preparations and Limits of Potency

§ 610.20 Standard preparations.

Standard preparations made available by the Bureau of Biologics shall be applied in testing, as follows:

(a) *Potency standards.* Potency standards shall be applied in testing for potency all forms of the following:

ANTIBODIES

Botulism Antitoxin, Type A.
Botulism Antitoxin, Type B.
Botulism Antitoxin, Type E.
Diphtheria Antitoxin.
Dysentery Antitoxin (Shiga).
Anti-Hemophilus Influenzae Type b Serum.
Histolyticus Antitoxin.
Oedematiens Antitoxin.
Perfringens Antitoxin.
Antipertussis Serum.
Antirabies Serum.
Scarlet Fever Streptococcus Antitoxin.
Sordellii Antitoxin.
Staphylococcus Antitoxin.
Tetanus Antitoxin.
Vibrion Septique Antitoxin.

ANTIGENS

Diphtheria Toxin for Schick Test.
Pertussis Vaccine.
Scarlet Fever Streptococcus Toxin.
Tuberculin, Old.
Tuberculin, Purified Protein Derivative.
Typhoid Vaccine.

BLOOD DERIVATIVE

Thrombin.

(b) *Opacity standard.* The U.S. Opacity Standard shall be applied in estimating the bacterial concentration of all bacterial vaccines. The assigned value of the standard when observed visually is 10 units. The assigned value of the standard when observed with a photometer is (i) 10 units when the wavelength of the filter is 530 millimicrons, (ii) 10.6 units when the wavelength of the filter is 650 millimicrons, and (iii) 9 units when the wavelength of the filter is 420 millimicrons.

§ 610.21 Limits of potency.

The potency of the following products shall be not less than that set forth below and products dispensed in the dried state shall represent liquid products having the stated limitations.

ANTIBODIES

Diphtheria Antitoxin, 500 units per milliliter.
Scarlet Fever Streptococcus Antitoxin, 400 units per milliliter.
Tetanus Antitoxin, 400 units per milliliter.
Tetanus Immune Globulin (Human), 50 units of tetanus antitoxin per milliliter.

ANTIGENS

Pertussis Vaccine, 12 units per total human immunizing dose.
Typhoid Vaccine, 8 units per milliliter.

Subpart D—Mycoplasma

§ 610.30 Test for *Mycoplasma*.

Except as provided otherwise in this subchapter, prior to clarification or filtration in the case of live virus vaccines

produced from in vitro living cell cultures, and prior to inactivation in the case of inactivated virus vaccines produced from such living cell cultures, each virus harvest pool and control fluid pool shall be tested for the presence of *Mycoplasma*, as follows:

Samples of the virus for this test shall be stored either (1) between 2° and 8° C. for no longer than 24 hours, or (2) at −20° C. or lower if stored for longer than 24 hours. The test shall be performed on samples of the viral harvest pool and on control fluid pool obtained at the time of viral harvest, as follows: No less than 2.0 ml. of each sample shall be inoculated in evenly distributed amounts over the surface of no less than 10 plates of at least two agar media. No less than 1.0 ml. of sample shall be inoculated into each of four tubes containing 10 ml. of a semisolid broth medium. The media shall be such as have been shown to be capable of detecting known *Mycoplasma* and each test shall include control cultures of at least two known strains of *Mycoplasma*, one of which must be *M. pneumoniae*. One half of the plates and two tubes of broth shall be incubated aerobically at 36° C. ±1° C. and the remaining plates and tubes shall be incubated anaerobically at 36° C. ±1° C. in an environment of 5–10 percent CO_2 in N_2. Aerobic incubation shall be for a period of no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than 4 additional plates and incubated aerobically. Anaerobic incubation shall be for no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than four additional plates and incubated anaerobically. All inoculated plates shall be incubated for no less than 14 days, at which time observation for growth of *Mycoplasma* shall be made at a magnification of no less than 300×. If the Dienes Methylene Blue-Azure dye or an equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of *Mycoplasma*. The presence of the *Mycoplasma* shall be determined by comparison of the growth obtained from the test samples with that of the control cultures, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for vaccine manufacture if none of the tests on the samples show evidence of the presence of *Mycoplasma*.

Subpart E—Hepatitis Requirements

§ 610.40 Test for hepatitis associated (Australia) antigen.

(a) *General.* Each donation of human blood, plasma, or serum to be used in preparing a biological product shall be tested for the presence of hepatitis associated (Australia) antigen. Such test shall be performed on blood, plasma, or serum taken from the donor at the time of donation or, for such material collected prior to the effective date of this section, upon removal from storage by the manufacturer. Only hepatitis associated antibody (anti-Australia antigen) licensed under this subchapter shall be used in performing the test and the test method(s) used shall be that for which the antibody product is specifically designed to be effective as recommended by the manufacturer in the package enclosure.

(b) *Restrictions on use*—(1) *Injectable biological products.* Blood, plasma, or serum that is reactive when tested for hepatitis associated (Australia) antigen shall not be used in manufacturing injectable biological products.

(2) *In vitro diagnostic biological products.* Blood, plasma, or serum that is reactive when tested for hepatitis associated (Australia) antigen may be used in manufacturing in vitro diagnostic biological products, provided that the package label of the biological products prepared from such blood, plasma, or serum conspicuously indicates that the product was prepared from material that was reactive when tested for hepatitis associated antigen and may transmit viral hepatitis.

§ 610.41 History of hepatitis associated (Australia) antigen.

A person testing positive, or known to have previously tested positive, for hepatitis associated (Australia) antigen may not serve as a donor of human blood, plasma, or serum to be used in preparing any injectable biological product, except that a person known to have previously tested positive for hepatitis associated (Australia) antigen may serve as a source of hepatitis associated antibody when such antibody is required for the manufacture of a licensed biological product provided such person meets the requirements of § 610.40 at the time of donation.

Subpart F—Dating Period Limitations

§ 610.50 Date of manufacture.

The date of manufacture shall be determined as follows:

(a) For products for which an official standard of potency is prescribed in either § 610.20 or § 610.21, or which are subject to official potency tests, the date of initiation by the manufacturer of the last valid potency test.

(b) For products which are not subject to official potency tests, (1) the date of removal from animals, (2) the date of extraction, (3) the date of solution, or (4) the date of cessation of growth, whichever is applicable.

§ 610.51 Periods of cold storage.

Except as otherwise provided in the regulations of this subchapter, products may be held in cold storage by the manufacturer as follows:

At a temperature not above 5°C.—1 year.
At a temperature not above 0°C.—2 years.

§ 610.52 Dating period.

The dating period for a combination of two or more products shall be no longer than the dating period of the component product with the shortest dating period. The dating period for a product shall begin on the date of manufacture, except that the dating period may begin on the date of issue from the manufacturer's cold storage, provided the product was maintained as prescribed in § 610.51. If held in the manufacturer's cold storage beyond the period prescribed, the dating period shall be reduced by a corresponding period.

§ 610.53 Dating periods for specific products.

The following dating periods are based on data relating to usage, clinical experience or laboratory tests that establish the period beyond which the product cannot be expected beyond reasonable doubt to yield its specific results and retain its safety, purity, and potency, provided the product is maintained at the recommended temperatures. The standards prescribed by the regulations in this subchapter, designed to insure continued safety, purity, and potency of the products, are based on the dating periods set forth below. Cold storage periods and temperatures prescribed in § 610.51 shall apply and outside labels shall recommend storage between 2° C. and 8° C., except when specifially provided otherwise. (Storage temperatures and storage periods are given in parentheses after the dating periods below when they differ from those specified in § 610.51.)

Adsorbed Anti-A Serum	One year.
Adenovirus and Influenza Virus Vaccines Combined Aluminum Hydroxide Adsorbed.	Six months (5° C., six months).
Adenovirus and Influenza Virus Vaccines Combined Aluminum Phosphate Adsorbed.	Six months (5° C., six months).
Adenovirus Vaccine	Six months (5° C., six months).
Aggregated Radio-Iodinated (I^{131}) Albumin (Human).	Thirty days. § 610.51 does not apply.
Allergenic Extracts	With 50 percent or more glycerin, three years (5° C., three years). With less than 50 percent glycerin, eighteen months (5° C., eighteen months). Products for which cold storage conditions are inappropriate, eighteen months, provided labeling recommends storage at no warmer than 30° C. § 610.51 does not apply. Powders and tablets, five years, provided labeling recommends storage at no warmer than 30° C. § 610.51 does not apply. Freeze dried products, five years (5° C., three years).
Allergenic Extracts, Alum Precipitated	Eighteen months (5° C., eighteen months).
Anthrax Vaccine, Adsorbed	One year (5° C., two years). § 610.51 does not apply.
Anti-A Blood Grouping Serum	*Liquid:* One year. *Dried:* Five years.

Anti-A, B Blood Grouping Serum	*Liquid:* One year. *Dried:* Five years.
Anti-B Blood Grouping Serum	*Liquid:* One year. *Dried:* Five years.
Anti-Di^a Serum (Anti-Diego)	One year.
Anti-Fy^a Serum (Anti-Duffy)	One year.
Anti-Fy^b Serum	One year.
Anti-Gr (Vw) Serum	One year.
Antihemophilic Factor (Human)	One year. § 610.51 does not apply.
Antihemophilic Globulin (Human)	One year.
Antihemophilic Plasma (Human)	Five years.
Anti-*Hemophilus influenzae* Type b Serum.	*Liquid:* Two years. *Dried:* Five years.
Anti-Human Chorionic Gonadotropic Serum.	One year. § 610.51 does not apply.
Anti-Human Serum	*Liquid:* One year. *Dried:* Five years.
Anti-I Serum	One year.
Anti-Influenza Virus Serum for the Hemagglutination Inhibition Test.	Two years.
Anti-Jk^a Serum (Anti-Kidd)	One year.
Anti-Jk^b Serum	One year.
Anti-Js^a Serum (Anti-Sutter)	One year.
Anti-k Serum (Anti-Cellano)	One year.
Anti-K Serum (Anti-Kell)	One year.
Anti-Kp^a Serum (Anti-Penney)	One year.
Anti-Kp^b and Anti-K Serum (Anti-Rautenberg and Anti-Kell).	One year.
Anti-Kp^b Serum (Anti-Rautenberg)	One year.
Anti-Le^a Serum (Anti-Lewis)	*Liquid:* One year. *Dried:* Five years.
Anti-Le^b Serum	*Liquid:* One year. *Dried:* Five years.
Anti-Lu^a Serum (Anti-Lutheran)	One year.
Anti-M Serum	One year.
Anti-M^g Serum	One year.
Anti-Mi^a Serum (Anti-Miltenberger)	One year.
Anti-N Serum	One year.
Anti-P Serum	One year.
Antirabies Serum	Two years.
Anti-Rh Typing Serum, Anti-hr$'$ (Anti-c).	One year.
Anti-Rh Typing Serum, Anti-hr$''$ (Anti-e).	One year.
Anti-Rh Typing Serum, Anti-hr^s (Anti-V).	One year.
Anti-Rh Typing Serum, Anti-rh$'$ (Anti-C).	*Liquid:* One year. *Dried:* Five years.
Anti-Rh Typing Serum, Anti-rh$''$ (Anti-E).	*Liquid:* One year. *Dried:* Five years.
Anti-Rh Typing Serum, Anti-Rh$_o$ (Anti-D).	*Liquid:* One year. *Dried:* Five years.
Anti-Rh Typing Serum, Anti-Rh$_o$$'$ (Anti-CD).	*Liquid:* One year. *Dried:* Five years.
Anti-Rh Typing Serum, Anti-Rh$_o$$''$ (Anti-DE).	One year.
Anti-Rh Typing Serum, Anti-Rh$_o$rh$'$rh$''$ (Anti-CDE).	One year.
Anti-Rh Typing Serum, Anti-Rh$_o$+ (Anti-D+D^u).	One year.
Anti-Rh Typing Serum, Anti-rh^w (Anti-C^w).	One year.
Anti-rh^w and Anti-K Serum (Anti-(C^w+Kell)).	One year.
Anti-s Serum	*Liquid:* One year. *Dried:* Five years.
Anti-S Serum	One year.
Antivenin (*Crotalidae*) Polyvalent	Five years with an initial 10 percent excess of potency, provided labeling recommends storage at no warmer than 37° C.
Antivenin (*Latrodectus mactans*)	Five years with an initial 10 percent excess of potency.
Antivenin (*Micrurus fulvius*)	Five years with an initial 10 percent excess of potency.
Anti-U Serum (Anti-Su)	One year.
Anti-Wr^a Serum (Anti-Wright)	One year.
B. histolyticus Antitoxin	Five years with an initial 20 percent excess of potency.
B. oedematiens Antitoxin	Five years with an initial 20 percent excess of potency.
B. sordellii Antitoxin	Five years with an initial 20 percent excess of potency.
BCG Vaccine	Six months (5° C., one year).
Blastomycin	Two years (5° C., one year).
Blood Group Specific Substances A and B.	Two years.
Blood Group Specific Substance A	Two years.
Blood Group Specific Substance B	Two years.
Botulism Antitoxin	Five years with an initial 20 percent excess of potency.
Chicken Pox Immune Serum (Human)	*Liquid:* One year. *Dried:* Five years.
Cholera Vaccine	Eighteen months (5° C., one year).
Cobra Venom with Silicic and Formic Acids.	Eighteen months (5° C., one year).
Cobra Venom Solution	Eighteen months (5° C., one year).
Coccidioidin	Three years (5° C., one year).
Collagenase	Eighteen months, provided labeling recommends storage at no warmer than 25° C. § 610.51 does not apply.
Cryoprecipitated Antihemophilic Factor (Human).	12 months from the date of collection of source blood, provided labeling recommends storage at not above −18° C. § 610.51 does not apply.
Diphtheria Antitoxin	*Liquid:* Five years with an initial 20 percent excess of potency. *Dried:* Five years with an initial 10 percent excess of potency.
Diphtheria and Tetanus Toxoids and Pertussis and Poliomyelitis Vaccines Adsorbed.	Four months (5° C., two months).
Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed and Poliomyelitis Vaccine.	(a) Four months (5° C., two months). (b) One year, provided the pertussis and poliomyelitis components unmixed when issued (5° C., one year).
Diphtheria and Tetanus Toxoids and Pertussis Vaccine.	Eighteen months (5° C., one year).
Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed.	Eighteen months (5° C., one year).
Diphtheria and Tetanus Toxoids and Poliomyelitis Vaccine.	One year (5° C., one year).

Diphtheria and Tetanus Toxoids	Two years (5° C., one year).
Diphtheria and Tetanus Toxoids Adsorbed.	Two years (5° C., one year).
Diphtheria Toxin for Schick Test	One year (5° C., one year).
Diphtheria Toxoid	Two years (5° C., one year).
Diphtheria Toxoid Adsorbed	Two years (5° C., one year).
Diphtheria Toxoid and Pertussis Vaccine	Eighteen months (5° C., one year).
Diphtheria Toxoid and Pertussis Vaccine Adsorbed.	Eighteen months (5° C., one year).
Dysentery Antitoxin, Shiga	Five years with an initial 20 percent excess of potency.
Equine Encephalomyelitis Vaccine (Eastern).	One year.
Equine Encephalomyelitis Vaccine (Western).	One year.
Fibrinogen (Human)	Five years.
Fibrinogen with Antihemophilic Factor (Human).	Five years.
Fibrinolysin (Human)	Two years.
Fibrinolysin and Desoxyribonuclease Combined (Bovine).	Three years, provided labeling recommends storage at no warmer than 30° C.
Fibrinolysin and Desoxyribonuclease Combined (Bovine) with Chloramphenical.	Three years, provided labeling recommends storage at no warmer than 30° C.
Gas Gangrene Polyvalent Antitoxin	Five years with an initial 20 percent excess of potency.
Haemophilus influenzae Typing Serum	One year.
Hepatitis Associated Antibody (Anti-Australia Antigen)	Six months (5° C., 6 months) except Iodinated (^{125}I) products.
	Iodinated (^{125}I) products, 45 days. § 610.51 does not apply.
Histamine Azoprotein	Two years.
Histoplasmin	Two years (5° C., one year).
Immune Serum Globulin (Human)	Three years (5° C., three years).
Influenza Virus Hemagglutinating Antigen.	Two years (5° C., one year).
Influenza Virus Vaccine	Eighteen months (5° C., one year).
Leukocyte Typing Serum (Dried)	Two years.
Anti-HL-A 1	Two years.
Anti-HL-A 2	Two years.
Anti-HL-A 3	Two years.
Anti-HL-A 5	Two years.
Anti-HL-A 7	Two years.
Anti-HL-A 8	Two years.
Anti-HL-A 9	Two years.
Anti-HL-A 10	Two years.
Anti-HL-A 11	Two years.
Anti-HL-A 12	Two years.
Anti-HL-A 13	Two years.
Lymphogranuloma Venereum Antigen	One year (5° C., one year).
Measles Immune Serum (Human)	*Liquid:* One year.
	Dried: Five years.
Measles Immune Globulin (Human)	Three years (5° C., three years).
Measles-Smallpox Vaccine, Live	One year. § 610.51 does not apply.
Measles Virus Vaccine, Inactivated	One year (5° C., one year).
Measles Virus Vaccine, Live, Attenuated	One year. § 610.51 does not apply.
Modified Plasma (Bovine)	Twenty months. § 610.51 does not apply.
Mumps Immune Serum (Human)	*Liquid:* One year.
	Dried: Five years.
Mumps Immune Globulin (Human)	Three years from date the dried or frozen bulk product is placed in final solution (5° C., three years).
Mumps Skin Test Antigen	Eighteen months (5° C., one year).
Mumps Vaccine	Eighteen months (5° C., one year).
Mumps Virus Vaccine, Live	One year. § 610.51 does not apply.
Normal Bovine Serum	Five years.
Normal Horse Serum	Five years.
Normal Human Plasma	*Liquid:* Three years provided product is maintained between 15° and 30° C., and labeling recommends storage between 15° and 30° C. § 610.51 does not apply.
	Dried: Seven years provided labeling recommends storage not above 37° C. § 610.51 does not apply.
Normal Human Serum	*Liquid:* Eighteen months.
	Dried: Five years.
	Frozen: Three years, provided labeling recommends storage at no warmer than −18° C. § 610.51 does not apply.
	Melted: One year after the date of melting. § 610.51 does not apply.
Normal Rabbit Serum	Five years.
Normal Serum Albumin (Human)	(a) Five years, provided labeling recommends storage between 2° and 10° C. (5° C., three years).
	or
	(b) Three years, provided labeling recommends storage at room temperature, no warmer than 37° C. (5° C., three years).
	or
	(c) Ten years, if in an hermetically sealed metal container and provided labeling recommends storage between 2° and 10° C. § 610.51 does not apply.
Oxophenarsine Hydrochloride	Three years (5° C., one year).
Perfringens Antitoxin	Five years with an initial 20 percent excess of potency.
Pertussis Immune Globulin (Human)	Three years from date the dried or frozen bulk product is placed in final solution (5° C., three years).
Pertussis Immune Serum (Human)	*Liquid:* One year.
	Dried: Five years.
Pertussis Vaccine	Eighteen months (5° C., one year).
Plague Vaccine	Eighteen months (5° C., one year).
Plasma Protein Fraction (Human)	(a) Five years (5° C., one year).
	(b) Three years, provided labeling recommends storage at no warmer than 30° C. (5° C., one year).
Pneumococcus Typing Serum	One year.
Poliomyelitis Immune Globulin (Human).	Three years (5° C., three years).
Poliomyelitis Vaccine	One year (5° C., one year).
Poliomyelitis Vaccine Adsorbed	One year (5° C., one year).
Poliovirus Vaccine, Live, Oral, Trivalent	*Frozen:* One year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state (−10° C., one year).
	Liquid: Thirty days, provided labeling recommends storage between 2° and 8° C. § 610.51 does not apply.
Poliovirus Vaccine, Live, Oral, Type I	*Frozen:* One year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state (−10° C.,

	one year). *Liquid:* Thirty days, provided labeling recommends storage between 2° and 8° C. § 610.51 does not apply.
Poliovirus Vaccine, Live, Oral, Type II	*Frozen:* One year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state (−10° C., one year). *Liquid:* Thirty days, provided labeling recommends storage between 2° and 8° C. § 610.51 does not apply.
Poliovirus Vaccine, Live, Oral, Type III	*Frozen:* One year, provided labeling recommends storage at a temperature which will maintain ice continuously in a solid state (−10° C., one year). *Liquid:* Thirty days, provided labeling recommends storage between 2° and 8° C. § 610.51 does not apply.
Polyvalent bacterial antigens with "No U.S. Standard of Potency."	*Liquid:* Eighteen months (5° C., one year). *Dried:* Five years (5° C., one year).
Polyvalent bacterial vaccines with "No U.S. Standard of Potency."	*Liquid:* Eighteen months (5° C., one year). *Dried:* Five years (5° C., one year).
Polyvalent modified bacterial antigens with "No U.S. Standard of Potency."	*Liquid:* Eighteen months (5° C., one year). *Dried:* Five years (5° C., one year).
Polyvalent sensitized bacterial vaccines with "No U.S. Standard of Potency."	*Liquid:* Eighteen months (5° C., one year). *Dried:* Five years (5° C., one year).
Profibrinolysin (Human)	Two years.
Pseudomonas Polysaccharide	Eighteen months.
Q Fever Vaccine	One year (5° C., one year).
Rabies Vaccine	*Liquid:* Six months (5° C., three months). *Dried:* Eighteen months.
Radio-Chromated (Cr^{51}) Serum Albumin (Human).	Sixty days from the date chromium is added. § 610.51 does not apply.
Radio-Iodinated (I^{125}) Serum Albumin (Human).	120 days from date iodination is completed. § 610.51 does not apply.
Radio-Iodinated (I^{131}) Serum Albumin (Human).	Thirty days from date iodination is completed. § 610.51 does not apply.
Reagent Red Blood Cells (Human)	Twenty-one days. § 610.51 does not apply.
Reagent Blood Group Specific Substances A and B.	Two years.
Red Blood Cells (Human)	(a) Twenty-one days from date of collection of source blood, provided labeling recommends storage between 1° and 10° C. and the hermetic seal is not broken during processing. § 610.51 does not apply. Twenty-four hours after plasma removal, provided labeling recommends storage between 1° and 10° C., if the hermetic seal is broken during processing. § 610.51 does not apply. (b) *Frozen:* Three years, provided labeling recommends storage at −65° C. or colder. Twenty-four hours after removal from storage at −65° C. or colder, provided labeling recommends storage between 1° and 10° C. § 610.51 does not apply.
Resuspended Red Blood Cells (Human)	Ten days. § 610.51 does not apply.
Rh$_0$ (D) Immune Globulin (Human)	Six months (5° C., six months).
Rocky Mountain Spotted Fever Vaccine	Eighteen months (5° C., one year).
Rubella Virus Vaccine, Live	One year. § 610.51 does not apply.
Russell Viper Venom	Five years.
Scarlet Fever Immune Serum (Human)	*Liquid:* One year. *Dried:* Five years.
Scarlet Fever Streptococcus Toxin for Dick Test.	One year (5° C., one year).
Scarlet Fever Streptococcus Toxin for Immunization.	One year (5° C., one year).
Shick Test Control	One year (5° C., one year).
Single Donor Plasma (Human)	(a) Five years, provided labeling recommends storage at not above −18° C. § 610.51 does not apply. (b) If used in coagulation defects, one year, provided labeling recommends storage at not above −18° C. § 610.51 does not apply.
Smallpox Vaccine	*Liquid:* Three months, provided labeling recommends storage at no warmer than 0° C. (−10° C., nine months, if product is maintained as glycerinated or equivalent vaccine in bulk or final containers). *Dried:* Eighteen months (5° C., six months).
Source Plasma (Human)	In lieu of an expiration date, the collection date shall appear on the label, as prescribed in § 640.69(e)(4) of this chapter.
Staphylococcus Antitoxin	Five years with an initial 20 percent excess of potency.
Staphylococcus Toxoid	Two years (5° C., one year).
Staphylococcus Toxoid and Bacterial Antigen made from Staphylococcus (Albus and Aureus).	Eighteen months (5° C., one year).
Staphylococcus Toxoid and Bacterial Vaccine made from Staphylococcus (Aureus).	Eighteen months (5° C., one year).
Staphylococcus Toxoid, Streptococcus Toxin, and Bacterial Vaccine made from Staphylococcus (Aureus), Streptococcus (Hemolyticus), Pneumococcus, *Hemophilus influenzae.*	One year (5° C., one year).
Streptococcus Erythrogenic Toxin	One year (5° C., one year).
Streptokinase	Eighteen months.
Streptokinase-Streptodornase	*Dried:* Two years (5° C., one year). *Tablets:* Eighteen months, provided labeling recommends storage at no warmer than 30° C. (5° C., six months).
Tetanus and Diphtheria Toxoids Adsorbed (For Adult Use).	Two years (5° C., one year).
Tetanus and Gas Gangrene Polyvalent Antitoxin.	Five years with an initial 20 percent excess of potency.
Tetanus Immune Globulin (Human)	Three years with an initial 10 percent excess of potency (5° C., one year).
Tetanus Antitoxin	*Liquid:* Five years with an initial 20 percent excess of potency. *Dried:* Five years with an initial 10 percent excess of potency.
Tetanus Toxoid	Two years (5° C., one year).
Tetanus Toxoid Adsorbed	Two years (5° C., one year).
Tetanus Toxoid and Pertussis Vaccine	Eighteen months (5° C., one year).
Thrombin	Three years.
Trichinella Extract	Eighteen months (5° C., one year).

Tuberculin	*Old, concentrated:* Containing 50 percent glycerin, five years. *Old diluted:* One year. *Purified Protein Derivative, concentrated:* Two years containing 50 percent glycerin (5° C., one year). *Purified Protein Derivative, diluted:* One year. § 610.51 does not apply. *Purified Protein Derivative, dried:* Five years. *Old, dried on multiple puncture device:* Two years, provided labeling recommends storage at no warmer than 30° C. (30° C., one year).
Typhoid and Paratyphoid Vaccine	Eighteen months (5° C., one year).
Typhoid Vaccine	Eighteen months (5° C., one year).
Typhus Vaccine	Eighteen months (5° C., one year).
Vibrion Septique Antitoxin	Five years with an initial 20 percent excess of potency.
Whole Blood (Human) collected in	(a) ACD solution—Twenty-one days, provided labeling recommends storage between 1° and 10° C. § 610.51 does not apply. (b) Heparin solution—Forty-eight hours, provided labeling recommends storage between 1° and 10° C. § 610.51 does not apply. (c) CPD solution—Twenty-one days, provided labeling recommends storage between 1° and 10° C. § 610.51 does not apply.
Yellow Fever Vaccine	One year, provided labeling recommends storage at no warmer than 5° C. (−20° C., one year).

Subpart G—Labeling Standards

§ 610.60 Container label.

(a) *Full label.* The following items shall appear on the label affixed to each container of a product capable of bearing a full label:

(1) The proper name of the product;

(2) The name, address, and license number of manufacturer;

(3) The lot number or other lot identification;

(4) The expiration date;

(5) The recommended individual dose, for multiple dose containers.

(b) *Package label information.* If the container is not enclosed in a package, all the items required for a package label shall appear on the container label.

(c) *Partial label.* If the container is capable of bearing only a partial label, the container shall show as a minimum the name (expressed either as the proper or common name), the lot number or other lot identification and the name of the manufacturer; in addition, for multiple dose containers, the recommended individual dose. Containers bearing partial labels shall be placed in a package which bears all the items required for a package label.

(d) *No container label.* If the container is incapable of bearing any label, the items required for a container label may be omitted, provided the container is placed in a package which bears all the items required for a package label.

(e) *Visual inspection.* When the label has been affixed to the container a sufficient area of the container shall remain uncovered for its full length or circumference to permit inspection of the contents.

§ 610.61 Package label.

The following items shall appear on the label affixed to each package containing a product:

(a) The proper name of the product;

(b) The name, address, and license number of manufacturer;

(c) The lot number or other lot identification;

(d) The expiration date;

(e) The preservative used and its concentration, or if no preservative is used and the absence of a preservative is a safety factor, the words "no preservative";

(f) The number of containers, if more than one;

(g) The amount of product in the container expressed as (1) the number of doses, (2) volume, (3) units of potency, (4) weight, (5) equivalent volume (for dried product to be reconstituted), or (6) such combination of the foregoing as needed for an accurate description of the contents, whichever is applicable;

(h) The recommended storage temperature;

(i) The words "Shake Well", "Do not Freeze" or the equivalent, as well as other instructions, when indicated by the character of the product;

(j) The recommended individual dose if the enclosed container(s) is a multiple-dose container;

(k) The route of administration recommended, or reference to such directions in an enclosed circular;

(l) Known sensitizing substances, or reference to an enclosed circular containing appropriate information;

(m) The type and calculated amount of antibiotics added during manufacture;

(n) The inactive ingredients when a safety factor, or reference to an enclosed circular containing appropriate information;

(o) The adjuvant, if present;

(p) The source of the product when a factor in safe administration;

(q) The identity of each microorganism used in manufacture, and, where applicable, the production medium and the method of inactivation, or reference to an enclosed circular containing appropriate information;

(r) Minimum potency of product expressed in terms of official standard of potency or, if potency is a factor and no U.S. standard of potency has been prescribed, the words "No U.S. standard of potency."

(s) For injectable products prepared from human blood, plasma, or serum, indication that the product was prepared from blood that was nonreactive when tested for hepatitis associated (Australia) antigen. In lieu of inclusion on the package label, such information may be included in a circular enclosed with the package.

§ 610.62 Proper name; package label; legible type.

(a) *Position.* The proper name of the product on the package label shall be placed above any trademark or trade name identifying the product and symmetrically arranged with respect to other printing on the label.

(b) *Prominence.* The point size and typeface of the proper name shall be at least as prominent as the point size and typeface used in designating the trademark and trade name. The contrast in color value between the proper name and the background shall be at least as great as the color value between the trademark and trade name and the background. Typography, layout, contrast, and other printing features shall not be used in a manner that will affect adversely the prominence of the proper name.

(c) *Legible type.* All items required to be on the container label and package label shall be in legible type. "Legible type" is type of a size and character which can be read with ease when held in a good light and with normal vision.

§ 610.63 Divided manufacturing responsibility to be shown.

If two or more establishments participate in the manufacture of a product, the name, address, and license number of each must appear on the package label, and on the label of the container if capable of bearing a full label.

§ 610.64 Name of selling agent or distributor.

The name and address of the selling agent or distributor of a product may appear on the label under the designation of "selling agent" or "distributor" provided that the name and address of the manufacturer is given precedence in prominence.

§ 610.65 Products for export.

Labels on packages or containers of products for export may be adapted to meet specific requirements of the regulations of the country to which the product is to be exported provided that in all such cases the minimum label requirements prescribed in § 610.60 are observed.

PART 620—ADDITIONAL STANDARDS FOR BACTERIAL PRODUCTS

Subpart A—Pertussis Vaccine

Sec.

AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES.—For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Pertussis Vaccine

§ 620.1 Pertussis Vaccine.

The proper name of this product shall be "Pertussis Vaccine", which shall be an aqueous preparation of either killed whole *Bordetella pertussis* bacteria or a fraction of *Bordetella pertussis* bacteria. The vaccine may be precipitated or adsorbed and may be combined with other antigens.

§ 620.2 Production.

(a) *Propagation of bacteria.* Human blood shall not be used in culture medium for propagating bacteria either for seed or for vaccine. The culture medium for propagating bacteria for vaccine shall not contain ingredients known to be capable of producing allergenic effects in human subjects, except blood or blood products from lower animals other than the horse. When blood or a blood product is used, it shall be removed by washing the harvested bacteria. The bacterial concentrate shall be free of extraneous bacteria, fungi, and yeasts, as demonstrated by microscopic examination and cultural methods.

(b) *Bacterial content.* (1) The opacity of the bacterial concentrate shall be determined in terms of the U.S. Opacity Standard not later than 2 weeks after the harvest of the bacteria and before any treatment capable of altering the opacity of the bacterial concentrate.

(2) The total immunizing dose of a vaccine prepared with whole bacteria shall contain (i) in the case of nonadsorbed vaccine no more bacteria than the equivalent of 60 opacity units and (ii) in the case of adsorbed vaccine no more than the equivalent of 48 opacity units.

(c) *Detoxification.* After removing a sample for purity testing, the bacteria shall be killed and detoxified either (1) by heating, (2) by addition of a chemical agent and appropriate aging, or (3) by any combination of the stated procedures. The procedure used shall be one that has been shown to have no adverse effect on required safety, purity, and potency.

(d) *Preservative.* The vaccine shall contain a preservative.

§ 620.3 U.S. Standard preparations.

(a) The U.S. Standard Pertussis Vaccine shall be used for determining the potency of Pertussis Vaccine.

(b) The U.S. Opacity Standard shall be used in estimating the bacterial content of the vaccine and of the challenge culture.

§ 620.4 Potency test.

The number of protective units of the total human immunizing dose shall be estimated for each lot of vaccine from the results of simultaneous intracerebral mouse protection tests of the vaccine under test and the U.S. Standard Pertussis Vaccine. The potency test shall be performed as follows:

(a) *Mice.* Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weight varying no more than 4 grams in a single test. In no event shall any of the mice weigh less than 10 grams or more than 20 grams. A system of randomization shall be used to distribute the mice into the groups, with respect to shelf position and to determine the order of challenge. There shall be at least 3 groups consisting of no less than 16 mice each, for each vaccine. In addition, there shall be at least 4 groups consisting of no less than 10 mice each, for control purposes: one group for the challenge dose and 3 groups for titrating the virulence of the challenge dose.

(b) *Vaccination.* (1) Five-fold serial dilutions of the vaccine to be tested and of the standard vaccine shall be made in 0.85 percent sodium chloride solution. The dilutions of the vaccine under test shall have the same protective unitage, based on an estimate of 12 units per total human immunizing dose, as the unitage of the corresponding dilution of the standard vaccine. Each mouse in each group for vaccination shall be injected intraperitoneally with 0.5 ml. of the appropriate dilution.

(2) The interval between vaccination and challenge shall be 14 to 17 days. At least 87.5 percent of the mice in each group shall survive the period between vaccination and challenge and each mouse challenged shall appear healthy.

(c) *The challenge.* (1) The challenge culture of *Bordetella pertussis* for each test shall be taken from a batch of cultures which have been maintained by a method, such as freeze-drying, that retains constancy of virulence.

(2) The challenge and virulence titration doses shall be prepared as follows: The bacteria shall be harvested from a 20 to 24 hour culture grown on Bordet-Gengou medium seeded from a rapidly growing culture less than 48 hours old and uniformly suspended in a solution containing 1.0 percent casein peptone and about 0.6 percent sodium chloride at pH 7.1±0.1. The suspension, freed from agar particles and clumps of bacteria, and adjusted to an opacity of 10 units, shall be diluted in the solution used for suspending the bacteria, to provide in a volume of 0.03 ml. (i) a challenge dose of 0.0001 opacity units (1: 3000) and (ii) virulence titration doses of $\frac{1}{50}$, $\frac{1}{250}$ and $\frac{1}{1250}$ respectively of the challenge dose.

(3) Each vaccinated mouse shall be injected intracerebrally with the challenge dose. The four groups of control mice shall be injected intracerebrally with the challenge dose and its three dilutions, respectively. The challenge-dose control mice shall be injected last. The interval between the removal of the bacteria from the culture medium and the injection of the last mouse shall not exceed 2½ hours.

(d) *Recording the results.* The mice shall be observed for 14 days. Mice dying within 72 hours after challenge shall be excluded from the test. Records shall be maintained of the number of mice that die after 72 hours and of the number of mice showing both paralysis and enlargement of the head at the end of 14 days. All mice that show both paralysis and enlargement of the head shall be considered as deaths for the purposes of determining the ED_{50}.

(e) *Validity of the test.* The test shall be valid provided (1) the ED_{50} of the vaccine under test and the standard vaccine is between the largest and smallest vaccinating doses; (2) the limits of one standard deviation of each ED_{50} fall within the range of 64 percent to 156 percent; (3) the protective response is graded in relation to the vaccinating doses; (4) the dose-response curves of the vaccine under test and the standard vaccine are parallel; (5) the challenge dose contains approximately 200 LD_{50}; (6) the LD_{50} contains no more than 300 colony forming units; and (7) the $\frac{1}{1250}$ dilution of the challenge dose contains no less than 10 and no more than 50 colony forming units.

(f) *Estimate of the potency.* The ED_{50} of each vaccine shall be calculated by a method that provides an estimate of the standard deviation. The protective unit value per total human immunizing dose of the vaccine under test shall be calculated in terms of the unit value of the standard vaccine.

(g) *Potency requirements.* The vaccine shall have a potency of 12 units per total human immunizing dose based upon either a single test estimate of no less than 8 units or a two-, three- or four-test geometric mean estimate of no less than 9.6, 10.8, or 12 units, respectively, except that for the vaccine in a multiple antigen product containing Poliomyelitis Vaccine, the estimate shall be no less than 14 units. In no event shall the estimate be more than 36 units.

(h) *Test design variation.* Variations in the design of the potency test may be permitted providing the results are demonstrated to be of equal or greater precision.

§ 620.5 Mouse toxicity test.

The final vaccine shall be demonstrated to be free from toxicity by the following test:

A group of no less than 10 mice, each mouse weighing 14 to 16 grams, shall have free access to food and water for no less than 2 hours before injection. The group weight of the mice shall be determined immediately prior to injection. Each mouse shall be injected intraperitoneally with a test dose of one-half of the largest recommended single human dose of the final vaccine in a volume of no less than 0.5 ml. nor more than 0.75 ml. The group weight of the mice shall be determined at the end of 72 hours and at the end of 7 days after injection. At the end of 72 hours the average weight per mouse may be no less than the average weight per mouse immediately preceding the injection; at the end of 7 days the average weight gain per mouse may be no less than 3.0 grams; and at the end of 7 days there may be vaccine-related deaths of no more than 5 percent of the total number of mice in all the toxicity tests performed.

§ 620.6 General requirements.

(a) *Safety.* The safety test prescribed in § 610.11 of this chapter shall be made on final container material except that the test shall consist of the intraperitoneal injection of no less than one-half of the largest individual human dose recommended into each of at least two mice weighing approximately 20 grams each, and either the intraperitoneal injection of no less than 3 times the largest individual human dose recommended or the subcutaneous injection of 5.0 ml., into each of at least two guinea pigs weighing approximately 350 grams each. The last sentence of § 610.11 of this chapter does not apply.

(b) *Dose.* These additional standards are based on a single injection of 0.5 ml., 1.0 ml., or 1.5 ml., and a total human immunizing dose of three single injections of a nonadsorbed vaccine, and two or three single injections of an adsorbed vaccine.

(c) *Product characteristics.* Recommendations shall be made through appropriate labeling that the product after issue should not be frozen and should be well shaken immediately prior to use.

(d) *Labeling.* In addition to the items required by other applicable labeling provisions of this part, the package label shall give the following information:

1. For a vaccine containing a precipitant or an adsorbent, the word "Adsorbed" shall follow the proper name in the same style of type and prominence as the proper name.
2. The total immunizing dose contains 12 units of pertussis vaccine.

(e) *Multiple antigen products.* The Pertussis Vaccine component of multiple antigen products shall be manufactured pursuant to these additional standards, except that the mouse toxicity test (§ 620.5) and the potency test (§ 620.4) shall be performed on the multiple antigen product.

(f) *Adsorbed vaccines.* Only aluminum compound reagents shall be introduced into the product to cause precipitation or adsorption of either Pertussis Vaccine or other antigens incorporated with Pertussis Vaccine.

(g) *Freezing prohibition.* Pertussis Vaccine and multiple antigen products of which Pertussis Vaccine is a component shall not be frozen at any time during storage.

(h) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014.

(1) A sample of no less than 20 milliliters of the final product for pertussis vaccine testing.

(2) Protocols showing summaries of the manufacturing processes and the results of all mouse toxicity (§ 620.5) and potency (§ 620.4) tests performed.

§ 620.7 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Pertussis Vaccine shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart B—Typhoid Vaccine

§ 620.10 Typhoid Vaccine.

The proper name of this product shall be Typhoid Vaccine which shall be an aqueous or dried preparation of killed *Salmonella typhosa* bacteria.

§ 620.11 Production.

(a) *Strain of bacteria.* Strain Ty 2 of *Salmonella typhosa* shall be used in the manufacture of Typhoid Vaccine.

(b) *Propagation of bacteria.* The culture medium for propagation of *S. typhosa* shall not contain ingredients known to be capable of producing allergenic effects in human subjects. The harvested bacteria shall be free of extraneous bacteria, fungi and yeasts, as demonstrated by microscopic examination and cultural methods.

(c) *Bacterial content.* (1) The number of bacteria in the concentrate of harvested bacteria shall be estimated not later than 2 weeks after harvest and before any treatment capable of altering the accuracy of the estimate.

(2) The number of *S. typhosa* bacteria in the vaccine shall not exceed 10^9 per ml.

(d) *Nitrogen content.* The total nitrogen content of the vaccine shall not exceed 0.035 mg./ml. for nonextracted bacteria preparations and shall not exceed 0.023 mg./ml. for acetone-extracted bacteria preparations.

(e) *Preservative.* Aqueous vaccine and the solution for reconstitution supplied with dried vaccine shall contain a preservative. Dried vaccine shall not contain a preservative.

§ 620.12 U.S. Standard preparations.

(a) The U.S. Standard Typhoid Vaccine shall be used for determining the potency of Typhoid Vaccine.

(b) The U.S. Opacity Standard shall be used to adjust the opacity of the suspension from which the challenge culture is prepared.

§ 620.13 Potency test.

The number of potency units per milliliter shall be estimated for each lot of vaccine from the results of simultaneous mouse protection tests of the vaccine under test and of the U.S. Standard Typhoid Vaccine. The test shall be performed as follows:

(a) *Mice.* Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weights between 13 and 16 grams. A system of randomization shall be used to distribute the mice into the groups, with respect to shelf position and to determine the order of challenge. There shall be at least three groups consisting of no less than 16 mice each, for each vaccine. In addition, there shall be at least four groups consisting of no less than 10 mice each, for control purposes; one group for the challenge dose and three groups for titrating the virulence of the challenge dose.

(b) *Inoculation of vaccine.* (1) Serial dilutions, no greater than 5-fold, of the vaccine to be tested and of the standard vaccine shall be made in saline (0.85 percent sodium chloride solution). The mid-dilution of each vaccine shall contain that amount of vaccine which will afford protection to approximately 50 percent of the mice. Each mouse in each group for inoculation shall be injected intraperitoneally with 0.5 ml. of the appropriate dilution.

(2) The interval between inoculation of the vaccine and challenge shall be no less than 7 days nor more than 14 days. At least 87.5 percent of the mice in each group shall survive the period between vaccine inoculation and challenge and each mouse challenged shall appear healthy.

(c) *The challenge.* (1) The challenge culture of Strain Ty 2 of *S. typhosa* for each test shall be taken from a batch of cultures maintained by a method, such as freeze-drying, that retains constancy of virulence.

(2) The challenge and virulence titration doses shall be prepared as follows: The bacteria shall be harvested from a 5- to 6-hour culture grown at 36°±1° C. on a nutrient agar medium which shall have been seeded from a 16- to 20-hour culture grown at 36°±1° C. on a nutrient agar medium, and the harvested bacteria then shall be uniformly suspended in saline. The suspension, freed from agar particles and clumps of bacteria and adjusted to an opacity of 10 units, shall be diluted in saline by 10-fold increments. The suspensions for the challenge and virulence titration doses shall be put into a sterile gastric mucin preparation. The challenge suspension shall be prepared from whichever bacterial dilution provides about 1,000 colony forming units for an 0.5 ml. challenge dose. The virulence titration suspensions shall be 10^1, 10^2, and 10^3 dilutions respectively of the challenge suspension.

(3) Each mouse inoculated with vaccine shall be injected intraperitoneally

with an 0.5 ml. dose of the challenge suspension. Each mouse in the four groups of control mice shall be injected intraperitoneally with an 0.5 ml. dose of the challenge suspension and its three dilutions, respectively. The challenge dose control mice shall be injected last. The interval between removal of the bacteria from the culture medium and the injection of the last mouse shall not exceed 2½ hours.

(d) *Recording the results.* The mice shall be observed daily for 3 days. A record shall be maintained of the number of mice that die. A record of the number of mice that survive shall be made at the end of the observation period.

(e) *Validity of the test.* The test is valid provided: (1) the ED_{50} of the vaccine under test and the Standard Vaccine is between the largest and smallest doses inoculated into the mice; (2) the limits of one standard deviation of the ED_{50} of each vaccine fall within the range of 61 percent to 163 percent; (3) a graded protective response is obtained in relation to the vaccine dilutions; (4) the dose response curves of the vaccine under test and the standard vaccine are parallel; (5) the challenge dose contains approximately 1,000 colony forming units; and (6) the LD_{50} of the challenge dose contains no more than 10 colony forming units.

(f) *Repeat tests.* If the test does not meet the criteria prescribed in paragraph (e) of this section, repeat tests may be performed, and the combined results of all tests shall meet the paragraph (e) criteria, except that the limits of one standard deviation of the ED_{50} shall be reduced in proportion to the total number of mice in a test group. Tests established as invalid pursuant to § 610.1 of this chapter may be disregarded.

(g) *Estimate of the potency.* The ED_{50} of each vaccine shall be calculated by a method that provides an estimate of the standard deviation. The protective unit value per milliliter of the vaccine under test shall be calculated in terms of the unit value of the standard vaccine.

(h) *Potency requirements.* The vaccine shall have a potency of 8 units per milliliter. Variations in potency unit estimates are acceptable provided the estimate is not less than 5.0 units per milliliter.

§ 620.14 General requirements.

(a) *Dose.* These standards are based on a human adult dose of 0.5 ml. for a single injection and a total immunizing dose of two injections of 0.5 ml. given at appropriate intervals.

(b) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, the package label shall state that the vaccine contains 8 units per milliliter.

(c) *Samples; protocols; official release.* For each lot of vaccine, the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014.

(1) A sample of no less than 40 ml. of the product distributed in no less than four containers.

(2) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

The product shall not be issued by the manufacturer until notification of official release is received from the Director, Bureau of Biologics, for each filling lot of dried vaccine and for each bulk lot of aqueous vaccine.

§ 620.15 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Typhoid Vaccine, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs, so finds and makes such finding a matter of official record.

Subpart C—Anthrax Vaccine, Adsorbed

§ 621.20 Anthrax Vaccine, Adsorbed.

The proper name of this product shall be Anthrax Vaccine, Adsorbed, which shall consist of an aqueous preparation of a fraction of *Bacillus anthracis* which contains the protective antigen adsorbed on aluminum hydroxide.

§ 620.21 Production.

(a) *Strain of bacteria.* A nonencapsulated, nonproteolytic, avirulent strain of *Bacillus anthracis* shall be used in the manufacture of anthrax vaccine.

(b) *Medium.* A chemically defined medium shall be used for the propagation of *Bacillus anthracis* which has protective-antigen promoting properties that are no less effective than the protective-antigen promoting properties of the Puziss and Wright 1095 medium as set forth in U.S. Patent No. 3,208,909, issued September 28, 1965, which patent is hereby incorporated by reference and deemed published herein. U.S. Patent No. 3,208,909 has been assigned to the Federal Government and copies will be provided to persons affected by the provisions of this subchapter upon request to the Director, Bureau of Biologics, or to the appropriate Information Center Officer listed in 45 CFR, Part 5. Copies also may be obtained upon request from the U.S. Patent Office, Washington, DC. The medium shall not contain ingredients known to be capable of producing allergenic effects in human subjects.

(c) *Propagation of bacteria.* The medium shall be inoculated with a 24-hour old vegetative culture seeded from a stock suspension of spores. The propagation culture, flushed with nitrogen, shall be incubated at 37° C.±1.0° C., agitated for approximately 27 hours, cooled to about 20° C., the pH adjusted to 8.0±0.1 and then filtered through a sterilizing filter(s) using nitrogen gas under pressure.

(d) *Adsorption of the protective antigen.* The sterile filtrate shall be adsorbed on sterile aluminum hydroxide gel and the recovered precipitate shall be resuspended and diluted in sterile 0.85 percent sodium chloride solution.

§ 620.22 U.S. Reference preparation.

The U.S. Reference Anthrax Vaccine distributed by the Bureau of Biologics shall be used for determining the potency of anthrax vaccine.

§ 620.23 Potency test.

The potency of each lot of vaccine shall be estimated from the results of simultaneous tests of the vaccine under test and the U.S. Reference Anthrax Vaccine. The test shall be performed as follows:

(a) *Guinea pigs.* Healthy guinea pigs shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weights between 325 and 350 grams. The diet of the guinea pigs shall be supplemented with vitamin C throughout the test period. At least three groups of no less than eight guinea pigs shall be used for each vaccine and at least one group of four guinea pigs shall be used for the challenge control.

(b) *Vaccination.* Serial dilutions, not greater than three-fold, of each vaccine shall be made in 0.85 percent sodium chloride solution. The mid-dilution of the vaccine under test shall contain that amount of vaccine which will afford protection to approximately 50 percent of the guinea pigs in the group vaccinated with that dilution. Each guinea pig in the test and reference vaccine groups shall be injected subcutaneously with 0.5 ml. of the appropriate dilution on the left side of the abdomen and about 2 cm. from the midline. The interval between vaccination and challenge shall be 14 days.

(c) *The challenge.* Each vaccinated and control guinea pig shall be injected intracutaneously on the right side of the abdomen with 0.1 ml. of a spore suspension of the virulent Vollum strain of *Bacillus anthracis* diluted in sterile distilled water to contain 10,000 spores per milliliter.

(d) *Recording the results.* The guinea pigs shall be observed daily for 10 days and the deaths recorded. The number of survivors shall be recorded at the end of the observation period.

(e) *Validity of the test.* The test shall be valid provided (1) the protective response to each vaccine is graded in relation to the amount of vaccine in the respective dilutions and (2) all control animals die within 10 days.

(f) *Potency requirement.* The potency of the product is satisfactory if the vaccine is no less potent than the reference. The potency of the product is considered to be equal to the reference when (1) the average time of death of the product-vaccinated guinea pigs is no less than the average time of death of the reference-vaccinated guinea pigs and the number of survivors of the product-vaccinated guinea pigs is no less than the number of survivors of the reference-vaccinated guinea pigs, or (2) the use of another statistical procedure, shown to be adequate for evaluating the potency of anthrax vaccine, demonstrates that

the product is no less potent than the reference.

§ 620.24 General requirements.

(a) *Dose.* These standards are based on a single human dose of 0.5 ml. and a total primary immunizing doses of three single doses, each given at appropriate intervals.

(b) *Product characteristics.* Recommendation shall be made through appropriate labeling that the product after issue should not be frozen.

(c) *Samples; protocols; official release.* For each lot of vaccine, the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville, Pike, Bethesda, MD 20014:

(1) A protocol which consists of a summary of the manufacture of each lot including all results of all tests for which test results are requested by the Director, Bureau of Biologics.

(2) A sample of no less than 40 ml. of the final product distributed in approximately equal amounts into four final containers.

The product shall not be issued by the manufacturer until notification of official release of the lot is received from the Director, Bureau of Biologics.

§ 620.25 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to anthrax vaccine, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such findings a matter of official record.

PART 630—ADDITIONAL STANDARDS FOR VIRAL VACCINES

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AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES.—For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Polimyelitis Vaccine

§ 630.1 Poliomyelitis Vaccine.

(a) *Proper name and definition.* The proper name of this product shall be "Poliomyelitis Vaccine", which shall consist of an aqueous preparation of poliovirus types 1, 2, and 3, grown in monkey kidney tissue cultures, inactivated by a suitable method.

(b) *Strains of virus.* Strains of poliovirus used in the manufacture of vaccine shall be identified by historical records, infectivity tests and immunological methods. Any strain of virus may be used that produces a vaccine meeting the requirements of §§ 630.2, 630.3, and 630.4, but the Commissioner of Food and Drugs may from time to time prohibit the use of any specific strain whenever he finds that it is practicable to use another strain of the same type that is potentially less pathogenic to man and that will produce a vaccine of at least equivalent safety and potency.

(c) *Monkeys; species permissible as source of kidney tissue.* Only Macaca or Cercopithecus monkeys, or a species found by the Director, Bureau of Biologics, to be equally suitable, which have met all requirements of §§ 600.11(f)(2) and 600.11(f)(8) of this chapter shall be used as a source of kidney tissue for the manufacture of Poliomyelitis Vaccine.

§ 630.2 Manufacture of Poliomyelitis Vaccine.

(a) *Cultivation of virus.* Virus for manufacturing vaccine shall be grown with aseptic techniques in monkey kidney cell cultures. Suitable antibiotics in the minimum concentration required may be used (§ 610.15(c) of this chapter).

(b) *Filtration.* Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered or clarified by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(c) *Virus titer.* The 50 percent endpoint ($TCID_{50}$) of the virus fluids after filtration shall be $10^{6.0}$ or greater as confirmed by comparison in a simultaneous test (using groups of 10 tubes at 1 log steps or groups of 5 tubes at 0.5 log steps) with a reference virus distributed by the Bureau of Biologics. Acceptable titrations of the reference virus shall not vary more than ± 0.5 $\log_{10}$ from its labeled titer using 0.5 milliliter inoculum in tissue culture.

(d) *Inactivation of virus.* The virus shall be inactivated, as evidenced by the tests described in § 630.4, through the use of an agent or method which has been demonstrated to be consistently effective in the hands of the manufacturer in inactivating a series of lots of poliovirus. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. solution of formaldehyde of 1:4000, and the inactivation conducted under controlled conditions of pH and time, at a temperature of 36° to 38° C. Three or more virus titers, suitably spaced to indicate rate of inactivation, shall be determined during the inactivation process. Filtration equivalent to that described in paragraph (b) of this section shall be performed after the estimated baseline time (time at which the 50 percent end-point reaches one tissue culture infective dose per milliliter), but prior to sampling for the first single strain tissue culture test required in § 630.4(b), except that this filtration may be omitted for strains of a virulence

for monkeys equal to or less than that of the MEF–1 Type 2 strain of poliovirus.

(e) *Additional processing.* Single strain or trivalent pools that have failed to pass safety tests prescribed in § 630.4 (b), (c), or (e) may be treated as follows:

(1) Filtration or clarification by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(2) Negative tests performed as described in § 630.4 (b) and (c) must be obtained on each of two successive samples taken so as to be separated by an interval of at least 3 days while the material is being subjected to treatment with 1:4000 U.S.P. formaldehyde solution and heat at 36° to 38° C. The first sample may be taken before incubation is begun and the second sample shall be taken after the incubation of at least 3 days is completed. For both single strain and trivalent pools the volume tested for each tissue culture safety test shall be equivalent to at least 1,500 human doses.

(3) Pools which are positive following such additional processing shall not be used for the manufacture of poliomyelitis vaccine.

(f) *Supplemental inactivation.* Supplemental inactivation employing a method capable of reducing the titer of a similarly produced virus suspension by a factor of 10^{-6} may be applied at any point after the filtration step described in paragraph (d) or (e)(1) of this section.

§ 630.3 Potency test.

Each lot of vaccine shall be subjected to a potency test which permits an estimation of the antigenic capacity of the vaccine. This is done by means of a simultaneous comparison of the serum antibody levels produced in monkeys by the vaccine under test with the antibody level of the reference serum distributed by the Bureau of Biologics. The potency test shall be performed on samples taken after all final processing of the product has been completed, including addition of preservative, except that when the final product contains material having an adjuvant effect an additional test shall be performed with a sample taken before the addition of the adjuvant material. The volume of the test sample for the additional test shall be adjusted to the equivalent volume of poliomyelitis vaccine in the final product. The test shall be conducted as follows:

(a) *Inoculation of monkeys.* A group of 12 or more Macaca monkeys, or a species found by the Director, Bureau of Biologics, to be equally suitable for the purpose, shall be used. Animals shall weigh between 4 and 8 pounds and shall be in overt good health. Animals that become ill and remain ill during the course of immunization shall be excluded from the group. The test shall not be valid unless at least 10 animals survive the test period and their preinoculation serum antibody levels are as prescribed in paragraph (d) of this section. The test vaccine shall be given intramuscularly to each monkey in 3 doses at 7-day intervals, each dose to be the recommended individual human dose. Only undiluted vaccine shall be used.

(b) *Serum samples.* A blood sample shall be taken from each monkey prior to vaccination and then again 7 days after the last injection. Serum shall be separated aseptically, and stored under refrigeration.

(c) *Serum-virus neutralization test.* The titers of individual monkey serums shall be determined in comparison with the reference serum in tests designed to include controls for all the variables of significance including the following:

(1) Serum toxicity control;

(2) Cell control and cell titration;

(3) Virus titration control (at least 4 tubes for each dilution at 0.5 log steps); and

(4) Serum controls using type-specific serums to identify the type of virus used in the neutralization test.

(d) *Interpretation of the test.* Animals showing preinoculation titers of 1:4 or over when tested against not more than 1,000 $TCID_{50}$ of virus, shall be excluded from the test. The geometric mean titer of antibody induced in the monkeys surviving the course of immunization and bleeding, shall be calculated. A comparison of the value so obtained shall be made with the value for the reference serum that was tested simultaneously and expressed as the ratio between the geometric mean titer value of the serums under test and the mean titer value of the reference serum.

(e) *Potency requirements.* A lot of vaccine tested against the reference serum shall be satisfactory if the geometric mean value of the group of individual monkey serums representing the lot of vaccine tested is at least 1.29 times the mean value of the reference serum for Type 1, at least 1.13 times for Type 2, and at least 0.72 times for Type 3.

§ 630.4 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer.

(a) *The virus pool—tests prior to inactivation*—(1) *B virus and Mycobacterium tuberculosis.* Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(2) *SV–40.* Prior to inactivation, the material shall be tested for the presence of SV–40 as follows (or by any other test producing equally reliable results): A sample of at least 5 ml. from the virus harvest or virus pool shall be neutralized by high titer specific antiserum of other than primate origin. A similar sample from the pool of tissue culture fluids from control vessels representing the tissue from which the virus was prepared may be tested in place of the virus sample. The sample shall be tested in primary cercopithecus tissue cultures or in a cell line demonstrated as at least equally susceptible to SV–40. Each tissue culture system shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days.

(3) *Test results.* The virus harvest or virus pool is satisfactory for poliomyelitis vaccine only if the tests produce no evidence of the presence of B virus, Mycobacterium tuberculosis or SV–40.

(b) *Single strain pool tissue culture tests for poliovirus.* (1) Before pooling to make the final poliomyelitis vaccine, during inactivation at 36° to 38° C., two samples of each monovalent bulk strain pool shall be tested for the presence of virus by tissue culture methods, the second sample to be taken at least 3 days after taking the first sample.

(2) Each sample shall be no smaller than the equivalent of 1,500 human doses and shall be subjected to the complete testing process and each test shall be performed on a different monkey kidney tissue culture cell preparation. The test sample for one of these tests may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV–40 and poliovirus. Each sample shall be inoculated into five or more tissue culture bottles of a suitable capacity, the ratio of the vaccine to the nutrient fluid being approximately 1:1 to 1:3, and the area of the surface growth of cells being at least 3 square centimeters per milliliter of sample. The tissue culture bottles shall be observed for at least 14 days.

(3) A first subculture shall be made at the end of 7 days from date of inoculation by planting at least 2 percent of the volume from each original bottle into suitable tissue culture vessels, followed by refeeding.

(4) A second subculture shall be made from each original bottle in the same manner at the end of 14 days from date of inoculation.

(5) Each of the first and second subcultures shall be observed for at least 7 days.

(6) If cytopathogenic effects occur either in the original bottles of the two tests or in the subcultures from them, or if cellular degeneration appears in the original bottles or in the subcultures before degeneration occurs in uninoculated cultures, the pool shall be held until the matter is resolved. If active poliovirus is indicated, the strain pool shall not be used for inclusion in a final vaccine unless effectively reprocessed as described in § 630.2(e). If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from other than the strain pool being tested.

(c) *Trivalent vaccine pool tissue culture test.* No less than 1,500 human doses of the trivalent vaccine pool, without final preservative, prepared by pooling the three type pools, each of which has passed all tests prescribed in paragraph (b) of this section, shall be subjected to the complete tissue culture test prescribed in such paragraph (b) in at least two approximately equal tests in separate monkey kidney tissue culture prep-

arations. This test sample may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV–40 and poliovirus.

(d) *Trivalent vaccine pool lymphocytic choriomeningitis test.* The final vaccine shall be shown to be free of lymphocytic choriomeningitis virus by intracerebral inoculation of the maximum volume tolerated into 10 or more mice which shall be observed daily for at least 21 days and a negative test shall not be valid unless at least eight mice survive for this period.

(e) *Test in monkeys for active virus.* (1) Vaccine from final containers selected at random from each filling of each lot shall be pooled to provide a test sample of at least 400 milliliters representing the various fillings. An equal volume of bulk vaccine may be substituted for test samples from each filling lot provided the procedure has been approved by the Director, Bureau of Biologics.

(2) A total of not less than 20 monkeys shall be inoculated with the test sample. A preinjection serum sample from each monkey must not contain neutralizing antibody against the three poliovirus types detectable in a dilution of 1:4 when tested against not more than 1,000 $TCID_{50}$ of virus. At least 80 percent of the test animals representing each filling or each bulk sample must survive the test period without significant weight loss, except that if at least 60 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of test animals. At least 80 percent of the animals used in the test must show microscopic evidence of inoculation trauma in the lumbar region of the spinal cord, and gross or microscopic evidence of inoculation trauma in the thalamic area. If less than 60 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals fail to meet the other criteria prescribed in this section, the test must be repeated.

(3) Vaccines shall be injected by combined intracerebral, intraspinal, and intramuscular routes into Macaca or Cercopithecus monkeys or a species found by the Director, Bureau of Biologics, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intracerebral injection shall consist of 0.5 milliliter of test sample into the thalamic region of each hemisphere. The intraspinal inpection shall consist of 0.5 milliliter of concentrated test sample into the lumbar spinal cord enlargement, the test sample to be concentrated 100 fold in the ultracentrifuge by a method demonstrated to recover at least 90 percent of the virus particles in the sediment after it has been resuspended in the same lot of unconcentrated test sample. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine pencillin (300,000 units) into the right arm muscles. The monkeys shall be observed for 17 to 19 days and signs suggestive of poliomyelitis shall be recorded.

(4) At the end of the observation period, samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification. Histological sections shall be prepared from both spinal cord enlargements and examined.

(5) Doubtful histopathological findings necessitate (i) examination of a sample of sections from several regions of the brain in question, and (ii) attempts at virus recovery from the nervous tissues previously removed from the animal. The test results must be negative. Test results are negative if the histological and other studies leave no doubt that poliomyelitis infection did not occur.

(f) *Tissue culture safety test for SV–40.* At least 500 human doses of each monovalent or trivalent pool of vaccine shall be tested for the presence of SV–40 using primary cercopithecus monkey tissue cultures or using a cell line demonstrated as at least equally susceptible to SV–40. The test shall be conducted as described in paragraph (b) of this section, except for the volume of test sample and except that one subculture of at least 2 percent of the volume of the fluids shall be made no less than 14 days from the date of inoculation and examined for at least 14 days from the date of subinoculation. The vaccine is satisfactory only if there is no evidence of the presence of SV–40 in any of the cultures or subcultures.

§ 630.5 General requirements.

(a) *Consistency of manufacture.* No lot of final vaccine shall be released unless it is one of a series of five consecutive lots produced by the same manufacturing process, all of which have shown negative results with respect to all tests for the presence of live poliovirus, and unless each of the monovalent pools of which a polyvalent final vaccine is composed similarly is one of a series of five consecutive monovalent pools of the same type of inactivated poliovirus, all of which have shown negative results in all tests for the presence of live poliovirus.

(b) *Dose.* These additional standards are based on a human dose of 1.0 milliliter for a single injection and a total human immunizing dose of three injections of 1.0 milliliter given at appropriate intervals.

(c) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A 2,500 milliliter sample, neutralized, not dialyzed, and without final preservative, taken at the latest possible stage of manufacturing before the addition of such preservative.

(2) A 200 milliliter bulk sample of the final vaccine containing final preservative.

(3) A total of not less than a 200 milliliter sample of the final vaccine in final labeled containers.

(4) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

§ 630.6 Equivalent methods.

Modification of any particular manufacturing method or procedure or the conditions under which it is conducted as set forth in the additional standards relating to poliomyelitis vaccine (§§ 630.1 to 630.5, inclusive) shall be permitted whenever the manufacturer presents evidence to demonstrate that such modification will provide equal or greater assurances of the safety, purity and potency of the vaccine as the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart B—Poliovirus Vaccine, Live, Oral

§ 630.10 Poliovirus Vaccine, Live, Oral.

(a) *Proper name and definition.* The proper name of this product shall be "Poliovirus Vaccine, Live, Oral," followed by a designation of the type. The vaccine shall be a preparation of one or more live, attenuated polioviruses grown in monkey kidney cell cultures, or a strain of human cell cultures found by the Director, Bureau of Biologics, to meet the requirements of § 630.12(b) and shall be prepared in a form suitable for oral administration.

(b) *Criteria for acceptable strains and acceptable seed virus.* (1) Strains of attenuated poliovirus Types 1, 2, and 3 used in the manufacture of the vaccine shall be identified by: (i) Historical records including origin and techniques of attenuation, (ii) antigenic properties, (iii) neurovirulence for monkeys, (iv) pathogenicity for other animals and tissue cultures of various cell types, and (v) established virus markers including rct/40, d, and other markers shown to be associated with strain virulence.

(2) Poliovirus strains shall not be used in the manufacture of Poliovirus Vaccine, Live, Oral, unless, (i) data are submitted to the Commissioner of Food and Drugs which establish that each such strain is free of harmful effect upon administration in the recommended dosage to at least 1 million people susceptible to poliomyelitis, under circumstances where adequate epidemiological surveillance of neurological illness has been maintained, and, (ii) each such strain produces a vaccine meeting the safety and potency requirements of §§ 630.11, 630.15, and 630.16(b). Susceptibility shall be demonstrated by §§ 630.11, 630.15, and 630.16 (b). Susceptibility shall be demonstrated by blood tests, stool examinations and other appropriate methods.

(3) Each seed virus used in manufacture shall be demonstrated to be free of extraneous microbial agents except for unavoidable bacteriophage.

(4) No seed virus shall be used for the manufacture of poliovirus vaccine unless its neurovirulence in Macaca monkeys

is no greater than that of the Reference Attenuated Poliovirus distributed by the Bureau of Biologics. The neurovirulence of the seed virus shall be demonstrated by the following tests to be performed by the manufacturer: (i) The test prescribed in § 630.16(b)(1) using seed virus as test material in place of monovalent virus pool material and (ii) the following comparative intramuscular neurovirulence test: Each of at least 10 monkeys shall be injected with a total of 5.0 ml. of the seed virus under test in one or more proximate locations of either a gluteus or gastrocnemius muscle. Similar injections shall be made in another group of 10 monkeys using the Reference Attenuated Poliovirus. Each monkey shall be injected intramuscularly with no less than $10^{7.7}$ $TCID_{50}$ of viral inoculum. All monkeys shall be observed for 17 to 21 days and a comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the Reference Attenuated Poliovirus, as prescribed in § 630.16(b)(1)(iii).

(5) Subsequent and identical neurovirulence tests shall be performed in monkeys whenever there is evidence of a change in the neurovirulence of the production virus, upon introduction of a new production seed lot, and as often as necessary otherwise to establish to the satisfaction of the Commissioner of Food and Drugs that the seed virus strains for vaccine manufacture have maintained their neurovirulence properties as set forth in § 630.16(b)(1)(iii).

(6) The Commissioner of Food and Drugs may, from time to time, prohibit the use of a specified strain whenever he finds it is practicable to use another strain of the same type which is potentially less pathogenic for man, and that it will produce a vaccine of greater safety and of at least equivalent potency.

§ 630.11 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design. Such clinical trials shall be conducted with five consecutive lots of poliovirus vaccine which have been manufactured by the same methods, each of which has shown satisfactory results in all prescribed tests. Type specific neutralizing antibody (from less than 1:4 before vaccine treatment, to 1:16 or greater after treatment) shall be induced in 80 percent or more of susceptibles when administered orally as a single dose, or in excess of 90 percent of susceptibles when administered orally after a series of doses. A separate clinical trial shall have been conducted for each monovalent and each polyvalent vaccine for which license application is made.

§ 630.12 Animal source; quarantine; personnel.

(a) *Monkeys*—(1) *Species permissible as source of kidney tissue.* Only Macaca found by the Director, Bureau of Biologics, to be equally suitable, which have met all the requirements of §§ 600.11(f)(2) and 600.11(f)(8) of this chapter shall be used as the source of kidney tissue for the manufacture of Poliovirus Vaccine, Live, Oral.

(2) *Experimental and test monkeys.* Monkeys that have been used previously for experimental or test purposes shall not be used as a source of kidney tissue in the processing of vaccine.

(3) *Quarantine; additional requirements.* Excluding deaths from accidents or causes not due to infectious diseases, if the death rate of any group of monkeys being conditioned in accordance with § 600.11(f)(2) of this chapter exceeds 5 percent per month, the remaining monkeys may be used for the manufacture of Poliovirus Vaccine only if they survive a new quarantine period.

(b) *Human cell culture strains.* Strains of human cell cultures used for the manufacture of Poliovirus Vaccine, Live, Oral shall be (1) identified by historical records, (2) demonstrated to be free of oncogenic properties in a suitable animal test and free of adventitious microbial agents, and (3) shown to be capable of producing a vaccine which, by experience in at least 10,000 persons, has been found to be safe and antigenic. The field studies shall be so conducted that at least 5,000 of the individuals must reside when given vaccine in areas where health related statistics are regularly compiled in accordance with procedures such as those used by the National Center for Health Statistics. Data in such form as will identify each person receiving vaccine shall be furnished to the Director, Bureau of Biologics.

(c) *Personnel.* All reasonably possible steps shall be taken to insure that personnel involved in processing the vaccine are immune to and do not excrete poliovirus.

§ 630.13 Manufacture of Poliovirus Vaccine, Live, Oral.

(a) *Virus passages.* Virus in the final vaccine shall represent no more than five tissue culture passages from the original strain, each of which shall have met the criteria of acceptability prescribed in § 630.10(b).

(b) *Virus propagated in monkey kidney cell cultures*—(1) *Continuous line cells.* When primary monkey kidney cell cultures are used in the manufacture of poliovirus vaccine, continuous line cells shall not be introduced or propagated in vaccine manufacturing areas.

(2) *Identification of processed kidneys.* The kidneys from each monkey shall be processed and the viral fluid resulting therefrom shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in the following subparagraph relating to that pair of kidneys shall have been withdrawn from the harvest.

(3) *Monkey kidney tissue production vessels prior to virus inoculation.* Prior to inoculation with the seed virus, the tissue culture growth in vessels representing each pair of kidneys shall be examined microscopically for evidence of cell degeneration at least 3 days after complete formation of the tissue sheet. If such evidence is observed, the tissue cultures from that pair of kidneys shall not be used for poliovirus vaccine manufacture. To test the tissue found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of four culture systems: (i) Macaca monkey kidney cells, (ii) Cercopithecus monkey kidney cells, (iii) primary rabbit kidney cells, and (iv) human cells from one of the systems described in § 630.16(a)(6), in the following manner: Aliquots of fluid from each vessel shall be pooled and at least 10 ml. of the pool inoculated into each system, with ratios of inoculum to medium being 1:1 to 1:3 and with the area of surface growth of cells at least 3 square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. At the end of the observation period, at least one subculture of fluid from the Cercopithecus monkey kidney cell cultures shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. If these tests indicate the presence in the tissue culture preparation of any viable microbial agent, the tissue cultures so implicated shall not be used for poliovirus vaccine manufacture.

(4) *Control vessels.* Before inoculation with seed virus, sufficient tissue culture vessels to represent at least 25 percent of the cell suspension from each pair of kidneys shall be set aside as controls. The control vessels shall be examined microscopically for cell degeneration for an additional 14 days. The cell fluids from such control vessels shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the same method prescribed for testing of fluids in subparagraph (b)(3) of this paragraph. In addition, the cell sheet in each control vessel shall be examined for presence of hemadsorption viruses by the addition of guinea pig red blood cells.

(5) *Virus harvest; interpretation of test results.* If the tissue culture in less than 80 percent of the control vessels is not free of cell degeneration at the end of the observation period, no tissue from the kidneys implicated shall be used for poliovirus vaccine manufacture. If the test results of the control vessels indicate the presence of any extraneous agent at the time of virus harvest, the entire virus harvest from that tissue culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in subparagraphs (3) or (4) of this paragraph demonstrate the presence in the tissue culture preparation of any microbial agent known to be capable of producing human disease, the virus grown in such tissue culture preparation shall not be used for poliovirus vaccine manufacture.

(6) *Kidney tissue production vessels after virus inoculation—temperature.* After virus inoculation, production vessels shall be maintained at a tempera-

ture not to exceed 35.0° C. during the course of virus propagation.

(7) *Kidney tissue virus harvests.* Virus harvested from vessels containing the kidney tissue from one monkey may constitute a monovalent virus pool and be tested separately, or viral harvests from more than one pair of kidneys may be combined, identified and tested as a monovalent pool. Each pool shall be mixed thoroughly and samples withdrawn for testing as prescribed in § 630.16(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at −60° C. or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(8) *Filtration.* After harvesting and removal of samples for the safety tests prescribed in § 630.16(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

(c) *Virus propagated in human cell cultures*—(1) *Use of continuous line cells.* When a human cell culture strain, previously found to be suitable by the Director, Bureau of Biologics, is used in the manufacture of poliovirus vaccine, no other continuous line cells or primary cell cultures shall be introduced or propagated in vaccine manufacturing areas.

(2) *Identification of human cell cultures.* The cell culture growth shall be charatcerized as to (i) identification as human cells, (ii) passage level, and (iii) karyology. Chromosome monitoring of the cell cultures used for vaccine production shall be made on permanent stained slide preparations which shall be maintained by the manufacturer as a permanent record. Monitoring shall be performed on each cell growth used for virus vaccine production. The karyologic determination shall include analysis of the exact chromosome count, karotype, polyploidy, chromosome breaks, structural chromosome abnormalities, other abnormalities such as despiralization or marked attenuations of the primary or secondary constrictions and the presence of minute chromosome. Findings based on these determinations shall not exceed the 95 percent confidence limits of the values established for the cell strain used. Cell cultures shall be processed in such a manner that the viral fluid resulting therefrom shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in the following subparagraph shall have been withdrawn from the harvest.

(3) *Human cell culture production vessels prior to virus inoculation.* Prior to inoculation with the seed virus, the cell culture growth shall be examined microscopically for evidence of cell degeneration after complete formation of the cell sheet. If such evidence is observed, the cell production lot shall not be used for poliovirus vaccine manufacture. To test the cell cultures found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of three culture systems: (i) Cercopithecus monkey kidney cells, (ii) primary rabbit kidney cells, and (iii) human cells from one of the systems described in § 630.16(a)(6), in the following manner. Aliquots of fluid from each vessel shall be pooled and at least 10 ml. of the pool inoculated into each system, with ratios of inoculum to medium being 1:1 to 1:3 and with the area of surface growth of cells at least 3 square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. At the end of the observation period, at least one subculture of fluid from the Cercopithecus monkey kidney cell cultures shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. If these tests indicate the presence in the tissue culture preparation of any viable microbial agent, the cell cultures so implicated shall not be used for poliovirus vaccine manufacture.

(4) *Control vessels.* Before inoculation with seed virus, a portion of the cell culture shall be set aside as control material. Such a portion either shall represent at least 25 percent of the cell suspension of a single cell growth or a volume of the fluid derived from the cell cultures equivalent to at least 25 percent of the volume of the final vaccine. The control vessels shall be examined microscopically for cell degeneration for an additional 14 days. The cell fluids from such control vessels shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the same method prescribed for testing of fluids in subparagraph (3) of this paragraph. In addition, the cell sheet in each control vessel shall be examined (i) for presence of hemadsorption viruses by the addition of guinea pig red blood cells and (ii) a pool of cell suspension containing at least 10^5 cells shall be tested in embryonated chicken eggs by the allantoic cavity route of inoculation for the presence of adventitious agents.

(5) *Virus harvest; interpretation of test results.* If more than 20 percent of the cell substrates in the control vessels demonstrate cell degeneration at the end of the observation period, the cells implicated shall not be used for poliovirus vaccine manufacture. If the test results of the control vessels indicate the presence of any extraneous agent at the time of virus harvest, the entire virus harvest from that cell culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in subparagraph (3) or (4) of this paragraph demonstrate the presence in the cell culture preparations of any microbial agent known to be capable of producing human disease, the virus grown in such cell culture preparation shall not be used for poliovirus vaccine manufacture.

(6) *Human cell culture production vessels after virus inoculation—temperature.* After virus inoculation, production vessels shall be maintained at a temperature not to exceed 35.0° C. during the course of virus propagation.

(7) *Virus harvest from human cell cultures.* Virus harvested from vessels representing a single cell growth may constitute a monovalent virus pool and be tested separately, or viral harvests from vessels representing more than one cell growth may be combined, identified and tested as a monovalent pool. Each pool shall be mixed thoroughly and samples withdrawn for testing for safety as prescribed in § 630.16(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at −60° C. or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(8) *Filtration.* After harvesting and removal of samples for the safety tests prescribed in § 630.16(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

§ 630.14 Reference strains.

The following reference viruses shall be obtained from the Bureau of Biologics.

Reference Poliovirus, Live, Attenuated, Type 1, as a control for correlation of virus titers in tissue cultures.

Reference Poliovirus, Live, Attenuated, Type 2, as a control for correlation of virus titers in tissue cultures.

Reference Poliovirus, Live, Attenuated, Type 3, as a control for correlation of virus titers in tissue cultures.

Reference Attenuated Poliovirus, Type 1, as a control for correlation of monkey neurovirulence tests.

§ 630.15 Potency test.

The concentration of live virus expressed as $TCID_{50}$ of each type in the vaccine shall constitute the measure of its potency. The accuracy of the titration to determine the concentration of live virus in the lot under test shall be confirmed by performing a titration with the Reference Poliovirus, Live, Attenuated of the appropriate type as a check on titration technique. The concentration of each type of live virus contained in the vaccine of the lot under test shall be between 200,000 and 500,000 $TCID_{50}$ per human dose.

§ 630.16 Test for safety.

(a) *Tests prior to filtration.* Monovalent virus pools shall contain no demonstrable viable microbial agent other than the attenuated live poliovirus intended except for unavoidable bacteriophage. The vaccine shall be tested for the absence of adventitious and other infectious agents including polioviruses of other types or strains, simian agents Mycobacterium tuberculosis, pox virus, lymphocytic choriomeningitis virus Echo viruses, Coxsackie viruses, and B virus. Testing of each monovalent pool shall include the following procedures:

(1) *Inoculation of rabbits.* A minimum of 100 ml. of each monovalent virus pool shall be tested by inoculation into at least 10 health rabbits, each weighing

1,500–2,500 grams. Each rabbit shall be injected intradermally in multiple sites, with a total of 1.0 ml. and subcutaneously with 9.0 ml., of the viral pool, and the animals observed for at least 3 weeks. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of B virus or any other viral infection.

(2) *Inoculation of adult mice.* Each of at least 20 adult mice, each weighing 15–20 grams, shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. of each monovalent virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of lymphocytic choriomeningitis virus or other viral infection.

(3) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old, shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the monovalent virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and all areas examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by the intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of Coxsackie or other viral infection.

(4) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighing 350–450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5.0 ml. of the monovalent virus pool to be tested. The animals shall be observed for at least 42 days and daily rectal temperatures recorded for the last 3 weeks of the test. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and its tissues shall be examined both microscopically and culturally for evidence of tubercle bacilli, and by passage of tissue suspensions into at least three other guinea pigs by the intracerebral and intraperitoneal routes of inoculation for evidence of viral infection. If clinical signs suggest infection with lymphocytic choriomeningitis virus, serological tests shall be performed on blood samples of the test guinea pigs to confirm the clinical observations. Animals that die or are sacrificed during the first 3 weeks after inoculation with poliovirus shall be examined for infection with lymphocytic choriomeningitis virus. Animals that die in the final 3 weeks shall be examined both microscopically and culturally for Mycobacterium tuberculosis. The monovalent virus pool is satisfactory for poliovirus vaccine only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with Mycobacterium tuberculosis, or any viral infection.

(5) *Inoculation of monkey kidney tissue cultures.* At least 500 doses or 50 ml., whichever represents a greater volume of virus, of each undiluted monovalent virus pool, or in equal proportions from individual harvests or subpools, shall be tested for simian viruses in Macaca, and the same volume in Cercopithecus, monkey kidney tissue cultures, in a ratio of inoculum to medium of from 1: 1 to 1: 3, and with the area of surface growth of cells at least 3 square centimeters per milliliter of test inoculum, after neutralization of the poliovirus by high titer specific antiserum of nonprimate origin. The immunizing antigens used for the preparation of antisera shall be grown in a human tissue culture cell line. The cultures shall be observed for no less than 14 days. At the end of the observation period at least one subculture of fluid from the Cercopithecus kidney cell culture shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all the tissue cultures fail to show evidence of the presence of simian viruses or any other viral infection.

(6) *Inoculation of human cell cultures.* At least 500 doses or 50 ml., whichever represents a greater volume of virus, taken from either a single monovalent pool, or in equal proportions from individual harvests or subpools, shall be tested in a ratio of inoculum to medium of 1:1 to 1:3, and with the area of surface growth of cells at least 3 square centimeters per milliliter of test inoculum, for the presence of measles virus in either (i) primary human amnion cells, (ii) primary human kidney cells, or (iii) any other cell system of comparable susceptibility to unmodified measles virus. The test material shall be neutralized with poliovirus antiserum of other than primate origin if the tissue culture cell system used is susceptible to poliovirus. The culture shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus or any other viral infection.

(7) *Inoculation of rabbit kidney tissue cultures.* At least 500 ml. of virus pool taken from either a single monovalent pool, or in equal proportions from individual harvests or subpools, shall be tested in a ratio of inoculum to medium of from 1:1 to 1:3, and with the area of surface growth of cells at least 3 square centimeters per milliliter of test inoculum, in primary rabbit kidney tissue culture preparations for evidence of B virus. The culture shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of B virus.

(b) *Tests after filtration.* The following tests relating to safety shall be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot):

(1) *Neurovirulence in monkeys.* Each monovalent virus pool or monovalent lot shall be tested in comparison with the Reference Attenuated Poliovirus for neurovirulence in Macaca mulatta (rhesus) monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1: 4 when tested against no more than 1,000 $TCID_{50}$ of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least $10^{7.6}$ $TCID_{50}$ per ml. when titrated in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days, under the supervision of a qualified pathologist, physician or veterinarian, and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(i) *Intrathalamic inoculation.* Each of at least 30 monkeys shall be injected intracerebrally by placing 0.5 ml. of virus pool material into the thalamic region of each hemisphere. Comparative evaluations shall be made with the virus pool under test and the Reference Attenuated Poliovirus. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate virus pool material into the thalamus, additional monkeys may be inoculated in order to reestablish the minimum number of 30 monkeys for the test.

(ii) *Intraspinal inoculation.* Each of a group of at least five monkeys shall be injected intraspinally with 0.2 ml. of virus pool material containing at least $10^{7.6}$ $TCID_{50}$ per ml. and each monkey in additional groups of at least five monkeys shall be injected intraspinally with 0.2 ml of a 1:1,000 and 1:10,000 dilution respectively, of the same virus pool material. Comparative evaluations shall be made with the virus pool under test and the reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as hav-

ing been injected satisfactorily. If on examination there is evidence of failure to inoculate intraspinally, additional animals may be inoculated in order to reestablish the minimum number of five animals per group.

(iii) *Determination of neurovirulence.* At the conclusion of the observation period comparative histopathological examinations shall be made of the lumbar cord, cervical cord, lower medulla, upper medulla, mesencephalon and motor cortex of each monkey in the groups injected with virus under test and those injected with the Reference Attenuated Poliovirus, except that for animals dying during the test period, these examinations shall be made immediately after death. If at least 60 percent of the animals of a group survive 48 hours after inoculation, those animals which did not survive may be replaced by an equal number of animals tested as prescribed in paragraph (b)(1) of this section. If less than 60 percent of the animals of a group survive 48 hours after inoculation, the test must be repeated. At the conclusion of the observation the animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristics of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the Reference Attenuated Poliovirus with respect to (a) the number of animals showing lesions characteristic of poliovirus infection, (b) the number of animals showing lesions other than those characteristic of poliovirus infection, (c) the severity of the lesions, (d) the degree of dissemination of the lesions, and (e) the rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrate that the neurovirulence of the test virus pool does not exceed that of the Reference Attenuated Poliovirus.

(iv) *Test with Reference Attenuated Poliovirus.* The Reference Attenuated Poliovirus shall be tested as prescribed in paragraph (b)(1) (i) and (ii) of this section at least once for every 10 production lots of vaccine, except that the interval between the test of the reference and the test of any lot of vaccine shall not be greater than 3 months. The test procedure shall be considered acceptable only if lesions of poliomyelitis are seen in monkeys inoculated with the reference material at a frequency statistically compatible with all previous tests with this preparation.

(2) *Test for virus titer.* The concentration of living virus in each monovalent virus pool or lot shall be determined, using the Reference Poliovirus Live, Attenuated of the same type as a control. The test shall be a 50 percent end-point titration calculation ($TCID_{50}$), performed with either groups of 10 tubes at 1 log dilution steps or groups of five tubes of 0.5 log dilution steps, or a test of demonstrated equivalent sensitivity. Acceptable titrations of the reference virus shall not vary more than ±0.5 log from its labeled titer.

(3) *Tests for In Vitro Markers.* A test shall be performed on each monovalent virus pool or each monovalent lot resulting therefrom, using the rct/40 Marker. A second test shall be performed using the d Marker or another marker method shown to be of value in identification of the attenuated strain. The test results shall demonstrate that the virus under test and the seed virus have substantially the same marker characteristics.

(i) *rct/40 Marker.* Attenuated strains which grow readily at 40° C. (±0.5° C.) are classified as rct/40 positive (+) in contrast to the rct/40 negative (−) strains which show an increased growth of at least 100,000 fold at 36° C. over that obtained at 40° C. Comparative determinations shall be made in either tube or bottle cultures.

(ii) *d Marker.* Attenuated strains which grow readily at low concentrations of bicarbonate under agar are classified as d positive (+) in contrast to the d negative (−) strains which exhibit delayed growth under the same conditions. The cultures shall be grown in a 36° C. incubator either in stoppered bottles or in plates in an environment of 5 percent CO_2 in air.

§ 630.17 General requirements.

(a) *Final container sterility test.* The final container sterility test need not be performed provided aseptic techniques are used in the filling process.

(b) *Consistency of manufacture.* No lot of vaccine shall be released unless each monovalent pool contained therein is one of a series of five consecutive pools of the same type, each pool having been manufactured by the same procedures, and each having met the criteria of neurovirulence for monkeys prescribed in § 630.16(b)(1), and of in vitro markers prescribed in § 630.16(b)(3).

(c) *Dose.* The individual human dose of vaccine shall contain from 200,000 to 500,000 $TCID_{50}$ of each type of virus in the final monovalent vaccine, and for polyvalent vaccine not more than 1,000,000 $TCID_{50}$ of Type 1 virus, 100,000 to 200,000 $TCID_{50}$ of Type 2 virus and 200,000 to 500,000 $TCID_{50}$ of Type 3 virus.

(d) *Labeling.* In addition to the items required by other applicable labeling provisions of this part, the final container label shall bear a statement indicating that liquid vaccine may not be used for more than 7 days after opening the container. Labeling may include a statement indicating that for frozen vaccine a maximum of 10 freeze-thaw cycles is permissible provided the total cumulative duration of thaw does not exceed 24 hours, and provided the temperature does not exceed 8° C. during the periods of thaw.

(e) *Samples and protocols.* For each lot of vaccine, the following materials shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(2) A 500 milliliter bulk sample of each final monovalent pool having a virus titer of no less than $10^{7.5}$ $TCID_{50}$ per milliliter, except that if the titer is greater, a correspondingly smaller volume may be submitted.

(3) A total of no less than 200 doses or no less than six final containers, whichever is the larger amount.

§ 630.18 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Poliovirus Vaccine, Live, Oral, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart C—Adenovirus Vaccine

§ 630.20 Adenovirus Vaccine.

(a) *Proper name and definition.* For the purpose of section 351(a)(2) of the act and § 600.3(k) of this chapter, the proper name of this product shall be "Adenovirus Vaccine" with a designation of the types of virus included in the vaccine. Such vaccine shall consist of an aqueous preparation of one or more adenoviruses grown in monkey kidney tissue cultures inactivated by a suitable method. Where more than one type of virus is used in the manufacture of the vaccine, equal proportions of each type shall be combined with a tolerance for each component of 5 percent of the total volume.

(b) *Strains of virus.* Strains of adenovirus used in the manufacture of the vaccine shall be identified by historical records, infectivity tests, and immunological methods. Only those strains of virus may be used that (1) produce a vaccine meeting the safety and potency requirements in §§ 630.23 and 630.24, (2) never had any passage in malignant cells of human or animal origin, and (3) have been maintained in monkey kidney cultures for at least 10 passages prior to use.

(c) *Monkey kidney tissue.* Only cynomolgus or rhesus monkeys or other species of equal suitability, in overt good health, that have reacted negatively to tuberculin within 2 weeks prior to use shall be used as a source of kidney tissue for the production of virus. Each animal shall be examined at necropsy under the supervision of a qualified pathologist for gross signs of disease. If there is any gross pathological lesion of any significance to their use in the manufacture of vaccine, the kidneys shall be discarded. Kidney tissue from monkeys that have been used previously for experimental purposes shall not be used, ex-

cept that monkeys in overt good health, used for the safety or potency tests of adenovirus vaccines with negative clinical findings (§§ 630.23 and 630.24) that have reacted negatively to tuberculin prior to such test, may be used within two weeks of the end of the test period. The monkeys shall not at any time have been housed in the same building where monkeys actually infected with or exposed to poliovirus are housed, and due precautions shall be taken to prevent cross-infection from any infected or potentially infected monkeys on the premises.

§ 630.21 General requirements.

(a) *Separate facilities.* The personnel, equipment and supplies used in the manufacture of adenovirus vaccine shall be separated from personnel, equipment or supplies used in connection with any other pathogenic virus to the extent necessary to prevent cross-contamination.

(b) *Final container tests.* Tests shall be made on final containers for safety, sterility and identity, in accordance with §§ 610.11, 610.12, and 610.14 of this chapter, respectively.

(c) *Release of vaccine.* A lot of vaccine shall not be released unless all required safety tests have given negative results.

(d) *Extraneous protein.* Extraneous protein capable of producing allergenic effects on human subjects shall not be added to the final virus production medium. If animal serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(e) *Nitrogen content.* The final vaccine shall have a protein nitrogen content of less than 0.02 milligram per milliliter.

(f) *Dose.* These additional standards for adenovirus vaccine are based on a human dose not exceeding 1.0 milliliter for a single injection.

(g) *Labeling.* In addition to compliance with the requirements of §§ 610.60 to 610.65 of this chapter inclusive, the label or package enclosure shall include an appropriate statement indicating the type and amount of each antibiotic added, if any. The preservative used shall be stated on the label, as well as allergenic substances added, if any, and the source, composition, and method of inactivation of the viruses.

(h) [Reserved]

(i) *Requirements for samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A 2,500-milliliter sample of the final vaccine taken at the latest possible stage of manufacture before the addition of preservative.

(2) A 200-milliliter bulk sample of the final vaccine containing all preservatives.

(3) A total of at least 200-milliliter sample of the final vaccine in final labelled containers.

(4) Protocol showing the history of the lot and the results of all of the tests which were carried out by the manufacturer.

§ 630.22 Manufacture of Adenovirus Vaccine.

(a) *Cultivation of virus.* Virus for manufacturing vaccine shall be grown with aseptic technique in monkey kidney cell cultures using a synthetic medium. Suitable antibiotics in the minimum concentration required may be used. If penicillin is used, not more than 200 units per milliliter may be added. Phenol red may not exceed a concentration of 0.002 percent.

(b) *Filtration.* Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered or clarified by a method having an efficiency at least equivalent to that of a Selas 02 type filter.

(c) *Virus titer.* The titer of each virus pool after filtration shall be determined by a suitable method. It shall also be demonstrated that each virus pool possesses adenovirus group antigen by the complement-fixing test.

(d) *Inactivation of virus.* The virus shall be inactivated, as evidenced by the test in tissue culture as set forth in § 630.23 through the use of an agent or method which has been demonstrated to be effective in the hands of the manufacturer in inactivating a series of at least 5 consecutive lots of adenovirus vaccine. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. formaldehyde solution of at least 1:4000. The inactivation shall be conducted under controlled conditions of pH and time at a temperature of 36° to 38° C. As an indication of inactivation, not less than two samples shall be removed during the inactivation process and treated as prescribed in § 630.23(b)(1). Regardless of the concentration of formaldehyde used, the total heating period shall be not less than 20 hours and at least three times the period required for the reduction of live virus to a point where no virus is detected in a 5 milliliter sample when tested in accordance with § 630.23(b)(1). At the end of the heating period a sample shall be removed for the single strain tissue culture safety test.

§ 630.23 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer:

(a) *The virus pool—(1) Tests prior to inactivation—(i) B virus and Mycobacterium tuberculosis.* Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(ii) *SV_{40}.* Prior to inactivation, the material shall be tested for the presence of SV_{40} as follows (or by any other test producing equally reliable results): A sample of at least five ml. from the virus harvest or virus pool or pool of tissue culture fluids from corresponding control vessels shall be neutralized by high titer antiserum of an origin other than human, chicken, or simian. The sample shall be tested in the same tissue culture system used for propagating the virus vaccine, and in primary cercopithecus tissue cultures or in a cell line demonstrated as equally susceptible to SV_{40}. Each tissue culture system shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and observed for an additional 14 days.

(iii) *Test results.* The virus harvest or virus pool is satisfactory for adenovirus vaccine only if the tests produce no evidence of the presence of B virus, Mycobacterium tuberculosis and SV_{40}.

(2) Each single strain virus pool shall be shown to be free of lymphocytic choriomeningitis virus and other mouse pathogens by intracerebral injection into 10 or more mice which shall be observed daily for at least 21 days. All mice which die during the observation period shall be studied as to the possible cause of death. A negative test shall not be valid unless at least 8 mice survive the full observation period and unless the virus pool was found free of agents pathogenic for mice; and

(3) An identity test shall be done on each virus pool using monovalent adenovirus serums free from poliomyelitis antibodies. Such serums shall have been prepared from animals immunized with virus grown in other than the tissue used for the neutralization test. The identity tests shall be done (i) in monkey kidney and (ii) in HeLa or other equally susceptible cells. The tissue cultures shall be observed for 7 days. Those showing cytopathogenic effect in the presence of type specific serum shall be subcultured in monkey kidney cells or HeLa cells. The subcultures shall be maintained for 7 days and observed for cytopathogenic effect. Only virus pools free of unidentified cytopathogenic agents and free of all viruses pathogenic to man other than adenoviruses may be used for vaccine manufacture.

(b) *Single strain tissue culture test for adenovirus.* (1) The samples specified in § 630.22(d) shall be placed immediately after sampling in contact with sodium bisulfite or a similar formaldehyde neutralizing substance that will stop the inactivation process. Each sample shall be dialyzed or rendered non-toxic to tissue culture cells by an appropriate method which does not affect the detection of live virus. An amount of fluid representing at least 5 milliliters of the original virus pool shall be inoculated into monkey kidney or other equally susceptible tissue cultures. The tissue cultures shall be maintained for 7 to 12 days and examined at intervals. At the end of the above period, the cell sheet shall be removed from each culture vessel, broken up by an appropriate means, suspended in a portion of its culture fluid equal to at least 10 percent of the volume which was present during incubation, and inoculated into corresponding fresh tissue culture preparations. Any fluids recovered prior to refeeding during original observation period shall be held at 2° to 5°C. A volume of each fluid representing at least 10 percent of the total volume shall be

subcultured to fresh tissue culture. All subcultures shall be examined for at least 7 days. This test shall be considered negative only if no cellular degeneraton occurs attributable to any virus.

(2) A sample of at least 500 milliliters of each single strain pool shall be fully subjected to the following testing procedure in tissue culture cells, with half the sample in monkey kidney cells and half in suitable human cells of demonstrated high susceptibility to adenovirus and poliovirus. The entire sample shall be dialyzed and rendered non-toxic for tissue culture cells. Each half of the sample shall be inoculated into 4 or more tissue culture bottles of suitable capacity so that direct observation of the culture cells is possible under conditions which assure the growth of adenovirus, poliovirus or simian viruses should infective particles of any of these viruses be present in the vaccine. The monkey kidney cell cultures shall be performed as described in § 630.4(b) except that a third subculture shall be included after 21 days of incubation of the initial culture and that this subculture shall be made by suspending the cell sheet. The initial human cell cultures shall be observed for at least 12 days. A subculture shall be made on each fluid at each refeeding and on the suspension of each cell sheet in the culture fluid removed at the end of the observation period. The inoculum for the subcultures shall be a volume of at least 2 percent of that of the fluid being studied. The subculture shall be examined frequently and refed as required, and maintained for a period of at least 12 days. If a cytopathogenic effect occurs during the test, the vaccine pool shall be held until the matter is resolved. If active poliomyelitis virus or adenovirus is indicated, the strain pool shall not be used for inclusion in a final vaccine. If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses did not originate from the strain pool being tested.

(c) *Final vaccine pool tissue culture test.* No less than 1,500 milliliters of the final vaccine pool without final preservative, prepared by pooling the individual single strain preparations, shall be tested in accordance with § 630.4 (b) and (c).

(d) *Final vaccine test for active virus in monkeys.* Final bulk vaccine shall be tested in monkeys as prescribed in § 630.4(e) except that the test may be applied to vaccine before it is placed in final containers, and the sample may be dialyzed in order to remove sodium bisulfite or the sodium bisulfite formaldehyde complex before injection intraspinally and intracerebrally into monkeys. In no case, however, shall dialyzed vaccine be used for the intramuscular injection of the monkeys. The test is considered negative if the histological and other studies leave no doubt that virus infections attributable to the vaccine did not occur.

(e) *Exclusion of certain pools from final vaccine.* Pools which fail to pass the tissue culture safety tests prescribed in this section shall not be included in final vaccine, unless it can be clearly shown that the cytopathogenic agent occurred in the test system and not in the vaccine pool. No pool shall be subjected to reprocessing.

§ 630.24 Potency test.

Each lot of vaccine shall be subjected to a potency test which permits an estimation of the antigenic capacity of the vaccine in comparison with a reference vaccine distributed by the Food and Drug Administration. This shall be done using at least 6 animals for each dilution of each vaccine tested and measuring the neutralizing antibody response of the animals receiving test vaccine and others receiving reference vaccine in simultaneous tests. The average antibody level for each type shall equal or exceed the corresponding value of the reference vaccine.

§ 630.25 Equivalent methods.

The provisions of § 630.6 permitting modifications in methods if found equivalent in assuring safety, purity, and potency, shall be applicable to the additional standards relating to adenovirus vaccine (§§ 630.20 to 630.24, inclusive).

Subpart D—Measles Virus Vaccine, Live, Attenuated

§ 630.30 Measles Virus Vaccine, Live, Attenuated.

(a) *Proper name and definition.* The proper name of this product shall be Measles Virus Vaccine, Live, Attenuated, which shall consist of a preparation of live, attenuated, measles virus.

(b) *Criteria for acceptable strains of attenuated measles virus.* Strains of attenuated measles virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation, (2) antigenic specificity as measles virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture of Measles Virus Vaccine, Live, Attenuated, shall have been shown to be safe and potent in man by field studies with experimental vaccines. Vaccine prepared from measles virus strains propagated in chick embyro or canine renal tissue cultures shall have been demonstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Vaccine prepared from measles virus strains propagated in canine renal tissue cultures shall also have been demonstrated to be free from harmful effects in not less than 100,000 persons. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents except for unavoidable bacteriophage.

(c) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated measles virus used in manufacture of measles virus vaccine. For this purpose, vaccine from each of the five consecutive lots (§ 630.31) used by the manufacturer to establish consistency of manufacture of the vaccine shall be tested separately in the following manner:

(i) Samples of each of the five lots of vaccine shall be tested in measles susceptible monkeys. Immediately prior to initiation of a test each monkey shall have been shown to be serologically negative for neutralizing antibodies by means of a tissue culture neutralization test with undiluted serum from each monkey tested at approximately 100 $TCID_{50}$ of Edmonston strain measles virus, or negative for measles virus antibodies as demonstrated by tests of equal sensitivity.

(ii) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(iii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Bureau of Biologics, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumber spinal cord enlargement.

(iv) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(v) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to subdivisions (i) through (iv) of this subparagraph. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(vi) At the end of the observation period, each surviving monkey shall (a) be bled and the serum tested for evidence of serum antibody conversion to measles virus and (b) be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identi-

fication if needed pursuant to subdivision (vii) of this subparagraph. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vii) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous systems tissues previously removed from the animal.

(viii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Wild virus controls.* As a check against the inadvertent introduction of wild measles virus, at least four uninoculated measles susceptible control monkeys shall be maintained as either cage mates to, or within the same immediate area of, the 20 inoculated test animals for each lot of vaccine for the entire period of observation (17–21 days) and an additional 10 days. Serum samples from these control contact monkeys drawn at the time of seed virus inoculation of the test animals, and again after completion of the test, shall be shown to be free of measles neutralizing antibodies.

(3) *Test results.* (i) For each lot of vaccine under test, at least 80 percent of the monkeys must show measles antibody serological conversion (1:4 or greater) when the serum as obtained from the monkey is tested and the control contact monkeys must demonstrate no immunological response indicative of measles virus infection.

(ii) The measles virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots (*a*) 90 percent of the monkeys survive the observation period, (*b*) the histological and other studies produce no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus, and (*c*) there is no evidence of the presence of extraneous neurotropic agents.

(4) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in § 630.32 (a), (b), and (c).

§ 630.31 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design, by subcutaneous administration of the product. Such clinical trials shall be conducted with five consecutive lots of measles virus vaccine which have been manufactured by the same methods, each of which has shown satisfactory results in all prescribed tests. There shall be a demonstration under circumstances wherein adequate clinical and epidemiological surveillance of illness has been maintained to show that the Measles Virus Vaccine, when administered as recommended by the manufacturer—i.e., either with or without human gamma globulin—is free of harmful effect upon administration to approximately 1,000 susceptible individuals, in that there were no detectable neutralizing antibodies before vaccination and there was serological conversion after vaccination. The five lots of vaccine used to qualify for consistency of vaccine manufacture shall be distributed as evenly as possible among the 1,000 individuals tested. Demonstration shall be made of immunogenic effect by the production of specific measles neutralizing antibodies (i.e., sero-conversion less than 1:4 to 1:8 or greater) in at least 90 percent of each of five groups of measles suspectible individuals, each having received the parenteral administration of a virus vaccine dose which is not greater than that which was demonstrated to be safe in field studies (§ 630.30(b)) when used under comparable conditions.

§ 630.32 Manufacture of live, attenuated Measles Virus Vaccine.

(a) *Virus cultures.* Virus shall be propagated in chick embryo tissue cultures or canine renal tissue cultures.

(b) *Virus propagated in chick embryo tissue cultures.* Embryonated chicken eggs used as the source of chick embryo tissue for the propagation of measles virus shall be derived from flocks certified to be free of Salmonella pullorum, avian tuberculosis, fowl pox, Rous sarcoma, avian leucosis and other adventitious agents pathogenic for chickens. If eggs are procured from flocks that are not so certified, tests shall be performed to demonstrate freedom of the vaccine from such agents. (See § 630.35(a)(8) for test for avian leucosis.)

(c) *Virus propagated in canine renal tissue cultures.* Only dogs in overt good health which have been maintained in quarantine in vermin-proof quarters for a minimum of six months, having had no exposure to other dogs or animals throughout the quarantine period, or dogs born to dogs while so quarantined, provided the progeny have been kept in the same type of quarantine continuously from birth, shall be used as a source of kidney tissue for the propagation of measles virus.

(1) *Dogs used for experimental purposes.* Dogs that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the manufacture of vaccine.

(2) *Quarantine and necropsy.* Each dog shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of dogs, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, infectious canine hepatitis, canine distemper, rabies, leptospirosis, and other diseases indigenous to dogs. If there are any such signs, symptoms, or other significant pathological lesions observed, tissue from such animals shall not be used in the manufacture of Measles Virus Vaccine, Live, Attenuated.

(d) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than ten tissue culture passages beyond the passage used to perform the clinical trials (§ 630.30 (b)) which qualified the manufacturer's vaccine strain for license.

(e) *Tissue culture preparation.* Only primary cell tissue cultures shall be used in the manufacture of Measles Virus Vaccine. Continuous cell lines shall not be introduced or propagated in Measles Virus Vaccine manufacturing areas.

(f) *Control vessels.* (1) From the tissue used for the preparation of tissue cultures for growing attenuated measles virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of tissue culture shall be used to prepare uninfected tissue control materials. This material shall be distributed in control vessels and observed microscopically for a period of no less than 14 days beyond the time of inoculation of the production vessels with measles virus; but if the production vessels are held for use in vaccine manufacture for more than 14 days, the control vessels shall be held and observed for the additional period. At the end of the observation period or at the time of virus harvest, whichever is later, fluids from the control cultures shall be tested for the presence of adventitious agents as follows:

Samples of fluid from each control vessel shall be collected at the same time as fluid is harvested from the corresponding production vessels. If multiple virus harvests are made from the same cell suspension, the control samples for each harvest shall be frozen and stored at −60° C. until the last viral harvest for that cell suspension is completed. The fluid from all the control samples from that suspension shall be pooled in proportionate amounts and at least five ml. inoculated into human and simian cell tissue culture systems and in the tissue culture system used for virus production. The cultures shall be observed for the presence of changes attributable to growth of adventitious viral agents including hemadsorption viral agents.

(2) The cell sheets of one quarter to one third of the control vessels shall be examined at the end of the observation period (14 days or longer) for the presence of hemadsorption viruses by the addition of guinea pig red blood cells. If the chick embryo cultures were not derived from a certified source (§ 630.32 (b)), the remaining tissue culture controls may be used to test for avian leucosis virus using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or a method of equivalent effectiveness.

(3) The test is satisfactory only if there is no evidence of adventitious viral agents and if at least 80 percent of the control vessels are available for observa-

tion at the end of the observation period (14 days or longer).

(g) *Test samples.* Samples of virus harvests or pools for testing by inoculation into animals, into tissue culture systems, into embryonated hens' eggs, and into bacteriological media, shall be withdrawn immediately after harvesting or pooling but prior to freezing except that samples of test materials frozen immediately after harvesting or pooling and maintained at −60° C. or below, may be tested upon thawing, provided no more than two freeze-thaw cycles are employed. The required tests shall be initiated without delay after thawing.

§ 630.33 Reference virus.

A U.S. Reference Measles Virus, Live, Attenuated, shall be obtained from the Bureau of Biologics as a control for correlation of virus titers.

§ 630.34 Potency test.

The concentration of live measles virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the U.S. Reference Measles Virus, Live, Attenuated or a calibrated equivalent strain as a titration control. The concentration of live measles virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 $TCID_{50}$ of the U.S. reference per human dose.

§ 630.35 Test for safety.

(a) *Tests prior to clarification of vaccine manufactured in chick embryo tissue cultures.* Prior to clarification, the following tests shall be performed on each virus pool of chick embryo tissue culture:

(1) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15–20 grams shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. amounts of each virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The virus pool may be used only if at least 80 percent of the original group of mice remain healthy and survive the observation period and if none of the mice show evidence of a transmissible agent or other viral infection, other than measles virus, attributable to the vaccine.

(2) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the original inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of a transmissible agent or viral infection, other than measles virus, attributable to the vaccine.

(3) *Inoculation of monkey tissue cell cultures.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 500 human doses or 50 ml., whichever represents a greater volume, shall be tested for adventitious agents in cercopithecus monkey kidney tissue culture preparations, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, and nonchicken origin. The immunizing antigen used for the preparation of the measles antiserum shall be grown in tissue culture cells that shall be free of extraneous viruses which might elicit antibodies that could inhibit growth of extraneous viruses present in the measles virus pool. The tissue culture of the virus pool shall be observed for no less than 14 days. The virus pool is satisfactory for measles virus vaccine only if all the tissue culture tests fail to show evidence of any extraneous transmissible agent other than measles virus attributable to the vaccine.

(4) *Inoculation of other cell cultures.* The measles virus pool shall be tested in the same manner as prescribed in subparagraph (3) in rhesus or cynomolgus monkey kidney, chick embryo, and human tissue cell cultures.

(5) *Inoculation of embryonated chicken eggs.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 100 doses or 10 ml., whichever represents a greater volume, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, nonchicken origin, shall be tested in embryonated eggs by the allantoic cavity route of inoculation and a separate group tested by the yolk sac route of inoculation, using 0.5 ml. of inoculum per egg. The virus pool is satisfactory if there is no evidence of adventitious agents.

(6) [Reserved]

(7) *Bacteriological tests.* Each virus pool shall be tested for sterility in accordance with § 610.12 of this chapter. In addition each virus pool shall be tested for the presence of M. tuberculosis, both avian and human, by appropriate culture methods.

(8) *Test for avian leucosis.* If the cultures were not derived from a certified source (§ 630.32(b)), and the control fluids were not tested for avian leucosis (§ 630.32(f)), at least 500 doses or 50 ml., whichever represents a greater volume of each undiluted vaccine pool, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or another method of equivalent effectiveness.

(b) *Tests prior to clarification of vaccine manufactured in canine renal tissue cultures.* Prior to clarification, the following tests shall be performed on each virus pool of canine renal tissue culture:

(1) *Inoculation of adult mice.* Virus grown in canine renal tissue cultures shall be tested in adult mice, as prescribed in paragraph (a)(1) of this section for virus grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(2) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and interperitoneally with 0.1 ml. of the canine renal tissue culture virus pool to be tested. The mice shall be observed daily for at least 28 days. Each mouse that dies after the first 48 hours of the test, or is sacrificed because of illness, shall be necropsied and all areas examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 28 days. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the originally inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of having been infected with rabies virus or any other transmissible agent or viral infection other than measles virus.

(3) *Inoculation of monkey tissue cell cultures.* Virus grown in canine renal tissue cultures shall be tested in monkey tissue cell cultures as prescribed in paragraph (a)(3) of this section for virus grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(4) *Inoculation of other cell cultures.* Virus grown in canine renal tissue cultures shall be tested in rhesus or cynomolgus monkey kidney tissue, canine renel tissue and human tissue cell cultures as prescribed in paragraph (a)(3) of this section for testing virus grown in chick embryo tissue culture in cercopithecus monkey kidney tissue culture preparations. Test result standards are those prescribed therein.

(5) *Inoculation of embryonated eggs.* Virus grown in canine renal tissue cultures shall be tested in embryonated eggs as prescribed in paragraph (a)(5) of this section for virus grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(6) [Reserved]

(7) *Bacteriological test.* Each virus pool shall be tested for sterility in accordance with § 610.12 of this chapter. In addition each virus pool shall be tested for M. tuberculosis, human, by appropriate culture methods.

(8) *Tests for adventitious agents.* Each virus pool shall be tested for the presence of such adventitious agents as canine distemper virus, canine hepati-

tis virus, leptospira and toxoplasma and the following fungi: coccidiomyces, histoplasma and blastomyces. The virus pool is satisfactory only if the results of all tests show no evidence of any extraneous agent attributable to the canine renal tissue or the vaccine.

(c) *Clarification.* After harvesting and removal of samples for testing as prescribed above in this section, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

§ 630.36 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b) [Reserved]

(c) [Reserved]

(d) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 $TCID_{50}$ of Measles Virus Vaccine, Live, Attenuated, expressed in terms of the assigned titer of the U.S. reference measles virus.

(e) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single-dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

(f) *Dried vaccine.* Measles Vaccine, Live, Attenuated, may be dried immediately after completion of processing to final bulk material and stored in the dried state, provided its residual moisture and other volatile substances content is not in excess of 2 percent, as provided in § 610.13(a) of this chapter.

(g) *Photochemical deterioration; protection.* Vaccine in multiple dose final containers shall be protected against photochemical deterioration. Such containers may be colored, or outside coloring or protective covering may be used for this purpose, provided (1) the method used is shown to provide the required protection, and (2) visible examination of the contents is not precluded. Vaccine in single dose containers may be protected in the same manner provided the same conditions are met.

(h) *Samples and protocols.* For each lot of vaccine, the following materials shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A protocol which consists of a summary of the history of the manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(2) A total of no less than 120 ml. in 10 ml. volumes, in a frozen state (−60° C.), of preclarification bulk vaccine containing no preservative or adjuvant, and no less than 100 ml. in 10 ml. volumes, in a frozen state (−60° C.), of post-clarification bulk vaccine containing stabilizer but no preservative or adjuvant, taken prior to filling into final containers.

(3) A total of no less than 200 recommended doses of the vaccine in final labeled containers distributed equally between the number of fillings made from each bulk lot, except that the representation of a single filling shall be no less than 30 final containers.

§ 630.37 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Measles Virus Vaccine, Live, Attenuated, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of officials record.

Subpart E—Measles Virus Vaccine, Inactivated

§ 630.40 Measles Virus Vaccine, Inactivated.

(a) *Proper name and definition.* The proper name of this product shall be Measles Virus Vaccine, Inactivated. The vaccine shall consist of a preparation of measles virus inactivated by an appropriate method.

(b) *Criteria for acceptable strains of measles virus.* Strains of measles virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation and (2) antigenic specificity as measles virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture of Measles Virus Vaccine, Inactivated, shall have been shown to be safe and potent in man by field studies with experimental vaccines. Vaccine prepared from measles virus strains propagated in chick embryo tissue cultures, monkey kidney tissue cultures or canine renal tissue cultures, shall have been demonstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Vaccine prepared from measles virus strains propagated in canine renal tissue cultures shall also have been demonstrated to be free from harmful effects in not less than 100,000 persons. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents.

§ 630.41 General requirements.

(a) [Reserved]

(b) *Extraneous protein.* The final vaccine shall have a protein nitrogen content of less than 0.02 milligram per individual human dose.

(c) *Dose.* These standards are based on an individual human dose of 1.0 ml. for a single injection.

(d) [Reserved]

(e) [Reserved]

(f) *Requirements for samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014.

(1) A sample of 1,500 doses of the vaccine taken after the last stage of manufacture before the addition of preservative or adjuvant.

(2) A sample of 100 doses of the final vaccine containing all preservatives.

(3) A sample of 200 doses of the final vaccine in final labeled containers.

(4) A protocol which consists of a summary of the history of the manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

§ 630.42 Manufacture of Measles, Virus Vaccine, Inactivated.

(a) *Virus cultures.* Virus shall be propagated in chick embryo tissue cultures, monkey kidney tissue cultures, or canine renal tissue cultures.

(b) *Virus propagated in chick embryo tissue cultures.* Embryonated chicken eggs used as a source of chick embryo tissue for the propagation of measles virus shall be derived from flocks certified to be free of Salmonella pullorum and avian tuberculosis, fowl pox, Rous sarcoma, avian leucosis and other adventitious agents pathogenic for chickens. If eggs are procured from flocks that are not so certified, tests shall be performed to demonstrate that the virus pool be free from such agents prior to inactivation.

(c) *Virus propagated in monkey kidney tissue cultures.* Only Macaca or Cercopithecus monkeys, or a species found by the Director, Bureau of Biologics, to be equally suitable, which have met all the quarantine requirements, shall be used as the source of kidney tissue for the manufacture of Measles Virus Vaccine, Inactivated.

(1) *Monkeys used for experimental purposes.* Monkeys that have been used previously for experimental purposes with microbiological agents shall not be used as a source of kidney tissue for the manufacture of vaccine. Monkeys that have been used previously for other experimental purposes may be used upon their return to a normal condition.

(2) *Quarantine.* Only monkeys that during the quarantine period, as provided by § 600.11(f)(2) of this chapter, have been tested with and have reacted negatively to tuberculin shall be used as a source of kidney tissue for vaccine manufacture.

(3) *Necropsy.* Each animal at necropsy shall be examined under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of monkeys, for the presence of signs or symptoms of ill health, particularly for (i) evidence of tuberculosis, (ii) presence of herpes-like lesions, including eruptions or plaques on or around the lips, in the buccal cavity or on the gums and (iii) signs of conjunctivitis. If any such signs or other

significant gross pathological lesions are present, the kidney shall not be used in the manufacture of Measles Virus Vaccine, Inactivated.

(d) *Virus propagated in canine renal tissue cultures.* Only dogs in overt good health which have been kept in quarantine in vermin-proof quarters for a minimum of six months, having had no exposure to other dogs or animals throughout the quarantine period, or dogs born to dogs while so quarantined, provided the progeny have been kept in the same type of quarantine continuously from birth, shall be used as a source of kidney tissue for the propagation of measles virus.

(1) *Dogs used for experimental purposes.* Dogs that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the manufacture of vaccine.

(2) *Quarantine and necropsy.* Each dog shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of dogs, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, infectious canine hepatitis, canine distemper, rabies, leptospirosis, and other diseases indigenous to dogs. If there are any such signs, symptoms, or other significant pathological lesions observed, the kidneys from such animals shall not be used in the manufacture of Measles Virus Vaccine, Inactivated.

(e) *U.S. Reference Measles Virus.* The following U.S. reference viruses shall be obtained from the Bureau of Biologics:

(1) U.S. Reference Measles Virus for titration.

(2) U.S. Reference Measles Vaccine for potency testing.

(f) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than ten tissue culture passages beyond the passage used to perform the clinical trials which qualified the vaccine strain for license (§ 630.40(b)), and the virus of that passage shall represent vaccine that shall have met the following criteria of acceptability:

(1) *Clinical safety.* The vaccine shall be free from harmful effects. Freedom from harmful effects shall be demonstrated by administration, as recommended by the manufacturer, and while maintaining adequate clinical and epidemiological surveillance of illness, to approximately 1,000 individuals, having no detectable neutralizing antibodies before vaccination and showing serological conversion after vaccination. Five consecutive lots of vaccine shall be used to qualify the vaccine for license and shall be distributed as evenly as possible among the 1,000 individuals tested.

(2) *Clinical potency.* The immunogenic effect (i.e., sero-conversion less than 1: 4 to 1: 8 or greater) shall be demonstrated in at least 90 percent of each of five groups of measles susceptible individuals, each group receiving vaccine from one of the five consecutive lots of vaccine which were used to qualify the vaccine for license, and each of which shall have met the safety standards prescribed in these regulations. The dose of vaccine shall be no greater than that which was demonstrated to be safe pursuant to subparagraph (1) of this paragraph and the vaccine shall be used under comparable conditions.

(g) *Types of tissue culture preparation permissible.* Measles Virus Vaccine, Inactivated, shall be produced only in primary cell tissue culture. Continuous line cells shall not be used and shall not be introduced into vaccine production areas.

(h) *Use of antibiotics.* Virus for manufacturing vaccine may be grown in cultures which contain minimum concentration of suitable antibiotics except that penicillin shall not be used in the tissue culture medium or added to the final product.

(i) *Clarification.* After harvesting, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

§ 630.43 Test for safety.

(a) *Tests prior to the inactivation process.* Samples of virus pools for testing by inoculation into animals or into bacteriological media shall be withdrawn immediately after pooling but prior to freezing or further processing, and tested, prior to the inactivation process, as provided in paragraphs (b) and (c) of this section except that samples of test materials frozen immediately after pooling and maintained at −60° C. or below, may be tested upon thawing, provided no more than two freeze thaw cycles are employed. The required tests shall be conducted without delay after thawing.

(1) *Measles virus propagated in chick embryo tissue cultures*—(i) *Inoculation of adult mice; test for adventitious agents.* Each chick embryo virus pool shall be shown to be free of contaminating agents pathogenic for mice by the intracerebral inoculation of 0.03 ml. and intraperitoneal inoculation of 0.5 ml. amounts of the pool into each of ten or more adult mice (15–20 gms.). The mice shall be observed for at least 21 days. The virus pool is satisfactory for measles virus vaccine only if at least 80 percent of the inoculated animals survive the observation period and none of the animals inoculated shows evidence of infection with extraneous transmissible agents attributable to the vaccine.

(ii) [Reserved]

(iii) *Bacteriological tests.* Each chick embryo virus pool shall be tested for bacteriological sterility in accordance with the procedures prescribed in § 610.12 of this chapter. In addition each virus pool shall be tested and found negative for the presence of M. tuberculosis, both avian and human, by appropriate culture methods.

(iv) *Test for avian leucosis.* The equivalent of at least 50 doses of final vaccine from each undiluted virus pool, or in proportionate amounts from individual harvests or subpools, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or a procedure of equivalent effectiveness. These tests may be performed on corresponding amounts of fluids from control vessels instead of on the undiluted virus pool or individual harvests of subpools.

(2) *Measles virus propagated in monkey kidney tissue cultures*—(i) *Inoculation of rabbits; test for B virus and other adventitious agents.* A minimum of 100 ml. of each monkey kidney virus pool shall be tested by inoculation into at least ten healthy rabbits, each weighing 1500–2500 grams. Each rabbit shall be injected intradermally at multiple sites with a total of 1.0 ml. and subcutaneously with 9.0 ml. of the virus, and the animals observed for at least three weeks. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool may be used for measles virus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if none of the rabbits used in the test shows lesions of any kind at the sites of inoculation or shows evidence of B virus or any other transmissible agent attributable to the vaccine.

(ii) *Inoculation of adult mice; test for adventitious agents.* Each virus pool grown in monkey kidney tissue culture shall be tested in adult mice. The test shall be performed and the results measured against the standards prescribed in subparagraph (1)(i) of this paragraph for chick embryo tissue culture.

(iii) *Inoculation of guinea pigs; test for M. tuberculosis.* Each of at least five guinea pigs, each weighing 350–450 grams shall be inoculated intraperitoneally with 5.0 ml. of the monkey kidney virus pool to be tested. The animals shall be observed for at least 42 days for death or signs of disease. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied. The tissues shall be examined both microscopically and culturally for evidence of M. tuberculosis. The virus pool is satisfactory for measles virus vacine only if at least 80 percent of the original group of guinea pigs remain healthy and survive the observation period, and if none of the animals used in the test shows evidence of infection with M. tuberculosis or any extraneous transmissible agent attributable to the vaccine.

(iv) *Bacteriological tests.* Each monkey kidney virus pool shall be tested for bacteriological sterility in accordance with the procedures prescribed in § 610.12 of this chapter. In addition each virus pool shall be tested for the presence of M. tuberculosis (human) by appropriate culture methods.

(v) *Tissue culture test for SV_{40}.* Each individual harvest or virus pool, or a pool

of tissue culture fluids from corresponding control vessels, shall be tested for the presence of SV_{40} either as follows or by a test producing equally reliable results: five ml. of a measles virus pool shall be neutralized by high titer antiserum of an origin other than human, chicken or simian. The sample shall be tested in the same tissue culture system used for propagating the virus vaccine, and in primary cercopithecus tissue cultures or in a cell line of demonstrated equal susceptibility to SV_{40}. The tissue cultures shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and the test continued for an additional 14 days. The virus harvest or virus pool is satisfactory for measles virus vaccine only if the test produces no evidence of the presence of SV_{40}.

(3) *Measles virus propagated in canine renal tissue cultures*—(i) *Inoculation of adult mice; test for adventitious agents.* Each virus pool prepared from canine renal tissue cultures shall be shown to be free from contaminating agents pathogenic for mice by the test prescribed in subparagraph (1)(i) of this section for chick embryo virus pools. Test result standards are those prescribed therein.

(ii) *Inoculation of suckling mice.* Suckling mice shall be inoculated as prescribed in § 630.35(b)(2) for virus (live, attenuated) grown in canine renal tissue cultures. Test result standards are those prescribed therein.

(iii) *Inoculation of monkey tissue cell cultures.* Monkey tissue cell cultures shall be inoculated as prescribed in § 630.35(a)(3) for virus (live, attenuated) grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(iv) *Inoculation of other cell cultures.* Virus grown in canine renal tissue cultures shall be tested in rhesus or cynomolgus monkey kidney tissue, canine renal tissue and human tissue cell cultures as prescribed in § 630.35(a)(3) for testing virus grown in chick embryo tissue cultures in cercopithecus monkey kidney tissue culture preparations. Test result standards are those prescribed therein.

(v) *Inoculation of embryonated chicken eggs.* Embryonated chicken eggs shall be inoculated as prescribed in § 630.35(a)(5) for virus (live, attenuated) grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(vi) [Reserved]

(vii) *Bacteriological test.* Each virus pool shall be tested for sterility in accordance with § 610.12 of this chapter. In addition each virus pool shall be tested for M. tuberculosis, human, by appropriate culture methods.

(viii) *Test for adventitious agents.* Each virus pool shall be tested for the presence of the adventitious agents enumerated in § 630.35(b)(8) for virus (live, attenuated) grown in canine renal tissue cultures. Test result standards are those prescribed therein.

(b) *Inactivation of virus.* The measles virus shall be inactivated through the use of an agent or method which the manufacturer has demonstrated to be effective in inactivating a series of at least five consecutive lots of measles virus vaccine. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. formaldehyde solution of a least 1:4,000. The inactivation shall be conducted under controlled conditions of pH and temperature. As an indication of inactivation not less than two samples shall be removed at the time of inactivation, and titrated in an appropriate tissue cell culture for viable measles virus. Regardless of the concentration of formaldehyde or other inactivating agent used, the total inactivation period shall be not less than three times the period demonstrated by the manufacturer to be necessary to reduce the concentration of live virus to a point where no virus is detectable in a 5.0 ml. sample.

(c) *Tests after inactivation for viable measles virus and adventitious agents*—(1) *Test in tissue cultures.* A sample representing the equivalent of at least 500 doses of final vaccine of each lot shall be rendered nontoxic for tissue culture cells and tested as follows: One half of the sample shall be tested in the same tissue culture system used for propagating the virus vaccine and one half of the sample shall be tested in primary cercopithecus monkey kidney tissue or another suitable cell line of demonstrated high susceptibility to measles virus, poliovirus, and SV_{40} or other adventitious viral agents. Each half of the sample shall be inoculated so that direct microscopic observation of the culture cells is possible under conditions which assure the growth of measles virus, poliovirus, and simian viruses which might have survived the inactivation procedure. After inoculation of the test sample, the tissue cultures shall be observed for at least 14 days. At the end of the observation period the fluids from all the culture bottles in a system shall be removed and pooled. At least two percent of each pool shall be subinoculated in the same cell system as that from which the pooled sample was drawn. The subcultures shall be observed for a period of at least 14 days and examined for cell changes indicative of viral growth. The lot of final vaccine is satisfactory for measles virus vaccine only if none of the tissue culture tests show evidence of viable measles virus or any extraneous transmissible agents attributable to the vaccine.

(2) *Test in embryonated chicken eggs.* For vaccine produced in chick embryo tissue culture, the equivalent of at least 100 doses of each vaccine lot shall be tested in embryonated eggs by the allantoic cavity route and of 100 doses by the yolk sac route of inoculation, using 0.5 ml. of inoculum per egg, and found negative for the presence of extraneous agents in the vaccine.

(3) *Test in monkeys for neurotropic agents.* Each lot of vaccine shall be tested for neurotropic agents following the procedure prescribed in § 630.4(e) except that antibody determinations for measles need not be performed, the test shall be performed before the product is placed in final containers and prior to the addition of an adjuvant, and that symptoms suggestive of all neurotropic agents shall be recorded during the observation period of 17 to 19 days. The lot is satisfactory only if the histological and other studies produce no clinical or histological evidence of central nervous system involvement attributable to the presence of a neurotropic agent in the vaccine.

§ 630.44 Potency test.

A potency test shall be performed on each lot of vaccine by determining the antigenic capacity of the vaccine under tests in comparison with a reference vaccine of antigenic capacity at least equal to that required for the clinical trials specified in § 630.42(h)(2). The test shall be performed using at least ten animals for each dilution of the test vaccine and of the reference vaccine. The average antibody levels of the animals injected with the vaccine under test shall equal or exceed the average antibody levels of the animals injected with the reference vaccine.

§ 630.45 Equivalent methods.

Modification of any particular method of process or the conditions under which it is conducted as set forth in the additional standards relating to Measles Virus Vaccine, Inactivated, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart F—Mumps Virus Vaccine, Live

§ 630.50 Mumps Virus Vaccine, Live.

(a) *Proper name and definition.* The proper name of this product shall be Mumps Virus Vaccine, Live, which shall consist of a preparation of live, attenuated mumps virus.

(b) *Criteria for acceptable strains of attenuated mumps virus.* Strains of attenuated mumps virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation, (2) antigenic specificity as mumps virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture of Mumps Virus Vaccine, Live, shall have been shown to be safe and potent in at least 5,000 susceptible individuals by field studies with experimental vaccines. Susceptibility shall be shown by the absence of neutralizing or other antibodies against mumps virus, or by other appropriate methods. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents except for unavoidable bacteriophage.

(c) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated mumps virus used in the manufacture of mumps vaccine. For this purpose, vaccine from each of the five consecutive lots (§ 630.51) used by the manufacturer to establish consistency of manufacture of the vaccine shall be tested separately in monkeys shown to be serologically negative for mumps virus antibodies in the following manner:

(i) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(ii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Bureau of Biologics, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumbar spinal cord enlargement.

(iii) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(iv) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to subdivisions (i) through (iii) of this subparagraph. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(v) At the end of the observation period, each surviving animal shall be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if needed pursuant to subdivision (vi) of this subparagraph. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vi) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous system tissues previously removed from the animals.

(vii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Test results.* The mumps virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots (i) 90 percent of the monkeys survive the observation period, (ii) the histological and other studies produce no evidence of changes in the central nervous system attributable to unusual neurotropism or replication of the seed virus and (iii) there is no evidence of the presence of extraneous neurotropic agents.

(3) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in § 630.52(a).

§ 630.51 Clinical trials to qualify for license.

To qualify for license, the antigenicity of Mumps Virus Vaccine, Live, shall be determined by clinical trials that follow the procedures prescribed in § 630.31 except that the immunogenic effect shall be demonstrated by establishing that a protective antibody response has occurred in at least 90 percent of each of the five groups of mumps susceptible individuals, each having received the parenteral administration of a virus vaccine dose which is not greater than that which was demonstrated to be safe in field studies (§ 630.50(b)) when used under comparable conditions.

§ 630.52 Manufacture of Mumps Virus Vaccine, Live.

(a) *Virus cultures.* Mumps virus shall be propagated in chick embryo cell cultures. The embryonated chicken eggs used as the source of chick embryo tissue for the propagation of mumps virus shall be derived from flocks certified or tested as prescribed in § 630.32(b).

(b) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than five cell culture passages beyond the passage used to perform the clinical trials (§ 630.50(b)) which qualified the manufacturer's vaccine strain for license.

(c) *Cell culture preparation.* Only primary cell cultures shall be used in the manufacture of mumps virus vaccine. Continuous cell lines shall not be introduced or propagated in mumps virus vaccine manufacturing areas.

(d) *Control vessels.* From the tissue used for the preparation of cell cultures for growing attenuated mumps virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of cell culture shall be used to prepare uninfected tissue control materials which shall be prepared and tested by following the procedures prescribed in § 630.32(f).

(e) *Test samples.* Test samples of mumps virus harvests or pools shall be withdrawn and maintained by following the procedures prescribed in § 630.32(g).

§ 630.53 Reference virus.

An NIH Reference Mumps Virus, Live, shall be obtained from the Bureau of Biologics as a control for correlation of virus titers.

§ 630.54 Potency test.

The concentration of live mumps virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the Reference Mumps Virus, Live, or a calibrated equivalent strain as a titration control. The concentration of live mumps virus contained in the vaccine of each lot under test shall be no less than the equivalent of 5,000 $TCID_{50}$ of the reference virus per human dose.

§ 630.55 Test for safety.

(a) *Tests prior to clarification.* Prior to clarification, the following tests shall be performed on each mumps virus pool prepared in chick embryo cell culture:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey cell cultures.* A mumps virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), and the virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The mumps virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), in rhesus or cynomolgus monkey kidney, in whole chick embryo and in human cell cultures. In addition, each virus pool shall be tested in chick embryo kidney and in chick embryo liver in the same manner except that the volume tested in each cell culture shall be equivalent to 250 human doses or 25 ml., whichever represents a greater volume. The mumps virus pool is satisfactory only if results equivalent to those in § 630.35(a)(3) are obtained.

(5) *Inoculation of embryonated chicken eggs.* A neutralized suspension of each undiluted mumps virus pool shall be tested in the volume and following the

procedures prescribed in § 630.35(a)(5), and the virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each mumps virus pool for the presence of M. tuberculosis, both avian and human, by appropriate culture methods. The virus pool is satisfactory only if found negative for M. tuberculosis, both avian and human.

(7) *Test for avian leucosis.* If the cultures were not derived from a certified source and control fluids were not tested for avian leucosis, the vaccine shall be tested in the volume and following the procedures prescribed in § 630.35(a)(8). The cultures are satisfactory for vaccine manufacture if found negative for avian leucosis.

(b) *Clarification.* The mumps virus fluids shall be clarified by following the procedures prescribed in § 630.35(c).

§ 630.56 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b) *Dose.* These standards are based on an individual human immunizing dose of no less than 5,000 $TCID_{50}$ of Mumps Virus Vaccine, Live, expressed in terms of the assigned titer of the Reference Mumps Virus, Live.

(c) *Labeling.* In addition to the items required by other applicable labeling provisions of this part, single dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

(d) *Dried vaccine.* Mumps Virus Vaccine, Live, may be dried immediately after completion of processing to final bulk material and stored in the dried state provided its residual moisture and other volatile substances content is not in excess of 2 percent when tested as prescribed in § 610.13(a) of this chapter.

(e) *Photochemical deterioration; protection.* Mumps Virus Vaccine, Live, in multiple dose containers, shall be protected against photochemical deterioration in accordance with the procedures prescribed in § 630.36(g).

(f) *Samples and protocols.* For each lot of vaccine, the following materials shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(2) A total of no less than a 500 ml. sample of bulk vaccine or an equivalent sample prior to addition of any preservative, stabilizer or adjuvant, in the frozen state (—60° C.) prior to filling into final containers.

(3) A total of no less than 200 recommended human doses of the vaccine in final labeled containers.

§ 630.57 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Mumps Virus Vaccine, Live, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart G—Rubella Virus Vaccine, Live

§ 630.60 Rubella Virus Vaccine, Live.

(a) *Proper name and definition.* The proper name of this product shall be Rubella Virus Vaccine, Live, which shall consist of a preparation of live, attenuated rubella virus.

(b) *Criteria for acceptable strains of attenuated rubella virus.* Strains of attenuated rubella virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation and (2) antigenic specificity as rubella virus as demonstrated by tissue culture neutralization tests.

(c) *Extraneous agents.* Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents except for unavoidable bacteriophage.

(d) *Field studies with experimental vaccines.* (1) Strains used for the manufacture of Rubella Virus Vaccine, Live, shall have been shown in field studies with experimental vaccines to be safe and potent in the group of individuals inoculated, which must include at least 10,000 susceptible individuals. Susceptibility shall be shown by the absence of neutralizing or hemagglutination-inhibiting antibodies against rubella virus or by other appropriate methods.

(2) The virus strain used in the field studies shall be propagated in the same cell culture system that will be used in the manufacture of the product.

(3) The field studies shall be so conducted that at least 5,000 of the susceptible individuals must reside when inoculated in areas where health related statistics are regularly compiled in accordance with procedures such as those used by the National Center for Health Statistics. Data in such form as will identify each inoculated person shall be furnished to the Director, Bureau of Biologics.

(4) Inoculated persons shall be shown not to be contagious for contacts through surveillance of rubella susceptible contacts of the inoculated persons.

(e) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated rubella virus used in the manufacture of rubella vaccine. For this purpose, vaccine from each of the five consecutive lots (§ 630.61) used by the manufacturer to establish consistency of manufacture of the vaccine shall be tested separately in monkeys shown to be serologically negative for rubella virus antibodies in the following manner:

(i) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(ii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Bureau of Biologics, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumbar spinal cord enlargement.

(iii) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(iv) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to subdivisions (i) through (iii) of this subparagraph. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(v) At the end of the observation period, each surviving animal shall be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if needed pursuant to subdivision (vi) of this subparagraph. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vi) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous system tissues previously removed from the animal.

(vii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to the presence of unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Test results.* The rubella virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots: (i) 90 percent of the monkeys survive the observation period, (ii) the histological and other studies produce no evidence of changes in the central nervous system attributable to the presence of unusual neurotropism or replication of the seed virus and (iii) there is no evidence of the presence of extraneous neurotropic agents.

(3) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in § 630.62(a), (b), (c), and (d).

§ 630.61 Clinical trials to qualify for license.

To qualify for license, the antigenicity of Rubella Virus Vaccine, Live, shall be determined by clinical trials that follow the procedures prescribed in § 630.31 except that the immunogenic effect shall be demonstrated by establishing that a protective antibody response has occurred in at least 90 percent of each of the five groups of rubella susceptible individuals, each having received the parenteral administration of a virus vaccine dose which is not greater than that which was demonstrated to be safe in field studies when used under comparable conditions.

§ 630.62 Production.

(a) *Virus cultures.* Rubella virus shall be propagated in duck embryo cell cultures, canine renal cell cultures or rabbit renal cell cultures.

(b) *Virus propagated in duck embryo tissue cell cultures.* Embryonated duck eggs used as a source of duck embryo tissue for the propagation of rubella virus shall be derived from flocks certified to be free of avian tuberculosis, the avian leucosis-sarcoma group of viruses and other agents pathogenic for ducks. Only ducks so certified and in overt good health and which are maintained in quarantine shall be used as a source of duck embryo tissue used in the propagation of rubella virus. Ducks in the quarantined flock that die shall be necropsied and examined for evidence of significant pathologic lesions. If any such signs or pathologic lesions are observed, eggs from that flock shall not be used for the manufacture of Rubella Virus Vaccine, Live. Control vessels shall be prepared, observed and tested as prescribed in § 630.32(f).

(c) *Virus propagated in canine renal tissue cell cultures.* When canine renal cell cultures are used for the propagation of rubella virus the renal tissue shall be obtained from dogs meeting the requirements specified in § 630.32(c). Control vessels shall be prepared, observed and tested as prescribed in § 630.32(f).

(d) *Virus propagated in rabbit renal tissue cell cultures.* Only rabbits in overt good health which have been maintained in quarantine individually caged in vermin-proof quarters for a minimum of 6 months, having had no exposure to other rabbits or animals throughout the quarantine period, or rabbits born to rabbits while so quarantined, provided the progeny have been kept in the same type of quarantine continuously from birth shall be used as a source of kidney tissue. Animals shall be free of antibodies for agents potentially pathogenic for man unless it has been demonstrated in the license application that the tests required by § 630.65(c) to be performed on each lot of vaccine are capable of detecting contamination of agents capable of producing such antibodies.

(1) *Rabbits used for experimental purposes.* Rabbits that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the production of vaccine.

(2) *Quarantine and necropsy.* Each rabbit shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of rabbits, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, myxomatosis, fibromatosis, rabbit pox, and other diseases indigenous to rabbits. If there are any such signs, symptoms or other significant pathological lesions observed, tissues from that colony shall not be used in the production of vaccine.

(3) *Control vessels.* Control vessels shall be prepared, observed and tested as prescribed in § 630.32(f).

(e) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than five cell culture passages beyond the passage used as the seed strain for the manufacture of the vaccines used to perform the field studies (§ 630.60(d)), which qualified the manufacturer's vaccine strain for license.

(f) *Cell cultures in vaccine production areas.* Only the cell cultures used in the propagation of rubella virus vaccine shall be introduced into rubella virus vaccine production areas.

(g) *Test samples.* Test samples of rubella virus harvests or pools shall be withdrawn and maintained by following the procedures prescribed in § 630.32(g).

§ 630.63 Reference virus.

A Reference Rubella Virus, Live, shall be obtained from the Bureau of Biologics as a control for correlation of virus titers.

§ 630.64 Potency test.

The concentration of live rubella virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, using either the Reference Rubella Virus, Live, or a calibrated equivalent strain as a titration control. The concentration of live rubella virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 $TCID_{50}$ of the reference virus per human dose.

§ 630.65 Test for safety.

(a) *Tests prior to clarification of vaccine manufactured in duck embryo cell cultures.* Prior to clarification, the following tests shall be performed on each rubella virus pool prepared in duck embryo cell cultures:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), in rhesus or cynomolgus monkey kidney, in chick embryo, duck embryo, and in human cell cultures, except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if results equivalent to those in § 630.35(a)(3) are obtained.

(5) *Inoculation of embryonated chicken eggs.* A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in § 630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Inoculation of embryonated duck eggs.* A suspension of each undiluted rubella virus pool shall be tested in embryonated duck eggs, in the volume and following the procedures prescribed in § 630.35(a)(5) except, that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(7) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of M. tuberculosis, both avian and human, by appropriate culture methods. The virus pool is satisfactory only if found negative for M. tuberculosis, both avian and human.

(8) *Test for avian leucosis.* The vaccine shall be tested for avian leucosis, in the volume and following the procedures prescribed in § 630.35(a)(8). The cultures are satisfactory for vaccine manufacture if found negative for avian leucosis.

(9) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in subparagraphs (3), (4), (5), and (6) of this paragraph shall be carried out also with rubella virus that has been neutralized by the addition of high titer antiserum of nonhuman, nonsimian and nonavian origin except that the volume of virus suspension of each undiluted virus pool tested shall be no less than 5 ml. The rubella antiserum shall have been prepared by using a rubella virus propagated in a cell culture system other than that used for the manufacture of the vaccine under test, and the cell culture system shall be free of extraneous agents which might elicit antibodies that could inhibit growth of any known extraneous agents which might be present in the vaccine under test. These tests may be performed either before or after clarification of the virus. The virus pool is satisfactory only if the results obtained are equivalent to those required in those subparagraphs.

(b) *Tests prior to clarification of vaccine manufactured in canine renal cell cultures.* Prior to clarification each rubella virus pool prepared in canine renal cell cultures shall be tested as follows:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(b)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The tests shall be performed in the volume and following the procedures prescribed in § 630.35(a)(3), in rhesus or cynomolgus monkey kidney tissue, canine renal tissue and human tissue cell cultures, except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(5) *Inoculation of embryonated chicken eggs.* The tests shall be performed in the volume and following the procedures prescribed in § 630.35(a)(5) except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(6) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of M. tuberculosis, human, by appropriate culture methods. The rubella virus pool is satisfactory only if found negative for M. tuberculosis, human.

(7) *Tests for adventitious agents.* Tests shall be performed for the presence of adventitious agents as prescribed in § 630.35(b)(8), and the rubella virus pool is satisfactory only if equivalent test results are obtained.

(8) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in subparagraphs (3), (4), and (5) of this paragraph shall be carried out also with rubella virus that has been neutralized following the procedures and in the volume prescribed in paragraph (a)(9) of this section. The virus pool is satisfactory only if the results obtained are equivalent to those required by that subparagraph.

(c) *Tests prior to clarification of vaccine manufactured in rabbit renal cell cultures.* Prior to clarification each rubella virus pool prepared in rabbit renal cell cultures shall be tested as follows:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The tests shall be performed in the volume and following the procedures prescribed in § 630.35(a)(3) in rhesus or cynomolgus monkey kidney tissue, rabbit renal tissue and human tissue cell cultures, except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(5) *Inoculation of embryonated chicken eggs.* A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in § 630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Inoculation of rabbits.* A minimum of 15 ml. of each virus pool shall be tested by inoculation into at least five healthy rabbits, each weighing 1500–2500 grams. Each rabbit shall be injected intradermally in multiple sites with a total of 1.0 ml. and subcutaneously with 2.0 ml., of the virus pool, and the animals observed for at least 30 days. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool is satisfactory only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of any viral infection.

(7) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighing 350–450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5 ml. of the undiluted virus pool. The animals shall be observed for at least 42 days. Each animal that dies after the first 24 hours of the test or is sacrificed because of illness, shall be necropsied. All remaining animals shall be sacrificed and necropsied at the end of the observation period. The virus pool is satisfactory only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with *M. tuberculosis* or any viral infection.

(8) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of *M. tuberculosis*, human, by appropriate culture methods. The rubella virus pool is satisfactory only if found negative for *M. tuberculosis*, human.

(9) *Tests for adventitious agents.* Each virus pool shall be tested for the presence of such known adventitious agents of rabbits as toxoplasma, encephalitozoon, herpes cuniculi, the vacuolating virus of rabbits, rabbit syncytial virus, myxoviruses and reoviruses. The virus pool is satisfactory only if the results of all tests show no evidence of any extraneous agent attributable to the rabbit renal tissue or the vaccine.

(10) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in subparagraphs (3), (4), and (5) of this paragraph shall be carried out also with rubella virus that has been neutralized by the addition of high titer antiserum of nonhuman, nonsimian and nonrabbit origin following the procedures and in the volume prescribed in paragraph (a)(9) of this section. The virus pool is satisfactory only if the results obtained are equivalent to those required by that paragraph.

(d) *Clarification.* The rubella virus fluids shall be clarified by following the procedures prescribed in § 630.35(c).

§ 630.66 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or on the bulk vaccine prior to filling.

(b) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 $TCID_{50}$ of Rubella Virus Vaccine, Live, expressed in terms of the assigned titer of the Reference Rubella Virus, Live.

(c) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to light.

(d) *Photochemical deterioration; protection.* Rubella Virus Vaccine, Live, in multiple dose containers, shall be protected against photochemical deterioration in accordance with the procedures prescribed in § 630.36(g).

(e) *Samples; protocols; official release.* For each lot of vaccine, the following shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD. 20014:

(1) A protocol which consists of a summary of the history of the manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(2) A total of no less than 120 ml. in 10 ml. volumes, in a frozen state (−60° C.), of preclarification bulk vaccine containing no preservative or adjuvant, and no less than 100 ml. in 10 ml. volumes, in a frozen state (−60° C.), of postclarification bulk vaccine containing stabilizer but no preservative or adjuvant, taken prior to filling into final containers.

(3) A total of no less than 200 recommended doses of the vaccine in final labeled containers distributed equally between the number of fillings made from each bulk lot, except that the representation of a single filling shall be no less than 30 single dose final containers or six multiple dose final containers.

The product shall not be issued by the manufacturer until notification of official release of the lot is received from the Director, Bureau of Biologics.

§ 630.67 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Rubella Virus Vaccine, Live, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart G—Smallpox Vaccine

§ 630.70 Smallpox Vaccine.

(a) *Proper name and definition.* The proper name of this product shall be Smallpox Vaccine, which shall be a preparation of live vaccinia virus obtained from inoculated calves or chicken embryos.

(b) *Strains of virus.* The strain of seed virus used in the manufacture of Smallpox Vaccine shall be identified by historical records including origin and manipulation, shall be sterile when tested by the procedure prescribed in § 610.12 of this chapter and shall be dermatropic according to the test prescribed in § 630.73(a). In addition, any new strain shall be shown not to produce a reactivity in man exceeding that produced by the Reference Smallpox Vaccine.

§ 630.71 Production.

Vaccinia virus used for the manufacture of vaccine shall be obtained from vesicles on the skin of an inoculated calf or from inoculated chorioallantoic membranes of chicken embryos, as set forth below:

(a) *Virus from calves*—(1) *Quarantine.* Only calves which, prior to being placed in quarantine have reacted negatively to tuberculin, were afebrile and free of ectoparasites, and which shall have met all other applicable quarantine requirements of § 600.11(f)(2)(i) of this chapter, shall be used for vaccinia virus production. The quarantine period shall be at least 14 days. During the last 7 days of the quarantine period daily morning and afternoon rectal temperatures shall be taken and calves that do not remain afebrile during that period shall not be used for virus production.

(2) *Inoculation.* A larger area of the calf than will be used for production purposes shall be prepared in a manner comparable to that appropriate for aseptic surgery, except that the area to be inoculated must be washed free of all antiseptics that may have a deleterious effect on virus propagation. The instrument and method used for scarification must produce a uniform penetration into the epidermis but must not extend through into the corium.

(3) *Incubation.* The inoculated calf shall remain in the incubation room confined to its stall and daily morning and afternoon rectal temperatures shall be taken to determine that only the expected febrile condition occurs. If any signs of disease other than vesiculation at the inoculation site occur, the virus from that calf shall not be used for vaccine manufacture.

(4) *Harvesting.* Before harvesting, the calf shall be anesthetized and killed by exsanguination. Prior to harvesting, the inoculated area shall be thoroughly cleansed by aseptic techniques. Only the vesicular material shall be harvested.

(5) *Necropsy.* A necropsy shall be made of each production calf. The harvested material shall not be used from any animal suspected of having an infection other than vaccinia.

(b) *Virus from embryonated chicken eggs*—(1) *Eggs for production.* Embryonated chicken eggs used for propagation of vaccinia virus shall be derived from flocks found to be free of, and continuously monitored for freedom from *Salmonella pullorum*, Mycoplasma species, avian tuberculosis, fowl pox, Newcastle disease virus, Rous sarcoma virus, avian leucosis complex of viruses, and other agents pathogenic for chickens, or appropriate tests shall be performed to demonstrate freedom of the vaccine from such agents.

(2) *Harvesting.* Aseptic techniques shall be used in harvesting the chorioallantoic membranes exhibiting vesicles characteristic of vaccinia infection.

§ 630.72 Reference vaccine.

Reference Smallpox Vaccine and reconstitution fluid shall be obtained from the Bureau of Biologics and shall be used in all tests for determining the potency of Smallpox Vaccine.

§ 630.73 Potency test.

Each filling of Smallpox Vaccine shall be tested for potency either by the "rabbit scarification" method or by the "pock count" method as follows:

(a) *Rabbit scarification*—(1) *Reconstitution of reference vaccine.* The Reference Smallpox Vaccine shall be reconstituted with the reconstitution fluid furnished by the Bureau of Biologics with the reference vaccine, and shall be used immediately after reconstitution.

(2) *Dilutions.* Dilutions shall be made starting with no less than 0.5 ml. each of the test vaccine and of the reference vaccine, including dilutions 1 : 3,000, 1 : 9,000, and 1 : 27,000. The same diluent shall be used for all the dilutions of both vaccines. The sample for vaccine in capillary tubes shall be obtained by pooling the contents of no less than 50 capillaries into a sterile container.

(3) *Preparation of test animals.* At least two rabbits with skin free of blemishes shall be used. The skin of the areas to be scarified must be free of hair, abrasions and virucidal and virustatic chemicals. Test sites measuring 2.5 x 5.0 cm. shall be marked off on the denuded skin of each rabbit without stretching the skin. All test sites shall be sacrificed uniformly.

(4) *Inoculation of test animals.* Immediately following thorough mixing, 0.2 ml. of each dilution of the test vaccine and of the reference shall be applied to the skin of each rabbit and rubbed into the appropriate scarified test area. After completion of all inoculations for each animal, the site shall be air dried with cool air and the animal then returned to its cage.

(5) *Recording the results.* The rabbits shall be observed daily. The reading shall be recorded at the height of reaction and such reading shall be used to calculate the maximum degree of reactivity for each dilution, which shall be determined by calculating the average percentage

reaction of at least two nonrefractive animals used in testing each lot. The arithmetic mean of the average reactions occurring at the 1 : 3,000, 1 : 9,000, and 1 : 27,000 dilutions shall be computed and used to determine the potency ratio between the test vaccine and the reference.

(6) *Potency requirements*—(i) *Vaccine intended for multiple pressure administration.* Vaccine intended for multiple pressure administration shall have a minimum potency ratio of 0.7 of the reference vaccine.

(ii) *Vaccine intended for jet injection.* One human dose of vaccine intended for administration by jet injector shall have a minimum potency ratio of 0.7 times that of 0.1 ml. of the reference vaccine, diluted 1 : 30.

(iii) *Heated liquid vaccine.* Samples of liquid vaccine from final containers taken at random shall be incubated at 35° to 37° C. for at least 18 hours, after which a 1:1,000 dilution of the heated sample and a 1:3,000 dilution of an unheated sample from the same lot shall be tested in parallel using the same rabbit, as prescribed in this paragraph. The vaccine is satisfactory if the potency of the heated sample is at least equal to that of the unheated sample.

(iv) *Heated dried vaccine.* Samples of dried vaccine from final containers taken at random shall be incubated at 35° to 37° C. for 30 days, after which a 1:1,000 dilution of the heated sample and a 1:3,000 dilution of an unheated sample from the same lot shall be tested in parallel using the same rabbit, as prescribed in this paragraph. The vaccine is satisfactory if the potency of the heated sample is at least equal to that of the unheated sample.

(b) *Pock counting in embryonated chicken eggs*—(1) *Dilutions.* Dilutions shall be made starting with no less than 0.5 ml. of the test vaccine and of the reference vaccine. The same diluent shall be used for all dilutions of both vaccines. The sample of vaccine in capillary tubes shall be obtained by pooling the contents of no less than 50 capillaries into a sterile vessel.

(2) *Inoculation of embryonated chicken eggs.* The chorioallantoic membranes of each of at least five embryonated chicken eggs shall be inoculated with 0.2 ml. for each virus dilution of the test vaccine and the reference vaccine, after which the eggs shall be incubated at 37° C. for 48 hours.

(3) *Estimation of potency.* Only membranes from living embryos shall be removed and the number of specific lesions thereon shall be counted and recorded. The number of pock forming units in 1.0 ml. of vaccine shall be calculated from the number of lesions, the dilution factor and the volume used, to determine the titer of the undiluted vaccine. The accuracy of the titration shall be confirmed in each test by performing simultaneously the same type of titration with the reference vaccine which shall demonstrate its assigned titer.

(4) *Potency requirements*—(i) *Vaccine intended for multiple pressure administration.* Vaccine intended for multiple pressure administration shall have a titer at least equivalent to the reference vaccine.

(ii) *Vaccine intended for jet injection.* Vaccine intended for administration by jet injector shall have a number of pock forming units in one human dose at least equivalent to that contained in 0.1 ml. of the reference vaccine diluted 1:30.

(iii) *Heated liquid vaccine.* Samples of liquid vaccine from final containers taken at random shall be incubated at 35° to 37° C. for at least 18 hours, after which the heated sample shall be tested in parallel with a sample of unheated vaccine of the same lot, as prescribed in this paragraph. The vaccine is satisfactory if the heated sample retains at least one tenth of the potency of the unheated sample.

(iv) *Heated dried vaccine.* Samples of dried vaccine from final containers taken at random shall be incubated at 35° to 37° C. for 30 days, after which the heated sample shall be tested in parallel with a sample of unheated vaccine of the same lot, as prescribed in this paragraph. The vaccine is satisfactory if the heated sample retains at least one-tenth of the potency of the unheated sample.

§ 630.74 Tests for safety.

(a) *Clostridium tetani.* A 10 ml. sample representative of the homogenized viral harvest or pool of several viral harvests shall be tested for the presence of *Clostridium tetani* in the following manner: Prior to the addition of preservatives other than glycerin, the test sample shall be inoculated into freshly heated Fluid Thioglycollate Medium or Smith fermentation tubes containing freshly heated Thioglycollate Broth Medium using a ratio of inoculum to culture medium sufficient for optimal bacterial growth. The test vessels shall be incubated at 35° to 37° C. and observed daily for at least 9 days for evidence of bacterial growth. Within 24–48 hours of an indication that there may be anaerobic growth, 1.0 ml. samples from each test vessel showing growth shall be injected subcutaneously into each of at least three mice, each weighing not more than 20 grams, or into each of at least three guinea pigs, each weighing not more than 350 grams, or into both such groups of mice and guinea pigs. The animals shall be observed daily for 6 days for signs of tetanus. If the animals show no signs of tetanus, additional groups of the same types and numbers of animals shall be injected 9 days after the original planting, with 1.0 ml. samples from each test vessel showing growth. The animals shall be observed daily for 6 days for signs of tetanus. If any animals die within 3 days without having shown signs of tetanus, the test shall be repeated within 18 hours of the deaths, with 0.1 ml. samples of the culture from which that animal was inoculated. Samples from the culture shall be injected into each of three additional test animals of the same species and the animals observed daily for 6 days. If there is any evidence of the presence of *Clostridium tetani*, the viral harvest may not be used in the manufacture of Smallpox Vaccine.

(b) *Anaerobes.* Prior to the addition of preservatives other than glycerin, a 10 ml. sample representative of the homogenized viral harvest or pool of viral harvests shall be inoculated into freshly heated Fluid Thioglycollate Medium or Smith fermentation tubes containing freshly heated Thioglycollate Broth Medium using a ratio of inoculum to culture medium sufficient for optimal bacterial growth. The test vessels shall be held at 65° C. for one hour, then incubated at 35° to 37° C. and observed daily for 10 days for evidence of bacterial growth. Within 24–48 hours of the first appearance of anaerobic growth, 1.0 ml. samples from each vessel showing growth shall be inoculated subcutaneously into each of at least three mice weighing not more than 20 grams and three guinea pigs weighing not more than 350 grams. Additional groups of animals shall be inoculated 9 days after the original planting if growth appears and provided the first set of test animals is negative. All test animals shall be observed daily for at least 6 days. If there is any evidence of the presence of heat resistant pathogenic anaerobes, the viral harvest may not be used in the manufacture of Smallpox Vaccine.

(c) *Coliform organisms.* A 5.0 ml. sample of bulk vaccine shall be tested for the presence of coliform organisms by the method published by the American Public Health Association, Inc., in "Standard Methods for the Examination of Water and Wastewater" (13th edition, 1971), section entitled "Multiple-Tube Fermentation Technic for Members of the Coliform Group," pages 662–678[1] and any amendments or revisions thereof, which section is hereby incorporated by reference and deemed published herein. Said publication is available at most medical and public libraries and copies of the pertinent section will be provided to any manufacturer affected by the provisions of this part upon request to the Director, Bureau of Biologics, or to the appropriate Information Center Officer listed in 45 CFR Part 5. In addition, an official historic file of the material incorporated by reference is maintained in the Office of the Director, Bureau of Biologics. A method different than that contained in the above cited section may be used to test for the presence of coliform organisms upon a showing that it is of equal or greater sensitivity. The ratio of the volume of inoculum to the volume of culture medium shall be such as will dilute the preservative to a level that does not inhibit growth of contaminating organisms. The vaccine is satisfactory if there is no evidence of coliform organisms.

(d) *Hemolytic streptococci and coagulase-positive staphylococci.* Each of three 1.0 ml. samples of bulk vaccine shall be spread uniformly on the surface of separate blood agar plates. The plates shall be incubated for 48 hours at 35° to 37° C. The vaccine is satisfactory if there is no evidence of the presence of either

[1] Copies may be obtained from: American Public Health Association, 1015 Eighteenth St. NW., Washington, DC 20036.

hemolytic streptococci or coagulase-positive staphylococci.

(e) *Viable bacteria*—(1) *Vaccine intended for multiple pressure administration.* Samples of each lot of both bulk and final container vaccine shall be tested for viable bacteria by a procedure designed to detect both aerobic and anaerobic growth through a period of 7 days. At least three 1.0 ml. samples of bulk vaccine and three 0.2 ml. samples of vaccine derived from not less than three final containers or dilutions thereof shall be inoculated into a volume of culture medium sufficient for optimal bacterial growth. The vaccine is satisfactory if it contains no more than 200 viable organisms per ml.

(2) *Vaccine intended for jet injection.* Samples of each lot of both bulk and final container vaccine shall be tested for viable bacteria in Fluid Thioglycollate Medium prepared in accordance with § 610.12(e)(1)(i) of this chapter for at least a 7-day test period. A sample of at least 10.0 ml. of bulk vaccine and 1.0 ml. from each of at least 20 final containers shall be tested. The ratio of the volume of the inoculum to the volume of culture medium shall be such as will dilute the preservative in the inoculum to a level that does not inhibit growth of contaminating micro-organisms. The vaccine is satisfactory if it contains no more than one organism per 100 doses of vaccine.

(f) *Sterile vaccine.* If any lot of smallpox Vaccine meets the sterility requirements prescribed in § 610.12 of this chapter the tests prescribed in paragraphs (b), (c), (d), and (e) of this section need not be performed.

§ 630.75 General requirements.

(a) *General safety.* Each lot of vaccine shall be tested for safety as prescribed in § 610.11 of this chapter and shall meet the safety requirements of that section, except that for liquid Smallpox Vaccine distributed in capillaries, the test may be performed with a sample of bulk vaccine taken at the time of filling into final containers.

(b) *Preservative.* A preservative that meets the requirements of § 610.15 of this chapter may be used, provided that if the preservative is phenol, its concentration shall not exceed 0.5 percent.

(c) *Labeling.* In addition to complying with all other applicable labeling provisions of this subchapter the package label shall bear the following:

(1) *Vaccine intended for jet injection.* (i) A conspicuous statement that the vaccine is intended for administration by jet injector.

(ii) A statement that the vaccine has been shown by appropriate test methods to contain not more than one organism per 100 doses or reference to an enclosed circular that contains such information, except that such a statement is not required for vaccine which meets the sterility requirements of § 610.12 of this chapter.

(2) *Vaccine intended for multiple pressure administration.* A statement that the vaccine has been shown by appropriate test methods to contain not more than 200 organisms per ml. or reference to an enclosed circular that contains such information, except that such a statement is not required for vaccine which meets the sterility requirements of § 610.12 of this chapter.

(d) *Samples; protocols; official release.* (1) For each lot of vaccine the following shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(i) A protocol which consists of a summary of the history of manufacture of each filling including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(ii) Three hundred capillaries from the first filling of a lot of liquid vaccine, and 200 capillaries from each subsequent filling.

(iii) Two 10 ml. samples of bulk liquid vaccine to be submitted along with the capillaries from the first filling and taken from the same vessel from which such capillaries were filled.

(iv) A sample from each drying, consisting of no less than the equivalent of 30 ml. of reconstituted vaccine, packaged in final containers, but in no event less than six filled final containers.

(2) Smallpox Vaccine shall not be issued by the manufacturer until notification of official release of the lot is received from the Director, Bureau of Biologics.

§ 630.76 Equivalent methods.

Modification of any particular manufacturing method or procedure or the conditions under which it is conducted as set forth in the additional standards relating to smallpox vaccine (§§ 630.70 to 630.75, inclusive) shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide equal or greater assurances of the safety, purity, and potency of the vaccine as the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

Subpart I—Measles-Smallpox Vaccine, Live

§ 630.80 Measles-Smallpox Vaccine, Live.

(a) *Proper name and definition.* The proper name of this product shall be Measles-Smallpox Vaccine, Live. The product shall consist of a preparation of live attenuated measles virus combined with live vaccinia virus.

(b) *Strains of virus.* Any strain of attenuated measles seed virus used in the manufacture of this product shall meet the requirements of § 630.30(b) and any strain of vaccinia seed virus used in the manufacture of this product shall meet the requirements of § 630.70(b).

(c) *Neurovirulence of measles virus and seed strain.* The neurovirulence of the measles virus seed strain shall be tested as prescribed in, and meet the requirements of, § 630.30(c).

§ 630.81 Clinical trials to qualify for license.

In addition to demonstrating that the measles component meets the requirements of § 630.31, the measles and smallpox antigenicity of the final product shall have been determined by clinical trials of adequate statistical design conducted with five consecutive lots of the final vaccine manufactured by the same methods and administered as recommended by the manufacturer. Such clinical trials shall include administration of the product to measles and smallpox susceptible individuals and to persons previously immunized with smallpox vaccine. At least 95 percent of the smallpox susceptible persons shall show a primary vaccination reaction and at least 95 percent of persons previously immunized with smallpox vaccine shall show a revaccination reaction. At least 90 percent of the measles susceptible individuals shall demonstrate measles neutralizing antibodies at the 1:8 dilution or greater. There shall also be a demonstration of the safety of the product, by administration as recommended by the manufacturer, under circumstances wherein adequate clinical and epidemiological surveillance of illness has been maintained.

§ 630.82 Production.

The measles vaccine component of this product shall be manufactured in accordance with, and meet the requirements of, § 630.32. The smallpox vaccine component of this product shall be manufactured in accordance with, and meet the requirements of, § 630.71, and in addition, prior to any filtration or dilution, shall be tested for potency in accordance with § 630.73 and shall have a potency at least equivalent to that of the Reference Smallpox Vaccine.

§ 630.83 Reference vaccines.

Reference Measles Virus Vaccine, Live, Attenuated, and Reference Smallpox Vaccine and reconstitution fluid shall be obtained from the Bureau of Biologics. The reference measles vaccine shall be used as a control for correlation of virus titers for the measles component of the product. The reference smallpox vaccine shall be used to determine the potency of the smallpox component of the product.

§ 630.84 Potency tests.

Each lot of Measles-Smallpox Vaccine, Live, shall be tested for potency, as follows:

(a) *Measles.* After neutralization of the vaccinia virus, each lot of the product shall be tested for, and shall meet the measles vaccine requirements of, potency prescribed in § 630.34.

(b) *Smallpox.* Each lot of the product shall be tested for potency as prescribed in § 630.73. The product is satisfactory if the vaccinia virus contained in one human dose is at least equivalent to that contained in 0.5 ml. of the Reference Smallpox Vaccine diluted 1:100.

(c) *Heated vaccine.* Samples of dried vaccine from final containers shall be taken at random and tested as prescribed

in, and shall meet the potency requirements of, § 630.73(a)(6)(iv) or (b)(4)(iv).

§ 630.85 Tests for safety.

The measles virus component of this product shall be tested for safety as prescribed in § 630.35. The smallpox component of this product shall be tested for safety as prescribed in § 630.74(a). The product is satisfactory if the safety test results meet the requirements of §§ 630.35 and 630.74(a), respectively.

§ 630.86 General requirements.

(a) *Sterility.* Each lot of vaccine shall be tested for, and meet the sterility requirements of, § 610.12 of this chapter, regardless of the source of the vaccinia virus.

(b) *Identity.* An immunological and virological identity test shall be performed either on each pool or the bulk vaccine prior to filling into final containers, or for each filling. If the immunological and virological identity test was performed only on the pool or bulk vaccine, a final container identity test must be performed pursuant to § 610.14 of this chapter.

(c) *Photochemical deterioration; protection.* Vaccine final containers shall be protected against photochemical deterioration. Such containers may be colored, or outside coloring or protective covering may be used for this purpose, provided (1) the method used is shown to provide the required protection, and (2) visible examination of the contents is not precluded.

(d) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, labeling shall contain a statement that the product is intended for administration only by jet injector and a description of the method of administration.

(e) *Samples; protocols; official release.* For each lot of vaccine the following materials shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014.

(1) A protocol which consists of a summary of the history of manufacture of each filling including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(2) A total of no less than 120 ml. in 10 ml. volumes, in a frozen state (−60° C.), of the bulk measles component prior to clarification and containing no preservative or adjuvant, and no less than 100 ml. in 10 ml. volumes, in a frozen state (−60°C.), of the bulk measles component after clarification and containing stabilizer but no preservative or adjuvant, taken prior to filling into final containers.

(3) A frozen 5 ml. sample of the smallpox component prior to any dilution or filtration.

(4) A frozen 5 ml. sample of the smallpox component taken subsequent to any dilution or filtration.

(5) A sample consisting of no less than the equivalent of 25 ml. of reconstituted vaccine packaged in no less than five final containers.

The product shall not be issued by the manufacturer until notification of official release of the filling is received from the Director, Bureau of Biologics.

§ 630.87 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Measles-Smallpox Vaccine, Live (§§ 630.80 to 630.86, inclusive), shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such finding a matter of official record.

PART 640—ADDITIONAL STANDARDS FOR HUMAN BLOOD AND BLOOD PRODUCTS

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AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Whole Blood (Human)

§ 640.1 Whole Blood (Human).

The proper name of this product shall be Whole Blood (Human). Whole Blood (Human) is defined as blood collected from human donors for transfusion to human recipients.

§ 640.2 General requirements.

(a) *Manufacturing responsibility.* All manufacturing of Whole Blood (Human), including donor examination, blood collection, laboratory tests, labeling, storage and issue, shall be done under the supervision and control of the same licensed establishment except that the Commissioner of Food and Drugs may approve arrangements, upon joint request of two or more licensed establishments, which he finds are of such a nature as to assure compliance otherwise with the provisions of this subchapter.

(b) *Periodic check on sterile technique.* Where blood is collected in an open system, that is, where the blood container is entered, at least one container of such blood that upon visual examination appears normal shall be tested each month between the 18th and 24th day after collection, as a continuing check on technique of blood collection, as follows:

The test shall be performed with a total sample of no less than 10 ml. of blood and a total volume of fluid thioglycollate or thioglycollate broth medium 10 times the volume of the sample of blood. The test sample shall be inoculated into one or more test vessels in a ratio of blood to medium of 1 to 10 for each vessel, mixed thoroughly, incubated for seven to nine days at a temperature of 30° to 32° C., and examined for evidence of growth of microorganisms every workday throughout the test period. On the third, fourth, or fifth day at least 1 ml. of material from each test vessel shall be subcultured in additional test vessels containing the same culture medium and in such proportion as will permit significant visual inspection, mixed thoroughly, incubated for seven to nine days at a temperature of 30° to 32° C. and examined for evidence of growth of microorganisms every workday throughout the test period. If growth is observed in any test vessel, the test shall be repeated to rule out faulty test procedure, using another sample of blood from either, (1) the container from which the initial test sample was taken, (2) the residual cells or plasma

from that blood, or (3) two different containers of blood, each 18 to 24 days old and each tested separately. The formula for fluid thioglycollate medium shall be as prescribed in § 610.12(e)(1) of this chapter and the formula for thioglycollate broth medium shall be as prescribed in § 610.12(f)(5) of this chapter. Media and design of container shall meet the requirements prescribed in § 610.12(e)(2)(i) of this chapter. In lieu of performing one test using an incubation temperature of 30° to 32° C., two tests may be performed, each in all respects as prescribed in this paragraph, one at an incubation temperature of 18° to 22° C. and one at an incubation temperature of 35° to 37° C. A different test may be performed provided that prior to the performance of such a test a manufacturer submits data which the Commissioner of Food and Drugs finds adequate to establish that the different test is equal or superior to the test herein prescribed as a check on sterile technique and makes the finding a matter of official record.

(c) *Final container.* The original blood container shall be the final container and shall not be entered prior to issue for any purpose except for blood collection. Such container shall be uncolored and transparent to permit visual inspection of the contents and any closure shall be such as will maintain an hermetic seal and prevent contamination of the contents. The container material shall not interact with the contents under the customary conditions of storage and use, in such a manner as to have an adverse effect upon the safety, purity, or potency of the blood.

(d) [Reserved]

(e) *Reissue of blood.* Blood that has been removed from storage controlled by a licensed establishment shall not be reissued by a licensed establishment unless the following conditions are observed:

(1) The container has a tamper-proof seal when originally issued and this seal remains unbroken;

(2) An original pilot sample is properly attached and has not been removed, except that blood lacking a pilot sample may be reissued in an emergency provided it is accompanied by instructions for sampling and for use within six hours after entering the container for sampling;

(3) The blood has been maintained continuously at 1° to 10° C.;

(4) The blood is held for observation until a significant inspection consistent with the requirements of § 640.5(e) can be made.

(f) *Issue prior to determination of test results.* Notwithstanding the provisions of § 610.1 of this chapter, blood may be issued by the licensee on the request of a physician, hospital, or other medical facility, before results of all tests prescribed in § 640.5 and the test for hepatitis associated (Australia) antigen prescribed in § 610.40 of this chapter have been determined where such issue is essential to allow time for transportation to assure arrival of the blood by the time when needed for transfusion of such blood provided (1) the blood is shipped directly to such physician or medical facility, (2) the records of the licensee contain a full explanation of the need for such issue, (3) the label on each container of such blood bears the information required by § 640.7(e), (4) the label does not bear results of tests other than those made on pilot samples of the blood to be shipped, taken at the time of its collection, and (5) the label does not bear the name or any other identification of the intended recipient.

§ 640.3 Suitability of donor.

(a) *Method of determining.* The suitability of a donor as a source of Whole Blood (Human) shall be determined by a qualified physician or by persons under his supervision and trained in determining suitability. Such determination shall be made on the day of collection from the donor by means of medical history, a test for hemoglobin level, and such physical examination as appears necessary to a physician who shall be present on the premises when examinations are made, except that the suitability of donors may be determined when a physician is not present on the premises, provided the establishment (1) maintains on the premises, and files with the Bureau of Biologics, a manual of standard procedures and methods, approved by the Director of the Bureau of Biologics, that shall be followed by employees who determine suitability of donors, and (2) maintains records indicating the name and qualifications of the person immediately in charge of the employees who determine the suitability of donors when a physician is not present on the premises.

(b) *Qualifications of donor; general.* Except as provided in paragraph (f), a person may not serve as a source of Whole Blood (Human) more than once in 8 weeks. In addition, donors shall be in good health, as indicated in part by:

(1) Normal temperature;

(2) Demonstration that systolic and diastolic blood pressures are within normal limits, unless the examining physician is satisfied that an individual with blood pressures outside these limits is an otherwise qualified donor under the provisions of this section;

(3) A blood hemoglobin level which shall be demonstrated to be no less than 12.5 gm. of hemoglobin per 100 ml. of blood;

(4) Freedom from acute respiratory diseases;

(5) Freedom from any infectious skin disease at the site of phlebotomy and from any such disease generalized to such an extent as to create a risk of contamination of the blood;

(6) Freedom from any disease transmissible by blood transfusion, insofar as can be determined by history and examinations indicated above; and

(7) Freedom of the arms and forearms from skin punctures or scars indicative of addiction to self-injected narcotics.

(c) *Additional qualifications of donor; viral hepatitis.* No individual shall be used as a source of Whole Blood (Human) if he has—

(1) A history of viral hepatitis;

(2) A history of close contact within six months of donation with an individual having viral hepatitis;

(3) A history of having received within six months human blood, or any derivative of human blood which the Food and Drug Administration has advised the licensed establishment is a possible source of viral hepatitis.

(d) *Therapeutic bleedings.* Blood withdrawn in order to promote the health of a donor otherwise qualified under the provisions of this section, shall not be used as a source of Whole Blood (Human) unless the container label conspicuously indicates the donor's disease that necessitated withdrawal of blood.

(e) *Immunized donors.* Blood withdrawn from donors known to have been immunized to human blood cell antigens shall not be used for Whole Blood (Human) unless the container label conspicuously indicates such information.

(f) *Qualifications; donations within less than 8 weeks.* A person may serve as a source of Whole Blood (Human) more than once in 8 weeks only if at the time of donation the person is examined and certified by a physician to be in good health, as indicated in part in paragraph (b).

§ 640.4 Collection of the blood.

(a) *Supervision.* Blood shall be drawn from the donor by a qualified physician or under his supervision by assistants trained in the procedure. A physician shall be present on the premises when blood is being collected, except that blood may be collected when a physician is not present on the premises, provided the establishment (1) maintains on the premises, and files with the Bureau of Biologics, a manual of standard procedures and methods, approved by the Director of the Bureau of Biologics, that shall be followed by employees who collect blood, and (2) maintains records indicating the name and qualifications of the person immediately in charge of the employees who collect blood when a physician is not present on the premises.

(b) *The donor clinic.* The pertinent requirements of §§ 600.10 and 600.11 of this chapter shall apply at both the licensed establishment and at any other place where the bleeding is performed.

(c) *Blood containers.* Blood containers and donor sets shall be pyrogen-free, sterile and identified by lot number. The amount of anticoagulant required for the quantity of blood to be collected shall be in the blood container when it is sterilized. In addition, all container and donor set surfaces that come in contact with blood used in the processing of Heparinized Whole Blood (Human) shall be water repellent.

(d) *The anticoagulant solution.* The anticoagulant solution shall be sterile and pyrogen-free. One of the following formulae shall be used in the indicated volumes:

(1) *Anticoagulant acid citrate dextrose solution (ACD).*

	Solution A	Solution B
Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$).	22.0 gm.	13.2 gm.
Citric acid ($C_6H_8O_7 \cdot H_2O$)	8.0 gm.	4.8 gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	24.5 gm.	14.7 gm.
Water for injection (U.S.P.) to make.	1,000 ml.	1,000 ml.
Volume per 100 ml. blood	15 ml.	25 ml.

(2) *Anticoagulant heparin solution.*

Heparin sodium (U.S.P.)	75,000 units.
Sodium chloride injection (U.S.P.) to make.	1,000 ml.
Volume per 100 ml. blood	6 ml.

A buffer to maintain stability shall be added, if necessary.

(3) *Anticoagulant citrate phosphate dextrose solution (CPD).*

Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$).	26.3 gm.
Citric acid ($C_6H_8O_7 \cdot H_2O$)	3.27 gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	25.5 gm.
Monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$).	2.22 gm.
Water for injection (U.S.P.) to make.	1,000 ml.
Volume per 100 ml. blood	14 ml.

(e) *Donor identification.* Each unit of blood shall be so marked or identified by number or other symbol as to relate it to the individual donor whose identity shall be established to the extent necessary for compliance with § 640.3.

(f) *Prevention of contamination of the blood.* The skin of the donor at the site of phlebotomy shall be prepared thoroughly and carefully by a method that gives maximum assurance of a sterile container of blood. The blood shall be collected by aseptic methods in a sterile system which may be closed or may be vented if the vent protects the blood against contamination.

(g) *Pilot samples for laboratory tests.* Pilot samples for laboratory tests shall meet the following standards:

(1) One or more pilot samples shall be provided with each unit of blood when issued or reissued except as provided in § 640.2(e)(2) and all pilot samples shall be from the donor who is the source of the unit of blood.

(2) All samples for laboratory tests performed by the manufacturer and all pilot samples accompanying a unit of blood shall be collected at the time of filling the final container by the person who collects the unit of blood.

(3) All containers for all samples shall bear the donor's identification before collecting the samples.

(4) All containers for pilot samples accompanying a unit of blood shall be attached to the whole blood container before blood collection, in a tamper-proof manner that will conspicuously indicate removal and reattachment.

(h) *Phlebotomy for Heparinized Whole Blood (Human).* Heparinized Whole Blood (Human) shall be collected with minimal damage to and minimal manipulation of the donor's tissue, and with a single, uninterrupted, free-flowing venipuncture.

(i) *Storage.* Immediately after collection, the blood shall be placed in storage within a 2° range between 1° and 6° C., unless it must be transported from the donor clinic to the processing laboratory. In the latter case the blood shall be placed in temporary storage having sufficient refrigeration capacity to cool the blood continuously toward a 2° range between 1° and 6° C. until it arrives at the processing laboratory where it shall be stored within a 2° range between 1° and 6° C.

§ 640.5 Testing the blood.

All laboratory tests shall be made on a pilot sample specimen of blood taken from the donor at the time of collecting the unit of blood, and these tests shall include the following:

(a) *Serological test for syphilis.* Whole Blood (Human) shall be negative to a serological test for syphilis.

(b) *Determination of blood group.* Each container of Whole Blood (Human) shall be classified as to ABO blood group. At least two blood group tests shall be made and the unit shall not be issued until grouping tests by different methods or with different lots of antiserums are in agreement. Only those Anti-A and Anti-B Blood Grouping Serums licensed under, or that otherwise meet the requirements of, the regulations of this subchapter shall be used, and the technique used shall be that for which the serum is specifically designed to be effective.

(c) *Determination of the Rh factors.* Each container of Whole Blood (Human) shall be classified as to Rh type on the basis of tests done on the pilot sample. The label shall indicate the extent of typing and the results of all tests performed. If the test, using Anti-Rh$_o$ (Anti-D) Typing Serum, is positive, the container may be labeled "Rh Positive". If this test is negative, the results shall be confirmed by further testing which may include tests for the Rh$_o$ variant (D^u) and for other Rh-Hr factors. Blood may be labeled "Rh Negative" if negative to tests for the Rh$_o$ (D) and Rh$_o$ variant (D^u) factors. If the test using Anti-Rh$_o$ (Anti-D) Typing Serum is negative, but not tested for the Rh$_o$ variant (D^u), the label must indicate that this test was not done. Only Anti-Rh Typing Serums licensed under, or that otherwise meet the requirements of, the regulations of this subchapter shall be used, and the technique used shall be that for which the serum is specifically designed to be effective.

(d) *Sterility test.* Whole Blood (Human) intended for transfusion shall not be tested for sterility by a method that entails entering the final container before the blood is used for transfusion.

(e) *Inspection.* Whole Blood (Human) shall be inspected visually during storage and immediately prior to issue. If the color or physical appearance is abnormal or there is any indication or suspicion of microbial contamination the unit of Whole Blood (Human) shall not be issued for transfusion.

§ 640.6 Modifications of Whole Blood (Human).

Upon approval by the Director, Bureau of Biologics, of an amendment to the product license application for Whole Blood (Human), a manufacturer may prepare Whole Blood (Human) from which the antihemophilic factor has been removed, provided the Whole Blood (Human) meets the applicable requirements of this subchapter and the following conditions are met:

(a) The antihemophilic factor shall be removed in accordance with paragraphs (a), (b), and (c) of § 640.52.

(b) Although the closed system between the red blood cells and plasma shall be maintained, the red blood cells shall be maintained between 1 and 6° C. at all times, including that time when the plasma is being frozen for removal of the antihemophilic factor.

(c) If containers for pilot samples are detached from the blood container during removal of the antihemophilic factor the pilot samples shall be reattached to the unit of Whole Blood (Human), modified, as soon as the plasma is returned to the red blood cells. The reattachement of the pilot samples shall be in a tamper-proof manner that will conspicuously indicate removal and reattachment.

§ 640.7 Labeling.

In addition to all other applicable labeling requirements, the following, except as prescribed in paragraphs (e) and (f) of this section, shall appear on the label of each container:

(a) *Anticoagulant*—(1) *Name.* The name of the anticoagulant immediately preceding and of no less prominence than the proper name, expressed as follows:

(i) either "ACD", or "acid citrate dextrose solution",

(ii) either "Heparinized" or "heparin solution",

(iii) either "CPD" or "citrate phosphate dextrose solution".

(2) *Quantity.* The quantity and kind of anticoagulant used and the volume of blood corresponding with the formula prescribed under § 640.4(d).

(b) *Serological test and test for hepatitis associated (Australia) antigen.* Indication of the method used for serological test for syphilis and the test for hepatitis associated (Australia) antigen, and the results.

(c) *Blood group and type.* Designation of blood group and Rh factors:

(1) The blood group and Rh factors shall be designated conspicuously.

(2) If a color scheme for differentiating the ABO blood groups is used, the color used to designate each blood group on the container shall be:

Blood Group A: Yellow.
Blood Group B: Pink.
Blood Group O: Blue.
Blood Group AB: White.

(d) *Additional information for labels of Group O Bloods.* Each Group O blood shall be labeled with a statement indicating whether or not isoagglutinin titers or other tests to exclude so-called "dan-

gerous" Group O bloods were performed, and indicating the classification based on such tests.

(e) *Issue prior to determination of test results.* The label on each container of blood that is issued pursuant to the provisions of § 640.2(f) shall bear the following information and instructions in lieu of the information specified in paragraphs (b), (c), and (d) of this section.

EMERGENCY SHIPMENT FOR USE ONLY BY

(Name of physician, hospital or other medical facility.)

CAUTION

BEFORE TRANSFUSION

1. Do not use until test results received from (name of licensee).
2. Perform crossmatch.

(f) *Whole Blood (Human), Modified.* The label on each container of blood that is issued pursuant to the provisions of § 640.6 shall bear, in addition to the other applicable labeling requirements, the following:

(1) Immediately following and in no less prominence than the proper name, the word "Modified."

(2) A prominent statement indicating that antihemophilic factor has been removed by cryoprecipitation. Such statement may appear on a separate label affixed to the container.

(3) Instructions not to use the unit of blood for patients requiring antihemophilic factor.

Subpart B—Red Blood Cells (Human)

§ 640.10 Red Blood Cells (Human).

The proper name of this product shall be Red Blood Cells (Human). The product is defined as red blood cells remaining after separating plasma from human blood.

§ 640.11 General requirements.

(a) *Check on sterile technique.* If Red Blood Cells (Human) are prepared in a vented or open system, a check on sterile technique shall be made each month by performing a test 20–28 hours after the preparation of at least one container of Red Blood Cells (Human), by the method prescribed in § 640.2(b).

(b) *Storage.* Immediately after processing, the Red Blood Cells (Human) shall be placed in storage and maintained within a 2° range between 1° and 6° C.

(c) *Inspection.* The product shall be inspected immediately after separation of the plasma, periodically during storage, and at the time of issue. The product shall not be issued if there is any abnormality in color or physical appearance or if there is any indication of microbial contamination.

§ 640.12 Suitability of donor.

The source blood for Red Blood Cells (Human) shall be obtained from a donor who meets the criteria for donor suitability prescribed in § 640.3.

§ 640.13 Collection of the blood.

(a) The source blood shall be collected as prescribed in § 640.4, except that paragraphs (d)(2), and (g), and (h) shall not apply.

(b) Source blood may also be derived from Whole Blood (Human) manufactured in accordance with applicable provisions of this subchapter.

§ 640.14 Laboratory tests.

A sample of source blood shall be taken from the donor at the time of collection and it shall be used for a serological test for syphilis, for tests to determine blood group and Rh factors, as prescribed in § 640.5 (a), (b), and (c).

§ 640.15 Pilot samples.

Pilot samples collected in integral tubing or in separate pilot tubes shall meet the following standards:

(a) One or more pilot samples of either the original blood or of the Red Blood Cells (Human) being processed shall be provided with each unit of Red Blood Cells (Human) when issued or reissued.

(b) Before they are filled, all pilot sample tubes shall be marked or identified so as to relate them to the donor of that unit of red cells.

(c) Before the final container is filled or at the time the final product is prepared, the pilot sample tubes to accompany a unit of cells shall be attached securely to the final container in a tamper proof manner that will conspicuously indicate removal and reattachment.

(d) All pilot sample tubes accompanying a unit of Red Blood Cells (Human) shall be filled at the time the blood is collected or at the time the final product is prepared, in each instance by the person who performs the collection or preparation.

§ 640.16 Processing.

(a) *Separation.* Red Blood Cells (Human) may be prepared either by centrifugation done in a manner that will not tend to increase the temperature of the blood, and no later than 6 days after the date of blood collection or by normal, undisturbed sedimentation no later than 21 days after the date of blood collection. A portion of the plasma sufficient to assure optimal cell preservation shall be left with the red cells except when a cryophylactic substance is added for prolonged storage.

(b) *Sterile system.* All surfaces that come in contact with the red cells shall be sterile and pyrogen-free. If an open system is used, that is, where the transfer container is not integrally attached to the blood container, and the blood container is entered after blood collection, the plasma shall be separated from the red blood cells with positive pressure maintained on the original container until completely sealed. If the method of separation involves a vented system, that is, when an airway must be inserted in the container for withdrawal of the plasma, the airway and vent shall be sterile and constructed so as to exclude microorganisms and maintain a sterile system.

(c) *Final containers.* Final containers used for Red Blood Cells (Human) shall be the original blood containers unless the method of processing requires a different container. The final container shall meet the requirements for blood containers prescribed in § 640.2(c). At the time of filling, if a different container is used, it shall be marked or identified by number or other symbol so as to relate it to the donor of that unit of red cells.

§ 640.17 Modifications for specific products.

Red Blood Cells (Human), Frozen: A cryophylactic substance may be added to the Red Blood Cells (Human) for extended manufacturer's storage at −65° C. or colder, provided the manufacturer submits data considered by the Director, Bureau of Biologics, as adequately demonstrating through in vivo cell survival and other appropriate tests that the addition of the substance, the materials used and the processing methods result in a final product that meets the required standards of safety, purity, and potency for Red Blood Cells (Human), and that the frozen product will maintain those properties for the prescribed dating period. Section 640.11 (b) and (c) do not apply while a cryophylactic substance is present.

§ 640.18 Labeling.

In addition to the items required by other applicable labeling provisions of this subchapter, labels for Red Blood Cells (Human) shall bear the following:

(a) The information required by § 640.7(a)(2), (b), and (c) for Whole Blood (Human), except the proper name.

(b) Immediately following or immediately below and in no less prominence than the proper name, appropriate words describing each approved variation applicable to the product in the final container; for example, Red Blood Cells (Human), Frozen, and Red Blood Cells (Human), Deglycerolized.

(c) Instructions to use a filter in the administration equipment.

(d) Where source blood has been derived from Whole Blood (Human), such fact and the name, address, and license number of the establishment.

Subparts C, D, and E—[Reserved]

Subpart F—Cryoprecipitated Antihemophilic Factor (Human)

§ 640.50 Cryoprecipitated Antihemophilic Factor (Human).

(a) *Proper name and definition.* The proper name of this product shall be Cryoprecipitated Antihemophilic Factor (Human) which shall consist of a preparation containing the antihemophilic factor obtained from a single unit of human blood.

(b) *Source.* Cryoprecipitated Antihemophilic Factor (Human) shall be prepared from human blood meeting the following criteria:

(1) *Suitability of the donor.* Blood for Cryoprecipitated Antihemophilic Factor (Human) shall be obtained only from a donor who meets the criteria for suitability prescribed in § 640.3.

(2) *Collection of the blood.* Blood for Cryoprecipitated Antihemophilic Factor

(Human) shall be collected as prescribed in § 640.4 except that paragraphs (d) (2), (g), and (h) shall not apply.

(3) *Testing the blood.* Blood for Cryoprecipitated Antihemophilic Factor (Human) shall be tested as prescribed in § 640.5 (a), (b), and (c).

§ 640.51 General requirements.

(a) *Diluent.* No diluent shall be added to the product by the manufacturer.

(b) *Storage.* Immediately after processing the product shall be placed in storage and maintained at −18° C. or colder.

(c) *Labeling.* In addition to the items required by other provisions of this subchapter, the package label shall bear the following:

(1) Designation of blood group and type of the source blood.

(2) A warning against using the product if there is evidence of thawing during storage.

(3) Instructions to thaw Cryoprecipitated Antihemophilic Factor (Human) in a water bath maintained at not warmer than 37° C.

(4) Instructions to store the product at room temperature after thawing, to use the product within 6 hours after thawing and within 2 hours of entering the container.

(5) Instructions to use a filter in the administration equipment.

(6) A statement indicating the volume of the source plasma and the type of anticoagulant solution present in the source plasma from which the product was prepared.

(7) Indication of the test method for hepatitis associated (Australia) antigen used and the result.

§ 640.52 Processing.

(a) *Separation of plasma.* The plasma shall be separated from the red blood cells in a closed sterile system within 4 hours after collection by centrifugation to obtain an essentially cell-free material.

(b) *Freezing the plasma.* The plasma shall be frozen within 2 hours after separation. A combination of dry ice and organic solvent may be used for freezing provided the procedure has been shown not to cause the solvent to penetrate the container or leach plasticizers from the container into the frozen plasma.

(c) *Separation of Cryoprecipitated Antihemophilic Factor (Human).* The Cryoprecipitated Antihemophilic Factor (Human) shall be separated from the plasma in a closed system by a procedure that precludes contamination and has been shown to produce a product which has demonstrated potency in patients having a factor VIII deficiency.

(d) *Final container.* Final containers used for Cryoprecipitated Antihemophilic Factor (Human) shall be uncolored and transparent to permit visual inspection of the contents and any closure shall be such as will maintain an hermetic seal and prevent contamination of the contents. The container material shall not interact with the contents under the customary conditions of storage and use, in such a manner as to have an adverse effect upon the safety, purity, and potency of the product. At the time of filling, the final container shall be marked or identified by number or other symbol so as to relate it to the donor.

Subpart G—Source Plasma (Human)

§ 640.60 Source Plasma (Human).

The proper name of this product shall be Source Plasma (Human). The product is defined as the fluid portion of human blood which has been stabilized against clotting, collected by plasmapheresis, and is intended as source material for further manufacture into blood derivatives (a portion of pooled plasma separable by chemical means) intended for injection.

§ 640.61 Informed consent.

The written consent of a prospective donor shall be obtained after a qualified licensed physician has explained the hazards of the procedure to the prospective donor. The explanation shall include the risks of a hemolytic transfusion reaction if he is given the cells of another donor, and the hazards involved if he is hyperimmunized. The explanation shall consist of such disclosure and be made in such a manner that intelligent and informed consent be given and that a clear opportunity to refuse is presented.

§ 640.62 Medical supervision.

A qualified licensed physician shall be on the premises when donor suitability is being determined, immunizations are being made, whole blood is being collected, and red blood cells are being returned to the donor.

§ 640.63 Suitability of donor.

(a) *Method of determining.* The suitability of a donor for Source Plasma (Human) shall be determined by a qualified licensed physician or by persons under his supervision and trained in determining donor suitability. Such determination shall be made on the day of collection from the donor by means of a medical history, tests, and such physical examination as appears necessary to the qualified licensed physician.

(b) *Initial medical examination.* Each donor shall be examined by a qualified licensed physician on the day of the first donation, or no more than one week prior to the first donation, and shall be certified to be in good health by the examining physician. The certification of good health shall be on a form supplied by the licensed establishment that indicates the certification is with respect to the suitability of the individual to be a plasmapheresis donor.

(c) *Qualification of donor.* Donors shall be in good health on the day of donation, as indicated in part by:

(1) Normal temperature;

(2) Demonstration that systolic and diastolic blood pressures are within normal limits, unless the examining physician is satisfied that an individual with blood pressures outside these limits is an otherwise qualified donor under the provisions of this section;

(3) A blood hemoglobin level of no less than 12.5 grams of hemoglobin per 100 milliliters of blood;

(4) A normal pulse rate;

(5) A total serum protein of no less than 6.0 grams per 100 milliliters of serum;

(6) Weight, which shall be at least 110 pounds;

(7) Freedom from acute respiratory diseases;

(8) Freedom from any infectious skin disease at the site of phlebotomy and from any such disease generalized to such an extent as to create a risk of contamination of the plasma;

(9) Freedom from any disease, other than malaria, transmissible by blood transfusion, insofar as can be determined by history and examinations indicated in this section;

(10) Freedom of the arms and forearms from skin punctures or scars indicative of addiction to self-injected narcotics;

(11) Freedom from a history of viral hepatitis;

(12) Freedom from a history of close contact within six months of donation with an individual having viral hepatitis;

(13) Freedom from a history of having received, within six months, human blood or any derivative of human blood which the Food and Drug Administration has advised the licensed establishment is a possible source of viral hepatitis, except for specific immunization performed in accordance with § 640.66 of this part.

(d) *General.* Any donor who, in the opinion of the interviewer, appears to be under the influence of any drug, alcohol, or for any reason does not appear to be providing reliable answers to medical history questions, shall not be considered a suitable donor.

§ 640.64 Collection of blood for Source Plasma (Human).

(a) *Supervision.* All blood for the collection of Source Plasma (Human) shall be drawn from the donor by a qualified licensed physician or by persons under his supervision trained in the procedure.

(b) *Blood containers.* Blood containers and donor sets shall be pyrogen-free, sterile and identified by lot number. The amount of anticoagulant required for the quantity of blood to be collected shall be in the blood container when it is sterilized.

(c) *The anticoagulant solution.* The anticoagulant solution shall be sterile and pyrogen-free. One of the following formulae shall be used in the indicated volumes:

(1) *Anticoagulant acid citrate dextrose solution (ACD).*

Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	22.0 grams
Citric acid ($C_6H_8O_7 \cdot H_2O$)	8.0 grams
Dextrose ($C_6H_{12}O_6H_2O$)	24.5 grams
Water for injection (U.S.P.) to make	1,000 milliliters
Volume per 100 milliliters blood	15 milliliters

(2) *Anticoagulant acid citrate dextrose dextrose solution (CPD).*

Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	26.3 grams
Citric acid ($C_6H_8O_7 \cdot H_2O$)	3.27 grams
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	25.5 grams
Monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$)	2.22 grams
Water for injection (U.S.P.) to make	1,000 milliliters
Volume per 100 milliliters blood	14 milliliters

(3) *Anticoagulant sodium citrate solution.*

Tri-sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	40 grams
Water for injection (U.S.P.) to make	1,000 milliliters
Volume per 100 milliliters of blood	10 milliliters

(d) *Donor identification.* Each unit of blood and plasma shall be so marked or identified by number or other symbol so as to relate it directly to the donor.

(e) *Prevention of contamination of the blood and plasma.* The skin of the donor at the site of phlebotomy shall be prepared thoroughly and carefully by a method that gives maximum assurance of a sterile container of blood. The blood shall be collected, the plasma separated, and the cells returned to the donor by aseptic methods in a sterile system which may be closed, or may be vented if the vent protects the blood cells and plasma against contamination.

§ 640.65 Plasmapheresis.

(a) *Procedure-general.* The plasmapheresis procedure, which is defined as that procedure in which blood is removed from a donor, the plasma separated from the formed elements and the formed elements returned to the donor, during a single visit to the establishment, shall be described in detail in the product license application.

(b) *Procedures-specific requirements.* The plasmapheresis procedure shall meet the following requirements:

(1) A sample of blood shall be drawn from each donor by a qualified licensed physician or by persons under his supervision and trained in such procedure on the day of the first plasmapheresis and at least every four months thereafter on which a serologic test for syphilis and a serum protein electrophoresis or quantitative immunodiffusion test for immunoglobulins to determine the immunoglobulin composition of the serum shall be performed. The results of the tests shall be reviewed by a qualified licensed physician within 10 days after the sample is drawn to determine whether or not the donor may continue on the program. If the plasma protein composition is not within normal limits established by the testing laboratory, the donor shall be removed from the program until these values return to normal. A donor with a reactive serologic test for syphilis shall not be plasmapheresed again until his serum tests nonreactive to a serologic test for syphilis.

(2) At least every four months, the accumulated laboratory data and collection records of each donor shall be reviewed by a qualified licensed physician to determine continuing suitability of the donor. Only those donors found suitable upon such a review shall remain in the plasmapheresis program. Such a review shall be signed by the reviewing physician.

(3) A donor identification system shall be established that positively identifies each donor and relates such donor directly to his blood and its components as well as to his accumulated records and laboratory data. Such system shall include either a photograph of each donor which shall be used on each visit to confirm the donor's identity, or some other method that provides equal or greater assurance of positively identifying the donor.

(4) The amount of whole blood, not including anticoagulant, removed from a donor during a plasmapheresis procedure or in any 48-hour period shall not exceed 1,000 milliliters unless the donor's weight is 175 pounds or greater, in which case the amount of whole blood, not including anticoagulant, removed from the donor during a plasmapheresis procedure or in any 48-hour period shall not exceed 1,200 milliliters.

(5) The amount of whole blood, not including anticoagulant, removed from a donor within a seven-day period shall not exceed 2,000 milliliters unless the donor's weight is 175 pounds or greater, in which case the amount of whole blood, not including anticoagulant, removed from the donor during a seven-day period shall not exceed 2,400 milliliters.

(6) No more than 500 milliliters of whole blood shall be removed from a donor at one time, unless the donor's weight is 175 pounds or greater, in which case no more than 600 milliliters of whole blood shall be removed from the donor at one time.

(7) The plasma shall be separated from the red blood cells immediately after blood collection. The maximum feasible volume of red blood cells shall be returned to the donor before another unit is collected.

§ 640.66 Immunization of donors.

If specific immunization of a donor is to be performed, the selection and scheduling of the injection of the antigen, and the evaluation of each donor's clinical response, shall be by a qualified licensed physician or physicians. The administration of the antigen may be performed by a licensed physician or a trained person under his supervision. Any material used for immunization shall be either a product licensed under section 351 of the Public Health Service Act for such purpose or one specifically approved by the Director, Bureau of Biologics, Food and Drug Administration. Immunization procedures shall be on file at each plasmapheresis center where immunizations are performed.

§ 640.67 Test for hepatitis B antigen.

Each unit of Source Plasma (Human) shall be nonreactive to a test for the hepatitis B antigen as prescribed in §§ 610.40 and 610.41 of this chapter.

§ 640.68 Processing.

(a) *Sterile system.* All surfaces that come in contact with the plasma shall be both sterile and pyrogen-free. If the method of separation involves a vented system (i.e., where an airway must be inserted into a container for withdrawal of the plasma), the airway and vent shall be sterile and constructed so as to exclude microorganisms and maintain a sterile system.

(b) *Final containers.* Final containers used for Source Plasma (Human), whether integrally attached or separated from the original blood container, shall not be entered prior to issuance for any purpose except for filling with the plasma. Such containers shall be uncolored and hermetically sealed, and shall permit clear visibility of the contents. Final containers and their components shall not interact with the plasma contents under conditions of storage and use so as to alter the safety, quality, purity, or potency of the plasma and shall provide adequate protection against external factors that may cause deterioration or contamination. Prior to filling, the final container shall be marked or identified by number or other symbol which will relate it directly to the donor.

(c) *Preservative.* Source Plasma (Human) shall not contain a preservative.

§ 640.69 General requirements.

(a) *Pooling.* Pooling of plasma by the manufacturer of Source Plasma (Human) from two or more donors is not permitted. Two units of plasma from the same donor may be pooled if such units are collected during one plasmapheresis procedure, provided the pooling is done by a procedure that gives maximum assurance of a sterile container of plasma.

(b) *Storage.* Immediately after filling, the plasma shall be stored at not warmer than −20° C., except for plasma collected as provided for in § 640.70.

(c) *Inspection.* Source Plasma (Human) shall be inspected at the time of issuance. If there is any evidence of thawing, the unit shall not be issued.

(d) *Pilot samples.* If pilot samples are provided, they shall meet the following standards:

(1) Prior to filling, all pilot samples shall be marked or identified so as to relate them directly to the donor of that unit of plasma.

(2) All pilot samples shall be filled at the time the final product is prepared by the person who prepares the final product.

(3) All pilot samples shall be representative of the contents of the final product.

(4) All pilot samples shall be collected in a manner that does not contaminate the contents of the final container.

(e) *Labeling.* In addition to the labeling requirements of § 610.62 of this chapter, and in lieu of the requirements in §§ 610.60 and 610.61 of this chapter, the following information shall appear on the label affixed to each container of Source Plasma (Human):

(1) The proper name of the product.
(2) Name, address, and license number of the manufacturer.
(3) Donor number.
(4) Collection date of the plasma.
(5) The statement: "Caution: For Manufacturing Use Only".
(6) The statement: "Store at −20° C. or colder".
(7) A statement as to whether the plasma was collected from normal donors or from immunized donors. In the case of immunized donors, the label shall state the immunizing antigen.
(8) The total volume of plasma and total quantity and type of anticoagulant used.
(9) The test for hepatitis B antigen used and the results.

(f) *Manufacturing responsibility.* All steps in the manufacture of Source Plasma (Human), including donor examination, blood collection, plasmapheresis, laboratory testing, labeling, storage, and issuing shall be performed by the establishment licensed to manufacture Source Plasma (Human), except that the following tests may be performed by a clinical laboratory licensed under section 353 of the Public Health Service Act, or by an establishment licensed for blood or blood derivatives under section 351 of the Public Health Service Act, provided such arrangements are approved by the Director, Bureau of Biologics, Food and Drug Administration:

(1) The test for hepatitis B antigen pursuant to § 640.67.
(2) The serum protein electrophoresis or quantitative immunodiffusion test for immunoglobulin as required by § 640.65 (b)(1).
(3) Such testing pursuant to paragraph (f) (1) and (2) of this section shall not be considered divided manufacturing, requiring two product licenses for source Plasma (Human), provided that:
(i) The results of such tests are maintained by the establishment licensed for Source Plasma (Human) whereby such results may be reviewed by a licensed physician as required in § 640.65(b)(2), and/or by authorized Food and Drug Administration inspectors.
(ii) The Source Plasma (Human) manufacturer has obtained a written agreement that the testing laboratory will permit authorized Food and Drug Administration inspectors to inspect their testing procedures and facilities during any reasonable business hours.
(iii) The testing laboratory will participate in any proficiency testing programs undertaken by the Bureau of Biologics, Food and Drug Administration.

(g) *Records.* In addition to the general recordkeeping requirements of § 600.12 of this chapter, every manufacturer of Source Plasma (Human) must keep for each donor a separate and complete record of all initial and periodic examinations, tests, laboratory data, interviews, etc., undertaken pursuant to §§ 640.63, 640.65, 640.66, and 640.67. This record must also contain the original or a clear copy of the donor's written consent for participation in the plasmapheresis program as required by § 640.61 and the certification of good health as prescribed in § 640.63(b). Each donor record must be directly cross-referenced to the unit(s) of Source Plasma (Human) associated with the donor.

§ 640.70 Modification of Source Plasma (Human).

(a) Upon approval by the Director, Bureau of Biologics, Food and Drug Administration, of an amendment to the product license for Source Plasma (Human), a manufacturer may prepare Source Plasma (Human) as a liquid product for a licensed blood derivative manufacturer who has indicated a need for a liquid product.

(b) Liquid Source Plasma (Human) shall meet all standards of the frozen Source Plasma (Human) except:

(1) Liquid Source Plasma (Human) shall be stored in nonleachable containers so that the containers and their components will not interact with the plasma contents under conditions of storage and use so as to alter the safety, quality, purity, or potency of the plasma and shall provide adequate protection against external factors that may cause deterioration or contamination.

(2) Liquid Source Plasma (Human) shall be shipped, stored and labeled for storage at a temperature of 10° C. or colder. An exception to the shipping or storage temperature shall be approved by the Director, Bureau of Biologics, Food and Drug Administration, based upon his receipt of substantial evidence to support another temperature. Such evidence may be submitted by either the product licensee of the liquid Source Plasma (Human) or the manufacturer of the final blood derivative product who has requested the liquid Source Plasma (Human).

(3) The label for the liquid Source Plasma (Human) shall be easily distinguished from that of the frozen product. Color coding shall not be used for this purpose.

(4) The label affixed to each container of liquid Source Plasma (Human) shall contain, in addition to the information required by § 640.69(e) but excluding § 640.69(e)(6) the name of the manufacturer of the final blood derivative product for whom is was prepared.

(5) Liquid Source Plasma (Human) shall be inspected immediately prior to issuance. If the color or physical appearance is abnormal, or there is any indication or suspicion of microbial contamination, the unit of liquid Source Plasma (Human) shall not be issued.

Subparts H and I—[Reserved]

Subpart J—Immune Serum Globulin (Human)

§ 640.100 Immune Serum Globulin (Human).

(a) *Proper name and definition.* The proper name of this product shall be Immune Serum Globulin (Human). The product is defined as a sterile solution containing antibodies derived from human blood.

(b) *Source material.* The source of Immune Serum Globulin (Human) shall be blood, plasma or serum from human donors determined at the time of donation to have been free of causative agents of diseases that are not destroyed or removed by the processing methods, as determined by the donor's history and from such physical examination and clinical tests as appear necessary for each donor at the time the blood was obtained. The source blood, plasma or serum shall not contain a preservative and shall be stored in a manner that will prevent contamination by microorganisms, pyrogens or other impurities.

(c) *Additives in source material.* Source blood, plasma or serum shall contain no additives other than citrate or acid citrate dextrose anticoagulant solution, unless it is shown that the processing method yields a product free of the additive to such an extent that the safety, purity and potency of the product will not be affected adversely.

§ 640.101 General requirements.

(a) *Heat stability test.* Approximately 2 ml. of completely processed material of each lot shall not show any visible sign of gelation after heating in a 12 x 75 mm. stoppered glass tube at 57° C. for 4 hours.

(b) *Hydrogen ion concentration.* The pH of final container material shall be 6.8±0.4 when measured in a solution diluted to 1 percent protein with 0.15 molar sodium chloride.

(c) *Turbidity.* The product shall be free of turbidity as determined by visual inspection of final containers.

(d) *Date of manufacture.* The date of manufacture is the date of initiating the last valid measles or poliomyelitis antibody test (§ 640.104(b) (2) and (3)) whichever date is earlier.

(e) *Labeling.* In addition to complying with all applicable labeling required in this subchapter, labeling shall indicate that:

(1) There is no prescribed potency for viral hepatitis antibodies.
(2) The product is not recommended for intravenous administration.
(3) The lot is or is not suitable for use with Measles Virus Vaccine, Live, Attenuated.
(4) The lot is or is not recommended for poliomyelitis.

(f) *Samples and protocols.* For each lot of Immune Serum Globulin (Human) the following material shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A 50 ml. sample of the final product.
(2) All protocols relating to the history of each lot and all results of all tests prescribed in these additional standards.

§ 640.102 Manufacture of Immune Serum Globulin (Human).

(a) *Processing method.* The processing method shall be one that has been shown: (1) To be capable of concentrating tenfold from source material at

least two different antibodies; (2) not to affect the integrity of the globulins; (3) to consistently yield a product which is safe for subcutaneous and intramuscular injection and (4) not to transmit viral hepatitis.

(b) *Microbial contamination.* Low temperatures or aseptic techniques shall be used to minimize contamination by microorganisms. Preservatives to inhibit growth of microorganisms shall not be used during processing.

(c) *Bulk storage.* The globulin fraction may be stored in bulk prior to further processing provided it is stored in clearly identified hermetically closed vessels. Globulin as either a liquid concentrate or a solid and containing alcohol or more than 5 percent moisture shall be stored at a temperature of −10° C. or lower. Globulin as a solid free from alcohol and containing less than 5 percent moisture, shall be stored at a temperature of 0° C. or lower.

(d) *Determination of the lot.* Each lot of Immune Serum Globulin (Human) shall represent a pooling of approximately equal amounts of material from not less than 1,000 donors.

(e) *Sterilization and heating.* The final product shall be sterilized promptly after solution. At no time during processing shall the product be exposed to temperatures above 45° C. and after sterilization the product shall not be exposed to temperatures above 30° to 32° C. for more than 72 hours.

§ 640.103 The final product.

(a) *Final solution.* The final product shall be a 16.5±1.5 percent solution of globulin containing 0.3 molar glycine and a preservative.

(b) *Protein composition.* At least 90 percent of the globulin shall have an electrophoretic mobility not faster than -2.8×10^{-5} centimeters^2 per volt per second, when measured at a 1 percent protein concentration in sodium diethylbarbiturate buffer at pH 8.6 and 0.1 ionic strength.

§ 640.104 Potency.

(a) *Antibody levels and tests.* Each lot of final product shall contain at least the minimum levels of antibodies for diphtheria, measles, and for at least one type of poliomyelitis. In the event the final bulk solution is stored at a temperature above 5° C. the antibody level tests shall be performed after such storage with a sample of the stored material.

(b) *Minimum levels.* The minimum antibody levels are as follows:

(1) No less than 2 units of diphtheria antitoxin per ml.

(2) A measles neutralizing antibody level of no less than 0.25 times the level of the reference measles serum, except that when recommended for use with Measles Virus Vaccine, Live, Attenuated, the measles antibody level shall be as prescribed in § 640.114.

(3) A poliomyelitis neutralizing antibody level of no less than 1.0 for Type 1, 1.0 for Type 2, and 2.5 for Type 3, times the antibody level of the reference poliomyelitis immune globulin.

(c) *Reference materials.* The following reference materials shall be obtained from the Bureau of Biologics:

(1) U.S. reference measles serum for correlation of measles antibody titers.

(2) U.S. reference poliomyelitis immune globulin for correlation of poliomyelitis antibody titers, Types 1, 2, and 3.

Subpart K—Measles Immune Globulin (Human)

§ 640.110 Measles Immune Globulin (Human).

(a) *Proper name and definition.* The proper name of the product shall be Measles Immune Globulin (Human). It shall consist of a sterile solution of 10 to 18 percent globulin derived from human blood, having a measles antibody level of 0.5 times the level of the U.S. measles reference serum. Measles Immune Globulin shall be made from a sterile 16.5±1.5 percent solution of human globulin.

(b) *Source material.* The source of Measles Immune Globulin (Human) shall be blood, plasma or serum from human donors determined at the time of donation to have been free of causative agents of diseases that are not destroyed or removed by the processing method, as determined by the donor's history and from such physical examination and clinical tests as appear necessary for each donor at the time the blood was obtained. The source blood, plasma or serum shall not contain a preservative and shall be stored in a manner that will prevent contamination by microorganisms, pyrogens or other impurities.

(c) *Additives in source material.* Source blood, plasma or serum shall contain no additives other than citrate or acid citrate dextrose anticoagulant solution, unless it is shown that the processing method yields a product free of the additive to such an extent that the safety, purity and potency of the product will not be affected adversely.

§ 640.111 General requirements.

(a) *Heat stability test.* Approximately 2 ml of final container material of each lot shall not show any visible sign of gelation after heating in a 12 x 75 mm. stoppered glass tube at 57° C. for four hours.

(b) *Hydrogen ion concentration.* The pH of final container material shall be 6.8±0.4 when measured in a solution diluted to 1 percent protein with 0.15 molar sodium chloride.

(c) *Turbidity.* The product shall be free of turbidity as determined by visual inspection of final containers.

(d) *Date of manufacture.* The date of manufacture is the date of initiating the last valid measles antibody test as required in § 640.114.

(e) [Reserved]

(f) [Reserved]

(g) *Samples and protocols.* For each lot of globulin, the following materials shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014.

(1) 30 ml of final product.

(2) All protocols relating to the history of the manufacture of each lot and all results of all tests prescribed in these additional standards.

§ 640.112 Manufacture of Measles Immune Globulin (Human).

(a) *Processing method.* The globulin shall be prepared by a processing method that (1) has been shown to be capable of concentrating tenfold from source material at least two different antibodies, (2) does not affect the integrity of the globulins and is capable of consistently yielding a product which is safe for subcutaneous and intramuscular injection and (3) will not transmit viral hepatitis.

(b) *Reference materials.* The following reference material shall be obtained from the Bureau of Biologics: U.S. reference measles serum for correlation of measles antibody titers with globulin products.

(c) *Microbial contamination.* Low temperatures or aseptic techniques shall be used to minimize contamination by microorganisms. Preservatives to inhibit growth of microorganisms shall not be used during processing.

(d) *Bulk storage.* The globulin fraction may be stored in bulk prior to further processing provided it is stored in well-marked hermetically closed vessels. Purified globulin as either a liquid concentrate or a solid and containing alcohol or more than 5 percent moisture shall be stored at a temperature not to exceed −10° C. Purified globulin as a solid free from alcohol and containing less than 5 percent moisture, shall be stored at temperatures not to exceed 0° C.

(e) *Determination of the lot.* Each lot of Measles Immune Globulin (Human) shall represent a pooling of material from not less than 1,000 donors.

(f) *Sterilization and dilution.* The product shall be prepared initially as a 16.5 percent solution and this preparation shall be sterilized promptly after solution. After sterilization the product shall not be exposed to temperatures above 45° C. for more than a total of 72 hours. Dilution of this sterile globulin solution shall be made only to adjust the required measles antibody level.

§ 640.113 The final product.

(a) *Final solution.* The final product shall be a 10 to 18 percent solution of globulin containing 0.3 molar glycine and a preservative.

(b) *Protein composition.* No less than 90 percent of the globulin shall have an electrophoretic mobility not faster than -2.8×10^{-5} centimeters^2 per volt per second, when measured at a 1 percent protein concentration in sodium diethylbarbiturate at pH 8.6 and 0.1 ionic strength.

§ 640.114 Potency.

Antibody levels and tests. Each lot of final product shall contain no less than the minimum levels of antibodies for diphtheria and measles as follows:

(a) The product shall contain no less than 2 units of diphtheria antitoxin per ml, adjusted for dilution from the 16.5 percent solution.

(b) Each lot of final product shall contain a measles antibody level of 0.5 times the level of the U.S. reference measles serum. The measles antibody potency shall be determined by simultaneous determinations of the neutralizing antibody titers of the globulin on tests and of a reference preparation against 100 $TCID_{50}$ (50–500 $TCID_{50}$ when based upon a single test) of measles virus in a tissue culture system. The potency test shall also include a determination of virus titer and controls for globulin toxicity and cell culture viability. Twofold serial dilutions of the globulin under test and of the reference preparation shall be employed in this determination. In applying these requirements a plus or minus variation of one twofold dilution is acceptable.

PART 650—ADDITIONAL STANDARDS FOR DIAGNOSTIC SUBSTANCES FOR DERMAL TESTS

Subpart A—Diphtheria Toxin for Schick Test

Sec.

Subpart B—Tuberculin

AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES.—For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Diphtheria Toxin for Shick Test

§ 650.1 Diphtheria Toxin for Shick Test.

The proper name of this product shall be Diphtheria Toxin for Schick Test, which shall be a preparation of a diphtheria toxin obtained from the growth of *Corynebacterium diphtheriae*.

§ 650.2 U.S. Standard preparation.

The U.S. Standard Diphtheria Toxin for Schick Test shall be used to determine the Schick test dose of the product. The Schick test dose of the standard is that amount of the standard, when mixed with 0.001 unit of the U.S. Standard Diphtheria Antitoxin and injected intradermally in a guinea pig, will induce an erythematous reaction of 10 mm. in diameter.

§ 650.3 Manufacture of Diphtheria Toxin for Shick Test.

(a) *Propagation of bacteria.* The culture medium for propagation of the *Corynebacterium diphtheriae* for preparation of the parent toxin shall not contain ingredients known to be capable of producing allergenic effects in human subjects.

(b) *The parent toxin.* Diphtheria Toxin for Schick Test shall be prepared from a parent toxin which has been demonstrated to be stable and which contains no less than 400 minimum lethal doses per milliliter or 400,000 minimum reaction doses per milliliter. A minimum lethal dose is the smallest amount of toxin that will kill a guinea pig weighing approximately 250 gm. on the fourth day after its subcutaneous injection. A minimum reaction dose is that amount of toxin which when injected intradermally into a guinea pig induces an erythematous reaction 10 mm. in diameter.

§ 650.4 Potency test.

The dermal reactivity of each lot of the product shall be determined from the results of simultaneous guinea pig intradermal potency tests of the product under test and of the standard. The test shall be performed as follows:

(a) *Guinea pigs.* At least four healthy female guinea pigs shall be used, all of the same strain and each of a size that will permit a random distribution of eight intradermal injections. The hair shall be removed from the back and both sides of each guinea pig without producing abrasions of the skin. The denuded skin of each animal shall be sectioned into four equal areas at right angles to the vertebral column to provide two injection sites in each of the four areas, one on each side of the vertebra. The test is not valid if the guinea pigs do not show a graded response to the graded dilutions of the Schick test dose of the standard toxin.

(b) *Preparation of the test doses.* Four dilutions, two of the product under test and two of the U.S. Standard Diphtheria Toxin for Schick Test, shall be prepared in sterile buffered saline pH 7.4 containing 0.2 percent gelatin. The low and high dilutions of the standard shall be those amounts of a Schick test dose of the standard which in a dose of 0.1 ml. are capable of eliciting graded erythematous dermal reactions between 10 mm. and 20 mm. in diameter. The low and high dilutions of the Schick test dose of the toxin under test shall be the same as those of the standard toxin and estimated to have the same dermal reactivity.

(c) *Inoculation.* The low and high dilutions of the product (chart designation P_L and P_H) and the low and high dilutions of the standard (chart designations S_L and S_H) shall be injected intradermally in a volume of 0.1 ml. into each of the four guinea pigs according to either the following scheme, or in another scheme, provided it will permit comparable randomization of injection sites:

Area	Guinea Pig Number							
	1		2		3		4	
	Left	Right	Left	Right	Left	Right	Left	Right
A	S_L	S_L	S_H	S_H	P_L	P_L	P_H	P_H
B	S_H	S_H	S_L	S_L	P_H	P_H	P_L	P_L
C	P_L	P_L	P_H	P_H	S_L	S_L	S_H	S_H
D	P_H	P_H	P_L	P_L	S_H	S_H	S_L	S_L

(d) *Calculation of test results.* Between 40 and 66 hours following injection, a diameter of the reaction for each injection site shall be calculated by averaging two diameters of the reaction measured at right angles to each other. The average reaction for each dilution for each animal shall be determined, then the average diameters of the reactions of all of the guinea pigs for each dilution shall be calculated. The ratios of the reactions are determined by dividing the average diameter of the low dilution of the product under test by the average diameter of the low dilution of the standard and by dividing the average diameter of the high dilution of the product by the average diameter of the high dilution of the standard.

(e) *Potency requirement.* The potency of the product under test is satisfactory if each calculated ratio of the reactions of the product under test and of the standard is 1.0. The potency of the lot under test is considered to be equal to that of the standard if the ratios are not lower than 0.77 or higher than 1.30, provided that in a single test the ratios are substantially the same.

§ 650.5 Stability test.

A sample of each lot of the product shall be held at 37° C. for not less than 24 hours and then tested for potency as prescribed in § 650.4. The stability of the product is satisfactory if test results of the sample meet the potency requirement prescribed in § 650.4(e).

§ 650.6 Samples; protocols; official release.

For each lot of the product, the following material shall be submitted to the Director, Bureau of Biologics:

(a) A protocol which consists of a summary of the history of manufacture of each lot including all results of all tests for which test results are requested by the Director, Bureau of Biologics.

(b) A sample of no less than 20 ml. of the product.

No lot of the product shall be issued by the manufacturer until notification of official release is received from the Director, Bureau of Biologics.

§ 650.7 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Diphtheria Toxin for Schick Test, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the product that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs so finds and makes such findings a matter of official record.

Subpart B—Tuberculin

§ 650.10 Tuberculin.

The proper name of this product shall be Tuberculin, which shall be a preparation derived from *Mycobacterium tuberculosis* or *M. Bovis*.

§ 650.11 General requirements.

(a) *General safety.* Each lot of Tuberculin shall be tested for safety as prescribed in § 610.11 of this chapter, except that the sample of tuberculin from multiple puncture devices shall be obtained by removing the tuberculin in a manner that will permit the injection of material from at least five devices into each of two guinea pigs and from at least two devices into each of two mice.

(b) *Labeling.* In addition to complying with all other applicable labeling provisions of this subchapter, the package label shall state the following:

(1) For Tuberculin for Mantoux testing, the number of U.S. units (TU) per dose.

(2) For Tuberculin for multiple puncture testing, a statement indicating that the activity per test is comparable to a stated number of U.S. units (TU) administered by the Mantoux method.

(3) The applicable type of Tuberculin placed immediately following and of no less prominence than the proper name, as follows:

(i) "Old," or

(ii) "Purified Protein Derivative" or "PPD."

(c) *Samples; protocols; official release.* For each lot of Tuberculin the following shall be submitted to the Director, Bureau of Biologics, Food and Drug Administration, Building 29A, 9000 Rockville Pike, Bethesda, MD 20014:

(1) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(2) Tuberculin distributed on a multiple puncture device, as follows:

(i) A total of no less than 100 devices,

(ii) A total of no less than 20 ml. of bulk tuberculin.

(3) A total of no less than 20 ml. of liquid tuberculin.

(4) Sufficient dried tuberculin in final containers so that upon reconstitution as recommended in labeling it will yield at least 20 ml.

The product shall not be issued by the manufacturer until notification of official release of the lot is received from the Director, Bureau of Biologics.

§ 650.12 U.S. Standard preparations.

(a) The U.S. Standard Tuberculin, Old, shall be used for determining the potency of nonfractionated tuberculins, as prescribed in § 650.14. One U.S. Tuberculin unit is 0.1 ml. of a 1:10,000 dilution of this standard.

(b) The U.S. Standard Tuberculin, Purified Protein Derivative, shall be used in determining the potency of tuberculins made from protein fractions, as prescribed in § 650.14. One U.S. Tuberculin unit is 0.1 ml. of a 1: 5,000 dilution of this standard.

§ 650.13 Production.

(a) *Propagation of mycobacteria.* The medium used for production of mycobacteria shall not contain ingredients known to be capable of producing allergenic effects in human subjects.

(b) *Tests for viable mycobacteria.* The culture filtrate from each strain in its most concentrated form shall be shown to be free of viable mycobacteria by the following tests:

(1) *Animal test.* A 1.0 ml. sample of the filtrate shall be injected intraperitoneally into each of at least three healthy guinea pigs weighing between 300 and 400 gm. At least two-thirds of the animals must survive an observation period of at least 6 weeks and must show a normal weight gain. After the observation period the animals shall be necropsied and examined for signs indicative of tuberculosis except that animals that die during the observation period shall be necropsied and examined as soon as feasible after death. The filtrate is satisfactory for Tuberculin manufacture if none of the animals in the test show evidence of tuberculosis infection.

(2) *Culture test.* A 2.0 ml. sample of the filtrate shall be inoculated onto Löwenstein-Jensen's egg medium or other media demonstrated to be equally capable of supporting growth. A control test on the culture medium shall be conducted simultaneously with the sample under test and shall be shown to be capable of supporting the growth of small numbers of the production strain(s). All the test vessels shall be incubated at a suitable temperature for a period of 6 weeks under conditions that will prevent drying of the medium, after which the cultures shall be examined for evidence of mycobacterial colonies. The filtrate is satisfactory for Tuberculin manufacture if the test shows no evidence of mycobacteria.

§ 650.14 Potency test.

The potency of each lot of Tuberculin shall be estimated from a comparison of the responses obtained by the intradermal injection into sensitized guinea pigs weighing over 500 gm. of a sample of the lot under test and of the appropriate standard preparation. The U.S. Standard Tuberculin, Old, shall be used in determining the potency of tuberculins made from the concentrated filtrate of the soluble products of the growth of the mycobacteria. The U.S. Standard Tuberculin, Purified Protein Derivative, shall be used in determining the potency of tuberculins made from protein fraction of the soluble products of the growth of the mycobacteria. The test shall be performed as follows:

(a) *Sensitization of test animals.* At least four white guinea pigs shall be sensitized with *M. tuberculosis* or *M. bovis*. The degree of sensitivity shall be such that an intradermal injection of one U.S. unit of the appropriate standard preparation will produce in each test animal an erythematous reaction approximately 100 mm^2 within 18–24 hours.

(b) *Test Procedure.* The hair shall be removed from both sides of the sensitized test animals without producing abrasions of the skin. Dilutions of the standard containing 0.5, 1, 2, and 4 U.S. units in the test dose of 0.1 ml. and four comparable levels of activity of the lot under test shall be injected intradermally into opposite and parallel sites of each animal. Only three dilutions need be used when the initial concentration of the lot under test does not contain four units in 0.1 ml. Within 18–24 hours following injection, measurements of the greater and lesser diameters of erythema measured to the closest millimeter shall be made at each site. The mean value of the product of the diameters for each dilution shall be calculated. The number of U.S. units in the lot under test shall be estimated from its relationship to the reactivity of the appropriate standard preparation.

(c) *Potency.* The potency of the lot is satisfactory if the test results are within limits, as follows:

(1) *Products for Mantoux testing.* ±20 percent of the labeled U.S. units.

(2) *Liquid products for multiple puncture testing.* ±20 percent of the U.S. units claimed by the manufacturer in the license application.

(3) *Products dried on multiple puncture devices.* ±50 percent of the U.S. units claimed by the manufacturer in the license application.

§ 650.15 Equivalent methods.

Modification of any particular method or process or the conditions under which it is conducted as set forth in the additional standards relating to Tuberculin, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the product that are equal to or greater than the assurances provided by such standards, and the Commissioner of Food and Drugs, so finds and makes such finding a matter of official record.

PART 660—ADDITIONAL STANDARDS FOR DIAGNOSTIC SUBSTANCES FOR LABORATORY TESTS

Subpart A—Hepatitis Associated Antibody (Anti-Australia Antigen)

AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Hepatitis Associated Antibody (Anti-Australia Antigen)

§ 660.1 Hepatitis Associated Antibody (Anti-Australia Antigen).

(a) *Proper name and definition.* The proper name of this product shall be Hepatitis Associated Antibody (Anti-Australia Antigen) which shall consist of a preparation of serum containing the hepatitis associated antibody.

(b) *Source.* The source of this product shall be plasma or blood, obtained aseptically from animals immunized with hepatitis associated (Australia) antigen which have met the applicable requirements of § 600.11 of this chapter or from human donors whose blood is positive for hepatitis associated antibody.

§ 660.2 General requirements.

(a) *Processing.* The processing method shall be one that has been shown to consistently yield a specific and potent final product free of properties which would adversely affect the test results when the product is tested by the methods recommended by the manufacturer in the package enclosure.

(b) *Ancillary reagents and materials.* All ancillary reagents and materials supplied in the package with the product shall meet generally accepted standards of purity and quality and shall be effectively segregated and otherwise manufactured in a manner (such as heating at 60° C. for 10 hours) that will reduce the risk of contaminating the product and other biological products. Ancillary reagents and materials accompanying the product which are used in the performance of the test as described by the manufacturer's recommended test procedures shall have been shown not to adversely affect the product within the prescribed dating period.

(c) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, the following shall also be included:

(1) Indication of the source of the product immediately following the proper name on both the final container and package label, e.g., human, guinea pig.

(2) Name of the test method(s) recommended for the product on the package label and on the final container label when capable of bearing a full label (see § 610.60(a) of this chapter).

(3) A warning on the package label and on the final container label if capable of bearing a full label (see § 610.60 (a) of this chapter) indicating that the product and antigen if supplied, shall be handled as if capable of transmitting hepatitis.

(4) If the product is dried, the final container label shall indicate "Reconstitution date: _____" and a statement indicating the period within which the product may be used after reconstitution.

(5) The package shall include a package enclosure providing (i) adequate instructions for use, (ii) a description of all recommended test methods, and (iii) warnings as to possible hazards, including hepatitis, in handling the product and any ancillary reagents and materials accompanying the product.

(d) *Final container.* Final containers shall be sterile, colorless, and transparent.

(e) *Date of manufacture.* The date of manufacture of Hepatitis Associated Antibody (Anti-Australia Antigen) that has been iodinated with radioactive iodine (I^{125}) shall be the day of labeling the antibody with the radionuclide.

(f) *Samples; protocols; official release*—(1) *Hepatitis Associated Antibody (Anti-Australia Antigen).* Except as provided otherwise in this paragraph, the following material for each filling of the product shall be submitted to the Director, Bureau of Biologics:

(i) A sample of each filling packaged as for distribution including all ancillary reagents and materials.

(ii) A protocol which consists of a summary of the history of manufacture of each filling, including all results of each test for which test results are requested by the Director, Bureau of Biologics.

(iii) No filling of the product shall be issued by the manufacturer until notification of official release of the filling is received from the Director, Bureau of Biologics.

(2) *Hepatitis Associated Antibody (Anti-Australia Antigen) iodinated with ^{125}I.* Hepatitis Associated Antibody (Anti-Australia Antigent) that has been iodinated with radioactive iodine (^{125}I) may be released by the manufacturer pursuant to the requirements of § 610.1 of this chapter without obtaining an official release from the Director, Bureau of Biologics provided:

(i) The manufacturer submits to the Bureau of Biologics a protocol of each master lot along with one sample of the lot, such material to be postmarked no more than 1 day following the manufacturer's release date.

(ii) At least two complete kits of each released lot will be retained as retention samples for no less than 90 days from the date of manufacture.

§ 660.3 Reference panel.

A Reference Hepatitis Associated Antigen (Australia Antigen) Panel shall be obtained from the Bureau of Biologics and shall be used for determining the potency and specificity of Hepatitis Associated Antibody (Anti-Australia Antigen).

§ 660.4 Potency test.

To be satisfactory for release each filling of Hepatitis Associated Antibody (Anti-Australia Antigen) shall be tested against the Reference Hepatitis Associated Antigen (Australia Antigen) Panel and shall be sufficiently potent to be able to detect the antigen in the appropriate sera of the reference panel by all test methods recommended by the manufacturer in the package enclosure.

§ 660.5 Specificity.

Each filling of the product shall be specific for hepatitis associated antibody as determined by specificity tests found acceptable to the Director, Bureau of Biologics.

Subpart B—Leukocyte Typing Serum

§ 660.10 Leukocyte Typing Serum.

(a) *Proper name and definition.* The proper name of this product shall be Leukocyte Typing Serum which shall consist of a preparation of serum containing an antibody or antibodies for identification of leukocyte antigens.

(b) *Source.* The source of this product shall be plasma or blood obtained aseptically from animals which have met the applicable requirements of § 600.11 of this chapter, or from human donors.

§ 660.11 Potency tests.

(a) *Test according to manufacturer's directions.* Each lot of the product intended for cytotoxicity testing shall produce an 80 percent or greater cell death with at least 85 percent of the positively reacting cell samples, and a 60 percent or greater cell death with the remaining 15 percent of the positvely reacting cell samples when tested by all methods recommended in the manufacturer's package enclosure against the manufacturer's panel of cells which shall have been approved by the Director, Bureau of Biologics, Food and Drug Administration. The antiserum shall maintain such level of reactivity throughout the dating period. The approved composition of the cell panel may be obtained from the Director, Bureau of Biologics, Food and Drug Administration, HFB–1, 5600 Fishers Lane, Rockville, MD 20852.

(b) *Test with diluted serum.* Each lot of the product, at a dilution of at least 1:2, shall produce a strong positive reaction of 80 percent or greater cell death for cytotoxic typing serums when tested with appropriate leukocytes by all methods recommended in the manufacturer's package enclosure.

(c) *Last valid potency test.* For purposes of determining the date of manufacture, the date of the last valid potency test shall be the date of initiation by the manufacturer of the test in paragraph (b) of this section.

§ 660.12 Specificity test.

Each lot of the product shall be specific for the antibody or antibodies indicated on the label when tested by all methods recommended in the manufacturer's package enclosure.

§ 660.13 Processing.

(a) *Method.* The processing method shall be one that has been shown to consistently yield a specific and potent final product free of properties which would adversely affect the product for its intended use.

(b) *Ancillary reagents and materials.* Ancillary reagents and materials accompanying the product, which are used in the performance of the test as described by the manufacturer's recommended test procedures, shall have been shown not to adversely affect the product within the prescribed dating period.

(c) *Color coding.* Color coding of labels, containers, or droppers supplied with the product shall not be used. The addition of coloring agents or dyes to the product or ancillary reagents to differentiate leukocyte antibodies is not permitted. A container of a vital stain for purposes of facilitating the reading of the test may be included in the testing kit.

(d) *Final containers.* Final containers shall be colorless, transparent, and shall have been sterilized and filled by aseptic procedures.

§ 660.14 Labeling.

In addition to the applicable requirements of §§ 610.60, 610.61, and 610.62 of this chapter, the following information shall be included in the labeling:

(a) The source of the product, if other than human, immediately following the proper name on both the final container and package label;

(b) The name of the specific antibody or antibodies present in the product immediately following the source when specified, or the proper name when the source is not specified. The antibody designation shall be of no less prominence than the proper name on all labeling;

(c) The name of the test method or methods recommended for the product on the package label and on the final container label when capable of bearing a full label;

(d) A package enclosure providing adequate instructions for use including:

(1) A description of all recommended test methods;

(2) A description of all supplementary reagents including a description of a suitable complement source;

(3) Necessary precautions, including a warning, against exposure to carbon dioxide;

(4) A caution to use more than one antiserum for each specificity;

(5) A caution not to dilute the antiserum;

(6) A caution that cross-reacting antigens exists.

(e) The package enclosure shall contain adequate directions for reconstitution which shall include the following instructions:

(1) Do not reconstitute with more than the recommended volume of diluent;

(2) Place the reconstituted material in small aliquots so that the product will undergo no more than two freeze-thaw cycles;

(3) Store all unused aliquots at −65° C. or colder within 8 hours of reconstitution;

(4) A statement that the frozen aliquots must be used within one year of reconstitution or prior to the expiration date appearing on the label of the product, whichever is earlier;

(5) A statement instructing the user to record the expiration date and the reconstitution date of the serum on the label of each multi-use aliquot stored in a small test tube, and to maintain similar information for the material stored in typing trays.

§ 660.15 Samples, protocols, official release.

(a) *Definition of a lot.* For release purposes, a lot is defined as uniform final container material identified by the manufacturer as having been thoroughly mixed in a single vessel and which has been dried in a single run. A lot may be retested upon expiration and assigned a new lot number provided all tests required of the initial lot are performed and a protocol of such tests and samples are submitted to the Bureau of Biologics, Food and Drug Administration, for release purposes. The protocol shall include identification of the lot number under which it was previously released and the date of release.

(b) *Sample size.* For each lot of product, four final containers packaged as for distribution shall be sent to the Director, Bureau of Biologics, Food and Drug Administration, Bldg. 29–A, 9000 Rockville Pike, Bethesda, MD 20014, for testing and release by the Bureau. In addition, 300 milligrams shall be submitted for a test to determine moisture content. Samples for moisture testing may be either (1) Final container material of the product, or (2) Dummy samples of material with the same protein concentration as the product, filled in the same size vials, with the same volume as the product. Such dummy samples shall be appropriately labeled and placed in random locations throughout the drying oven.

(c) *Protocols and release.* A protocol which consists of a summary of the history of manufacture of each lot, including all results of all tests required by regulations, shall be submitted for each lot of product to be released. The product shall not be issued by the manufacturer until notification of official release of the lot is received from the Director, Bureau of Biologics, Food and Drug Administration.

PART 680—ADDITIONAL STANDARDS FOR MISCELLANEOUS PRODUCTS

Subpart A—Allergenic Products

Subpart B—Trivalent Organic Arsenicals

AUTHORITY: Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see 39 CFR Parts 124 and 125, esp. § 125.2.

Subpart A—Allergenic Products

§ 680.1 Allergenic Products.

(a) *Definition.* Allergenic Products are products that are administered to man for the diagnosis, prevention or treatment of allergies.

(b) *Criteria for source material.* Only specifically identified allergenic source materials which contain no more than 1 percent of detectable foreign materials, shall be used in the manufacture of an Allergenic Product. Source materials such as feathers, hairs, and danders shall be free from blood and serum.

§ 680.2 Manufacture of Allergenic Products.

(a) *Extraneous allergenic substances.* All manufacturing steps shall be performed so as to insure that the product will contain only the allergenic and other substances intended to be included in the final product.

(b) *Cultures derived from microorganisms.* Culture media into which organisms are inoculated for the manufacture of Allergenic Products shall contain no allergenic substances other than those necessary as a growth requirement. Neither horse protein nor any allergenic derivative of horse protein shall be used in culture media.

(c) *Liquid products for oral administration.* Liquid products intended for oral administration that are filled in multiple dose final containers shall contain a preservative in a concentration adequate to inhibit microbial growth.

(d) *Residual pyridine.* Products for which pyridine is used in manufacturing shall have no more residual pyridine in the final product than 25 micrograms per milliliter.

§ 680.3 Tests.

(a) *Identity.* When a specific identity test meeting the provisions of § 610.14 of this chapter cannot be performed, the manufacture of each lot shall be separated from the manufacture of other products in a manner that will preclude adulteration, and records made in the course of manufacture shall be in sufficient detail to verify the identity of the product.

(b) *Safety.* A safety test shall be performed on the contents of a final container of each lot of each product as prescribed in § 610.11 of this chapter, except for the following:

(1) For lots consisting of no more than 20 final containers or 20 sets of individual dilutions, or where the final container contains no more than one intended human dose, the safety test need

not be performed on the contents of a final container provided the safety test is performed on each lot of stock concentrate and on each lot of diluent contained in the final product. Only stock concentrates and diluents which have passed the general safety test shall be kept in the work areas used for the manufacture of Allergenic Products. A stock concentrate is an extract derived from a single allergenic source and used in the manufacture of more than one lot of product, and from which final dilutions or mixtures are prepared directly.

(2) For powders for scratch tests, a sample shall be suspended in a suitable diluent and injected into each animal, and the sample size shall be the single human dose recommended.

(c) *Sterility.* A sterility test shall be performed on each lot of each Allergenic Product as prescribed in § 610.12 of this chapter, with the following exceptions:

(1) When bulk material is not prepared, the sterility test prescribed for bulk material shall be performed on each container of each stock concentrate at the time a stock concentrate is prepared, and the test sample shall be no less than 1 ml. from each stock concentrate container.

(2) For lots consisting of no more than 5 final containers, the final container test shall be performed in accordance with § 610.12(f)(7) of this chapter using the sample therein prescribed or using a sample of no less than 0.25 ml. of product from each final container, divided in approximately equal proportions for testing in Fluid Thioglycollate and Fluid Sabouraud's media. The test sample in the latter alternative method may be an overfill in the final container.

(3) For products prepared in sets of individual dilution series, a test sample of 0.25 ml. shall be taken from a final container of each dilution, which samples may be pooled and one half of the pooled material used for the test with fluid Thioglycollate medium and one-half used for the test with fluid Sabouraud's medium.

(4) Tablets and capsules need not be tested for sterility provided aseptic techniques are employed in their manufacture.

Subpart B—Trivalent Organic Arsenicals

§ 680.10 Tests prior to release.

Tests required to be made, prior to the release of each lot of a licensed product, shall be supplemented in the case of the trivalent organic arsenicals by tests for:

(a) Stability,
(b) Solubility,
(c) Arsenic content,
(d) Moisture,
(e) Relative nontoxicity.

§ 680.11 Pretesting by Bureau; sample of each lot.

Prior to the release of any lot of the product, the manufacturer shall forward to the Director, Bureau of Biologics, no less than 15 ampoules of the largest single-dose size in such lot, together with protocols showing the results of each test required prior to release.

§ 680.12 Expiration date.

Notification from the Director, Bureau of Biologics, that lot samples forwarded in accordance with § 680.11 have satisfactorily passed prescribed tests shall indicate a date which may be taken as the date of manufacture for the purpose of fixing the expiration date. The date of issue shall be the same as the date of manufacture.

§ 680.13 Composition of product.

Solutions or solutions of mixtures in the concentrations recommended for clinical administration shall be of such hydrogen ion value and tonicity as to be physiologically compatible with human blood.

§ 680.14 Container.

The product shall be hermetically sealed under vacuum or under a dry nonoxidizing gas in glass ampoules. The contents of any final container shall not exceed 10 maximum human doses.

§ 680.15 Final container label.

In addition to the labeling requirements stated in § 610.60 of this chapter, the final container label of the trivalent organic arsenicals shall bear the statements required in § 680.16 (b) and (c) and an additional statement giving the amount of the drug contained in the ampoule.

§ 680.16 Outside label.

The outside label, in addition to the complete proper name and all other items required for products generally shall show conspicuously: (a) If the product is dispensed as a mixture or solution, the name of all admixed substances,

(b) If the ampoule is a multiple dose container, the fact that it is a multiple dose container.

(c) Specific method of preparation, if any, required prior to administration, as, for example alkalinization.

NOTE.—Incorporation by reference provisions approved by the Director of the FEDERAL REGISTER, December 12, 1972.

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