(2) Unsanitary practices such as, but not limited to, eating, smoking, or expectorating on the floors or otherwise creating a nuisance in any room, compartment, or place in which biological products are prepared, handled, or stored at licensed establishments are prohibited.

(Approved by the Office of Management and Budget under control number 0579–0013)

[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 114.8 Outline of Production required.

An Outline of Production shall be on file with Animal and Plant Health Inspection Service for each licensed biological product or for each biological product authorized to be imported into the United States for Distribution and Sale. Preparation of a biological product in a licensed establishment shall be in accordance with the Outline of Production for such product filed with Animal and Plant Health Inspection Service as provided in this section, but subject to changes as may be required under § 114.8(f).

(a) The Outline of Production shall be prepared as prescribed in § 114.9 and submitted to Animal and Plant Health Inspection Service for filing. When objectionable features, if any, are corrected and no further exceptions are taken by Animal and Plant Health Inspection Service to an Outline of Production for a biological product, such Outline of Production shall be approved for filing.

(b) Each page shall be stamped as filed on the date such action was taken in the bottom right hand corner. Although the filed outline may be referred to as an approved outline, approval for filing constitutes no endorsement by Animal and Plant Health Inspection Service of such biological product or the methods and procedures used to prepare such biological product.

(c) The original and two copies shall be retained by Animal and Plant Health Inspection Service and the remaining copies returned.

(d) Each licensee shall review each Outline of Production for accuracy and sufficiency not less frequently than once a year. Revisions necessary to bring an Outline of Production into compliance with the regulations shall be submitted to Animal and Plant Health Inspection Service.

(e) When a list of licensed products to be continued in production at a licensed establishment is requested by the Administrator in accordance with § 102.5(d) of this subchapter, the licensee shall supplement the list with information for each product as follows:

(1) The Outline of Production currently being used shall be identified as to the date when last revised and filed with Animal and Plant Health Inspection Service and the date of the last review made by the licensee.

(2) The Outline of Production to be kept in the active file shall be designated. If more than one has been filed for a product, only the Outline of Production currently being used shall be included.

(f) The Administrator may, upon the basis of information not available to him at the time the current Outline of Production for a biological product was filed, object to the methods or procedures being used in the preparation of such biological product and notify the licensee to modify the filed Outline of Production to eliminate such objections. If the licensee does not comply with the notice, the Administrator may, after affording opportunity for a hearing to the licensee, suspend the product license for the biological product involved; in which case, the licensee shall not prepare such product until subsequent notice of withdrawal of the suspension is given to the licensee.

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[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 66784, Dec. 26, 1991]

§ 114.9 Outline of Production guidelines.

Each Outline of Production shall be prepared in accordance with the applicable directions provided in this section.

(a) *General requirements.* (1) The original and not more than four copies of each Outline of Production or special outline or revised pages of either shall

be prepared on heavy paper (8.5″ x 11″) of a type receptive to permanent stamp ink.

(2) The name of the biological product (or component), the establishment license number, and the date prepared shall appear on a front cover page and each page of the Outline of Production or special outline. The name of the licensee (or foreign manufacturer) shall appear on the front cover page.

(3) The pages shall be numbered in the upper center. At least 1½ inch margin shall be left at the top of the first page and a 2 inch margin at the bottom of each page for the Animal and Plant Health Inspection Service stamp.

(4) Amended pages shall be numbered the same as those being superseded. They shall bear the date prepared and refer to the date on the pages being superseded. If one replacement page supersedes more than one page, the new page shall indicate same, but if several replacement pages are added to supersede one page, the page number followed by letters shall be used.

(5) The last page of the original and one copy of either a new or a completely rewritten Outline of Production and each page revised separately shall be signed in the lower left corner by the authorized representative of the licensee (or foreign producer). Stamped or facsimile signatures are not acceptable.

(6) A summary of changes shall appear on an attached page and refer to each page, paragraph, or subparagraph being changed.

(7) Transmittal forms shall be used for the original and subsequent revisions. Blank forms shall be available upon request to Animal and Plant Health Inspection Service.

(b) *Special outline.* An outline describing the preparation of a component of a biological product or an operation performed in the preparation of a biological product may be required if such special outline could be referred to in Outlines of Production to eliminate repetition. Each special outline shall be identified by number and shall not be used until accepted and filed by Animal and Plant Health Inspection Service.

(c) Outline of Production for antiserum, antitoxin, and normal serum shall be written according to the following:

OUTLINE GUIDE FOR PRODUCTION OF ANTISERUM AND ANTITOXIN AND NORMAL SERUM

License No. Name of Product Date

I. *Serum animals.* A. Species, conditions, age, and general health.

B. Examination, preparation, care, quarantine, tests, and treatment of animals before injections are started.

C. Holding, handling, exercising, and monitoring the condition of animals after injections are started.

II. *Antigens.* A. Composition and character of the antigen.

1. Micro-organisms.

2. Source and date of accession of each micro-organism.

3. Strains.

4. Proportions of each micro-organism and strain.

B. Identification methods used for each micro-organism and frequency with which these methods are applied.

C. Virulence and purity of cultures or antigen and the determination and maintenance thereof. Range of subcultures or passages to be used in production.

D. Attenuation, if any, before use for production purposes.

E. Character, size, and shape of containers used for growing micro-organisms.

F. Media used for stock, seed, and antigen cultures (composition and reaction of). May refer to a special outline by number.

G. Preparation of the antigen or toxin and toxoid. Complete and full description of each step and its manner of accomplishment and number these steps in sequence. Include all tests for each antigen, and the specifications for character, identity, virulence, concentration, and standardization.

III. *Immunization of animals.* A. Outline fully with special attention given to the following:

1. Character and dose of the antigen.

2. Method and frequency of injections.

3. Time required for immunization or hyperimmunization.

4. Preliminary bleedings and tests, if any, to ascertain quality of serum.

5. All other similar matters, including treatments between bleedings.

B. Period of time elapsing between last injection and first bleeding; and between bleedings.

C. Technique of bleeding operations; volume of blood collected at each bleeding; and period of rest.

IV. *Preparation of the biological product.* A. Describe fully and show each step of preparation from the first bleeding to the completion of the preserved product in bulk containers prior to filling of final containers.

B. Composition of the preservative and proportions used. Indicate at which step of production, and the method used in adding the preservative.

C. Agglutination and complement-fixation titers and the methods of their determinations.

D. Disposition of unsatisfactory biological products and infective materials not used in production.

E. Assembly of units to make a serial; volume of the average serial; and the volume of the maximum serial.

V. *Testing*. Indicate the stages in the preparation of the biological product at which samples are collected. Refer to all applicable Standard Requirements. Outline all additional tests in detail and state minimum requirements for each satisfactory test.

A. Purity.

B. Safety.

C. Potency.

D. Other tests.

VI. *Post preparatory steps*.

A. Form and size of final containers in which the product is to be distributed.

B. Methods and techniques of filling final containers. Volume of fill for each size final container.

C. Collection, storage, and submission of representative samples. Indicate at which steps in the production these samples are taken.

D. Expiration date based on the earliest date of harvest and the date of the last satisfactory potency test.

E. Use, dosage, and route of administration for each animal species for which it is recommended.

F. Include any additional pertinent information.

(d) Outline of Production for *vaccines*, *bacterins*, *antigens*, and *toxoids* shall be written according to the following:

OUTLINE GUIDE FOR VACCINES, BACTERINS, ANTIGENS, AND TOXOIDS

License No. Name of Product Date

I. *Composition, etc., of the product*. A. Micro-organisms used. Give the isolation and passage history.

B. Source and date of accession of each micro-organism.

C. Strains.

D. Proportions of each strain.

II. *Cultures*. A. Brief description of methods of identifying each micro-organism and the frequency with which these methods are applied.

B. Virulence and purity of cultures and the determination and maintenance thereof. Range of subcultures or passages to be used in production.

C. Composition and reaction of media used for seed and production cultures. Include the source of eggs, tissue, or cell cultures, and the tests to determine that eggs, tissues, and cells are free of contamination.

D. Character, size, and shape of containers used for growing cultures.

E. Storage conditions of seed cultures.

F. Methods of preparing suspensions for seeding or inoculation.

G. Technique of inoculating (1) seed media; (2) production media. Titer or concentration of inoculum, and the volume of medium for each size and type of culture container.

H. Period of time and conditions for incubation and degree of temperature used for each micro-organism or group of micro-organisms.

I. Character and amount of growth; observation as to contamination of growth.

J. Method of attenuation, if any, before used for production purposes.

III. *Harvest*. A. Handling and preparation of cultures and media (including eggs) before removal of micro-organisms or tissues for production purposes.

B. Minimum and maximum period of time elapsing from time of inoculation until harvest.

C. Technique of harvesting micro-organisms or tissues (specify) for production purposes.

D. Specifications for acceptable harvest material.

E. Handling of discarded material not used in production.

F. Include any additional pertinent information.

IV. *Preparation of the product*. Describe fully and show each step of preparation from harvest of antigen containing tissues or production cultures to the completion of the finished product in final containers. In describing the preparation of the product, emphasize the following:

A. Method of inactivation, attenuation, or detoxification.

B. Composition of preservative, adjuvant or stabilizer, and proportions used stated in such a manner that the concentration can be calculated; stage and method of addition.

C. Method and degree of concentration.

D. If product is standardized to give concentration of antigen, show procedures and calculations.

E. 1. Assembly of units to make a serial (illustrate by example).

2. Volume of average serial.

3. Volume of maximum serial.

4. Any other pertinent information.

F. Volume of fill for each size vial. Type of vial if unusual.

G. Method and technique of filling and sealing of final containers.

H. Desiccation, including moisture control. Give maximum percent moisture.

I. Amount of antigenic material per dose or doses in final container.

V. *Testing*. Indicate the stages in the preparation of the biological product at which the samples are collected. Refer to all applicable Standard Requirements. Outline all additional tests in detail and state the minimum requirement for each satisfactory test.

A. Purity.

B. Safety.

C. Potency.

D. Moisture, if desiccated.

E. Any other tests.

VI. *Post preparatory steps*. A. Form and size of final containers in which the product is to be distributed.

B. Collection, storage, and submission of representative samples. Indicate at which steps in the production these samples are taken.

C. Expiration date based on the earliest date of harvest and the date of the last satisfactory potency test. If applicable, give the date of lyophilization.

D. Use, dosage, and route of administration for each animal species for which the biological product is recommended.

(e) Outlines of Production for allergenic extracts shall be written according to the following:

OUTLINE GUIDE FOR ALLERGENIC EXTRACTS

License No. Name of Product Date

I. *Composition of the product*. A. Source and type of raw material.

B. Weight/volume concentration.

II. *Preparation of the product*. A. Describe fully and show each step of preparation to the completion of the finished product in true containers. In describing the preparation of the product, emphasize the following:

1. Method of extraction.

2. Composition of preservative, adjuvant or stabilizer, and proportions used; stage and method of addition.

3. Method and degree of concentration.

4. Standardization of the product.

5. (a) Assembly of units to make a serial.

(b) Volume of average serial.

(c) Maximum serial.

6. Volume of fill for each size vial.

7. Method and technique of filling and sealing of final containers.

8. Amount material per dose or doses in final container.

III. *Testing*. Indicate the stages in the preparation of the biological product at which the samples are collected. Refer to all applicable Standard Requirements. Outline all additional tests in detail and state the minimum requirement for each satisfactory test.

A. Purity.

B. Safety.

C. Potency.

D. Any other tests.

E. Include any additional pertinent information.

IV. *Post preparatory steps*. A. Form and size of final containers in which the product is to be distributed.

B. Collection, storage, and submission of representative samples. Indicate at which steps in the production these samples are taken.

C. Expiration date based on the earliest date of harvest and the date of the last satisfactory potency test.

D. Use, dosage, and route of administration for each animal species for which the biological product is recommended.

(f) Outlines of Production for diagnostic test kits based on antigen-antibody reactions, and other diagnostics whose production methods are amenable to description as described herein shall be written according to the following requirements:

OUTLINE GUIDE FOR DIAGNOSTIC TEST KITS

License No. Name of product Date

Introduction

Provide a brief description of the kit as follows:

1. Principle of the test (ELISA, latex agglutination, etc.).

2. Antigen or antibody detection test.

3. Sample(s) used for testing (serum, whole blood, tears, etc.).

4. List reagents, references, and equipment included.

5. Identify materials obtained under split manufacturing agreements.

6. General description of test interpretations and their limitations, including followup tests.

I. *Antibody Components*

A. Production of polyclonal antibody components.

1. If purchased, list suppliers, criteria for acceptability, and describe all tests performed after receipt to determine that specifications have been met.

2. If produced in-house, describe the species, age, weight, conditions, and general health of all animals used in antiserum production.

a. Preinjection considerations:

Describe the examination, preparation, care, quarantine procedures, and treatments administered before immunization(s). Describe all tests used to determine suitability for use. Describe the preparation of any standard negative serum(s) collected prior to immunization.

b. Immunization of animals.

i. Describe the character and dose of the antigen; if adjuvant is used provide details on its preparation. If commercial product is used include its true name as shown on the

label, the manufacturer, serial number, and expiration date.

ii. Describe the method and schedule for immunizations.

iii. Describe the method for harvesting and evaluating the immunization product, including tests for acceptability.

iv. Provide number and intervals between harvests, volume obtained, and any other pertinent information.

B. Production of Monoclonal Antibody Components.

1. Hybridoma components:

a. If hybridoma components are purchased, list suppliers and criteria for acceptability; if tests are performed after receipt, describe fully.

b. If hybridomas are prepared inhouse, identify the antigen(s) used, describe the immunization scheme, and the species of animal used.

c. Identify the tissue of origin, and the procedures for harvesting, isolating, and identifying the immune cells.

d. Describe the source, identity, and the product secreted (light or heavy chain) by the parent Myeloma Cell Line.

e. Summarize cloning and recloning procedures, including clone characterization and propagation, if appropriate.

f. If appropriate, describe procedures for establishing and maintaining seed lots.

g. Describe any other pertinent tests or procedures performed on the hybridoma cell line.

2. Antibody production:

a. Describe the production method. If produced in cell culture, animal serum additives must conform to 9 CFR 113.53. If produced in animals, describe fully including husbandry practices and passage procedures.

b. Provide the criteria for acceptable monoclonal antibody, including tests for purity.

c. Describe all tests or other methods used to ensure uniformity between production lots of monoclonal antibody. Include all reaction conditions, equipment used, and reactivity of the component.

d. Describe all characterization procedures and include the expected reactivity of all reference monoclonal antibodies.

II. *Antigen Preparation*

A. Identify the microorganism(s) or antigen being used. If previously approved Master Seed virus, bacteria, or antigen derived therefrom is used, provide pertinent information on the testing performed, and details of dates of United States Department of Agriculture confirmatory tests and approval, as appropriate.

B. Describe all propagation steps, including identification of cell cultures, media ingredients, cell culture conditions, and harvest methods. For antigen produced in eggs, give the egg source, age, and route of inoculation. If cell lines are being used, give dates of testing and approval as specified in 9 CFR 113.52.

C. Describe procedures used for extracting and characterizing the antigen.

D. Describe the method used to standardize the antigen.

E. If the antigen is purchased, identify the supplier and describe the criteria for acceptable material, including all tests performed by the producer and/or the recipient to determine acceptability.

III. *Preparation of Standard Reagents*

A. Describe the positive and negative controls included in the kit. If purchased, list suppliers and criteria for acceptance.

B. Describe the preparation and standardization of the conjugate(s). If purchased, list suppliers and criteria for acceptance.

C. Describe the preparation and standardization of the substrate(s). If purchased, list suppliers and criteria for acceptance.

D. Identify buffers, diluents, and other reagents included in the kit. The preparation of these components may be described in this section or in filed Special Outlines.

IV. *Preparation of the Product*

Fully describe methods used to standardize antigens, reference standards, positive control serum, negative control serum, and standard reagents from production/purchase to completion of finished product in final containers, including the following:

1. Composition and quantity of preservative in each.

2. Method of filling, plating, or attaching the antigen or antibody component to a solid phase.

3. Minimum and maximum acceptable fill volumes for each final container of reagent included in the kit.

4. The disposition of unsatisfactory material.

V. *Testing*

Refer to all applicable standard requirements.

A. Purity.

Describe all tests of the kit for purity or specify the exemption as provided in 9 CFR 113.4.

B. Safety.

In vitro products are exempt from safety tests.

C. Potency.

Provide details of tests used to determine the relative reactivity of the kit including minimum requirements for a satisfactory test. Reference standards and control serum used for this purpose should be identified by unique codes or lot numbers.

VI. *Postpreparatory Steps*

A. Describe the form and size of final containers of each reagent/component included in the kit.

B. Describe the collection, storage, and submission of representative samples. Refer to 9 CFR 113.3(b)(7).

C. Specify the expiration date. Refer to 9 CFR 114.13.

D. Provide details of recommendations for use, including all limitations, qualifications, and interpretation of results.

E. Submit confidentiality statement identifying specific parts of the outline containing information, the release of which would cause harm to the submitter.

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[39 FR 16869, May 10, 1974, as amended at 48 FR 57473, Dec. 30, 1983; 56 FR 20124, May 2, 1991; 56 FR 66784, Dec. 26, 1991]

§ 114.10 Antibiotics as preservatives.

Antibiotics are authorized for use as preservatives for biological products if used within the limitations as to kinds and amounts prescribed in this section.

(a) When an antibiotic or combination of antibiotics, with or without a fungistat is to be used in the preparation of a biological product, the kind(s) and amount(s) of each shall be specified in the outline for such product in such a way that the concentration in the final product may be calculated. Except as may be approved by the Administrator, only those individual antibiotics or combinations of antibiotics listed in paragraphs (b) and (c) of this section shall be used.

(b) Permitted individual antibiotics:

(1) The antibiotic level of a specified individual antibiotic in one ml. of a biological product, when prepared as recommended for use, shall not exceed the amounts listed in this paragraph: *Provided*, That in the case a desiccated biological product is to be used with an indefinite quantity of water or other menstruum, the determination shall be based on 30 ml. per 1,000 dose vial or equivalent.

(2) Except as prescribed in paragraph (c) of this section, only one antibiotic shall be used as a preservative in a biological product. The kind and maximum amount per ml. of such antibiotic shall be restricted to:

Amphotericin B	2.5 mcg.
Nystatin (Mycostatin)	30.0 units
Tetracyclines	30.0 mcg.
Penicillin	30.0 units
Streptomycin	30.0 mcg.
Polymyxin B	30.0 mcg.
Neomycin	30.0 mcg.
Gentamicin	30.0 mcg.

(c) Permitted combinations:

(1) Penicillin and streptomycin.

(2) Either amphotericin B or nystatin, but not both, may be used with one of the other antibiotics listed in paragraph (b) of this section, or with a combination of penicillin and streptomycin, or with a combination of polymyxin B and neomycin.

(3) The maximum amount of each antibiotic in a combination shall be the amount prescribed for such antibiotic in paragraph (b) of this section.

(d) Antibiotics used in virus seed stock purification are not restricted as to kind or amounts provided carryover into the final product is controlled and specified in outlines of production.

[39 FR 16869, May 10, 1974, as amended at 56 FR 66784, Dec. 26, 1991]

§ 114.11 Storage and handling.

Biological products at licensed establishments shall be protected at all times against improper storage and handling. Completed product shall be kept under refrigeration at 35 °to 45 °F. (2 °to 7 °C.) unless the inherent nature of the product makes storage at a different temperature advisable, in which case, the proper storage temperature shall be specified in the filed Outline of Production. All biological products to be shipped or delivered shall be securely packed.

§ 114.12 Expiration date required.

Each serial or subserial of biological product prepared in a licensed establishment shall be given an expiration date determined in accordance with the requirements provided in § 114.13 or § 114.14. A licensed biological product shall be considered worthless under the Virus-Serum-Toxin Act subsequent to the expiration date appearing on the label.

[41 FR 44687, Oct. 12, 1976]

§ 114.13 Expiration date determination.

Unless otherwise provided for in a Standard Requirement of filed Outline of Production, the expiration date for