United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

1400 Independence Ave, SW

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM NO. 800.206

TO: Veterinary Services Leadership Team
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: for Jack A. Shere
Deputy Administrator

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SUBJECT: General Licensing Considerations: Preparing Outlines of Production for Vaccines, Bacterins, Antigens, Toxoids, and Diagnostic Test Kits

I. PURPOSE

This memorandum provides guidance on preparing Outlines of Production for vaccines, bacterins, antigens, toxoids, and diagnostic test kits in compliance with title 9, *Code of Federal Regulations* (9 CFR), part 114.9.

II. CANCELLATION

This memorandum replaces Veterinary Services (VS) Memorandum No. 800.206 dated March 26, 2014.

III. BACKGROUND

The Animal and Plant Health Inspection Service (APHIS) regulations in 9 CFR 114.9 provide broad subject headings for Outlines of Production. These standardized headings ensure consistent placement of key information used by the Center for Veterinary Biologics (CVB) in regulating veterinary biologics. The headings are broad to accommodate the diversity of today's biological products, but this also results in considerable variation in content provided by individual licensees and permittees. This memorandum provides guidance regarding writing an effective Outline of Production.

IV. GUIDANCE ON PREPARING OUTLINES OF PRODUCTION

A. General Considerations

1. Use accurate terms

Write Outlines in a manner that ensures consistent production and testing methods. Pay particular attention to terms that may allow for variability in production or testing. Ensure the language used is appropriate for the purpose.

a. "*Should*" means the procedure or value is recommended, but not mandatory. Deviations from the described procedure are not necessarily out of compliance with the filed Outline of Production. Likewise, "*may*" permits a procedure, but does not require it.

b. "*Shall," "must,"* or *"will*" means that the procedure or action is mandatory. Deviations are out of compliance.

c. "*And*" versus "*or." "And*" means that both conditions must be met. "*Or*" means that either condition (but not necessarily both) must be met.

Avoid language that allows variability in production or testing. Manufacturing and testing procedures should not be subject to undue variability. Alternatives may be acceptable to document minor variations between domestic and exported versions of a product, or to allow equivalent alternatives in domestic production where such alternatives are necessary.

If the language allows variability, clearly describe the decision criteria to determine the option to use for an individual serial. CVB may request data to support the equivalence of alternative methods.

2. Avoid duplicated text

The codified section headings for Outlines may appear to promote some overlap in content among various sections. To the practical extent, avoid duplicating text in sections. This reduces the potential for introducing errors when revising repetitive sections.

a. Include text in the most applicable section and cite this section (e.g., "See section III.A") in other sections where overlap occurs.

b. Direct the reader to the source of the full text. Avoid "citation chains" that direct the reader to a location that, in turn, directs them to a third location.

c. If citations refer to another Outline, ensure that the Outline is for an actively licensed product. Do not cite Outlines for terminated or prelicense products. Cite which sections of the Outline apply.

d. If citations refer to a document CVB published (e.g., supplemental assay methods), refer to the "current version" of the document rather than a specific version number. This prevents referring to obsolete documents.

3. Subsection identification

Adequate numbering and subsection identification facilitate review of Outlines and references to specific text. When text for a single section or subsection spans more than one page of a *paper* document, identify the section and subsection number at the top of each page. Alternatively, include the complete identifier in the format for each subheading level (i.e., write "V.C.3" instead of "3"). This avoids returning to a previous page to find the section number.

For electronic Outlines, follow the portal-specific guidance for creating navigable headings as an acceptable means of subsection identification.

a. Add subsections beyond those specified in 9 CFR 114.9 as needed, but add them at the end of the existing subsections. Do not insert them in a location that requires renumbering the existing subsections.

b. If a section or subsection exceeds more than a brief paragraph or two, consider dividing the text into additional subsections. This helps reviewers refer to specific text in their comments.

4. Describe variable parameters

Describe parameters with inherent variability as an acceptable range rather than an absolute value. Examples include time and temperature (e.g., 20-25 °C instead of 23 °C) and ingredients added to a target concentration instead of by direct measurement.

a. Avoid vague, subjective descriptors such as "overnight" or "room temperature."

b. Use mechanical ranges within the accuracy of the equipment (i.e., reasonable for the normal negligible variability that one might expect to encounter in adequately controlled daily production).

c. Limit other ranges to concentrations reasonably expected in the course of routine production. Ranges must reflect consistent manufacture.

d. Define upper and lower limits; avoid open-ended ranges.

5. Note equivalent materials

In general, Outlines of Production should have enough detail to ensure consistent manufacture of all serials. This may necessitate including manufacturing sources and product identification numbers to describe component materials adequately. In certain cases, the licensee may use equivalent materials from a number of sources. When using equivalent materials, specify the preferred product source in the Outline, followed by the phrase "or equivalent." Specifying the preferred material provides a basis for determining equivalency of any alternative material that may be used.

6. Document exemptions

If CVB grants an exemption to a codified requirement, document the exemption in the applicable section of the Outline. Include the CVB approval date and the CVB mail log number under which the approval was granted, if known. If the product was initially licensed under a different establishment number, indicate the establishment number under which the exemption was granted.

7. Document pivotal study approvals

When documenting pivotal regulatory study approvals in an Outline or special outline, include the study identifier (if applicable), the CVB approval date, and the CVB mail log number (if known). If the product was initially licensed under a different Establishment number, indicate the Establishment number under which the study was granted.

8. Use of Special Outlines

Use Special Outlines to document processes or tests that pertain to more than one product. Cite the single Special Outline at the appropriate location in each applicable Outline of Production. In addition, use Special Outlines when a lengthy detailed description is required for a product or test. Moving these descriptions to a separate document can improve the overall readability and flow of the Outline.

Structure Special Outlines in the same way as Outlines of Production. Include a cover page, appropriate section and subsection identification, and document any changes. Submit Special Outlines in the same manner as Outlines of Production.

9. Include summary of changes

   a. For each change include the specific reason for the change. If the change is justified by data on file with CVB, cite the study number or other data identifier and CVB mail log number/date of CVB acceptance. If a change was made in response to prior CVB correspondence, indicate the CVB mail log number and date of the correspondence. CVB may return Outlines unprocessed at the discretion of the CVB reviewer, if adequate justifications are not provided.

   b. National Center for Animal Health (NCAH) portal submitters: Use the "track changes" functionality of the word processing software to show where insertions and deletions are made. Use the "comment" feature to insert the reason and justification for the change next to the applicable edited text. No separate "summary" of changes is required, but may be prepared *in addition to* tracked changes and contextual comments, if desired.

   c. Paper submitters: List every change made to the Outline in the summary of changes (see 9 CFR 114.9(a)(6)). Cite the page and paragraph in which each change occurs.

B. Section-specific considerations

See appendix I for a section-by-section discussion of Outline content for vaccines, bacterins, antigens, toxoids, and immunomodulators. See appendix II for additional information regarding products involved in split manufacture agreements. See appendix III for guidance regarding Outlines for diagnostic test kits.

V. GUIDANCE ON SUBMITTING OUTLINES OF PRODUCTION

A. Format

Configure all Outlines according to the headings described in 9 CFR 114.9. Do not change headings or insert new headings, except as discussed in section IV.A.3.a of this memorandum.

Submit electronic Outlines via the NCAH Portal *only* on the template provided by CVB or another CVB-approved template created by your firm. See portal-related guidance for details on necessary electronic template requirements.

B. Number of Copies to Submit

1. Submission by the NCAH portal: Follow portal-specific guidance on the CVB web site.
2. Paper submissions: Submit an original and at least one copy. The original and first copy must each bear an original signature, preferably in a different ink color (e.g., blue) than the text.

C. Transmittal Forms

1. NCAH portal submitters. Submit Outlines with APHIS Form 2049. Do not use APHIS Form 2015. Submit only complete revisions.

2. Paper submitters

   a. Submit Outlines with APHIS Form 2015, "Transmittal of Labels and Circulars or Outlines."

   b. Ensure the date submitted (block 4) corresponds to the preparation date of the Outline (or date of submitted pages). If the mailing date of the submission differs from the preparation date of the Outline, indicate the mailing date of the submission in block 13, "Comments."

   c. Enter the preparation date of the last complete revision in block 12, "Date of Previous Outline." Do not use the date of individual pages amended after the last complete revision.

3. Do not use an APHIS Form 2015 to submit Outlines of Production for products exported under the Food and Drug Administration's Export Reform and Enhancement Act of 1996 (FDA-EREA). Submit FDA-EREA Outlines as correspondence with a cover letter regardless of whether submitting by portal or paper.

D. Complete Revisions versus Page Amendments

1. NCAH portal submitters. Submit only Complete Revisions. Because of the electronic nature of the document, CVB does not accept individual page amendments that need to be collated with older pages.

2. Paper submitters. Update Outlines by submitting complete revisions or individual page amendments. Either approach is acceptable in many cases. However, CVB recommends a complete revision in lieu of individual page amendments when at least half the pages in the document have been amended since the last complete revision.

E. Date of Superseded Version.

If an Outline submission replaces a previously-filed version, state the date of the superseded version ("supersedes date;" see 9 CFR 114.9(a)(4)).

1. If the Outline is new, state, "New" on the cover page and all subsequent page headers.

   When an Outline is part of a merger or acquisition, it may be "new" only to the establishment acquiring the product. In such cases, specify the document on which the new Outline is based. Place this information on the "Supersedes" line of the cover page. Example: Establishment Z submits a "new" Outline for Product B. This product was formerly licensed to Establishment X as Product A. State on the cover page of the "new Outline" for Est. Z, "Supersedes: Identical to Est X, Product A Outline of Production" or equivalent. Including this cross reference on the cover page does *not* automatically inactivate the cited document.

2. Update the supersedes date on a *paper* cover page only when submitting a complete revision of the Outline. Use the preparation date of the last complete revision, rather than a later date when individual pages were amended.

3. For the supersedes date on an individual *paper* page header, show the preparation date of the previous version of that page if updated after the last complete revision. For a complete *paper* revision, state "Complete Revision" instead of a supersedes date on the individual page headers.

4. When resubmitting an Outline that CVB returned unprocessed, use the preparation date of the last filed Outline for the supersedes date, rather than the date of the unprocessed submission. However, on the APHIS Form 2015 for the current submission, note the preparation dates of unprocessed outlines in block 13, "Comments."

F. Version number (NCAH Portal Submissions only)

   Follow the portal-specific guidance for electronic Outlines found on the CVB website.

VI. IMPLEMENTATION/APPLICABILITY

This memorandum applies to all products, licensed and unlicensed, as of the date of this memorandum. For licensed products, Outlines of Production should comply within a year from the date of this memorandum.

Appendices

Appendix I

Section-Specific Guidelines for Preparing Outlines of Production: Vaccines, Bacterins, Antigens, and Toxoids

Cover Page

Each Outline and special outline must have a cover page, containing the information specified in title 9 of the *Code of Federal Regulations* (9 CFR), part 114.9(a)(2).

Paper submitters only: In general, licensees and permittees submitting paper copy should replace the cover page only when submitting a complete revision, not for individual page changes. When there is a necessary cover page change, (e.g., a name change for the existing establishment) but no other revisions warranting a complete revision, submit an updated cover page without submitting a complete revision. Continue to list the date of the last complete revision on the cover page. Underneath this date, add a statement "cover page updated [date]."

The following guidance applies to vaccines, bacterins, antigens, and toxoids in general. See VS Memorandums 800.213 and 800.214 for additional guidance specific to platform and prescription platform product Outlines.

Section I. Composition of the product

Complete this section in each Outline. Do not cite another related Outline.

A. *Microorganisms used.* For each microorganism used to produce the product, provide the following information:

- Organism name.
- Complete lot number (or other unique identifier), exactly as it appears on the stored Master Seed container and licensee's records.
- Isolation and known preparation history, including the number of passages since isolation. Specify the type of medium, cell culture, or animal used for each passage.
- The date CVB approved the Master Seed for use in production. Include the CVB Mail Log number of the approval letter, if known.
- For viruses and other obligate intracellular organisms, approvals are specific for one or more animal species, depending on the extraneous agent testing performed (9 CFR 113.47). List each of the animal species for which the approval applies.

- Name and license number of the firm at the time CVB approved the Master Seed for use in production. Do **not** list the date that CVB licensed the product containing the Seed.

If inventories of the original Master Seed are depleted and CVB has authorized the use of a passage of the original Seed as the "new" Master Seed, list the lot identification and approval date for the current Master Seed lot, not the depleted original lot.

B. *Source and date of accession of each microorganism.* For each microorganism used to produce the product, indicate the date and from whom the current licensee obtained the Master Seed. If the Master Seed was obtained through a merger or sublicensing agreement with a current or former U.S. Department of Agriculture-licensed firm, provide the name and license number of the firm and the effective date of the relevant company merger or agreement.

C. *Strains.* Provide the strain or isolate designation for each microorganism used to produce the product. If the microorganism is a generally recognized strain, specify the strain. Otherwise, provide the isolate designation as it appears on containers used to store the Master Seed.

D. *Proportions of each strain.*

- If the product contains only one strain or isolate for each fraction, mark this section "Not Applicable."
- If a fraction of the product contains more than one strain or isolate, but the strains or isolates can be distinguished in the serial release potency test, mark this section "Not Applicable."
- If a fraction contains more than one strain or isolate that cannot all be distinguished in the serial release potency test, indicate the proportion of each strain or isolate in the fraction. Base the proportions of the strains in that fraction on antigenic mass, not on volume of harvest fluids or other criteria. For each fraction, the sum of the proportions must total 100 percent. For other fractions in the product that meet either of the first two criteria, indicate "Not Applicable."

Section II. Cultures

Antigen production methods are often common to multiple products. If desired, compile the information in this section in a Special Outline and cite the Special Outline in each applicable Outline of Production. This is preferable in lieu of maintaining product licenses for monovalent products solely for the purpose of citing the Outline for the production of a particular antigen.

A. *Methods of identifying each microorganism.* Specify the stages when identity testing is performed (e.g., working seeds or production cultures). Specify the tests that will be performed. Include the frequency with which methods are applied (e.g., when each working seed is established).

B. *Virulence and purity of cultures and the determination and maintenance thereof. Range of subcultures and passages to be used in production.* Specify the number of passages beyond Master Seed allowed in production. This is determined by the number of passages used to produce the serial with which product efficacy was demonstrated. Typically, it is limited to five passages beyond Master Seed.

The portion of the heading pertaining to the virulence and purity of the culture and maintenance thereof is largely obsolete since the implementation of the Master Seed system. Historically, the virulence of some cultures was maintained by periodic passage through animals. This is no longer an acceptable practice. Seeds recovered after animal passage are now considered to be "new" Master Seeds and are subject to full testing before use.

C. *Composition and reaction of media used for seed and production cultures.* Provide information on Master Cell Stocks and eggs, tissues, and primary cells in each Outline. Do not cite related Outlines.

This section must include a statement regarding the source of any ingredients of animal origin. The preferred wording can be found in VS Memorandum No. 800.51, section IV.A.2.

1. *Master Cell Stocks.* For each cell line used to produce the product, provide the following information:

- Name of cell line.
- Complete lot number (or other unique identifier), exactly as it appears on the stored Master Cell Stock container and the licensee's records.
- Source and history.
- Maximum approved passage level.
- Date when CVB approved the Master Cell Stock for use in production and the CVB mail log number of the approval letter, if known.
- Master Cell Stock approvals are specific for one or more animal species, depending on the extraneous agent testing performed (9 CFR 113.47). Include each of the animal species to which the approval applies.
- Name and license number of the firm at the time CVB approved the Master Cell Stock for use in production.

- Antigens in this product that are propagated with this cell.

2. *Eggs, tissue, or primary cells.* For each type used, specify:

   - Type of cellular substrate. Indicate the age of embryos, as applicable.
   - VS Memorandum No. 800.65 provides guidance on eggs used in production. If eggs are used in production, include a statement in the Outline that ensures compliance with this memorandum.
   - Tests to determine that eggs, tissues, and cells are free of contamination. Include the stage at which the tests are performed and criteria for a satisfactory test.
   - Antigens propagated with each substrate.

3. *Composition of media.* For each microbiological medium used in manufacture, include the following:

   - Name of medium.
   - Composition (ingredients and amounts): The composition of Seed media used during scale-up may be subject to some lot-to-lot variation, depending on the immediate needs of individual cultures. If certain ingredients are expressed as a range to allow for customization, ensure that the upper and lower limits reflect concentrations reasonably used in routine production. However, prepare final production media in a consistent manner.
   - Storage conditions, temperature, and dating period.
   - How medium is used (for example, as production culture for *Mannheimia haemolytica* fraction).
   - Exemptions, if any, to 9 CFR 113.53 (testing ingredients of animal origin).

D. *Character, size, and shape of containers used for growing cultures.* Specify the following:

   - Culture system (e.g., stationary flasks, roller bottles, or bioreactors).
   - Range of vessel sizes that reasonably may be used.
   - Vessel composition (e.g., glass or plastic).
   - Vessel configuration (e.g., height-to-diameter ratio and baffled or unbaffled tank).
   - Type of mixing system.

- Type and number of agitation impellers, if applicable.
- How aeration or other gas is provided to the culture, if applicable.
- Type of sealing used with agitation system to ensure integrity of the vessel.
- Closure systems used on containers and vessels.

E. *Storage conditions of Seed cultures.* Indicate how the Master Seed is stored. If Working Seeds are prepared for long-term storage, indicate how they are stored.

F. *Methods of preparing suspensions for seeding or inoculation.* Indicate how the Seed is recovered from scale-up cultures for inoculation into subsequent cultures.

G. *Technique of inoculation.* Describe the following:

- Acceptable range of inoculum concentration (or multiplicity of infection).
- Cell density, if cellular cultures are involved.
- Volume of medium for each size and type of culture container.

If these parameters vary depending on the type of passage (scale-up versus final production passage), clarify the differences in subsections II.G.1 and II.G.2.

H. *Conditions for incubation used for each microorganism or group of microorganisms.* For each antigen, describe:

- Incubation conditions (time, temperature, atmospheric conditions). If the conditions vary depending on the type of passage, clarify the differences.
- Handling that may occur during the incubation period (e.g., adding nutrients, candling eggs).
- List critical parameters under process control. Briefly describe how the parameters are measured.

I. *Character and amount of growth; observation as to contamination of growth.* For each antigen, describe:

- Parameters (e.g., optical density, percent of cells with cytopathic effect) that determine when incubation is complete.
- Tests performed at this stage in production to evaluate purity of the culture.

J. *Method of attenuation before use in production.* APHIS wrote this section heading before implementing the Master Seed concept. Procedures performed to attenuate a Seed organism before establishment of the Master Seed lot should be described in section I.A. APHIS no longer accepts attenuation of post-Master

Seed cultures on a serial-by-serial basis. Thus, in most cases, section II.J should be marked "Not Applicable."

Section III. Harvest

A. *Handling and preparation of cultures and media before removal of microorganisms or tissues for production purposes.* Describe any procedures or testing performed on a completed culture while it is still in the culture vessel or system. If egg culture is involved, indicate whether dead eggs are discarded or handled separately. Describe conditions (e.g., refrigeration) to maintain eggs before harvest.

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

- Specify the incubation time for each individual culture, not the total time elapsed from inoculation of the first scale-up culture to the harvest of the final production culture.
- Clarify any differences in incubation time that may exist between scale-up cultures and the final production culture.

A. *Technique of harvesting microorganisms or tissues for production purposes.* Describe how the microorganisms are removed from the culture vessel and any processing that occurs at this point in manufacture:

- If the organism is propagated in eggs, indicate which parts (e.g., chorioallantoic membrane, embryo, allantoic fluid) are retained.
- Describe any grinding or coarse filtration.

B. *Specification for acceptable harvest material.* Include all critical parameters, including minimum antigen concentration and purity. Include the evaluation methods used to determine acceptability of the culture. If a spectrophotometer is used to measure antigen concentration, specify the wavelength and type of spectrophotometer used.

C. *Handling of discarded material not used in production.* VS Memorandum No. 800.56 describes acceptable methods to discard material. Cite this memorandum if one of the described methods is used. Fully describe any exceptions.

D. *Additional pertinent information.* Use this section to describe any critical information regarding manufacture not covered under another heading.

Section IV. Preparation of the product

A. *Method of inactivation, attenuation, or detoxification.* Include:

- Procedure for inactivation or detoxification.
- Test method to confirm completeness of inactivation or detoxification.
- If applicable, additional procedures to be performed if the initial test demonstrates incomplete inactivation.
- Documentation of CVB acceptance of inactivation kinetics data to support the inactivation time and procedure. For products licensed before 2013, and for which there is no explicit date on which inactivation data were accepted, the acceptance date is the date of the Outline in effect at the time of product licensure.

B. *Composition of preservative, adjuvant, or stabilizer.*

1. *Adjuvant.* Include:

- Chemical composition of adjuvant (and trade name, if applicable). Include any antibiotics or preservatives added to the adjuvant.
- Proportion of adjuvant used, stated so that the concentration can be calculated.

2. *Preservative.*

- If a preservative (antibiotics and/or other) is added at batching/serial assembly, specify the identity and the actual concentration added. Do not merely cite 9 CFR 114.10.
- If the final product may contain residual amounts of antibiotics or other preservatives because of upstream processes (e.g., growing organisms in media containing antibiotics or thimerosal), indicate which substances may be present in residual amounts and identify the stages at which they were added. You do not need to estimate the concentration of residual antibiotic or preservative in the final product if there is no material risk that this level exceeds codified maximum amounts (e.g., 9 CFR 114.10).

C. *Method and degree of concentration.* If the microbial harvests may be concentrated before batching/serial assembly, specify:

- Criteria that determine whether concentration is necessary.
- Method of concentration, including specific parameters (such as g-force or filter molecular weight cutoff).

- Maximum permissible concentration factor.

D. *Standardization of product.* If the product is standardized for antigen concentration, describe the procedures and calculations.

E. *Assembly of units to make a serial.*

1. Provide an example of a typical serial, including all components and proportions added. If the product may be administered at different dose volumes, depending on indications for use, base calculations on the most common dose volume.

2. Volume of average serial.

3. Volume of maximum serial.

4. Other pertinent information.

F. *Volume of fill.* Include an acceptable range for the fill volume for each size of container.

G. *Method and technique of filling and sealing final containers.* Include exemptions to 9 CFR 114.6, if applicable.

H. *Desiccation, including moisture control.* If the product is lyophilized, include:

- How live product is handled between harvest and desiccation to maintain viability.
- Procedure for desiccation.
- Permissible temperature ranges during desiccation.
- Permissible time period for desiccation, including the longest time the product may be held at maximum temperature.
- Test method for residual moisture (9 CFR 113.29).
- Allowable percent residual moisture in finished product.

I. *Amount of antigenic material per dose in final container.* Describe, in the most objective terms possible (e.g., preinactivation cell count, spectrophotometry or nephelometer readings) the minimum antigen content in the product at the time of serial assembly. Avoid characterizing the amount of antigen as the amount sufficient to pass serial release potency tests, or as a volume of non-standardized antigen.

Section V. Testing

Include stepwise procedures in sufficient detail so a laboratory technician experienced in general laboratory techniques could perform the assay. Many standard requirements specify only general assay architecture (e.g., perform a serum neutralization test), so additional detail beyond the 9 CFR citation is often required. If testing is performed by a third party under contract, include the information specified in VS Memorandum No. 800.115.

Mark section V as "not applicable" in Outlines of Production for combination packages (i.e., two or more individually licensed component products that may be marketed with a recommendation to combine the components before administration) because the combination package product is not tested. Do not refer the reader to Outlines for the component products.

Stepwise procedures for section V tests may be described in Special Outlines. If Special Outlines are used, retain key information regarding the assay and interpretation of results in each Outline of Production so the information can be retrieved easily during the serial release process. Information to remain in section V when a Special Outline is used includes:

- Criteria for a valid test
- Criteria for a satisfactory test
- Criteria for an inconclusive test
- Lot number of the current potency reference preparation, if applicable, and the original approval date and current expiration date.
- Preparation specific to a particular lot of potency reference (such as working dilution).

Where the Special Outline refers to the above information, direct the reader to "see the Outline of Production for the product being tested." Do not duplicate this information in the Special Outline.

A. *Purity.*

- Specify the media volumes to be used when testing according to 9 CFR 113.26 or 9 CFR 113.27. Determine these volumes according to the dilution of preservative procedure in 9 CFR 113.25(d) and Supplemental Assay Method 903. Document CVB acceptance of the dilution of preservative study supporting the specified media volume.

- Other purity tests (e.g., *Mycoplasma*, *Salmonella*, lymphoid leukosis, chicken anemia virus) may be required depending on product ingredients and manufacturing method. List any exemptions to these requirements.

B. *Safety.*

- If an animal safety test is required, specify a single consistent vaccination route (e.g., subcutaneous).
- If an exemption to animal safety testing has been granted, per VS Memorandum No. 800.116, document the exemption but retain a description of the safety tests that have been exempted. This is necessary so that if the exemption is rescinded for individual high-risk serials according to the provisions in VS Memorandum No. 800.116, the testing to be completed is documented.
- Test poultry and aquaculture products in the species for which the product is recommended or in a suitable surrogate species.

C. *Potency*

- Include a potency test for every antigenic fraction associated with a label claim.
- Document CVB acceptance of the pivotal immunogenicity and efficacy study for each antigenic fraction (live or killed). Include the age and species of the animals and the route of administration used to establish product efficacy. If the fraction is a live organism, specify the titer of the efficacy serial.
- If multiple immunogenicity and efficacy studies were performed to support claims for a specific antigenic fraction (such as short-term efficacy versus duration of immunity claims or claims for different disease syndromes caused by a single agent), include the above information on each approved study.
- If software is required to calculate relative potency, document the procedure for using the software in the Outline (or Special Outline) or in a separate standard operating procedure submitted for CVB review. Specify the software version(s) allowed, and if a specific template/protocol file is also used, specify its version-controlled file name. Document the procedure in sufficient detail that a user in an independent laboratory, possibly using different software, would be able to reproduce the calculation. Such details include pertinent software options and settings, the data model, validity criteria, and (if necessary) the region of the response curves that will be utilized to estimate relative potency.

D. *Moisture* (if desiccated). Describe the assay procedure for moisture testing, as well as criteria for acceptable results, in section IV.H. In section V, direct the user to see section IV.H.

Section VI. Post-preparatory steps

A. *Form and size of final containers.* Specify the composition and nominal fill volumes of the final containers.

This section also provides a uniform location for miscellaneous information.

1. Diluent: If the product is packaged with a diluent, indicate the name of the diluent. If the diluent is purchased, specify the supplier. If it is made in-house, specify the Special Outline containing the production method. This is not applicable to combination packages.

2. International testing: In general, do not include testing conducted solely for the purpose of international distribution in the Outline of Production filed with APHIS. If, however, the inclusion of this testing facilitates export, place international testing requirements in section VI.A. Filed Outlines with such testing in section V should be revised at the time of the next annual review.

Include the following disclaimer for any international testing that appears in section VI.A: "The testing described in this section is conducted solely for international purposes. It is not used by APHIS in serial release determinations."

B. *Collection, storage, and submission of representative samples.*

- Indicate the production steps where samples are taken.
- For samples taken during the fill process in accordance with 9 CFR 113.3, include any applicable information regarding specific authorizations to submit partial-fill samples or quantities other than those described in 9 CFR.
- If samples are shipped to designated recipients before APHIS release, list the approved recipients and maximum number/volume of samples to be shipped. Describe any approvals required before shipping samples.

C. *Expiration date.*

- According to 9 CFR 114.12 the expiration date for each serial is computed from the date of the initiation of the first potency test. The start date is considered to be the date the serial is introduced into the test system (the first day the serial is injected into the animal or the first day the serial is added to an *in vitro* test plate). Format this section to read: "The expiration date is (or will not exceed) XX months after the date of initiation of the first potency test."

- 9 CFR 114.13 requires real-time stability data to confirm the acceptability of product dating. Document the CVB acceptance of the supporting study. If the dating has not yet been confirmed, indicate that appropriate data are being collected. Update the Outline when the CVB accepts the stability data.
- If a product is a combination package, format section VI.C to read:

  "The expiration date of the combination package is the earliest expiration date of the individual product components in accordance with 9 CFR 114.13. This combination package is composed of released product of Codes _____."

  Do not list the dating period for the component products in the combination package Outline.

D. *Use, dosage, and route of administration.*

Include the following:

- All APHIS-approved prophylactic and therapeutic efficacy claims, even if not currently stated on product labeling. Format these claims in accordance with the provisions in VS Memorandum No. 800.54.
- All APHIS-approved safety claims (such as whether the product is safe for use in pregnant animals).
- Dose volume.
- Animal species in which the product is used.
- Routes of administration.
- Number of doses in primary vaccination series and dosing interval.
- Minimum age for administration.
- Timing of administration, if applicable (for example, vaccination 4 to 6 weeks before breeding).
- Revaccination recommendations (beyond primary vaccination series).
- Duration of immunity.
- Slaughter withdrawal period (for livestock, equine, poultry, and aquaculture products).
- All antibiotics or other preservatives (e.g., thimerosal) in the product, including those that may be present in trace amounts. If antibiotics added to preliminary manufacturing components are reduced by downstream

processing to undetectable levels in the final product and CVB has accepted supporting data, indicate the date CVB accepted the data.

- APHIS-mandated warnings or cautionary statements.
- Any other product-specific instructions for use.

Many establishments reproduce the complete product label text in the Outline. This is not required, but it helps ensure all required elements on the label have been included in the Outline.

E. *Site of Manufacture.* If the licensee has licensed premises in multiple geographically separated locations (i.e., cities), specify the location at which each step of production occurs. Specify one location for each step unless the step *routinely* occurs in more than one location. This does not preclude moving production to other licensed premises, with APHIS approval, as production needs dictate. Follow the guidance in VS Memorandum No. 800.87 when transferring locations.

Appendix II

Guidance for Outlines for Products Involved in Split Manufacture

I. If a product is part of a split manufacturing agreement (i.e., a For Further Manufacture (FFM) product or a Final-Use Product (FUP) that contains FFM components), detail in each Outline in the manufacturing chain only those processes done by that manufacturer at that point in production.

   A. In each section of an Outline utilizing manufacturing precursors provided by a FFM manufacturer, cite the Establishment Number and Product Code of the FFM product for details of upstream processes conducted by the FFM establishment. Do not reproduce the information found in the FFM Outline.

   B. In FFM Outlines, do not describe how the product is processed after leaving the FFM manufacturer. Mark sections of the FFM Outline covering downstream processes "Not Applicable."

II. Indicate in section III.F (Other Pertinent Information) of Outlines for products that use FFM components:

   A. Minimum specifications for acceptance of each FFM component.

   B. Tests performed to ensure the incoming product meets these specifications.

III. Include in section VI.A of Outlines for FFM products:

   A. Name (and U.S. Veterinary Establishment Number, if applicable) of each recipient of the FFM product.

   B. Type and size of containers in which the FFM product is shipped.

   C. Shipping conditions, including the required shipping temperature range. Include any monitoring procedures that are in place during shipment.

   D. Responsible party (e.g., commercial carrier, transfer by establishment employees) for shipping between establishments

IV. Frequently, the FFM manufacturer is involved in serial release testing of split-manufactured products. In such situations:

   A. Describe in section IV.J of the FFM Outline any serial release testing performed by the FFM manufacturer

B. When the FFM manufacturer performs a serial release test, indicate in section V of the FUP Outline that the FFM provider conducts the test and cite section IV.J of the FFM Outline for the details of the test procedure. Section V of the FUP should include all items expected to be in section V of a product completely manufactured by a single firm. The items listed in V.C. of appendix I that are routinely kept in the main Outline of Production (i.e., reference information, criteria for valid test, criteria for satisfactory test, retest criteria) apply to a FUP outline, as well as the outline of production of a product completely manufactured by a single firm.

V. If the FFM product is inactivated, the results of testing to confirm complete inactivation must be reported on APHIS Form 2008 for each FFM serial. In section V of the FFM Outline, describe the inactivation confirmation test procedure, the test validity criteria, the criteria for satisfactory results, the date of CVB acceptance, and CVB mail log number, if known. For products licensed before 2013 and for which there is no explicit date on which inactivation data were accepted, list the date of the Outline in effect at the time of licensure.

Appendix III

Guidance for Preparing Outlines of Production for Diagnostic Test Kits

Introduction

Briefly specify the type of assay and describe how it works. Specify the analyte detected (e.g., antibody, antigen), the sample types (e.g., serum) used, the list of kit components, any equipment included (e.g., droppers), any specialized equipment required, and items that are licensed under a split manufacturing agreement. Include recommendations regarding use, result interpretations, and test result limitations. The kit description should not specify or imply the test kit is a quantitative assay. Do not use statements such as "proportional to the OD value," unless the kit is a poultry antibody test kit. Such statements are allowed only for poultry antibody test kits.

Section I: Antibody Components

Every kit in which ingredients of animal origin are used in manufacture must contain a statement regarding the origin of these ingredients. The recommended statement is in VS Memorandum No. 800.51, section IV.A.2. Place the statement at the beginning of section I for consistency, even if the kit does not contain antibody components. If there are no ingredients of animal origin, state that there are no such ingredients.

Antibody may be purchased or prepared on licensed premises. If antibody is purchased, specify the identity, vendor and catalog number, country of origin, acceptance criteria, and any acceptance testing performed on each lot. The statement, "accepted under a Certificate of Analysis" is not acceptable. If the antibody is prepared on licensed premises, describe production steps and specify acceptance criteria. Details of production should include species, age, weight, conditions, and general health of any animals used. See 9 CFR 114.9(f) regarding required pre-injection, immunization, and harvest information.

1. Purchased Monoclonal Antibody

   Purchased monoclonal antibodies must be full characterized. In addition to the information listed above, specify the clone designation or reacting epitope.

2. Hybridomas

   Hybridomas used in the preparation of monoclonal antibodies prepared on licensed premises must meet the applicable Master Cell requirements in 9 CFR 113.52. Include the information specified in appendix I, section II.C.1, in the Outline.

Section II: Antigen Preparation, including PCR Primers, Master Seed and Master Cell Stock

Antigen may be purchased or prepared on licensed premises.

1. Purchased Antigen

If antigen is purchased, specify the antigen identity, vendor and catalog number, country of origin, and lot acceptance criteria for each lot. Include testing performed by the vendor and testing performed on licensed premises. The statement "accepted under a Certificate of Analysis" is not acceptable.

2. Antigen Prepared on Licensed Premises

Antigen prepared on licensed premises must be prepared from Master Seed. Include in the Outline all information specified in appendix I, sections I.A through I.C. Also specify suppliers, catalog numbers, acceptance criteria, and shelf life for media and media ingredients. If media are prepared on licensed premises, describe the formulation and production steps.

3. Synthetic Antigen or Sequences

When synthetic antigens or oligonucleotides are used, specify the amino acid sequence, nucleotide sequence, or carbohydrate composition, as applicable, along with any other critical structural specifications and criteria necessary to ensure quality. Synthetic antigens or oligonucleotides may be purchased. If so, specify the product identity, vendor and catalog number, country of origin, lot acceptance criteria, and any testing done on licensed premises.

Section III: Preparation of Standard Reagents

Test kit components are subject to the requirements and restrictions indicated in the following chart.

Component	Source	Same Lot for Entire Serial	Source Identified and/or Formulae in Outline	Submit Data Before Changing Production Method	Component Dating Cannot Exceed Dating of Kit Serial
Anti-species Antibody or Conjugate	Licensed Premises or Vendor	Required	Required	Required	Required
Agent Antigen or Antibody	Licensed Premises or Vendor	Required	Required	Required	Required
PCR Master Mix	Licensed Premises	Required	Required	Required	Required
Sample Diluent	Licensed Premises or Vendor	Not Required	Required	Required	Required
Controls	Licensed Premises or Vendor	Required	Required	Required	Required
Stop Solution	Licensed Premises or Vendor	Not Required	Required	Required	Not Required
Coating of Solid Phase	Licensed Premises	Required	Required	Required	Required

1. Controls

Describe the manufacture of all controls in the kit. The controls may be purchased. If so, specify the identity, vendor and catalog number, country of origin, lot acceptance criteria, and testing performed on licensed premises for each lot. The statement "accepted under a Certificate of Analysis" is not acceptable. Specify control acceptance criteria in this section.

2. Conjugate

The conjugate is any reagent used to amplify or report an antigen-antibody reaction. This category includes anti-species antibody, immunoglobulin binding proteins (such

as A, G, or L; colloidal gold; biotin), or enzyme-labeled versions of any of these. Conjugate does not have to be prepared on licensed premises, but acceptance criteria for each lot must be specified. If conjugate is prepared on licensed premises, describe the production process.

3. Substrate

The substrate is the substance that undergoes a color change or other detectable reaction when catalyzed by an enzyme-labeled kit component. It may be purchased. If so, specify the identity, vendor and catalog number, acceptance criteria, and testing performed on each lot. If substrate is prepared on licensed premises, describe the production process.

4. Buffers, Diluents, or Other Reagents Included in the Kit

Specify all buffers, diluents, and other reagents included in the kit. These may be purchased or manufactured on licensed premises. If manufactured on licensed premises, describe the preparation and acceptance criteria. Describe the source and formula of each buffer, diluent, or other reagent in the kit. If purchased, specify the vendor, catalog number, and acceptance criteria for each lot. Specify methods used to stabilize these liquids against bacterial contamination, including the maximum time between manufacture and stabilization.

Section IV: Preparation of the Product

1. Preservatives

List the chemical name (e.g., sodium azide) or the full Trade Name (e.g., Proclin™ 950) of each preservative used in kit manufacture. If purchased, specify the vendor, catalog number, and acceptance criteria for each lot. If preservatives are formulated on licensed premises, describe the formulation.

2. Solid Phase

When coated solid-phase components (e.g., immunoassay plates, beads, or membranes) are prepared, identify the solid-phase component type and the coating process. Each lot of coated solid-phase component should be prepared with a single lot of coating reagent and a single lot of solid-phase substrate. Include the formula for reagents used to prepare the solid-phase components.

3. Fill volume

List the minimum and maximum fill volumes for each final container to ensure there is enough of the component to adequately perform the test(s).

4. Disposal

Describe the method(s) used to dispose of unsatisfactory material. Alternatively, the Outline may refer to VS Memorandum No. 800.56.

Section V: Testing for Serial Release

1. Test kits are exempt from the sterility and purity tests described in 9 CFR 113.26, 113.27, and 113.28, and from animal safety tests. Specify the exemptions in the Outline. Include the statement, "Test kits are exempt from purity tests," to section V.A. Include the statement, "Test kits are exempt from animal safety tests," to section V.B.

2. See VS Memorandum No. 800.73 for guidance on the reports and data needed for CVB approval of potency tests. Potency is typically evaluated using a panel of well-characterized samples approved by CVB. Include the identifier for each panel member, along with criteria for valid and satisfactory tests. Include storage conditions. Include plate diagrams where applicable. Include the assay protocol.

   As a minimum, include panel members that serve as examples of:

   - Samples from negative/uninfected animals.
   - Samples from strongly positive animals.
   - Samples from weakly positive animals.

Section VI: Post-preparatory Steps

1. List the number of final component containers in each kit box. Multiple presentations, with various quantities of kit components, are acceptable.

2. Describe the collection of samples from each serial to be retained and submitted to CVB. See 9 CFR 113.3(b)(7), 113.3(e)(1) and VS Memorandum No. 800.59. Specify conditions for storage and shipment of the kits, in accordance with 9 CFR 114.11.

3. Each lot of each component in the kit shall be assigned an expiration date based on the stability of the individual component. The expiration date may be indicated on the component label. The expiration date of the serial shall be calculated from the date of initiation of the first potency test, but shall not exceed the expiration date of any of the components. The dating for test kits shall not exceed 12 months unless real-time stability data to justify a longer interval have been approved by CVB. Indicate the date CVB approved the real-time data confirming the dating period.

4. Information in section VI.D should be consistent with the user instructions

provided in regulated labeling. Include all recommendations, qualifications, limitations, and interpretations. Describe the acceptable qualities and the potential impact for a test sample to be suitable for analysis in the test kit. All potentially infective material must be appropriately labeled. Include chemical safety and disposal instructions.

5. Section VI.E: Site of Manufacture. See appendix 1, section VI.E for additional information.