DIAGNOSTIC TEST KITS

Introduction

The Virus-Serum-Toxin Act provides for the regulation of diagnostic test kits, but there are few regulations currently in the 9 CFR (except an Outline guide in 9 CFR 114.9) to address them. Detailed guidance is provided in Veterinary Services Memorandum 800.73. This chapter serves to explain the content of the memo and 9 CFR 114.9.

The CVB regulates complete kits (i.e., all critical reagents needed for testing, packaged together with instructions for use and interpretation of results). The CVB does not regulate individual reagents. Very few foreign countries regulate kits. Test kits must claim to detect or diagnose the existence of, or susceptibility to, potential disease causing agents in animals. Test kits which do not claim to detect or diagnose an infectious disease (e.g. bacterial typing or metabolic disease kits) are not regulated by the CVB. Tests developed and used in-house by contract testing services are not endorsed or regulated by the CVB.

Reviewing Required Studies

Diagnostic Sensitivity/Specificity (Se/Sp) Studies:
Similar to efficacy studies, Se/Sp studies should be conducted using one serial, following the proposed assay procedure described in the product insert and Section V of the Outline of Production. Se/Sp studies should be conducted for each sample type. For test kits in which the results are determined to be positive or negative based on a visual read, each test should be read independently by multiple individuals who are blinded as to the status of the samples. Samples used in the Se/Sp must be evaluated against an acceptable "gold standard" assay (See Appendices 1 and 2). Generally, the "gold standard" assay should not be another licensed test kit. There is no set number of positive and negative samples needed to evaluate the Se/Sp study. The sample size should be as large as possible but will depend upon the disease and availability of samples [redacted] The samples should be of U.S. origin, obtained from a variety of geographical locations, and should include all known relevant serotypes or genotypes. The CVB will not allow the use of experimentally infected animals to establish sensitivity and specificity, alone. Such data may be used as a subset but may not be used as a sole source for a particular agent or serovar. A range of reactivities, including weak and strong positives, should be covered. The samples should be obtained, prepared, and stored in a manner similar to that used in the field. The test kit results are evaluated against the "gold standard" results in a 2x2 table:

	Gold STD Positive	**Gold STD Negative**
Test Kit Positive	True Positive (TP)	False Positive (FP)
Test Kit Negative	False Negative (FN)	True Negative (TN)

The sensitivity percentage is calculated: 100 * TP/(TP+FN)
The specificity percentage is calculated: 100 * TN/(FP+TN)

[redacted] Test kits should not be licensed with quantitative label claims.

Reproducibility/Repeatability/Suitability Studies:
These studies are used to evaluate the test kit under field conditions using cooperating laboratories. The cooperating laboratories should be representative of the intended end-user of the test kit after licensure. The laboratories should be located in different relevant geographical locations or should routinely obtain samples from such regions. These studies should be conducted under 9 CFR 103.3 authorization [redacted]

The manufacturer/permittee will send a blinded panel of positive and negative samples (typically ~20 per analyte) to at least three cooperative laboratories. The panel should consist of known weak, moderate, and strong positive samples and negative samples, and be composed of different sample types, if applicable. Some of the panel members (especially weak positives) should be provided as blinded duplicates or triplicates to test for within-test run repeatability. The cooperative laboratory should be asked to repeat the panel testing on separate days (repeatability). The results from each laboratory are evaluated to determine the precision between laboratories (reproducibility). In addition, each cooperative laboratory may be asked to test well-characterized samples taken from their own inventory and evaluate the clarity of procedural instructions in the product insert (suitability).

Ruggedness Studies:
Manufacturers should submit data demonstrating the ruggedness of their test kit. Ruggedness (sometimes called robustness) measures the capacity of the assay to remain unaffected by deliberate small variations in method parameters. The tested variations should reflect situations likely to be encountered in the field. Possible examples are failure to let reagents come to recommended room temperature before use (i.e., compare results using refrigerated vs. room temperature reagents) or failure to stop an incubation period in the recommended time frame (i.e., compare results with incubation as recommended vs. incubation as recommended + 1 hour).

Outline of Production

See the Outline of Production chapter of this manual and VS Memorandum 800.73 for general guidance on reviewing the Outline of Production for kits. The Outline template for diagnostic test kits is found in 9 CFR 114.9(f). There are seven sections within the Outline of Production for a diagnostic kit:

Introduction: This section contains the principle of the test, recommended test samples, reagents included in the kit, products obtained via split manufacture, and the test interpretations and limitations. The split manufacture section (subsection 5) should include only licensed FFM biologics. Most of the information in this section and Section VI.D will be included in the product Circular Label.

Sections I and II. Antibody Components and Antigen Preparation:
These sections should include information on the preparation of antibody, antigen, and peptide components used for capture, detection, and for the positive and negative controls. For PCR test kits, information regarding the PCR primers should be included in Section II, as should information regarding nucleic acid positive and negative controls. Firms are permitted to use purchased antibody, antigen, or peptide reagents or PCR primers in the manufacture of their kit if properly identified with the source and catalogue number. Purchased reagents are not required to be obtained under an FFM license. The firm's criteria for accepting the reagent into their facility should be listed. The production processes used by the reagent supplier should not be included in the Outline of Production.

If firm produces specific antibody (polyclonal or monoclonal) or antigens, they must follow the Master Seed and Master Cell concept and describe the production process. If synthetic peptides or nucleic acids are used, the firm must follow the Master Sequence concept. The target specificity of antibodies (e.g., recognized epitope, protein, or glycoprotein) should be stated. If a monoclonal antibody is prepared, the Outline of Production should not contain the production steps that were used to produce the hybridoma.

Section III. Preparation of Standard Reagents:
This section lists the preparation of the positive and negative controls, conjugate, substrate, inert buffers, diluents, and other reagents directly packaged as kit components. Buffers and diluents used in the manufacture of kit components (e.g., coating buffer used to prepare coated plates) should be listed in the appropriate Section (I, II, or IV) where they are utilized.

Section IV. Preparation of the Product:
The template for Section IV, as listed in 9 CFR 114.9, is a bit confusing. Section IV.A lists the kit preservatives. Section IV.B describes the filling, plating, or attachment of antibody or antigen to the solid phase . Section IV.C describes the filling method and minimum and maximum fill volumes of the kit components. Section IV.D describe the handling of unsatisfactory materials.

Section V. Testing:
See VS Memorandum 800.73, Section VII.F, for guidance on serial release testing for kits.

Section V.C should include a stepwise description of the assay procedure for performing the potency test; in most cases, this is the same procedure included in the product insert. It also should include a sample-by-sample listing of the approved potency test panel and lot numbers, along with acceptable performance values. [redacted]

It is helpful to list in this section the CVB approval date(s) for pivotal sensitivity/specificity studies which determined test kit cut-off values.

Section VI. Postpreparatory Steps:
Section VI.A should list the number of component containers and plates or test devices contained in the finished box. A firm may have multiple package sizes for a finished product, and must list all configurations. In Section VI.B, firms should select two kits as samples but are only required to submit one to the CVB prior to serial release, as stated in 9 CFR 113.3(b)(7). If the CVB does not request the remaining selected sample, it may be returned to product inventory upon serial release. Partial kits may be submitted if full kits include more testing capacity than needed for confirmatory testing; in accordance with 9 CFR 113.3(b)(7), the quantity of each kit component submitted must be specified. Retention samples are selected in accordance with 9 CFR 113.3(e)(1).

If the storage temperature of the test kit differs from the specifications in 9 CFR 114.11, the storage and shipping temperatures must be specified. Some kits components may be stored at different temperatures, in which case the storage temperature for each component should be specified. The temperature maintained at the firm may differ from the procedure used during transit to the end user. If so, specify the shipping temperature range. If an exemption to the 9 CFR was granted, the date of the exemption should be given.

Section VI.D, like the analogous section in the Outline for a vaccine, should include all pertinent information approved for inclusion on labeling. Some firms may elect to include label text verbatim in this section. Some of the information required in this section is redundant with the Introduction,; it is permissible to cite the Introduction rather than retype the text in this section.

Components Within a Serial

Upon designation of a serial, all kits must contain the same manufactured lot of critical components. Generally, solid phase component (plates, membranes), antibodies or antigens, conjugates, and PCR master mixes are considered critical components, as are controls used in the

calculation of cutoff values. Wash buffers and diluents are not critical components. Within a serial, there may be several production lots of noncritical components.

Label Claims

As with any biologic, all label claims must be specific and supported by data filed with the CVB. Kits typically have claims to detect antigens or monitor serological status. For diagnostic test kits, labeling should specify:

1. Species of animal: this must be specific (e.g., cattle), not general categories (e.g., ruminants)

2. Specific sample type: e.g., whole blood, serum, or plasma; retropharyngeal lymph node or popliteal lymph node.

3. If the kit claims to differentiate vaccinated and infected animals, the specific vaccines may be stated if apropriate.

4. If the kit is specifically approved as a screening test or as a confirmatory test, a clear indication statement should be included.

5. Other specifications may be warranted, depending on the data submitted and the intended use. For example, it may be prudent to specify the time period after vaccination during which the kit is adequately sensitive.

6. The use of symbols on labels are permitted under CVB policy with certain considerations. A list of symbols approved for use on APHIS regulated veterinary biologics labeling may be found at:
https://www.aphis.usda.gov/animal_health/vet_biologics/publications/ISOsymbolsLabels.pdf
7.

Restrictions

If you are working with a kit for an exotic disease or a disease which is part of a declared or undeclared APHIS eradication and control program, it is likely the license will be restricted to avoid potential misuse. Such products may be restricted to use under the supervision of APHIS or State officials.

Appendix 1

Gold Standard Assays to Determine the Diagnostic Sensitivity and Specificity of Antigen Test Kits

Agent	**Gold Standard**
Avian Influenza Virus	Virus Isolation
Avian Leukosis Virus	COFAL
Bovine Virus Diarrhea	Virus Isolation/ rtPCR
Canine Parvovirus	HA/HI
Classical Swine Fever	Virus Isolation
Feline Immunodeficiency Virus	Virus Isolation
Foot-and-Mouth Disease Virus	Virus Isolation
Giardia lamblia	fecal wet mount
Infectious Bovine Rhinotracheitis Virus	Virus Isolation
Parvovirus (canine)	Virus Isolation / fecal hemagglutination
Heartworm (canine or feline)	necropsy worm count, with breakdown into the number of male and female worms (acceptable to compare to a licensed kit for negative samples, to avoid sacrificing healthy animals)
Mycobacterium avium spp. paratuberculosis	fecal culture
Transmissible Spongiform Encephalopathies	immunohistochemistry on obex

Appendix 2

Gold Standard Assays to Determine the Diagnostic Sensitivity and Specificity of Antibody Test Kits

Antibody specific for	**Gold Standard**		**Antibody specific for**	**Gold Standard**
Anaplasma phagocytophilum	IFA, samples from the Northeast and upper Midwest		Foot-and-Mouth Disease	SN
Anaplasma platys	IFA, samples from the mid-South and Southwest		Infectious Bovine Rhinotracheitis	SN
Avian Encephalomyelitis	SN		Infectious Bronchitis	SN
Avian Influenza	SN/HI		Infectious Bursal Disease	SN
Avian Reovirus	AGID		Infectious Laryngotracheitis	SN
Avian Rhinotracheitis	SN		*Leptospira canicola, L. grippotyphosa, L. icterohaemorrhagiae, L. pomona* (combined)	MAT
Babesiosis	CF		Tuberculosis (*Mycobacterium bovis*)	caudal fold test/agent isolation
Bluetongue	CF		Johne's Disease (*Mycobacterium avium* spp. *paratuberculosis*)	fecal culture
Bovine Leukemia	SN		*Mycoplasma gallisepticum*	Agglutination
Borrelia burgdorferi	IFA		*Mycoplasma meleagridis*	Agglutination
Caprine Arthritis-Encephalitis	AGID		*Mycoplasma synoviae*	Agglutination
Canine *Leptospira*	micro-agglutination		*Neospora caninum*	SN
Chicken Anemia Virus	SN/IFA		Newcastle Disease Virus	HI
Egg Drop Syndrome	HI		*Ornithobacterium rhinotracheale*	ELISA
Ehrlichia canis	IFA		Porcine Reproductive & Respiratory Syndrome	SN / immunoperoxidase monolayer assay
Ehrlichia ewingii	species-specific ELISA assay [redacted]		Pseudorabies	SN
Epizootic Hemorrhagic Disease	SN		Swine influenza	SN
Equine Infectious Anemia	AGID			
Feline Infectious Peritonitis	SN			
Feline Immunodeficiency	SN			

Antibody specific for	Gold Standard		Antibody specific for	Gold Standard
Virus				

SN=serum neutralization; HI=hemagglutination inhibition; AGID=agar gel immunodiffusion; IFA=indirect fluorescent antibody; ELISA=enzyme-linked immunosorbent assay; MAT=microscopic agglutination test