



Veterinary Diagnostics: Concept to Market

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Authorities, Regulations & Guidance



- Virus-Serum-Toxin Act
 - Authorizes Sec of Agriculture to regulate manufacture and distribution of biologics (assays, test kits) intended for use in diagnosis of disease (not physiologic status) in animals.
- 9 Code of Federal Regulations 101-118
- VS Memorandum 800.73

Regulated Test Kits

- Complete diagnostic test kit contains
 - All unique reagents required to run test
 - Instructions for running the test
 - Instructions for interpretation of test results
 - Claims, uses, and limitations
- Used to diagnose existence of susceptibility to potential disease-causing agents
- Reagents alone are not regulated

Licensing Requirements



- To qualify for licensure, diagnostic test kits should with reasonable certainty yield the results intended when used according to label/insert instructions
- The architecture/design, recommendations, claims, target disease, target animal, sample source, and intended uses determine specific requirements

Kit Components

- Master Seeds, Cells or Sequences
- Ingredients of Animal Origin
- Agent Antigen or Antibody
- Anti-Species Antibody or Conjugate
- Coated Solid-Phase Components
- Substrates
- Buffer(s)

Pre-license Assessment of Kits

- Accuracy and precision
- Diagnostic sensitivity
 - Dynamic range
 - Analytical sensitivity
- Diagnostic specificity
- Ruggedness, repeatability, suitability
- Predictive values for specific disease prevalence (espec. for Program Diseases)

Pre-license Validation

- Test large number of known positive and known negative samples covering a range of reactivities and all sample types
- Determine onset of detection of disease
- Evaluate - in at least 3 laboratory settings
 - Serum panel (ruggedness)
 - Well-characterized patient samples
 - Against standard assay
 - Suitability of test kit
 - Adequacy of instructions
- Authorization 9CFR 103.3

License Requirements

- Must be prepared in licensed facilities following an approved Outline of Production using approved components
 - Antigen(s) must be prepared from approved Master Seed(s) or each lot must be approved
 - Antibodies must be prepared from approved Master Seed(s) – monoclonal or well-characterized polyclonal
 - Conjugates may be purchased from approved vendor
 - All ingredients must be free from contamination

Key Licensing Requirements

- Sensitivity - probability of correctly identifying infected animals
 - Established minimum reactivity
- Specificity - probability of correctly identifying uninfected animals
 - Cross-reactivity identified
- Reproducibility
- Suitability/Ruggedness
 - Performed in 3 labs

Manufacturing Controls

- Aim is to minimize within serial and serial-to-serial variation by ensuring consistency of production
- Master Seed/Master Cell concept
- Validation of new lots of antibody/antisera
- Serial release testing with range of reactivities/cross-reactivities/near-neighbor
- Confirms ability of serial to achieve expected result when used appropriately

Intended Use(s) May Impact Licensing Requirements



- Herd/population status vs. individual animal tests
- Screening vs. Confirmatory Test
- Trade implications - clearance for export
- Vaccination or disease status vs. susceptibility to disease vs. exposure to agent/microbe

Intended Use(s) May Impact Licensing Requirements



- Matched diagnostic kits and companion vaccines
- State/Federal animal health control programs
 - May have different recommendations, uses, interpretations for different populations, species, and program needs
 - Use may be controlled by APHIS



Diagnostic Test Kit Licensing

- USDA controlled manufacturing of all components and kits
 - Minimal variation within and between serials
- USDA evaluation of test kit before and after licensure
- Ongoing monitoring of field performance and investigations



Diagnostic Test Kit Licensing

- Presentation credits:
 - Center for Veterinary Biologics
 - Veterinary Services
 - Animal and Plant Health Inspection Service
 - United States Department of Agriculture
 - Kansas Biosciences Authority
 - Kansas City Animal Health Corridor
- Questions? www.aphis.usda.gov/vs/cvb/

Thank you

Qualities of Diagnostic Test Kits

- Accuracy
- Precision: degree of scatter
- Ruggedness (robustness)
- Diagnostic Sensitivity
- Diagnostic Specificity
- Dynamic Range
- Analytical Sensitivity
- Analytical Specificity

Qualities of Diagnostic Test Kits



- Ruggedness (robustness)
 - Capacity to remain unaffected by small variations
- Diagnostic sensitivity
 - Ability to correctly identify samples from positive animals
- Diagnostic specificity
 - Ability to correctly identify samples from negative animals

Qualities of Diagnostic Test Kits



- Dynamic Range
 - Interval between upper and lower levels of analyte for which assay has suitable precision and accuracy
- Analytical Sensitivity
 - Lower limit of analyte detected with suitable precision and accuracy
- Analytical Specificity
 - Ability to distinguish target from nontarget in sample

Test	Infected Animal	Uninfected Animal
Positive	True Pos (TP)	False Pos (FP)
Negative	False Neg (FN)	True Neg (TN)

- Specificity: $TN / (FP + TN)$
- Sensitivity: $TP / (TP + FN)$
- Accuracy: $(TP + TN) / (TP + FP + FN + TN)$
- Prevalence: $(TP + FN) / (TP + FP + FN + TN)$
- Positive Predictive Value: $PV (+) = TP / (TP + FP)$
- Negative Predictive Value: $PV (-) = TN / (TN + FN)$

Other Components

- Ingredients of animal origin
 - 9CFR Parts 104, 113.50, 113.53 and 122
 - Sourced from countries with low or risk of BSI (NCIE and 9CFR 94.18)
- Agent antigen or antibody
 - Master Seed or each lot approved
 - Significant changes in strain/passage
 - May require sensitivity/specificity studies
 - Must be approved

Other Components

- Anti-species antibody or conjugate
 - Antibody; protein-A, -G, -L; biotin; etc
 - May be purchased (established criteria)
 - Single lot antibody or conjugate/serial
- Coated solid-phase components
 - Single lot of coating reagent/serial
- Substrates
 - May be purchased (established criteria)
 - Single lot/serial
- Buffers must be clean and stable

Factors Impacting Diagnostic Test Kit Licensing



- Difficulty in identifying true status of animals complicates assessment of kits
 - Johnes Disease
- Foreign animal disease issues
 - False positives high in population of 0 or very low prevalence
 - Positives should be confirmed by gold standard test prior to diagnosis/action

Factors Impacting Diagnostic Test Kit Licensing



- Use of kit in State/Federal animal health control programs
 - Disease prevalence
 - Screening vs. confirmatory
 - Sensitivity/specificity and stage of control program
 - APHIS diagnostic evaluation prior to use
 - National Veterinary Services Laboratories

Other Properties of Interest

- Accuracy
 - Correct identification (%) of disease status in individual animals
- Positive predictive value
 - Probability that a positive test result is correct
- Negative predictive value
 - Probability that a negative test result is correct

Serial Release Testing Based on Serum Panel



- APHIS provides for some of diseases
- Consist of multiple samples
 - Negatives
 - Weak positives (assure sensitivity)
 - Strong positives (assure ruggedness)
 - Cross-reactives (assure specificity)
- Confirms ability of serial to achieve expected result when used appropriately