CVB Regulatory Perspective - 3R Implementation & Example

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Regulatory Authority

Virus-Serum-Toxin Act

- Title 9 of the Code of Federal Regulation (9 CFR)
  - Section 113.5: General Testing-Product released only after testing to establish pure, safe, potent and efficacious veterinary biologic

- Veterinary Services Memorandum

- Center for Veterinary Biologics Notices

- Other Guidance Documents: Supplemental Assay Methods, Testing Protocols, Reagent Data Sheets

- Product Specific: Outline of Production
  - Section V (Final Product Testing Requirements)
Importance of Animal Welfare

❖ CVB supports implementation of 3RS in release testing.

❖ The goal of potency testing is to protect the animals in the field. All new assays must provide the same confidence as those currently approved in order to prevent their suffering.

C. septicum: Courtesy of the Department of Pathobiology, University of Guelph.

C. novyi: Courtesy of Dr. Henry Stämpfli.

https://www.merckvetmanual.com/generalized-conditions/clostridial-diseases/malignant-edema
Validation provides evidence the assay does what it is intended to do for assays.

- VSM 800.112 divides the validation process into several phases:
  - Conceptualization
  - Development
  - Optimization
  - Verification
  - Monitoring
Protocols and Reagents

❖ Supplemental Assay Methods (SAMs)
  • SAMs 624 – 627 posted on public website

❖ Monoclonal Antibodies
  • *L. canicola* = 4DB-001
  • *L. grippotyphosa* = LGF02-002
  • *L. icterohaemorrhagiae* = 294-004
  • *L. pomona* = 2D7

❖ Polyclonal Antibodies
Communication


- Vaccine manufacturers encouraged to initiate or continue product-specific validation with ELISAs
- The CVB encouraged to re-examine the necessity of back-titration animals in the hamster challenge assay
- International harmonization of alternative potency methods was recommended by all

❖ Focus workshops on *Leptospira* vaccine potency testing at AHI and AVBC meetings
Overview

- **Vaccinate**
- Wait 14 - 18 days
- **Challenge w/Leptospira**
- Observe 14 days
- Count survivors

Animal Numbers

<table>
<thead>
<tr>
<th>Vaccinates</th>
<th>Challenge Controls</th>
<th>Concurrent Challenge (LD50 Titration)</th>
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<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>20</td>
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</table>
Codified Potency Tests for Leptospira Bacterins

❖ Vaccine Disposition
  • Satisfactory: 80% Vaccinates Survive

❖ Validity Requirements
  • 80% Death in Challenge Controls
  • LD50:10 – 10,000
9 CFR Potency Tests for Leptospira

Overview

Vaccinate

Wait
14 - 18 days

Challenge w/Leptospira

Observe
14 days

Count survivors

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Grippotyphosa

### Experiment 1
- 37 °C Bead Bath
- 37 °C Water Bath
- Bench (20–25 °C)

### Experiment 2
- 37 °C Bead Bath
- 37 °C Water Bath
- Bench (20–25 °C)

- Bench (20–25 °C)
- Bench (20–25 °C)

#### Hamster Fractional Loss

- **Flash freeze**
- **Overnight**

#### Days

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Validate ELISAs for serial release

Published two notices exempting back-titration hamsters from the codified assays: Notice 15-13 and 17-06

Long-term maintenance of virulent challenge

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**Hamsters Per Doses Released**

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<th>Year</th>
<th>Hamsters per 100,000 Doses</th>
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National Centers for Animal Health, Ames, Iowa, USA