Opportunities and Strategies to Further Reduce Animal Use for Leptospira Vaccine Potency Testing

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Safeguarding Animal Health
Animal Use

- Regulatory Potency Testing
  - 9CFR 113.101, 113.102, 113.103, 113.104

- Culture Maintenance
Regulatory Testing

Ideal

- Replacement of hamster vaccination-challenge model with an *in vitro* test
  - USDA CVB ELISAs
  - CVB Notice 09-16
## Regulatory Potency Testing

<table>
<thead>
<tr>
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<th>9 CFR</th>
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<tbody>
<tr>
<td>Vaccinates</td>
<td>10</td>
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<tr>
<td>Challenge Controls</td>
<td>10</td>
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<tr>
<td>Back-titration</td>
<td>20</td>
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<tr>
<td>Total animals/serial (1 serial tested)</td>
<td>40</td>
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<tr>
<td>Total animals/serial (2 serial tested)</td>
<td>25</td>
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<tr>
<td>Total animals/serial (3 serial tested)</td>
<td>20</td>
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Challenge Maintenance

Ideal

- *In vitro* culture method
  - Virulent
  - Stable
  - Highly prolific
  - “Master Seed” Challenge
Challenge Maintenance

Reality

❖ Lots of variables affect virulence
  ❖ pH
  ❖ Osmolarity
  ❖ Temperature
  ❖ BSA Source
  ❖ Others

Current Protocols in Microbiology

USDA
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Challenge Maintenance

Strain specificity

- 250+ pathogenic serovars
  - Variations among leptospiral strains

Photo from The Leptospirosis Information Center
Challenge Maintenance

- *In vivo passage*
  - Continuous passage of virulent *Leptospira* through hamsters
  - Proposed: Intermittent passage of virulent *Leptospira* through hamsters
    - Virulence
    - Minimal time to initiate testing
    - Continuous supply of challenge strains
Challenge Maintenance

Current

- **1 day**: Remove from liquid N₂ and inoculate semi-solid medium
- **14 - 30+ days**: Growth in semi-solid medium
- **1 day**: Inoculate hamsters
- **~10 days**: Three passages in hamsters
- **1 day**: Challenge culture for *in vivo* potency testing
- **~30 days**: Completing *in vivo* potency testing
- **1 day**: Cryostorage in liquid N₂ (optional)

Proposed

- **1 day**: Remove from liquid N₂ and inoculate hamsters
- **~10 days**: Three passages in hamsters
- **1 day**: Challenge culture for *in vivo* potency testing
- **1 day**: Inoculation of shipping media for distribution
- **~30 days**: Completing *in vivo* potency testing
- **1 day**: Advanced Reagent identity verification (PFGE and MAT) & Culture Growth
- **~10 days**: Reagent identity (ELISA)
Culture Maintenance

Parameters
- Quality control of sample
- Speed of Freezing
- Cryopreservative
- Thaw → Inoculation

Evaluation
- Virulence after short-term and long-term storage
  - Three passages through hamsters
  - Challenge in 10 hamsters
Preliminary Study

Example

Infected Hamster Liver (i.e. L. canicola)

1% BSA diluent

- 2.5% glycerol
- 5% glycerol
- 7.5% glycerol

-70°C 2 hrs  -70°C 16 hrs

P-80 semi-solid diluent

- 2.5% glycerol
- 5% glycerol
- 7.5% glycerol

-70°C 2 hrs  -70°C 16 hrs
Culture Maintenance:

Preliminary Results

- Quality control of sample
  - Spirochete Counts

- Speed of Freezing
  - Short-freezes ≥ Gradual Freeze

- Cryopreservative
  - Glycerol > DMSO
  - < 5% Glycerol

- Thaw → Inoculation

Fluorescent antibody detection of *L. interrogans* serovar *pomona*. Photograph courtesy of Richard Hornsby
Summary

❖ Regulatory Potency Testing
  ➢ Ideal: ELISAs developed by CVB
  ➢ Reduced Animal Use
    ▪ Shared Controls
    ▪ Re-evaluating back-titrations

❖ Culture Maintenance
  ➢ Ideal: *In vitro* culture
    ▪ Not feasible at this time
  ➢ Reduced animal use: Intermittent passage
Thank you

- Geetha Srinivas
- Renee Olsen
- Dave Alt
- Mark Wilson

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Questions?