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

Angela Walker, DVM, PhD
Center for Veterinary Biologics
Veterinary Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa  USA
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>
<th></th>
<th>9 CFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>10</td>
</tr>
<tr>
<td>Challenge Controls</td>
<td>10</td>
</tr>
<tr>
<td>Back-titration</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total animals/serial</strong></td>
<td></td>
</tr>
<tr>
<td><em>(1 serial tested)</em></td>
<td>40</td>
</tr>
<tr>
<td><em>(2 serial tested)</em></td>
<td>25</td>
</tr>
<tr>
<td><em>(3 serial tested)</em></td>
<td>20</td>
</tr>
</tbody>
</table>
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
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
- Inoculation of shipping media for distribution

~30 days
- Completing in vivo potency testing
- Reagent identity verification & Culture Growth

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
- Inoculation of shipping media for distribution

~30 days
- Completing in vivo potency testing
- Advanced Reagent identity verification (PFGE and MAT) & Culture Growth
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

---

**USDA**

Safeguarding Animal Health

---

Veterinary Services
Center for Veterinary Biologics
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

USDA
Safeguarding Animal Health

Veterinary Services Center for Veterinary Biologics
Culture Maintenance:

Preliminary Results

- Quality control of sample
  - Spirochete Counts

- Speed of Freezing
  - Short-freeses $\geq$ Gradual Freeze

- Cryopreservative
  - Glycerol $>$ DMSO
  - $< 5\%$ Glycerol

- Thaw $\rightarrow$ 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