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>(1 serial tested)</td>
<td>40</td>
</tr>
<tr>
<td>(2 serial tested)</td>
<td>25</td>
</tr>
<tr>
<td>(3 serial tested)</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

Current Protocols in Microbiology

USDA
Safeguarding Animal Health
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 \( N_2 \) 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 \( N_2 \) (optional)

Proposed

1 day
Remove from liquid \( N_2 \) 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

Safeguarding Animal Health

USDA
Veterinary Services
Center for Veterinary Biologics
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
  -70°C 2 hrs
  -70°C 16 hrs

- 5% glycerol
  -70°C 2 hrs
  -70°C 16 hrs

- 7.5% glycerol
  -70°C 2 hrs
  -70°C 16 hrs

P-80 semi-solid diluent

- 2.5% glycerol
  -70°C 2 hrs
  -70°C 16 hrs

- 5% glycerol
  -70°C 2 hrs
  -70°C 16 hrs

- 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
Questions?