

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



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Animal Use



❖ Regulatory Potency Testing

- 9CFR 113.101, 113.102, 113.103, 113.104

❖ Culture Maintenance



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Regulatory Testing

Ideal

- ❖ Replacement of hamster vaccination-challenge model with an *in vitro* test
 - USDA CVB ELISAs
- ❖ CVB Notice 09-16



Regulatory Potency Testing

9 CFR	
Vaccinates	10
Challenge Controls	10
Back-titration	20
Total animals/serial (1 serial tested)	40
Total animals/serial (2 serial tested)	25
Total animals/serial (3 serial tested)	20



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Challenge Maintenance

Ideal

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

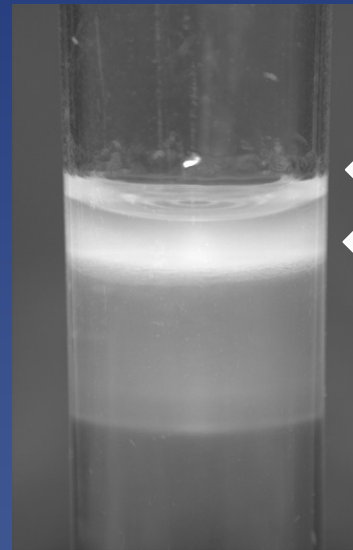


Challenge Maintenance

Reality

❖ Lots of variables affect virulence

- ❖ pH
- ❖ Osmolarity
- ❖ Temperature
- ❖ BSA Source
- ❖ Others



← Air – Medium Interface
← Dinger Zone

Current Protocols in
Microbiology

Challenge Maintenance

Strain specificity

- ❖ 250+ pathogenic serovars
 - Variations among leptospiral strains

Photo from The Leptospirosis
Information Center

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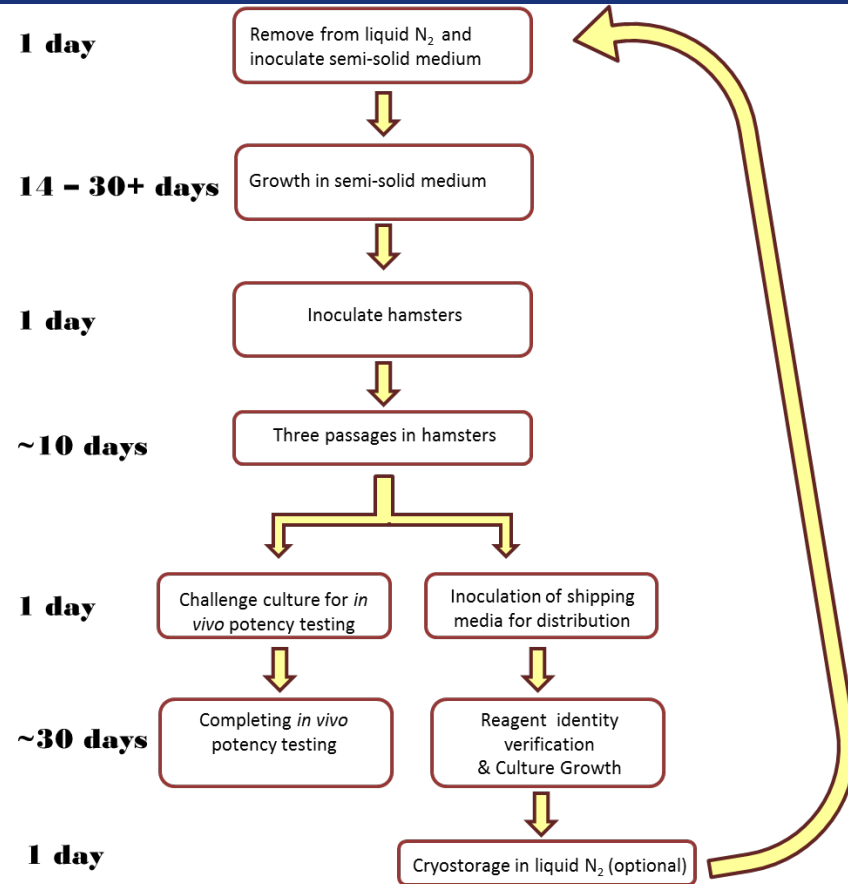
Challenge Maintenance

❖ *In vivo* passage

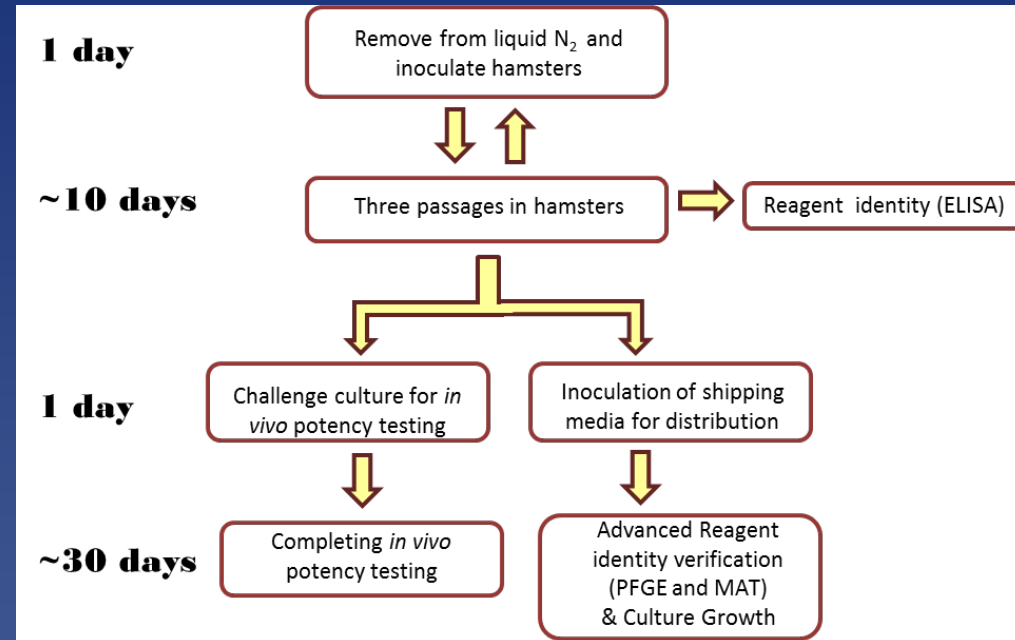
- Continuous passage of virulent *Leptospira* through hamsters
- Proposed: Intermittent passage of virulent *Leptospira* through hamsters
 - Virulence
 - Minimal time to initiate testing
 - Continuous supply of challenge strains

Challenge Maintenance

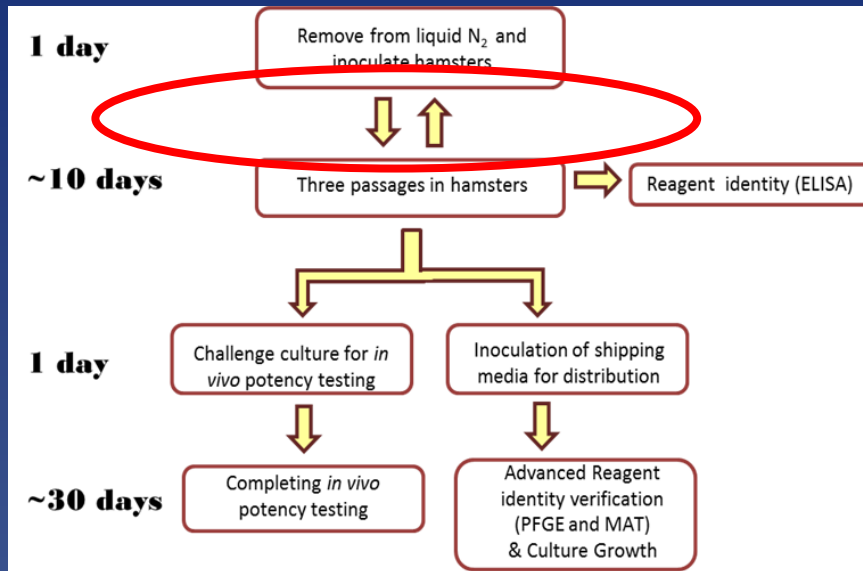
Current



Proposed



Culture Maintenance



Parameters

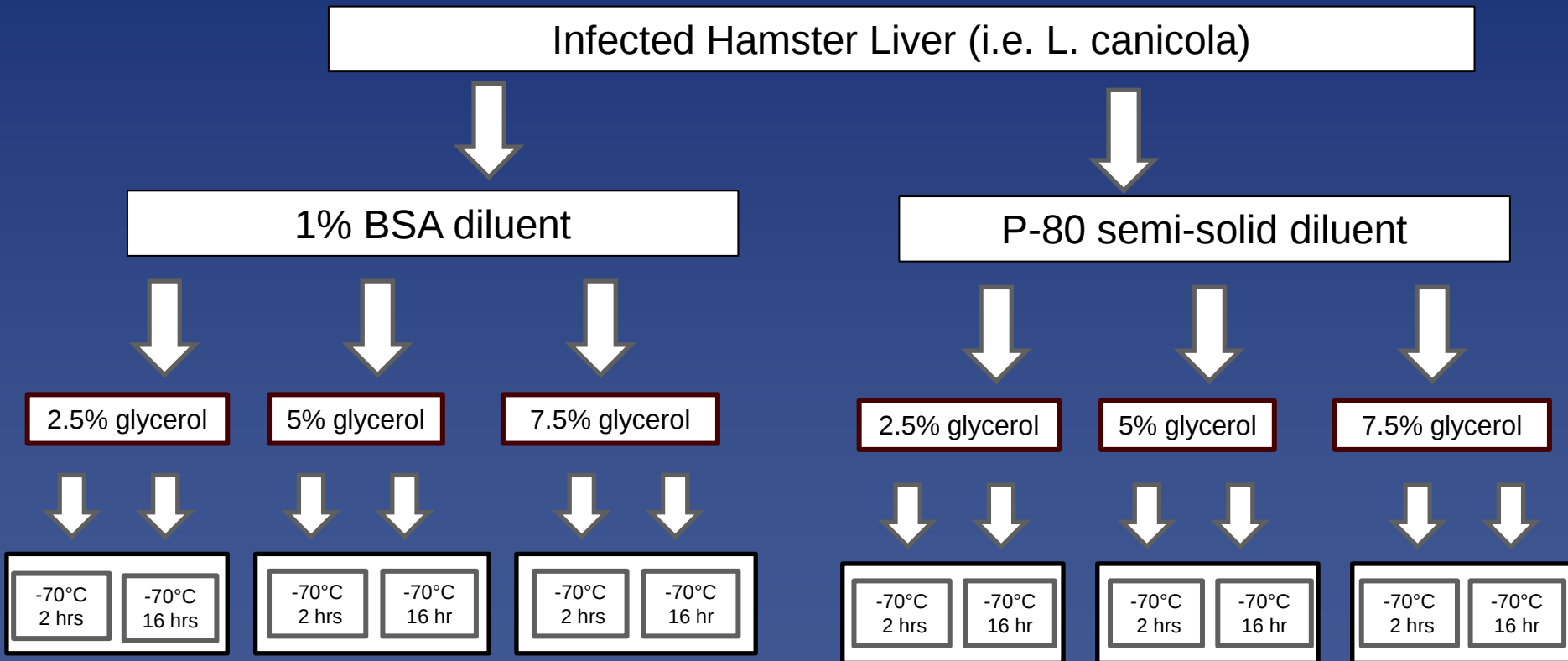
- ❖ Quality control of sample
- ❖ Speed of Freezing
- ❖ Cryopreservative
- ❖ Thaw → Inoculation

Evaluation

- ❖ Virulence after short-term and long-term storage
 - Three passages through hamsters
 - Challenge in 10 hamsters

Preliminary Study

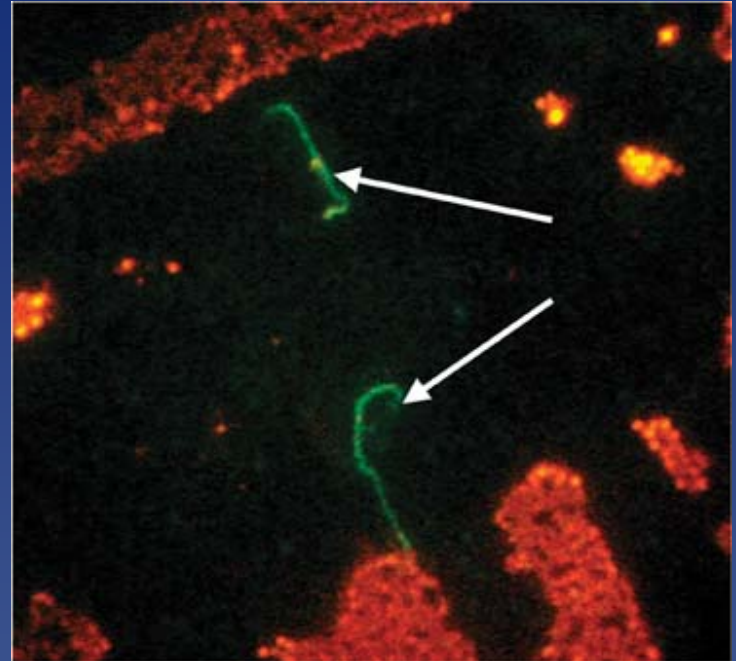
Example



Culture Maintenance:

Preliminary Results

- ❖ Quality control of sample
 - Spirochete Counts
- ❖ Speed of Freezing
 - Short-freezes $\geq$ 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



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Thank you

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



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Questions?



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