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

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# 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

| | 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 |

# Challenge Maintenance

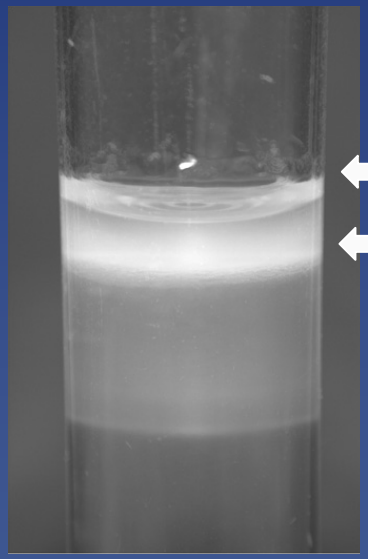
## Ideal

- *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

# Challenge Maintenance

## Strain specificity

- 250+ pathogenic serovars
  - Variations among leptospiral strains

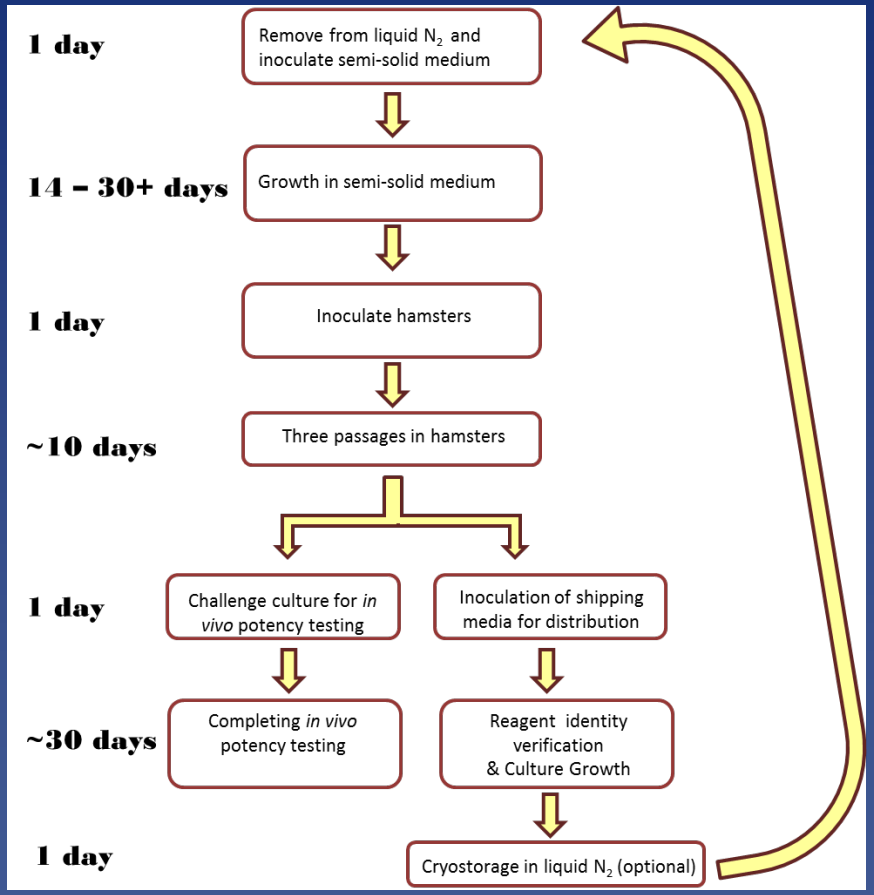
Photo from The Leptospirosis Information Center

# Challenge Maintenance

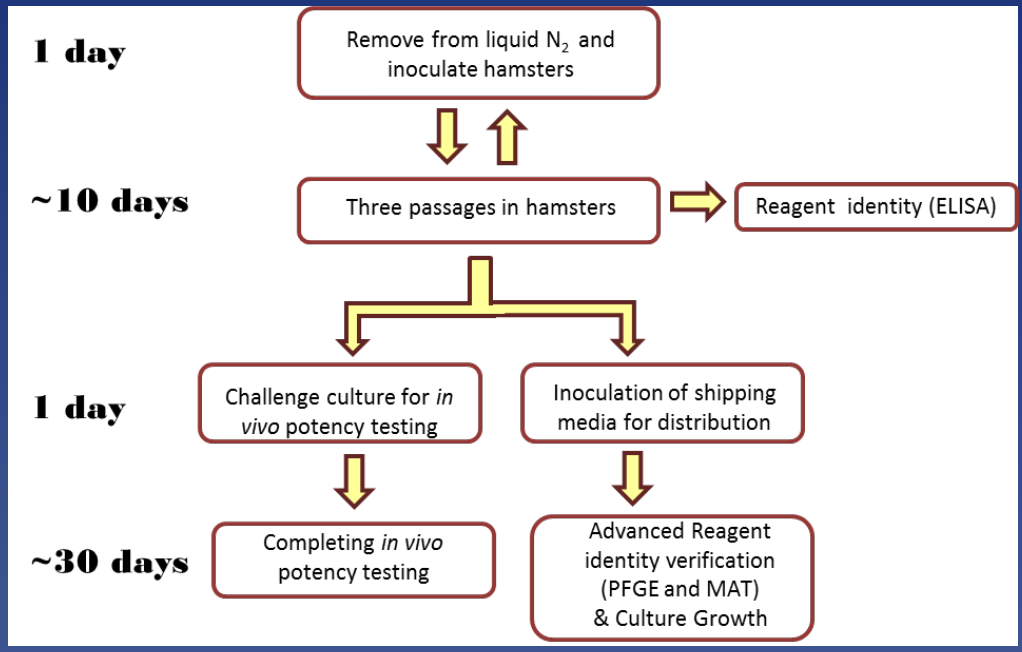
- *In vivo passage*
  - Continuous passage of virulent *Leptospira* through hamsters
  - Proposed: Intermittent passage of virulent *Leptospira* through hamsters
    - Virulence
    - Minimal time to initiate testing
    - Continuous supply of challenge strains

# Challenge Maintenance

## Current




## Proposed

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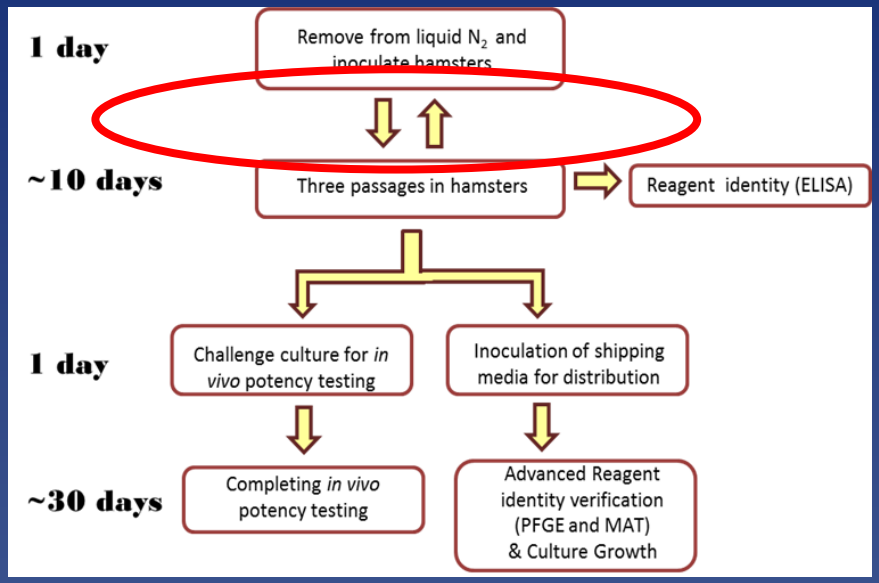
# Culture Maintenance




## Parameters

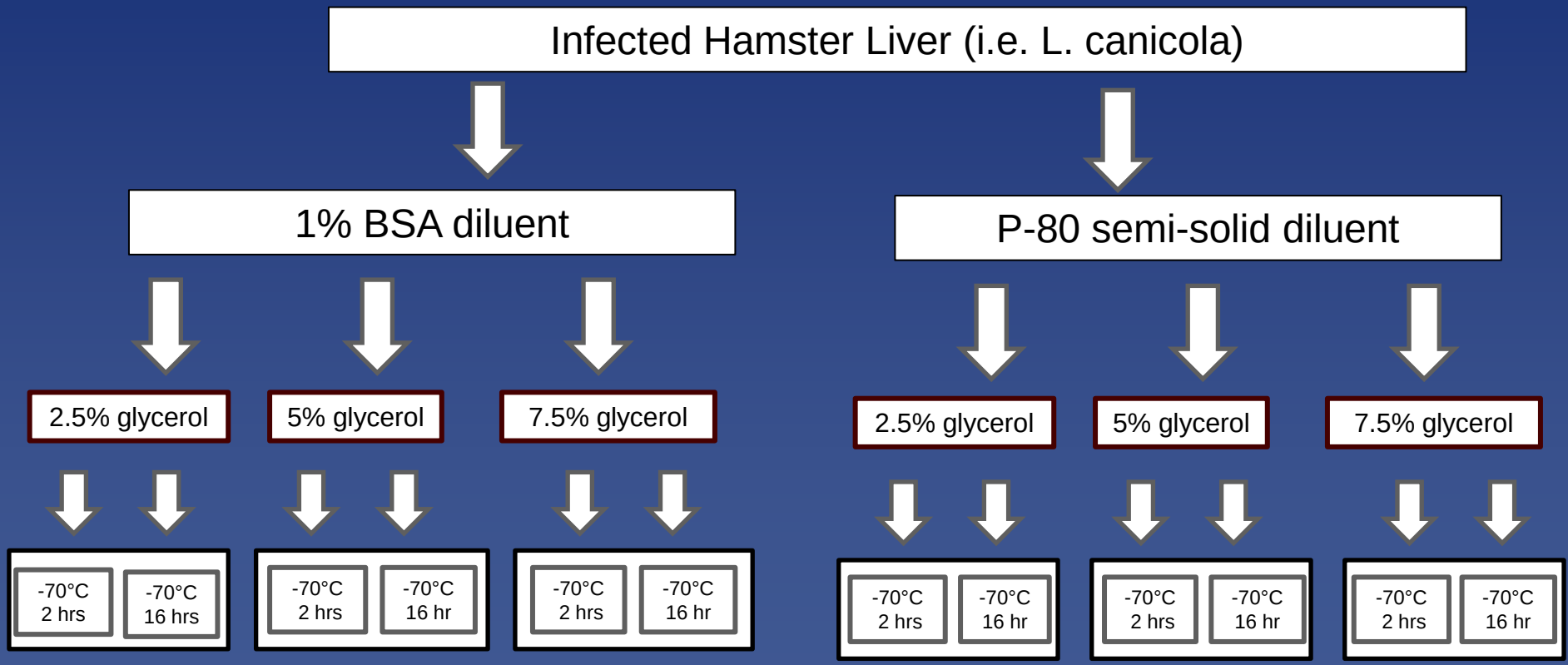
- Quality control of sample
- Speed of Freezing
- Cryopreservative
- Thaw → Inoculation

## Evaluation

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

# Preliminary Study

## Example

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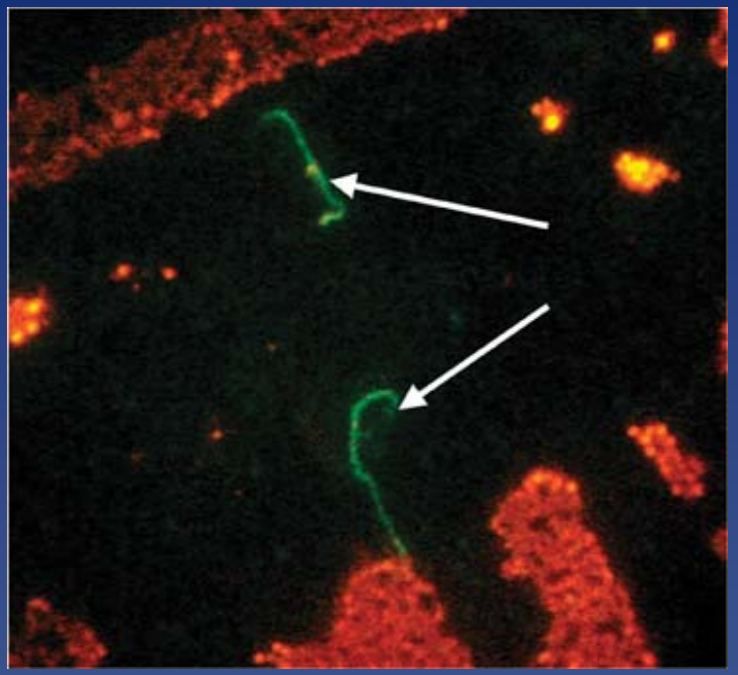
# Culture Maintenance:

## 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?