

# 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



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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

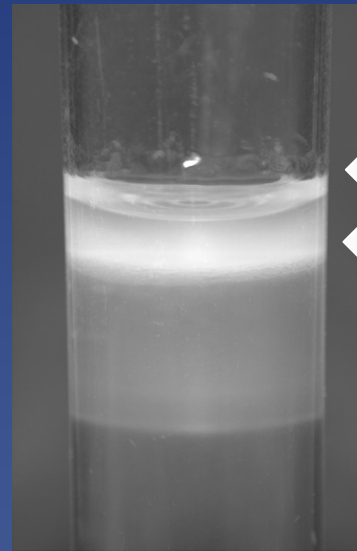
- ❖ *In vitro* culture method
  - Virulent
  - Stable
  - Highly prolific
  - “Master Seed” Challenge



# 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



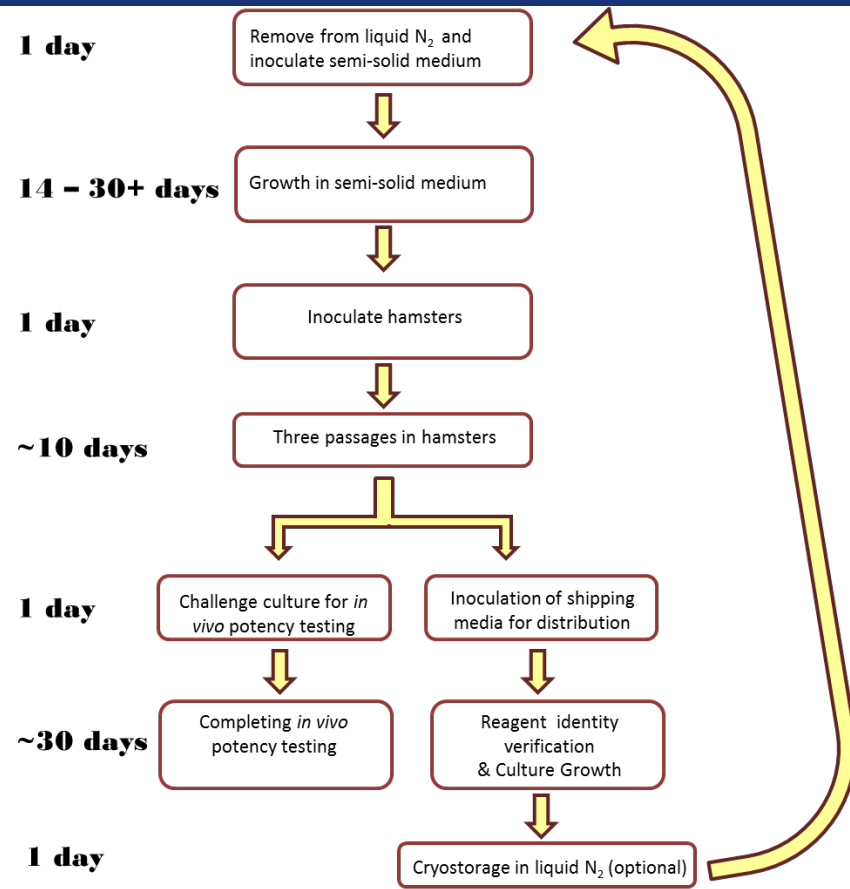
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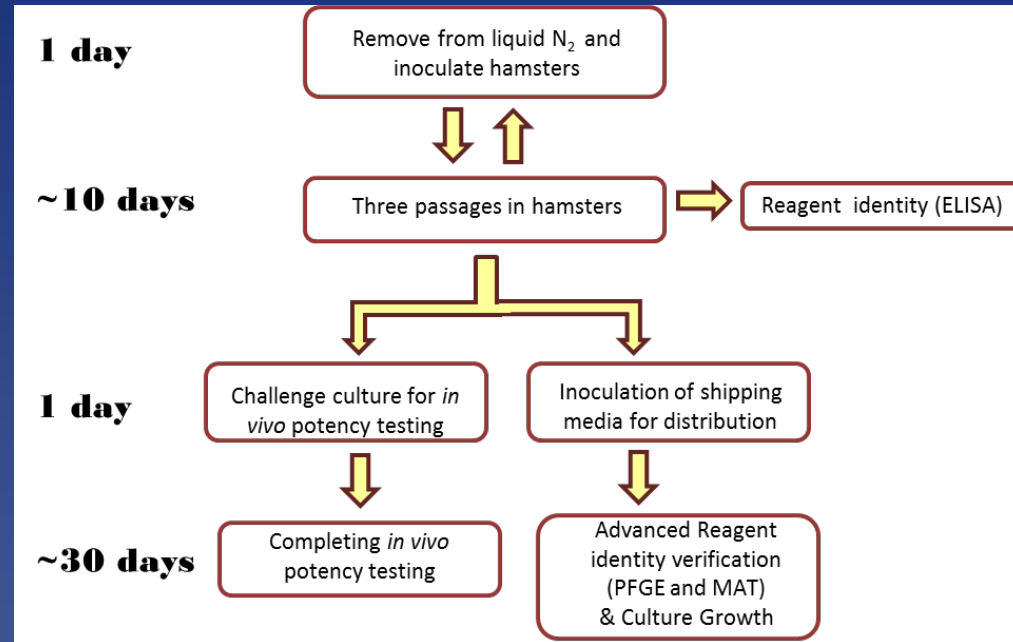


# Challenge Maintenance

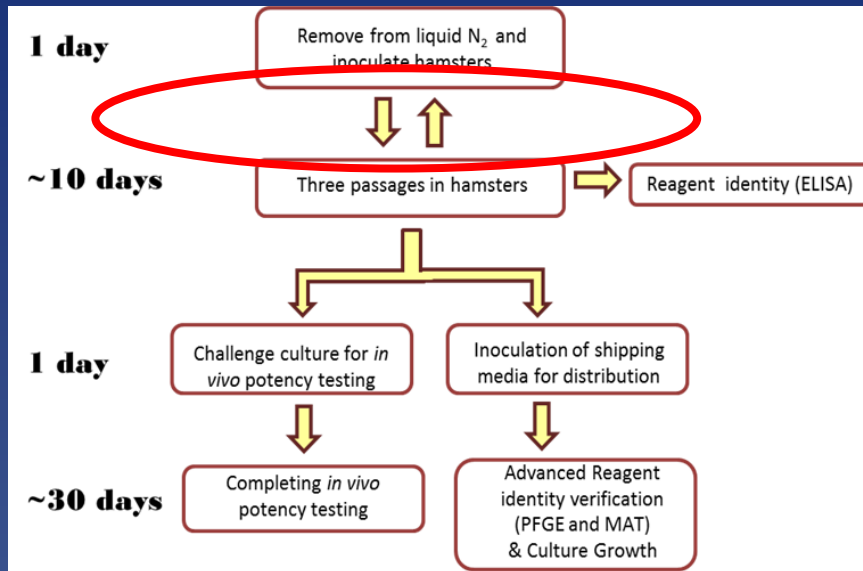
## Current



## Proposed



# Culture Maintenance



## Parameters

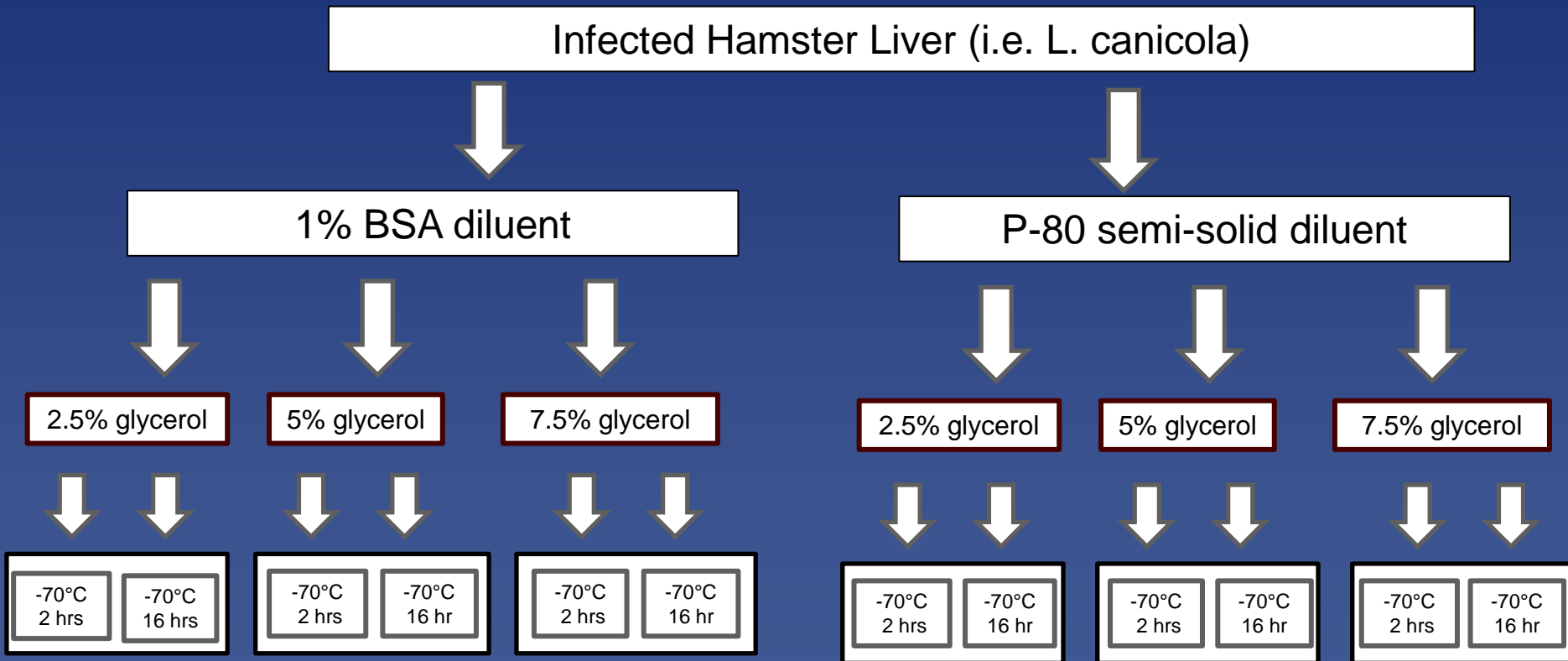
- ❖ Quality control of sample
- ❖ Speed of Freezing
- ❖ Cryopreservative
- ❖ Thaw → Inoculation

## Evaluation

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

# Preliminary Study

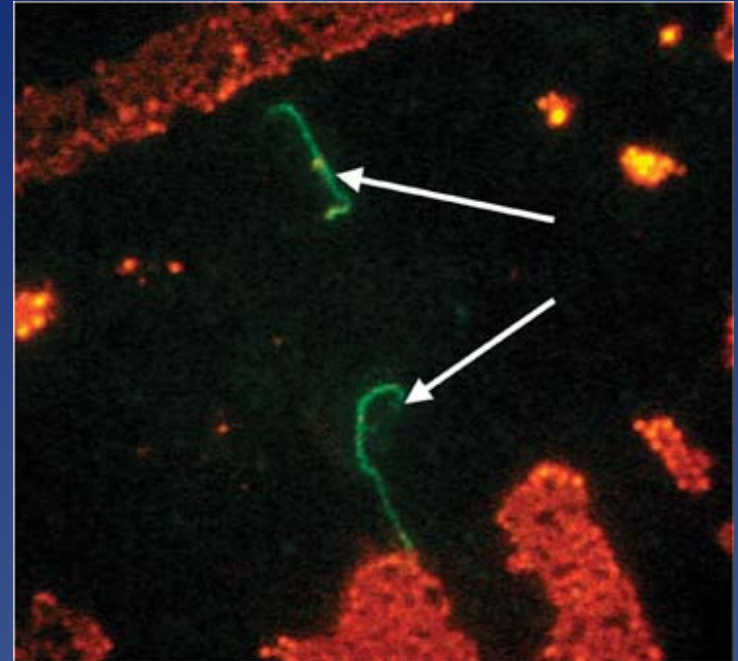
## Example



# 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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