



# Reduction in Animal Use in Veterinary *Leptospira* Vaccine Potency Testing through USDA Regulatory Changes

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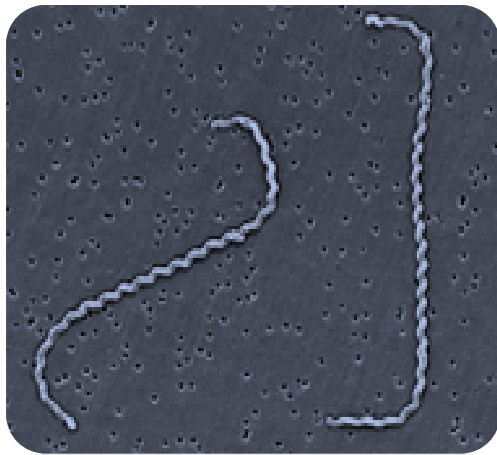
# Leptospirosis

## ❖ Health Risk

- Potential to infect all mammals including man



## ❖ Financial Impact in Agriculture



# Regulatory Oversight

## ❖ Potency Test

- Conducted on every serial (numbered lot) marketed in the United States

## ❖ Potency Test Options

- Codified Potency Assays
  - Hamster vaccination – challenge assays
  - 9 CFR 113.101 – 113.104: Canicola, Grippotyphosa, Icterohaemorrhagiae, and Pomona
- *In vitro* Assays



# 9 CFR Potency Tests for Leptospira Vaccines

## ❖ Overview



## ❖ Animal Numbers

Vaccinates	Challenge Controls	Concurrent Challenge (LD <sub>50</sub> Titration)
10	10	20

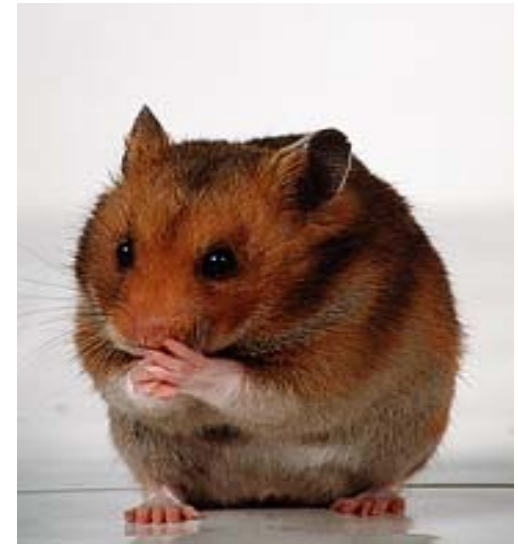
# Codified Potency Test for Leptospira Bacterins

## ❖ Vaccine Disposition

- Satisfactory: 80% Vaccinates Survive
- Second-stage test: 60 – 70% Vaccinates Survive

## ❖ Validity Requirements

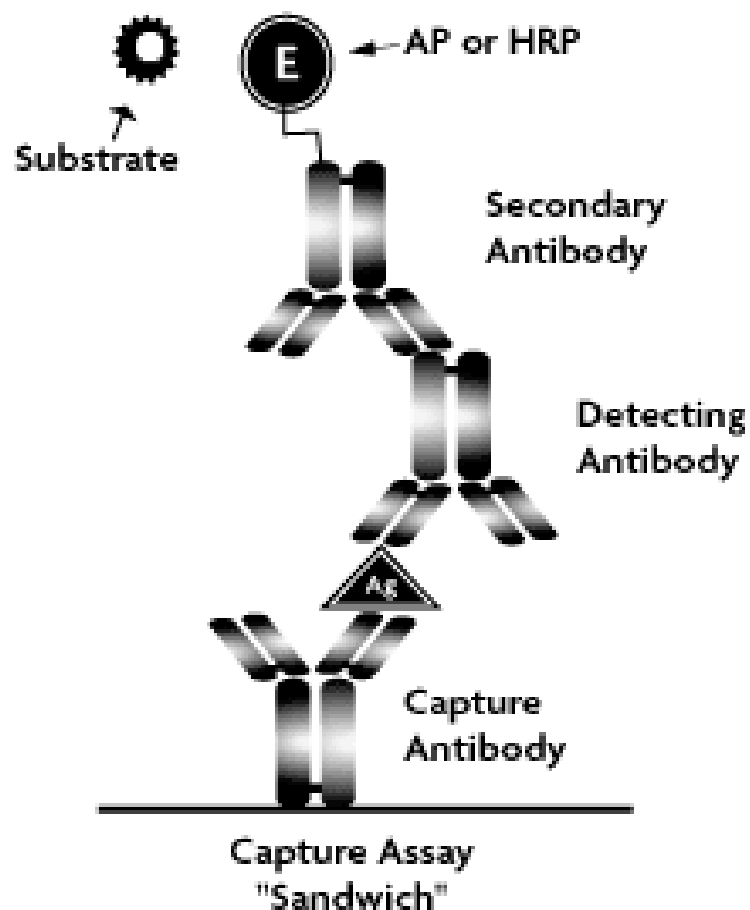
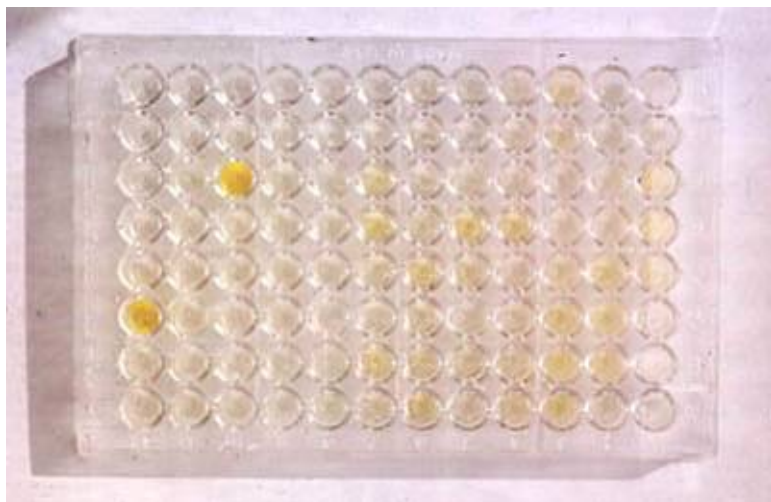
- 80% Death in Challenge Controls
- LD<sub>50</sub>: 10 – 10,000



# Disadvantages of Hamster Potency Assay

- ❖ Animal welfare
- ❖ Cost
- ❖ Time
- ❖ Labor
- ❖ Personnel exposure

# Enzyme-linked Immunosorbent Assay (ELISA)



# Advantages of the ELISA

## ❖ Animal welfare

## ❖ Cost

- ELISA: ~\$4.00 per test Codified assay: > \$1200 per test

## ❖ Time

- ELISA: ~2 days per test Codified assay: ~ 35 days per test

## ❖ Labor

- ELISA: Technician Codified assay: Technician & caretakers

## ❖ Personnel exposure



# Availability of Protocols and Reagents

## ❖ Supplemental Assay Methods (SAMs)

- SAMs 624 – 627 posted on public website

## ❖ Monoclonal Antibodies

- *L. canicola* = 4DB-001
- *L. grippotyphosa* = LGF02-002
- *L. icterohaemorrhagiae* = 294-004
- *L. pomona* = 2D7

## ❖ Polyclonal Antibodies

# The Reference Obstacle

- ❖ *In vitro* assay requires a reference standard
- ❖ Qualification of a reference requires host animal studies
  - Large study in pigs, dogs, and/or cattle
  - Stability monitoring over time



# The Reference Obstacle: Response

- ❖ National Reference Standards (NRSs)
- ❖ Correlated host response to hamster vaccination challenge assay for reference qualification and stability monitoring





# Guidance Documents

## ❖ CVB Notice No. 07-02

- Qualification of *Leptospira grippotyphosa* and *Leptospira icterohaemorrhagiae* Reference Bacterins for Products Intended for Use in Dogs

## ❖ CVB Notice No. 07-12

- Qualification of *Leptospira pomona* and *Leptospira canicola* Reference Bacterins for Products Intended for Use in Dogs

## ❖ CVB Notice No. 09-16

- Qualification of *Leptospira Canicola*, *Leptospira Grippotyphosa*, *Leptospira Icterohaemorrhagiae*, and *Leptospira Pomona* Reference Bacterins for Products Intended for Use in Swine and/or Cattle

## ❖ VSM 800.112:

- Guidelines for Validation of In Vitro Potency Assay

# Communication

- ❖ International Workshop on Alternative Methods for *Leptospira* Vaccine Potency Testing: State of the Science and the Way Forward
  - Vaccine manufacturers encouraged to initiate or continue product-specific validation with ELISAs
  - The CVB encouraged to re-examine the necessity of back-titration animals in the hamster challenge assay
  - International harmonization of alternative potency methods was recommended by all
  
- ❖ Focus workshops on *Leptospira* vaccine potency testing at AHI and AVBC meetings

# Other Reduction Options: Removing Back-titration Animals

- ❖ Specific products may be difficult to validate
  - Adjuvant
  - Serovar

Experimental group	Number of animals	<u>Categories D and E animals</u>	
		(%)	Avg number
Vaccinates	10	2.3	0.2
Challenge controls	10	93.4	9.3
Back-titration	20	61.4	12.3

# Other Reduction Options: Removing Back-titration Animals

- ❖ Plan: Examine the impact of over-challenge on subpotent serial disposition
- ❖ Studies underway at the CVB January 2013
  - 3 experiments per serogroup (4 serogroups)
  - Tentative Plan: Data collection completed by early 2014



# Conclusion

## ❖ ELISAs

## ❖ Reagents

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## ❖ CVB Notices and SAMs

- Biologics Regulations and Guidance Public Website:

[http://www.aphis.usda.gov/animal\\_health/vet\\_biologics/vb\\_regs\\_and\\_guidance.shtml](http://www.aphis.usda.gov/animal_health/vet_biologics/vb_regs_and_guidance.shtml)

## ❖ Communication and On-going Projects





United States Department of Agriculture

THANKS

Questions ???