

USDA Regulatory Guidelines and Practices for Veterinary *Leptospira* Vaccine Potency Testing

Geetha B. Srinivas, DVM, PhD
Section Leader, Bacteriology
Center for Veterinary Biologics
USDA/APHIS/VS



Safeguarding Animal Health



Potency Test for Leptospira

- ❖ USDA Codified Potency Test
- ❖ *In vitro* Assay
- ❖ Guidance Documents
- ❖ Reagents

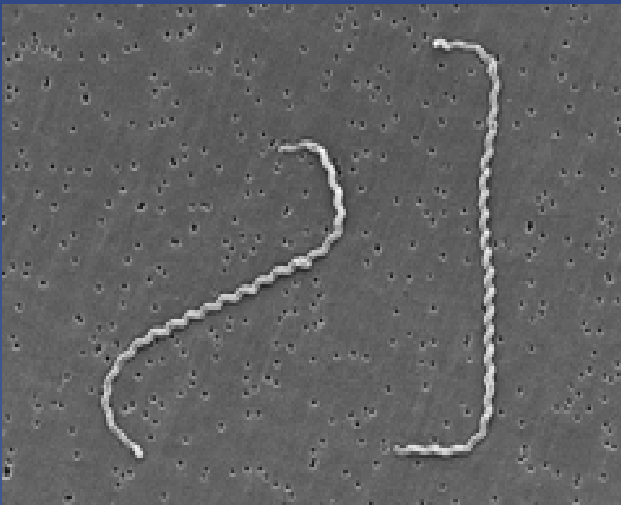


Safeguarding Animal Health



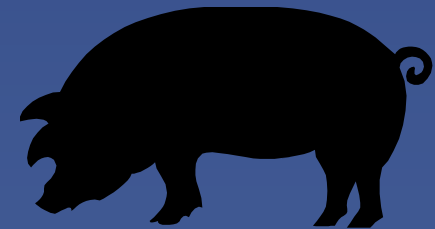
Leptospirosis

- ❖ Natural hosts - feral and domestic mammals
- ❖ Incidental host – humans



Leptospira Serovars

- ❖ L. pomona, canicola, grippotyphosa and icterohemorrhagiae
- ❖ L. hardjo
- ❖ L. bratislava
- ❖ Vaccines
- ❖ VSTA



Safeguarding Animal Health



Current 9CFR Potency Tests for Leptospira Bacterins

Serogroup	9CFR Potency Test	Number of Animals
L. pomona	Hamster vac- chal 113.101	10 Vaccinates 10 Controls 20 Back-titrations
L. ictero- haemorrhagiae	Hamster vac- chal 113.102	10 Vaccinates 10 Controls 20 Back-titrations
L. canicola	Hamster vac- chal 113.103	10 Vaccinates 10 Controls 20 Back-titrations
L. grippotyphosa	Hamster vac- chal 113.104	10 Vaccinates 10 Controls 20 Back-titrations

Codified Potency Test for Leptospira Bacterins

❖ Hamster vaccination-challenge assay (9CFR)

- Vaccination
- Virulent challenge
- Observation
- Validity criteria



Disadvantages of Hamster Potency Assay

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



Safeguarding Animal Health



Leptospira Project Goals

❖ 3R

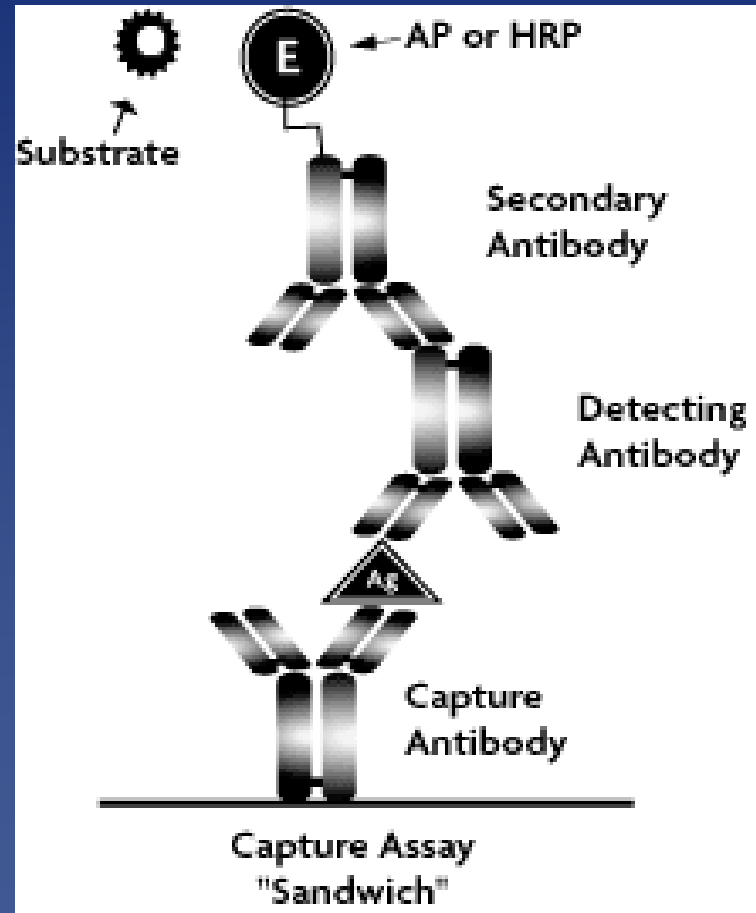
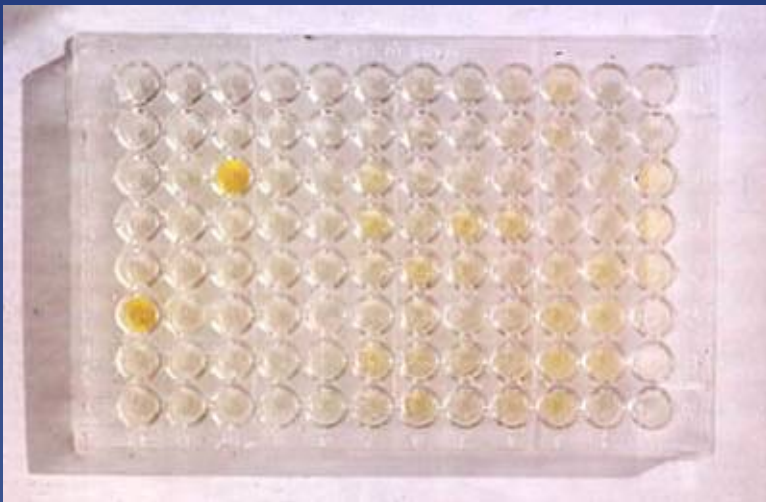
- ❖ Replace hamster test with *in-vitro* tests - 4
- ❖ Validate assay for each named antigen
- ❖ Establish national reference bacterins



Safeguarding Animal Health



Enzyme-linked Immunosorbent Assay (ELISA)



Advantages of the ELISA

- ❖ Measures a relevant antigen
- ❖ No hamsters involved (few cultures to maintain)
- ❖ Less expensive
 - \$4 per test
- ❖ Personnel are not exposed to a human pathogen



Safeguarding Animal Health



Supplemental Assay Methods

- ❖ SAM 624 - *L. pomona*
- ❖ SAM 625 - *L. canicola*
- ❖ SAM 626 - *L. grippotyphosa*
- ❖ SAM 627 – *L. icterohemorrhagiae*



Safeguarding Animal Health



Reagents for In Vitro Assay

❖ Standard Reference Bacterins (SRB)

- IRP 523 (05): *L. grippotyphosa* SRB
- IRP 524 (05): *L. pomona* SRB
- IRP 542 (06): *L. icterohaemorrhagiae* SRB
- IRP 555 (07): *L. canicola* SRB

❖ Monoclonal Antibodies

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

❖ Polyclonal Antibodies



Safeguarding Animal Health



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 Assays



Safeguarding Animal Health



Requirements for Exemption of Codified Test

❖ Identification of a Reference Bacterin

- Standard reference bacterin
- Product-matched reference bacterin and qualify it via the codified hamster potency assay
- Product-matched reference bacterin and qualify it via a host animal vaccination-challenge study

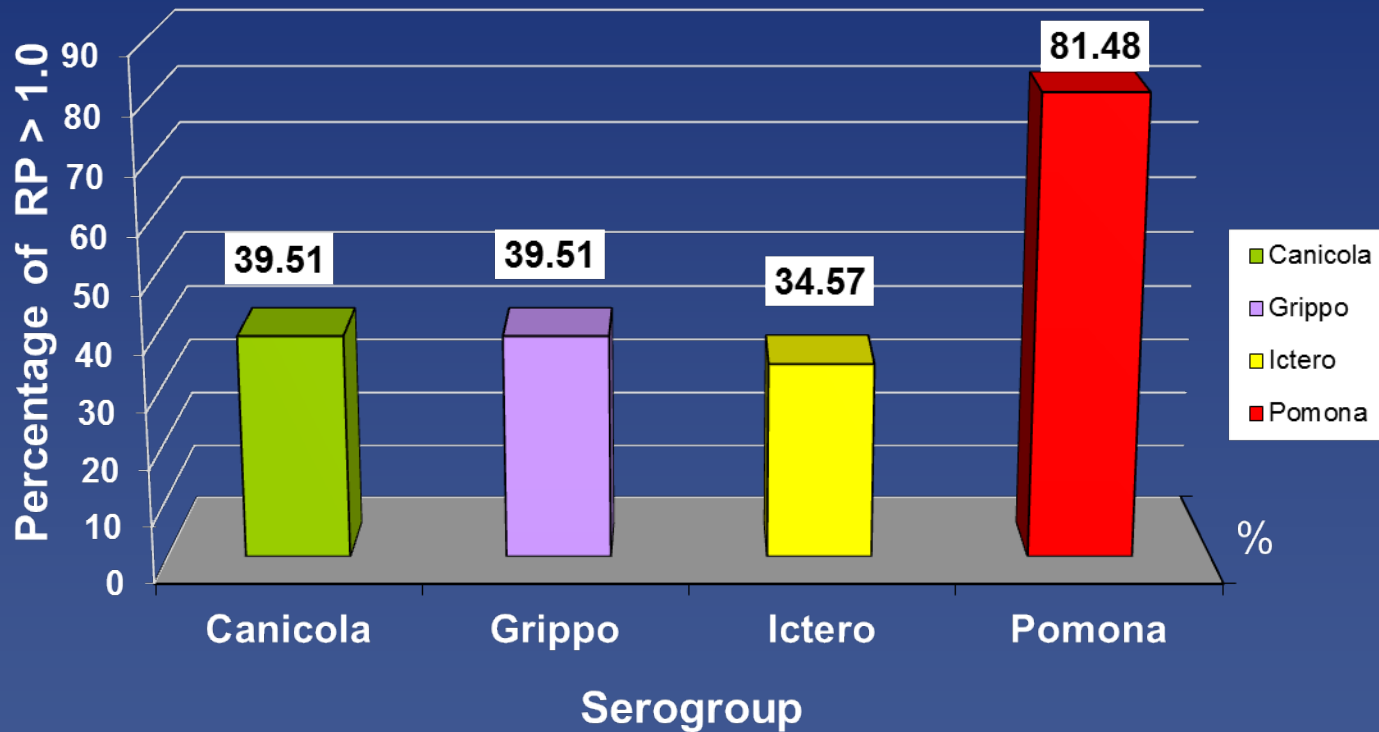
❖ Assay Validation Data



Safeguarding Animal Health



Preliminary Evaluation - ELISA Results



Safeguarding Animal Health



Requirements for Exemption of Codified Test

❖ Identification of a Reference Bacterin

- Standard reference bacterin
- Product-matched reference bacterin and qualify it via the codified hamster potency assay
- Product-matched reference bacterin and qualify it via a host animal vaccination-challenge study



Safeguarding Animal Health



Requirements for Exemption of Codified Test – Contd.

❖ *In vitro* Assay Validation

- Specificity
- Reproducibility
- Dose response
- Parallelism



Safeguarding Animal Health



Other Serovars - Potency Tests

Serovar	Host Animal	Potency
L. hardjo	Bovine	Per Outline Method
L. bratislava	Porcine	Per Outline Method



Safeguarding Animal Health



Conclusion

- ❖ Hamster potency test
- ❖ ELISA
- ❖ Reagents
 - ❖ Geetha Srinivas: Geetha.B.Srinivas@aphis.usda.gov
 - ❖ Janet Wilson: Janet.M.Wilson@aphis.usda.gov
- ❖ CVB Notices and SAMs
 - Biologics Regulations and Guidance Public Website:
http://www.aphis.usda.gov/animal_health/vet_biologics/vb_regs_and_guidance.shtml



Safeguarding Animal Health



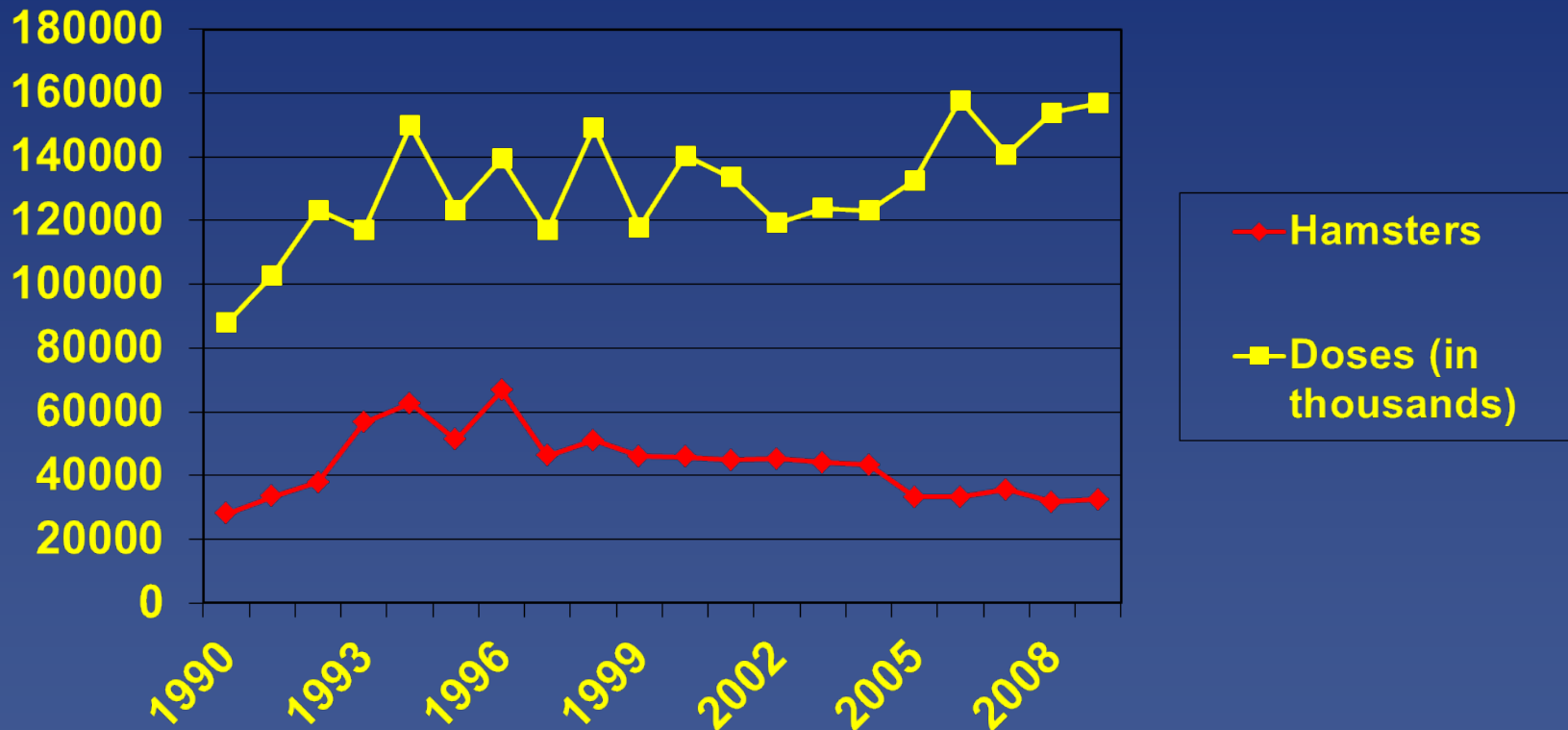
THANKS



Questions??

National Centers for Animal Health, Ames, Iowa, USA

Outcome of CVB's 2/2 Efforts



Safeguarding Animal Health

