



2014 USDA Updates

Efforts to Reduce
Hamster Usage in
Leptospirosis Vaccine
Potency Testing

Progress 2013-2014



- Set goal
- Identified Challenges
- Set strategies to meet the challenges

Our Goal

- Reduce hamster usage in leptospirosis vaccine potency testing
 - ❖ Monitor trend in usage over 5 years
 - ❖ Provide yearly updates on progress

Live animal test

- Potency testing is mandated under 9 CFR §113.101 through 113.104
- Syrian Hamsters are very sensitive to *Leptospira* challenge:
 - ❖ Regulatory potency testing of vaccines
 - ❖ Maintenance of virulent challenge strains of *Leptospira* for those potency tests
 - 'Maintenance' animals: Create the challenge inoculum



Vaccination with test product

- 10 hamsters vaccinated with 0.25 mL of diluted product by the recommended route for the host animals. Additional 10 hamsters serve as unvaccinated controls.
- Diluted product:
 - ✓ L. canicola & L. icterohaemorrhagiae 1/80
 - ✓ L. grippotyphosa & L. pomona 1/800

Challenge with Inoculum

- Performed 14-18 days post-vaccination
- Administered to test & control animals

Validity requirements

- Back-titration hamsters are used to determine adequate level of challenge inoculum:
 - ❖ 4 groups of 5 hamsters injected with serial dilutions of the serovar to estimate LD₅₀
 - ❖ Challenge inoculum must contain 10-10,000 LD₅₀ equivalents of the virulent serovar

Observation 14 Days Post- Challenge

- A valid test requires mortality in at least 80% of controls
 - ❖ Symptoms: seizures, ataxia, bleeding, dehydration, renal & hepatic complications, death
- The vaccine test batch is deemed potent if 80% of the vaccinates survive

9 CFR Potency Tests for Leptospira Vaccines

❖ Overview

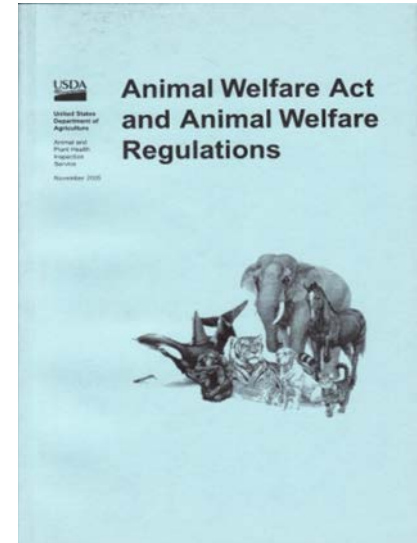


❖ Animal Numbers

Vaccinates	Challenge Controls	Concurrent Challenge (LD ₅₀ Titration)
10	10	20

Consideration of Alternatives

- The PI is to *consider* alternatives to painful procedures
 - ❖ Animal Welfare Regulations §2.31d
- Enforced by APHIS Animal Care
 - ❖ Policy 12 provides guidance which includes regulatory/ mandatory testing



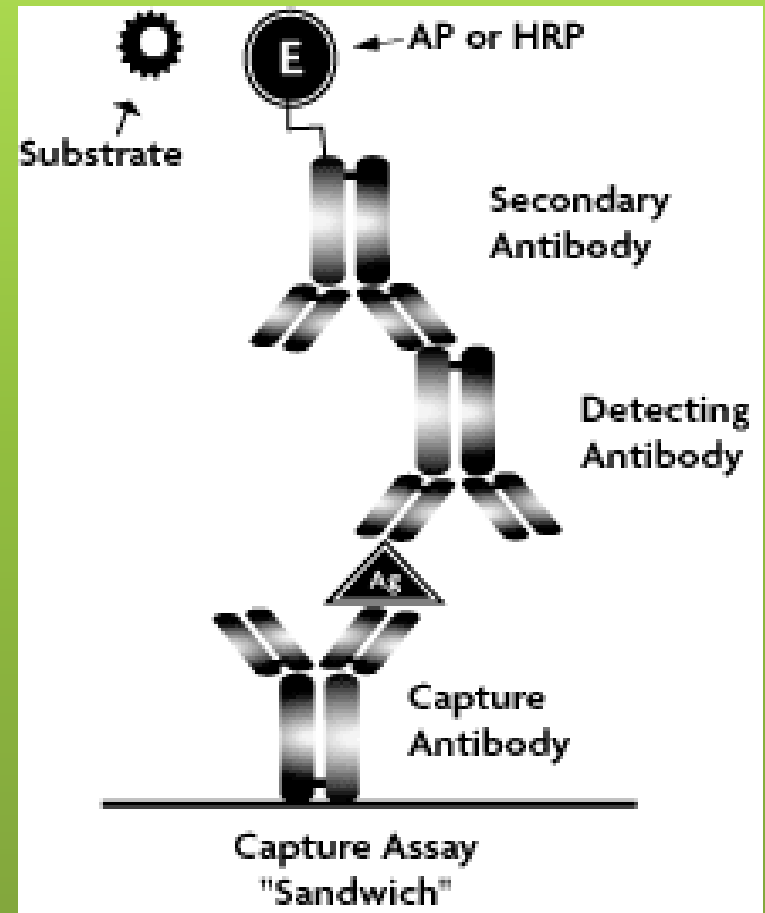
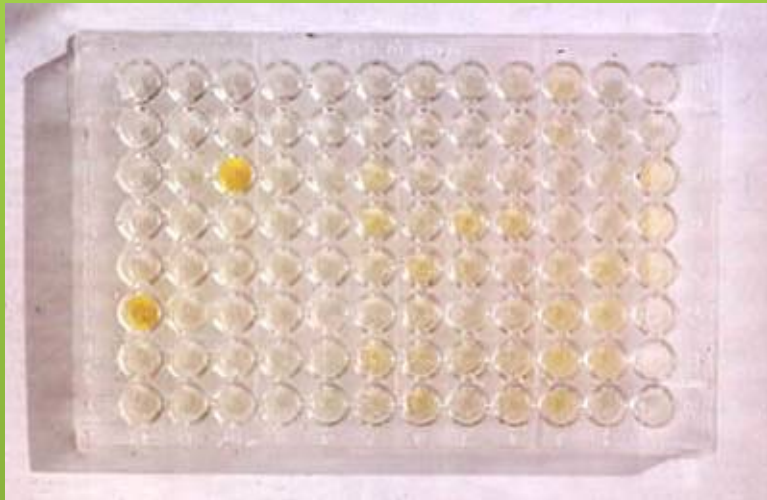
Alternative: ELISA Test

In vitro ELISA antigen quantification methods have been developed and validated by CVB for

- *L. interrogans*:
- *L. pomona*
- *L. canicola*
- *L. icterohaemorrhagiae*
- *L. kirschneri* Serogroup *grippotyphosa*

Note: CVB can supply the *Leptospira*-specific antibodies and National Reference Standards necessary to conduct the test

Enzyme-linked Immunosorbent Assay (ELISA)



Manufacturer Exemptions

- Manufacturers can obtain an exemption from CVB for the live animal hamster potency test.
- Guidance document :VS Memo 800.102
 - ❖ Firm must validate the ELISA tests for each product
 - ❖ Product matched references may also need to be produced and qualified by the firm in order to utilize the ELISA tests

Identified Challenges to Goals

- Cannot require companies to use ELISA test
- CVB confirmatory testing of vaccine potency must be performed the same way as the firm
 - ❖ Live animal test may be used
- Making searches to alternatives more effective
- Monitoring annual report
 - ❖ Obtaining specific information on animal usage under painful categories (D and E)

Offsetting the Challenges

- Animal and Plant Health Inspection Agency
 - Center for Veterinary Biologics (CVB)
 - Animal Care (AC)
- The National Agricultural Library
 - Animal Welfare Information Center (AWIC)

Center for Veterinary Biologics

- Working towards global acceptance of ELISA
- Exploring ways to reduce hamsters usage
 - ✓ To reduce serial passage in hamsters for challenge maintenance. Investigating using cryopreserved virulent *Leptospira* stock
 - ✓ Examining the impact of removing back titration from the testing regimen

Animal Care



- Modifying ACIS database to better retrieve information regarding hamster usage
 - ❖ § 2.36 Animal Welfare Regulations
 - ✓ Can monitor trends in hamster usage in category D & E
 - ✓ It is hoped a decrease will occur with use of ELISA and successful CVB efforts regarding back titration

Animal Welfare Information Center (AWIC)

Education and Outreach

- Serves as a resource: § 2143e Animal Welfare Act
 - ✓ Training sessions on literature searches for alternatives offered regularly
 - ✓ Exploring the provision of a 'canned' alternative search on *Leptospira* regulatory testing & monitoring public use
- Website provides links to ICCVAM validated tests
 - ✓ Link to the 2012 International Conference on Alternative Methods to Leptospirosis Vaccine Potency Testing

Monitoring Progress

- The USDA ICCVAM representative will continue discussions with CVB and AWIC to monitor progress
 - ✓ AWIC and CVB have persons who serve as alternates to ICCVAM