



2015
USDA Updates for
SACATM Meeting

Hamster Usage in
Leptospirosis Vaccine
Potency Testing

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Our Goal

- Reduce hamster usage in leptospirosis vaccine potency testing by:
 - ❖ Promoting the *in vitro* test alternative
 - ❖ Reducing animals needed to maintain virulent strains
 - ❖ Reducing animal numbers in vaccination - challenge assays

Team Approach

Animal Plant Health Inspection Service (APHIS)

- ❖ Center for Veterinary Biologics (CVB)
- ❖ Animal Care (AC)

The National Agricultural Library (NAL)

- ❖ Animal Welfare Information Center (AWIC)

Live animal test



- Leptospira bacterin potency testing is mandated under 9 CFR §113.101- 113.104
 - ❖ Virus-Serum-Toxin Act, enforced by CVB
- Syrian Hamsters are very sensitive to Leptospira
 - ❖ Used for regulatory potency testing of vaccine
 - ❖ Used to maintain virulent challenge strains
[Maintenance hamsters]

Clarification

Maintenance

Maintain virulent strains

- 4-6 hamsters given *Leptospira* IP
- Illness in 3-4 days
- Spirochetes in liver
- Liver homogenates used to inject another set of animals

Back-titration

Determination of LD 50

- Account for 50% of animals used during the regulatory test

Live Animal Test: Step #1

- 10 hamsters vaccinated with 0.25 mL of diluted vaccine product
 - ❖ *L. canicola* & *L. icterohaemorrhagiae* 1/80
 - ❖ *L. grippotyphosa* & *L. pomona* 1/800
- Additional 10 hamsters serve as unvaccinated controls

Step #2: Create Challenge Inoculum

Validity Requirements:

- 'Back-titration' hamsters used to determine adequate level of challenge inoculum
- 4 groups of 5 hamsters injected with serial dilutions of the serovar to estimate LD₅₀
 - ❖ As per Assay methods outlined by CVB



Step # 3: Administer Challenge Inoculum

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

Step #4: Observation 14 Days Post- Challenge

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

9 CFR Live Animal Potency Tests for Leptospira Vaccines

❖ Overview



❖ Animal Numbers

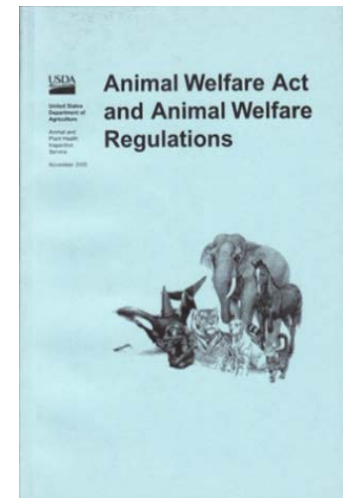
Vaccinates	Challenge Controls	Concurrent Challenge (LD ₅₀ Titration)
10	10	20

Hamster Numbers Add Up!

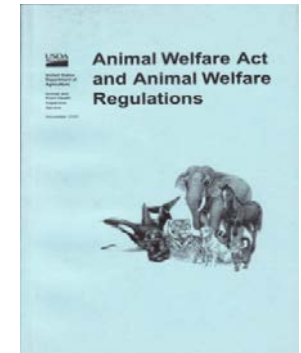
- Maintenance Hamsters
 - ❖ To keep virulent strains on-hand
- Vaccine Potency testing
 - ❖ Done for each serovar within a single vaccine
 - ❖ Example: A vaccine with 3 serovars
 - 40 hamsters x 3 serovars = 120 hamsters

The Animal Welfare Act (AWA)

- Is enforced by Animal Care (AC)
- Requires an Annual Report on animal usage
- Requires a search for alternatives



Search for Alternatives



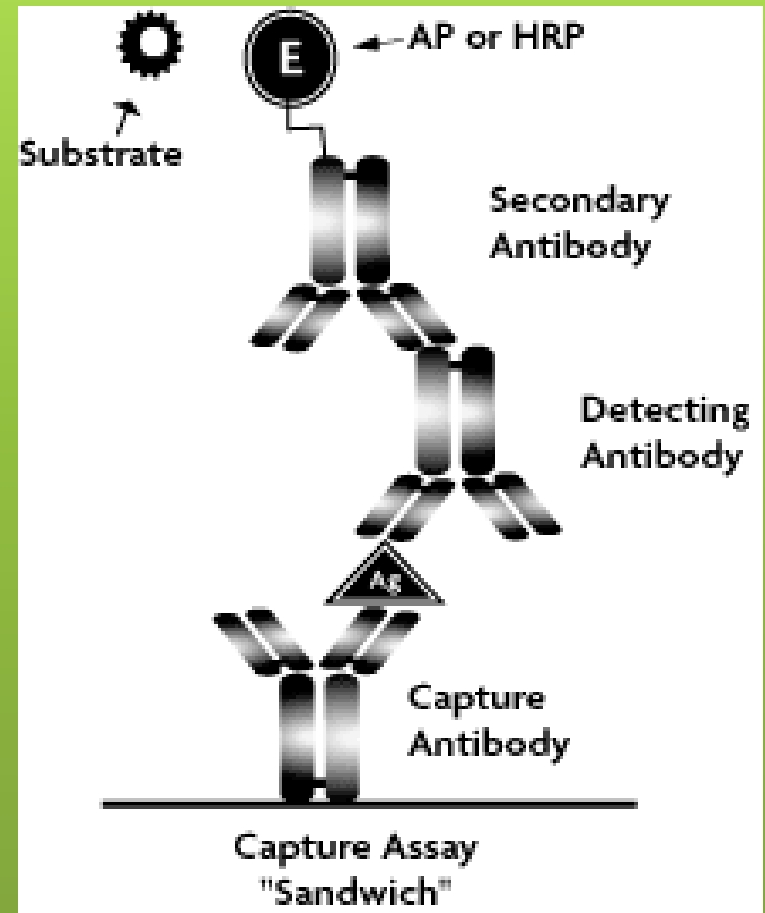
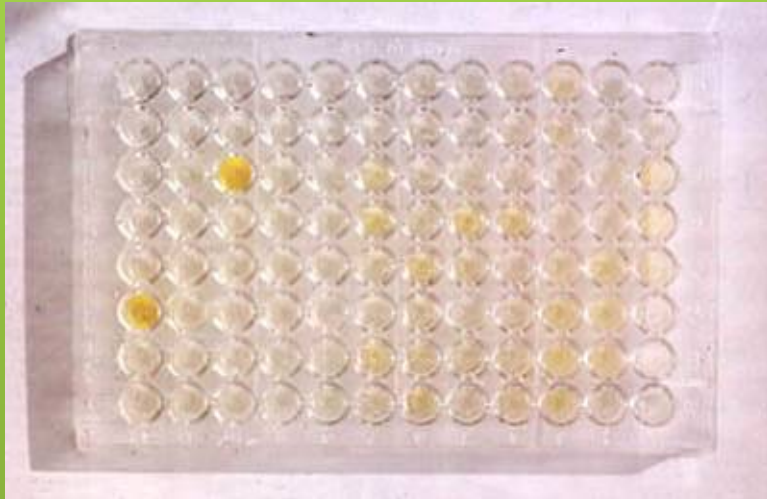
- Wording under the AWA:
 - ❖ Investigator to seek ways to eliminate the use of animals or reduce pain/distress in testing and experimentation
 - ❖ Establish an information service at NAL to provide instruction on finding alternatives
 - ✓ AWIC

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*

Enzyme-linked Immunosorbent Assay (ELISA)



VETERINARY SERVICES: MEMORANDUM NO. 800.102

- Guidance for an exemption to the regulations for Leptospira bacterin potency testing in hamsters. [2013]
 - ❖ Provides information on alternative in vitro potency assays (SAM)
 - ❖ Provides information on reagents available from CVB

Supplemental Assay Methods (SAM)

Protocols for *in vitro* potency assays using the ELISA as compared to a suitable non-expired reference

- ❖ SAM 624: *L. interrogans* serogroup *pomona*
- ❖ SAM 625: *L. interrogans* serogroup *canicola*
- ❖ SAM 626: *L. kirschneri* serogroup *grippotyphosa*
- ❖ SAM 627: *L. interrogans* serogroup *icterohaemorrhagiae*

Exemption requirements

- Firms are to obtain an exemption from CVB before using the ELISA.
 - ❖ Use of ELISA is optional
 - ❖ CVB does not track exemptions
- Firms must validate the potency assays for their products and submit data to CVB for acceptance

FYI about Europe

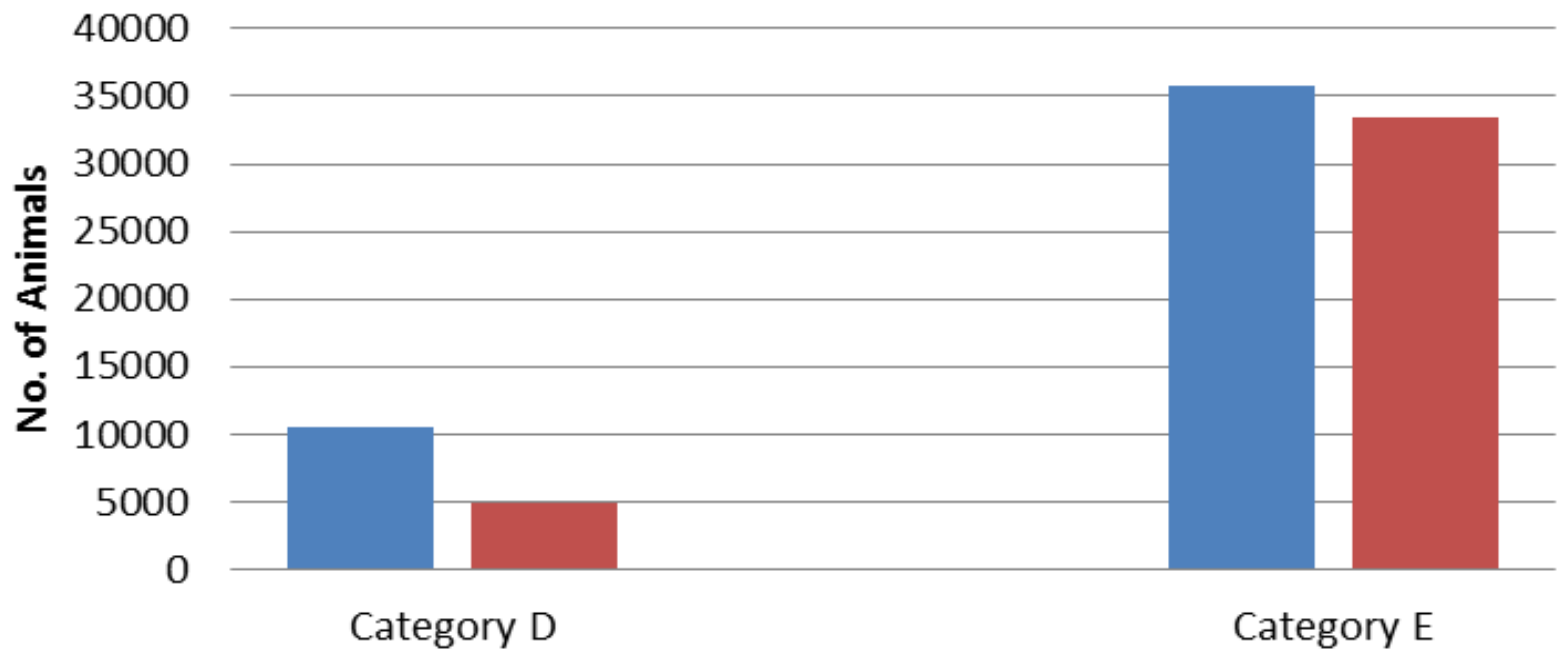
- The EU Pharmacopeia eliminated the hamster potency test but still requires a guinea pig vaccination-serology potency test.
- ❖ The ELISA provides a complete elimination of animals provided relative potency assays have been properly validated.

Monitoring Hamster Usage

- AC will monitor Annual Reports for facilities listed in the Product Code Book
 - ❖ Annual Reports & Product Code Book are publically available
- The Annual Report:
 - ❖ Category D: pain/distress relieved
 - ❖ Category E: pain /distress not relieved
 - ✓ requires a written explanation

Hamster Usage

■ 2013 ■ 2014



Promoting the ELISA

- CVB

- ❖ Promoting global use of the ELISA test

- AWIC

- ❖ Has a link on potency testing that provides canned alternative searches in Agricola, Google Scholar, and Pub Med
- ❖ <http://awic.nal.usda.gov/alternatives-biologics-testing>

More Progress

- CVB has prepared a Notice to eliminate the hamster back titration for 2 *Leptospira* serovars.
- Draft Notice was made available for comment in August 2015.
- Impact will be significant when finalized

Challenges

For CVB:

- ❖ Cannot require companies to use ELISA
- ❖ Exploring the use of cryopreserved virulent *Leptospira* stock instead of creating fresh stock in maintenance hamsters

For AC:

- ❖ Annual report provides no specifics on usage in Category D listings

Conclusion



- AWIC, Animal Care, and CVB working towards reducing hamster usage in Leptospirosis vaccine potency testing
- Impact of efforts can be monitored using hamster numbers on the Annual Report
- Elimination of back-titration hamsters can make a significant impact