



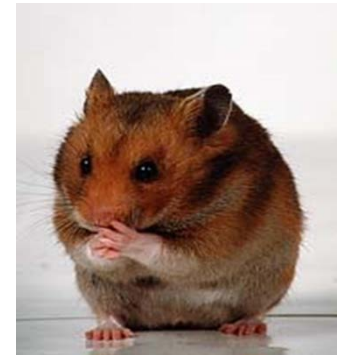
United States Department of Agriculture

Reducing Hamster Usage In *Leptospira* Vaccine Potency Testing

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Live Animal Test

- **Virus-Serum-Toxin Act**
 - Enforced by APHIS-Center for Veterinary Biologics CVB
 - *Leptospira* bacterin potency testing is mandated under 9 CFR §113.101- 113.104

- Syrian Hamsters are very sensitive to *Leptospira*
 - Used as part of the standard regulatory test for *Leptospira* vaccine potency



Creation of Challenge Inoculum For Potency Testing

Back-titration hamsters:

- *In vivo* passaging: 4 groups of 5 hamsters injected with serial dilutions of a serogroup to estimate LD₅₀
 - Symptoms: Hemorrhage (nasal and urine), and death.
 - 5% display neurologic ataxia as a result of hepatic failure.
 - 33% of the animals display no symptoms before becoming moribund.

9 CFR Live Animal Potency Tests for Leptospira Vaccines

❖ Overview



❖ Animal Numbers

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



14 Days Post-Challenge

- A valid test requires 80% mortality in controls
- The vaccine test batch is deemed potent if 80% of the vaccinates survive



Hamster Numbers Add Up!

- **Example: A canine vaccine vial with 3 serogroups**
 - 20 Back-titration hamsters x 3 serogroups = 60
 - 20 hamsters for live animal test x 3 serogroups = 60

Total= 120 hamsters
- ***Now consider the hundreds of vials needed for batch testing***



Our Goal

- Reduce hamster usage in potency testing through initiatives begun by the APHIS-CVB:
 - **2013:** Exemption to use *in vitro* ELISA test developed by APHIS-CVB instead of standard test
 - **2015:** Exemption by CVB to eliminate back-titration hamsters for *L. canicola* & *L. icterohaemorrhagiae*
 - **2017:** Exemption by CVB to eliminate back-titration hamsters for *L. pomona* & *L. grippotyphosa*

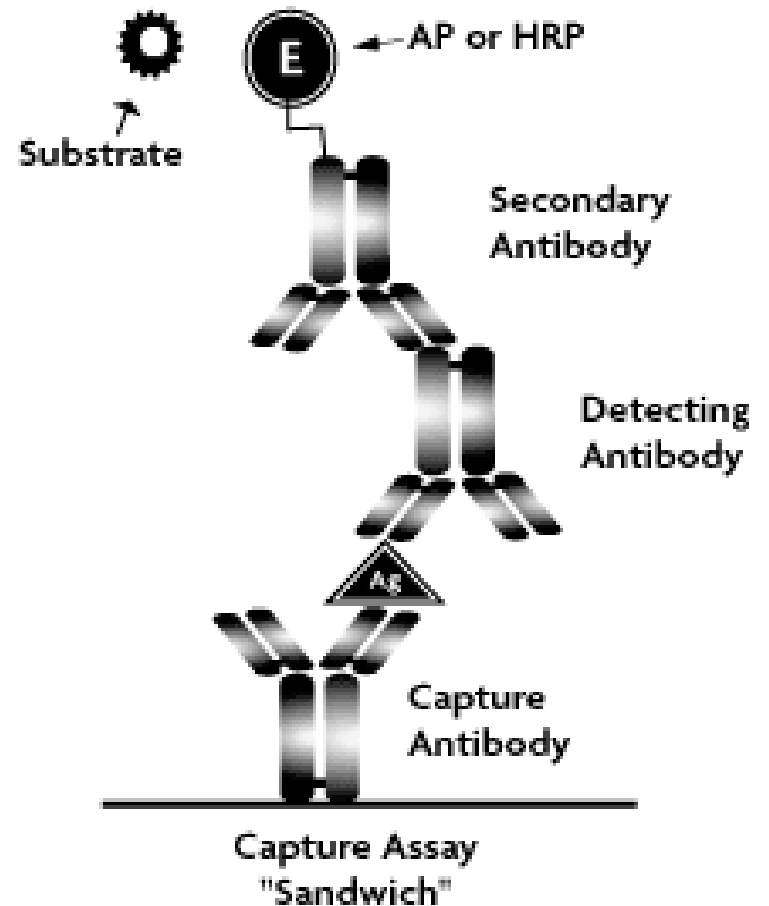
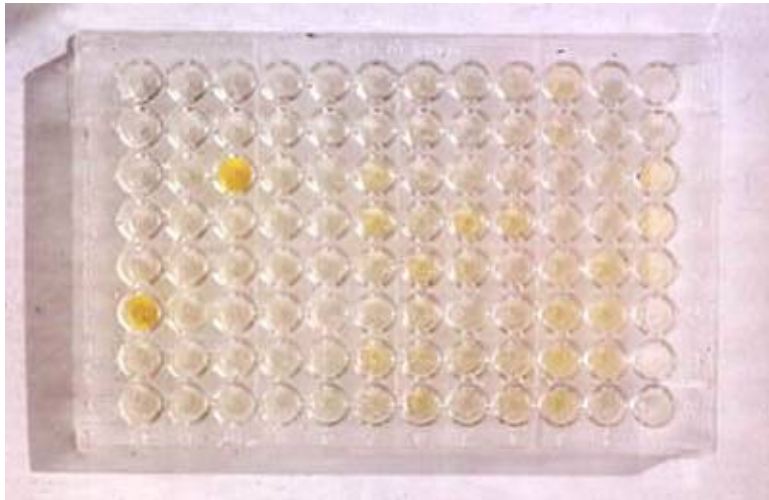


Alternative: ELISA Test Replacement of the Live Animal

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

- *L. pomona*
- *L. canicola*
- *L. icterohaemorrhagiae*
- *L. grippotyphosa*

Enzyme-linked Immunosorbent Assay (ELISA)





United States Department of Agriculture

Veterinary Services: Memorandum No. 800.102

- **2013:** Guidance to use the ELISA test in *Leptospira* bacterin potency testing in hamsters.
 - Provides information on alternative *in vitro* potency assays
 - 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
- Firms must validate the potency assays for their products and submit data to CVB for acceptance



Alternative: Elimination of Back-Titration Hamsters Reduction in Live Animals

- **2015 APHIS-CVB Notice # 15-13:**
 - Provided biologic licensees, permittees and applicants the option to remove back-titration hamsters from *in vivo* potency tests for *Leptospira* serogroups *icterohaemorrhagiae* and *canicola*
 - CVB would allow an exemption from the titration requirement in vaccination-challenge potency assays for these serogroups
- *Removal of the back-titration hamsters could reduce animal use by 50% when potency testing these two fractions*



Alternative: Elimination of Back-Titration Hamsters Reduction in Live Animals


- **2017 APHIS-CVB Notice # 17-06:**
 - Provided biologic licensees, permittees and applicants the option to remove back-titration hamsters from *in vivo* potency tests for *Leptospira* serogroups pomona and grippotyphosa
 - CVB would allow an exemption from the titration requirement in vaccination-challenge potency assays for these serogroups
- *Removal of the back-titration hamsters could reduce animal use by 50% when potency testing these two fractions*





USDA Team Effort


National Agricultural Library- Animal Welfare Information Center (NAL-AWIC)


- Will inform the public about the exemptions
- Mandated under the Animal Welfare Act to provide information on improved methods of animal experimentation,
 - includes methods which could reduce or replace animal use or refine a study to minimize pain and distress to animals (3Rs alternatives)


Laws and Guidelines 

Training 

Alternatives Literature Searching 

3Rs Alternatives: Technologies, and Approaches 

Housing, Care and Welfare 

Institutional Animal Care and Use Committees 

Routine Procedures

Organizations

Animal Welfare Information Center

The **Animal Welfare Information Center (AWIC)** is mandated by the Animal Welfare Act (AWA) to provide information for improved animal care and use in research, testing, and teaching.




Workshop on Meeting the Information Requirements of the Animal Welfare Act

AWIC provides training on how to effectively search the literature for 3Rs alternatives.

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News Events

[EPA Launches Voluntary Pilot Program to Reduce Animal Testing](#) 
Wednesday, January 04, 2017
DHHS. NIH. NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).



USDA Team Effort

APHIS- Animal Care

- Will track impact of exemptions using the Annual Report submitted by manufacturers of a *Leptospira* biological
- The facilities are listed in the Product Code Book (on-line)



According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0036. The time required to complete this information collection is estimated to average 2 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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No. 0180-DOA-AN

Fiscal Year 2016

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE ANNUAL REPORT OF RESEARCH FACILITY (TYPE OR PRINT)	1. REGISTRATION NUMBER
	2. HEADQUARTERS RESEARCH FACILITY (Name, address, and telephone number as registered with USDA, include ZIP Code)

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets, if necessary.)

FACILITY LOCATIONS (Sites)	

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets, if necessary, or use APHIS FORM 7023A.)

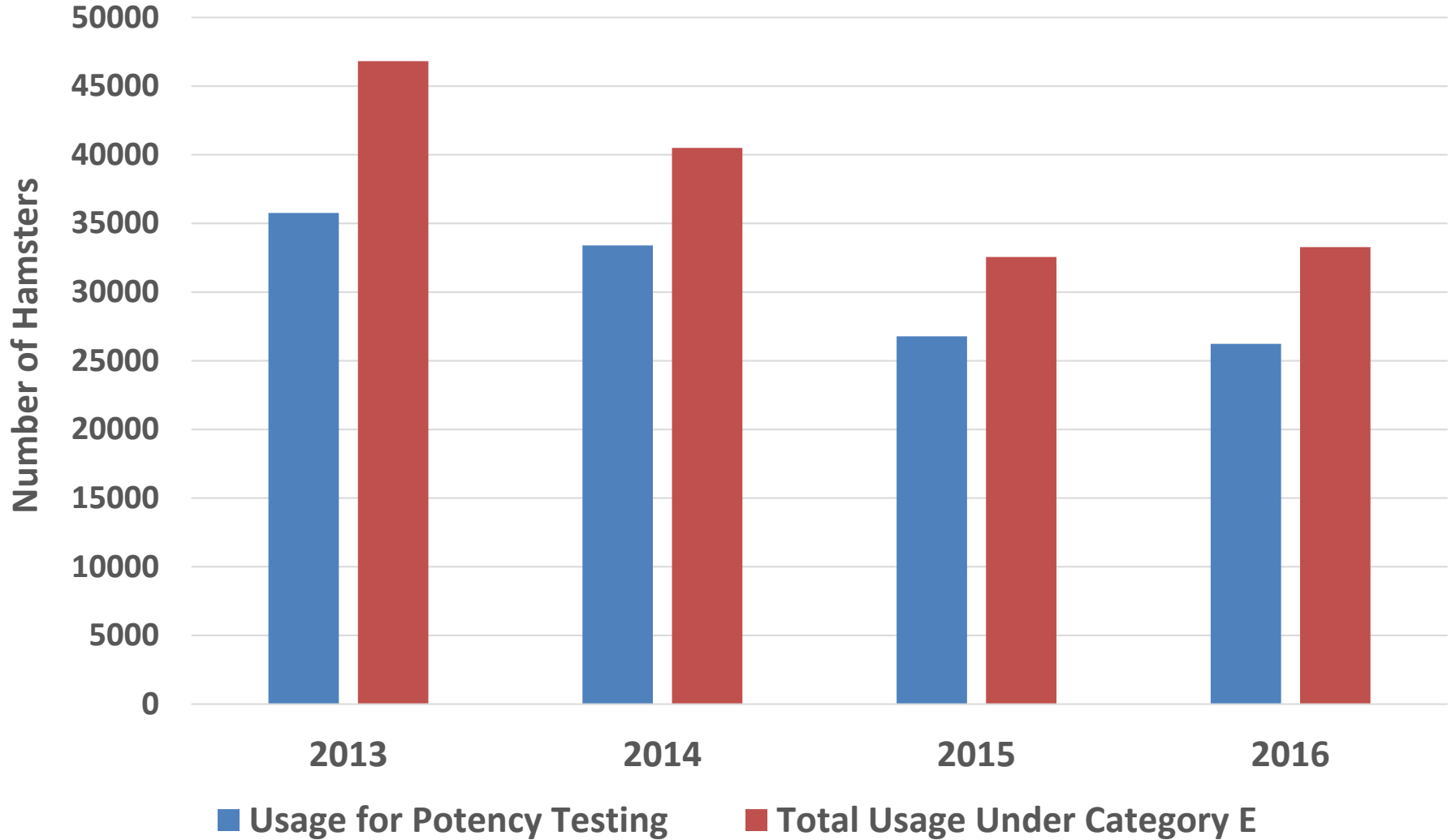
A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be attached to this report.)	F. TOTAL NUMBER OF ANIMALS (Cols. C + D + E)
4. Dogs					0
5. Cats					0
6. Guinea Pigs					0
7. Hamsters					0
8. Rabbits					0
9. Non-human Primates					0
10. Sheep					0
11. Pigs					0
12. Other Farm Animals					0
13. Other Animals					0
					0
					0
					0

#7- Hamsters
Category E:

- Pain unrelieved
- Explanation required



Hamster Usage Under Category E





Challenges

- USDA cannot require companies to use the alternatives
- More work needs to be done
 - CVB exploring the use of cryopreserved *Leptospira* stock to eliminate maintenance of virulent challenge through continuous transfer in live animals

Conclusion



- The USDA continuously works towards reducing hamster usage in *Leptospira* vaccine potency testing by:
 - Providing approved exemptions to the standard live animal test i.e. ELISA test and elimination of back-titration hamsters
 - Providing Information on available alternatives
 - Monitoring impact of exemptions via the Annual Reports
 - Researching use of cryopreserved *Leptospira* strains



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United States Department of Agriculture

THANK YOU