



United States Department of Agriculture

# ***Leptospira* Vaccine Potency Testing**

## 2018 Updates on Reducing Hamster Usage

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USDA-Animal Care



# 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

# 4 Groups of Hamsters

- Controls
- Test animals
- Back-titration animals
  - To create virulent challenge strains
- Those to maintain 'fresh' virulent strains





# Creation of Challenge Inoculum

## Back-titration hamsters:

- *In vivo* passaging: 4 groups of 5 hamsters injected with serial dilutions of a serogroup to estimate LD<sub>50</sub>
  - **Symptoms:** Hemorrhage (nasal and urine), and death
    - 5% display neurologic ataxia as a result of hepatic failure
    - 33% display no symptoms before becoming moribund

# Live Animal Potency Test of *Leptospira* Vaccines

## ❖ Overview



## ❖ Animal Numbers

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



# Results: 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 numbers of hamsters needed for batch testing vials of vaccines for dogs, cattle, and swine!*

# Maintenance of Virulent Strains



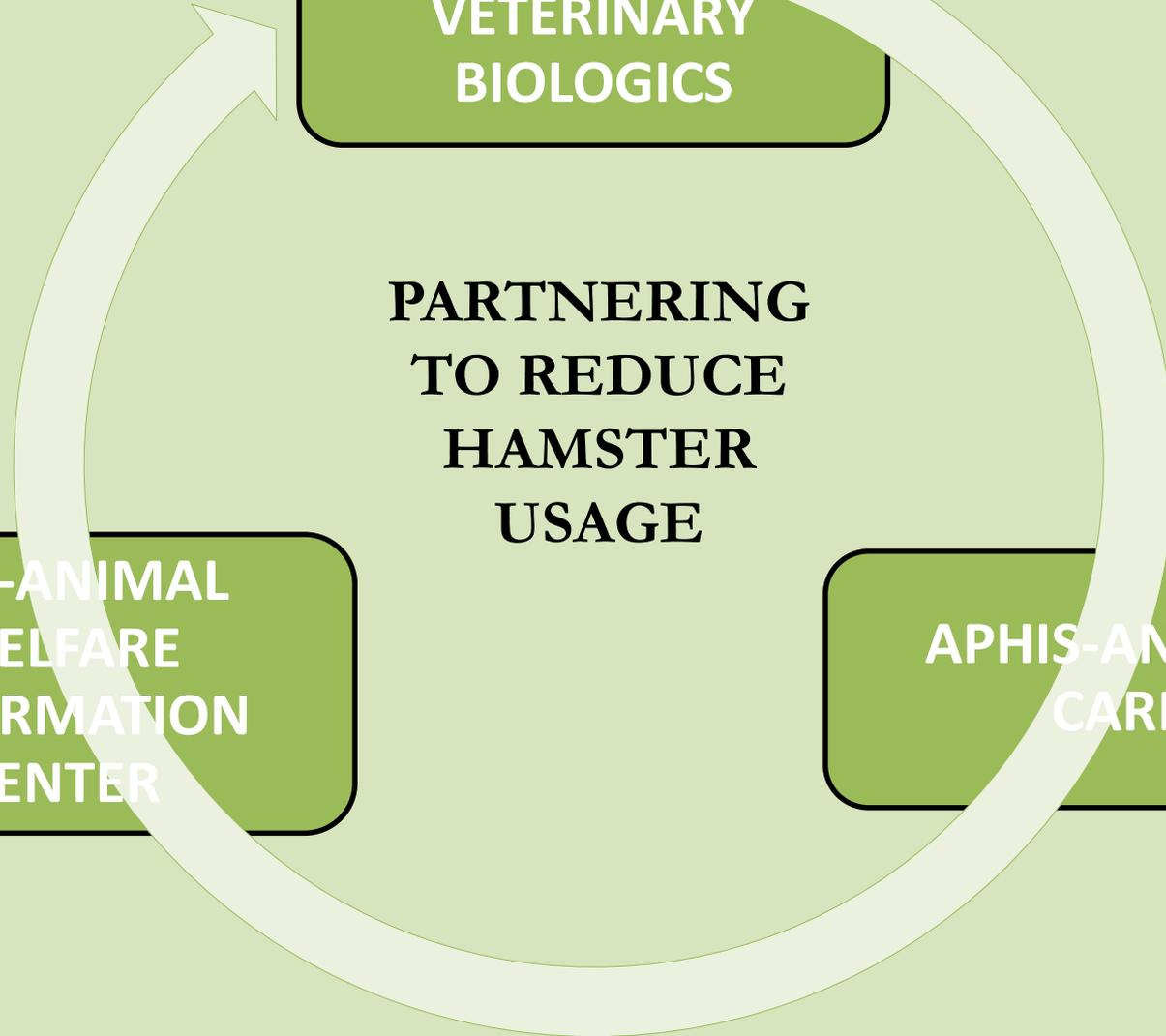
- Note: Once virulent strains are created, they need to be maintained in a live animal so ‘freshly’ prepared virulent cultures can be made
  - Approximately 46-50 hamsters/week

**APHIS-CENTER FOR  
VETERINARY  
BIOLOGICS**

**PARTNERING  
TO REDUCE  
HAMSTER  
USAGE**

**NAL-ANIMAL  
WELFARE  
INFORMATION  
CENTER**

**APHIS-ANIMAL  
CARE**





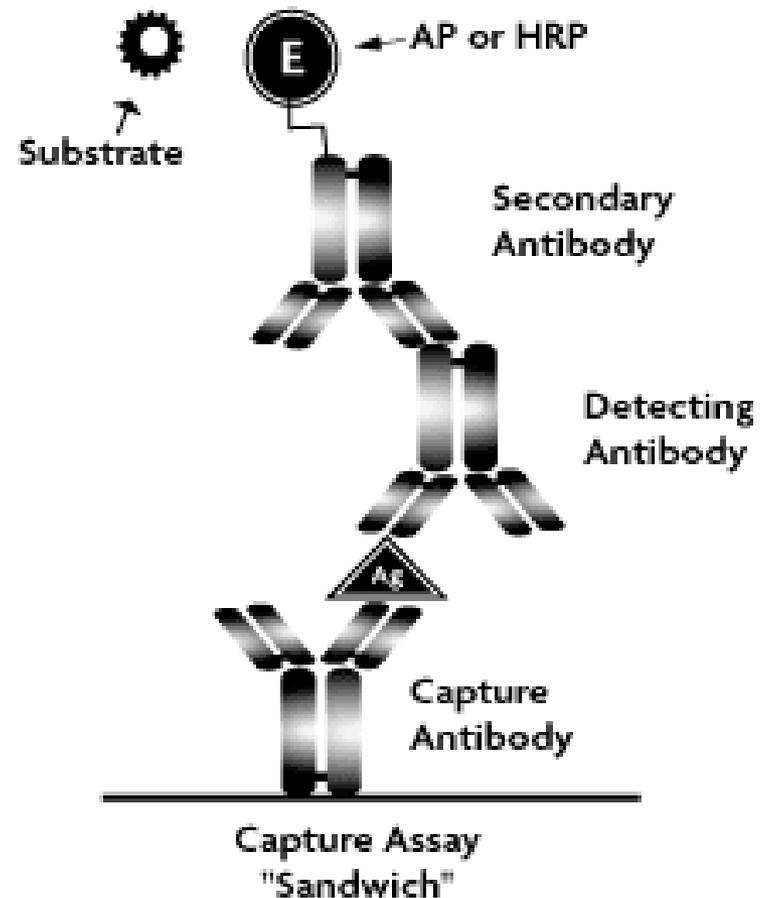
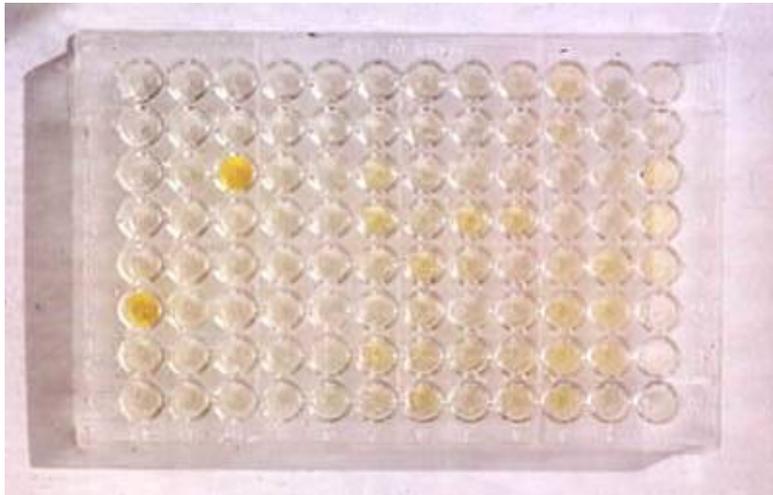
# Center for Veterinary Biologics (CVB)

- Initiatives that support the 3Rs (*replace, refine, reduce*)
  - **2013**: Exemption to use *in vitro* ELISA test developed by CVB instead of the codified test
  - **2015**: Exemption to eliminate back-titration hamsters for *L. canicola* & *L. icterohaemorrhagiae*
  - **2017**: Exemption to eliminate back-titration hamsters for *L. pomona* & *L. grippotyphosa*
  - **2018**: Cryopreservation of virulent challenge serotypes

# Enzyme-linked Immunosorbent Assay (ELISA)

- Replaces the codified *in vivo* test
  - *No live animals needed*
- *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





United States Department of Agriculture

# Veterinary Services: Memorandum No. 800.102

- **2013:** Guidance on using the ELISA test
  - Provides information on this alternative to *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 must 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



# Elimination of Back-titration Hamsters

## 2015 APHIS-CVB Notice # 15-13

- Option to remove back-titration hamsters from *in vivo* potency tests for serogroups *L. canicola* and *L. icterohaemorrhagiae*
- Must apply to CVB for an exemption to the *in vivo* test's titration requirement

## 2017 APHIS-CVB Notice # 17-06

- Option to remove back-titration hamsters from *in vivo* potency tests for serogroups *L. pomona* and *L. grippotyphosa*
- Must apply to CVB for an exemption to the *in vivo* test's titration requirement

*Removal of back-titration hamsters can reduce animal use by 50%*



# 2018: Cryopreservation of Virulent Serogroups

## Cryopreserved Serogroups

CVB has made available virulent serogroups *L. canicola*, *L. pomona*, *L. grippotyphosa*, and *L. icterohaemorrhagiae* such that the live animal is not needed to maintain strains

[https://www.aphis.usda.gov/animalhealth/vetbiologics/publications/vb\\_reagent\\_catalog.pdf](https://www.aphis.usda.gov/animalhealth/vetbiologics/publications/vb_reagent_catalog.pdf)

## Cryopreservation Protocol

CVB has created a protocol to be used by any company that wishes to cryopreserve strains on their own.

[https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/biologics-regulations-and-guidance/ct\\_vb\\_pros\\_bb](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/biologics-regulations-and-guidance/ct_vb_pros_bb)

*Potential elimination of ~ 2600 animals/yr./facility  
15-20 yr. use of cryopreserved strains*



# Animal Care

Currently monitoring hamster usage of companies that produce an APHIS approved *Leptospira* biological

- Part of USDA commitment to ICCVAM
- Monitoring via the Annual report (APHIS form 7023)
- Companies manufacturing APHIS approved products
  - The Veterinary Biologics Product Catalogue
  - [https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/ct\\_vb\\_licensed\\_products](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/ct_vb_licensed_products)



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OMB APPROVED  
0579-0036  
Exp.: 10/31/2018

This report is required by law (7 U.S.C. 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

Interagency Report Control  
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

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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.)

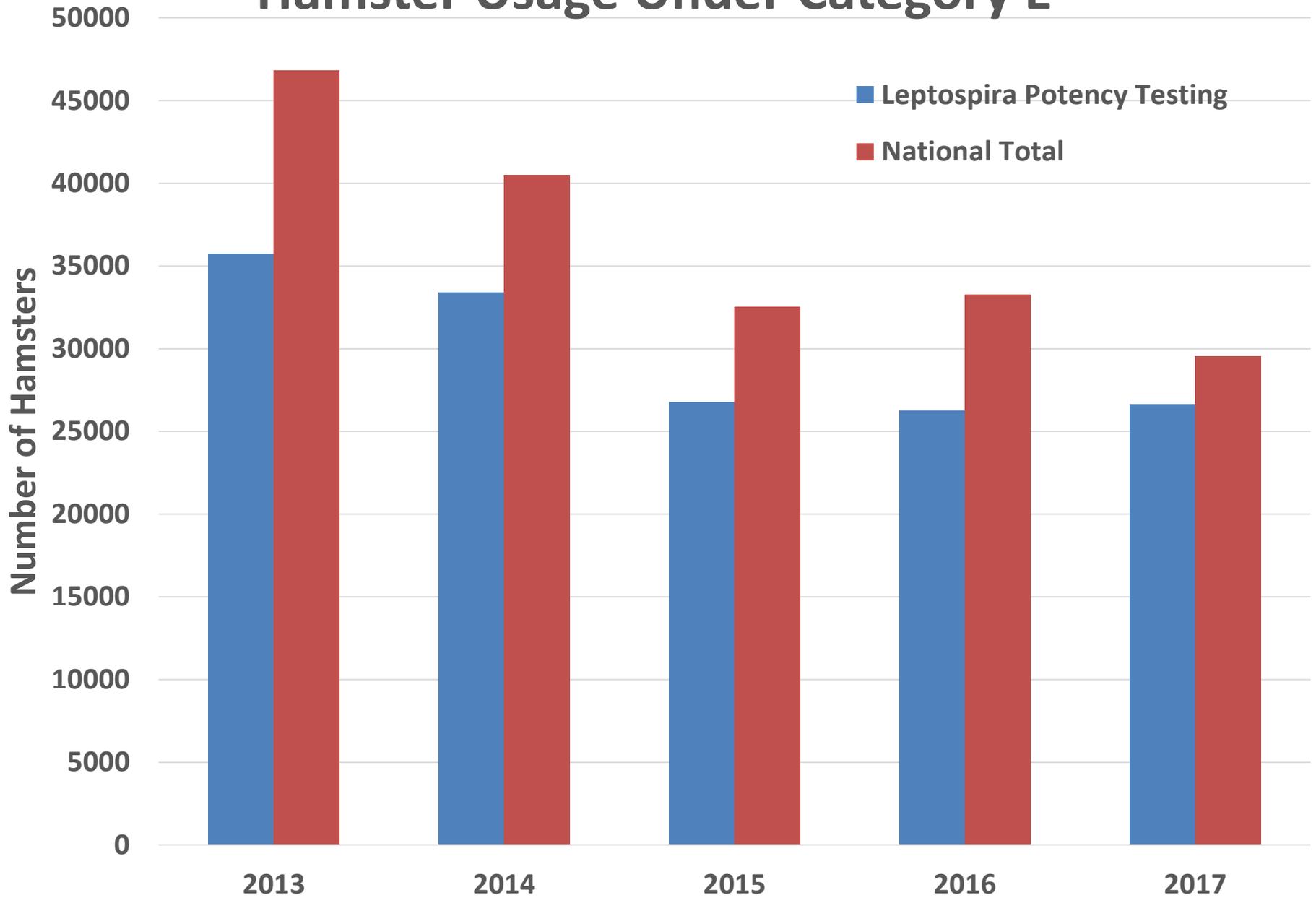
A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving 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
					0
13. Other Animals					0
					0
					0
					0

ASSURANCE STATEMENTS

#7- Hamsters  
Category E:  

- Pain unrelieved
- Explanation required

# Hamster Usage Under Category E





# Animal Welfare Information Center (AWIC)

- The Animal Welfare Act lists the National Agricultural Library (NAL) as a resource for information on improved methods of animal experimentation
- AWIC is the division of NAL that informs the research community about methods which could reduce or replace animals, or refine a study to minimize pain and distress
- CVB exemptions are listed on the web site <https://www.nal.usda.gov/awic/biologics-and-vaccine-testing>

Home » Information Centers » Animal Welfare Information Center » 3Rs Alternatives: Technologies, and Approaches » Mandatory and Regulatory Testing » Biologics and Vaccine Testing

About AWIC +

Laws and Guidelines +

Training +

Alternatives Literature Searching +

3Rs Alternatives: Technologies, and Approaches -

Alternatives in Education: Veterinary Medicine

Experimental Design

Funding Opportunities

Mandatory and Regulatory Testing -

Biologics and Vaccine Testing

## Biologics and Vaccine Testing

Leptospira vaccine potency testing

USDA. Animal and Plant Health Inspection Service (APHIS). Center for Veterinary Biologics (CVB).

- [Guidance for obtaining an exemption to the requirement for testing Leptospira bacterins for potency in hamsters, Veterinary Services \(VS\) Memorandum No. 800.102 \(2013\)](#) -- provide information on alternative in vitro potency assays (SAM) and on reagents available from the Center for Veterinary Biologics (CVB). Use of ELISA is optional. Firms are to obtain an exemption from CVB before using the ELISA. Firms must validate the potency assays for their products and submit data to CVB for acceptance.
- [Option to Remove Back-titration Hamsters from In Vivo Potency Tests for Leptospira Serogroups Canicola and Icterohaemorrhagiae, CVB Notice #15-13 \(2015\)](#) -- provides 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 serogroups.
- [Option to Remove Back-titration Hamsters from In Vivo Potency Tests for Leptospira Serogroups Pomona and Grippityphosa, CVB Notice #17-06 \(2017\)](#) -- provides biologic licensees, permittees and applicants the option to remove back-titration hamsters from in vivo potency tests for Leptospira serogroups pomona and grippityphosa. CVB would allow an exemption from the titration requirement in vaccination-challenge potency assays for these serogroups.

# 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
  - Allowing the use of cryopreserved virulent strains
  - Providing information on alternatives in potency testing
  - Monitoring impact of CVB initiatives via annual reports of hamster usage



# Acknowledgements

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**THANK YOU**