

PRODUCT SPECIFIC VALIDATION OF AN *IN VITRO* ASSAY FOR *LEPTOSPIRA* POTENCY TESTING: AN INDUSTRY PERSPECTIVE

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Testing: State of the Science and the Way Forward

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Presentation Overview

- Background
- Current Options for firms
- Implementation of SAM methods
- Industry hurdles
- Closing review

Background

- Chapter 9, Federal Code of Regulations allows for potency testing of *Leptospira* serovars in hamsters:
- 9 CFR 113.101 *L. pomona*
- 9 CFR 113.102 *L. icterohaemorrhagiae*
- 9 CFR 113.103 *L. canicola*
- 9 CFR 113.104 *L. grippotyphosa*

Background

- Each serovar test requires at a minimum:
 - 10 Vaccinates
 - 10 Controls
 - 20 Back titrations
 - Passage hamsters
- Total 40 hamsters to run test
- 28-32 total length of test

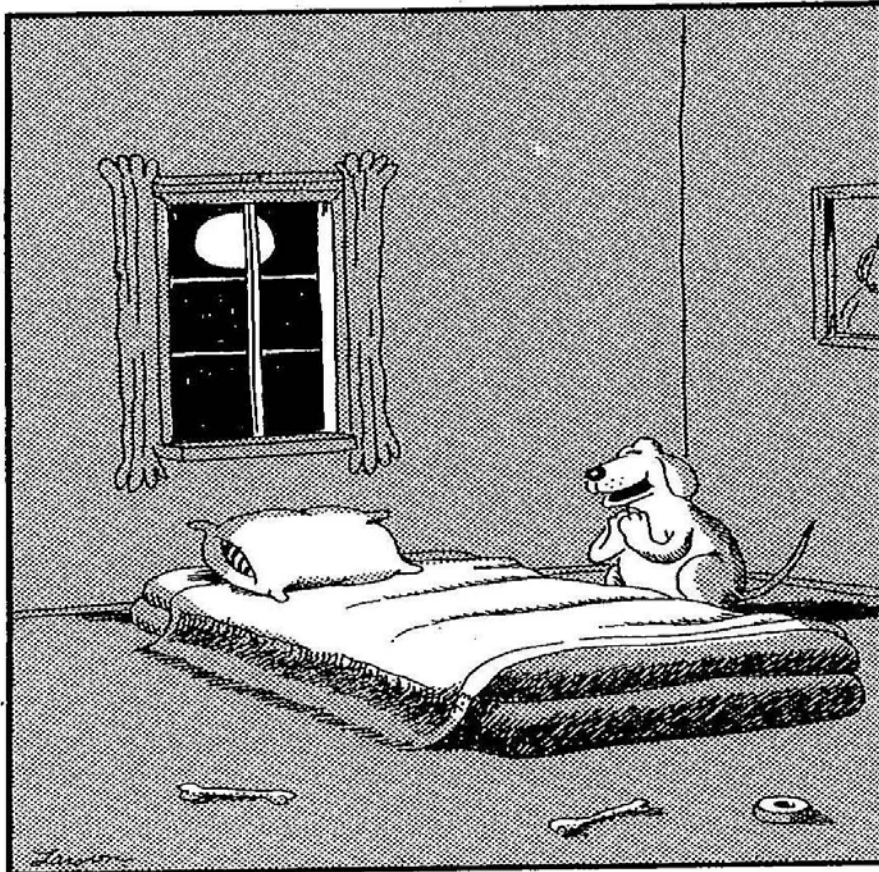


Background

◎ Category E

- Procedures producing pain or distress unrelieved by analgesics
- testing where death of an animal is an endpoint
- Lepto testing in hamsters is Category E

Background



The Far Side®
·2007·
JULY
27
Friday

“...And please let Mom, Dad, Rex, Ginger, Tucker,
me, and all the rest of the family be negative controls

Background

- USDA has developed the following ELISA Supplemental Assay Methods (SAM) for 4 Lepto Serovars applicable to multiple host species:
 - SAM 624 – *L. pomona*
 - SAM 625 – *L. canicola*
 - SAM 626 – *L. grippityphosa*
 - SAM 627 – *L. icterohaemorrhagiae*

Background

- ⦿ USDA also has qualified reference vaccines for the same 4 Lepto serovars:
 - ⦿ *L. pomona* – IRP524
 - ⦿ *L. canicola* – IRP555
 - ⦿ *L. grippotyphosa* – IRP523
 - ⦿ *L. icterohaemorrhagiae* – IRP542
- ⦿ Note – these are non adjuvanted master references

Current Options

- The tools are in the toolbox if we chose to use them.



Current Options

- Methods
 - Use 9 CFR test – Current Industry practice
 - Use of USDA SAM ELISA methods
 - Develop and Validate methods by firm (ELISA or other technology)
- References
 - Use USDA available references
 - Use “as is” or formulate to match product specific
 - Validate references in house via challenge

Implementation of SAM Methods

● Validation of SAM methods

- Evaluate if antigen extraction is needed
- Selection of Reference Vaccine
- Method validation must meet requirements of VS Memorandum 800.112

Industry Hurdles

◎ Specific firm responses

- From internal discussions, the various firms represented are at different developmental stages in regards to converting testing from *in-vivo* to *in-vitro*. **None of the firms surveyed are using the ELISA for product release in the US.** Here are some general comments and concerns / hurdles regarding conversion using the SAM methods.
- Primary antibodies from SAM methods not detecting serovars used by some firms
- Problems eluting / extracting product adjuvant

Industry Hurdles

- Specific Firm responses (continued)
 - High background response
 - Unsatisfactory reproducibility
 - Concerns regarding reagent availability
 - Increased antigen input when using CVB references

Industry Hurdles

- ◎ Specific firm responses (continued)
 - Unable to meet 800.112 for validation
 - Unsatisfactory threshold of detection
 - Testing adjuvanted product vs an unadjuvanted reference vaccine
 - Can bulk vaccine be tested prior to adjuvanting and blending?
 - Extraction of adjuvant? What will work. Does it effect the parallelism and response of the assay?

Industry Hurdles

- Specific firm responses (continued)
 - Return on investment? Validation of these assays requires considerable resources. Time, money and people are tight for all of us. We are already able to release product with the hamster test. Would our efforts be better spent on new R&D projects?

Closing Review

- ⦿ Industry is at varying stages in conversion
 - Some firms are using ELISA for product release outside of the US
- ⦿ Meeting VS 800.112 validation requirements problematic for most firms
- ⦿ Questions still remain regarding handling the use of unadjuvanted references

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