

# NVSL National Johne's Working Group Update

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Brucella and Mycobacterium Reagents  
Team

# NVSL 2006 Update

- 2005 Serologic Proficiency Testing Update
- Timeline for 2006 Proficiency Testing Process
- Low Positive Controls for ELISA based tests
- Johnin PPD Developmental Project

# 2005 Serologic Proficiency Test Update

# 2005 Serologic Proficiency Test Update

- **New Z-score grading scheme was implemented**
  - Previous method – Positive / Negative identification of samples
  - Z-score method
    - **Qualitative** (Pos/Neg ID) – Critical samples
      - Labs **must** correctly ID all critical samples – any wrong and Lab fails PT
    - **Quantitative** (SP ratio/ELISA score)
      - Labs could miss 2 samples and pass PT

# 2005 Serologic Proficiency Test Update

- **Labs reported results electronically**
  - Greatly reduced risk of transcription errors during data evaluation

# 2005 Serologic Proficiency Test Update

## Determining critical sample vs. Z-score sample

Test Result	IDEXX S/P Ratio	Biocor ELISA Score	Qualitative Score (Positive/Negative)	Quantitative Score (Z-Scored Samples)
Negative	$\leq 0.09$	$\leq 0.49$	100% must be correct	N/A
Inconclusive Positive	0.10 to 0.99	0.50 to 3.49	N/A	$-3 \geq Z\text{-Score} \geq 3$ (Can miss 2 Samples)
Strong Positive	$\geq 1.0$	$\geq 3.50$	100% must be correct	N/A

Note: Biocor ELISA score = (sample OD – mean neg) x 10

# 2005 Serologic Proficiency Test Update

- **IDEXX panel**
  - 15 Critical samples
  - 10 Z-score samples
- **BIOCOR panel**
  - 18 Critical samples
  - 7 Z-score samples

# 2005 Serologic Proficiency Test Update

- 72 Participating US Laboratories
- 9 International Laboratories
  - Canada, Chile, Netherlands
- Some labs had multiple participants:
  - BIOCOR – 43 individuals / 34 labs
  - IDEXX – 57 individuals / 49 labs
  - Other – 3 assays / 2 labs

# 2004 Proficiency Test Results

**NOTE: AFTER  
retests were  
complete**

	<b>BioCor</b>	<b>IDEXX</b>	<b>Other</b>
<b>Number of Individuals Tested</b>	37	53	6
<b>Number of Individuals Receiving a Passing Score</b>	37	53	4
<b>Percentage Receiving a Passing Score</b>	100	100	66.7

# 2005 Proficiency Test Results

**NOTE: AFTER retests were complete**

	<b>BioCor</b>	<b>IDEXX</b>	<b>Other</b>
<b>Number of Individuals Tested</b>	43	57	3
<b>Number of Individuals Receiving a Passing Score</b>	43	56	1
<b>Percentage Receiving a Passing Score</b>	100%	98.2%	33.3%

# 2006 Serologic Proficiency Test

- Timeline
  - Currently working on sample selection
    - Trying to find more z-scored samples
  - Obtain Test Kit orders by mid May 06
  - Distribution of Test Kits in late June 06
  - Report Results in late August 06
- Obtain feedback from labs on new scoring and reporting system

# Low Positive Controls for ELISA Based Tests

# Low Positive Controls for ELISA Based Tests

- Serum Distributed (Jan05 – Dec05)
  - IDEXX low positive control - **123 vials**
  - BIOCOR low positive control - **101 vials**
- Serum Distributed (Jan06 – Mar06)
  - IDEXX low positive control - **82 vials**
  - BIOCOR low positive control - **20 vials**
- 60 Laboratories have ordered low positive controls
  - 38 laboratories since Jan 2005

# Low Positive Controls for ELISA Based Tests

- Continued work...
  - Identify and obtain supply of low positive controls
    - NVSL purchased one cow to keep on site
    - Potential to purchase 2 more for on site
      - Working with Iowa State & Fed vets to find more
    - Working with CVB, NADC, CEAH, and Colorado State University to obtain large volumes of serum and feces for NVSL inventory
    - **Please contact us if other sources are available!!!**

# Low Positive Controls for ELISA Based Tests

- Continued work...
  - Collaborate with NJWG, CEAH, and CVB
  - Encourage laboratories to submit data for analysis
    - Potential to develop incentive program
    - Serum at no charge if data is submitted to CEAH quarterly
  - Work with kit manufacturers to review data

# Johnin PPD Developmental Project

# Johnin PPD Project

## ■ History

- 1998 – 2000 : NVSL / ISU collaborative production of 4 batches of Johnin PPD
- TCA precipitation method
- Evaluation of batches has continued and shown variable results
  - Protein analysis
  - In vivo cattle and sheep studies
  - Guinea pig potency assay

# Johnin PPD Project

- 2005 USAHA Johnine's Committee
  - USAHA Resolution No. 20
    - NVSL develop a systematic protocol for the production of a consistent, quality Johnin PPD
    - NVSL produce Johnin PPD for research evaluation
  - Potential Use for further research
    - Skin test product
    - INF- $\gamma$  protein

# Johnin PPD Project

- Planning Meeting
  - USDA / NVSL
    - Capsel, Marquardt, Carter, Kinker, Bunn, Schleicher
  - USDA / NADC
    - Stabel, Paustian
  - ISU
    - Robbe-Austerman, Thoen

# Johnin PPD Project

- Developing Protocol
  - Randy Capsel & Janet Marquardt
  - Considerations:
    - Heat kill vs. sterile filtration of culture
    - Evaluation methods??
      - **Protein Characterization**
      - **Guinea Pig Potency Assay**
      - **In vivo evaluation - cattle & sheep**
      - **International reference for comparison**

# Johnin PPD Project

- Next Steps...
  - Determine protocol
  - Produce small batches for evaluation
  - Identify optimal production batch
  - Attempt larger scale production
  - Determine Use???
    - Need more research and evaluation before determination of potential use in the Johne's Program

# Questions??

# Contact Information

## ■ BMRT

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