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## NATIONAL BRUCELLOSIS STANDARD TESTING PROTOCOL

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SURVEILLANCE PREPAREDNESS AND RESPONSE  
SERVICES  
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## Overview

- Remind everyone again
- Reasons for national testing protocol
- Benefits of standardization
- Protocol for cattle, bison and cervids
- 2013 National SOP published
- New VS Guidance
- What if you don't use it?
- Questions?





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## Historical Serologic Protocols

- Not consistent across labs
- Different numbers and types of tests used in each state
- Can they all be equivalent quality?

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*“Where’s the rivanol? I love the rivanol!”*

*“What the Hell do you know about Bangs testing?”*

*“The Bangs is coming back!!”*

*“What? No card test!”*

*“You idiot!!”*



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## Loss of Control

- *“I don’t mind if everyone uses the same protocol as long as ...the protocol we use is mine!”*



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## Historical Serologic Protocols

Test	Number of states using test (out of 32 unique state responses)	Percent
BAPA	23	71.9%
RST	1	3.1%
RAP	19	59.4%
CARD	8	25.0%
CITE	1	3.1%
FPA	2	6.3%
Other	1	3.1%

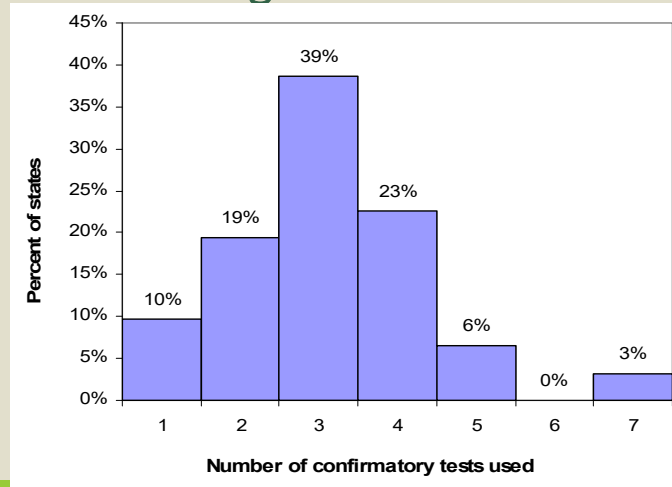
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## Historical Serologic Protocols



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## Situation Prior to Standardization

- Prior to 2007, not consistent across labs
- Different numbers and types of tests used in each state
- Interpretation of tests varies?
  - Parallel testing increases sensitivity
  - Series testing increases specificity
- Can they all be equivalent quality?

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## National Standardized Testing Protocol

Test	Studies	N	Sensitivity (Mean)	Specificity (Mean)	LR(+)
BAPA	15	60,634	95.4%	97.7%	<b>37</b>
Card	11	6434	90%	55%	2
Rivanol	12	4,845	89%	63%	2.4
FPA	7	39,934	97.5%	99%	<b>97.5</b>
CF	38	28,537	89%	83.5%	5.4

Source: Gall D and K Nielson. Rev. sci. tech. Off. Int. Epiz. 2004, 23(3), 989-1002



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## National Standardized Testing Protocol

	Total number tested	1,000,000
10 infected	Prevalence	0.0010%
Test name	Parallel interpretation	
RAP	Sensitivity	99.99%
FPA	Specificity	80.68%
CF	Number false negative	0
	Number false positive	193,177

Source: Eric Ebel, VS Epidemiologist, 2002



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## National Standardized Testing Protocol

	Total number tested	1,000,000
10 infected	Prevalence	0.0010%
Test name	<b>Series interpretation</b>	
RAP	Sensitivity	82.78%
FPA	Specificity	99.996%
CF	Number false negative	2
	Number false positive	42

Source: Eric Ebel, VS Epidemiologist, 2002

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## National Standardized Testing Protocol

	Total number tested	1,000,000
10 infected	Prevalence	0.0010%
Test name	<b>'Current' interpretation (2007 confirmatory parallel)</b>	
RAP	Sensitivity	95.14%
FPA	Specificity	99.60%
CF	Number false negative	0
	Number false positive	4,006

Source: Eric Ebel, VS Epidemiologist, 2002

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## National Standardized Testing Protocol

	Total number tested	1,000,000
10 infected	Prevalence	0.0010%
Test name	RAP > FPA in Series alone	
RAP	Sensitivity	93.02%
FPA	Specificity	99.97%
CF	Number false negative	1
	Number false positive	253

Note: CF is supplemental information for classification

Source: Eric Ebel, VS Epidemiologist, 2002

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## National Standardized Testing Protocol

- Due to extremely low prevalence the US program requires very high specificity (minimize costs of false positives)
- Standardized Testing protocol (2014) =
  - BAPA/RAP >> FPA in series
  - to maximize specificity, minimize cost
- Screening test, if positive, followed by confirmatory test

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## National Standardized Testing Protocol

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Classification of Brucellosis Tests in US testing protocol:

- **Screening Test**
  - BAPA
  - RAP
- **Primary Confirmatory Test**
  - FPA
- **Secondary Confirmation Test**
  - Complement Fixation
- **Supplemental Test**
  - 8% Card
  - ELISA/BRT/HIRT
  - Plate/Standard Plate Test
  - Rivanol
  - Tube/Standard Tube Test
  - Western Blot

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## National Standardized Testing Protocol

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- We calculated an expected responder rate of about 25 per 100,000 samples tested should be seen with our current protocol...
- ...but we actually only detect about one half or less than that at slaughter = ~10 responders per 100,000.

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## National Standardized Testing Protocol

### 2013 Review of Brucellosis Slaughter Lab Responder rates

Lab	Testable Samples	Total FPA tests	FPA(+) Rate*
2012			
KY Lab	1,181,166	24	2.0
TX Lab	1,515,444	122	8.1
2016			
KY Lab	855,522	21	2.4
TX Lab	628,305	71	11

\* FPA response rates are reported in FPA positives per 100,000 samples

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## National Standardized Testing Protocol

- The average responder rate of the national testing protocol is roughly .01% or 10 per 100,000 or a specificity of around 99.99%.
- Significant variations away from 10 responders per 100,000 will trigger further investigation
- 2016 responder rate = 6.2 per 100,000


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- Published in 2013
- Official protocol for cattle, bison and cervids

Standard Operating Procedures for Submission and Testing of Brucellosis Serological Specimens  
APHIS/Veterinary Services Approved Brucellosis Laboratories



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### 7.8.2 Non-Negative FPA Specimens

- If a specimen yields a non-negative FPA test interpretation, *the specimen shall be forwarded to NVSL for confirmation....*
- *Disease classification will be made based on official protocol using all available epi info*

Standard Operating Procedures for Submission and Testing of Brucellosis Serological Specimens  
APHIS/Veterinary Services Approved Brucellosis Laboratories



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*NVSL guidance for performing confirmatory testing on a sample from a brucellosis lab that did NOT follow the national testing protocol.*

- If a specimen yields a non-negative test result from a non-approved brucellosis testing protocol, *then the specimen shall be forwarded to NVSL where the official brucellosis protocol will be used*
- *Classification based on standard protocol + epi*

Standard Operating Procedures for Submission and Testing of Brucellosis Serological Specimens  
APHIS/Veterinary Services  
Approved Brucellosis Laboratories



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*NVSL guidance for performing confirmatory testing on a sample from a brucellosis lab that followed the national testing protocol.*

- If a specimen yields a non-negative test result to the screening test only, the NVSL will perform the primary confirmatory test (FPA).
- NVSL will perform secondary confirmatory test (CF) if FPA is non-negative.

Standard Operating Procedures for Submission and Testing of Brucellosis Serological Specimens  
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*NVSL guidance for performing confirmatory testing on a sample from a brucellosis lab that followed the national testing protocol.*

- If a specimen yields non-negative test results to the screening and confirmatory tests, then NVSL will rerun both confirmatory tests if initial test results are provided on 10-4 form.
- If initial test results not provided to NVSL, then confirmatory tests in series.

Standard Operating  
Procedures for Submission  
and Testing of Brucellosis  
Serological Specimens  
APHIS/Veterinary Services  
Approved Brucellosis  
Laboratories



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*NVSL guidance for performing supplemental brucellosis testing*

- Supplemental testing can be completed at NVSL according to the *Standard Operating Procedure* upon request... use VS form 10-4 "Examinations requested."

Standard Operating  
Procedures for Submission  
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## Summary

- There is a national brucellosis testing protocol:  
BAPA/RAP >> FPA
- There is a published brucellosis testing SOP and a new guidance document
- All non-negatives must be confirmed at NVSL
- Follow the protocol!



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Questions?

