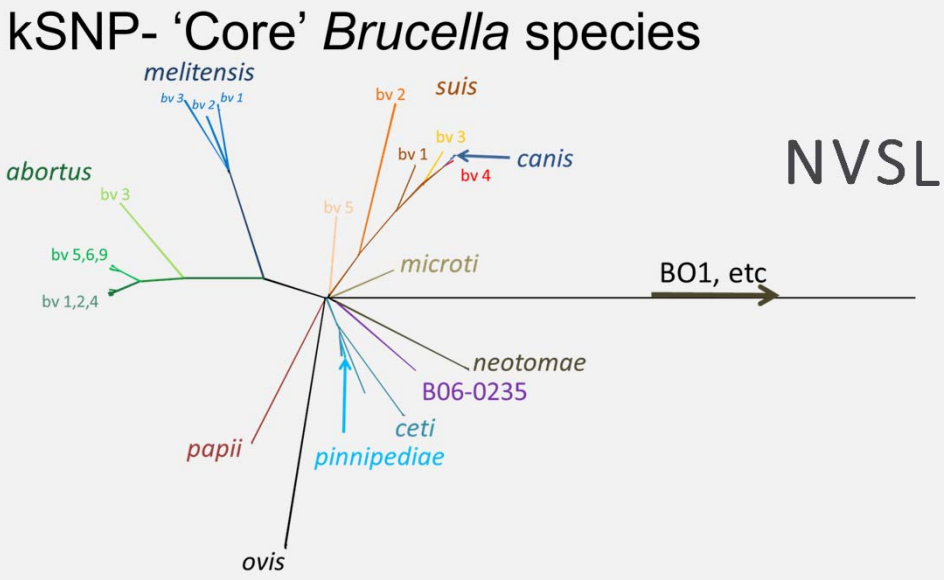
United States Department of Agriculture



NVSL BRUCELLOSIS UPDATE

SUELEE ROBBE-AUSTERMAN
DIRECTOR,
DIAGNOSTIC BACTERIOLOGY AND PATHOLOGY LABORATORY
NATIONAL VETERINARY SERVICES LABORATORIES
U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
10/22/2018

Discussion points

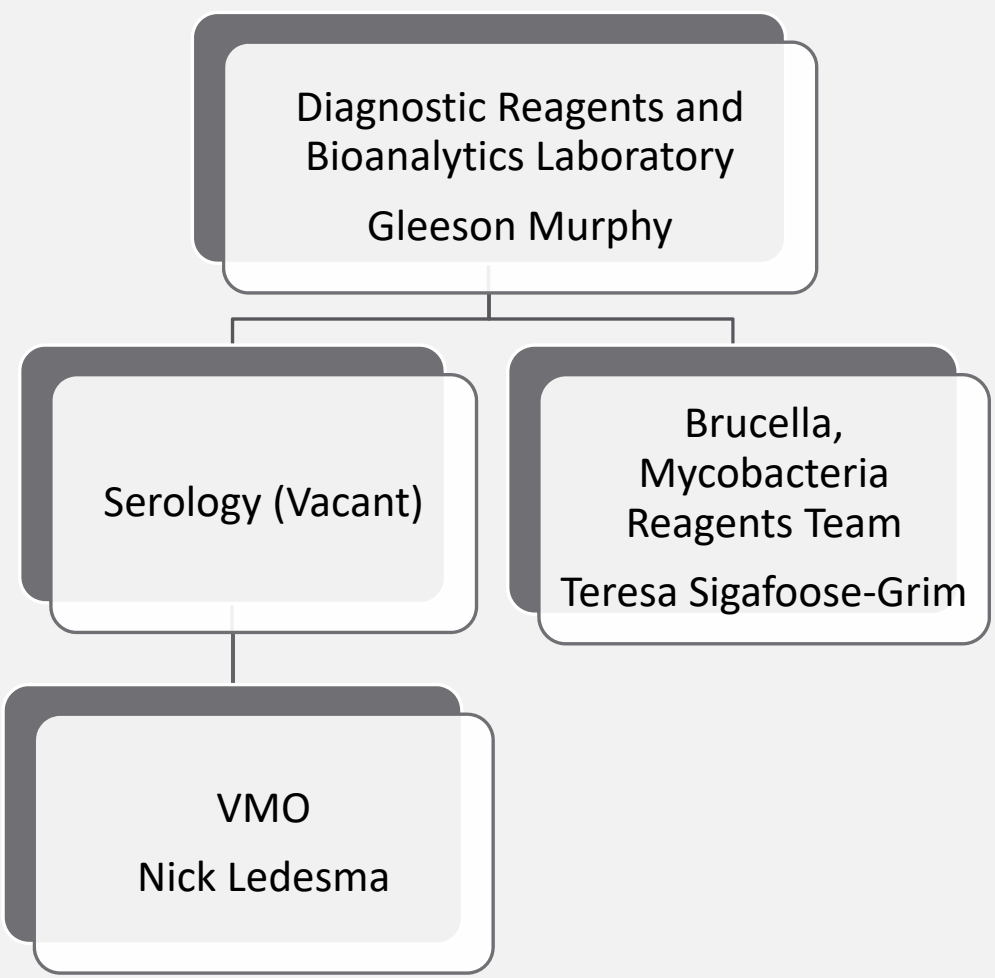
Staff introductions

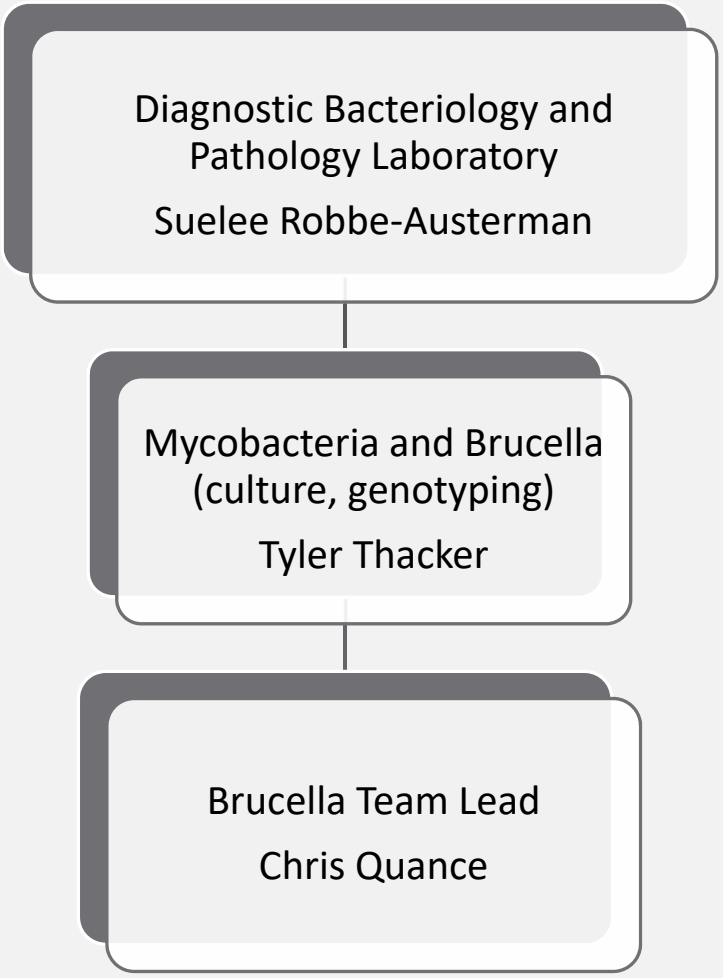
Direct PCR tissue assay at NVSL

Whole genome sequencing – what we can learn about transmission dynamics

Reagent production – Characterizing our reagent strains

Brucellosis testing and reagents at NVSL



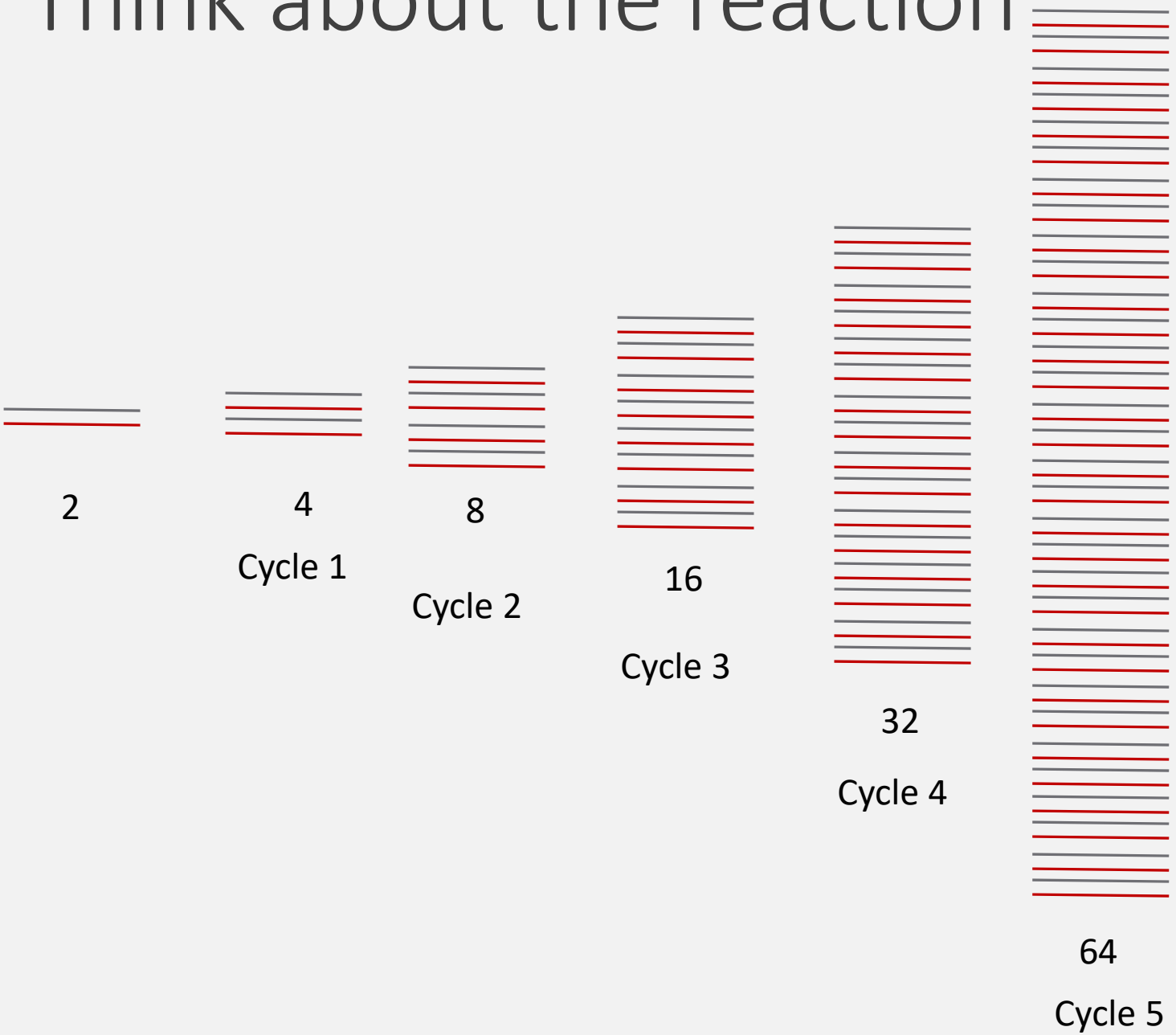


Direct PCR from tissues for Brucella

NVSL has a standard IS711 PCR assay for direct tissue/sample detection using real time PCR.

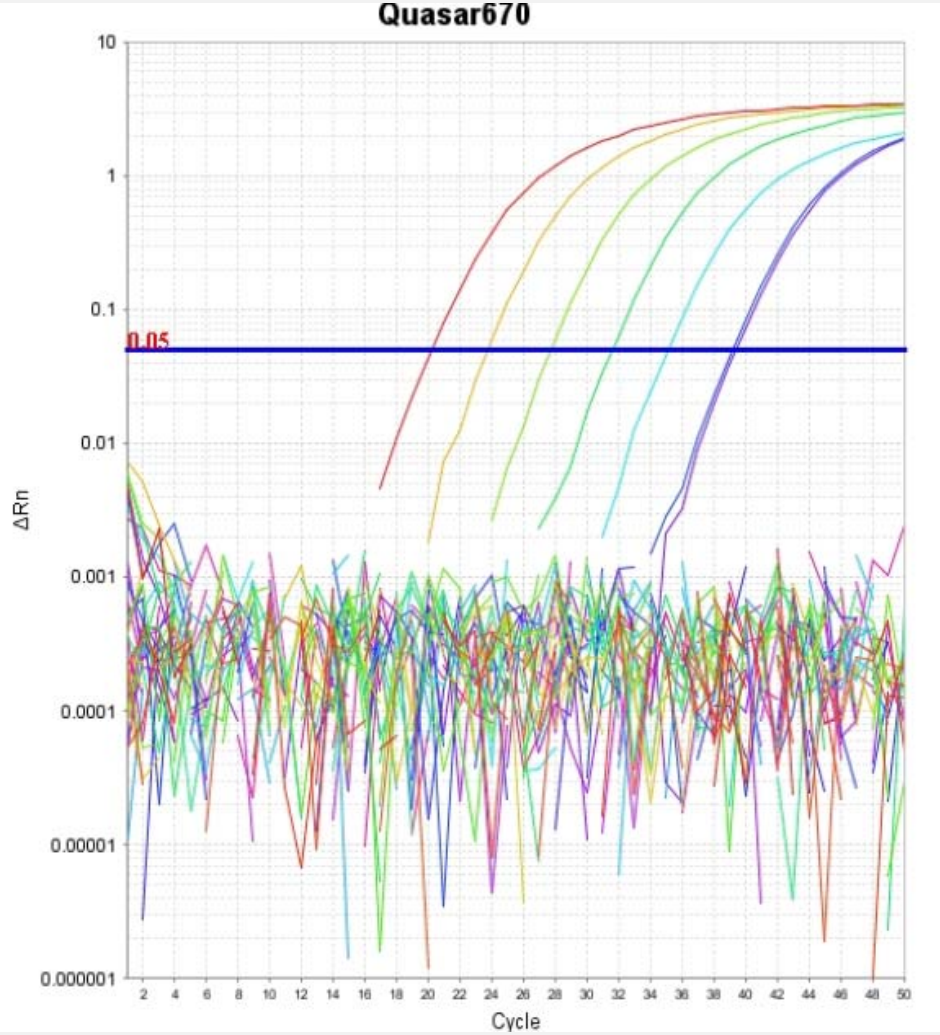
- Real time PCR provides results as a semi quantitative Ct value
  - The lower the Ct, the more DNA

Think about the reaction



	2
Cycle 1	4
Cycle 2	8
Cycle 3	16
Cycle 4	32
Cycle 5	64
Cycle 6	128
Cycle 7	256
Cycle 8	512
Cycle 9	1,024
Cycle 10	2,048
Cycle 11	4,096
Cycle 12	8,192
Cycle 13	16,384
Cycle 14	32,768
Cycle 15	65,536
Cycle 16	131,072
Cycle 17	262,144
Cycle 18	524,288
Cycle 19	1,048,576
Cycle 20	2,097,152
Cycle 21	4,194,304
Cycle 22	8,388,608
Cycle 23	16,777,216
Cycle 24	33,554,432
Cycle 25	67,108,864
Cycle 26	134,217,728
Cycle 27	268,435,456
Cycle 28	536,870,912
Cycle 29	1,073,741,824
Cycle 30	2,147,483,648
Cycle 31	4,294,967,296
Cycle 32	8,589,934,592
Cycle 33	17,179,869,184
Cycle 34	34,359,738,368
Cycle 35	68,719,476,736
Cycle 36	137,438,953,472
Cycle 37	274,877,906,944
Cycle 38	549,755,813,888
Cycle 39	1,099,511,627,776
Cycle 40	2,199,023,255,552

1 Log change = ~3.2 cycles



	2
Cycle 1	4
Cycle 2	8
Cycle 3	16
Cycle 4	32
Cycle 5	64
Cycle 6	128
Cycle 7	256
Cycle 8	512
Cycle 9	1,024
Cycle 10	2,048
Cycle 11	4,096
Cycle 12	8,192
Cycle 13	16,384
Cycle 14	32,768
Cycle 15	65,536
Cycle 16	131,072
Cycle 17	262,144
Cycle 18	524,288
Cycle 19	1,048,576
Cycle 20	2,097,152
Cycle 21	4,194,304
Cycle 22	8,388,608
Cycle 23	16,777,216
Cycle 24	33,554,432

Direct PCR from tissues for Brucella

PCR detection using real time PCR

- Semi quantitative Ct value, the lower the Ct, the more DNA

Significant improvements in chemistry post 2005 that allow for more accuracy and better DNA extraction.

Whole genome sequencing has greatly improved our understanding and the ability to find targets.

But! There is still significant error that can occur within workflows that requires caution!

Performance of TB direct PCR at NVSL

Performed on borate tissue instead of culture for slaughter surveillance

Histopathology is completed in parallel, direct PCR is not reported to the field.

Discrepant samples are reviewed, all samples positive on either test are cultured.

We do perform PCR on culture isolates on the same plate.

Document Number: FM-MB-0080.05
Title: Sample Preparation, Extraction, and Mycobacterial DNA detection by Direct PCR

ead Processing Technician: L Date: 10/17/2018 Page 12 of 15

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 Neg.Cont.	2 Pos.Cont.	3 18-036136	4 18-036137	5 18-036140	6 18-036141	7 18-036147	8 Neg.Cont.	9 Pos.Cont.	10 18-036152	11 18-036156	12 18-036158
B	13 18-036163	14 18-036167	15 Neg.Cont.	16 Pos.Cont.	17 18-036176	18 18-036178	19 18-036182	20 18-036189	21 18-036191	22 Neg.Cont.	23 Pos.Cont.	24 18-036195
C	25 18-036197	26 18-036200	27 18-036202	28 18-036215	29 Neg.Cont.	30 Pos.Cont.	31 18-036216	32 18-036217	33 18-036223	34 18-036225	35 18-036226	36 18-036227
D	37 18-036231	38 18-036235	39 18-036238	40 18-036258	41 Neg.Cont.	42 Pos.Cont.	43 18-036264	44 18-036265	J7 TB18-08903	J8 TB18-08904	J9 TB18-08905	
E												
F	M1 Neg. MGIT	M2 TB18-06288	M3 TB18-06796	M4 TB18-06796	M5 TB18-07276	M6 TB18-07373	M7 Neg. MGIT	M8 TB18-07411	M9 TB18-07452	M10 TB18-08161	M11 TB18-08190	M12 TB18-08245
G	M13 Neg. MGIT	J1 TB18-07665	J2 TB18-07666	J3 TB18-07667	J4 TB18-07668	J5 TB18-07669	J6 TB18-07670					
H												96 M.bovis

Performance of TB direct PCR at NVSL

Performed on borate tissue instead of culture for slaughter surveillance

Histopathology is completed in parallel, direct PCR is not reported to the field.

Discrepant samples are reviewed, all samples positive on either test are cultured.

We do perform PCR on culture isolates on the same plate.

From January 1, 2018 thru Oct 5, 2018

- **4, 543** Slaughter surveillance samples were tested for direct PCR.
- During these runs, we had **952** negative controls on the plates.
- Not one negative control identified cross contamination!
- **22** false positives occurred, **0.48%** false positive rate, **99.52%** observed Sp.
- **15** of those occurred on 1 day.

Negative controls – Effectiveness of identifying false positive samples

Document Number: FM-MB-0080.05
Title: Sample Preparation, Extraction, and Mycobacterial DNA detection by Direct PCR

Analysis by: Kevin Stokes Date: 3/9/2018

File name: 180308_1_MTBC

Print, Process, and Copy to Outside Forms | Print & Process Current Form Only | Print Full Report | U= Undet. | Ct | Valid Run

Well	Accession #	Re-Test	Notes	IC	Target	Result	Redo (X)	L3 (X)
A1	TW Neg.Cont.		TW Neg.Cont.	32.6	U	Not Detected		
A2	TB18-01849		Fluid-Sediment	28.7	U	Not Detected		
A3	TB18-01849		Fluid-Inoculum	29.1	U	Not Detected		
A4	TB18-01850		Fluid-Sediment	28.1	U	Not Detected		
A5	TB18-01850		Fluid-Inoculum	28.1	U	Not Detected		
A6	TB18-01851		Fluid-Sediment	27.9	U	Not Detected		
A7	TB18-01851		Fluid-Inoculum	28.3	37.1	Detected		
A8	TB18-01852		Fluid-Sediment	27.8	U	Not Detected		
A9	TB18-01852		Fluid-Inoculum	28.0	U	Not Detected		
A10	Neg.Cont.			31.8	U	Not Detected		
A11	Pos.Cont.			33.5	31.6	Detected		
A12	18-007967			33.6	U	Not Detected		
B1	18-007970			34.5	U	Not Detected		
B2	18-007974			34.3	28.8	Detected		X
B3	18-007977			32.8	U	Not Detected		
B4	18-007980			33.6	U	Not Detected		
B5	Neg.Cont.			32.0	U	Not Detected		
B6	Pos.Cont.			32.5	31.2	Detected		
B7	18-007981			33.4	U	Not Detected		
B8	18-007984			33.2	37.7	Detected	X	
B9	18-007988			33.7	U	Not Detected		
B10	18-007990			34.2	37.2	Detected	X	
B11	18-007996			33.2	U	Not Detected		
B12	Neg.Cont.			33.0	U	Not Detected		
C1	Pos.Cont.			33.2	32.5	Detected		
C2	18-007997			35.7	U	Not Detected		
C3	18-007999			35.0	U	Not Detected		
C4	18-008001			33.2	36.8	Detected	X	
C5	18-008002			32.0	36.7	Detected	X	
C6	18-008005			33.1	36.8	Detected	X	
C7	Neg.Cont.			31.5	U	Not Detected		
C8	Pos.Cont.			33.5	31.2	Detected		
C9	18-008006			35.0	U	Not Detected		
C10	18-008008			33.9	35.5	Detected	X	
C11	18-008010			34.1	27.8	Detected		X

Negative controls – Effectiveness of identifying false positive samples

Document Number: FM-MB-0080.05
Title: Sample Preparation, Extraction, and Mycobacterial DNA detection by Direct PCR

Analysis by: Kevin Stokes Date: 3/9/2018

File name: 180308_1_MTBC

Print, Process, and Copy to Outside Forms | Print & Process Current Form Only | Print Full Report | U= Undet. | Valid Run

Well	Accession #	Re-Test	Notes	IC Ct	Target Ct	Result	Redo (X)	L3 (X)
A1	TW Neg.Cont		TW Neg.Cont.	32.6	U	Not Detected		
A2	TB18-01849		Fluid-Sediment	28.7	U	Not Detected		
A3	TB18-01849		Fluid-Inoculum	29.1	U	Not Detected		
A4	TB18-01850		Fluid-Sediment	28.1	U	Not Detected		
A5	TB18-01850		Fluid-Inoculum	28.1	U	Not Detected		
A6	TB18-01851		Fluid-Sediment	27.9	U	Not Detected		
A7	TB18-01851		Fluid-Inoculum	28.3	37.1	Detected		
A8	TB18-01852		Fluid-Sediment	27.8	U	Not Detected		
A9	TB18-01852		Fluid-Inoculum	28.0	U	Not Detected		
A10	Neg.Cont.			31.8	U	Not Detected		
A11	Pos.Cont.			33.5	31.6	Detected		
A12	18-007967			33.6	U	Not Detected		
B1	18-007970			34.5	U	Not Detected		
B2	18-007974			34.3	28.8	Detected		X
B3	18-007977			32.8	U	Not Detected		
B4	18-007980			33.6	U	Not Detected		
B5	Neg.Cont.			32.0	U	Not Detected		
B6	Pos.Cont.			32.5	31.2	Detected		
B7	18-007981			33.4	U	Not Detected		
B8	18-007984			33.2	37.7	Detected	X	
B9	18-007988			33.7	U	Not Detected		
B10	18-007990			34.2	37.2	Detected	X	
B11	18-007996			33.2	U	Not Detected		
B12	Neg.Cont.			33.0	U	Not Detected		
C1	Pos.Cont.			33.2	32.5	Detected		
C2	18-007997			35.7	U	Not Detected		
C3	18-007999			35.0	U	Not Detected		
C4	18-008001			33.2	36.8	Detected	X	
C5	18-008002			32.0	36.7	Detected	X	
C6	18-008005			33.1	36.8	Detected	X	
C7	Neg.Cont.			31.5	U	Not Detected		
C8	Pos.Cont.			33.5	31.2	Detected		
C9	18-008006			35.0	U	Not Detected		
C10	18-008008			33.9	35.5	Detected	X	
C11	18-008010			34.1	27.8	Detected		X

False positive summary- NVSL TB

From January 1, 2018 thru Oct 5, 2018

- **4, 543** Slaughter surveillance samples were tested for direct PCR.
- During these runs, we had **952** negative controls on the plates.
- Not one negative control identified cross contamination!
- **22** false positives occurred, **0.48%** false positive rate, **99.52%** observed Sp.
- **15** of those occurred on 1 day.

Error occurs all the time, this is not routinely reported in the literature.

Here is one example:

Pertussis Pseudo-outbreak linked to specimens contaminated by Bordetella pertussis DNA From clinic surfaces.

- Pediatrics. 2012 Feb;129(2):e424-30. doi: 10.1542/peds.2011-1710. Epub 2012 Jan 16.

Collection of samples – instruments a source of cross contamination?

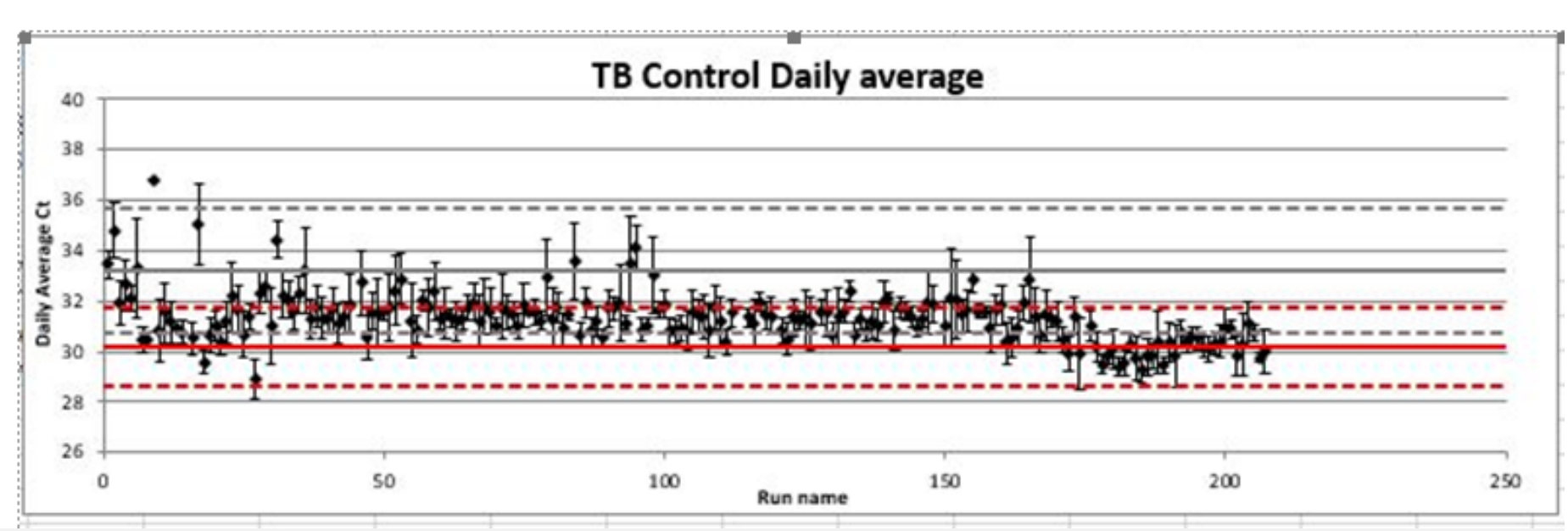
Baja CA, >90% of the samples are histology and culture positive, and we have more PCR discrepancies than expected

F5	negcont		Culture Isolate; TB; Other; cPCR Only;	32.9	U	Not Detected	
F6	TB18-06989		Culture Isolate; TB; Other; cPCR Only;	30.9	18.7	Detected	
F7	negcont		Culture Isolate; TB; Other; cPCR Only;	32.3	U	Not Detected	
F8	TB18-06990		Culture Isolate; TB; Other; cPCR Only;	31.5	29.1	Detected	
F9	negcont		Culture Isolate; TB; Other; cPCR Only;	31.9	U	Not Detected	
F10	TB18-06991		Culture Isolate; TB; Other; cPCR Only;	30.6	16.7	Detected	
F11	negcont		Culture Isolate; TB; Other; cPCR Only;	30.6	U	Not Detected	
F12	TB18-06992		Culture Isolate; TB; Other; cPCR Only;	30.3	16.8	Detected	
G1	negcont		Culture Isolate; TB; Other; cPCR Only;	32.5	U	Not Detected	
G2	TB18-06993		Culture Isolate; TB; Other; cPCR Only;	34.1	30.5	Detected	
G3	negcont		Culture Isolate; TB; Other; cPCR Only;	32.0	U	Not Detected	
G4	TB18-06994		Culture Isolate; TB; Other; cPCR Only;	30.8	31.5	Detected	
G5	negcont		Culture Isolate; TB; Other; cPCR Only;	31.8	U	Not Detected	
G6	TB18-06995		Culture Isolate; TB; Other; cPCR Only;	31.2	20.8	Detected	
G7	TB18-07135		Culture Isolate; TB; Other; cPCR Only;	32.0	U	Not Detected	
G8	negcont		Culture Isolate; TB; Other; cPCR Only;	31.6	U	Not Detected	
G9	TB18-07136		Culture Isolate; TB; Other; cPCR Only;	33.9	U	Not Detected	
G10	negcont		Culture Isolate; TB; Other; cPCR Only;	30.5	U	Not Detected	
G11	TB18-07137		Culture Isolate; TB; Other; cPCR Only;	37.4	28.3	Detected	
G12	negcont		Culture Isolate; TB; Other; cPCR Only;	U	U	Not Detected	
H1	TB18-07138		Culture Isolate; TB; Other; cPCR Only;	32.6	15.1	Detected	
H2	negcont		Culture Isolate; TB; Other; cPCR Only;	32.8	U	Not Detected	
H3	TB18-07139		Culture Isolate; TB; Other; cPCR Only;	32.4	27.8	Detected	
H4	negcont		Culture Isolate; TB; Other; cPCR Only;	31.3	U	Not Detected	
H5	TB18-07140		Culture Isolate; TB; Other; cPCR Only;	31.7	35.1	Detected	
H6	negcont		Culture Isolate; TB; Other; cPCR Only;	32.6	U	Not Detected	

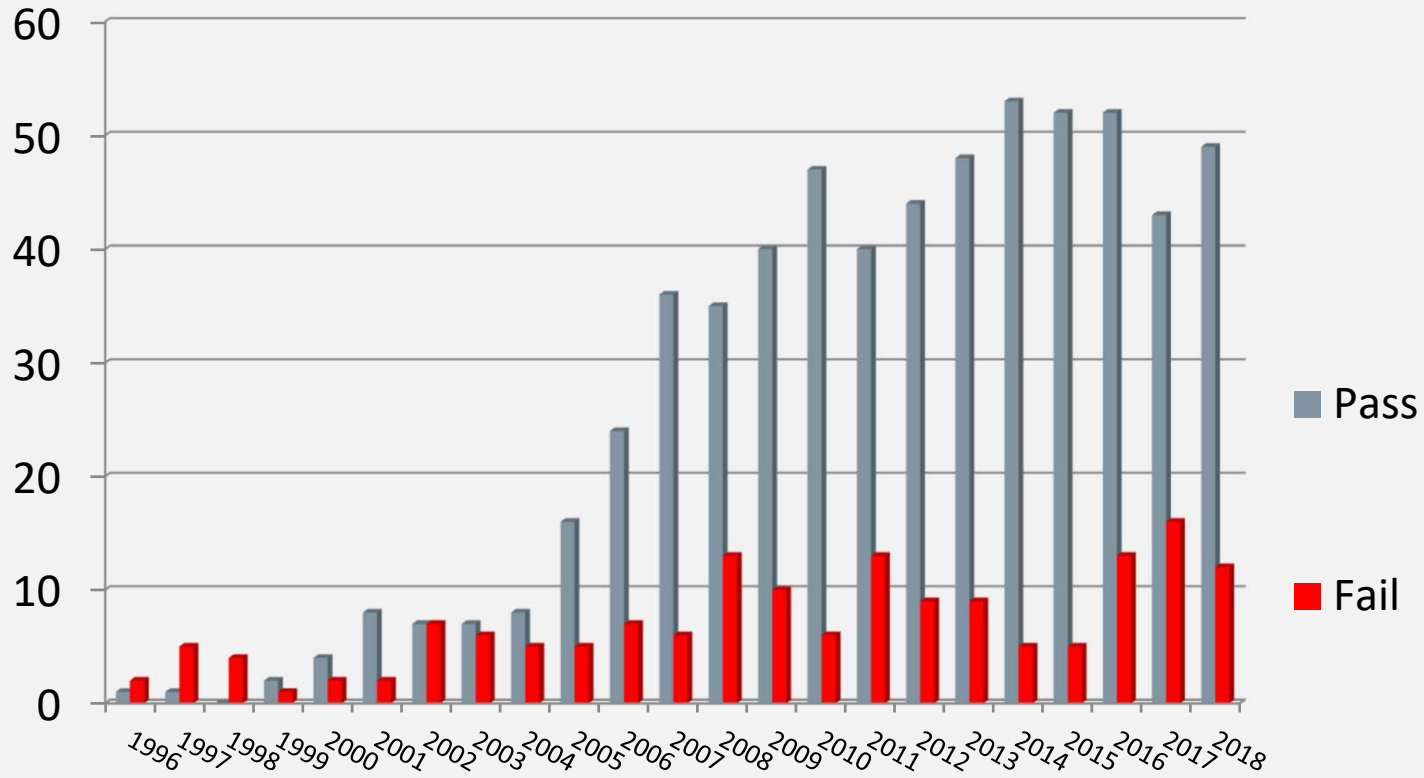
Environmental BSC testing – TB, Ct values

	Cleaning		
	Before	**After**	**Difference**
317 GLOVE	***27.49***	30.38	-2.88
317 Hood Floor	35.36	34.18	**1.18**
317 SLEEVE	***26.16***	31.51	-5.35
316 GLOVE	31.08	32.12	-1.04
316 Hood Floor	33.20	**Not detected**	-33.20
316 SLEEVE	31.21	32.61	-1.40
317 Hood Floor	***22.25***	**Not detected**	-22.25
317 Right Glove	***28.26***	32.36	-4.11
317 Left Glove	31.57	***28.53***	**3.05**
316 Hood Floor	**Not detected**	**Not detected**	0.00
316 Left Glove	34.60	35.59	-0.99
316 Right Glove	33.33	35.32	-1.99
317 Hood Floor	37.69	36.59	**1.10**
317 Left Glove	35.48	36.67	-1.19
317 Right Glove	34.63	34.79	-0.16
316 Hood Floor	39.33	**Not detected**	-39.33
316 Left Glove	32.85	32.93	-0.08
316 Right Glove	32.67	35.67	-3.00
317 Hood Floor	38.88	**Not detected**	-38.88

Track and trend



Johne's direct PCR – false positives continue to be the primary reason for failure



Summary of direct PCR for Brucella

Open PCR platforms are at high risk for sporadic false positives that are difficult to detect.

- Consequently, robust evaluations must be done at each laboratory that implements a direct PCR. (Se/Sp estimations will be lab specific)
- Our evaluation at NVSL suggests we have an unacceptably high false positive rate for direct detection of Brucella based on the suggested protocols.

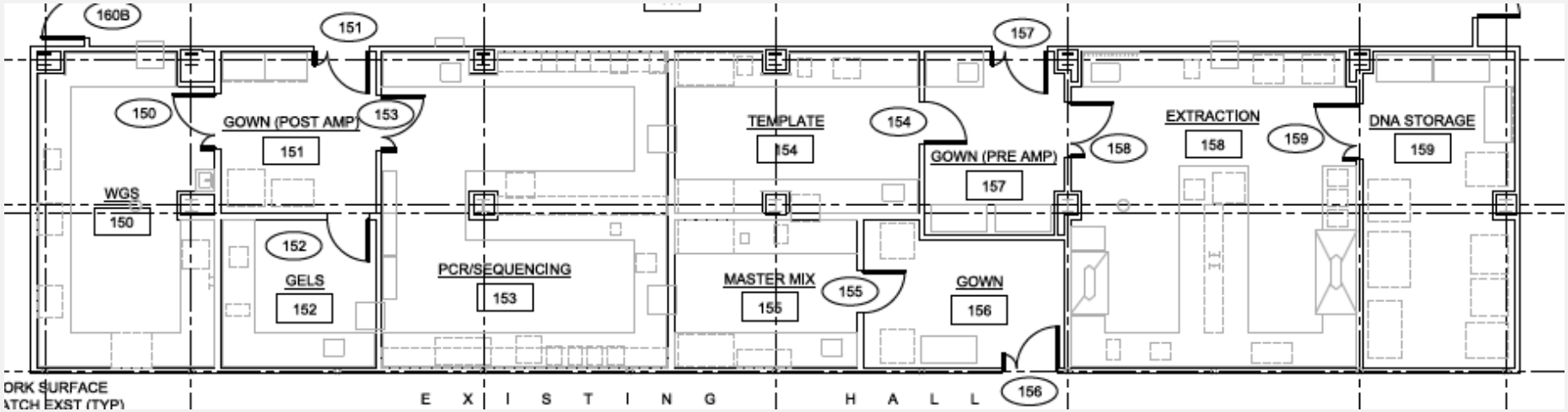
Testing multiple tissues or samples from the same animal compounds the false positive rate.

It is critical to continue the research and evaluation of direct PCR

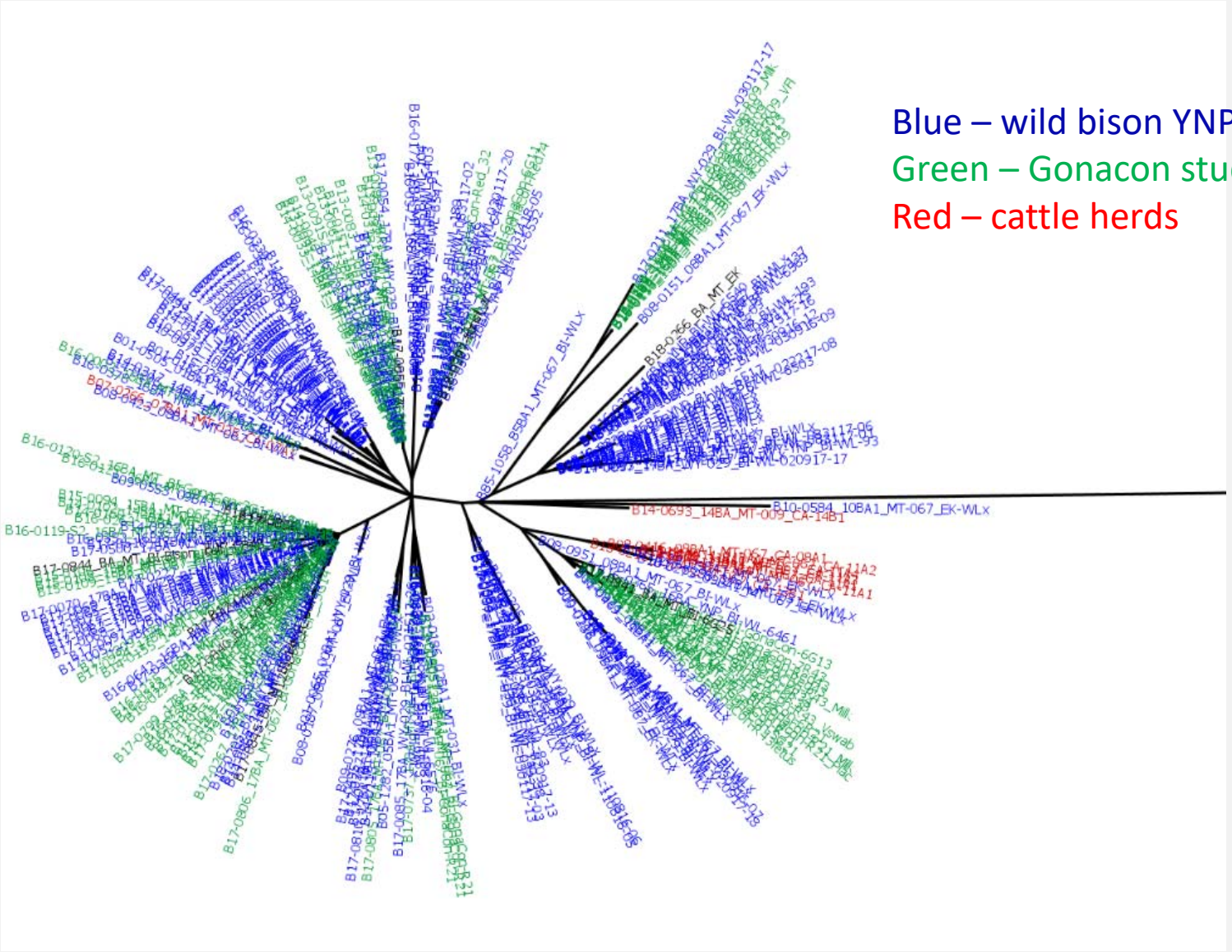
- Robust method of identifying false positive samples by adjusting study designs. (checker board designs)
- Consider more robust (simple) workflows and innovative closed platforms

NVSL will continue to support this work where ever possible.

NVSL Future – dedicated molecular suite



Transmission Dynamics



Blue – wild bison YNP, ELK- MT
Green – Gonacon study
Red – cattle herds

The GonaCon study – opportunity to evaluate transmission patterns using WGS

Landmark study – The most thorough multi-year (2013-2017) brucellosis study in USDA history.

- Serology 2x year
- At calving: milk, vaginal swabs, calf ocular swabs, feces cultured.
- Abortions were submitted for culture
- Bison were euthanized and submitted for culture when death occurred or in 2017.

Results

BANGLE TAG	Age/DOB	2013	2014	2015	2016	2017	Necropsy
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

5/13 (38%) seropositive cows had normal calving events for 5 years

1 became seronegative

1 was Necropsy culture positive with a unique genotype (others are pending)

1/5 seronegative did not have an abortion or weak calf

Results

BANGLE TAG	Age/DOB	2013	2014	2015	2016	2017	Necropsy
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

3/13 bison (23%) had multiple confirmed Brucella abortions

Results

BANGLE TAG	Age/DOB	2013	2014	2015	2016	2017	Necropsy
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

76 calving events, 20 abortion/weak calf events (26% abortion rate)

15 Brucella positive abortions/weak calves (20% confirmed Brucella abortions)

56 normal calving events, 7 (12.5%) Brucella culture positive

Results

		WGS					
BANGLE TAG	**Age/DOB**	**2013**	**2014**	**2015**	**2016**	**2017**	**Necropsy**
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

Genotype 1 was isolated from 5 bison

Results

		WGS					
BANGLE TAG	**Age/DOB**	**2013**	**2014**	**2015**	**2016**	**2017**	**Necropsy**
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

Genotype 3 was isolated from 4 bison

Results

		WGS					
BANGLE TAG	**Age/DOB**	**2013**	**2014**	**2015**	**2016**	**2017**	**Necropsy**
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

Genotype 2 was isolated from 2 bison

Results

		WGS					
BANGLE TAG	**Age/DOB**	**2013**	**2014**	**2015**	**2016**	**2017**	**Necropsy**
Green 08	8, 2009	Calf -	Calf -	no calf	Calf -	Calf -	
Green 09	6, 2010	WC = Geno 1	Open	Calf -	Calf -		Neg
Green 10	6, 2009	AB = Geno 1	AB -				
Green 15	7, 2010	AB -	AB = Geno 1	Calf -	Calf -	Calf -	
Green 14	8, 2009	Calf -	AB = Geno 3	AB Geno3+1,2	AB = Geno3+1	Calf = Geno 3-2	
Red 03	8, 2009	AB-Geno 1	Calf = Geno 1	Calf = Geno 1	Calf -	Calf -	
Red 06	7, 2010	No calf	Calf -	AB = Geno 3	Calf = Geno 3	Open	
Red 08	58 2009	Calf -	AB = Geno 3	AB = Geno 3+1	Calf -	AB -	
Red 09	6, 2011	Open	Open	AB = Geno 2	AB = Geno 2	AB -	
Red 13	7, 2010	Calf = Geno 3	Calf -	Calf -	AB = Geno 2	Calf -	
Red 21	8, 2009	Calf = Geno 5	Calf -	AB = Geno 6-1	Calf =Geno 6-2	Calf -	
Red 16	7, 2010	AB - Geno 1	Calf -	Calf -	Calf -	Calf -	
Red 20	8, 2009	Calf -	Open	AB -	No calf	Calf -	
Red 07	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 22	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 24	8, 2009	Calf -	Calf -	Calf -	Calf -	Calf -	
Red 26	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	
Red 18	8, 2009	Calf -	Open	Calf -	Calf -	Calf -	Geno 4

Genotype 5, 6 were isolated from the same bison 2 years apart

Reagent production

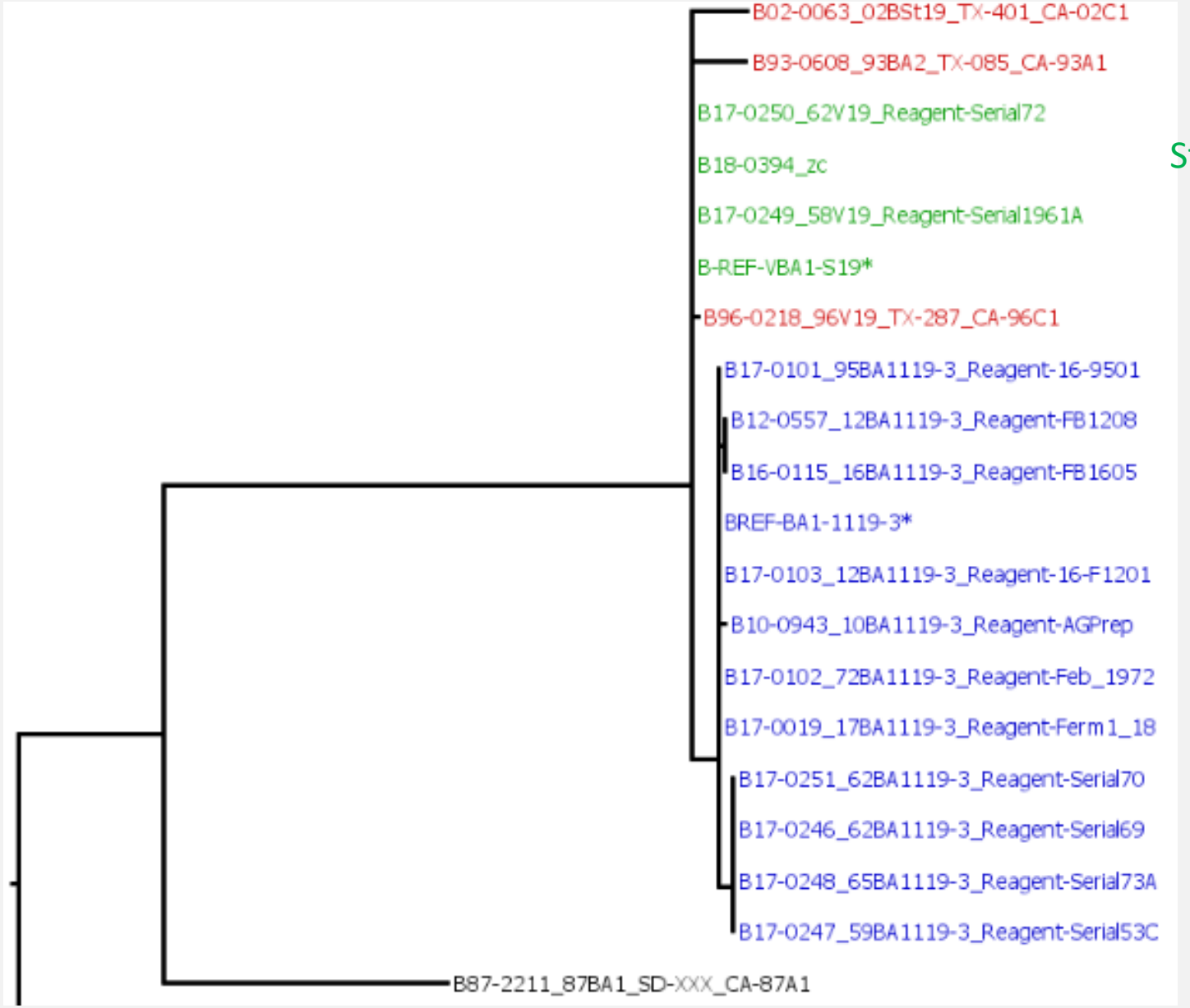
OIE allows Brucella reagents to be produced from two strains of Brucella:

- Strain S99 produced by AHVLA
- Strain 1119-3 produced by NVSL

Part of upgrading our quality and strain documentation, NVSL has been characterizing seed strains, reference strains and reagent production strains.

Strain 19 and our reagent production strain 1119-3 has been indistinguishable on all tests for years.

Reagent production

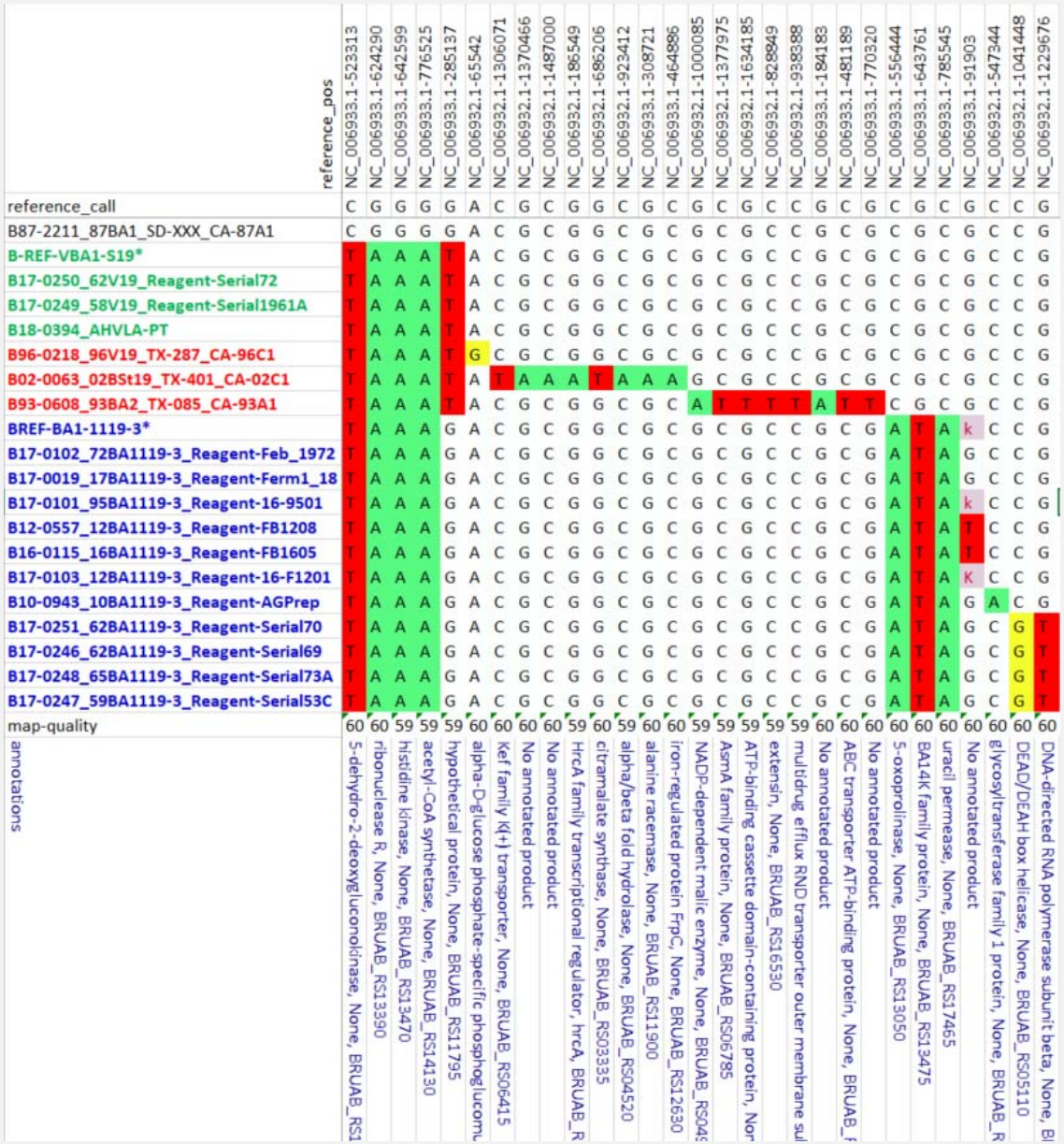


S-19 from vaccinated cattle

Strain 19

Strain 1119-3

Reagent production



reference_pos	NC_006933.1-523313	NC_006933.1-624290	NC_006933.1-642599	NC_006933.1-776525	NC_006933.1-285137	NC_006932.1-65542	NC_006932.1-1306071	NC_006932.1-1370466	NC_006932.1-1487000	NC_006932.1-186549	NC_006932.1-686206	NC_006932.1-923412	NC_006933.1-308721	NC_006933.1-464886	NC_006932.1-1000085	NC_006932.1-1377975	NC_006932.1-1634185	NC_006932.1-828849	NC_006932.1-938388	NC_006933.1-184183	NC_006933.1-481189	NC_006933.1-770320	NC_006933.1-556444	NC_006933.1-643761	NC_006933.1-785545	NC_006933.1-91903	NC_006932.1-547344	NC_006932.1-1041448	NC_006932.1-1229676
reference_call	C	G	G	G	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B87-2211_87BA1_SD-XXX_CA-87A1	C	G	G	G	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B-REF-VBA1-S19*	T	A	A	A	T	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B17-0250_62V19_Reagent-Serial72	T	A	A	A	T	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B17-0249_58V19_Reagent-Serial1961A	T	A	A	A	T	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B18-0394_AHVLA-PT	T	A	A	A	T	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B96-0218_96V19_TX-287_CA-96C1	T	A	A	A	T	G	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B02-0063_02BSt19_TX-401_CA-02C1	T	A	A	A	T	A	T	A	A	A	T	A	A	A	G	C	G	C	C	G	C	G	C	G	C	G	C	C	G
B93-0608_93BA2_TX-085_CA-93A1	T	A	A	A	T	A	C	G	C	G	G	C	G	C	A	T	T	T	T	A	T	T	C	G	C	G	C	C	G
BREF-BA1-1119-3*	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	k	C	C	G
B17-0102_72BA1119-3_Reagent-Feb_1972	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	C	C	G
B17-0019_17BA1119-3_Reagent-Ferm1_18	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	C	C	G
B17-0101_95BA1119-3_Reagent-16-9501	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	k	C	C	G
B12-0557_12BA1119-3_Reagent-FB1208	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	T	C	C	G
B16-0115_16BA1119-3_Reagent-FB1605	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	T	C	C	G
B17-0103_12BA1119-3_Reagent-16-F1201	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	K	C	C	G
B10-0943_10BA1119-3_Reagent-AGPrep	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	A	C	G
B17-0251_62BA1119-3_Reagent-Serial70	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	C	G	T
B17-0246_62BA1119-3_Reagent-Serial69	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	C	G	T
B17-0248_65BA1119-3_Reagent-Serial73A	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	C	G	T
B17-0247_59BA1119-3_Reagent-Serial53C	T	A	A	A	G	A	C	G	C	G	G	C	G	C	G	C	G	C	C	G	C	G	A	T	A	G	C	G	T
map-quality	60	60	59	59	59	60	60	60	60	59	60	59	60	60	59	59	59	59	59	60	60	60	60	60	60	60	60	60	60
annotations	5-dehydro-2-deoxygluconokinase, None, BRUAB_RS1	ribonuclease R, None, BRUAB_RS13390	histidine kinase, None, BRUAB_RS13470	acetyl-CoA synthetase, None, BRUAB_RS14130	hypothetical protein, None, BRUAB_RS11795	alpha-D-glucose phosphate-specific phosphoglucomu	Kef family K(+) transporter, None, BRUAB_RS06415	No annotated product	No annotated product	HrcA family transcriptional regulator, hrcA, BRUAB_R	citramalate synthase, None, BRUAB_RS03335	alpha/beta fold hydrolase, None, BRUAB_RS04520	alanine racemase, None, BRUAB_RS11900	iron-regulated protein FrpC, None, BRUAB_RS12630	NADP-dependent malic enzyme, None, BRUAB_RS045	AsmA family protein, None, BRUAB_RS06785	ATP-binding cassette domain-containing protein, Nor	extensin, None, BRUAB_RS16530	multidrug efflux RND transporter outer membrane su	No annotated product	ABC transporter ATP-binding protein, None, BRUAB_f	No annotated product	5-oxoprolinase, None, BRUAB_RS13050	BA14K family protein, None, BRUAB_RS13475	uracil permease, None, BRUAB_RS17465	No annotated product	glycosyltransferase family 1 protein, None, BRUAB_R	DEAD/DEAH box helicase, None, BRUAB_RS05110	DNA-directed RNA polymerase subunit beta, None, Bl

Reagent production

What does this mean?

- Strain 1119-3 is a subculture of Strain 19. All evidence suggests it was a subculture from the beginning.
- History of this is not clear…. It appears that S1119-3 was evaluated at the time and found to be “superior” in reagent production.
- S99 the other OIE approved reagent production strain is a wild-type strain.
- USDA successfully eradicated Brucellosis in cattle and commercial swine using Strain 1119-3.
- Based on this work, Strain 1119-3 should be an exempt Select Agent Strain 19.
- But what about our international partners? Should they still use Strain 1119-3, or move to AHVLA Strain 99?
- Serology Evaluated reagents produced from both:

Serology Results

- Preliminary Data
  - 2000 presumed negative cattle sera
  - Cold complement fixation method (more sensitive)
  - Screened at 1:5 and 1:10 dilutions
- Complement fixation antigen comparison
  - NVSL Strain 1119-3 & AHVLA Strain 99
  - 1981 tested sera were in concordance
  - 9 samples were discrepant
  - 9 samples were anticomplementary

Serology Brucella Testing Scheme

Cattle/Swine/Cervids

- Screening with BAPA (or RAP)
  - Non-negatives confirmed by FPA
  - Non-negative FPA (including those run by state labs) confirmed by CF
  - Screening testing not repeated

Sheep/Goats

- Screening with 3% card is recommended (not 8%)
  - Mikolon, Andrea B., et al. "Evaluation of North American antibody detection tests for diagnosis of brucellosis in goats." Journal of clinical microbiology 36.6 (1998): 1716-1722.
- Non-negatives confirmed by FPA*
- Non-negative FPA confirmed by CF*

*Assays not currently validated for these species

Suelee Robbe-Austerman
Director, DBPL
National Veterinary Services Laboratories
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
Suelee.Robbe-Austerman@usda.gov

Nick Ledesma
VMO – Serology Section, DBRL
National Veterinary Services Laboratories
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
Nicholas.A.Ledesma@usda.gov