2022 Johne's Disease Fecal Proficiency Test
General Summary
October 31, 2022

Overview

A total of 53 laboratories ordered panels during the 2022 Johne's Disease Fecal Proficiency Test (6 Canadian, 3 European Union, 1 Australian, 1 Japanese and 42 USA laboratories). Table 1 details the number of individual and pooled panels shipped and the overall pass/fail status for each method. A total of 134 panels were requested. None of the individual or pooled panels were reported to be incomplete or missing individual samples. Results were not returned for 5 individual and 4 pooled panels. Upon receipt of results, labs were notified of their preliminary pass/fail status. If preliminary results indicated the laboratory failed, the laboratory was given the opportunity to retake the proficiency panel provided the results were submitted by September 30th, 2022. The results provided in Table 1 include the retests. Laboratories that used reagents for DNA isolation and PCR from a single manufacturer are listed by manufacturer. Laboratories that use either in-house reagents or mixed commercial reagents for DNA isolation and PCR are listed under the "In-House" category. Proficiency panels used for liquid culture are divided into two categories TREK and Other (in-house made liquid media or other commercially available liquid culture systems). Laboratories that purchased solid media or made their own solid media for Johne's culture are grouped together as "HEY Solid Media." All samples were considered valid because "… at least 70 percent of the laboratories participating in the fecal culture …" reported the same result.

Table 1. Summary results of the 2022 Johne's Disease Fecal Proficiency Test. In order to pass, results must meet the criteria listed in the 2010 Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program.

2022	# passed 1st attempt (%)	# failed 1st attempt (%)	# passed 2nd attempt (%)	# failed 2nd attempt (%)	# Panels not retested	Total Shipped	Total shipped in 2021 (%change)	Total shipped in 2020 (%change)
Individual Panel								
Direct PCR (all)	52 (%)	3 (%)	2 (%)		1	60	67 (-10%)	63 (+6%)
Tetracore	11 (%)	1 (%)			1	12	19 (-37%)	17 (+12%)
Thermo Fisher	24 (%)	1 (%)	1 (%)			26	25 (+4%)	28 (-11%)
In-House	17 (%)	1 (%)	1 (%)			19	19 (+0%)	16 (+19%)
Liquid Systems (all)	8 (%)	2 (%)			2	11	10 (+10%)	12 (-17%)
Other	1 (%)	1 (%)			1	2	0 (+200%)	2 (-100%)
TREK	7 (%)	1 (%)			1	8	8 (+0%)	9 (-11%)
HEY Solid Media (all)	4 (%)					5	6 (-17%)	6 (+0%)
Individual Panel Total	**64 (%)**	**5 (%)**	**2 (%)**		3	**76**	**83 (-8%)**	**81 (+2%)**
Pooling Panel								
Direct PCR (all)	44 (%)	2 (%)	1 (%)		1	49	50 (-2%)	48 (+4%)
Liquid	6 (%)	1 (%)			1	7	7 (+0%)	11 (-36%)
Solid	1 (%)					2	3 (-33%)	3 (+0%)
Pooled Panel Total	**51 (%)**	**3 (%)**	**1 (%)**		**2**	**58**	**60 (-3%)**	**57 (+5%)**

Individual Panel Description

Each individual panel consisted of 25 blinded samples and 1 positive control. Positive samples were collected from naturally infected cows and negative samples were from individual animals residing in uninfected herds. When possible, approximately 4 liters of fecal material was collected rectally per animal, shipped to the National Veterinary Services Laboratories (NVSL), aliquoted into individual vials, and stored at -70°C until panels were distributed. Fecal material from moderate shedding animals could not be obtained for use. To approximate moderate shedding animals, three samples were produced by diluting feces from a high shedding cow with material from a culture negative cow. These samples are 18-05419A (NE), 18-05419C (NE), and 18-05419D (NE). The name reflects the sample ID of the positive material used. Panels were assembled in lots of 20 with each lot having a different order of samples. (See Appendix 1 at the end of this report for the key). Table 2 shows the categorical (positive/negative) performance for each identification method by animal ID. According to the 2010 Uniform Program Standards, a laboratory receives a passing score when: all samples from non-shedding and high-shedding animals are correctly classified; and 70% of the remaining samples (low and moderate shedding animals) are correctly classified. All samples performed as expected.

Table 2. Composition of the 2022 Johne's Disease Fecal Proficiency Panels, and the overall categorical summary results per cow for each method performed by laboratories.

				Percent of Samples Correctly Classified					
					Liquid Media		Direct PCR		
Cow ID	# Vials /Panel	Shedding Status[1]	All Panels 72[2]	HEY 4	TREK 8	Other 2	Thermo F. 26	Tetracore 12	In-House 20
18-01900 (IA)	1	Critical- Neg	99%	100%	100%	100%	100%	92%	100%
18-01901 (IA)	1	Critical- Neg	99%	100%	100%	50%	100%	100%	100%
21-02542 (IA)	3	Critical- Neg	99%	100%	96%	83%	99%	100%	100%
21-02543 (IA)	1	Critical- Neg	99%	100%	100%	100%	100%	100%	95%
20-00154 (WI)	2	Low	98%	100%	88%	100%	100%	96%	100%
20-00153 (WI)	1	Low	100%	100%	100%	100%	100%	100%	100%
12-03432 (ND)	1	Moderate	100%	100%	100%	100%	100%	100%	100%
20-08637 (NE)	2	Moderate	95%	100%	100%	100%	100%	75%	98%
18-05419C (NE)[4]	1	Moderate	100%	100%	100%	100%	100%	100%	100%
18-05419D (NE)[4]	1	Moderate	100%	100%	100%	100%	100%	100%	100%
18-05422 (NE)[3]	3	Mod-High	99%	100%	100%	100%	100%	97%	98%
18-05419A (NE)[4]	1	Mod-High	100%	100%	100%	100%	100%	100%	100%
18-06468 (NE)	2	Mod-High	100%	100%	100%	100%	100%	100%	100%
12-03432 (NE)	3	Critical- High	100%	100%	100%	100%	100%	100%	100%
18-06467 (NE)	3	Critical- High	100%	100%	100%	100%	100%	100%	100%

[1]In order to pass, laboratories must correctly classify critical samples. A critical sample is any negative sample or a sample that is identified as a heavy shedder by more than 50% of the laboratories using solid media.

[2]Number of proficiency panels submitted per method.

[3]The positive control was one of the three from this animal.

[4] Positive sample diluted with negative material.

Samples from 13 animals were also used in the previous year's panel. Their performance is compared in Table 3 showing the respective year panels' performance for each identification method. These samples performed similarly between years.

Table 3. Comparison of animals used in the 2021 and 2022 Johne's Disease Fecal Proficiency Panels with the overall results for each method performed by laboratories.

					Percent of Samples Correctly Classified					
						Liquid Media			Direct PCR		
				All Panels	HEY	TREK	Other	Thermo F.	Tetracore	In-House
	Panel	# Vials	Shedding	2021	77[1]	6	8	0	25	19	19
Cow ID	Year	/Panel	Status	2022	72	4	8	2	26	12	20
18-01900 (IA)	2022	1	Critical- Neg		99%	100%	100%	100%	100%	92%	100%
18-01900 (IA)	2021	1	Critical- Neg		99%	100%	100%	0%	96%	100%	100%
18-01901 (IA)	2022	1	Critical- Neg		99%	100%	100%	50%	100%	100%	100%
18-01901 (IA)	2021	1	Critical- Neg		100%	100%	100%	0%	100%	100%	100%
21-02542 (IA)	2022	3	Critical- Neg		99%	100%	96%	83%	99%	100%	100%
21-02542 (IA)	2021	2	Critical- Neg		99%	100%	94%	0%	100%	97%	100%
21-02543 (IA)	2022	1	Critical- Neg		99%	100%	100%	100%	100%	100%	95%
21-02543 (IA)	2021	1	Critical- Neg		100%	100%	100%	0%	100%	100%	100%
20-00154 (WI)	2022	2	Low		98%	100%	88%	100%	100%	96%	100%
20-00154 (WI)	2021	3	Low		97%	94%	100%	0%	97%	100%	93%
20-00153 (WI)	2022	1	Low		100%	100%	100%	100%	100%	100%	100%
20-00153 (WI)	2021	3	Low		98%	89%	100%	0%	100%	100%	97%
12-03432 (ND)	2022	1	Moderate		100%	100%	100%	100%	100%	100%	100%
12-03432 (ND)	2021	3	Critical- High		99%	100%	100%	0%	100%	100%	97%
20-08637 (NE)	2022	2	Moderate		95%	100%	100%	100%	100%	75%	98%
20-08637 (NE)	2021	1	Mod-High		95%	100%	100%	0%	100%	78%	100%
18-05419C (NE)	2022	1	Moderate		100%	100%	100%	100%	100%	100%	100%
18-05419C (NE)	2021	2	Moderate		99%	100%	100%	0%	98%	100%	98%
18-05419D (NE)	2022	1	Moderate		100%	100%	100%	100%	100%	100%	100%
18-05419D (NE)	2021	2	Critical- High		100%	100%	100%	0%	100%	100%	100%
18-05422 (NE)	2022	3	Mod-High		99%	100%	100%	100%	100%	97%	98%
18-05422 (NE)	2021	2	Moderate		99%	100%	100%	0%	100%	100%	95%
18-05419A (NE)	2022	1	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-05419A (NE)	2021	2	Low		100%	100%	100%	0%	100%	100%	100%
18-06468 (NE)	2022	2	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-06468 (NE)	2021	1	Critical- High		100%	100%	100%	0%	100%	100%	100%

[1]Number of proficiency panels submitted per method.

Table 4 shows the average values reported for each of the testing methods summarized by animal. It is interesting to note that the Tetracore method of PCR showed a 5 Ct value difference on average for the sample 20-08637 (NE) compared to the Thermo Fisher or In-House methods otherwise performed comparably for the rest of the samples. This is a similar result to last year's testing on this sample.

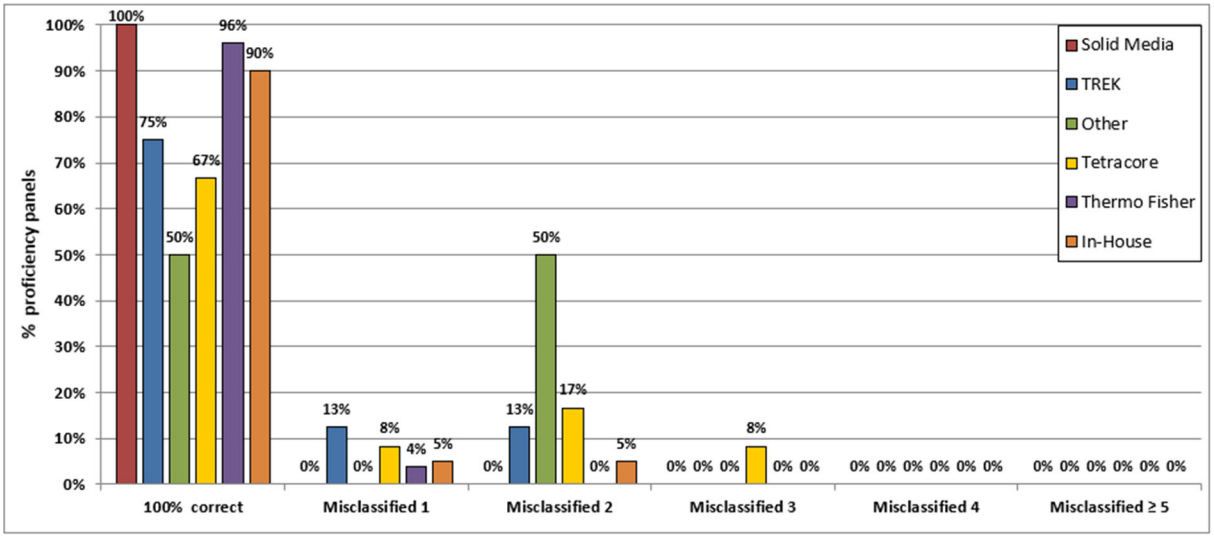
Table 4. A comparison of the averaged result values among the three methods for shedding animals. Total panel numbers for each method are listed below the method title.

		Average Result Values for Shedding Animals					
		Liquid Media			Direct PCR		
		HEY[1]	TREK	Other	Thermo F.	Tetracore	In-House
Cow ID	Shedding Status	Colonies per Tube	Days to Positive	Days to Positive	Ct	Ct	Ct
		4	8	2	26	12	20
20-00154 (WI)	Low	6.5	33	25	30.4	28.7	30.1
20-00153 (WI)	Low	2.4	32	25	28.7	27.6	28.2
12-03432 (ND)	Moderate	13.7	23	24	24.5	23.5	24.5
20-08637 (NE)	Moderate	15.1	29	24	29.3	34.6	29.4
18-05419C (NE)	Moderate	11.3	33	24	30.1	28.2	29.4
18-05419D (NE)	Moderate	16.5	28	24	29.3	27.6	28.8
18-05422 (NE)3	Mod-High	8.8	28	24	26.4	25.0	26.3
18-05419A (NE)	Mod-High	15.0	27	24	28.8	26.7	28.4
18-06468 (NE)	Mod-High	28.0	24	24	25.2	23.9	24.6
12-03432 (NE)	Critical- High	19.5	26	24	26.5	25.1	26.5
18-06467 (NE)	Critical- High	15.7	24	24	24.0	22.4	23.4

[1]Results shown include reported values only. Reports that do not include Ct values for direct PCR, days-to-positive for Liquid culture, colonies per tube or list Too-Numerous-To-Count (TNTC) for solid culure are not included; this skews the values down for the solid culture of high-shedding animals.

The performance of each method was further evaluated by determining the number of samples that were misclassified (Figure 1). The TREK system sample classification declined since last year, decreasing 13%. The other liquid culture systems were not used last year but were 100% in 2020. Laboratories using solid media correctly classified 100% of the samples, a large increase over last year (67%). The performance of all the direct PCR methods remained constant compared to last year.

Figure 1. Percentage of 2022 Johne's disease fecal proficiency panels by number of samples misclassified for the three culture (solid media, TREK liquid media, and other liquid media) and three direct PCR (Tetracore, Thermo Fisher and In-House) methods. A panel consisted of 25 fecal samples.



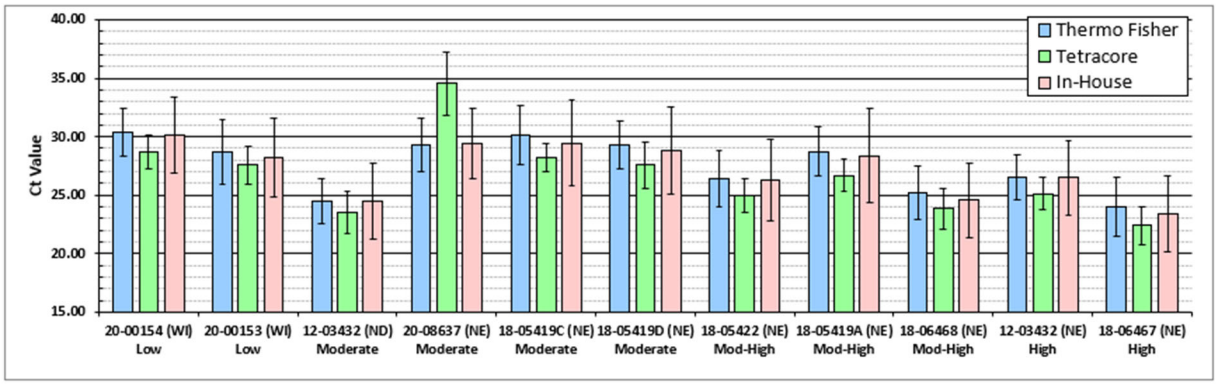
According to the 2010 Johne's Disease Uniform Program Standards, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and correctly identify 70% or more of the remaining, valid, non-critical samples (can miss ~3 samples). Table 5 lists the reasons laboratories failed to pass the proficiency panel for each method. As in previous years the most common reason for failure is misclassifying a negative sample as positive. Multiple laboratories are represented in this table.

Table 5. Reasons laboratories failed the 2022 Johne's Disease Fecal Proficiency Panel.

2022	Direct PCR (Tetracore)	Direct PCR (Thermo F.)	Direct PCR (In-House)	TREK liquid media	Other liquid media	HEY solid media
Misclassified a negative sample as positive	1	1	1	1	1	
Missed 4 or more low / moderate shedders (lack of sensitivity)						
Misclassified a high shedding sample as negative						
Multiple reasons cited above						
Total failed panels	**1 (8%)**	**1 (4%)**	**1 (5%)**	**1 (13%)**	**1 (50%)**	**0 (0%)**
Total panels tested	**12**	**26**	**20**	**8**	**2**	**4**

Because direct PCR is the most common detection method used, the performance of that assay across laboratories is critical to consistent application of the program across the US. Variation in reported cycle threshold (Ct) of the direct PCR methods was investigated (see Figure 2) by comparing the average reported Ct for positive samples. Ct values from each panel were used in this comparison and include samples categorized as negative, but that had Ct scores reported (e.g. negative, but a Ct of 39.9). The overall means of all three methods for each animal were statistically similar.

Figure 2. The average and 1 standard deviation from reported Ct values were plotted for the three direct PCR methods (Thermo Fisher, Tetracore, and In-house). Shedding status is listed below the animal ID. Animal numbers ending in letters "18-05419A (NE)" are diluted samples.



False positive results continue to be the most common cause of failure. Table 6 examines the number of negative samples reported with Ct values by PCR method; this includes laboratories that reported Ct values and correctly classified them as negative. Errors were generally distributed amongst the negative animals that were used in this year's panel when considering the number of vials included. There was a total of 4 laboratories that reported Ct values on at least one negative sample, an increase from last year. Of those 4 laboratories, 3 failed the proficiency test (see Table 5) by calling a negative sample positive and is an increase from last year's proficiency testing. False positive results by PCR can be caused by a number of factors such as cross-contamination within the lab, problems with primer/probe design, etc. The data collected and reported here are not sufficient to determine the cause of the false negative results.

Table 6. The number of samples from non-infected cows reported with Ct values (regardless of their categorical positive/negative results) by direct PCR method.

	Tetracore	Thermo F.	In-House	Total
18-01900 (IA)	0	1	0	1
18-01901 (IA)	0	0	1	1
21-02542 (IA)	1	0	0	1
21-02543 (IA)	0	0	1	1
Num. panels reporting Ct	1	1	2	4

Pooling Panel Description

For the pooled panels, 25 individual samples were provided with instructions regarding which 5 samples to pool together, for a total of 5 pooled samples. Table 7 lists the contents of each pool, and Appendix 2 lists the pool numbers associated with each lot. To pass, laboratories were required to correctly classify the negative pools and the two pools that contained a high-shedding animal. Laboratories were allowed to pass even if they misclassified the other pool.

Table 7. Composition of the 2022 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	18-06468 (NE)	32
1 High, 4 Negative samples	18-05422 (NE)	TNTC
2 Low-Mod, 3 Negative samples	12-03432 (NE)	16
	18-05419B (NE)	16
5 Negative samples		
5 Negative samples		

*Refers to the positive samples, not the pooled sample.

Table 8 describes the performance of each method used to test the pooled samples. All but 1 laboratory using solid and liquid culture passed. Though 2 pooled direct PCR panels are reported as failed due to calling negative pools positive, all but 1 laboratory passed the pooled panel using direct PCR.

Table 8. Performance of each method used in the Johne's Disease 2022 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

2022		No. panels		
		Direct PCR	Liquid media	Solid media
Panels that failed	Identified the negative pool as positive		1	
	Identified a high -shedding pool as negative			
	Two non-critical pools were identified as negative			
	Failed due to multiple criteria	2		
Panels that passed	One non-critical pool was misidentified as negative			
	All 5 pools were identified correctly	45	6	1
	Total Failed Pooled Panels	**2 (4%)**	**1 (14%)**	**0 (0%)**
	Total	**47**	**7**	**1**

A current listing of all the approved laboratories is available in the NVLS web site: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/sa_approved_labs/ct_approved_labs.

Remaining sample vials from the 2022 Proficiency Panel are available to laboratories for validation or research purposes. Available samples can be viewed in the reagents catalog under Johne's positive/negative fecal samples on the NVSL web site Reagent Catalog at https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/sa_reagents/ct_reagents

Appendix 1. 2022 Johne's Disease Individual Fecal Proficiency Panel key by panel number. Samples are coded by color according to shedding status as follows: Critical - Negative samples, Non-critical positive samples, Critical – high shedding samples. Sample 26 was the positive control.

Vial #	1-20	21-40	41-60	61-80	81-100
1	18-05422 (NE)	21-02543 (IA)	18-01901 (IA)	18-05419D (NE)	12-03432 (NE)
2	18-01900 (IA)	12-03432 (ND)	20-00154 (WI)	18-05419A (NE)	18-01901 (IA)
3	20-00154 (WI)	18-05422 (NE)	20-08637 (NE)	12-03432 (NE)	18-06467 (NE)
4	18-05419C (NE)	18-06467 (NE)	18-06468 (NE)	12-03432 (NE)	21-02543 (IA)
5	18-06468 (NE)	21-02542 (IA)	12-03432 (ND)	18-01901 (IA)	18-05422 (NE)
6	18-06467 (NE)	12-03432 (NE)	18-05422 (NE)	21-02542 (IA)	18-01900 (IA)
7	21-02542 (IA)	20-08637 (NE)	18-01900 (IA)	18-06467 (NE)	20-00153 (WI)
8	12-03432 (NE)	18-05419A (NE)	18-05419D (NE)	20-00154 (WI)	18-05419C (NE)
9	21-02542 (IA)	12-03432 (NE)	21-02542 (IA)	18-05422 (NE)	21-02542 (IA)
10	18-06467 (NE)	20-00154 (WI)	12-03432 (NE)	18-01900 (IA)	12-03432 (NE)
11	18-05422 (NE)	20-08637 (NE)	20-00154 (WI)	12-03432 (NE)	18-05419A (NE)
12	12-03432 (ND)	18-05419C (NE)	18-05419A (NE)	12-03432 (ND)	12-03432 (NE)
13	18-06467 (NE)	18-06468 (NE)	18-06467 (NE)	21-02543 (IA)	18-06467 (NE)
14	21-02543 (IA)	18-05419D (NE)	21-02542 (IA)	20-08637 (NE)	18-06468 (NE)
15	18-05419A (NE)	21-02542 (IA)	18-05419C (NE)	18-05422 (NE)	20-08637 (NE)
16	12-03432 (NE)	20-00153 (WI)	18-06467 (NE)	20-00154 (WI)	21-02542 (IA)
17	20-08637 (NE)	18-06467 (NE)	18-06468 (NE)	21-02542 (IA)	12-03432 (ND)
18	12-03432 (NE)	18-05422 (NE)	12-03432 (NE)	20-08637 (NE)	20-00154 (WI)
19	18-01901 (IA)	18-01900 (IA)	12-03432 (NE)	18-06468 (NE)	18-05419D (NE)
20	20-08637 (NE)	12-03432 (NE)	21-02542 (IA)	20-00153 (WI)	18-05422 (NE)
21	20-00153 (WI)	18-06468 (NE)	20-08637 (NE)	18-05419C (NE)	18-06467 (NE)
22	18-05419D (NE)	18-06467 (NE)	18-05422 (NE)	18-06467 (NE)	18-06468 (NE)
23	18-06468 (NE)	21-02542 (IA)	18-06467 (NE)	21-02542 (IA)	20-00154 (WI)
24	20-00154 (WI)	18-01901 (IA)	20-00153 (WI)	18-06467 (NE)	21-02542 (IA)
25	21-02542 (IA)	20-00154 (WI)	21-02543 (IA)	18-06468 (NE)	20-08637 (NE)
26	18-05422 (NE)	18-05422 (NE)	18-05422 (NE)	18-05422 (NE)	18-05422 (NE)

Appendix 2. 2022 Johne's Disease Pooled Fecal Proficiency Panel key by panel number.

	Sample Pool Number			
Pool Description	Panel #1-20	Panel #21-40	Panel #41-60	Panel #61-70
5 Negative samples	2	4	1	5
5 Negative samples	3	1	2	4
2 Low-Mod (18-05419B & 12-03432), 3 Negative samples	4	3	4	2
1 High (18-05422), 4 Negative samples	1	5	5	3
1 High (18-06468), 4 Negative samples	5	2	3	1

Any questions or comments can be directed to the Diagnostic Bacteriology and Pathology Laboratory at 515.337.7388.

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