2019 Johne's Disease Fecal Proficiency Panel
General Summary
October 2, 2019

Overview

A total of 60 laboratories participated in the 2019 Johne's Disease Fecal Proficiency Panel (7 Canadian, 3 European Union, 1 New Zealand, 2 Australian and 47 USA laboratories). Table 1 details the number of individual and pooled panels shipped and the overall pass/fail status for each method. Laboratories could order multiple panels for each method and were notified of their preliminary pass/fail status upon submission of their results. A total of 157 panels were requested, with 1 replaced due to a defective panel. Results were not returned for 5 Individual and 3 Pooled panels. If preliminary results indicated the laboratory failed, it was given the opportunity to retake the proficiency panel provided the results were completed by September 30th, 2019. The results provided in Table 1 include these retests. Laboratories that only used reagents from a single manufacturer, either Tetracore or Thermo Fisher Sci. (used AM1840 or MagMax CORE for extraction), are listed separately. Laboratories that use either in-house reagents or mix commercial reagents from multiple manufacturers are listed under the "In-House" category. The laboratory that used in-house liquid culture reagents is grouped with the laboratories using the MGIT system. All laboratories using solid media were grouped together regardless if they purchased media or used in-house media (1 laboratory).

Table 1. Summary results of the 2019 Johne's Disease Fecal Proficiency Panel. 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.

2019	# passed 1st attempt (%)	# failed 1st attempt (%)	# passed 2nd attempt (%)	# failed 2nd attempt (%)	# Kits not retested	Total Shipped	Total shipped in 2018 (%change)
Individual Panel							
Direct PCR (all)	56 (90%)	6 (10%)	3 (100%)		3	68	69 (-1%)
Tetracore	15 (88%)	2 (12%)	1 (100%)		1	18	21 (-14%)
Thermo Fisher	24 (89%)	3 (11%)	1 (100%)		2	28	26 (+8%)
In-House	17 (94%)	1 (6%)	1 (100%)			19	20 (-5%)
Liquid Systems (all)	11 (92%)	1 (8%)			1	13	16 (-19%)
MGIT 960	2 (67%)	1 (33%)			1	3	4 (-25%)
TREK	9 (100%)					9	11 (-18%)
HEY Solid Media (all)	6 (100%)					6	7 (-14%)
Individual Panel Total	**73 (91%)**	**7 (9%)**	**3 (100%)**		4	**87**	**92 (-5%)**
Pooling Panel							
Direct PCR (all)	49 (98%)	1 (2%)	1 (100%)			51	51 (+0%)
Liquid	11 (92%)	1 (8%)			1	12	13 (-8%)
HEY	3 (100%)					3	4 (-25%)
Pooled Panel Total	**63 (97%)**	**2 (3%)**	**1 (100%)**		**1**	**66**	**68 (-3%)**

Individual Panel Description

Each individual panel consisted of 25 blinded samples and one positive control. Positive samples were collected from naturally infected cows, and negative samples were from individual animals residing in non-infected herds. When possible, approximately 4 liters of fecal material were collected rectally per animal, shipped to NVSL, aliquoted in to individual vials, and stored at -70˚C until kits were distributed. Because we lacked moderate shedding samples, five samples were produced where material from a high shedding cow was diluted with material from a culture negative cow and thoroughly mixed. These samples are 18-05419A (NE), 18-05419B (NE), 18-05419C (NE), 18-05419D (NE), and 15-00471D (FL) reflecting the animal that the positive material originated. Panels were assembled in groups, each with a different key (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 they correctly classify 70% of the remaining samples (low and moderate shedding animals). All samples performed as expected except 17-03492. The 2010 Uniform Program Standards states that an animal is considered valid "by a consensus of at least 70 percent of the laboratories participating in the fecal culture check testing process." Animal 17-03492 (WI) had 33% of all vials correctly classified (direct PCR and culture) and had 69.4% correctly classified by laboratories using culture methods, making it an invalid animal. Designating it an invalid animal did not affect the pass/fail result of any laboratory.

Table 2. Composition of the 2019 Johne's Disease Fecal Proficiency Panel, 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 Kits 83[2]	HEY 6	TREK 9	MGIT 3	Thermo F. 28	Tetracore 18	In-House 19
18-01897 (IA)	2	Critical- Neg	99%	100%	100%	83%	100%	100%	97%
18-01898 (IA)	2	Critical- Neg	99%	100%	100%	100%	100%	94%	100%
18-01899 (IA)	3	Critical- Neg	98%	100%	100%	100%	96%	100%	98%
17-03492 (WI)	2	Low	33%	58%	78%	67%	21%	19%	29%
16-01645 (IA)[3]	2	Moderate	99%	100%	100%	100%	96%	100%	100%
18-05419A (NE)[4]	2	Moderate	99%	100%	100%	100%	96%	100%	100%
18-05419B (NE)[4]	2	Mod-High	99%	100%	100%	100%	96%	100%	100%
18-05419C (NE)[4]	1	Mod-High	100%	100%	100%	100%	100%	100%	100%
18-05419D (NE)[4]	2	Mod-High	99%	100%	100%	100%	98%	100%	100%
15-00471D (FL)[4]	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-03210 (FL)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-02487 (FL)	2	Critical- High	99%	100%	100%	100%	100%	97%	100%
17-03143 (FL)	2	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 two from this animal.

[4]Positive sample diluted with negative material.

Samples from 6 animals were used in previous years and their performance was compared. Table 3 shows the respective year panels' categorical (positive/negative) performance for each identification method by animal ID. Note that animal 17-03492(WI) was valid in 2017 and 2018 but was invalid in 2019.

Table 3. Comparison between six animals used in the 2016, 2017, 2018, and 2019 Johne's Disease Fecal Proficiency Panels with the overall categorical summary results per cow for each method performed by laboratories.

				Percent of Samples Correctly Classified						
					Liquid Media			Direct PCR		
				All Kits	HEY	TREK	MGIT	Thermo F.	Tetracore	In-House
			2016	102[1]	10	13	3	26	27	18
			2017	95	9	12	5	28	23	18
	Panel	# Vials	2018	87	7	11	4	26	21	18
Cow ID	Year	/Panel	Shedding Status 2019	83	6	9	3	28	18	19
17-03492 (WI)	2017	2	Low-Mod	44%	72%	92%	30%	25%	39%	39%
17-03492 (WI)	2018	2	Low	33%	71%	73%	75%	21%	17%	20%
17-03492 (WI)	2019	2	Low	33%	58%	78%	67%	21%	19%	29%
16-01645 (IA)	2016	2	Mod-High	99%	100%	97%	100%	98%	100%	97%
16-01645 (IA)	2017	2	Mod-High	99%	100%	92%	100%	100%	100%	100%
16-01645 (IA)	2018	2	Mod-High	99%	100%	100%	100%	96%	100%	100%
16-01645 (IA)	2019	2	Mod-High	99%	100%	100%	100%	96%	100%	100%
15-00471D (FL)	2016	2	High	100%	100%	97%	100%	100%	100%	100%
15-00471D (FL)	2017	2	Critical- High	99%	100%	100%	100%	100%	98%	100%
15-00471D (FL)	2018	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
15-00471D (FL)	2019	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-03210 (FL)	2018	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-03210 (FL)	2019	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-02487 (FL)	2017	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-02487 (FL)	2018	3	Critical- High	99%	100%	91%	100%	100%	100%	100%
17-02487 (FL)	2019	2	Critical- High	99%	100%	100%	100%	100%	97%	100%
17-03143 (FL)	2018	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-03143 (FL)	2019	2	Critical- High	100%	100%	100%	100%	100%	100%	100%

[1]Number of proficiency panels submitted per method.

Table 4 shows the averaged value reported for each of the methods summarized by animal. Interestingly, the reported values for animal 17-03143 (FL) differ between methods with the averaged values most similar to the high-shedding animals for liquid culture and direct PCR. However, it is more similar to a low-shedding animal using solid culture. These results are similar to those found in 2018 for this animal and 2014 and 2016 with animal 12-00956 (KS), which had a mixed infection of both cattle and 'bison' strains of *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Reviewing whole genome sequence data from our laboratory indicates animal 17-03143 (FL) likely also has a mixed infection with both cattle and 'bison' strains of MAP, which is consistent with the results shown in Table 4 since 'bison' MAP strains do not grow well, if at all, on HEY media. For those laboratories conducting strain differentiation assays, the 'bison' MAP strain should be the dominant strain recovered from the liquid culture systems and the cattle strain from the HEY media.

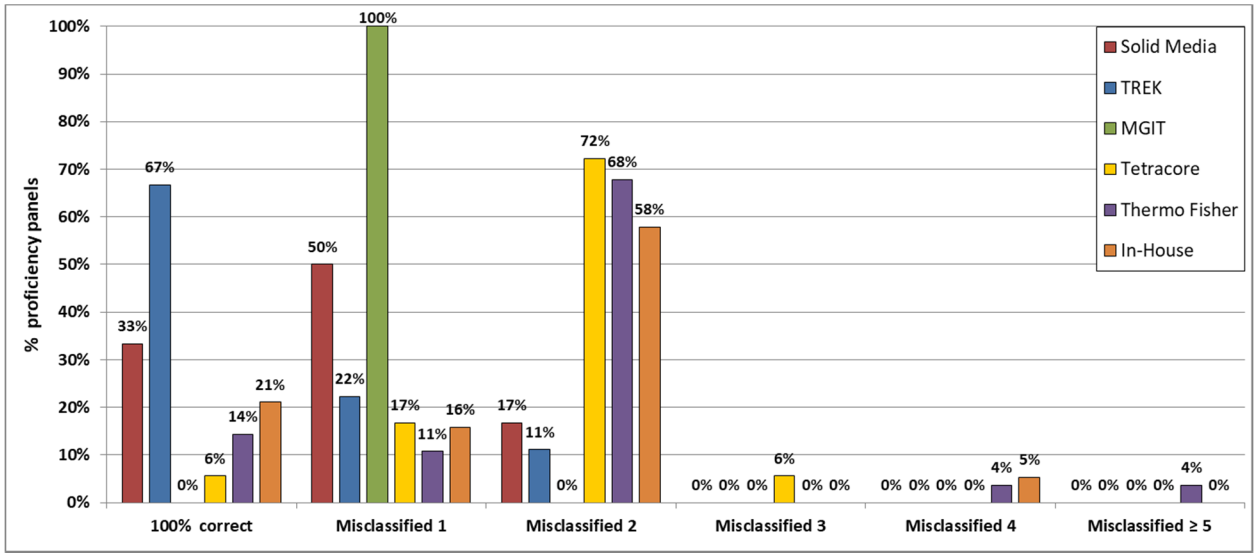
Table 4. A comparison of the averaged result values among the three methods for shedding animals.

		Average Result Values for Shedding Animals					
		Liquid Media			Direct PCR		
		HEY[1]	TREK	MGIT	Thermo F.	Tetracore	In-House
	Shedding	Colonies per Tube	Days to Positive	Days to Positive	Ct	Ct	Ct
Cow ID	Status	6	9	3	28	18	19
17-03492 (WI)	Low	0.7	35	47	37.3	37.6	35.6
16-01645 (IA)	Moderate	23.3	25	29	31.5	29.5	29.8
18-05419A (NE)	Moderate	18.3	24	15	30.7	28.2	28.6
18-05419B (NE)	Mod-High	25.8	28	27	29.8	28.4	28.3
18-05419C (NE)	Mod-High	36.4	23	17	29.1	27.2	27.5
18-05419D (NE)	Mod-High	28.1	22	9	28.7	26.5	26.5
15-00471D (FL)	Critical- High	29.4	20	22	26.4	25.4	24.6
17-03210 (FL)	Critical- High	TNTC	16	17	23.6	22.5	21.9
17-02487 (FL)	Critical- High	TNTC	17	15	23.1	22.0	21.4
17-03143 (FL)	Critical- High	2.5	15	13	21.7	19.7	19.7

[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 especially 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) using all 25 samples. In this analysis 67% of laboratories using the TREK system correctly classified all the samples. For the laboratories using solid media 33% correctly classified all the samples. The performance of the three direct PCR methods was similar to last year with slightly more laboratories correctly calling all the samples, but many still had difficulty with samples from animal 17-03492 (WI). Twenty-one percent of laboratories using In-house direct PCR methods correctly classified all the samples.

Figure 1. Percentage of 2019 Johne's disease fecal proficiency panels by number of samples misclassified for the three culture (TREK liquid media, solid media and MGIT 960 liquid media) and three direct PCR (Tetracore, Thermo Fisher and In-House) methods. A panel consisted of 25 fecal samples.



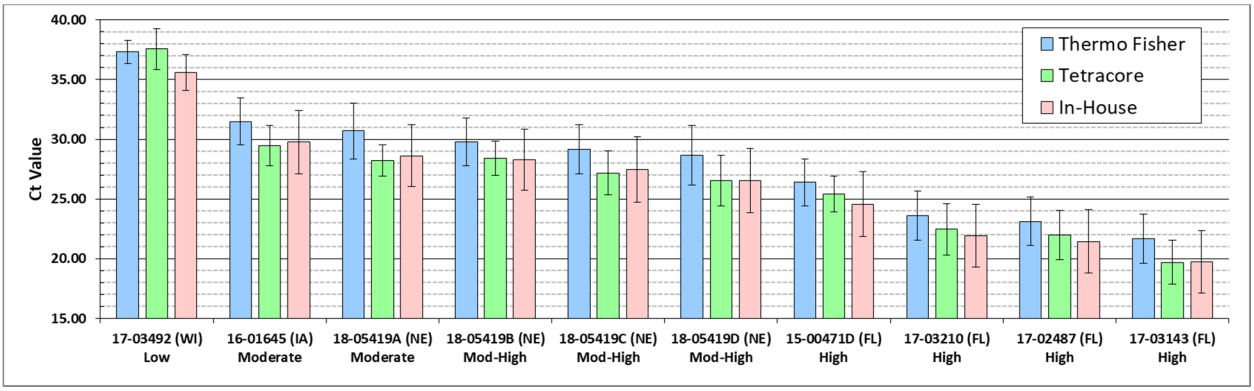
According to the 2010 Johne's Disease Uniform Program Standards, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and misidentify 70% (~3 samples) or fewer of the remaining, valid, non-critical samples. Table 5 lists the specific reasons 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.

Table 5. Reasons laboratories failed the 2019 Johne's Disease Fecal Proficiency Panel.

2019	Direct PCR (Tetracore)	Direct PCR (Thermo F.)	Direct PCR (In-House)	TREK liquid media	MGIT liquid media	HEY solid media
Misclassified a negative sample as positive	1	2	1		1	
Missed 4 or more low / moderate shedders (lack of sensitivity)		1				
Misclassified a high shedding sample as negative						
Multiple reasons cited above	1					
Total failed kits	**2 (11%)**	**3 (11%)**	**1 (5%)**	**0 (0%)**	**1 (33%)**	**0 (0%)**
Total kits tested	**18**	**28**	**19**	**9**	**3**	**6**

Because direct PCR is now the most common organism detection assay offered, the performance of that assay across laboratories becomes more important. Variation in reported cycle threshold (Ct) of the direct PCR methods was investigated in Figure 2 by comparing the average reported Ct for the positive samples. Only valid Ct values from each panel were used in this comparison and include samples categorized as negative but that had valid 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. The diluted samples, 18-05419A (NE), 18-05419A (NE), 18-05419A (NE), 18-05419A (NE), 15-00471D (FL), all performed similar to natural shedding animals.

Figure 2. Average, and 1 standard deviation, reported Ct of 2019 Johne's disease fecal proficiency panel animals 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 with either direct fecal PCR or confirmatory culture PCR continues 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. Also shown are the number of panels where at least one Ct is reported. Errors were generally evenly distributed amongst the three negative animals that were used in this year's panel when considering the number of vials included. Interestingly, laboratories using In-House methods accounted for 6 of 8 Ct values from animal 18-01899 (IA). There were a total of 9 laboratories that reported Ct values on at least one negative sample. Of those 9 laboratories, 5 failed the PT (see Table 5) by calling a negative sample positive and is a reduction from last year's panel (12 of 15 failed). Although fewer laboratories reported Ct values for negative samples, it continues to be an issue. Almost half the laboratories reporting Ct values called them negative.

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
18-01897 (IA)			1	1
18-01898 (IA)	2		1	3
18-01899 (IA)		3	6	9
Num. panels reporting Ct	2	3	7	9

Pooling Panel Description

Twenty five 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 of panels. To pass, laboratories were required to correctly classify the negative pool and the two pools that contained a high-shedding animal (17-03210 & 17-02487). Laboratories were allowed to pass even if they misclassified one of the other pools (17-04003 or 17-04070).

Table 7. Composition of the 2019 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	17-03210	~2500
1 High, 4 Negative samples	17-02487	~1000
1 Moderate, 4 Negative samples	17-04003	30
1 Moderate, 4 Negative samples	18-05419A	18
5 Negative samples		

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

Table 8 describes the performance of each method used to test the pool. It is commendable that all laboratories using solid culture passed. All but one laboratory passed the pooled panel using direct PCR and liquid culture methods. Three laboratories using liquid culture misclassified the negative pool and another misclassified multiple pools.

Table 9. Performance of each method used in the Johne's Disease 2018 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

2019		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	1		
	Two non-critical pools were identified as negative			
	Failed due to multiple criteria			
Panels that passed	One non-critical pool was misidentified as negative			
	All 5 pools were identified correctly	49	11	3
	Total Failed Pooled Kits	**1 (2%)**	**1 (8%)**	**0 (0%)**
	Total	**50**	**12**	**3**

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 2019 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. 2019 Johne's Disease Individual Fecal Proficiency Panel key by kit number. Samples are coded by color according to shedding status as follows: Negative, Non-critical positive samples, Critical – high shedding samples. Sample 26 was the positive control.

Vial #	1-25	26-50	51-75	76-100	100-105
1	18-05419A (NE)	18-05419D (NE)	18-05419B (NE)	18-05419C (NE)	18-05419A (NE)
2	18-05419B (NE)	17-02487 (FL)	17-03210 (FL)	18-05419D (NE)	18-05419C (NE)
3	15-00471D (FL)	17-03786 (IA)	17-03787 (IA)	18-05419D (NE)	17-02487 (FL)
4	17-03787 (IA)	17-04259 (IA)	18-05419A (NE)	17-03143 (FL)	17-03786 (IA)
5	17-03143 (FL)	17-03210 (FL)	17-02487 (FL)	17-04259 (IA)	17-03787 (IA)
6	17-03492 (WI)	17-03787 (IA)	17-03786 (IA)	16-01645 (IA)	17-03143 (FL)
7	18-05419B (NE)	15-00471D (FL)	17-03210 (FL)	18-05419B (NE)	17-03787 (IA)
8	18-05419A (NE)	17-03787 (IA)	17-04259 (IA)	17-02487 (FL)	17-03210 (FL)
9	18-05419D (NE)	17-03210 (FL)	17-03143 (FL)	17-03786 (IA)	17-03492 (WI)
10	17-02487 (FL)	18-05419B (NE)	16-01645 (IA)	17-03492 (WI)	18-05419D (NE)
11	17-04259 (IA)	16-01645 (IA)	18-05419C (NE)	15-00471D (FL)	17-03143 (FL)
12	18-05419C (NE)	17-03143 (FL)	15-00471D (FL)	17-03787 (IA)	18-05419B (NE)
13	17-03210 (FL)	17-03492 (WI)	17-03786 (IA)	17-03210 (FL)	16-01645 (IA)
14	17-03786 (IA)	18-05419D (NE)	17-04259 (IA)	17-04259 (IA)	18-05419A (NE)
15	15-00471D (FL)	18-05419A (NE)	17-03492 (WI)	17-03143 (FL)	18-05419D (NE)
16	17-04259 (IA)	18-05419B (NE)	15-00471D (FL)	17-04259 (IA)	15-00471D (FL)
17	18-05419D (NE)	17-03143 (FL)	17-04259 (IA)	18-05419A (NE)	17-04259 (IA)
18	16-01645 (IA)	17-03492 (WI)	18-05419D (NE)	17-02487 (FL)	17-03210 (FL)
19	17-02487 (FL)	18-05419C (NE)	18-05419A (NE)	17-03786 (IA)	17-03492 (WI)
20	17-03786 (IA)	15-00471D (FL)	17-02487 (FL)	17-03210 (FL)	18-05419B (NE)
21	17-04259 (IA)	17-04259 (IA)	17-03492 (WI)	17-03787 (IA)	17-02487 (FL)
22	17-03143 (FL)	18-05419A (NE)	18-05419D (NE)	18-05419B (NE)	17-03786 (IA)
23	17-03787 (IA)	17-02487 (FL)	18-05419B (NE)	15-00471D (FL)	17-04259 (IA)
24	17-03210 (FL)	17-03786 (IA)	17-03143 (FL)	17-03492 (WI)	15-00471D (FL)
25	17-03492 (WI)	17-04259 (IA)	17-03787 (IA)	18-05419A (NE)	17-04259 (IA)
26	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)

Appendix 2. 2019 Johne's Disease Pooled Fecal Proficiency Panel key by kit number.

	Pool Sample Number			
Pool Description	Kit# 1-20	Kit# 21-40	Kit# 41-60	Kit# 61-70
5 Negative samples	5	2	4	1
1 mod (18-05419A), 4 Negative samples	4	3	1	5
1 mod-high (17-04003), 4 Negative samples	2	4	3	2
1 high (17-02487), 4 Negative samples	1	5	2	3
1 high (17-03210), 4 Negative samples	3	1	5	4

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

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