



2020 Johne's Disease Fecal Proficiency Test General Summary November 2, 2020

Overview

A total of 60 laboratories ordered panels during the 2020 Johne's Disease Fecal Proficiency Test (7 Canadian, 3 European Union, 1 New Zealand, 1 Australian and 48 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 143 panels were requested with 2 pooled panels reported to be incomplete but did not need replacements. Results were not returned for 6 Individual panels and 1 Pooled panel. 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, 2020. The results provided in [Table 1](#) include these retests. Laboratories that only used reagents for DNA isolation and PCR from a single manufacturer are listed separately. Laboratories that use either in-house reagents or mixed commercial reagents for DNA isolation and PCR are listed under the "In-House" category. The laboratory that used in-house liquid culture reagents to test for Johne's is grouped with the laboratory using the MGIT system. Laboratories that purchased solid media or made their own solid media for Johne's culture are grouped together.

Table 1. Summary results of the 2020 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.

2020	# passed	# failed	# passed	# failed	# Panels	Total Shipped	Total shipped in 2019 (%change)
	1st attempt (%)	1st attempt (%)	2nd attempt (%)	2nd attempt (%)	not retested		
Individual Panel							
Direct PCR (all)	52 (96%)	2 (4%)	2 (100%)			63	68 (-7%)
Tetracore	16 (94%)	1 (6%)	1 (100%)			17	18 (-6%)
Thermo Fisher	27 (96%)	1 (4%)	1 (100%)			28	28 (+0%)
In-House	18 (100%)					16	19 (-16%)
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Liquid Systems (all)	11 (100%)					12	13 (-8%)
MGIT 960	2 (100%)					2	3 (-33%)
TREK	9 (100%)					9	9 (+0%)
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HEY Solid Media (all)	5 (100%)					6	6 (+0%)
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Individual Panel Total	68 (97%)	2 (3%)	2 (100%)			81	87 (-7%)
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Pooling Panel							
Direct PCR (all)	47 (98%)	1 (2%)			1	48	51 (-6%)
Liquid	11 (100%)					11	12 (-8%)
HEY	3 (100%)					3	3 (+0%)
Pooled Panel Total	61 (98%)	1 (2%)			1	62	66 (-14%)



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 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 panel sample use. To approximate moderate shedding animals, four samples were produced by diluting feces from a high shedding cow with material from a culture negative cow. These samples are 18-05419A (NE), 18-05419B (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 except 20-00152 (WI). 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 20-00152 (WI) had 68% of all vials correctly classified (direct PCR and all culture methods) and had 56.3% correctly classified by laboratories using culture methods, making it invalid. Designating the samples from Animal 20-00152 (WI) invalid did not affect the pass/fail result of any laboratory.

Table 2. Composition of the 2020 Johne’s Disease Fecal Proficiency Panels, and the overall categorical summary results per cow for each method performed by laboratories.

Cow ID	# Vials /Panel	Shedding Status ¹	All Panels 79 ²	Percent of Samples Correctly Classified					
				Liquid Media			Direct PCR		
				HEY 5	TREK 9	MGIT 2	Thermo F. 28	Tetracore 17	In-House 18
18-01899 (IA)	1	Critical- Neg	100%	100%	100%	100%	100%	100%	100%
18-01900 (IA)	2	Critical- Neg	99%	100%	100%	100%	96%	100%	100%
18-01901 (IA)	2	Critical- Neg	100%	100%	100%	100%	100%	100%	100%
19-00486 (IA)	1	Critical- Neg	99%	100%	100%	100%	100%	94%	100%
20-00152 (WI)	2	Low	68%	40%	56%	100%	63%	76%	81%
20-00154 (WI)	2	Low-Mod	98%	100%	94%	100%	100%	100%	94%
20-00153 (WI) ³	2	Low-Mod	100%	100%	100%	100%	100%	100%	100%
18-05419B (NE) ⁴	2	Mod-High	100%	100%	100%	100%	100%	100%	100%
18-05419C (NE) ⁴	1	Mod-High	100%	100%	100%	100%	100%	100%	100%
18-05419A (NE) ⁴	1	Mod-High	100%	100%	100%	100%	100%	100%	100%
18-05419D (NE) ⁴	2	Mod-High	99%	100%	94%	100%	100%	100%	100%
18-06468 (NE)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
18-05422 (NE)	2	Critical- High	99%	100%	100%	100%	100%	94%	100%
12-03432 (ND)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
18-05419 (NE)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%

¹In 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.

²Number of proficiency panels submitted per method.

³The positive control was one of the two from this animal.

⁴Positive sample diluted with negative material.



Samples from 5 animals were also used in previous years. Their performance is compared in [Table 3](#) showing the respective year panels' performance for each identification method.

Table 3. Comparison of animals used in the 2019 and 2020 Johne's Disease Fecal Proficiency Panels with the overall results for each method performed by laboratories.

Cow ID	Panel Year	# Vials /Panel	Shedding Status	2019 2020	All Panels 83 ¹ 79	Percent of Samples Correctly Classified					
						Liquid Media			Direct PCR		
						HEY	TREK	MGIT	Thermo F.	Tetracore	In-House
18-01899 (IA)	2019	3	Critical- Neg		98%	6	9	3	28	18	19
18-01899 (IA)	2020	1	Critical- Neg		100%	5	9	2	28	17	18
18-05419A (NE)	2019	2	Moderate		99%	100%	100%	100%	96%	100%	100%
18-05419A (NE)	2020	1	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-05419B (NE)	2019	2	Mod-High		99%	100%	100%	100%	96%	100%	100%
18-05419B (NE)	2020	2	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-05419C (NE)	2019	1	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-05419C (NE)	2020	1	Mod-High		99%	100%	94%	100%	100%	100%	100%
18-05419D (NE)	2019	2	Mod-High		99%	100%	100%	100%	98%	100%	100%
18-05419D (NE)	2020	2	Critical- High		100%	100%	100%	100%	100%	100%	100%

¹Number of proficiency panels submitted per method.

[Table 4](#) shows the averaged values reported for each of the testing methods summarized by animal.

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

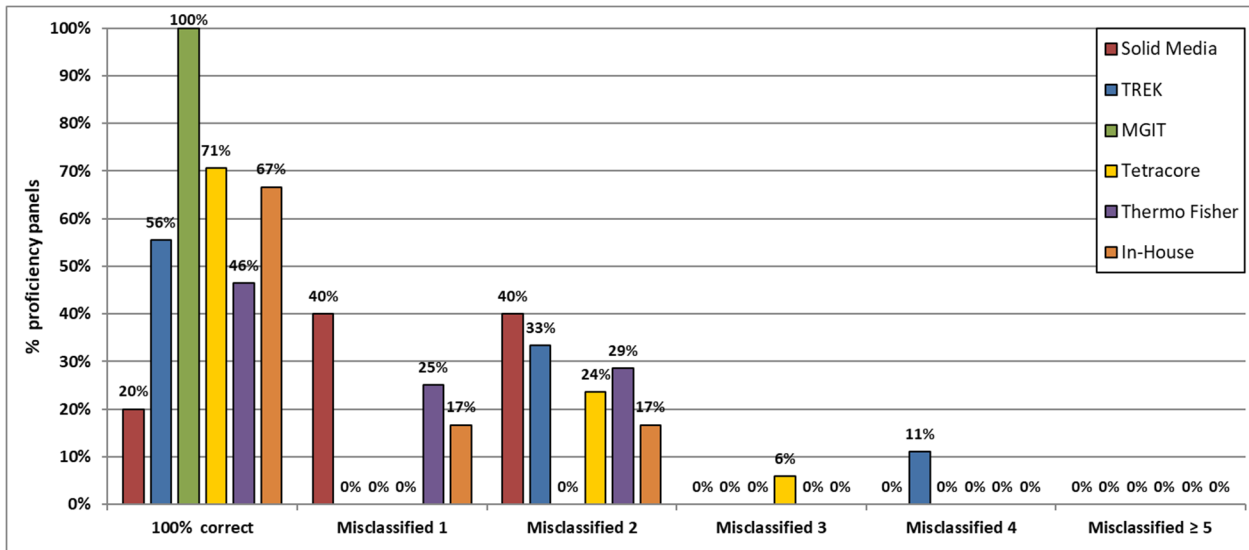
Cow ID	Shedding Status	Average Result Values for Shedding Animals					
		Liquid Media			Direct PCR		
		HEY ¹	TREK	MGIT	Thermo F.	Tetracore	In-House
		Colonies per Tube	Days to Positive	Days to Positive	Ct	Ct	Ct
		5	9	2	28	17	18
20-00152 (WI)	Low	1.3	27	16	35.8	34.2	34.4
20-00154 (WI)	Low-Mod	16.9	28	22	29.8	28.6	29.4
18-05419B (NE)	Low-Mod	24.9	23	24	30.0	28.4	29.2
18-05419C (NE)	Mod-High	37.0	26	15	29.3	28.1	28.1
18-05419D (NE)	Mod-High	44.1	25	18	28.8	27.0	28.1
20-00153 (WI)	Mod-High	17.8	20	14	28.3	27.9	27.2
18-05419A (NE)	Mod-High	17.5	20	12	28.1	26.2	27.5
18-05422 (NE)	Critical- High	26.0	18	12	26.0	24.6	25.4
18-06468 (NE)	Critical- High	40.0	16	11	24.6	23.2	23.5
12-03432 (ND)	Critical- High	7.5	18	12	23.5	22.0	22.7
18-05419 (NE)	Critical- High	TNTC	16	11	22.6	20.7	21.7

¹Results 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 culture 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). Laboratories using the TREK system correctly classified 56% of the samples; whereas, 100% of laboratories using the MGIT system correctly classified all the samples. Though only 2 labs were included in the MGIT data, this is the first time in over 10 years that the MGIT outperformed the TREK system. Laboratories using solid media correctly classified 20% of the samples, a decrease from last year. The performance of the three direct PCR methods improved compared to last year with more laboratories correctly calling all samples. Sixty-seven percent of laboratories using In-house direct PCR methods correctly classified all the samples compared to 21% who correctly called all the samples last year.

Figure 1. Percentage of 2020 Johnne’s disease fecal proficiency panels by number of samples misclassified for the three culture (solid media, TREK liquid media, and MGIT 960 liquid media) and three direct PCR (Tetracore, Thermo Fisher and In-House) methods. A panel consisted of 25 fecal samples.





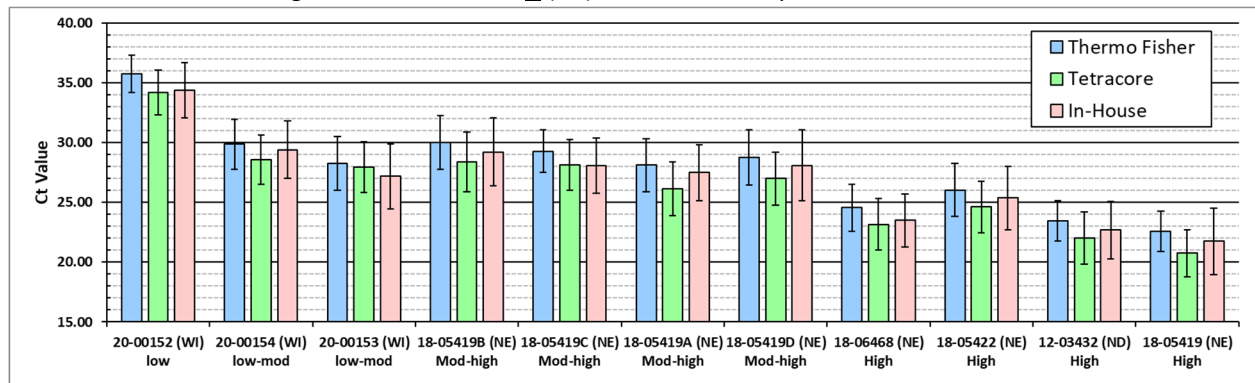
According to the 2010 Johne’s Disease Uniform Program Standards, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and correctly identify 70% or more of the remaining, valid, non-critical samples (can miss ~3 samples). [Table 5](#) lists 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.

Table 5. Reasons laboratories failed the 2020 Johne’s Disease Fecal Proficiency Panel.

2020	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		2				
Misclassified a high shedding sample as negative						
Missed 4 or more low / moderate shedders (lack of sensitivity)						
Multiple reasons cited above	1					
Total failed panels	1 (6%)	2 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total panels tested	17	28	18	9	2	5

Because direct PCR is now the most common organism detection assay used, the performance of that assay across laboratories becomes more important. 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. 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.

Figure 2. Average, and 1 standard deviation, reported Ct of 2020 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 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 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 9 laboratories that reported Ct values on at least one negative sample. Of those 9 laboratories, only 2 failed the PT (see [Table 5](#)) by calling a negative sample positive and is a reduction from last year’s proficiency testing results. Although fewer laboratories reported Ct values for negative samples, it continues to be an issue. 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
18-01899 (IA)			1	1
18-01900 (IA)	2	2	1	5
18-01901 (IA)	1			1
19-00486 (IA)	2			2
Num. panels reporting Ct	5	2	2	9

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 both negative pools and the two pools that contained a high-shedding animal (18-05419). Laboratories were allowed to pass even if they misclassified the other pool (17-12249 & 17-01709).

Table 7. Composition of the 2020 Johne’s Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	18-05419 (NE)	~4500
1 High, 4 Negative samples	18-05419 (NE)	~4500
1 Mod, 1 Low, 3 Negative samples	17-12249 (WI)	50.5
	17-01709 (IA)	2
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. It is commendable that all laboratories using solid and liquid culture passed. All but one laboratory passed the pooled panel using direct PCR.

Table 8. Performance of each method used in the Johne’s Disease 2020 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

2020		No. panels		
		Direct PCR	Liquid media	Solid media
Panels that failed	Identified the negative pool as positive			
	Identified a high -shedding pool as negative			
	Two non-critical pools were identified as negative			
	Failed due to multiple criteria	1		
Panels that passed	One non-critical pool was misidentified as negative	1		
	All 5 pools were identified correctly	45	12	3
	Total Failed Pooled Panels	1 (2%)	0 (0%)	0 (0%)
	Total	47	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 2020 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. 2020 Johne's Disease Individual Fecal Proficiency Panel key by panel number. Samples are coded by color according to shedding status as follows: **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	20-00152 (WI)	18-05419B (NE)	18-05422 (NE)	20-00154 (WI)	12-03432 (ND)
2	18-01900 (IA)	18-05419 (NE)	18-01901 (IA)	18-05419C (NE)	18-01899 (IA)
3	20-00153 (WI)	18-01900 (IA)	18-05419B (NE)	18-01901 (IA)	18-05419 (NE)
4	18-05419C (NE)	20-00154 (WI)	20-00152 (WI)	12-03432 (ND)	18-01900 (IA)
5	18-05419 (NE)	18-05419D (NE)	12-03432 (ND)	18-01899 (IA)	20-00154 (WI)
6	18-01900 (IA)	18-05422 (NE)	18-01899 (IA)	18-05419B (NE)	18-05422 (NE)
7	12-03432 (ND)	18-01901 (IA)	18-06468 (NE)	18-05419 (NE)	20-00152 (WI)
8	18-01899 (IA)	18-05419B (NE)	19-00486 (IA)	18-01900 (IA)	18-06468 (NE)
9	18-05419D (NE)	12-03432 (ND)	20-00154 (WI)	18-05422 (NE)	19-00486 (IA)
10	18-06468 (NE)	18-01899 (IA)	18-05419 (NE)	20-00152 (WI)	18-01900 (IA)
11	19-00486 (IA)	20-00153 (WI)	18-01900 (IA)	18-06468 (NE)	18-05419B (NE)
12	18-05419A (NE)	18-06468 (NE)	20-00154 (WI)	18-05419D (NE)	18-01901 (IA)
13	18-05419 (NE)	18-05419C (NE)	18-05422 (NE)	12-03432 (ND)	18-05419D (NE)
14	18-05419B (NE)	18-01901 (IA)	18-05419D (NE)	20-00152 (WI)	20-00154 (WI)
15	18-01901 (IA)	12-03432 (ND)	18-01900 (IA)	18-05419A (NE)	18-05419 (NE)
16	18-05419D (NE)	20-00152 (WI)	18-05419A (NE)	18-06468 (NE)	20-00153 (WI)
17	20-00152 (WI)	18-05419A (NE)	20-00153 (WI)	19-00486 (IA)	18-05419A (NE)
18	12-03432 (ND)	18-01900 (IA)	18-05419 (NE)	18-05419B (NE)	12-03432 (ND)
19	20-00154 (WI)	18-05422 (NE)	18-05419C (NE)	18-05422 (NE)	18-05419D (NE)
20	18-05419B (NE)	18-05419D (NE)	20-00152 (WI)	18-01901 (IA)	18-05419C (NE)
21	18-05422 (NE)	20-00154 (WI)	18-06468 (NE)	18-01900 (IA)	20-00152 (WI)
22	18-01901 (IA)	18-06468 (NE)	18-05419B (NE)	20-00153 (WI)	18-05422 (NE)
23	18-06468 (NE)	19-00486 (IA)	18-05419D (NE)	18-05419 (NE)	18-01901 (IA)
24	20-00154 (WI)	18-05419 (NE)	18-01901 (IA)	18-05419D (NE)	18-06468 (NE)
25	18-05422 (NE)	20-00152 (WI)	12-03432 (ND)	20-00154 (WI)	18-05419B (NE)
26	20-00153 (WI)	20-00153 (WI)	20-00153 (WI)	20-00153 (WI)	20-00153 (WI)

Appendix 2. 2020 Johne's Disease Pooled Fecal Proficiency Panel key by kit number.

Pool Description	Sample Pool Number			
	Panel #1-20	Panel #21-40	Panel #41-60	Panel #61-70
5 Negative samples	2	4	3	1
5 Negative samples	4	2	4	3
1 Mod (17-12249), 1 Low (17-01709), 3 Negative samples	1	5	2	4
1 high (18-05419), 4 Negative samples	3	1	5	2
1 high (18-05419), 4 Negative samples	5	3	1	5

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

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