



## 2021 Johne's Disease Fecal Proficiency Test General Summary October 21, 2021

### Overview

A total of 56 laboratories ordered panels during the 2021 Johne's Disease Fecal Proficiency Test (7 Canadian, 3 European Union, 1 Australian and 45 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. One individual panel was lost in shipping and replaced. 7 individual panels and 2 pooled panels were reported to be incomplete, missing individual samples were sent to make the panels complete. Results were not returned for 5 individual and 3 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 30<sup>th</sup>, 2021. The results provided in [Table 1](#) include these retests. One sample, 20-08637 (NE), was included in the individual panels as a critical positive from initial culture and PCR testing data; however direct PCR results, submitted by participating laboratories showed a larger spread and higher Ct values than initial testing. According to testing procedures, a valid proficiency panel sample will be determined "by a consensus of at least 70 percent of the laboratories participating in the fecal culture check testing process." As culture testing did not identify a validity issue with the sample, but because the reported PCR data had such high variation of Ct values, it was decided to re-classify the sample from a critical positive to a moderate positive classification instead of removing it from the grading scheme. The results provided in [Table 1A](#) include test results with removal of retests from panels that would have passed if this sample was originally classified as a moderate positive sample. 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. Laboratories that purchased solid media or made their own solid media for Johne's culture are grouped together.

**Table 1.** Summary results of the 2021 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.

2021	# passed	# failed	# passed	# failed	# Panels	Total Shipped	Total shipped in 2020 (%change)	Total shipped in 2019 (%change)
	1st attempt (%)	1st attempt (%)	2nd attempt (%)	2nd attempt (%)	not retested			
<b>Individual Panel</b>								
Direct PCR (all)	50 (86%)	8 (14%)	4 (80%)	1 (20%)	3	67	63 (+6%)	68 (-7%)
Tetracore	10 (67%)	5 (33%)	3 (75%)	1 (25%)		19	17 (+12%)	18 (-6%)
Thermo Fisher	22 (92%)	2 (8%)	1 (100%)			25	28 (-11%)	28 (+0%)
In-House	18 (95%)	1 (5%)			1	19	16 (+19%)	19 (-16%)
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Liquid Systems (all)	7 (88%)	1 (13%)			1	10	12 (-17%)	13 (-8%)
Other						0	2 (-100%)	3 (-33%)
TREK	7 (88%)	1 (13%)			1	8	9 (-11%)	9 (+0%)
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HEY Solid Media (all)	6 (100%)					6	6 (+0%)	6 (+0%)
<hr/>								
<b>Individual Panel Total</b>	<b>63 (88%)</b>	<b>9 (13%)</b>	<b>4 (80%)</b>	<b>1 (20%)</b>	<b>4</b>	<b>83</b>	<b>81 (+2%)</b>	<b>87 (-7%)</b>
<hr/>								
<b>Pooling Panel</b>								
Direct PCR (all)	47 (98%)	1 (2%)			1	50	48 (+4%)	51 (-6%)
Liquid	6 (100%)					7	11 (-36%)	12 (-8%)
Solid	2 (67%)	1 (33%)			1	3	3 (+0%)	3 (+0%)
<b>Pooled Panel Total</b>	<b>55 (96%)</b>	<b>2 (4%)</b>			<b>2</b>	<b>60</b>	<b>57 (+5%)</b>	<b>66 (-14%)</b>



**Table 1A.** Summary results of the 2021 individual John’s Disease Fecal Proficiency Test panels with sample reclassification.

2021	# passed 1st attempt (%)	# failed 1st attempt (%)	# passed 2nd attempt (%)	# failed 2nd attempt (%)	# Panels not retested	Total Shipped *	Total shipped in 2020 (%change)	Total shipped in 2019 (%change)
<b>Individual Panel</b>								
Direct PCR (all)	55 (95%)	3 (5%)	2 (100%)		1	64	63 (+2%)	68 (-7%)
Tetracore	14 (93%)	1 (7%)	1 (100%)			16	17 (-6%)	18 (-6%)
Thermo Fisher	23 (96%)	1 (4%)	1 (100%)			25	28 (-11%)	28 (+0%)
In-House	18 (95%)	1 (5%)			1	19	16 (+19%)	19 (-16%)
Liquid Systems (all)	7 (88%)	1 (13%)			1	10	12 (-17%)	13 (-8%)
Other						0	2 (-100%)	3 (-33%)
TREK	7 (88%)	1 (13%)			1	8	9 (-11%)	9 (+0%)
HEY Solid Media (all)	6 (100%)					6	6 (+0%)	6 (+0%)
<b>Individual Panel Total</b>	<b>68 (94%)</b>	<b>4 (6%)</b>	<b>2 (100%)</b>		<b>2</b>	<b>80</b>	<b>81 (+2%)</b>	<b>87 (-7%)</b>

\*-By reclassifying sample 20-08637 (NE), 6 panels passed that would have failed originally. Retests from these laboratories have been excluded from these calculations

### 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 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 20-08637 (NE) as previously described. Reclassifying the sample from animal 20-08637 (NE) allowed 6 panels to pass that would have failed with the original classification and allowed 1 laboratory to pass the proficiency test.



**Table 2.** Composition of the 2021 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 <sup>1</sup>	All Panels 77 <sup>2</sup>	Percent of Samples Correctly Classified					
				Liquid Media			Direct PCR		
				HEY 6	TREK 8	MGIT 0	Thermo F. 25	Tetracore 19	In-House 19
18-01900 (IA)	1	Critical- Neg	99%	100%	100%	0%	96%	100%	100%
18-01901 (IA)	1	Critical- Neg	100%	100%	100%	0%	100%	100%	100%
21-02542 (IA)	2	Critical- Neg	99%	100%	94%	0%	100%	97%	100%
21-02543 (IA)	1	Critical- Neg	100%	100%	100%	0%	100%	100%	100%
20-00154 (WI)	3	Low	97%	94%	100%	0%	97%	100%	93%
18-05419A (NE) <sup>4</sup>	2	Low	100%	100%	100%	0%	100%	100%	100%
20-00153 (WI) <sup>3</sup>	3	Low	98%	89%	100%	0%	100%	100%	97%
18-05419B (NE) <sup>4</sup>	2	Mod	99%	100%	100%	0%	98%	100%	100%
18-05422 (NE)	2	Mod	99%	100%	100%	0%	100%	100%	95%
18-05419C (NE) <sup>4</sup>	2	Mod	99%	100%	100%	0%	98%	100%	98%
20-08637 (NE)	1	Mod-High	95%	100%	100%	0%	100%	78%	100%
18-06468 (NE)	1	Critical- High	100%	100%	100%	0%	100%	100%	100%
18-05419D (NE) <sup>4</sup>	2	Critical- High	100%	100%	100%	0%	100%	100%	100%
12-03432 (ND)	3	Critical- High	99%	100%	100%	0%	100%	100%	97%

<sup>1</sup>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.

<sup>2</sup>Number of proficiency panels submitted per method.

<sup>3</sup>The positive control was one of the three from this animal.

<sup>4</sup>Positive sample diluted with negative material.

Samples from 10 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 2020 and 2021 Johne’s Disease Fecal Proficiency Panels with the overall results for each method performed by laboratories.

Cow ID	Panel Year	# Vials /Panel	Shedding Status	2020 2021	All Panels 79 <sup>1</sup> 77	Percent of Samples Correctly Classified					
						Liquid Media			Direct PCR		
						HEY 5 6	TREK 9 8	MGIT 2 0	Thermo F. 28 25	Tetracore 17 19	In-House 18 19
18-01900 (IA)	2020	2	Critical- Neg		99%	100%	100%	100%	96%	100%	100%
18-01900 (IA)	2021	1	Critical- Neg		99%	100%	100%	0%	96%	100%	100%
18-01901 (IA)	2020	2	Critical- Neg		100%	100%	100%	100%	100%	100%	100%
18-01901 (IA)	2021	1	Critical- Neg		100%	100%	100%	0%	100%	100%	100%
20-00154 (WI)	2020	2	Low-Mod		98%	100%	94%	100%	100%	100%	94%
20-00154 (WI)	2021	3	Low		97%	94%	100%	0%	97%	100%	93%
18-05419A (NE)	2020	1	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-05419A (NE)	2021	2	Low		100%	100%	100%	0%	100%	100%	100%
20-00153 (WI)	2020	2	Low-Mod		100%	100%	100%	100%	100%	100%	100%
20-00153 (WI)	2021	3	Low		98%	89%	100%	0%	100%	100%	97%
18-05419B (NE)	2020	2	Mod-High		100%	100%	100%	100%	100%	100%	100%
18-05419B (NE)	2021	2	Moderate		99%	100%	100%	0%	98%	100%	100%
18-05422 (NE)	2020	2	Critical- High		99%	100%	100%	100%	100%	94%	100%
18-05422 (NE)	2021	2	Moderate		99%	100%	100%	0%	100%	100%	95%
18-05419C (NE)	2020	1	Mod-High		99%	100%	94%	100%	100%	100%	100%
18-05419C (NE)	2021	2	Moderate		99%	100%	100%	0%	98%	100%	98%
18-05419D (NE)	2020	2	Critical- High		100%	100%	100%	100%	100%	100%	100%
18-05419D (NE)	2021	2	Critical- High		100%	100%	100%	0%	100%	100%	100%
12-03432 (ND)	2020	2	Critical- High		100%	100%	100%	100%	100%	100%	100%
12-03432 (ND)	2021	3	Critical- High		99%	100%	100%	0%	100%	100%	97%
18-05419D (NE)	2020	2	Critical- High		100%	100%	100%	100%	100%	100%	100%
18-05419D (NE)	2021	2	Critical- High		100%	100%	100%	0%	100%	100%	100%
12-03432 (ND)	2020	2	Critical- High		100%	100%	100%	100%	100%	100%	100%
12-03432 (ND)	2021	3	Critical- High		99%	100%	100%	0%	100%	100%	97%

<sup>1</sup>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 5-6 Ct value difference on average for the reclassified sample 20-08637 (NE) compared to the Thermo Fisher or In-House methods.





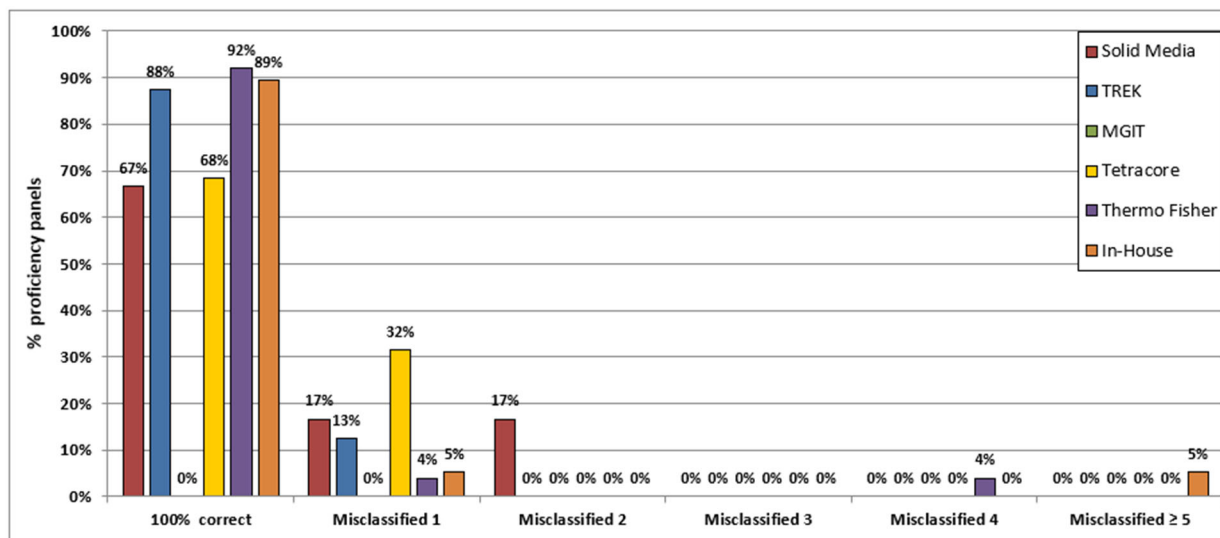
**Table 4.** A comparison of the averaged result values among the three methods for shedding animals.

		Average Result Values for Shedding Animals					
		Liquid Media			Direct PCR		
		HEY <sup>1</sup>	TREK	MGIT	Thermo F.	Tetracore	In-House
		Colonies per Tube	Days to Positive	Days to Positive	Ct	Ct	Ct
Cow ID	Shedding Status	6	8	0	25	19	19
20-00154 (WI)	Low	11.1	32	N/A	29.6	29.0	30.3
18-05419A (NE)4	Low	30.4	26	N/A	28.4	27.2	28.5
20-00153 (WI)	Low	13.5	30	N/A	28.4	28.4	28.5
18-05419B (NE)4	Mod	16.0	30	N/A	30.1	28.7	30.4
18-05422 (NE)	Mod	27.8	24	N/A	26.0	25.2	26.5
18-05419C (NE)4	Mod	17.1	33	N/A	29.4	28.6	29.8
20-08637 (NE)	Mod-High	15.7	26	N/A	28.7	34.5	29.3
18-06468 (NE)	Critical- High	31.6	23	N/A	24.6	23.8	25.0
18-05419D (NE)4	Critical- High	21.3	25	N/A	29.2	27.8	29.2
12-03432 (ND)	Critical- High	15.7	23	N/A	23.7	22.6	24.0

<sup>1</sup>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 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](#)). The TREK system sample classification improved since last year, increasing from 56% to 88%. Laboratories using solid media correctly classified 67% of the samples, a significant increase over last year (20%). The performance of the Thermo Fisher and In-house direct PCR methods improved compared to last year with more laboratories correctly calling all samples, but the performance of the Tetracore method decreased by 3%.

**Figure 1.** Percentage of 2021 Johne’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 5A](#) lists the reasons laboratories failed to pass the proficiency panel for each method with sample 20-08637 (NE) reclassified.

**Table 5.** Reasons laboratories failed the 2021 Johne’s Disease Fecal Proficiency Panel.

2021	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	1		1		
Missed 4 or more low / moderate shedders (lack of sensitivity)		1	1			
Misclassified a high shedding sample as negative	5		1			
Multiple reasons cited above			1			
<b>Total failed panels</b>	<b>6 (32%)</b>	<b>2 (8%)</b>	<b>2 (10%)</b>	<b>1 (13%)</b>	<b>0</b>	<b>0 (0%)</b>
<b>Total panels tested</b>	<b>19</b>	<b>25</b>	<b>20</b>	<b>8</b>	<b>0</b>	<b>6</b>

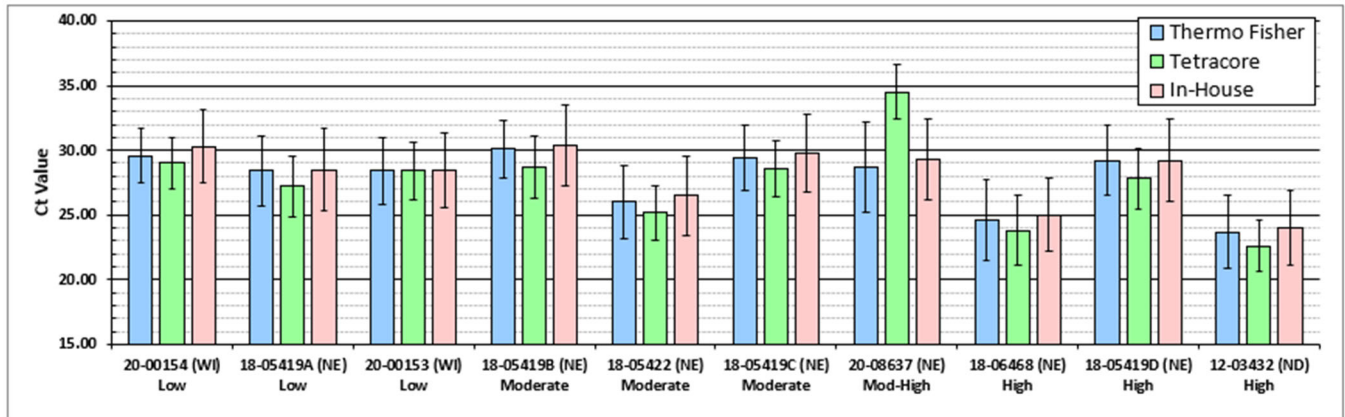
**Table 5A.** Reasons laboratories failed the 2021 Johne’s Disease Fecal Proficiency Panel with sample reclassification.

2021	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	1		1		
Missed 4 or more low / moderate shedders (lack of sensitivity)						
Misclassified a high shedding sample as negative						
Multiple reasons cited above			1			
<b>Total failed panels</b>	<b>1 (5%)</b>	<b>1 (4%)</b>	<b>1 (5%)</b>	<b>1 (13%)</b>	<b>0</b>	<b>0 (0%)</b>
<b>Total panels tested</b>	<b>19</b>	<b>25</b>	<b>20</b>	<b>8</b>	<b>0</b>	<b>6</b>

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, except for animal 20-08637 (NE).



**Figure 2.** Average, and 1 standard deviation, reported Ct of 2021 John’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 3 laboratories that reported Ct values on at least one negative sample, a decrease from last year. Of those 3 laboratories, 2 failed the PT (see [Table 5](#)) by calling a negative sample positive and is the same 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
18-01900 (IA)	1			1
18-01901 (IA)		1		1
21-02542 (IA)		1		1
21-02543 (IA)				0
Num. panels reporting Ct	1	2	0	3

### 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 pool and the two pools that contained a high-shedding animal (18-06468). Laboratories were allowed to pass even if they misclassified one of the other pools.

**Table 7.** Composition of the 2021 Johne’s Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	18-06468 (NE)	40
1 High, 4 Negative samples	18-06468 (NE)	40
1 Mod, 1 Low, 3 Negative samples	18-05419B (NE)	25
	20-00154 (WI)	17
2 Low, 3 Negative samples	20-00153 (WI)	18
	20-00154 (WI)	17
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 2021 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

2021		No. panels		
		Direct PCR	Liquid media	Solid media
Identified the negative pool as positive				
Panels that failed	Identified a high -shedding pool as negative	1		
	Two non-critical pools were identified as negative			1
Failed due to multiple criteria				
Panels that passed	One non-critical pool was misidentified as negative	1		
	All 5 pools were identified correctly	47	6	2
<b>Total Failed Pooled Panels</b>		<b>1 (2%)</b>	<b>0 (0%)</b>	<b>1 (33%)</b>
<b>Total</b>		<b>49</b>	<b>6</b>	<b>3</b>





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](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/sa_approved_labs/ct_approved_labs).



Remaining sample vials from the 2021 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](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/sa_reagents/ct_reagents)





**Appendix 1.** 2021 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	12-03432 (ND)	18-05419D (NE)	20-00153 (WI)	12-03432 (ND)	20-08637 (NE)
2	18-01900 (IA)	18-01901 (IA)	20-00154 (WI)	21-02543 (IA)	21-02542 (IA)
3	20-00153 (WI)	18-06468 (NE)	20-08637 (NE)	18-05419A (NE)	12-03432 (ND)
4	20-08637 (NE)	21-02542 (IA)	18-01900 (IA)	20-00153 (WI)	18-01901 (IA)
5	20-00154 (WI)	12-03432 (ND)	18-05419A (NE)	18-05419D (NE)	20-00154 (WI)
6	21-02542 (IA)	21-02543 (IA)	18-05419B (NE)	20-00154 (WI)	18-05419A (NE)
7	18-05419D (NE)	20-00153 (WI)	12-03432 (ND)	18-05422 (NE)	21-02543 (IA)
8	18-01901 (IA)	20-00154 (WI)	18-05422 (NE)	18-05419B (NE)	20-00153 (WI)
9	18-06468 (NE)	18-05419B (NE)	20-00154 (WI)	18-01900 (IA)	18-05419D (NE)
10	18-05419A (NE)	12-03432 (ND)	18-05419D (NE)	21-02542 (IA)	18-05422 (NE)
11	20-00154 (WI)	18-05422 (NE)	21-02543 (IA)	20-00153 (WI)	18-05419B (NE)
12	18-05419B (NE)	18-01900 (IA)	18-06468 (NE)	18-05419A (NE)	12-03432 (ND)
13	18-05419C (NE)	18-05419A (NE)	21-02542 (IA)	18-05419C (NE)	20-00154 (WI)
14	12-03432 (ND)	18-05419D (NE)	18-05422 (NE)	12-03432 (ND)	18-01900 (IA)
15	18-05419C (NE)	18-05419C (NE)	20-00153 (WI)	18-01901 (IA)	18-06468 (NE)
16	18-05422 (NE)	20-00154 (WI)	12-03432 (ND)	18-05419D (NE)	18-05419C (NE)
17	20-00153 (WI)	18-05422 (NE)	18-05419A (NE)	20-00154 (WI)	21-02542 (IA)
18	21-02543 (IA)	20-08637 (NE)	18-05419C (NE)	18-05419B (NE)	18-05419C (NE)
19	18-05419B (NE)	21-02542 (IA)	20-00154 (WI)	20-08637 (NE)	12-03432 (ND)
20	20-00154 (WI)	18-05419A (NE)	21-02542 (IA)	18-05419C (NE)	18-05422 (NE)
21	18-05422 (NE)	20-00153 (WI)	18-05419C (NE)	12-03432 (ND)	20-00154 (WI)
22	18-05419A (NE)	18-05419C (NE)	18-05419D (NE)	18-05422 (NE)	18-05419B (NE)
23	18-05419D (NE)	20-00154 (WI)	18-01901 (IA)	18-06468 (NE)	20-00153 (WI)
24	12-03432 (ND)	18-05419B (NE)	12-03432 (ND)	21-02542 (IA)	18-05419A (NE)
25	21-02542 (IA)	12-03432 (ND)	18-05419B (NE)	20-00154 (WI)	18-05419D (NE)
26	20-00153 (WI)	20-00153 (WI)	20-00153 (WI)	20-00153 (WI)	20-00153 (WI)

**Appendix 2.** 2021 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
2 Low (20-00153 & 20-00154), 3 Negative samples	1	5	2	4
1 Mod (18-05419B), 1 Low (20-00154), 3 Negative samples	4	2	4	3
1 High (18-06468), 4 Negative samples	3	1	5	2
1 High (18-06468), 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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