2017 Johne's Disease Fecal Proficiency Panel
General Summary
October 2, 2017

Overview

A total of 62 laboratories participated in the 2017 Johne's Disease Fecal Proficiency Panel (7 Canadian, 4 European Union, 1 New Zealand, 1 Australian and 49 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 170 panels were requested, with none needing to be replaced. Results were not returned for 6 Individual and 4 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, 2017. The results provided in Table 1 include these retests. Laboratories that only used reagents from a single manufacturer, either Tetracore or Life Technologies, are listed separately. Laboratories that use either in-house reagents, other commercial kits not marketed in the US, or mix commercial reagents are listed under the "In House" category. One laboratory used in-house liquid culture reagents and 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.

Table 1. Summary results of the 2017 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.

	# passed 1st attempt (%)	# failed 1st attempt (%)	# passed 2nd attempt (%)	# failed 2nd attempt (%)	# Kits not retested	Total Shipped	Total shipped in 2016 (%change)
Individual Panel							
Direct PCR (all)	43 (73%)	16 (27%)	9 (90%)	1 (10%)	6	71	71 (+0%)
Tetracore	14 (74%)	5 (26%)	3 (75%)	1 (25%)	1	23	27 (-15%)
Life Technologies	19 (79%)	5 (21%)	4 (100%)		1	28	26 (+8%)
In-House	10 (63%)	6 (38%)	2 (100%)		4	18	18 (+0%)
Liquid Systems (all)	15 (88%)	2 (12%)			2	20	22 (-9%)
MGIT 960	5 (100%)					5	4 (+25%)
TREK	10 (83%)	2 (17%)			2	12	17 (-29%)
HEY Solid Media (all)	8 (89%)	1 (11%)			1	10	11 (-9%)
Individual Panel Total	**66 (78%)**	**19 (22%)**	**9 (90%)**	**1 (10%)**	9	**101**	**104 (-3%)**
Pooling Panel							
Direct PCR (all)	45 (98%)	1 (2%)			1	48	48 (+0%)
Liquid	11 (73%)	4 (27%)			4	16	16 (+0%)
HEY	4 (100%)					5	3 (+67%)
Pooled Panel Total	**60 (92%)**	**5 (8%)**			**5**	**69**	**67 (+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 as soon as possible in individual vials, and stored at -70°C until kits were distributed. Because we lacked moderate shedding samples, two samples were produced where material from a high shedding cow was diluted with material from a culture negative cow and mixed thoroughly. These samples are labeled 17-02487D (FL) and 15-00471D (FL), reflecting the animal that the positive material originated. Panels were assembled in groups, each with a different key (See Table 9 at the end of this report for the key). Vial #18 in the second lot of panels (#26 thru #50) did not meet our quality criteria and therefore was omitted from the analysis. Table 2 shows the categorical (positive/negative) performance for each identification method by animal ID. According to the 2010 Uniform Program Standards a sample is considered valid "by a consensus of at least 70 percent of the laboratories participating in the fecal culture check testing process." Animal 15-00627 (IA) did not meet this criteria and was not included in the official grading. Removal of samples from this animal left 5 samples from valid low and moderate shedding animals. Instead of requiring laboratories to correctly classify 70% of these non-critical valid samples (miss up to 1 sample to pass) we allowed up to 3 misclassifications to pass.

Table 2. Composition of the 2017 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 95[2]	HEY 9	TREK 12	MGIT 5	Life Tech 28	Tetracore 23	In-House 18
17-03780 (IA)	1	Critical- Neg	98%	100%	100%	100%	96%	100%	94%
17-03785 (IA)	1	Critical- Neg	97%	100%	100%	100%	100%	91%	94%
17-03784 (IA)	2	Critical- Neg	96%	100%	96%	100%	98%	93%	94%
17-03787 (IA)	2	Critical- Neg	99%	100%	100%	100%	100%	100%	94%
17-03786 (IA)	2	Critical- Neg	95%	94%	100%	100%	95%	93%	92%
15-00627 (IA)	2	Low	52%	39%	50%	20%	38%	70%	69%
17-03492 (WI)	2	Low-Mod	44%	72%	92%	30%	25%	39%	39%
17-02487D (FL)[3]	2	Mod	98%	100%	92%	90%	100%	100%	97%
16-01645 (IA)[4]	2	Mod-High	99%	100%	92%	100%	100%	100%	100%
15-00471D (FL)[3]	2	Critical- High	99%	100%	100%	100%	100%	98%	100%
16-01648 (IA)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
16-01646 (IA)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
17-02487 (FL)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
15-00471 (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]Positive sample diluted with negative material.

[4]The positive control was one of the two from this animal.

Samples from 5 animals were also used in 2016 and their performance compared. Table 3 shows the respective year panels' categorical (positive/negative) performance for each identification method by animal ID.

Table 3. Comparison between five animals used in the 2017 and 2016 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	Life Tech	Tetracore	In-House
	Panel	# Vials	Shedding	2016 102[1]	10	13	3	26	27	18
Cow ID	Year	/Panel	Status	2017 95	9	12	5	28	23	18
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%
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%
16-01648 (IA)	2016	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
16-01648 (IA)	2017	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
16-01646 (IA)	2016	2	Critical- High	100%	100%	100%	100%	100%	100%	97%
16-01646 (IA)	2017	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
15-00471 (FL)	2016	2	Critical- High	99%	100%	100%	88%	100%	98%	100%
15-00471 (FL)	2017	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 and shedding animals.

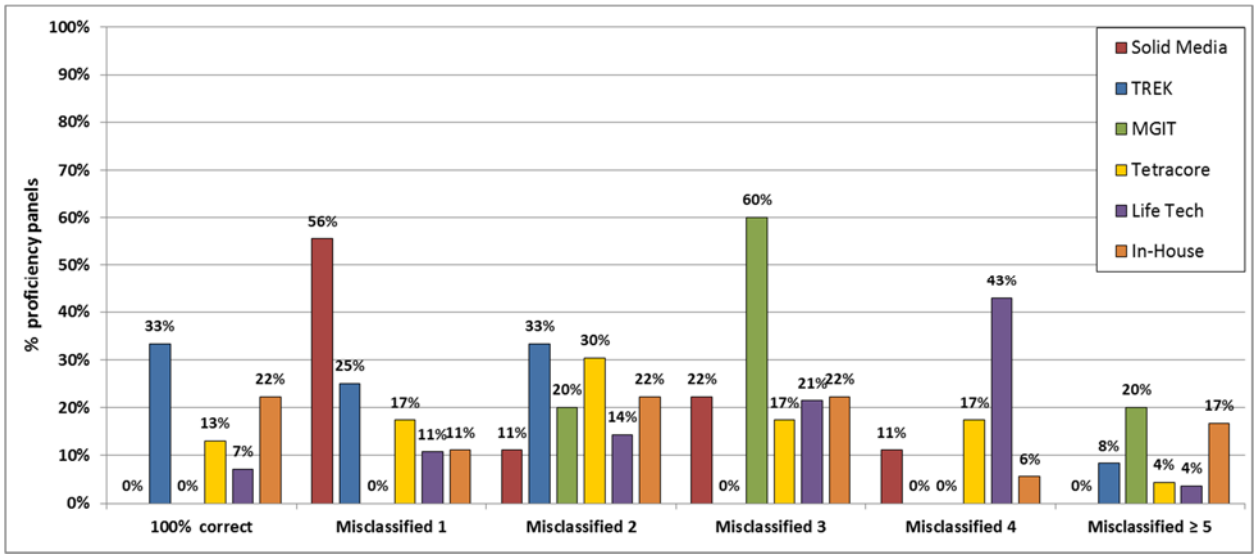
Table 4. A comparison of the averaged result values among the three methods for shedding animals.

		Average Result Values for Shedding Animals					
		Liquid Media			Direct PCR		
		HEY[1]	TREK	MGIT	Life Tech	Tetracore	In-House
	Shedding	Colonies per Tube	Days to Positive	Days to Positive	Ct	Ct	Ct
Cow ID	Status	9	12	5	28	23	18
15-00627 (IA)	Low	0.3	35	40	36.7	35.8	35.3
17-03492 (WI)	Low-Mod	0.8	34	40	37.7	36.3	36.4
17-02487D (FL)	Mod	2.3	31	35	32.4	30.9	31.3
16-01645 (IA)	Mod-High	24.3	26	30	32.1	29.7	30.5
15-00471D (FL)	Critical- High	36.0	22	22	26.8	25.7	26.0
16-01648 (IA)	Critical- High	51.4	18	17	26.1	23.9	24.1
16-01646 (IA)	Critical- High	TNTC	13	13	21.9	27.1	22.1
17-02487 (FL)	Critical- High	15.5	16	17	23.9	22.9	21.9
15-00471 (FL)	Critical- High	38.0	16	16	23.2	22.9	22.1

[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). Please note that this analysis included all 25 samples, including the two samples from cow 15-00627 (IA) that were not included in the official grading. In this analysis 33% of laboratories using the TREK system correctly classified all the samples. No laboratory using solid media correctly classified all the samples but most misclassified only 1 sample. The performance of the three direct PCR methods were more variable than expected when compared to the previous two years. Twenty-two percent of laboratories using In-house direct PCR methods correctly classified all the samples.

Figure1. Percentage of 2017 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, Life Technologies, and In-House) methods. A panel consisted of 25 fecal samples.



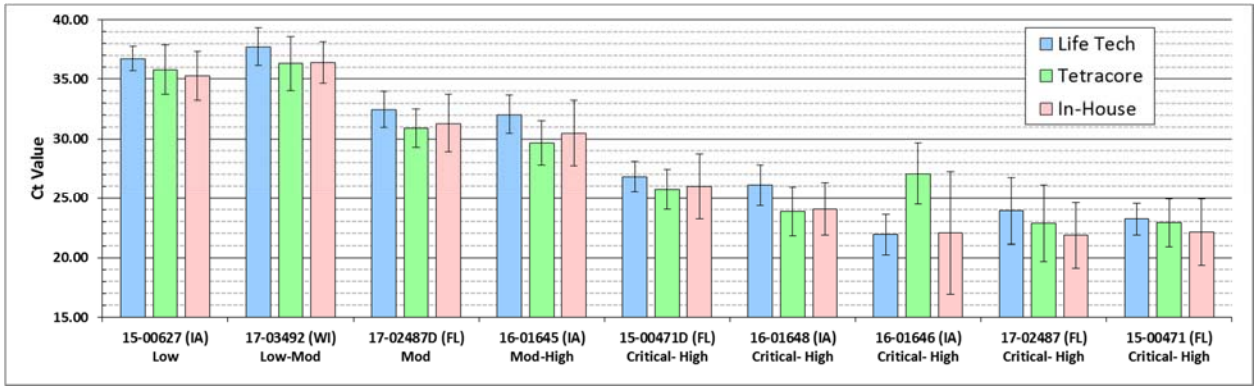
According to the 2010 Johne's Disease Uniform Program Standards, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and misidentify 3 or fewer of the remaining, valid, non-critical samples (modified for this year, see discussion above). 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. Interestingly, it seemed to be a bigger problem for laboratories using direct PCR methods than liquid or solid culture.

Table 5. Reasons laboratories failed the 2017 Johne's Disease Fecal Proficiency Panel.

	Direct PCR (Tetracore)	Direct PCR (Life Tech)	Direct PCR (In-House)	TREK liquid media	MGIT liquid media	HEY solid media
Misclassified a negative sample as positive	5	5	6	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	**6 (26%)**	**5 (18%)**	**6 (33%)**	**2 (17%)**	**0**	**1 (11%)**
Total kits tested	**23**	**28**	**18**	**12**	**5**	**9**

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 groups were statistically similar, except 16-01646 where a statistically significant difference is seen between laboratories using Life Technologies and Tetracore reagents.

Figure 2. Average reported Ct of 2017 Johne's disease fecal proficiency panel animals for the three direct PCR methods (ABI, Tetracore, and In House). Shedding status is listed below the animal ID.



False positive results with either direct fecal PCR or confirmatory culture PCR 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 had Ct values but correctly reported them as negative. Also shown are the number of panels where at least one Ct is reported. Errors were relatively evenly distributed amongst the five negative animals that were used in this year's panel when considering the number of vials included. There were a total of 19 laboratories that reported Ct values on at least one negative sample. Of those 19 laboratories, 17 failed the PT (see Table 5) by calling a negative sample positive and this is a very significant increase from year's past.

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	ABI	In-House
17-03780 (IA)		2	1
17-03785 (IA)	2	1	2
17-03784 (IA)	4	3	4
17-03787 (IA)	1	2	3
17-03786 (IA)	4	4	4
Num. panels reporting Ct	7	5	7

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 Table 9 lists the pool numbers associated with each lot of panels. Laboratories were required to correctly classify the negative pool and the two pools that contained a high-shedding animal (15-00471& 13-08115) in order to pass. Laboratories were allowed to misclassify one of the other pools (16-01645 & 15-00427) and still pass the panel.

Table 7. Composition of the 2017 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	15-00471	~1250
1 High, 4 Negative samples	13-08115	~500
1 Moderate, 4 Negative samples	16-01645	25
1 Moderate, 4 Negative samples	15-00427	11
5 Negative samples		

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

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

Table 8. Performance of each method used in the Johne's Disease 2017 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

		No. panels		
		Direct PCR	Liquid media	Solid media
Panels that failed	Identified the negative pool as positive		3	
	Identified a high -shedding pool as negative	1		
	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			
	All 5 pools were identified correctly	41	10	4
	Total Failed Pooled Kits	**1 (2%)**	**4 (27%)**	
	Total	**45**	**15**	**4**

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 2017 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

Table 9. 2017 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# 61-65
5 Negative samples	4	2	1	5
1 low-mod (15-00427), 4 Negative samples	1	5	3	2
1 mod-high (16-01645), 4 Negative samples	5	3	4	1
1 high (13-08115), 4 Negative samples	2	4	5	3
1 high (15-00471), 4 Negative samples	3	1	2	4

Table 10. 2017 Johne's Disease Individual Fecal Proficiency Panel key by kit number. Samples are coded by color according to shedding status as follows: Negative, Noncritical positive samples, Critical – high shedding samples. Vial 18 in panels 26-50 was omitted from grading, see discussion above for details. Sample 26 was the positive control.

Vial #	1-25	26-50	51-75	76-100	100-105
1	15-00627 (IA)	16-01648 (IA)	15-00627 (IA)	17-03784 (IA)	17-03492 (WI)
2	15-00471D (FL)	15-00627 (IA)	17-03785 (IA)	15-00471 (FL)	17-02487D (FL)
3	16-01645 (IA)	17-03784 (IA)	17-02487D (FL)	17-03786 (IA)	17-03780 (IA)
4	17-03492 (WI)	15-00471 (FL)	17-03786 (IA)	17-03787 (IA)	15-00471D (FL)
5	16-01646 (IA)	17-03786 (IA)	17-02487 (FL)	17-03492 (WI)	17-02487 (FL)
6	17-02487 (FL)	17-03780 (IA)	17-03784 (IA)	17-02487D (FL)	17-03786 (IA)
7	17-03786 (IA)	16-01648 (IA)	15-00471 (FL)	16-01645 (IA)	17-03785 (IA)
8	17-03784 (IA)	17-03787 (IA)	17-03787 (IA)	16-01646 (IA)	15-00627 (IA)
9	15-00471 (FL)	17-02487D (FL)	15-00471D (FL)	17-02487 (FL)	15-00471D (FL)
10	16-01648 (IA)	15-00627 (IA)	17-03492 (WI)	16-01648 (IA)	17-02487 (FL)
11	17-03787 (IA)	17-03785 (IA)	16-01645 (IA)	15-00627 (IA)	16-01648 (IA)
12	16-01648 (IA)	17-03492 (WI)	15-00627 (IA)	17-03784 (IA)	17-03784 (IA)
13	17-02487 (FL)	17-03784 (IA)	17-03492 (WI)	17-03780 (IA)	15-00471 (FL)
14	17-03492 (WI)	17-02487D (FL)	17-02487 (FL)	15-00471D (FL)	17-03492 (WI)
15	17-03780 (IA)	15-00471 (FL)	16-01648 (IA)	17-03786 (IA)	17-03787 (IA)
16	17-02487D (FL)	15-00471D (FL)	17-03784 (IA)	17-02487 (FL)	16-01646 (IA)
17	17-03784 (IA)	17-03786 (IA)	16-01646 (IA)	16-01648 (IA)	16-01645 (IA)
18	15-00627 (IA)	Omitted	15-00471 (FL)	17-03492 (WI)	17-02487D (FL)
19	15-00471D (FL)	17-03787 (IA)	17-03786 (IA)	15-00471D (FL)	17-03784 (IA)
20	17-03785 (IA)	16-01646 (IA)	17-03787 (IA)	15-00471 (FL)	16-01648 (IA)
21	16-01646 (IA)	16-01645 (IA)	15-00471D (FL)	17-03787 (IA)	17-03786 (IA)
22	17-03786 (IA)	16-01646 (IA)	17-03780 (IA)	17-02487D (FL)	15-00471 (FL)
23	15-00471 (FL)	17-03492 (WI)	17-02487D (FL)	15-00627 (IA)	17-03787 (IA)
24	17-03787 (IA)	17-02487 (FL)	16-01646 (IA)	17-03785 (IA)	16-01646 (IA)
25	17-02487D (FL)	15-00471D (FL)	16-01648 (IA)	16-01646 (IA)	15-00627 (IA)
26	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)

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