2016 Johne's Disease Fecal Proficiency Panel
General Summary
October 3, 2016

Overview

A total of 62 laboratories participated in the 2016 Johne's Disease Fecal Proficiency Panel (7 Canadian, 4 European Union, 1 New Zealand, 1 Australian and 49 USA laboratories). Compared to 2015, the number of individual proficiency panel requesting laboratories increased for direct PCR, decreased for liquid, and remained the same for solid culture methods. Requests for pooled proficiency panels increased for direct PCR, decreased for liquid, and remained constant solid culture methods. 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 172 panels were requested, with one reported to be incomplete and needing replaced. Results were not returned for 3 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, 2016. 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, including one laboratory that used in-house solid media.

Table 1. Summary results of the 2016 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 2015 (%change)
Individual Panel							
Direct PCR (all)	52 (80%)	13 (20%)	5 (83%)	1 (17%)	7	71	60 (+18%)
Tetracore	21 (84%)	4 (16%)	2 (100%)		2	27	25 (+8%)
Life Technologies	21 (88%)	3 (13%)	1 (50%)	1 (50%)	1	26	21 (+24%)
In-House	10 (63%)	6 (38%)	2 (100%)		4	18	13 (+38%)
Liquid Systems (all)	16 (76%)	5 (24%)			5	22	24 (-8%)
MGIT 960	3 (75%)	1 (25%)			1	4	5 (-20%)
TREK	13 (76%)	4 (24%)			4	17	17 (+0%)
HEY Solid Media (all)	10 (100%)					11	12 (-8%)
Individual Panel Total	**78 (81%)**	**18 (19%)**	**5 (83%)**	**1 (17%)**	12	**104**	**96 (+8%)**
Pooling Panel							
Direct PCR (all)	46 (98%)	1 (2%)	1 (100%)			48	44 (+9%)
Liquid	14 (88%)	2 (13%)			2	16	17 (-6%)
HEY	2 (100%)					3	4 (-25%)
Pooled Panel Total	**62 (95%)**	**3 (5%)**	**1 (100%)**		**2**	**67**	**63 (+6%)**

Individual Panel Description

Each individual panel consisted of 25 unknown 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. 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. This year, material from two animals was mixed with material from a different shedding animal before being aliquoted. These two animals were 15-00427 (ID) that was mixed with material from 15-00426 (ID) and 15-00628 (IA) that was mixed with material from 15-00471 (FL). Although animal 15-00628 (IA) is characterized as a high shedder, it was included in the non-critical shedding animal category since a full evaluation of the mixture was not completed before assembly of the panels. Panels were assembled in groups, each with a different key (See Table 9 at the end of this report for the key). Table 2 shows the categorical (positive/negative) performance for each identification method by animal ID. This year all animals met the required 70% pass rate to be considered valid.

Table 2. Composition of the 2016 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 102[2]	HEY 10	TREK 13	MGIT 3	Life Tech 26	Tetracore 27	In-House 18
13-00352 (IA)	1	Critical- Neg	98%	100%	100%	100%	100%	96%	94%
13-00353 (IA)	1	Critical- Neg	98%	100%	100%	100%	96%	100%	94%
14-02869 (IA)	4	Critical- Neg	97%	100%	94%	100%	96%	99%	97%
14-02870 (IA)	2	Critical- Neg	99%	100%	100%	100%	100%	100%	100%
12-00956 (KS)	2	Moderate	100%	100%	100%	100%	100%	100%	100%
15-00427 (ID)	2	Moderate	97%	100%	97%	100%	96%	96%	97%
16-01645 (IA)[3]	2	Mod-High	99%	100%	97%	100%	98%	100%	97%
15-00628 (IA)	2	High	100%	100%	97%	100%	100%	100%	100%
15-00426 (ID)	2	Critical- High	97%	100%	97%	100%	98%	100%	89%
13-08115 (ID)	2	Critical- High	100%	100%	100%	100%	100%	98%	100%
16-01648 (IA)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
15-00471 (FL)	2	Critical- High	99%	100%	100%	88%	100%	98%	100%
16-01646 (IA)	2	Critical- High	100%	100%	100%	100%	100%	100%	97%

[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.

Samples from 2 animals have been used in prior years, 1 in 2014 and 1 in 2015, 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 two animals used in the 2014 and 2015 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
			2014	105[1]	13	22	7	19	24	17
	Panel	# Vials	Shedding	2015	93	12	17	5	21	25	13
Cow ID	Year	/Panel	Status	2016	102	10	13	3	26	27	18
12-00956 (KS)	2014	2	Moderate		99%	100%	100%	100%	100%	98%	97%
12-00956 (KS)	2016	2	Moderate		100%	100%	100%	100%	100%	100%	100%
13-08115 (ID)	2015	2	Low		99%	100%	100%	100%	100%	98%	100%
13-08115 (ID)	2016	2	Low		100%	100%	100%	100%	100%	98%	100%

[1]Number of proficiency panels submitted per method.

Table 4 shows the averaged value reported for each of the methods and shedding animals. Interestingly, the reported values for animal 12-00956 (KS) differ between methods. The averaged values of animal 12-00956 (KS) are most similar to the high-shedding animals for liquid culture and direct PCR. However, it is more similar to a low- or moderate-shedding animal using solid culture. These results are similar to those found in 2014 when this animal was also used in the panels and is included at the bottom of Table 4. In our laboratory we isolated both cattle and 'bison' strains of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from 12-00956 (KS), 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.

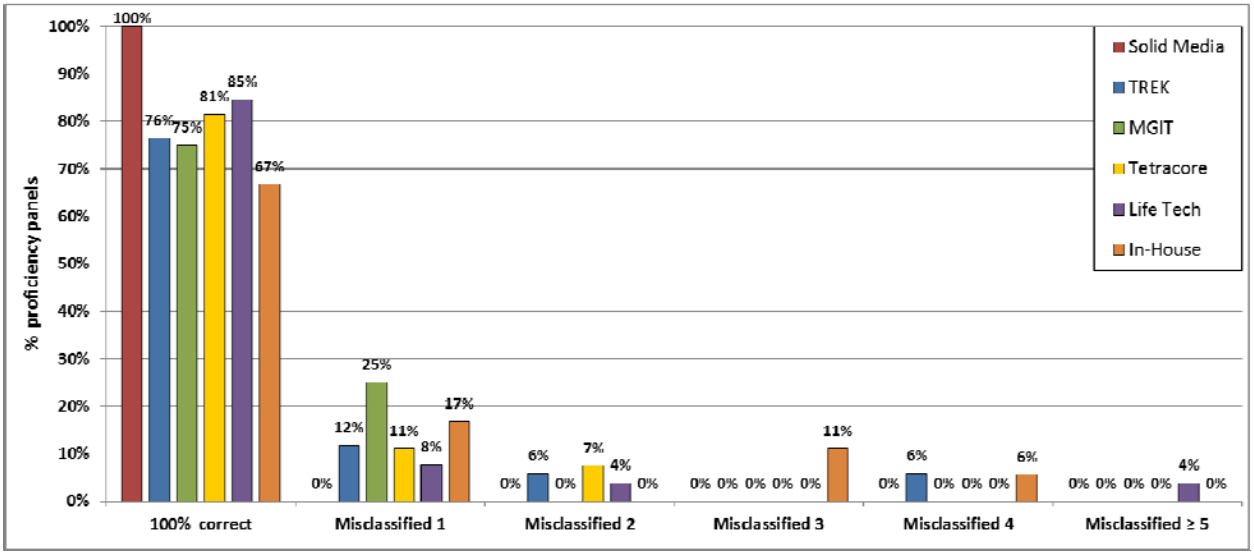
Table 4. A comparison of the averaged result values among the three methods for shedding animals.

		Average Result Values for Shedding Animals					
			Liquid Media		Direct PCR		
		HEY[1]	TREK	MGIT	Life Tech	Tetracore	In-House
		Colonies	Days to	Days to			
	Shedding	per Tube	Positive	Positive	Ct	Ct	Ct
Cow ID	Status	10	13	3	26	27	18
12-00956 (KS)	Moderate	11.2	22	12	27.2	25.7	26.3
15-00427 (ID)	Moderate	11.0	32	28	32.8	30.1	31.2
16-01645 (IA)	Mod-High	24.6	25	26	32.0	29.5	29.7
15-00628 (IA)	High	43.8	21	19	27.4	25.3	25.6
15-00426 (ID)	Critical- High	22.7	23	17	29.7	29.0	26.9
13-08115 (ID)	Critical- High	TNTC	20	16	28.2	26.7	26.5
16-01648 (IA)	Critical- High	30.0	18	15	26.3	23.1	23.3
15-00471 (FL)	Critical- High	TNTC	15	13	23.6	22.0	21.6
16-01646 (IA)	Critical- High	TNTC	14	10	22.3	26.8	20.4
2014 12-00956 (KS)	Moderate	10.5	20	18	27.1	27.2	27.5

[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). In this analysis 100% of the laboratories using solid media correctly classified all samples. All three direct PCR methods and TREK performed comparably. Eighty-five percent of laboratories using Life Technologies direct PCR method correctly classified all the samples and 76% of laboratories using the TREK system correctly classified all samples.

Figure1. Percentage of 2016 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.



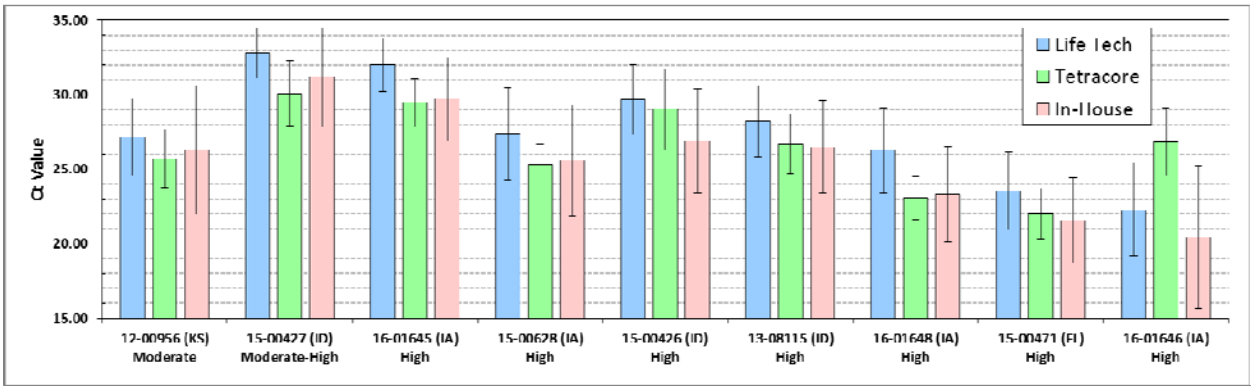
According to the 2010 Johne's Disease Uniform Methods and Rules, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and misidentify less than 30% of the remaining, valid, non-critical samples. Table 5 lists the specific reasons laboratories failed to pass the proficiency panel for each method.

Table 5. Reasons laboratories failed the 2016 Johne's Disease Fecal Proficiency Panel. Laboratories were required to correctly identify all the negative samples as negative and all the critical high shedding samples as positive (critical samples). They also were required to correctly classify at least 70% of the remaining samples.

	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	2	3	4	3	0	0
Missed 4 or more low / moderate shedders (lack of sensitivity)	0	0	0	1	0	0
Misclassified a high shedding sample as negative	1	0	2	0	1	0
Multiple reasons cited above	1	1	0	0	0	0
Total failed kits	**1 (4%)**	**4 (15%)**	**6 (33%)**	**4 (24%)**	**1 (25%)**	**0 (0%)**
Total kits tested	**27**	**26**	**18**	**17**	**4**	**10**

As more laboratories use direct PCR as their primary organism detection assay, 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 with the average Ct score between the methods for each animal differing by less than 3, except 16-01646 that varies by >6. Despite life technologies having the most laboratories correctly classify all of the samples, this method resulted in generally higher mean Ct values.

Figure 2. Average reported Ct of 2016 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 four negative animals that were used in this year's panel when considering the number of vials included. There were a total of 12 laboratories that reported Ct values on at least one negative sample. Of those 12 laboratories, 11 failed the PT (see Table 5) by calling a negative sample positive and this is a 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
13-00352 (IA)	1		2
13-00353 (IA)		1	1
14-02869 (IA)	1	4	4
14-02870 (IA)	1	1	3
Num. panels reporting Ct	3	5	4

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 two negative pools and the two pools that contained a high-shedding animal (13-08115 & 16-01648) in order to pass. Laboratories were allowed to misclassify the other pool (16-01645) and still pass the panel.

Table 7. Composition of the 2016 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	16-01648	~4000
1 High, 4 Negative samples	13-08115	~500
1 Moderate, 4 Negative samples	16-01645	29
5 Negative samples		
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 but three laboratories passed the pooled panel. The laboratory that failed using a direct PCR method misclassified a negative pool. Of the laboratories using liquid culture one misclassified the negative pool and another misclassified a pool with a high shedding animal.

Table 8. Performance of each method used in the Johne's Disease 2016 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	1	1	0
	Identified a high -shedding pool as negative	0	1	0
	Two non-critical pools were identified as negative	0	0	0
	Failed due to multiple criteria	0	0	0
Panels that passed	One non-critical pool was misidentified as negative	1	1	0
	All 5 pools were identified correctly	46	13	2
	Total Failed Pooled Kits	**1 (2%)**	**2 (13%)**	**0 (0%)**
	Total	**48**	**16**	**2**

A current listing of all the approved laboratories is available in the NVLS web site: Approved laboratories.

Remaining sample vials from the 2016 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.

Table 9. 2016 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	2	1	2	3
5 Negative samples	3	2	5	2
1 mod-high (16-01645), 4 Negative samples	4	5	4	1
1 high (13-08115), 4 Negative samples	5	3	1	4
1 high (16-01648), 4 Negative samples	1	4	3	5

Table 10. 2016 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. Sample 26 was the positive control.

Vial #	1-25	26-50	51-75	76-100	100-105
1	13-08115 (ID)	14-02870 (IA)	15-00628 (IA)	15-00426 (ID)	14-02870 (IA)
2	13-00352 (IA)	16-01645 (IA)	15-00426 (ID)	15-00471 (FL)	16-01646 (IA)
3	14-02870 (IA)	15-00427 (ID)	16-01646 (IA)	14-02869 (IA)	14-02869 (IA)
4	15-00471 (FL)	13-08115 (ID)	14-02869 (IA)	12-00956 (KS)	16-01646 (IA)
5	14-02869 (IA)	13-00353 (IA)	15-00427 (ID)	15-00628 (IA)	15-00426 (ID)
6	15-00426 (ID)	15-00471 (FL)	15-00471 (FL)	16-01646 (IA)	15-00628 (IA)
7	15-00471 (FL)	14-02869 (IA)	14-02870 (IA)	14-02869 (IA)	15-00427 (ID)
8	14-02869 (IA)	16-01648 (IA)	16-01645 (IA)	16-01648 (IA)	14-02870 (IA)
9	16-01645 (IA)	15-00628 (IA)	15-00471 (FL)	14-02870 (IA)	12-00956 (KS)
10	15-00427 (ID)	12-00956 (KS)	14-02869 (IA)	15-00426 (ID)	13-08115 (ID)
11	15-00628 (IA)	15-00426 (ID)	15-00628 (IA)	12-00956 (KS)	13-00353 (IA)
12	16-01648 (IA)	16-01646 (IA)	12-00956 (KS)	13-08115 (ID)	16-01648 (IA)
13	14-02869 (IA)	14-02869 (IA)	13-08115 (ID)	13-00353 (IA)	14-02869 (IA)
14	16-01648 (IA)	15-00426 (ID)	13-00352 (IA)	16-01646 (IA)	16-01648 (IA)
15	13-00353 (IA)	13-08115 (ID)	16-01646 (IA)	14-02870 (IA)	15-00628 (IA)
16	13-08115 (ID)	13-00352 (IA)	14-02869 (IA)	15-00628 (IA)	15-00427 (ID)
17	12-00956 (KS)	15-00471 (FL)	13-08115 (ID)	16-01645 (IA)	16-01645 (IA)
18	14-02870 (IA)	14-02869 (IA)	13-00353 (IA)	15-00427 (ID)	14-02869 (IA)
19	15-00427 (ID)	15-00628 (IA)	16-01648 (IA)	15-00471 (FL)	15-00471 (FL)
20	15-00628 (IA)	16-01646 (IA)	14-02869 (IA)	14-02869 (IA)	15-00426 (ID)
21	15-00426 (ID)	14-02869 (IA)	16-01648 (IA)	13-08115 (ID)	13-00353 (IA)
22	16-01646 (IA)	12-00956 (KS)	14-02870 (IA)	13-00352 (IA)	15-00471 (FL)
23	14-02869 (IA)	15-00427 (ID)	15-00426 (ID)	16-01648 (IA)	12-00956 (KS)
24	16-01646 (IA)	14-02870 (IA)	15-00427 (ID)	14-02869 (IA)	13-00353 (IA)
25	12-00956 (KS)	16-01648 (IA)	12-00956 (KS)	15-00427 (ID)	13-08115 (ID)
26	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)	16-01645 (IA)

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

Report was prepared by:
Kevin D. Stokes, PhD
USDA/APHIS/STAS/NVSL
Mycobacteria /Brucella Section
Kevin.D.Stokes@USDA.APHIS.GOV