2015 Johne's Disease Fecal Proficiency Panel
General Summary
October 8, 2015

Overview

A total of 61 laboratories participated in the 2015 Johne's Disease Fecal Proficiency Panel (7 Canadian, 4 European Union, 1 New Zealand, 1 Australian and 48 USA laboratories). Compared to 2014, 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, and remained constant for liquid and 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 160 panels were requested; results were not returned for 5. None of the kits were reported to be faulty this year. If preliminary results indicated that the laboratory had failed, it was given the opportunity to retake the proficiency panel provided the results were completed by September 30th, 2015. 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 reagent, which is grouped with the laboratories using the MGIT system. All laboratories using solid media were grouped together even though two laboratories used in-house solid media.

Table 1. Summary results of the 2015 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 2014 (%change)
Individual Panel							
Direct PCR (all)	52 (91%)	5 (9%)	2 (100%)		3	60	63 (-5%)
Tetracore	21 (91%)	2 (9%)	2 (100%)			25	24 (+4%)
Life Technologies	20 (95%)	1 (5%)				21	19 (+11%)
In-House	11 (85%)	2 (15%)				13	17 (-24%)
Liquid Systems (all)	21 (95%)	1 (5%)			1	24	29 (-17%)
MGIT 960	4 (80%)	1 (20%)				5	7 (-29%)
TREK	17 (100%)					17	22 (-23%)
HEY Solid Media (all)	11 (92%)	1 (8%)			1	12	13 (-8%)
Individual Panel Total	**84 (92%)**	**7 (8%)**	**2 (100%)**		5	**96**	**105 (-9%)**
Pooling Panel							
Direct PCR (all)	40 (95%)	2 (5%)	1 (100%)		1	44	41 (+7%)
Liquid	16 (100%)					17	18 (-6%)
HEY	3 (100%)					3	4 (-25%)
Pooled Panel Total	**59 (97%)**	**2 (3%)**	**1 (100%)**		**1**	**64**	**63 (+2%)**

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. 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. Numbers in red indicate percentages that were less than the required 70%.

Table 2. Composition of the 2015 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 93[2]	HEY 12	TREK 17	MGIT 5	Life Tech 21	Tetracore 25	In-House 13
14-02868 (IA)	2	Critical- Neg	100%	100%	100%	100%	100%	100%	100%
13-01420 (IA)	3	Critical- Neg	99%	100%	100%	100%	98%	100%	97%
13-00354 (IA)	1	Critical- Neg	99%	92%	100%	75%	100%	100%	100%
13-00349 (IA)	1	Critical- Neg	98%	100%	100%	100%	100%	92%	100%
14-02866 (IA)	1	Critical- Neg	99%	100%	100%	100%	98%	98%	100%
12-03917 (ND)	2	Low	98%	100%	97%	63%	98%	98%	100%
12-03913 (ND)	2	Low	96%	83%	91%	75%	98%	100%	88%
12-03914 (ND)	2	Moderate	99%	100%	100%	100%	100%	100%	92%
13-06801 (IA)	2	Moderate	99%	100%	100%	100%	100%	100%	100%
14-03358 (IA)	2	Critical- High	99%	100%	100%	88%	98%	98%	100%
13-08115 (ID)[3]	2	Critical- High	99%	100%	100%	100%	100%	98%	100%
11-09754 (MT)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
12-03428 (ND)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
12-03916 (ND)	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.

Samples from 6 animals had been used in prior years, 2 in 2013 and 4 in 2014, and their performance compared. Table 3 shows the respective year panels' categorical (positive/negative) performance for each identification method by animal ID.

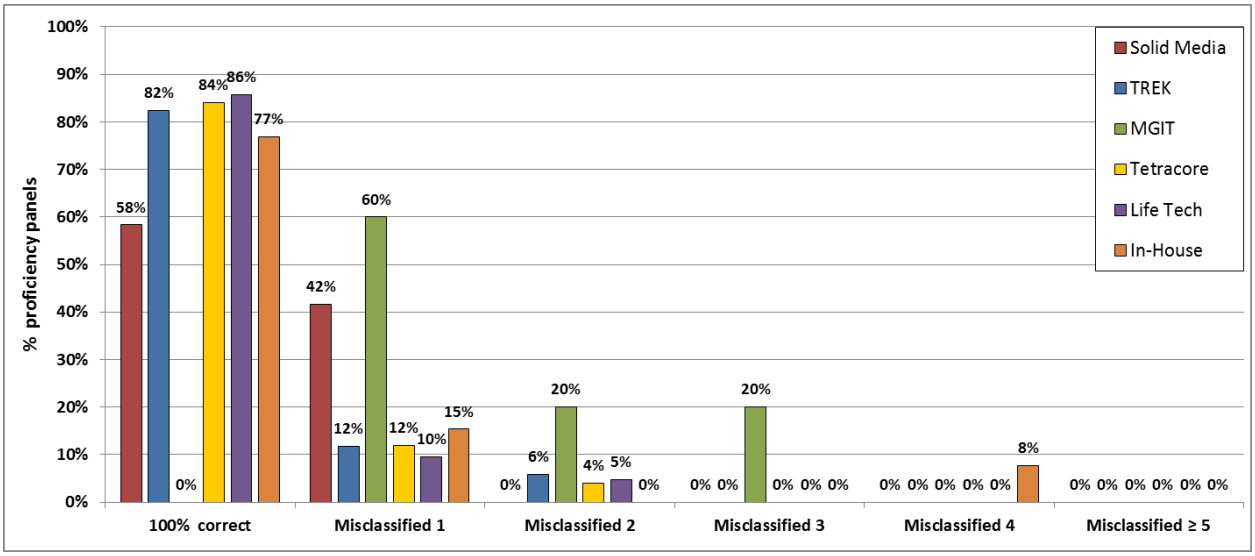
Table 3. Comparison between four animals used in both the 2012 and 2013 Johne's Disease Fecal Proficiency Panels with the overall categorical summary results per cow for each method performed by laboratories.

				Percent of Samples Correctly Classified						
					Liquid Media			Direct PCR		
				All Kits	HEY	TREK	MGIT	Life Tech	Tetracore	In-House
			2013	109[1]	19	19	11	16	25	19
	Panel	# Vials	Shedding	2014	105	13	22	7	19	24	17
Cow ID	Year	/Panel	Status	2015	93	12	17	5	21	25	13
13-00349 (IA)	2014	2	Critical-Neg		99%	100%	100%	100%	97%	100%	97%
13-00349 (IA)	2015	1	Critical-Neg		98%	100%	100%	100%	100%	92%	100%
12-03917 (ND)	2014	2	Low		96%	92%	100%	75%	97%	98%	97%
12-03917 (ND)	2015	2	Low		98%	100%	97%	63%	98%	98%	100%
12-03913 (ND)	2014	2	Low		89%	85%	100%	63%	92%	90%	88%
12-03913 (ND)	2015	2	Low		96%	83%	91%	75%	98%	100%	88%
12-03914 (ND)	2013	2	Low		96%	97%	97%	86%	100%	100%	89%
12-03914 (ND)	2015	2	Moderate		99%	100%	100%	100%	100%	100%	92%
11-09754 (MT)	2014	2	Critical-High		99%	100%	100%	100%	100%	98%	100%
11-09754 (MT)	2015	2	Critical-High		100%	100%	100%	100%	100%	100%	100%
12-03428 (ND)	2013	2	Critical-High		99%	100%	100%	95%	100%	100%	97%
12-03428 (ND)	2015	2	Critical-High		100%	100%	100%	100%	100%	100%	100%

[1]Number of proficiency panels submitted per method.

The performance of each method was further evaluated by determining the number of samples that were misclassified (Figure 1). In this analysis all three direct PCR methods and TREK performed comparably and very well. Eighty-six percent of laboratories using Life Technologies direct PCR method correctly classified all the samples. Eighty-two percent of laboratories using the TREK system correctly classified all samples, and 58% of the laboratories using solid media correctly classified all samples.

Figure1. Percentage of 2015 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.



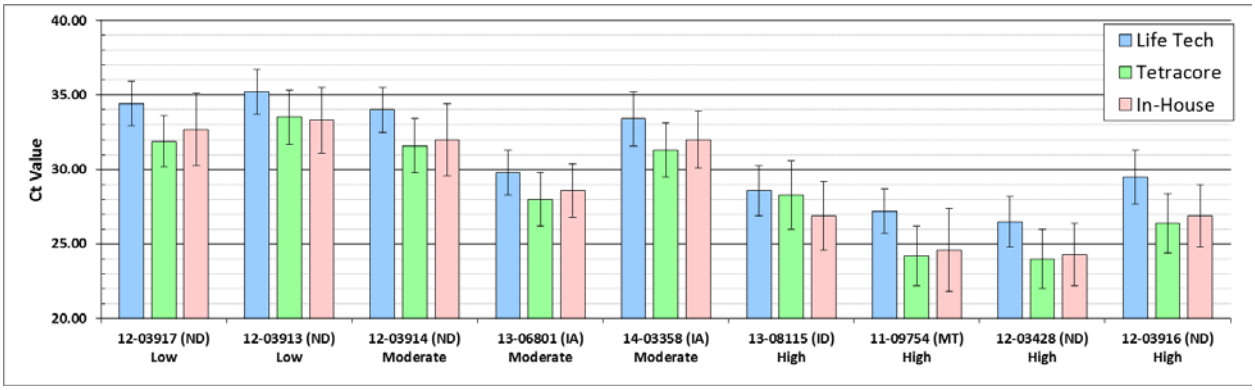
According to the 2010 Johne's Disease Uniform Methods and Rules, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and misidentify less than 30% of the remaining, valid, non-critical samples. Table 4 lists the specific reasons laboratories failed to pass the proficiency panel for each method.

Table 4. Reasons laboratories failed the 2015 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	0	2	1	0	1	1
Missed 4 or more low / moderate shedders (lack of sensitivity)	1	0	0	0	0	0
Misclassified a high shedding sample as negative	0	0	0	0	0	0
Multiple reasons cited above	1	0	0	0	0	0
Total failed kits	**2 (8%)**	**2 (10%)**	**2 (8%)**	**0 (0%)**	**1 (20%)**	**1 (8%)**
Total kits tested	**25**	**21**	**13**	**17**	**5**	**12**

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. Despite life technologies having the most laboratories correctly classify all of the samples, this method resulted in higher mean Ct values.

Figure 2. Average reported Ct of 2015 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. While animal 13-00349 (IA) is the only non-infected cow used in previous check tests, fecal material from animals in this herd has been used in previous years' proficiency panels. This year the highest shedding animals we included were one animal that contained 8,500 CFU per tube (2 samples) and another that contained 5,700 CFU per tube (2 samples). Table 5 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. Errors were relatively evenly distributed amongst three of the negative animals that were used in this year's panel. There were a total of 5 laboratories that reported Ct values one negative sample each. Of those 5 laboratories, 4 failed the PT (see Table 4) by calling the negative sample positive, which is half the number of failing laboratories as the last three years.

Table 5. 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
13-01420 (IA)	1	0	1
13-00354 (IA)	0	0	1
13-00349 (IA)	0	2	0

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 6 lists the contents of each pool, and Table 8 lists the pool numbers associated with each lot of kits. Laboratories were required to correctly classify the negative pool and the two pools that contained a high-shedding animal (12-03427 & 13-08115) in order to pass. Laboratories were allowed to misclassify one of the other two pools (12-00953 & 14-03358) and still pass the panel.

Table 6. Composition of the 2015 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	12-03427	3,350
1 High, 4 Negative samples	13-08115	500
1 Moderate, 4 Negative samples	12-00953	44
1 Moderate, 4 Negative samples	14-03358	38
5 Negative samples		

*Refers to the positive samples, not the pooled sample

Table 7 further describes the performance of each method used in the pooled proficiency test. It is commendable that all but two laboratories passed the pooled panel. The two laboratories that failed were using a direct PCR method; one laboratory misclassified the negative pool and other misclassified a pool with a high shedding animal.

Table 7. Performance of each method used in the Johne's Disease 2015 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	0	0
	Identified a high -shedding pool as negative	1	0	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	2	0	0
	All 5 pools were identified correctly	39	16	3
	Total Failed Pooled Kits	**2 (5%)**	**0 (0%)**	**0 (0%)**
	Total	**43**	**16**	**3**

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

Remaining sample vials from the 2015 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 8. 2015 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# 61-80**
5 Negative samples	4	1	3	5
1 moderate (14-03358), 4 Negative samples	5	4	1	2
1 moderate (12-00953), 4 Negative samples	2	5	4	3
1 high (13-08115), 4 Negative samples	3	2	5	1
1 high (12-03427), 4 Negative samples	1	3	2	4

Table 9. 2015 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
1	12-03428 (ND)	14-03358 (IA)	13-01420 (IA)	12-03916 (ND)
2	13-00349 (IA)	12-03428 (ND)	12-03913 (ND)	12-03914 (ND)
3	13-01420 (IA)	14-02868 (IA)	12-03917 (ND)	13-00354 (IA)
4	12-03917 (ND)	12-03916 (ND)	12-03916 (ND)	13-06801 (IA)
5	13-08115 (ID)	13-01420 (IA)	12-03914 (ND)	12-03917 (ND)
6	13-06801 (IA)	12-03913 (ND)	14-03358 (IA)	14-02868 (IA)
7	12-03917 (ND)	12-03914 (ND)	13-08115 (ID)	13-06801 (IA)
8	14-02868 (IA)	12-03917 (ND)	11-09754 (MT)	13-01420 (IA)
9	12-03913 (ND)	13-00349 (IA)	13-00354 (IA)	12-03913 (ND)
10	11-09754 (MT)	11-09754 (MT)	12-03913 (ND)	12-03428 (ND)
11	13-06801 (IA)	13-08115 (ID)	12-03917 (ND)	11-09754 (MT)
12	12-03914 (ND)	14-03358 (IA)	13-06801 (IA)	12-03913 (ND)
13	14-02868 (IA)	13-06801 (IA)	13-00349 (IA)	13-01420 (IA)
14	14-02866 (IA)	13-01420 (IA)	12-03914 (ND)	11-09754 (MT)
15	14-03358 (IA)	12-03913 (ND)	14-02868 (IA)	12-03917 (ND)
16	13-01420 (IA)	13-00354 (IA)	12-03428 (ND)	14-02868 (IA)
17	11-09754 (MT)	12-03917 (ND)	11-09754 (MT)	14-02866 (IA)
18	12-03916 (ND)	14-02868 (IA)	14-03358 (IA)	14-03358 (IA)
19	12-03913 (ND)	14-02866 (IA)	14-02868 (IA)	13-08115 (ID)
20	14-03358 (IA)	12-03914 (ND)	14-02866 (IA)	12-03916 (ND)
21	13-00354 (IA)	11-09754 (MT)	13-06801 (IA)	13-01420 (IA)
22	12-03916 (ND)	13-01420 (IA)	13-01420 (IA)	12-03428 (ND)
23	13-01420 (IA)	12-03428 (ND)	12-03916 (ND)	12-03914 (ND)
24	12-03428 (ND)	13-06801 (IA)	13-01420 (IA)	13-00349 (IA)
25	12-03914 (ND)	12-03916 (ND)	12-03428 (ND)	14-03358 (IA)
26	13-08115 (ID)	13-08115 (ID)	13-08115 (ID)	13-08115 (ID)

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