2013 Johne's Disease Fecal Proficiency Panel
General Summary
October 3, 2013

Overview

A total of 63 laboratories participated in the 2013 Johne's Disease Fecal Proficiency Panel (7 Canadian, 3 European Union, 1 New Zealand, 1 Australian and 51 USA laboratories). Overall, the number of laboratories that requested individual proficiency panels for direct PCR and liquid culture methods increased from 2012 and decreased for solid culture methods. Requests for pooled proficiency panels increased for direct PCR and solid culture methods, and decreased for liquid 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 176 panels were requested; results were not returned for 3 of them. No panels were reported to be faulty this year. If preliminary results indicated that the laboratory had failed, they were given the opportunity to retake the proficiency panel provided the results be completed by September 30th, 2013. The results provided in Table 1 include these retests. Laboratories that only used reagents from a single manufacturer, either Tetracore or Applied Biosystems, 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.

Table 1. Summary results of the 2013 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 2012 (%change)
Individual Panel							
Direct PCR (all)	48 (84%)	9 (16%)	2 (67%)	1 (33%)	4	61	62 (-2%)
Tetracore	22 (92%)	2 (8%)	1 (100%)			25	23 (9%)
Life Technologies	15 (94%)	1 (6%)				16	25 (-36%)
In-House	11 (65%)	6 (35%)	1 (50%)	1 (50%)		19	13 (46%)
Liquid Systems (all)	23 (82%)	5 (18%)	2 (100%)		3	30	31 (-3%)
MGIT 960	5 (56%)	4 (44%)	2 (100%)			11	8 (38%)
TREK	18 (95%)	1 (5%)				19	22 (-14%)
HEY Solid Media (all)	18 (95%)	1 (5%)			1	19	21 (-10%)
Individual Panel Total	**89 (86%)**	**15 (14%)**	**4 (80%)**	**1 (20%)**	8	**110**	**114 (-4%)**
Pooling Panel							
Direct PCR (all)	36 (97%)	1 (3%)	1 (100%)			39	36 (8%)
Liquid	19 (95%)	1 (5%)			1	21	23 (-9%)
HEY	6 (100%)					6	6 (0%)
Pooled Panel Total	**61 (97%)**	**2 (3%)**	**1 (100%)**		**1**	**66**	**65 (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. Samples from cow 10-08285 were very challenging (red numbers) with only 59% of the samples classified correctly. Because 10-08285 samples failed to meet the required 70% pass rate, they were excluded from the official panel grading, but are still included in this analysis.

Table 2. Composition of the 2012 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 109[2]	HEY 19	TREK 19	MGIT 11	Life Tech 16	Tetracore 25	In-House 19
09-02559 (ND)	1	Critical- Neg	99%	100%	95%	100%	100%	100%	95%
10-05134 (OH)	2	Critical- Neg	99%	100%	100%	95%	100%	98%	97%
10-06315 (OH)	2	Critical- Neg	98%	100%	100%	100%	97%	100%	92%
10-08285 (OH)	2	Low	59%[3]	78%	87%	55%	38%	50%	45%
11-06357 (IA)	1	Low	83%	94%	100%	64%	81%	76%	74%
11-09383 (MT)	2	Low	84%	92%	95%	68%	84%	80%	82%
12-03915 (ND)	2	Low	96%	100%	95%	82%	100%	100%	92%
12-03914 (ND)	2	Low	96%	97%	97%	86%	100%	100%	89%
12-00957 (KS)	2	Moderate	99%	100%	100%	91%	100%	100%	97%
12-02530 (MT)	2	Moderate	96%	100%	100%	86%	100%	98%	89%
12-00953 (KS)[4]	2	Moderate	99%	100%	100%	95%	100%	98%	100%
12-03430 (ND)	2	Critical- High	99%	100%	100%	91%	100%	100%	97%
12-03427 (ND)	2	Critical- High	99%	97%	100%	91%	100%	100%	100%
12-03428 (ND)	2	Critical- High	99%	100%	100%	95%	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]In order to be considered valid, more than 70% of the samples from an animal must be correctly classified.

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

Samples from 4 animals were used in both the 2012 and 2013 panels and their performance compared. Table 3 shows the 2012 and 2013 categorical (positive/negative) performance for each identification method by animal ID. There is a reduced percent of samples correctly classified this year compared to 2012 using direct PCR and MGIT methods in the samples from the low shedding animals, while solid culture improved and TREK held steady. The constant or slight increase seen with the HEY and TREK methods for the samples from low shedding animals suggests there was no degradation in the quality of the samples over the year.

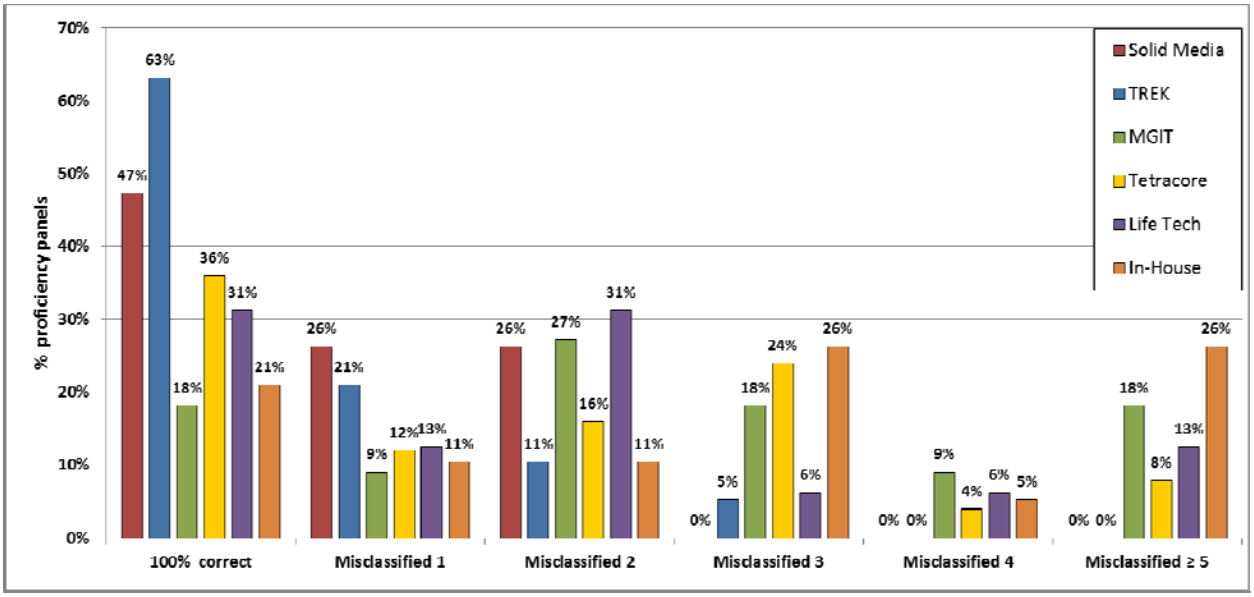
Table 3. Comparison between four animals used in both the 2012 and 2013 Johne's Disease Fecal Proficiency Panels with the overall categorical summary results per cow for each method performed by laboratories.

				Percent of Samples Correctly Classified					
	# Vials	Shedding			Liquid Media		Direct PCR		
	/Panel	Status	All Kits	HEY	TREK	MGIT	Life Tech	Tetracore	In-House
	2012		112[1]	21	22	8	23	25	13
Cow ID	2013		109	19	19	11	16	25	19
11-06357 (IA)	3	Low	88%	92%	100%	79%	87%	79%	90%
11-06357 (IA)	1	Low	83%	94%	100%	64%	81%	76%	74%
11-09383 (MT)	2	Low	91%	88%	95%	81%	91%	90%	96%
11-09383 (MT)	2	Low	84%	92%	95%	68%	84%	80%	82%
12-00957 (KS)	2	Moderate	100%	100%	100%	94%	100%	100%	100%
12-00957 (KS)	2	Moderate	99%	100%	100%	91%	100%	100%	97%
12-02530 (MT)	3	Moderate	99%	100%	100%	100%	97%	97%	100%
12-02530 (MT)	2	Moderate	96%	100%	100%	86%	100%	98%	89%

[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). Please note that this analysis included all 25 samples, including the two samples from cow 10-08285 that were not included in the official grading. In this analysis TREK outperformed all other methods with 63% of the kits correctly classifying all 25 samples. Thirty-six percent of laboratories using Life Technologies direct PCR correctly classified all samples, and 47% of the laboratories using solid media correctly classified all samples.

Figure1. Percentage of 2013 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.



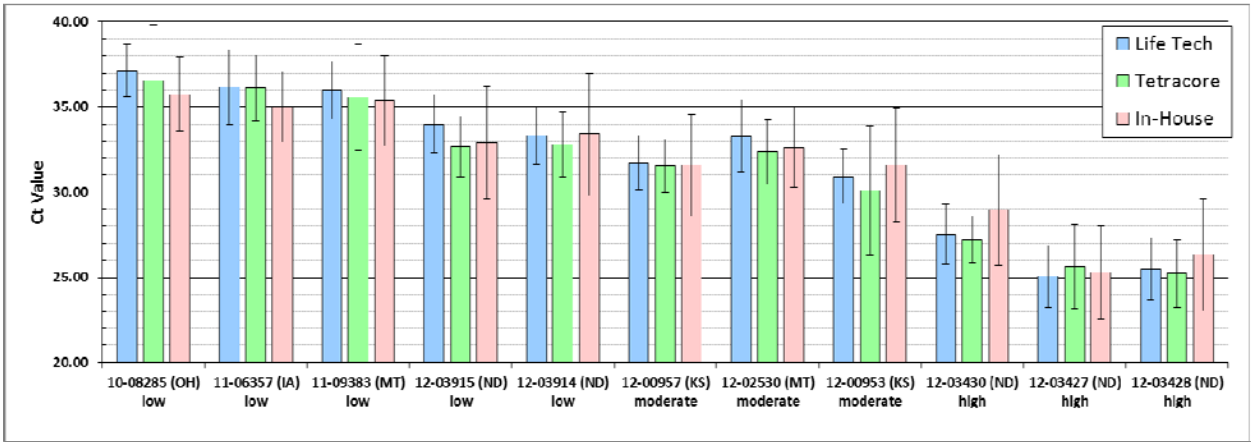
According to the 2010 Johne's Disease Uniform Methods and Rules, laboratories must correctly classify all critical high shedding samples as positive, all negative samples as negative and misidentify less than 30% of the remaining, valid, non-critical samples. Table 4 lists the specific reasons laboratories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

Table 4. Reasons laboratories failed the 2013 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	1	1	3	1	1	0
Missed 3 or more low / moderate shedders (lack of sensitivity)	1	0	2	0	0	0
Misclassified a high shedding sample as negative	0	0	2	0	2	1
Multiple reasons cited above	0	0	0	0	1	0
Total failed kits	**2 (8%)**	**1 (6%)**	**7 (37%)**	**1 (5%)**	**4 (36%)**	**1 (5%)**
Total kits tested	**25**	**16**	**19**	**19**	**11**	**19**

As more and 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 times (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). However, any Ct reported as a range (e.g. >38) or as a text entry (e.g. undetermined) were excluded from this analysis. The overall means of all three groups were remarkably similar with the average Ct score between the methods for each animal differing by less than 1.75. These differences were not statistically significant. Not only were averages similar but standard deviations were also similar among the methods for each animal. Of note is that the standard deviations for the In-House method group ranged from 7-13 in 2012 but ranged from 2-3 this year. This was the biggest improvement among the methods from last year and makes all three direct PCR methods very similar. As stated above, samples from four animals were used in both the 2012 and 2013 panels. Comparing the average Ct from year to year for each of the four animals and method showed that each differed by less than 1 Ct, and most were less than 0.5, which is remarkably consistent. This consistency also suggests the quality of these samples is very consistent from last year to this year.

Figure 2. Average reported Ct of 2012 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 none of the non-infected cows have been used in the check test previously, fecal material from animals in these herds has been used as negative samples in the proficiency panel in previous years. Although we did not include any samples from animals that were shedding over 7,000 CFU per tube this year, we did have one that contained 3,350 CFU per tube (2 samples) and another that contained 5700 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 evenly distributed amongst the samples. There were a total of 5 laboratories that reported Ct values for negative samples; of those two reported more than one negative sample with Ct values. Two kits were tested using SYBR Green technology, which resulted in Ct values due to non-specific amplification in most of the samples. The false-positive results

for these two kits were not included in Table 5. There were a total of 8 laboratories that failed the PT (see Table 4) by calling negative samples positive, the same as last year. Three of those laboratories chose to retake the PT and 2 successfully passed the panel on the retest. The only laboratory that failed the retest identified different animals as negative.

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 (60 tested)	ABI (120 tested)	In-House (120 tested)
09-02559 (ND)	0	0	2
10-05134 (OH)	1	0	1
10-06315 (OH)	0	1	3

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 vial numbers associated with each pool. Laboratories were required to correctly classify the negative pool and the two pools that contained a high-shedding animal (10-08425 & 12-03427) in order to pass. Laboratories were allowed to misclassify one of the other two pools (moderate or mod-high) and still pass the panel.

Table 6. Composition of the 2013 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 Very High, 4 Negative samples	10-08425	>10000
1 High, 4 Negative samples	12-03427	3350
1 Mod-High, 4 Negative samples	11-06361	86
1 Moderate, 4 Negative samples	12-02530	20
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. No laboratory failed because they misclassified negative pools as positive or classified pools with a high shedding animal as negative. It is commendable that no laboratory misclassified the negative pool and that only two laboratories failed out of 64 kits. All laboratories that submitted results using solid culture methods passed, with all but one of those correctly classifying all the pools. Direct PCR and Liquid culture methods had one laboratory each fail due to misclassifying both non-critical pools.

Table 7. Performance of each method used in the Johne's Disease 2013 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

		Direct PCR	Liquid media	Solid media
Panels that failed	Identified the negative pool as positive	0	0	0
	Identified a high -shedding pool as negative	0	0	0
	Two non-critical pools were identified as negative	1	1	0
	Failed due to multiple criteria	0	0	0
Panels that passed	One non-critical pool was misidentified as negative	10	3	1
	All 5 pools were identified correctly	27	16	5
	Total Failed Pooled Kits	**1 (3%)**	**1 (5%)**	**0 (0%)**
	Total	**38**	**20**	**6**

A current listing of all the approved laboratories is available in the NVLS web site: http://www.aphis.usda.gov/animal_health/lab_info_services/approved_labs.shtml .

Remaining sample vials from the 2013 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: http://www.aphis.usda.gov/animal_health/lab_info_services/reagents.shtml.

Table 8. 2013 Johne's Disease Pooled Fecal Proficiency Panel key by kit number

	Pool Sample Number		
Pool Description	**Kit# 1-25**	**Kit# 25-50**	**Kit# 51-75**
5 Negative samples	2	5	3
1 moderate (12-02530), 4 Negative samples	4	1	5
1 mod-high (11-06361), 4 Negative samples	3	4	1
1 high (12-03427), 4 Negative samples	1	2	4
1 very high (10-08425), 4 Negative samples	5	3	2

Table 9. 2013 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	101-125
1	12-03430 (ND)	10-08285 (OH)	10-05134 (OH)	12-00953 (KS)	12-03915 (ND)
2	11-09383 (MT)	12-03430 (ND)	12-03428 (ND)	12-00957 (KS)	10-08285 (OH)
3	10-05134 (OH)	12-03427 (ND)	11-09383 (MT)	09-02559 (ND)	10-06315 (OH)
4	12-03428 (ND)	10-05134 (OH)	12-02530 (MT)	12-03428 (ND)	12-03427 (ND)
5	12-03914 (ND)	12-03915 (ND)	12-03430 (ND)	12-03427 (ND)	12-02530 (MT)
6	12-02530 (MT)	12-02530 (MT)	10-06315 (OH)	10-05134 (OH)	12-00953 (KS)
7	10-08285 (OH)	12-03914 (ND)	12-03914 (ND)	10-08285 (OH)	12-03914 (ND)
8	12-03915 (ND)	12-03428 (ND)	12-00953 (KS)	12-03430 (ND)	12-00957 (KS)
9	10-05134 (OH)	09-02559 (ND)	12-00957 (KS)	12-03915 (ND)	10-05134 (OH)
10	12-03428 (ND)	12-03914 (ND)	12-03430 (ND)	11-06357 (IA)	12-03428 (ND)
11	10-06315 (OH)	12-00957 (KS)	10-08285 (OH)	12-03427 (ND)	10-06315 (OH)
12	12-03430 (ND)	12-03915 (ND)	11-09383 (MT)	10-06315 (OH)	11-06357 (IA)
13	12-03915 (ND)	12-00957 (KS)	12-00957 (KS)	12-03914 (ND)	12-00957 (KS)
14	12-00957 (KS)	12-03430 (ND)	09-02559 (ND)	11-09383 (MT)	11-09383 (MT)
15	12-00957 (KS)	10-05134 (OH)	12-03427 (ND)	12-02530 (MT)	10-05134 (OH)
16	12-03427 (ND)	11-09383 (MT)	12-03915 (ND)	12-03430 (ND)	12-03427 (ND)
17	10-06315 (OH)	12-02530 (MT)	11-06357 (IA)	10-05134 (OH)	12-02530 (MT)
18	11-06357 (IA)	11-09383 (MT)	12-03428 (ND)	12-02530 (MT)	12-03430 (ND)
19	12-03914 (ND)	12-03428 (ND)	10-05134 (OH)	10-08285 (OH)	10-08285 (OH)
20	12-00953 (KS)	10-06315 (OH)	12-03914 (ND)	12-03915 (ND)	12-03428 (ND)
21	12-02530 (MT)	10-08285 (OH)	10-08285 (OH)	12-03914 (ND)	09-02559 (ND)
22	12-03427 (ND)	12-00953 (KS)	12-03915 (ND)	10-06315 (OH)	12-03430 (ND)
23	09-02559 (ND)	11-06357 (IA)	12-02530 (MT)	12-03428 (ND)	12-03914 (ND)
24	10-08285 (OH)	12-03427 (ND)	10-06315 (OH)	12-00957 (KS)	11-09383 (MT)
25	11-09383 (MT)	10-06315 (OH)	12-03427 (ND)	11-09383 (MT)	12-03915 (ND)
26	12-00953 (KS)	12-00953 (KS)	12-00953 (KS)	12-00953 (KS)	12-00953 (KS)

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