2014 Johne's Disease Fecal Proficiency Panel
General Summary
October 8, 2014

Overview

A total of 59 laboratories participated in the 2014 Johne's Disease Fecal Proficiency Panel (7 Canadian, 4 European Union, 1 New Zealand, 1 Australian and 46 USA laboratories). Compared to 2013, the number of requesting laboratories increased for individual proficiency panels for direct PCR and decreased for liquid and solid culture methods. Requests for pooled proficiency panels increased for direct PCR, and decreased 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 168 panels were requested; results were not returned for 6 of them. There was 1 kit 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 were completed by September 30th, 2014. 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.

Table 1. Summary results of the 2014 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 2013 (%change)
Individual Panel							
Direct PCR (all)	53 (91%)	5 (9%)	2 (100%)		3	63	61 (3%)
Tetracore	23 (96%)	1 (4%)			1	24	25 (-4%)
Life Technologies	16 (89%)	2 (11%)	1 (100%)		1	19	16 (19%)
In-House	14 (88%)	2 (13%)	1 (100%)		1	17	19 (-11%)
Liquid Systems (all)	24 (92%)	2 (8%)	1 (100%)		1	29	30 (-3%)
MGIT 960	7 (100%)					7	11 (-36%)
TREK	17 (89%)	2 (11%)	1 (100%)		1	22	19 (16%)
HEY Solid Media (all)	12 (92%)	1 (8%)			1	13	19 (-32%)
Individual Panel Total	**89 (92%)**	**8 (8%)**	**3 (100%)**		5	**105**	**110 (-5%)**
Pooling Panel							
Direct PCR (all)	37 (93%)	3 (8%)	1 (100%)		2	41	39 (5%)
Liquid	17 (100%)					18	21 (-14%)
HEY	4 (100%)					4	6 (-33%)
Pooled Panel Total	**58 (95%)**	**3 (5%)**	**1 (100%)**		**2**	**63**	**65 (-3%)**

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 11-09382 were very challenging (red numbers) with only 46% of the samples classified correctly. Because 11-09382 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 2014 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 105[2]	HEY 13	TREK 22	MGIT 7	Life Tech 19	Tetracore 24	In-House 17
10-05134 (OH)	2	Critical- Neg	99%	100%	100%	100%	100%	98%	100%
10-06315 (OH)	2	Critical- Neg	98%	100%	92%	100%	100%	100%	97%
13-01419 (IA)	2	Critical- Neg	99%	96%	100%	100%	97%	100%	100%
13-00349 (IA)	2	Critical- Neg	99%	100%	100%	100%	97%	100%	97%
11-09382 (MT)	2	Low	46%[3]	42%	58%	13%	42%	44%	56%
12-03913 (ND)	2	Low	89%	85%	100%	63%	92%	90%	88%
12-03917 (ND)	2	Low	96%	92%	100%	75%	97%	98%	97%
12-00956 (KS)	2	Moderate	99%	100%	100%	100%	100%	98%	97%
12-00953 (KS)	2	Moderate	97%	100%	100%	100%	100%	94%	94%
11-07393 (IA)	2	High	98%	92%	100%	88%	100%	100%	100%
12-03430 (ND)[4]	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
11-09754 (MT)	2	Critical- High	99%	100%	100%	100%	100%	98%	100%
12-03427 (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]In order to be considered valid, more than 70% of the samples from an animal must be correctly classified.

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

Reported values for animal 12-00956 (KS) differ between methods and should be noted. Table 3 shows the averaged value reported for each of the methods and shedding animals. 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. 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 3 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 3. A comparison of the averaged result values among the three methods for shedding animals.

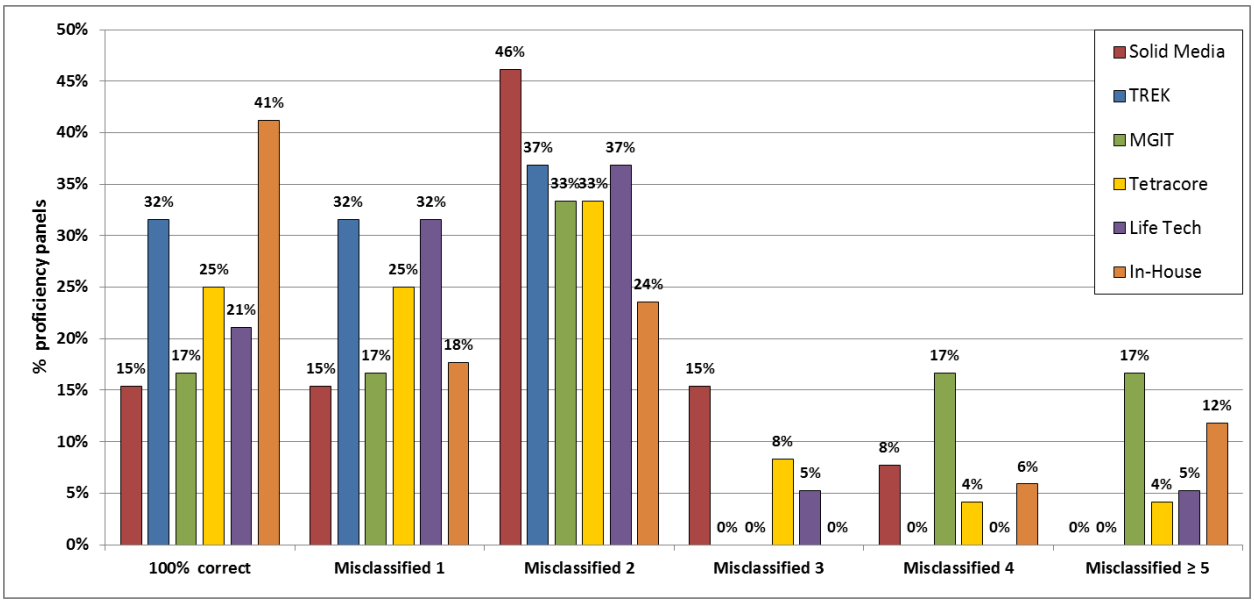
		Average Result Values for Shedding Animals					
		Liquid Media			Direct PCR		
		HEY[1]	TREK	MGIT	Life Tech	Tetracore	In-House
	Shedding	Colonies per Tube	Days to Positive	Days to Positive	Cτ	Cτ	Cτ
Cow ID	Status	13	22	7	19	24	17
11-09382 (MT)	Low	0.3	41	41	37.6	37.6	36.6
12-03913 (ND)	Low	2.3	36	37	34.8	34.7	33.6
12-03917 (ND)	Low	6.0	34	32	33.9	33.6	32.5
12-00956 (KS)	Moderate	10.5	20	18	27.1	27.2	27.5
12-00953 (KS)	Moderate	21.3	26	24	31.5	30.5	30.3
11-07393 (IA)	High	10.6	18	14	24.6	27.6	25.0
12-03430 (ND)	Critical- High	8.6	22	15	28.0	27.3	27.4
11-09754 (MT)	Critical- High	10.7	19	16	26.4	25.0	24.6
12-03427 (ND)	Critical- High	4.9	18	13	25.4	25.0	24.5

[1]Results shown include reported values only. Reports that do not include Cτ 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.

When evaluating laboratory performance for each animal there was an unusual level of variability noticed among duplicate samples from animal 11-07393 (IA). While this animal was classified as a critical-high shedder due to NVSL's CFU counts, several laboratories reported one sample as high shedding and the other as low to moderate. This finding was independent of method and appeared to be inherent in the sample. Because of this unusual level of variability reported, we reclassified these samples as non-critical high. This reclassification allowed 2 laboratories who would have failed the proficiency test to pass. Unfortunately this variability was not detected until roughly 50% of the laboratories reported their results.

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 11-09382 (MT) that were not included in the official grading. In this analysis the In House Direct PCR methods outperformed all other methods with 41% of the kits correctly classifying all 25 samples. Thirty-two percent of laboratories using the TREK system correctly classified all samples, and 15% of the laboratories using solid media correctly classified all samples.

Figure1. Percentage of 2014 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.



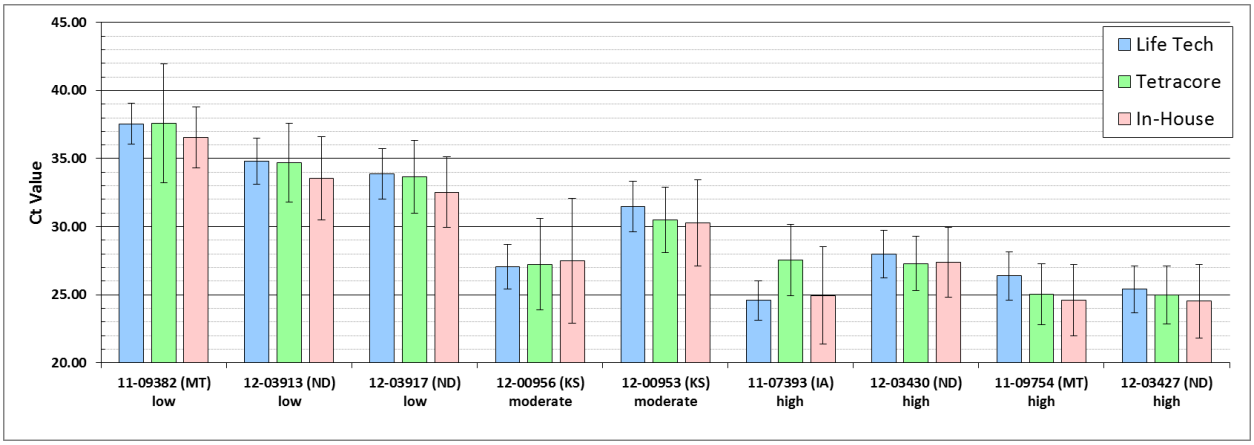
According to the 2010 Johne's Disease Uniform Methods and Rules, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and misidentify less than 30% of the remaining, valid, non-critical samples. Table 4 lists the specific reasons laboratories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

Table 4. Reasons laboratories failed the 2014 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	2	2	2	0	1
Missed 3 or more low / moderate shedders (lack of sensitivity)	0	0	0	0	0	0
Misclassified a high shedding sample as negative	1	0	0	0	0	0
Multiple reasons cited above	1	0	0	0	0	0
Total failed kits	**1 (4%)**	**2 (11%)**	**2 (12%)**	**2 (11%)**	**0 (0%)**	**1 (8%)**
Total kits tested	**24**	**19**	**17**	**20**	**6**	**13**

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). However, any Ct reported as a range (e.g. >38) or as a text entry (e.g. undetermined) were excluded from this analysis. Except for animal 11-07393 (IA), the animal where inconsistencies were noted among the samples, 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 1.8. These differences were not statistically significant, even for 11-07393 (IA) with a difference of 2.97.

Figure 2. Average reported Ct of 2014 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 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 5,700 CFU per tube (2 samples) and another that contained 3,350 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 the samples. There were a total of 8 laboratories that reported Ct values for negative samples; of those two reported more than one negative sample with Ct values. There were a total of 8 laboratories that failed the PT (see Table 4) by calling negative samples positive, the same as the last two years. Although a larger percentage of laboratories correctly called all the samples using In House methods, the laboratories using the same In House methods also reported more Ct values for the negative samples.

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
10-05134 (OH)	2	0	0
10-06315 (OH)	0	0	2
13-01419 (IA)	0	1	1
13-00349 (IA)	1	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 (11-06361 & 12-00953) 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 2014 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	11-09754	1,950
1 Mod-High, 4 Negative samples	11-06361	86
1 Moderate, 4 Negative samples	12-00953	43
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. Interestingly, the only laboratories that failed misclassified a pool containing a high shedding animal and were using a direct PCR method. It is commendable that no laboratory misclassified the negative pool and that only three laboratories failed out of 63 kits. All laboratories that submitted results using liquid and solid culture methods passed and also correctly classified all the pools.

Table 7. Performance of each method used in the Johne's Disease 2014 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	0	0	0
	Identified a high -shedding pool as negative	3	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	0	0	0
	All 5 pools were identified correctly	38	16	4
	Total Failed Pooled Kits	**3 (7%)**	**0 (0%)**	**0 (0%)**
	Total	**41**	**16**	**4**

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

Remaining sample vials from the 2014 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. 2014 Johne's Disease Pooled Fecal Proficiency Panel key by kit number

	Pool Sample Number		
	Kit#	**Kit#**	**Kit#**
Pool Description	**1-25**	**25-50**	**51-75**
5 Negative samples	5	1	3
1 moderate (12-00953), 4 Negative samples	2	4	1
1 mod-high (11-06361), 4 Negative samples	1	2	4
1 high (11-09754), 4 Negative samples	3	5	2
1 very high (12-03427), 4 Negative samples	4	3	5

Table 9. 2014 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	13-00349 (IA)	12-03917 (ND)	12-03427 (MT)	10-06315 (OH)	11-09754 (ND)
2	11-09754 (ND)	11-07393 (ND)	12-03913 (ND)	10-05134 (KS)	13-01419 (IA)
3	10-05134 (KS)	13-01419 (IA)	11-09382 (MT)	11-09754 (ND)	11-09382 (MT)
4	11-07393 (ND)	12-03430 (IA)	10-05134 (KS)	13-00349 (IA)	12-00956 (KS)
5	10-06315 (OH)	10-06315 (OH)	12-00956 (KS)	11-07393 (ND)	12-03917 (ND)
6	11-09382 (MT)	12-00953 (OH)	11-09754 (ND)	12-03913 (ND)	12-03427 (MT)
7	12-03427 (MT)	12-00956 (KS)	10-05134 (KS)	12-00953 (OH)	13-01419 (IA)
8	13-01419 (IA)	12-03913 (ND)	12-00953 (OH)	12-03917 (ND)	11-07393 (ND)
9	12-03917 (ND)	11-09382 (MT)	12-03430 (IA)	11-09754 (ND)	10-06315 (OH)
10	12-00953 (OH)	13-00349 (IA)	13-01419 (IA)	13-00349 (IA)	12-03427 (MT)
11	12-00956 (KS)	11-09754 (ND)	12-03427 (MT)	12-00956 (KS)	13-00349 (IA)
12	12-03913 (ND)	10-05134 (KS)	13-00349 (IA)	11-07393 (ND)	12-03913 (ND)
13	12-00953 (OH)	11-09754 (ND)	11-07393 (ND)	10-05134 (KS)	12-03917 (ND)
14	12-03917 (ND)	13-00349 (IA)	10-06315 (OH)	11-09382 (MT)	12-03430 (IA)
15	11-07393 (ND)	12-00956 (KS)	13-01419 (IA)	12-03913 (ND)	10-06315 (OH)
16	13-01419 (IA)	12-03913 (ND)	12-03917 (ND)	12-03427 (MT)	12-00953 (OH)
17	12-00956 (KS)	12-03917 (ND)	11-09754 (ND)	13-01419 (IA)	11-09754 (ND)
18	12-03427 (MT)	12-00953 (OH)	10-06315 (OH)	12-00953 (OH)	10-05134 (KS)
19	10-06315 (OH)	11-07393 (ND)	11-09382 (MT)	12-03917 (ND)	11-07393 (ND)
20	11-09382 (MT)	13-01419 (IA)	12-00956 (KS)	12-03430 (IA)	10-05134 (KS)
21	12-03913 (ND)	12-03427 (MT)	13-00349 (IA)	13-01419 (IA)	11-09382 (MT)
22	13-00349 (IA)	10-05134 (KS)	11-07393 (ND)	11-09382 (MT)	12-03913 (ND)
23	12-03430 (IA)	12-03427 (MT)	12-00953 (OH)	12-00956 (KS)	13-00349 (IA)
24	10-05134 (KS)	10-06315 (OH)	12-03913 (ND)	12-03427 (MT)	12-00953 (OH)
25	11-09754 (ND)	11-09382 (MT)	12-03917 (ND)	10-06315 (OH)	12-00956 (KS)
26	12-03430 (IA)	12-03430 (IA)	12-03430 (IA)	12-03430 (IA)	12-03430 (IA)

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