2012 Johne's Disease Fecal Proficiency Panel
General Summary
October 4, 2012

Overview

A total of 59 laboratories participated in the 2012 Johne's Disease Fecal Proficiency Panel (7 Canadian, 3 European Union, 1 New Zealand and 48 USA laboratories). Overall, the number of laboratories that requested individual proficiency panels for direct PCR, liquid culture, and solid culture testing slightly decreased from 2011. Requests for pooled proficiency panels decreased slightly for direct PCR, held steady for liquid culture, and slightly increased for solid culture. 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 179 panels were requested; results were not returned for 3 of them, and one was faulty and replaced. If preliminary results indicated that the laboratory had failed, they were given the opportunity to retake the proficiency panel provided the results could be completed by September 28th, 2012. 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 2012 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 (%)	# labs not retesting	Total Shipped	Total shipped in 2011 (%change)
Individual Panel							
Direct PCR (all)	44 (83%)	9 (17%)	7 (88%)	1 (13%)	1	62	63 (-2%)
Tetracore	15 (79%)	4 (21%)	4 (100%)			23	31 (-26%)
Applied Biosystems	18 (86%)	3 (14%)	3 (75%)	1 (25%)		25	19 (32%)
In-House	11 (85%)	2 (15%)				13	13 (0%)
Liquid Systems (all)	28 (97%)	1 (3%)	1 (100%)			31	32 (-3%)
MGIT 960	8 (100%)					8	9 (-11%)
TREK	20 (95%)	1 (5%)	1 (100%)			22	23 (-4%)
HEY Solid Media (all)	18 (86%)	3 (14%)			3	21	25 (-16%)
Individual Panel Total	**91 (84%)**	**13 (13%)**	**8 (89%)**	**1 (11%)**	**4**	**114**	**120 (-5%)**
Pooling Panel							
Direct PCR (all)	28 (88%)	4 (13%)	2 (50%)	2 (50%)		36	38 (-5%)
Liquid	21 (95%)	1 (5%)			1	23	23 (0%)
HEY	5 (100%)					6	4 (50%)
Pooled Panel Total	**54 (92%)**	**5 (8%)**	**2 (50%)**	**2 (50%)**	**1**	**65**	**65 (0%)**

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 8 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 43% 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. All laboratories performing MGIT culture passed, the only method to do so. However, this method was the least sensitive for low shedding samples.

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 112[2]	HEY 21	TREK 22	MGIT 8	Applied Biosystems 23	Tetracore 25	In-House 13
10-04922 (OH)	1	Critical- Neg	98%	100%	100%	100%	100%	96%	92%
10-04923 (OH)	3	Critical- Neg	99%	100%	98%	100%	97%	99%	100%
10-04999 (OH)	2	Critical- Neg	99%	100%	100%	100%	100%	98%	96%
11-09382 (MT)	2	Low	43%[3]	36%	59%	13%	26%	62%	42%
11-06357 (IA)	3	Low	88%	92%	100%	79%	87%	79%	90%
11-09383 (MT)	2	Low	91%	88%	95%	81%	91%	90%	96%
11-06361 (IA)	2	Low	100%	100%	100%	100%	100%	100%	100%
12-00957 (KS)	2	Moderate	100%	100%	100%	94%	100%	100%	100%
12-02530 (MT)	3	Moderate	99%	100%	100%	100%	97%	97%	100%
11-09381 (MT)	2	Critical- High	99%	95%	100%	100%	100%	100%	100%
11-06359 (IA)	2	Critical- High	100%	100%	100%	100%	100%	100%	100%
12-00954 (KS)	2	Critical- High	100%	100%	100%	100%	98%	100%	100%

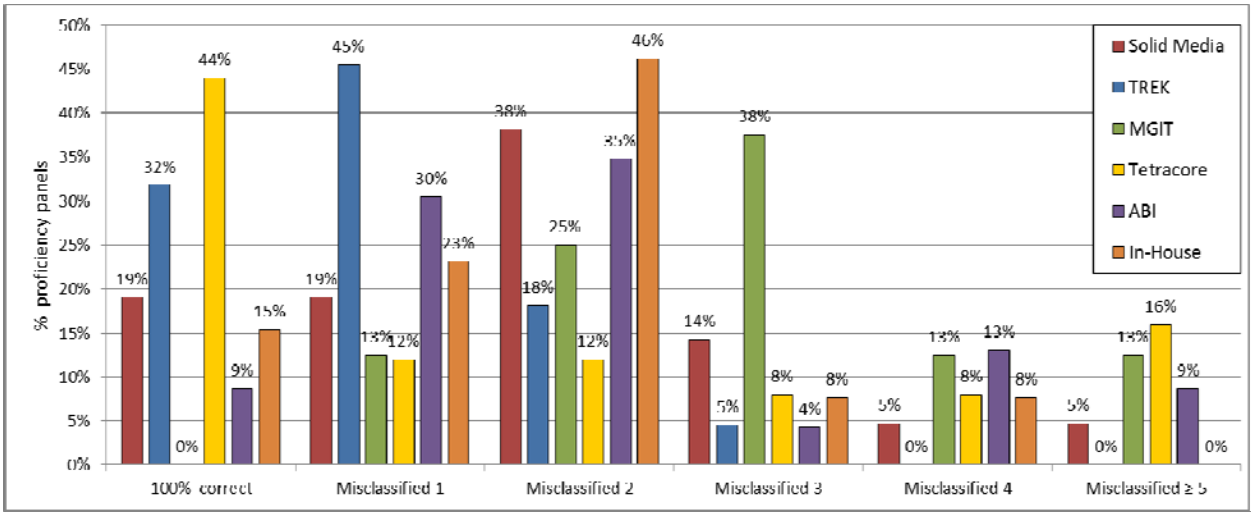
[1]In order to pass, laboratories must correctly classify critical samples. A critical sample is any negative sample or a sample that is identified as a heavy shedder by more than 50% of the laboratories using solid media.

[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.

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 that were not included in the official grading. In this analysis Tetracore outperformed all other methods with 44% of the kits correctly classifying all 25 samples. This is the first year where a direct PCR method out performed liquid culture. Thirty-two percent of laboratories using TREK liquid culture correctly classified all samples, and 19% of the laboratories using solid media correctly classified all samples.

Figure1. Percentage of 2012 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, ABI, 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 3 lists the specific reasons laboratories failed to pass the proficiency panel for each method.

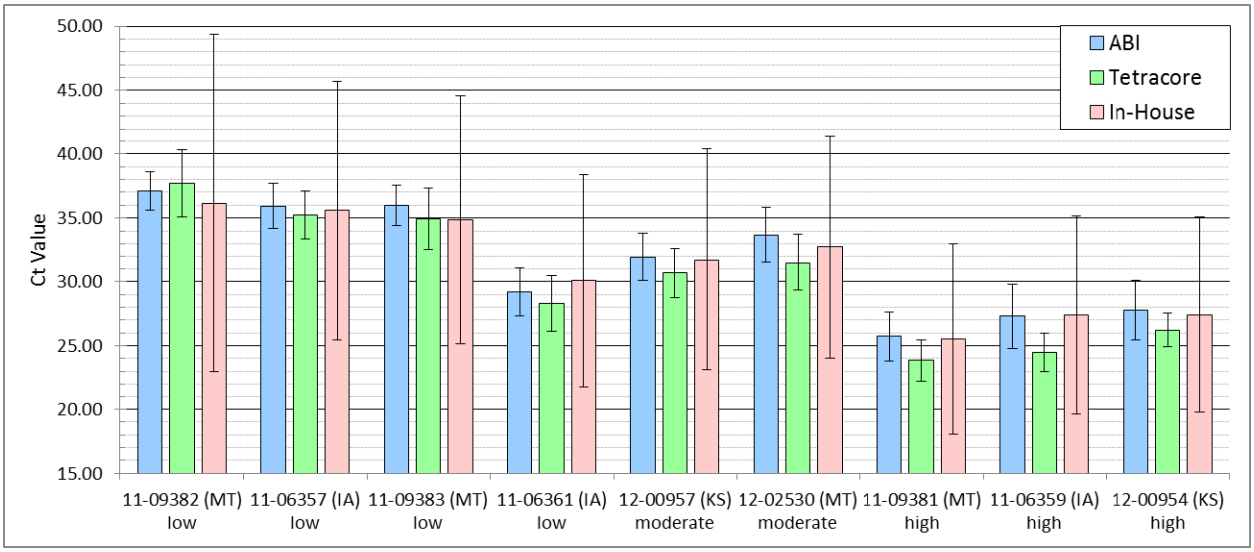
Table 3. Reasons laboratories failed the 2012 Johne's Disease Fecal Proficiency Panel. Laboratories were required to correctly identify all the negative samples as negative and all the critical high shedding samples as positive (critical samples). They also were required to correctly classify at least 70% of the remaining samples.

	Direct PCR (Tetracore)	Direct PCR (ABI)	Direct PCR (In-House)	TREK liquid media	MGIT liquid media	HEY solid media
Misclassified a negative sample as positive	3	2	2	1	0	0
Missed 3 or more low / moderate shedders (lack of sensitivity)	1	1	0	0	0	1
Misclassified a high shedding sample as negative	0	1	0	0	0	2
Multiple reasons cited above	0	0	0	0	0	0
Total failed kits	**4 (16%)**	**4 (17%)**	**2 (15%)**	**1 (5%)**	**0 (0%)**	**3 (14%)**
Total kits tested	**25**	**23**	**13**	**22**	**8**	**21**

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 includes samples categorized as negative but 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. The averages differed

by less than 2 Ct for samples from each animal except 11-02530 (2.1 Ct) and 11-06359 (2.9 Ct) but the differences were not statistically significant. Not only were averages similar but standard deviations were also similar for the ABI and Tetracore methods, which indicates these two methods performed remarkably consistently between laboratories and technicians. The standard deviations of the In-House methods were much larger and somewhat concerning even though there are a wide range of methods categorized within this group. Laboratories using these methods may want to look at ways to reduce variation if it occurred, which can be found in their individual laboratory reports released separately.

Figure2. 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 the non-infected 10-04922 and 10-04923 cows had not been used in the check test previously, 10-04999 has been. Also, 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 2,600 CFU per tube (2 samples) and another that contained 4000 CFU per tube (2 samples). Table 4 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 8 laboratories (7 with direct PCR and one with the confirmatory PCR) that failed the initial PT (see table 3) by calling negative samples positive as compared to 9 last year. Seven of those laboratories chose to retake the PT and 5 successfully passed the panel on the retest. The only laboratory that failed the retest identified different animals as negative.

Table 4. The number of samples from non-infected cows reported with Ct values (regardless of their categorical positive/negative results) by direct PCR method.

	Tetracore (138 tested)	ABI (150 tested)	In-House (78 tested)
10-04922 (OH)	1	0	2
10-04923 (OH)	1	2	0
10-04999 (OH)	1	0	2

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 5 lists the contents of each pool and Table 7 lists the vial numbers associated with each pool. Laboratories were required to correctly classify the two negative pools and the one pool that contained the high-shedding animal (10-08425). Laboratories were allowed to misclassify one of the other two pooled samples and still pass the panel.

Table 5. Composition of the 2012 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 high, 4 Negative samples	10-08282	2,275
1 moderate, 4 Negative samples	12-02530	20
2 low, 3 Negative samples	10-08285	1
5 Negative samples		
5 Negative samples		

*Refers to the positive samples, not the pooled sample

Table 6 further describes the performance of each method used in the pooled proficiency test. For direct PCR, 4 laboratories failed because they misclassified negative pools as positive, one failed because they misclassified both non-critical pools, and one misclassified both negative samples and the two non-critical pools. Only 1 laboratory failed using a liquid culture method and misclassified a negative pool. None of the laboratories using solid media failed the proficiency panel.

Table 6. Performance of each method used in the Johne's Disease 2012 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 a negative pool as positive	4	1	0
	Identified the high -shedding pool as negative	0	0	0
	Two non-critical pools were identified as negative	1	0	0
	Failed due to multiple criteria	1	0	0
Panels that passed	One non-critical pool was misidentified as negative	11	3	3
	All 5 pools were identified correctly	19	18	2
	Total Failed Pooled Kits	**6 (17%)**	**1 (5%)**	**0 (0%)**
	Total	**36**	**22**	**5**

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 the2012 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 7. 2012 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	1	1	1
5 Negative samples	4	4	4
2 low (10-08285), 3 Negative samples	5	5	5
1 moderate (12-02530), 4 Negative samples	2	2	2
1 high (10-08282), 4 Negative samples	3	3	3

Table 8. 2012 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	11-06361 (IA)	11-06357 (IA)	11-09383 (MT)	11-09382 (MT)	12-02530 (MT)
2	10-04923 (OH)	11-09383 (MT)	10-04923 (OH)	10-04999 (OH)	10-04923 (OH)
3	11-06359 (IA)	12-00954 (KS)	11-06359 (IA)	12-00954 (KS)	11-09381 (MT)
4	12-00957 (KS)	10-04999 (OH)	11-09381 (MT)	11-06357 (IA)	11-06361 (IA)
5	11-06357 (IA)	11-09383 (MT)	10-04923 (OH)	11-06359 (IA)	11-09382 (MT)
6	12-02530 (MT)	12-02530 (MT)	11-06361 (IA)	10-04999 (OH)	10-04922 (OH)
7	11-09383 (MT)	10-04923 (OH)	12-00957 (KS)	12-02530 (MT)	12-00954 (KS)
8	10-04923 (OH)	11-09381 (MT)	11-06357 (IA)	11-06361 (IA)	11-06359 (IA)
9	11-06359 (IA)	11-06359 (IA)	12-00954 (KS)	11-09383 (MT)	10-04923 (OH)
10	11-06357 (IA)	10-04922 (OH)	10-04999 (OH)	12-00957 (KS)	11-09382 (MT)
11	11-09381 (MT)	11-09382 (MT)	12-02530 (MT)	10-04923 (OH)	11-09381 (MT)
12	10-04922 (OH)	12-00957 (KS)	12-00957 (KS)	11-09381 (MT)	10-04999 (OH)
13	11-09381 (MT)	10-04999 (OH)	11-06357 (IA)	11-06359 (IA)	12-00957 (KS)
14	12-02530 (MT)	11-09381 (MT)	11-09382 (MT)	10-04922 (OH)	11-06357 (IA)
15	10-04999 (OH)	11-09382 (MT)	12-02530 (MT)	11-06361 (IA)	11-09383 (MT)
16	11-06357 (IA)	11-06361 (IA)	11-06359 (IA)	11-09382 (MT)	11-06357 (IA)
17	11-09382 (MT)	12-00957 (KS)	10-04922 (OH)	12-02530 (MT)	12-00957 (KS)
18	12-00954 (KS)	11-06357 (IA)	11-09381 (MT)	11-06357 (IA)	12-02530 (MT)
19	10-04999 (OH)	11-06361 (IA)	10-04999 (OH)	12-00957 (KS)	11-06361 (IA)
20	12-00957 (KS)	12-02530 (MT)	11-09383 (MT)	12-02530 (MT)	12-02530 (MT)
21	12-02530 (MT)	11-06359 (IA)	11-06357 (IA)	11-06357 (IA)	11-06359 (IA)
22	11-06361 (IA)	10-04923 (OH)	12-02530 (MT)	11-09383 (MT)	10-04999 (OH)
23	11-09382 (MT)	12-02530 (MT)	11-06361 (IA)	10-04923 (OH)	11-09383 (MT)
24	11-09383 (MT)	11-06357 (IA)	11-09382 (MT)	11-09381 (MT)	11-06357 (IA)
25	10-04923 (OH)	10-04923 (OH)	10-04923 (OH)	10-04923 (OH)	10-04923 (OH)
26	12-00954 (KS)	12-00954 (KS)	12-00954 (KS)	12-00954 (KS)	12-00954 (KS)

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