2011 Johne's Disease Fecal Proficiency Panel
General Summary
October 18, 2011

Overview

A total of 62 laboratories participated in the 2011 Johne's Disease Fecal Proficiency Panel (6 Canadian, 3 European Union, 1 New Zealand and 52 USA laboratories). Overall, the number of laboratories requesting proficiency panels for direct PCR and pooled testing slightly increased from 2010 and held steady or decreased for liquid and solid media 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. 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 30th, 2011. The results provided in Table 1 include these retests.

Table 1. Summary results of the 2011 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 2010 (%change)
Individual Panel							
Direct PCR (all)	40 (75%)	13 (25%)	8 (80%)	2 (20%)	3	63	58 (+9%)
Tetracore	17 (68%)	8 (32%)	5(83%)	1 (17%)	2	31	29 (+7%)
Applied Biosystems	16 (89%)	2 (11%)	1		1	19	14 (+26%)
In House / Other	7 (70%)	3 (30%)	2 (67%)	1 (33%)		13	14 (-8%)
Liquid Systems (all)	29 (94%)	2 (6%)	1		1	32	33 (-3%)
MGIT 960	9 (100%)					9	9 (0%)
TREK	20 (91%)	2 (9%)	1		1	23	23 (0%)
HEY Solid Media (all)	22 (92%)	2 (8%)	1			25	26 (-8%)
Individual Panel Total	**91 (84%)**	**17 (16%)**	**10**	**2**	**4**	**120**	**117 (+3%)**
Pooling Panel							
Direct PCR (all)	35 (95%)	2 (5%)	1		1	38	34 (+12%)
MGIT 960	7 (100%)					7	6 (+17%)
TREK	15(94%)	1 (6%)			1	16	14 (+13%)
HEY	4 (100%)					4	6 (-33%)
Pooled Panel Total	**61 (95%)**	**3 (5%)**	**1**		**2**	**65**	**60 (+8%)**

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 7 at the end of this report for the key). Table 2 shows the categorical (positive/negative) performance for each identification method by animal ID. Interestingly the most difficult positive sample was different for the direct PCR assays than the liquid culture assays. Overall 79/126 (63%) of direct PCR tests identified cow 11-04682 correctly, where liquid culture identified that sample 61/64 (95%) correctly. The sample from cow 10-08079 proved problematic using liquid culture with 55/64 (86%) tests correctly identifying that cow as positive, compared to 120/126 (95%) for direct PCR. Correct classification using solid media culture was problematic with the samples from both cows.

Table 2. Composition of the 2011 Johne's Disease Fecal Proficiency Panel, and the overall categorical summary results per cow for each method performed by laboratories.

			Liquid Media			Direct PCR		
Cow ID	# Vials/ Panel	Shedding Status[1]	HEY 24[2]	TREK 23	MGIT 7	Tetracore 29	Applied Biosystems 21	In House 13
10-04921 (OH dairy cow)	2	Critical- Neg	98%	98%	100%	95%	98%	**88%**
09-02557 (ND beef cow)	2	Critical- Neg	100%	100%	100%	95%	98%	96%
10-04999 (OH beef cow)	2	Critical- Neg	100%	100%	100%	95%	98%	100%
11-04682 (IA dairy cow)	2	Low	**63%**	93%	100%	**64%**	**64%**	**58%**
10-08079 (IA dairy cow)	2	Low	**69%**	**89%**	**78%**	97%	95%	92%
11-04681 (IA dairy cow)	3	Low	97%	99%	100%	97%	100%	92%
09-07866 (IA dairy cow)	1	High	96%	96%	100%	100%	100%	100%
10-08282 (OH dairy cow)	2[3]	Critical- High	100%	96%	100%	98%	100%	92%
10-08284 (OH dairy cow)	2	Critical- High	100%	98%	100%	98%	98%	100%
11-01804 (IA dairy cow)	2	Critical- High	98%	98%	100%	100%	100%	96%
09-01151 (IA beef cow)[4]	1	Critical- High	100%	94%	100%	92%	94%	100%
10-08425 (OH dairy cow)	2	Critical- High	96%	98%	100%	100%	100%	100%
10-08283 (OH dairy cow)	3	Mod-High	99%	97%	100%	95%	100%	95%

[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]This cow was represented in the kit twice, except for kits 101-125 where she also replaced 09-01151.

[4]Sample identified as having PCR inhibition (see text for details)

In 2010, samples from a clinical beef cow, 09-01151, caused significant inhibition in several laboratories using Tetracore and some in house assays. Over 15 laboratories would have failed the proficiency panel if those would have been considered critical samples. Consequently, NVSL chose not to fail laboratories, and instead strongly recommend an internal control be incorporated into the routine testing of clinical samples. This year NVSL had enough vials from 09-01151 to include one sample in kit # 1-100. Only 2 laboratories using Tetracore, and one using Applied Biosystems (who used an in house confirmatory test because of specificity concerns) failed to correctly identify that animal as positive. This is significant progress.

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 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 2011 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 (AB)	Direct PCR (In house)	TREK liquid media	MGIT liquid media	HEY solid media
Misclassified a negative sample as positive	6	1	2	1	0	1
Missed 4 or more low/ moderate shedders (lack of sensitivity)	1	0	1	1	0	1
Misclassified a high shedding sample as negative	1	1	0	0	0	1
A critical sample was contaminated	NA	NA	NA	0	0	0
Multiple reasons cited above	1	0	1	0	0	0
Total failed kits	**9 (29%)**	**2 (11%)**	**4 (31%)**	**2 (9%)**	**0**	**2 (8%)**
Total kits tested	**31**	**19**	**13**	**23**	**9**	**25**

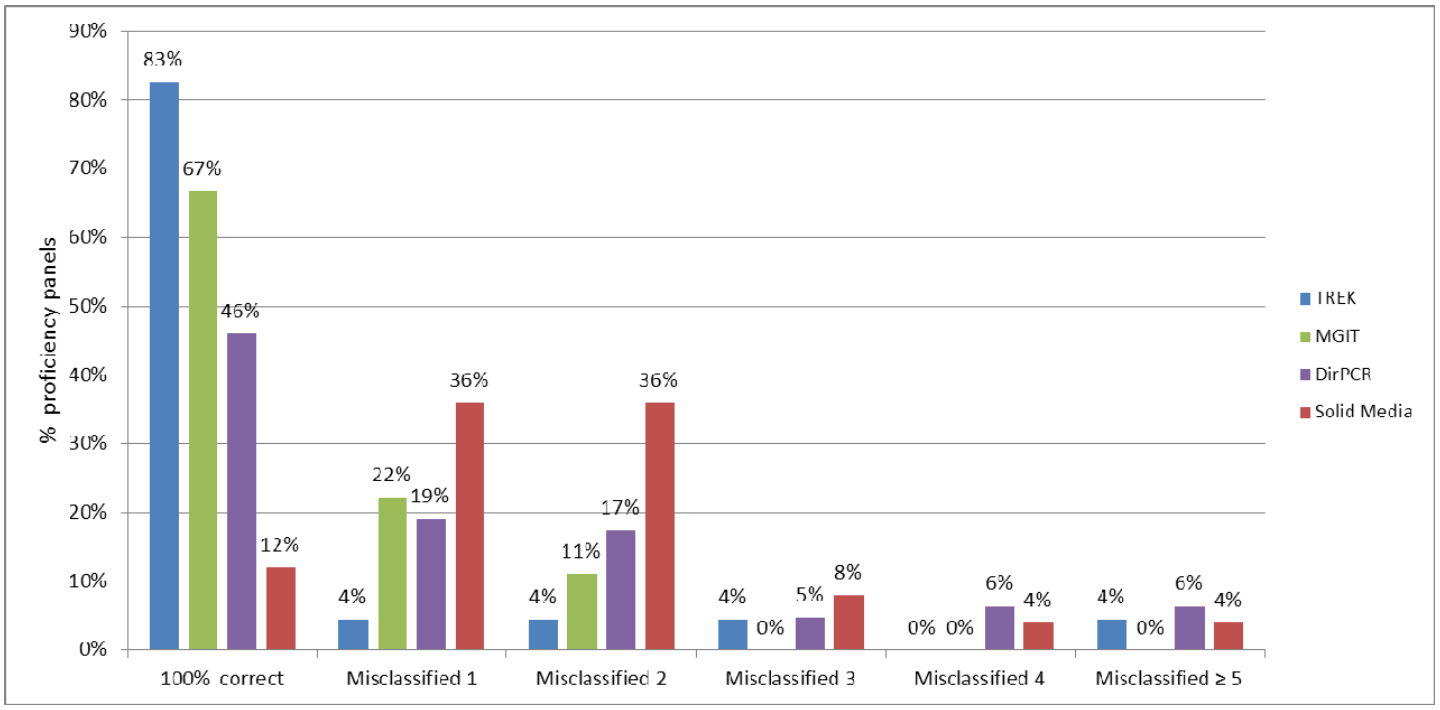
A disturbing trend continued from previous years, and that is an increasing number of laboratories using direct PCR misclassified negative samples as positive. While the 2011 non-infected cows had not been used in the check test previously, the herds have been. The major difference in this panel from previous years is the extreme number of organisms contained in 6 samples. Three of the cows (2 samples/cow) represented in the panel this year were shedding over 7,000 CFU per tube, compared to 1 animal in previous years. This emphasizes the importance of having strict procedures to prevent cross contamination and amplicon contamination. Table 4 examines the number of negative samples reported with Ct values by PCR method. Interestingly, 28/47 (60%) of laboratories that had undetermined Ct values for all negative samples correctly classified all of the positive samples. In contrast, only 5/16 (31%) of the laboratories that reported Ct values in some of the negative samples were able to correctly classify all the positive samples. There were a total of 9 laboratories that failed the initial PT (see table 3) by calling negative samples positive. Six of those laboratories chose to retake the PT, of those only 2 identified the same cow as positive, which was expected by random chance alone.

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 (58 tested)	AB (42 tested)	In house (26 tested)
10-04921 (OH dairy cow)	6	1	3
10-04999 (OH beef cow)	3	1	1
09-02557 (ND beef cow)	5	1	2

Figure 1 compares the performance of each method by the number of samples misclassified per panel. TREK continues to have the highest percentage of laboratories that correctly classify all the samples at 83% (19/23). This year, MGIT is a close second with 67% (6/9), followed by direct PCR with 46% (29/63). Only 3 of 25 kits submitted using solid media were able to correctly classify all of the samples.

Figure1. Percentage of 2010 Johne's disease fecal proficiency panels by number of samples misclassified for the three culture methods (TREK liquid media, solid media and MGIT 960 liquid media) and direct PCR. A panel consisted of 25 fecal samples.



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. Depending on the key (see Table 8 at the end of this report) the vial numbers associated with each pool varied. Laboratories were required to correctly classify the negative pool and the one pool that contained the very high shedding animal (10-08425). Laboratories were allowed to misclassify one of the other three pooled samples.

Table 5. Composition of the 2011 Johne's Disease Fecal Pooling Proficiency Panel

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 Very High, 4 Negative samples	10-08425	>10,000
1 High, 4 Negative samples	08-04828	480
1 high (bison strain), 4 Negative samples	10-01627	N/A
1 mod/high, 4 Negative samples	10-08283	75
5 Negative samples		

*Refers to the positive sample, not the pooled sample

Table 6 further describes the performance of each method used in the pooled proficiency test. For direct PCR, 2 laboratories failed because they both misclassified the pools containing 10-01627 (bison stain), and 10-08283. For the 6 passing laboratories that misclassified one sample, 4 missed the lowest shedding pool containing 10-08283, and 2 missed the pool containing the bison strain (10-1627). The 1 laboratory that failed using TREK misclassified the pools containing 10-08283 and 08-04828. As expected the 4 laboratories that used solid media misclassified the pool containing the sample with the bison strain (10-01627).

Table 6. Performance of each method used in the Johne's Disease 2011 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

		No. panels			
		Direct PCR	**MGIT**	**TREK**	**Solid media**
Panels that failed	Identified the negative pool as positive	0	0	0	0
	A high shedding pool was identified as negative	0	0	0	0
	Two non-critical pools were identified as negative	2	0	1	0
Panels that passed	One non-critical pool was misidentified as negative	7	0	0	4
	All 5 pools were identified correctly	29	7	15	0
	Total	**38**	**7**	**16**	**4**

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 the2011 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. 2011 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	09-02557 (ND beef cow)	10-08079 (IA dairy cow)	10-04921 (OH dairy cow)	09-01151 (IA beef cow)	10-08282 (OH dairy cow)
2	10-04921 (OH dairy cow)	10-04921 (OH dairy cow)	10-08079 (IA dairy cow)	10-04921 (OH dairy cow)	10-04921 (OH dairy cow)
3	10-08079 (IA dairy cow)	10-04921 (OH dairy cow)	09-01151 (IA beef cow)	10-08079 (IA dairy cow)	10-08079 (IA dairy cow)
4	09-01151 (IA beef cow)	09-01151 (IA beef cow)	10-08284 (OH dairy cow)	11-04681 (IA dairy cow)	10-08284 (OH dairy cow)
5	10-08284 (OH dairy cow)	10-08284 (OH dairy cow)	09-02557 (ND beef cow)	10-08284 (OH dairy cow)	09-02557 (ND beef cow)
6	10-08282 (OH dairy cow)	10-08282 (OH dairy cow)	10-08282 (OH dairy cow)	09-02557 (ND beef cow)	10-08079 (IA dairy cow)
7	09-02557 (ND beef cow)	09-02557 (ND beef cow)	10-08079 (IA dairy cow)	10-08079 (IA dairy cow)	11-04681 (IA dairy cow)
8	10-04921 (OH dairy cow)	10-08284 (OH dairy cow)	11-04681 (IA dairy cow)	10-08282 (OH dairy cow)	09-02557 (ND beef cow)
9	10-08079 (IA dairy cow)	10-08079 (IA dairy cow)	10-08282 (OH dairy cow)	09-02557 (ND beef cow)	10-04921 (OH dairy cow)
10	11-04681 (IA dairy cow)	11-04681 (IA dairy cow)	09-02557 (ND beef cow)	10-04921 (OH dairy cow)	10-08284 (OH dairy cow)
11	10-08284 (OH dairy cow)	10-08282 (OH dairy cow)	10-04921 (OH dairy cow)	10-08284 (OH dairy cow)	10-08282 (OH dairy cow)
12	10-08282 (OH dairy cow)	09-02557 (ND beef cow)	10-08284 (OH dairy cow)	10-08282 (OH dairy cow)	10-08282 (OH dairy cow)
13	11-04682 (IA dairy cow)	11-04681 (IA dairy cow)	10-08425 (OH dairy cow)	11-04681 (IA dairy cow)	11-04681 (IA dairy cow)
14	10-04999 (OH beef cow)	10-04999 (OH beef cow)	11-04682 (IA dairy cow)	11-01804 (IA dairy cow)	11-01804 (IA dairy cow)
15	10-08425 (OH dairy cow)	10-08425 (OH dairy cow)	11-04681 (IA dairy cow)	11-04682 (IA dairy cow)	11-04682 (IA dairy cow)
16	11-04681 (IA dairy cow)	10-08283 (OH dairy cow)	10-04999 (OH beef cow)	11-04681 (IA dairy cow)	11-04682 (IA dairy cow)
17	11-04681 (IA dairy cow)	09-07866 (IA dairy cow)	10-08425 (OH dairy cow)	10-04999 (OH beef cow)	11-04681 (IA dairy cow)
18	09-07866 (IA dairy cow)	11-01804 (IA dairy cow)	09-07866 (IA dairy cow)	10-08425 (OH dairy cow)	10-04999 (OH beef cow)
19	11-01804 (IA dairy cow)	10-08425 (OH dairy cow)	11-01804 (IA dairy cow)	09-07866 (IA dairy cow)	10-08425 (OH dairy cow)
20	10-08425 (OH dairy cow)	11-01804 (IA dairy cow)	11-01804 (IA dairy cow)	11-01804 (IA dairy cow)	09-07866 (IA dairy cow)
21	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)
22	11-01804 (IA dairy cow)	11-04682 (IA dairy cow)	11-04682 (IA dairy cow)	11-04682 (IA dairy cow)	10-04999 (OH beef cow)
23	10-08283 (OH dairy cow)	10-04999 (OH beef cow)	10-04999 (OH beef cow)	10-04999 (OH beef cow)	11-01804 (IA dairy cow)
24	11-04682 (IA dairy cow)	11-04682 (IA dairy cow)	10-08283 (OH dairy cow)	10-08425 (OH dairy cow)	10-08283 (OH dairy cow)
25	10-04999 (OH beef cow)	11-04681 (IA dairy cow)	11-04681 (IA dairy cow)	10-08283 (OH dairy cow)	10-08425 (OH dairy cow)
26	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)	10-08283 (OH dairy cow)

Table 8. 2011 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	2
1 mod/high (10-08283), 4 Negative samples	2	3	4
1 High-bison strain (10-01627), 4 Negative samples	4	5	1
1 High (08-4828), 4 Negative samples	3	4	5
1 Very High (10-08425), 4 Negative samples	1	2	3

Any questions or comments can be directed to the Diagnostic Bacteriology Laboratory at 515.337.7388 or 515.337.7568.

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