2010 Johne's Disease Fecal Proficiency Panel
General Summary
October 4, 2010

Overview

A total of 61 laboratories participated in the 2010 Johne's Disease Fecal Proficiency Panel (5 Canadian, 3 European Union, 1 New Zealand and 52 USA laboratories). In the USA, laboratories must order separate panels and demonstrate proficiency for each method they wish to use for the Johne's Disease National Program. Overall, the number of laboratories requesting proficiency panels for PCR and pooled testing increased from 2009. Concurrently, there was a sharp decline in the number of laboratories requesting individual panels for both 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. Therefore, results provided in Table 1 include these retests. The TREK liquid media culture system was the only method where all laboratories passed the individual panel. This year, no laboratory used the Bactec 460 liquid media culture system.

Table 1. Summary results of the 2010 Johne's Disease Fecal Proficiency Panel. In order to pass results must meet the criteria listed in the 2006 Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program.

	# passed (%)	# not passing (%)	# passed on retest (%)	# not passing on retest (%)	# not returned (%)	Total Shipped	Total shipped in 2009 (%change)
Individual Panel							
Direct PCR (all)	47 (81%)	6 (10%)	3 (5%)	1 (2%)	1 (2%)	58	52 (+12%)
Tetracore	25 (86%)	3 (10%)*	1(3%)[A]			29	27 (+7%)
Applied Biosystems	11 (79%)	1 (7%)[C]	1 (7%)[B]	1 (7%)[B]		14	6 (+133%)
In House / Other	11 (78%)	2 (14%)	1 (7%)			14	17 (-18%)
Liquid Systems (all)	29 (88%)	3 (9%)			1 (3%)	33	43 (-23%)
MGIT 960	6 (43%)	3 (29%)				9	14 (-36%)
TREK	23 (100%)					23	27 (-15%)
HEY Solid Media (all)	21 (81%)	2 (8%)	1 (4%)		2 (8%)	26	36 (-28%)
Individual Panel Total	**106 (81%)**	**18 (14%)**			**7 (5%)**	**117**	**131 (-11%)**
Pooling Panel							
Direct PCR (all)	27 (79%)	4 (12%)	2 (6%)		1 (3%)	34	26 (+31%)
MGIT 960	5 (83%)	1 (17%)				6	3 (+100%)
TREK	13 (93%)	1 (7%)				14	16 (-13%)
HEY	5 (83%)	1 (17%)				6	6 (0%)
Pooled Panel Total	**50 (83%)**	**7 (12%)**	**2 (3%)**		**1 (2%)**	**60**	**51 (+15%)**

*All 3 laboratories requested a retest, 1 retested with Tetracore[A] the other 2 retested with Applied Biosystems[B]

[C]This laboratory did not request a retest.

Individual Panel Description

Each individual panel consisted of 26 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. All 117 individual panels contained the same set of samples. Panels were assembled in groups, each with a different key (See Table 6 at the end of this report for the key). Table 2 shows the categorical (positive/negative) summary performance for each identification method by animal ID. Two cows (0901, 09-07865) were shedding at such low levels less than 70% of samples were identified as positive by participating laboratories; consequently these samples were removed for official grading purposes. Cow 10-01627 was shedding a 'bison strain' of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), as determined by IS1311 restriction endonuclease assay (Whittington *et. al*, *Mol Cell Probe*, 2001). Consistent with previous literature, laboratories had difficulty isolating this strain using HEY media (5 out of 48 positive), yet this cow was shedding at a high level based on PCR and days to positive using routine liquid culture protocols.. NVSL has isolated this strain from bison originating from Montana and Oregon, elk from Oregon, and now cattle from Idaho.

Table 2. Composition of the 2010 Johne's Disease Fecal Proficiency Panel, and the overall categorical summary results per cow for each method performed by laboratories.

			% Samples Correctly Classified					
				Liquid Media		Direct PCR		
Cow ID	# Vials/ Panel	Shedding Status[1]	HEY	TREK	MGIT	Tetracore	Applied Biosystems	In House
			24[2]	23	9	29	14	14
247 (ND beef cow)	2	Critical- Neg	98%	100%	100%	98%	96%	96%
10-01088 (OH beef cow)	2	Critical- Neg	100%	100%	100%	98%	93%	93%
10-02775 (OH beef cow)	2	Critical- Neg	100%	100%	100%	98%	89%	93%
0901 (IA beef cow)	2	Low	50%	59%	28%	26%	25%	46%
09-07865 (IA dairy cow)	2	Low	6%	67%	0%	69%	68%	54%
420 (IA dairy cow)	2	Low	88%	98%	67%	90%	100%	86%
311 (NY dairy cow)	1	High	100%	100%	100%	93%	100%	100%
318 (NY dairy cow)	1	Critical- High	100%	100%	100%	97%	100%	100%
09-07866 (IA dairy cow)	2	High	100%	100%	72%	98%	100%	100%
10-01627 (ID dairy cow)[3]	2	High	10%	98%	100%	100%	100%	96%
460 (IA dairy cow)	2	Critical- High	100%	100%	78%	97%	100%	100%
09-01151 (IA beef cow)[4]	2	High	100%	100%	100%	78%	100%	75%
477 (IA dairy cow)	2	Critical- High	98%	100%	94%	97%	100%	100%
233 (IA dairy cow)	2	Critical- High	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]This cow was infected with a 'bison strain' of MAP.

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

Inhibition of the PCR reaction is always a potential cause for false negative results, especially when conducting PCR directly from fecal material. While there may have been sporadic inhibition in past proficiency panels, this year was the first year systemic inhibition problems were reported for multiple

laboratories. Cow 09-01151 was a clinical beef cow identified as a heavy shedder in all of the culture methods; yet 12 laboratories using either a commercial (Tetracore) or in house DNA extraction method classified at least one sample from this cow as negative. None of these laboratories were using an internal control which would have detected PCR inhibition. The 2 commercially available PCR methods for Johne's disease in the United States either haven an internal control automatically provided (Applied Biosystems) or one is available upon request (Tetracore). An internal control should be added to each sample, preferably before DNA extraction. Positive results for the internal control then confirm the validity of the PCR reaction. Because this identified a widespread problem, NVSL requested that laboratories misclassifying these samples rerun the samples incorporating the internal control and further permanently alter their protocols to include this step. Therefore, NVSL strongly recommends that all direct PCR methods use an internal control in each test well, regardless of source of PCR reagents.

According to the 2006 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. Because 4 samples were removed for official grading, laboratories were allowed to misclassify up to 3 of the remaining noncritical samples, including the samples with the 'bison strain' of MAP. Even though this put laboratories using solid media at a slight disadvantage, no laboratory failed as a direct result of the 'bison' MAP samples. Table 3 lists the specific reasons laboratories failed to pass the proficiency panel for each method. Misclassifying negative samples as positive continues to be the most common reason for failing a proficiency test. Reports of contamination overgrowth were low and sporadic.

Table 3. Reasons laboratories failed the 2010 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.

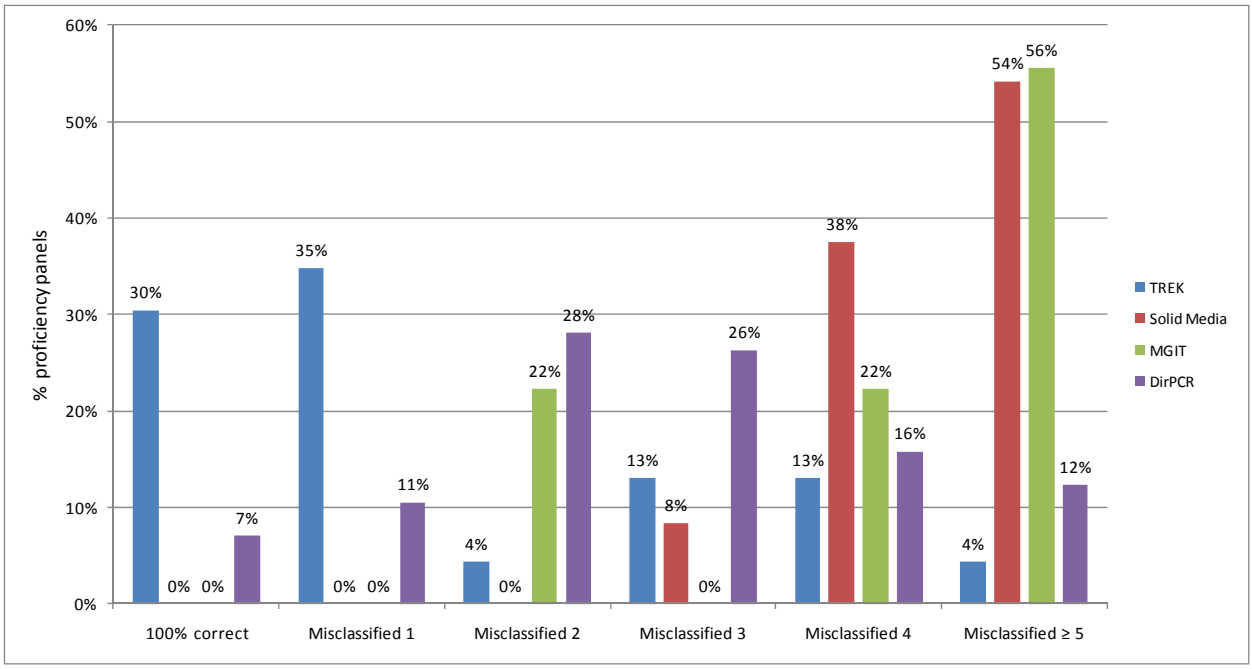
	Direct PCR (Tetracore)	Direct PCR (AB)	Direct PCR (In house)	TREK liquid media	MGIT liquid media	HEY solid media
Misclassified a negative sample as positive	0	2	1	0	0	0
Missed 5 or more low/ moderate shedders (lack of sensitivity)	2	0	0	0	0	0
Misclassified a high shedding sample as negative	0	0	0	0	1	1
A critical sample was contaminated	NA	NA	NA	0	1	0
Multiple reasons cited above	1	0	1	0	1	1
Total failed kits	**3 (10%)**	**2 (14%)**	**2 (14%)**	**0**	**3 (33%)**	**2 (8%)**
Total kits tested	**29**	**14**	**14**	**23**	**9**	**24**

Figure 1 compares the performance of each method by the number of samples misclassified per panel. For completeness, all samples were included in this analysis. Similar to last year, TREK media performed very well, with 65% (15/23) of laboratories correctly identifying 100% (25/25) or 96% (24/25) of all samples in the proficiency panel. Direct PCR was the next best performing method, with 18%

(10/57) of all laboratories misclassifying 1 or fewer samples per panel. The 4 very low shedding samples and the bison strain samples significantly affected the performance of solid media, as over half of the laboratories using this method misclassified 5 or more samples per panel.

Five of nine laboratories using the MGIT 960 media system misclassified five or more samples. These laboratories were not able to identify any of the low shedding samples from cow 09-07865, and only 5/18 of cow 0901. Laboratories using this media system also had difficulty with cow 420. The same collection from this cow was used in the 2009 proficiency panel. Laboratories using the MGIT system in 2009 misclassified this cow 3/20 times (85% correct); whereas this year laboratories misclassified this cow 6/18 times (67% correct). In contrast, the performance of the TREK media for this sample remained unchanged at 98% correct for both 2009 and 2010.

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 4 lists the contents of each pool. Depending on the key (see Table 7 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 two pools that contained heavy shedding samples. Laboratories were allowed to misclassify one of the two pooled samples containing only low shedding samples.

Table 4. Composition of the 2010 Johne's Disease Fecal Pooling Proficiency Panel

	Positive sample(s) description	
	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	460	400
1 High, 4 Negative samples	455	1775
1 Low, 4 Negative samples	420	4
1 Low, 4 Negative samples	14	3
5 Negative samples		

*Refers to the positive sample, not the pooled sample

Table 5 further describes the performance of each method used in the pooled proficiency test. Even though this pooling panel had 2 pools consisting of 4 negative samples and 1 low shedding sample, only 1 laboratory using solid media misclassified both pools. TREK media detected the highest percentage of positive pools. For direct PCR, 2 laboratories identified a pool with the low shedding samples as suspect or inconclusive. For the purposes of Table 5, these pools were not considered misclassified.

Table 5. Performance of each method used in the Johne's Disease 2010 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel. All laboratories achieved a passing score.

		No.panels*			
		Direct PCR	**MGIT**	**TREK**	**Solid media**
Panels that failed	Identified the negative pool as positive	1	0	1	0
	A high shedding pool was identified as negative	3	1	0	0
	Both low shedding pools were identified as negative	0	0	0	1
Panels that passed	One low shedding pool was misidentified as negative	7	3	0	3
	All 5 pools were identified correctly	22	2	13	2
	Total	**33**	**6**	**14**	**6**

Individual detailed results and statistics for each panel will be provided to individual laboratories before October 20, 2010. Certificates of approval will be mailed in November, 2010. 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 the2010 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.

Any questions or comments can be directed to:

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Table 6. 2010 Johne's Disease Individual Fecal Proficiency Panel key by kit number. Samples are coded by color according to shedding status as follows: Negative, Low – eliminated from official grading, Noncritical positive samples, Critical – high shedding samples, Positive control.

Vial #	1-20	21-40	41-60	61-80	81-100	101-120
1	09-07865 (IA dairy cow)	10-01088 (OH beef cow)	10-01627 (ID dairy cow)	311 (NY dairy cow)	09-01151 (IA beef cow)	247 (ND beef cow)
2	10-01627 (ID dairy cow)	10-01627 (ID dairy cow)	10-01088 (OH beef cow)	10-01627 (ID dairy cow)	10-01627 (ID dairy cow)	09-01151 (IA beef cow)
3	10-01088 (OH beef cow)	10-01627 (ID dairy cow)	311 (NY dairy cow)	10-01088 (OH beef cow)	10-01088 (OH beef cow)	10-01627 (ID dairy cow)
4	311 (NY dairy cow)	311 (NY dairy cow)	247 (ND beef cow)	318 (NY dairy cow)	247 (ND beef cow)	10-01088 (OH beef cow)
5	247 (ND beef cow)	247 (ND beef cow)	09-07865 (IA dairy cow)	247 (ND beef cow)	09-07865 (IA dairy cow)	247 (ND beef cow)
6	09-01151 (IA beef cow)	09-01151 (IA beef cow)	09-01151 (IA beef cow)	09-07865 (IA dairy cow)	10-01088 (OH beef cow)	09-07865 (IA dairy cow)
7	09-07865 (IA dairy cow)	09-07865 (IA dairy cow)	10-01088 (OH beef cow)	10-01088 (OH beef cow)	318 (NY dairy cow)	10-01088 (OH beef cow)
8	10-01627 (ID dairy cow)	247 (ND beef cow)	318 (NY dairy cow)	09-01151 (IA beef cow)	09-07865 (IA dairy cow)	09-07865 (IA dairy cow)
9	10-01088 (OH beef cow)	10-01088 (OH beef cow)	09-01151 (IA beef cow)	09-07865 (IA dairy cow)	10-01627 (ID dairy cow)	10-01627 (ID dairy cow)
10	318 (NY dairy cow)	318 (NY dairy cow)	09-07865 (IA dairy cow)	10-01627 (ID dairy cow)	247 (ND beef cow)	09-01151 (IA beef cow)
11	247 (ND beef cow)	09-01151 (IA beef cow)	10-01627 (ID dairy cow)	247 (ND beef cow)	09-01151 (IA beef cow)	311 (NY dairy cow)
12	09-01151 (IA beef cow)	09-07865 (IA dairy cow)	247 (ND beef cow)	09-01151 (IA beef cow)	311 (NY dairy cow)	318 (NY dairy cow)
13	0901 (IA beef cow)	460 (IA dairy cow)	477 (IA dairy cow)	460 (IA dairy cow)	460 (IA dairy cow)	420 (IA dairy cow)
14	09-07866 (IA dairy cow)	09-07866 (IA dairy cow)	0901 (IA beef cow)	420 (IA dairy cow)	420 (IA dairy cow)	0901 (IA beef cow)
15	477 (IA dairy cow)	477 (IA dairy cow)	460 (IA dairy cow)	0901 (IA beef cow)	0901 (IA beef cow)	460 (IA dairy cow)
16	460 (IA dairy cow)	10-02775 (OH beef cow)	09-07866 (IA dairy cow)	460 (IA dairy cow)	0901 (IA beef cow)	09-07866 (IA dairy cow)
17	460 (IA dairy cow)	233 (IA dairy cow)	477 (IA dairy cow)	09-07866 (IA dairy cow)	460 (IA dairy cow)	477 (IA dairy cow)
18	233 (IA dairy cow)	420 (IA dairy cow)	233 (IA dairy cow)	477 (IA dairy cow)	09-07866 (IA dairy cow)	420 (IA dairy cow)
19	420 (IA dairy cow)	477 (IA dairy cow)	420 (IA dairy cow)	233 (IA dairy cow)	477 (IA dairy cow)	0901 (IA beef cow)
20	477 (IA dairy cow)	420 (IA dairy cow)	420 (IA dairy cow)	420 (IA dairy cow)	233 (IA dairy cow)	09-07866 (IA dairy cow)
21	10-02775 (OH beef cow)	10-02775 (OH beef cow)	10-02775 (OH beef cow)	10-02775 (OH beef cow)	10-02775 (OH beef cow)	10-02775 (OH beef cow)
22	420 (IA dairy cow)	0901 (IA beef cow)	0901 (IA beef cow)	0901 (IA beef cow)	09-07866 (IA dairy cow)	10-02775 (OH beef cow)
23	10-02775 (OH beef cow)	09-07866 (IA dairy cow)	09-07866 (IA dairy cow)	09-07866 (IA dairy cow)	420 (IA dairy cow)	477 (IA dairy cow)
24	0901 (IA beef cow)	0901 (IA beef cow)	10-02775 (OH beef cow)	477 (IA dairy cow)	10-02775 (OH beef cow)	460 (IA dairy cow)
25	09-07866 (IA dairy cow)	460 (IA dairy cow)	460 (IA dairy cow)	10-02775 (OH beef cow)	477 (IA dairy cow)	233 (IA dairy cow)
26	233 (IA dairy cow)	233 (IA dairy cow)	233 (IA dairy cow)	233 (IA dairy cow)	233 (IA dairy cow)	233 (IA dairy cow)

Table 7. 2010 Johne's Disease Pooled Fecal Proficiency Panel key by kit number

	Pool Sample Number			
Pool Description	Kit# 1-20	Kit# 21-40	Kit# 41-50	Kit# 51-60
1 High (cow 460), 4 Negative samples	4	3	5	1
1 High (cow 455), 4 Negative samples	5	4	2	3
1 Low (cow 420), 4 Negative samples	3	2	1	4
1 Low (cow 14), 4 Negative samples	1	5	4	2
5 Negative samples	2	1	3	5