2009 Johne's Disease Fecal Proficiency Panel
General Summary
October 6, 2009

Overview

A total of 64 laboratories participated in the 2009 Johne's Disease Fecal Proficiency Panel (6 Canadian, 3 European Union and 55 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. Table 1 details the number of individual and pooled panels shipped and the pass/fail status for each method. Laboratories were allowed to order multiple panels for each method and were notified of their preliminary pass/fail status upon submission of their results. They were given the opportunity to retake a failed proficiency panel and nearly all chose to do so. Results in Table 1 include retests.

Table 1. Summary results* of the 2009 Johne's Disease Fecal Proficiency Panel.

	# passed (%)	# not passing (%)	# not returned (%)	Total Shipped
Individual Panel				
Direct PCR (all)	40 (77%)	10 (19%)	2 (4%)	52
Tetracore	24 (89%)	3 (11%)		27
Applied Biosystems	6 (100%)	0		6
In House / Other	10 (59%)	7 (41%)		17
Liquid Systems (all)	34 (79%)	5 (12%)	4 (9%)	43
BACTEC 460	2 (100%)	0		2
MGIT 960	6 (43%)	4 (29%)	4 (29%)	14
TREK	26 (96%)	1 (4%)		27
HEY Solid Media (all)	32 (89%)	3 (8%)	1 (3%)	36
Individual Panel Total	**106 (81%)**	**18 (14%)**	**7 (5%)**	**131**
Pooling Panel				
Direct PCR	25 (96%)	0	1 (4%)	26
Liquid	20 (100%)	0	0	20
HEY	6 (100%)	0	0	6
Pooled Panel Total	**51 (98%)**	**0**	**1 (2%)**	**52**

* 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.

Individual Panel Description

Each individual panel consisted of 26 samples with one sample identified as a 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 per animal and aliquoted as soon as possible in individual vials then stored at -70°C. All 131 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 of each method by cow ID. The culture/PCR error rates were similar with the exception of the low shedding samples from cows 3000 and 86 where liquid media outperformed both direct PCR and solid culture. Despite similar levels of bacteria recovered using solid or liquid media from cow 3000 and 86 (liquid culture average days to positive was 36.4 and 37.0 respectively), direct PCR failed to detect over 50% of samples from cow 86.

Table 2. Composition of the 2009 Johne's Disease Fecal Proficiency Panel, and the overall categorical summary results per cow for each method performed by laboratories.

Cow ID	# Vials / Panel	Date Collected	State of Origin	Shedding Status	Avg. CFU/ Tube[1]	% Samples Correctly Classified[2]		
						Direct PCR	Solid	Liquid
ST10	2	Apr-08	GA	Neg	0	96%	98%	97%
247	2	Oct-08	ND	Neg	0	98%	100%	96%
492903	2	Apr-08	MT	Neg	0	98%	100%	97%
492922[3]	1	Apr-08	MT	Neg	0	96%	100%	97%
3000	1	Apr-08	IA	Low	1.5	72%	69%	87%
86	2	Oct-08	IA	Low	2	47%	73%	88%
14	2	Apr-08	NY	Low	3	96%	92%	91%
420	2	Apr-08	IA	Low	4	92%	89%	95%
311	2	Apr-08	NY	High	325	99%	98%	100%
339	2	Apr-08	NY	High	1000	99%	94%	97%
455	2	Apr-08	IA	High	1775	99%	98%	99%
5	2	Apr-08	IA	High	3350	98%	97%	99%
446[4]	2	Apr-08	IA	High	5625	100%	100%	100%
392	2	Apr-08	IA	High	7275	100%	97%	99%

[1] Colony counts were determined by NVSL, averaging results from 3 cultures for each cow. For high shedders the inoculum was diluted 10^{-x} until colony counts were under 100 per tube.

[2] Samples were classified as positive or negative for MAP by the laboratories. If the sample was represented twice in the panel, it was counted twice. For example Cow ST10 had 2 samples in each panel and 50 panels were submitted using direct PCR (2x50 = 100).

[3] Sample was spiked with *Mycobacterium fortuitum*.

[4] One of the two samples from this cow was identified as the positive control.

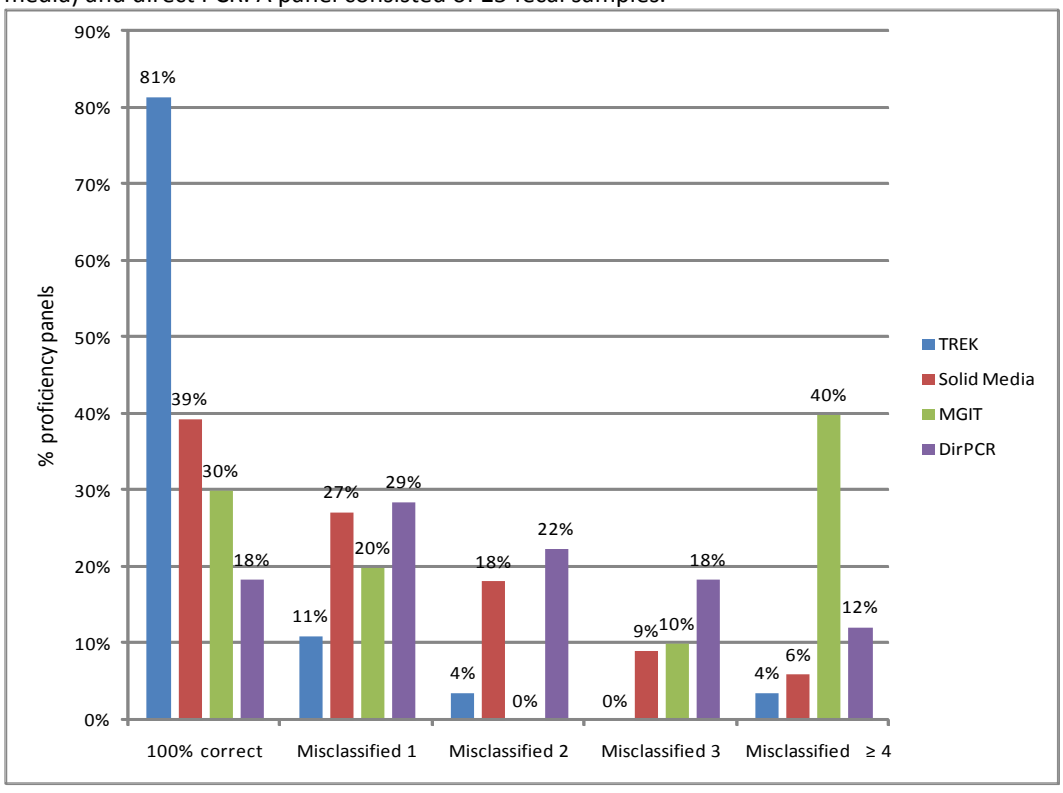
According to the 2006 Johne's Disease Uniform Methods and Rules, laboratories must correctly classify all high shedding samples as positive, all negative samples as negative and misidentify 4 or fewer (<30%) non-critical samples. Table 3 lists the specific reasons laboratories failed to pass the proficiency 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.

Figure 1 compares the performance of each method by the number of samples misclassified. TREK media had the highest number of panels, 22/27 (81%) that correctly classified all 25 samples followed by solid media 13/33 (39%); MGIT 3/10 (30%); and direct PCR 9/49 (18%). Both solid media and direct PCR tended to indentify low shedding samples as negative suggesting those samples were near the detection limit for these methods.

Table 3. Reasons laboratories failed the 2009 Johne's Disease Fecal Proficiency Panel. Laboratories were required to correctly identify all the negative samples as negative and all the high shedding samples as positive (critical samples). They also were required to correctly classify at least 70% of all 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	2	0	3	1	1	0
Missed 5 or more low/ moderate shedders (lack of sensitivity)	1	0	1	0	1	1
Misclassified a high shedding sample as negative	0	0	1	0	0	1
A critical sample was contaminated	NA	NA	NA	0	0	1
Multiple reasons cited above	0	0	2	0	2	0
Total failed kits	**3 (11%)**	**0**	**7 (41%)**	**1 (4%)**	**4 (40%)**	**3 (9%)**
Total kits tested	**27**	**6**	**17**	**27**	**10**	**35**

Figure1. Percentage of 2009 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 samples were provided with instructions to pool 5 samples 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 shedders. Laboratories were allowed to misclassify one of the two pooled samples containing only medium or low shedding samples. All laboratories submitting results in 2009 achieved a passing score.

Table 4. Composition of the 2009 Johne's Disease Fecal Pooling Proficiency Panel.

	Positive sample(s) description	
Pool Description	Cow ID	Avg. CFU/ tube*
1 High, 4 Negative samples	392	7275
1 Moderately High, 4 Negative samples	311	325
2 Moderate, 3 Negative samples	18	14
2 Low, 3 Negative samples	86	2
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. While all laboratories passed, liquid media continues to detect the highest number of positive pools. The only pool that was misclassified was the pool with 2 low shedding (cow 86) and 3 negative samples.

Table 5. Performance of each method used in the Johne's Disease 2009 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel. All laboratories achieved a passing score.

	100% correctly classified	Misclassified 1 sample	**Total**
Liquid Media	**19 (95%)**	**1 (5%)**	**20**
TREK	16	0	16
MGIT	2	1	3
Bactec	1	0	1
Solid Media	**5 (83%)**	**1 (17%)**	**6**
Direct PCR	**15 (60%)**	**10 (40%)**	**25**
Tetracore	12	5	17
Applied Biosystems	1	3	4
In House	2	2	4

Individual detailed results and statistics for each panel will be provided to individual laboratories around October 20, 2009. Certificates of approval will be mailed in November, 2009. 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 the2009 Proficiency Panel have been made 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:

Beth Harris, M.S., Ph.D.
USDA/APHIS/NVSL
Head, Mycobacteria /Brucella Section
Office: 515.663.7362
Beth.N.Harris@aphis.usda.gov

Suelee Robbe-Austerman, DVM, PhD
USDA/APHIS/NVSL
VMO, Mycobacteria/Brucella Section
Office: 515.663.7837
Suelee.Robbe-Austerman@aphis.usda.gov

Table 6. 2009 Johne's Disease Individual Fecal Proficiency Panel key by kit number

Vial #	1-20	21-40	41-60	61-80	81-100	101-120	121-140
1	14	420	86	247	339	ST10	492922*
2	339	455	ST10	311	492903	420	392
3	247	86	**446**	ST10	14	492922*	455
4	311	ST10	14	420	339	392	ST10
5	ST10	**446**	339	455	247	455	420
6	455	14	247	86	311	247	392
7	247	5	311	ST10	ST10	311	492903
8	311	446	ST10	446	420	420	339
9	339	420	455	14	492922*	392	247
10	492903	392	247	5	392	492903	311
11	3000	492903	311	339	455	339	420
12	5	3000	339	420	247	492903	492903
13	86	5	492903	392	311	14	311
14	492922*	86	3000	492903	420	339	5
15	392	492922*	5	3000	392	247	14
16	14	392	86	5	492903	311	339
17	5	455	492922*	86	3000	5	247
18	446	247	392	492922*	5	446	ST10
19	420	311	14	392	86	455	3000
20	392	339	5	455	446	86	5
21	492903	492903	446	247	14	ST10	86
22	420	14	420	311	5	3000	446
23	455	339	392	339	ST10	5	14
24	86	247	492903	492903	455	86	86
25	ST10	311	420	14	86	14	455
26	**446**	ST10	455	**446**	**446**	**446**	**446**

* Sample was spiked with *Mycobacterium fortuitum* .
The bolded 446 sample was identified as the positive control

Table 7. 2009 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, 4 Negative samples	1	4	5	2
1 Moderately High, 4 Negative samples	3	3	4	1
2 Moderate, 3 Negative samples	5	2	3	4
2 Low, 3 Negative samples	2	5	1	3
5 Negative samples	4	1	2	5