# 2018 Johne's Disease Fecal Proficiency Panel
## General Summary
October 5, 2018

## Overview

A total of 60 laboratories participated in the 2018 Johne's Disease Fecal Proficiency Panel (7 Canadian, 4 European Union, 1 New Zealand, 2 Australian and 46 USA laboratories). 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 164 panels were requested, with 4 replaced due to import/export issues. Results were not returned for 3 Individual and 3 Pooled panels. If preliminary results indicated the laboratory failed, it was given the opportunity to retake the proficiency panel provided the results were completed by September 30th, 2018. The results provided in Table 1 includes 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. One laboratory used in-house liquid culture reagents and is grouped with the laboratories using the MGIT system. All laboratories using solid media were grouped together, regardless if they purchased media or used in-house media (1 laboratory).

Table 1. Summary results of the 2018 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.

| 2018 | # passed 1st attempt (%) | # failed 1st attempt (%) | # passed 2nd attempt (%) | # failed 2nd attempt (%) | # Kits not retested | Total Shipped | Total shipped in 2017 (%change) |
|---|---|---|---|---|---|---|---|
| **Individual Panel** | | | | | | | |
| Direct PCR (all) | 49 (80%) | 12 (20%) | 4 (67%) | 2 (33%) | 6 | 69 | 71 (-3%) |
| Tetracore | 14 (74%) | 5 (26%) | 1 (50%) | 1 (50%) | 3 | 21 | 23 (-9%) |
| Life Technologies | 20 (83%) | 4 (17%) | 2 (100%) | | 2 | 26 | 28 (-7%) |
| In-House | 15 (83%) | 3 (17%) | 1 (50%) | 1 (50%) | 1 | 20 | 18(11%) |
| Liquid Systems (all) | 14 (93%) | 1 (7%) | | | 1 | 16 | 20 (-20%) |
| MGIT 960 | 4 (100%) | | | | | 4 | 5 (-20%) |
| TREK | 10 (91%) | 1 (9%) | | | 1 | 11 | 12 (-8%) |
| HEY Solid Media (all) | 7 (100%) | | | | | 7 | 10 (-30%) |
| **Individual Panel Total** | **70 (84%)** | **13 (16%)** | **4 (67%)** | **2 (33%)** | 7 | **92** | **101 (-9%)** |
| **Pooling Panel** | | | | | | | |
| Direct PCR (all) | 45 (92%) | 4 (8%) | | | 4 | 51 | 48 (+6%) |
| Liquid | 11 (92%) | 1 (8%) | | | 1 | 13 | 16 (-19%) |
| HEY | 4 (100%) | | | | | 4 | 5 (-20%) |
| **Pooled Panel Total** | **60 (92%)** | **5 (8%)** | | | **5** | **68** | **69 (-1%)** |

## Individual Panel Description

Each individual panel consisted of 25 blinded 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. When possible, approximately 4 liters of fecal material were collected rectally per animal, shipped to NVSL, aliquoted in to individual vials, and stored at -70˚C until kits were distributed. Because we lacked moderate shedding samples, three samples were produced where material from a high shedding cow was diluted with material from a culture negative cow and mixed thoroughly. These samples are 15-00426D (ID), 15-00471D (FL), and 17-02487D (FL), reflecting the animal that the positive material originated. Panels were assembled in groups, each with a different key (See Table 10 at the end of this report for the key). Table 2 shows the categorical (positive/negative) performance for each identification method by animal ID. According to the 2010 Uniform Program Standards, a laboratory receives a passing score when: all samples from non-shedding and high shedding animals are correctly classified; and they correctly classify 70% of the remaining samples (low and moderate shedding animals). This year laboratories were allowed up to 3 misclassifications (67% correctly classified) to pass. All samples performed as expected except 17-03492. The 2010 Uniform Program Standards states that an animal is considered valid "by a consensus of at least 70 percent of the laboratories participating in the fecal culture check testing process." Animal 17-03492 (WI) had 33% of all vials correctly classified (direct PCR and culture) but had 73% correctly classified by laboratories using culture methods, making it a valid animal.

Table 2. Composition of the 2018 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 89[2] | HEY 7 | TREK 11 | MGIT 4 | Life Tech 26 | Tetracore 21 | In-House 20 |
| 17-03787 (IA) | 1 | Critical- Neg | 94% | 100% | 100% | 100% | 100% | 81% | 95% |
| 17-03786 (IA) | 1 | Critical- Neg | 94% | 100% | 100% | 100% | 96% | 86% | 95% |
| 17-04259 (IA) | 3 | Critical- Neg | 97% | 100% | 100% | 100% | 98% | 93% | 100% |
| 18-01896 (IA) | 1 | Critical- Neg | 98% | 100% | 100% | 100% | 94% | 98% | 95% |
| 17-03492 (WI) | 2 | Low | 33% | 71% | 73% | 75% | 21% | 17% | 20% |
| 18-00942 (ID) | 2 | Low | 98% | 95% | 97% | 100% | 96% | 98% | 100% |
| 17-02487D (FL)[3] | 3 | Low | 97% | 100% | 91% | 100% | 100% | 100% | 95% |
| 15-00426D (ID)[3] | 1 | Moderate | 99% | 100% | 100% | 100% | 98% | 100% | 98% |
| 16-01645 (IA)[4] | 2 | Mod-High | 99% | 100% | 100% | 100% | 96% | 100% | 100% |
| 15-00471D (FL)[3] | 2 | Critical- High | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 17-03210 (FL) | 2 | Critical- High | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 17-02487 (FL) | 3 | Critical- High | 99% | 100% | 91% | 100% | 100% | 100% | 100% |
| 15-00471 (FL) | 1 | Critical- High | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 17-03143 (FL) | 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]Positive sample diluted with negative material.

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

Samples from 9 animals were used in either 2016, 2017, or both and their performance was compared. Table 3 shows the respective year panels' categorical (positive/negative) performance for each identification method by animal ID.

Table 3. Comparison between nine animals used in the 2016, 2017, and 2018 Johne's Disease Fecal Proficiency Panels with the overall categorical summary results per cow for each method performed by laboratories.

| | | | | | Percent of Samples Correctly Classified | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Liquid Media | | | Direct PCR | |
| | | | | All Kits | HEY | TREK | MGIT | Life Tech | Tetracore | In-House |
| | | | 2016 | 102[1] | 10 | 13 | 3 | 26 | 27 | 18 |
| | Panel | # Vials | Shedding | 2017 | 95 | 9 | 12 | 5 | 28 | 23 | 18 |
| Cow ID | Year | /Panel | Status | 2018 | 87 | 7 | 11 | 4 | 26 | 21 | 18 |
| 17-03787 (IA) | 2017 | 2 | Critical- Neg | | 99% | 100% | 100% | 100% | 100% | 100% | 94% |
| 17-03787 (IA) | 2018 | 1 | Critical- Neg | | 94% | 100% | 100% | 100% | 100% | 81% | 95% |
| 17-03786 (IA) | 2017 | 2 | Critical- Neg | | 95% | 94% | 100% | 100% | 95% | 93% | 92% |
| 17-03786 (IA) | 2018 | 1 | Critical- Neg | | 94% | 100% | 100% | 100% | 96% | 86% | 95% |
| 17-03492 (WI) | 2017 | 2 | Low-Mod | | 44% | 72% | 92% | 30% | 25% | 39% | 39% |
| 17-03492 (WI) | 2018 | 2 | Low | | 33% | 71% | 73% | 75% | 21% | 17% | 20% |
| 17-02487D (FL) | 2017 | 2 | Moderate | | 98% | 100% | 92% | 90% | 100% | 100% | 97% |
| 17-02487D (FL) | 2018 | 3 | Low | | 97% | 100% | 91% | 100% | 100% | 100% | 95% |
| 15-00426D (ID) | 2016 | 2 | Moderate | | 97% | 100% | 97% | 100% | 96% | 96% | 97% |
| 15-00426D (ID) | 2018 | 1 | Moderate | | 99% | 100% | 100% | 100% | 98% | 100% | 98% |
| 16-01645 (IA) | 2016 | 2 | Mod-High | | 99% | 100% | 97% | 100% | 98% | 100% | 97% |
| 16-01645 (IA) | 2017 | 2 | Mod-High | | 99% | 100% | 92% | 100% | 100% | 100% | 100% |
| 16-01645 (IA) | 2018 | 2 | Mod-High | | 99% | 100% | 100% | 100% | 96% | 100% | 100% |
| 15-00471D (FL) | 2016 | 2 | High | | 100% | 100% | 97% | 100% | 100% | 100% | 100% |
| 15-00471D (FL) | 2017 | 2 | Critical- High | | 99% | 100% | 100% | 100% | 100% | 98% | 100% |
| 15-00471D (FL) | 2018 | 2 | Critical- High | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 17-02487 (FL) | 2017 | 2 | Critical- High | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 17-02487 (FL) | 2018 | 3 | Critical- High | | 99% | 100% | 91% | 100% | 100% | 100% | 100% |
| 15-00471 (FL) | 2016 | 2 | Critical- High | | 99% | 100% | 100% | 88% | 100% | 98% | 100% |
| 15-00471 (FL) | 2017 | 2 | Critical- High | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 15-00471 (FL) | 2018 | 1 | Critical- High | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

[1]Number of proficiency panels submitted per method.

Table 4 shows the averaged value reported for each of the methods summarized by animal. Interestingly, the reported values for animal 17-03143 (FL) differ between methods with the averaged values most similar to the high-shedding animals for liquid culture and direct PCR. However, it is more similar to a low-shedding animal using solid culture. These results are similar to those found in 2014 and 2016 with animal 12-00956 (KS), which had a mixed infection of both cattle and 'bison' strains of *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Reviewing whole genome sequence data from our laboratory indicates animal 17-03143 (FL) likely also has a mixed infection with both cattle and 'bison' strains of MAP, which is consistent with the results shown in Table 4 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.

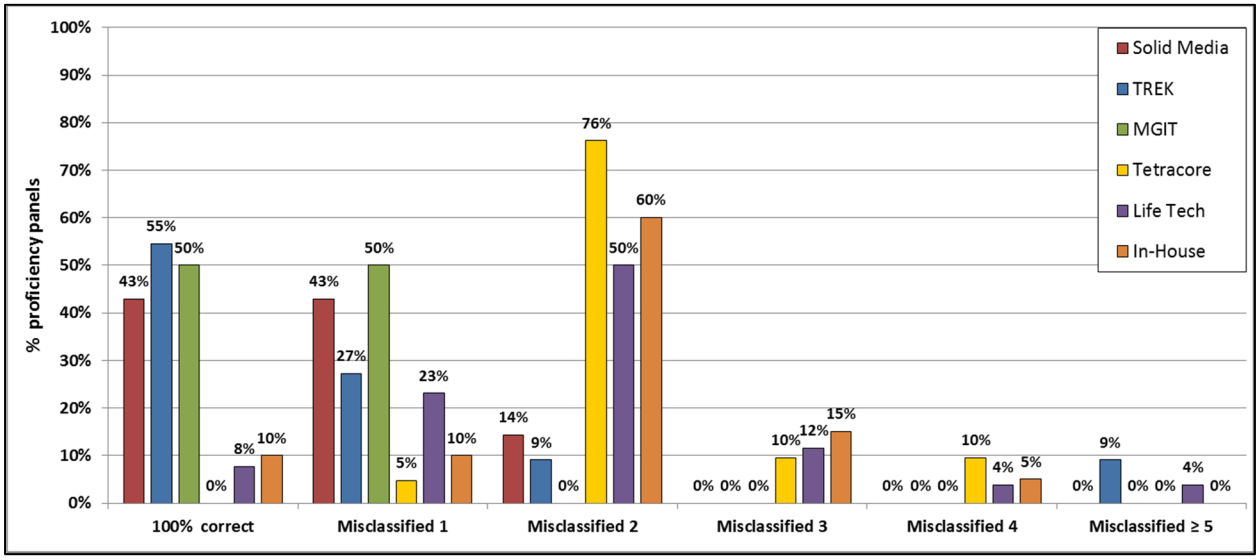
Table 4. A comparison of the averaged result values among the three methods for shedding animals.

| | | 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 | Ct | Ct | Ct |
| Cow ID | Status | 9 | 12 | 5 | 28 | 23 | 18 |
| 17-03492 (WI) | Low | 0.5 | 33 | 34 | 37.4 | 37.7 | 35.9 |
| 18-00942 (ID) | Low | 2.4 | 32 | 30 | 32.1 | 30.9 | 31.4 |
| 17-02487D (FL) | Low | 8.5 | 30 | 27 | 32.9 | 30.9 | 31.8 |
| 15-00426D (ID) | Moderate | 5.1 | 31 | 33 | 33.1 | 30.5 | 31.0 |
| 16-01645 (IA) | Mod-High | 23.8 | 25 | 27 | 31.7 | 29.8 | 30.0 |
| 15-00471D (FL) | Critical- High | 39.5 | 21 | 16 | 26.6 | 25.6 | 25.4 |
| 17-03210 (FL) | Critical- High | TNTC | 18 | 15 | 23.6 | 22.5 | 22.2 |
| 17-02487 (FL) | Critical- High | 44.0 | 16 | 14 | 23.1 | 22.2 | 22.1 |
| 15-00471 (FL) | Critical- High | TNTC | 16 | 13 | 23.0 | 22.1 | 21.7 |
| 17-03143 (FL) | Critical- High | 2.6 | 16 | 12 | 21.9 | 19.8 | 20.4 |

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

The performance of each method was further evaluated by determining the number of samples that were misclassified (Figure 1) using all 25 samples. In this analysis 55% of laboratories using the TREK system correctly classified all the samples. For the laboratories using solid media 43% correctly classified all the samples. The performance of the three direct PCR methods was similar to last year with even fewer laboratories correctly calling all the samples, mainly due to the difficulty of samples from animal 17-03492 (WI). Ten percent of laboratories using In-house direct PCR methods correctly classified all the samples.

Figure1. Percentage of 2018 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.



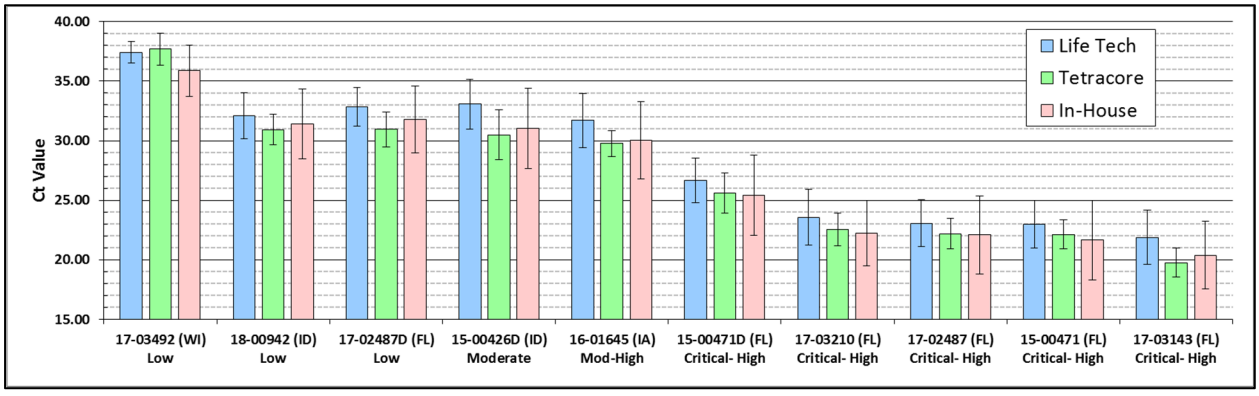
According to the 2010 Johne's Disease Uniform Program Standards, laboratories must correctly classify all critical-high shedding samples as positive, all negative samples as negative and misidentify 3 or fewer of the remaining, valid, non-critical samples. Table 5 lists the specific reasons laboratories failed to pass the proficiency panel for each method. As in previous years the most common reason for failure is misclassifying a negative sample as positive. Interestingly, only laboratories using direct PCR methods misclassified negative samples as positive.

Table 5. Reasons laboratories failed the 2018 Johne's Disease Fecal Proficiency Panel.

| 2018 | 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 | 6 | 3 | 4 | | | |
| Missed 4 or more low / moderate shedders (lack of sensitivity) | | 1 | | | | |
| Misclassified a high shedding sample as negative | | | | 1 | | |
| Multiple reasons cited above | | | | | | |
| **Total failed kits** | **6 (29%)** | **4 (15%)** | **4 (20%)** | **1 (9%)** | **0 (0%)** | **0 (0%)** |
| **Total kits tested** | **21** | **26** | **20** | **11** | **4** | **7** |

Because direct PCR is now the most common organism detection assay offered, 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). The overall means of all three methods for each animal were statistically similar. The diluted samples, 15-00471D (FL), 17-00426D (FL), and 17-02487D (FL), all performed similar to natural shedding animals.

Figure 2. Average, and 1 standard deviation, reported Ct of 2018 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. Animal numbers ending in "D" are diluted samples.



False positive results with either direct fecal PCR or confirmatory culture PCR continue to be the most common cause of failure. Table 6 examines the number of negative samples reported with Ct values by PCR method; this includes laboratories that had Ct values and correctly reported them as negative. Also shown are the number of panels where at least one Ct is reported. Errors were relatively evenly distributed amongst the four negative animals that were used in this year's panel when considering the number of vials included. There were a total of 15 laboratories that reported Ct values on at least one negative sample. Of those 15 laboratories, 12 failed the PT (see Table 5) by calling a negative sample positive and is a slight reduction from last year's panel (17 of 19 failed). This continues to be a significant issue.

Table 6. 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 | Total |
|---|---|---|---|---|
| 17-03787 (IA) | 4 | | 1 | 5 |
| 17-03786 (IA) | 4 | 1 | 2 | 7 |
| 17-04259 (IA) | 5 | 5 | 2 | 12 |
| 18-01896 (IA) | 1 | 1 | 1 | 3 |
| Num. panels reporting Ct | 6 | 4 | 5 | 15 |

## 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 7 lists the contents of each pool, and Table 9 lists the pool numbers associated with each lot of panels. To pass, laboratories were required to correctly classify the negative pool and the two pools that contained a high-shedding animal (15-00471& 17-02487). Laboratories were allowed to misclassify one of the other pools (16-01645) and still pass the panel.

Table 7. Composition of the 2018 Johne's Disease Fecal Pooling Proficiency Panel.*

| | Positive sample(s) description | |
|---|---|---|
| | Cow ID | Avg. CFU/ tube* |
| 1 High, 4 Negative samples | 15-00471 | ~1250 |
| 1 High, 4 Negative samples | 17-02487 | ~1000 |
| 1 Moderate, 4 Negative samples | 16-01645 | 25 |
| 1 Moderate, 4 Negative samples | 16-01645 | 25 |
| 5 Negative samples | | |

*Refers to the positive samples, not the pooled sample.

Table 8 further describes the performance of each method used in the pooled proficiency test. It is commendable that all laboratories using solid culture passed. All but one laboratory passed the pooled panel using direct PCR methods. Three laboratories using liquid culture misclassified the negative pool and another misclassified multiple pools.

Table 8. Performance of each method used in the Johne's Disease 2018 Fecal Pooling Proficiency Panel. A total of 5 pooled samples were in each panel.

| 2018 | | No. panels | | |
|---|---|---|---|---|
| | | **Direct PCR** | **Liquid media** | **Solid media** |
| **Panels that failed** | Identified the negative pool as positive | 1 | | |
| | Identified a high -shedding pool as negative | 1 | 1 | |
| | Two non-critical pools were identified as negative | | | |
| | Failed due to multiple criteria | 2 | | |
| **Panels that passed** | One non-critical pool was misidentified as negative | 2 | | |
| | All 5 pools were identified correctly | 43 | 11 | 4 |
| | **Total Failed Pooled Kits** | **4 (8%)** | **1 (8%)** | **0 (0%)** |
| | **Total** | **49** | **12** | **4** |

A current listing of all the approved laboratories is available in the NVLS web site: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/sa_approved_labs/ct_approved_labs.

Remaining sample vials from the 2018 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 at https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/sa_reagents/ct_reagents

Table 9. 2018 Johne's Disease Pooled Fecal Proficiency Panel key by kit number.

| | Pool Sample Number | | | |
|---|---|---|---|---|
| **Pool Description** | **Kit# 1-20** | **Kit# 21-40** | **Kit# 41-60** | **Kit# 61-70** |
| 5 Negative samples | 3 | 1 | 4 | 5 |
| 1 mod-high (16-01645), 4 Negative samples | 1 | 3 | 2 | 2 |
| 1 mod-high (16-01645), 4 Negative samples | 4 | 5 | 3 | 4 |
| 1 high (17-02487), 4 Negative samples | 5 | 2 | 1 | 3 |
| 1 high (15-00471), 4 Negative samples | 2 | 4 | 5 | 1 |

Table 10. 2018 Johne's Disease Individual Fecal Proficiency Panel key by kit number. Samples are coded by color according to shedding status as follows: Negative, Non-critical positive samples, Critical – high shedding samples. Sample 26 was the positive control.

| Vial # | 1-25 | 26-50 | 51-75 | 76-100 |
|---|---|---|---|---|
| 1 | 17-02487D (FL) | 16-01645 (IA) | 17-02487 (FL) | 15-00426D (ID) |
| 2 | 17-03143 (FL) | 17-02487D (FL) | 17-04259 (IA) | 18-00942 (ID) |
| 3 | 17-03786 (IA) | 17-03492 (WI) | 15-00471D (FL) | 17-02487 (FL) |
| 4 | 17-03210 (FL) | 17-02487 (FL) | 18-00942 (ID) | 17-04259 (IA) |
| 5 | 17-02487 (FL) | 17-04259 (IA) | 17-03143 (FL) | 17-03210 (FL) |
| 6 | 17-04259 (IA) | 15-00471D (FL) | 17-03786 (IA) | 17-03492 (WI) |
| 7 | 15-00471D (FL) | 17-02487D (FL) | 16-01645 (IA) | 17-02487D (FL) |
| 8 | 17-03492 (WI) | 15-00471 (FL) | 17-03492 (WI) | 17-03143 (FL) |
| 9 | 17-02487 (FL) | 17-03787 (IA) | 17-02487D (FL) | 18-01896 (IA) |
| 10 | 17-04259 (IA) | 17-03210 (FL) | 17-02487 (FL) | 15-00471D (FL) |
| 11 | 16-01645 (IA) | 18-00942 (ID) | 17-04259 (IA) | 17-02487D (FL) |
| 12 | 17-03143 (FL) | 17-03143 (FL) | 17-03210 (FL) | 16-01645 (IA) |
| 13 | 18-01896 (IA) | 17-03786 (IA) | 17-02487D (FL) | 17-02487D (FL) |
| 14 | 17-02487D (FL) | 17-02487 (FL) | 15-00426D (ID) | 15-00471 (FL) |
| 15 | 15-00426D (ID) | 17-04259 (IA) | 17-03492 (WI) | 17-03787 (IA) |
| 16 | 17-03492 (WI) | 15-00471D (FL) | 17-03143 (FL) | 17-02487 (FL) |
| 17 | 18-00942 (ID) | 17-02487D (FL) | 18-01896 (IA) | 17-04259 (IA) |
| 18 | 15-00471 (FL) | 15-00426D (ID) | 17-02487 (FL) | 15-00471D (FL) |
| 19 | 17-03787 (IA) | 18-00942 (ID) | 17-04259 (IA) | 17-03492 (WI) |
| 20 | 15-00471D (FL) | 17-02487 (FL) | 17-03210 (FL) | 17-03143 (FL) |
| 21 | 17-02487D (FL) | 17-04259 (IA) | 17-02487D (FL) | 17-03786 (IA) |
| 22 | 17-02487 (FL) | 17-03210 (FL) | 15-00471 (FL) | 17-03210 (FL) |
| 23 | 17-04259 (IA) | 17-03143 (FL) | 17-03787 (IA) | 18-00942 (ID) |
| 24 | 17-03210 (FL) | 18-01896 (IA) | 15-00471D (FL) | 17-02487 (FL) |
| 25 | 18-00942 (ID) | 17-03492 (WI) | 18-00942 (ID) | 17-04259 (IA) |
| 26 | 16-01645 (IA) | 16-01645 (IA) | 16-01645 (IA) | 16-01645 (IA) |

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