

**The National Veterinary Services  
Laboratories Johne's Disease  
Decontamination Protocol  
A Validation Study: Preliminary Data**

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# Reasons for the Project

- Validate NVSL decontamination protocol for Johne's disease culture in other labs
- Compare BBL HEY tubes vs BBL HEY flasks
- Compare incubation time – 8 wks vs 16 wks

# Participants and Items Sent

- 11 NVSL Johne's disease approved labs volunteered to participate
  - Labs located in CO, ID, MO, NE, OR, SD, TN, WA, WI,
- NVSL Johne's disease lab also participated
- 2 tubes of BBL HEY media with Mycobactin J from different lots from BD
- 1 flask of BBL HEY media with Mycobactin J (experimental lot from BD)



# BBL Mycoflasks



# Project Protocol Day 1

- $2 \pm 0.1$  grams of feces are placed into 35 ml of sterile distilled water in a centrifuge tube.
- The tube is shaken vigorously by hand to break up the large clumps.
- The tube is then placed on a horizontal shaker for 30 minutes.
- After shaking the tubes are allowed to sit upright undisturbed for at least 30 minutes.

# Project Protocol Day 1

- 5 mls of liquid are removed from the upper 1/3 of the original centrifuge tube and placed into a new 50 ml centrifuge tube containing 25 ml of 0.9% HPC in 1/2 x BHI broth.
- The new centrifuge tube containing 5 mls of liquid is incubated at  $37^{\circ} \pm 2^{\circ}\text{C}$  for 18-24 hours (overnight).

# Project Protocol Day 2

- Incubated sample is centrifuged at 900 x g for  $30 \pm 2$  minutes
- Supernatant is discarded and the pellet is re-suspended in 1ml of BHI broth containing 100 $\mu$ g/ml nalidixic acid, 100 $\mu$ g/ml vancomycin and 50 $\mu$ g/ml amphotericin B (Antibiotic Brew)
- Sample is shaken or vortexed for 15 seconds
- Incubate at  $37^\circ \pm 2^\circ\text{C}$  overnight

# Project Protocol Day 3

- Shake or vortex inoculum for 15 seconds prior to inoculating the media
- Inoculate each HEY tube and HEY flask with 200 $\mu$ l of inoculum
- Inoculum is rolled to ensure that the surfaces are covered
- Media is incubated at  $37^{\circ} \pm 2^{\circ}\text{C}$  for 8 wks

# Methods Used in the Study

- All low, medium, and high samples were from three single sources
- HEY media without Mycobactin J was not used since there were no “trick samples”
- More than 50 colonies observed are called TNTC

# Points to Observe in the Data

- Flasks have greater colony counts than tubes for the same inoculum
- Most labs have similar results but there are lab to lab variations for the same protocol

# 8 Week Results

Kit#	1				2			
	Colony Counts from Solid Media				Colony Counts from Solid Media			
KEY	SAMPLE	T1	T2	Flask	SAMPLE	T1	T2	Flask
Neg	4	0	0	0	4	0	0	0
Neg	8	0	0	0	6	0	0	0
Neg	11	0	0	0	10	0	0	0
Neg	18	0	0	0	15	0	0	0
Neg	20	0	0	0	19	0	0	0
Low	5	5	6	17	1	1	1	23
Low	6	4	3	18	8	2	0	44
Low	13	13	3	13	11	1	1	17
Low	14	4	1	15	12	0	0	17
Low	15	5	9	24	13	0	0	14
Med	1	TNTC	TNTC	TNTC	2	2	2	7
Med	2	TNTC	TNTC	TNTC	7	41	40	TNTC
Med	9	27	30	TNTC	16	15	6	TNTC
Med	12	TNTC	TNTC	TNTC	17	0	1	TNTC
Med	16	11	13	36	20	12	5	TNTC
High	3	5	5	22	3	1	0	7
High	7	7	3	17	5	3	0	13
High	10	6	7	13	9	0	0	27
High	17	7	7	9	14	3	0	50
High	19	6	4	9	18	1	0	TNTC

Good example of more growth on flasks.

No growth on tubes when it is expected.

# 8 Week Results

3				Kit#	4			
Colony Counts from Solid Media					Colony Counts from Solid Media			
SAMPLE	T1	T2	Flask	KEY	SAMPLE	T1	T2	Flask
2	0	0	0	Neg	8	0	0	0
4	0	0	0	Neg	10	0	0	0
8	0	0	0	Neg	12	0	0	0
14	0	0	0	Neg	14	0	0	0
20	0	0	0	Neg	16	0	0	0
6	0	0	22	Low	4	4	5	12
10	0	0	8	Low	5	4	3	18
11	0	0	10	Low	6	1	2	4
15	0	0	8	Low	17	0	0	0
16	0	0	1	Low	18	0	0	0
5	0	0	TNTC	Med	1	8	14	TNTC
9	1	0	14	Med	2	10	20	TNTC
12	0	0	40	Med	3	33	42	TNTC
17	0	0	TNTC	Med	19	25	8	TNTC
18	0	0	TNTC	Med	20	8	27	TNTC
1	0	0	11	High	7	1	2	9
3	0	0	8	High	9	1	0	8
7	0	0	4	High	11	0	3	4
13	0	0	14	High	13	2	0	3
19	0	0	0	High	15	3	3	9

Minimal growth on tubes.

No growth when it is expected.

# 8 Week Results

5				6				Kit#
Colony Counts from Solid Media				Colony Counts from Solid Media				
SAMPLE	T1	T2	Flask	SAMPLE	T1	T2	Flask	KEY
4	0	0	0	4	0	0	0	Neg
8	0	0	0	6	0	0	0	Neg
11	0	0	0	10	0	0	0	Neg
18	0	0	0	15	0	0	0	Neg
20	0	0	0	19	0	0	0	Neg
5	0	8	25	1	8	TNTC	19	Low
6	4	1	14	8	13	11	31	Low
13	7	7	20	11	11	5	49	Low
14	4	10	17	12	13	10	50	Low
15	9	9	22	13	18	11	19	Low
1	28	39	TNTC	2	TNTC	TNTC	40	Med
2	TNTC	33	TNTC	7	TNTC	TNTC	TNTC	Med
9	30	24	TNTC	16	TNTC	TNTC	TNTC	Med
12	TNTC	26	TNTC	17	TNTC	TNTC	TNTC	Med
16	41	35	TNTC	20	TNTC	TNTC	TNTC	Med
3	3	6	20	3	18	5	26	High
7	7	5	24	5	23	29	22	High
10	10	2	19	9	14	13	30	High
17	2	20	23	14	43	29	TNTC	High
19	12	13	33	18	13	21	35	High

# 8 Week Results

Kit#	7				8			
KEY	SAMPLE	Colony Counts from Solid Media			Colony Counts from Solid Media			
		T1	T2	Flask	SAMPLE	T1	T2	Flask
Neg	2	0	0	0	8	0	0	0
Neg	4	0	0	0	10	0	0	0
Neg	8	0	0	0	12	0	0	0
Neg	14	0	0	0	14	0	0	0
Neg	20	0	0	0	16	0	0	0
Low	6	5	7	14	4	17	16	28
Low	10	7	17	25	5	22	17	23
Low	11	10	8	15	6	11	16	45
Low	15	1	6	20	17	2	7	17
Low	16	9	8	20	18	14	14	32
Med	5	30	35	TNTC	1	TNTC	TNTC	TNTC
Med	9	50	50	TNTC	2	TNTC	TNTC	TNTC
Med	12	50	50	TNTC	3	TNTC	TNTC	TNTC
Med	17	14	30	TNTC	19	TNTC	TNTC	TNTC
Med	18	TNTC	TNTC	TNTC	20	TNTC	TNTC	TNTC
High	1	7	17	23	7	18	19	37
High	3	16	12	40	9	24	22	55
High	7	20	12	50	11	26	17	37
High	13	10	10	20	13	9	12	17
High	19	6	12	15	15	39	23	40

# 8 Week Results

9					Kit#	10			
Colony Counts from Solid Media						Colony Counts from Solid Media			
SAMPLE	T1	T2	Flask	KEY	SAMPLE	T1	T2	Flask	
4	0	0	0	Neg	4	0	0	0	
8	0	0	0	Neg	6	0	0	0	
11	0	0	0	Neg	10	0	0	0	
18	0	0	0	Neg	15	0	0	0	
20	0	0	0	Neg	19	0	0	0	
5	15	10	30	Low	1	2	TNTC	10	
6	7	10	20	Low	8	3	22	12	
13	10	10	30	Low	11	1	1	3	
14	6	6	16	Low	12	63	0	4	
15	20	25	30	Low	13	1	4	1	
1	20	20	40	Med	2	TNTC	31	TNTC	
2	40	40	45	Med	7	31	TNTC	TNTC	
9	23	15	25	Med	16	TNTC	58	TNTC	
12	TNTC	TNTC	TNTC	Med	17	55	50	62	
16	TNTC	TNTC	TNTC	Med	20	25	18	97	
3	34	26	32	High	3	5	3	9	
7	20	10	20	High	5	2	2	8	
10	20	18	25	High	9	6	5	18	
17	17	15	30	High	14	2	3	19	
19	10	7	15	High	18	5	9	21	

Lab variability is seen here.

# 8 Week Results

Kit#	11				12			
KEY	Colony Counts from Solid Media				Colony Counts from Solid Media			
	SAMPLE	T1	T2	Flask	SAMPLE	T1	T2	Flask
Neg	2	0	0	0	8	0	0	0
Neg	4	0	0	0	10	0	0	0
Neg	8	0	0	0	12	0	0	0
Neg	14	0	0	0	14	0	0	0
Neg	20	0	0	0	16	0	0	0
Low	6	1	1	8	4	18	16	41
Low	10	1	1	2	5	26	20	41
Low	11	1	2	3	6	4	6	20
Low	15	3	0	6	17	9	9	16
Med	16	3	4	24	18	9	5	37
Med	5	10	13	41	1	TNTC	TNTC	TNTC
Med	9	25	28	TNTC	2	TNTC	TNTC	TNTC
Med	12	45	9	TNTC	3	TNTC	TNTC	TNTC
Med	17	29	1	TNTC	19	TNTC	TNTC	TNTC
High	18	17	5	22	20	TNTC	TNTC	TNTC
High	1	1	4	TNTC	7	11	10	31
High	3	4	2	10	9	30	28	TNTC
High	7	3	0	18	11	10	14	33
High	13	3	7	16	13	24	16	TNTC
High	19	0	6	28	15	9	10	28

# Results

- All labs had no growth on negative samples
- 2 labs had no growth on samples that should have been positive
- 1 lab had no growth on all HEY tubes (except one tube and 1 colony), flask colony counts were similar to what other labs counted
- All labs except two had growth on at least one of the two HEY tubes for this inoculation volume when growth was expected

# Results

- Most labs had between 2-3X the amount of growth on flasks vs. tubes for the same inoculum
- Most labs cultured about the same amount of MAP for the low, medium, and high count feces
- High count feces was not as high as was originally, but shows how effective the flasks were at allowing more MAP to grow

# Results

	Neg Tubes	Neg Flasks	Low Tubes	Low Flasks	Med Tubes	Med Flasks	High Tubes	High Flasks
Average Colonies Counted	0.00	0.00	7.72	19.07	36.62	58.02	9.25	24.90
Standard Deviation	0.00	0.00	10.60	12.34	22.86	16.41	9.35	17.65

- High count feces was lower than previously tested
- For quantifying these results, 60 colonies was used for TNTC
- Results include colony counts from all of the tubes and flasks used in this study
- Variability is noted in the amount of colonies counted

# Comments-Reading Flasks

- Flasks are easier to read overall than the tubes
- Glare and condensation made the flasks a bit difficult to read
- The flask was superior to the tube(s) in the reduced time to spot visible MAP colonies, greater number of colonies, and ease of handling and observation
- Found it hard to read through the glass flasks

# Comments-Condensation

- Had to wipe off a film to see some of the colonies
- Flasks have thick glass and are harder to read with any condensation than the tubes
- Flasks are too thick to avoid high condensation
- If no condensation, the flasks are easier to read and count

# Comments-General

- Flasks are too thick to view under a microscope
- Color change is indicative of egg yolk consumption by MAP growth
- Flasks are a great space saver in the incubator
- The flask was superior to the tube(s) in the reduced time to spot visible MAP colonies, greater number of colonies, and ease of handling and observation
- More difficult to cover surface of flask with 200 $\mu$ l of inoculum than the tubes
- Overall we prefer the flasks over the tubes

# Summary

- Flasks have greater colony counts than tubes for the same inoculum
- Variation was noted from lab to lab with counts
  - Lab technique?
  - Reagents? (source, age of reagent, storage conditions)
  - Shaking out condensation in tubes before inoculation?
  - Equipment used?
- Recovery of MAP is good at 8 weeks
- 16 week results still pending

# Acknowledgements

- BD- for supplying the flasks for this study
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***Any Questions?***