

# VFM Replacement Media

## June 2019

VFM Working Group

D. Bade, M. Papich, S. Simjee, J. Watts, C. Knapp, S. Killian,  
J. Dustin Loy, A. Thacil, N. Holliday, M. Traczewski, T. Shryock, C.C. Wu

# STATUS

- Testing is complete and data has been analyzed.
- The VFM Working Group has reviewed and has made suggestions.
- This presentation to the VAST is to accept and vote on the results of the testing.
- The final step would be to publish the new media and accepted ranges in the VET01 and VET08 documents.

# Testing Procedure

- As was presented to VAST, and agreed to, in January 2016
  - VFM (as a comparator for QC)
    - 10 replicates of *A. pleuropneumoniae* (ATCC 27090)
    - 10 replicates of *H. somni* (ATCC 700025)
    - Test in CO<sub>2</sub> at 7 labs
    - One lot of VFM
  - MHF-Y (Mueller Hinton Fastidious broth with Yeast Extract, Modification of EUCAST MHF media)
    - 10 replicates of *A. pleuropneumoniae* (ATCC 27090)
    - 10 replicates of *H. somni* (ATCC 700025)
    - Test in CO<sub>2</sub> and aerobically at 7 labs
    - 100 clinical APP and 100 clinical HS – Testing to be split among labs.
    - Three lots of MHF-Y for QC and one lot for clinical isolates
      - Note: clinical isolates were not tested in VFM

# Testing Procedure

Antimicrobial	Approved CLSI Breakpoint (µg/mL)				CLSI QC MIC in VFM (µg/mL)		MIC Testing Range (µg/mL)
	Animal Species	S	I	R	<i>Histophilus somni</i> (ATCC 700025)	<i>Actinobacillus pleuropneumoniae</i> (ATCC 27090)	
Ampicillin	Swine (GWG)	≤0.5	1	≥2	N/A	N/A	0.03-16
Cefovecin	None				0.001-0.008	0.008-0.03	0.0002-0.5
Cefquinome	Cattle (Sponsor approved only)	≤2	4	≤8	0.002-0.008	0.004-0.03	0.001-16
Ceftiofur	Swine	≤2	4	≤8	0.0005-0.004	0.004-0.016	0.0002-8
	Cattle	≤2	4	≤8			
Danofloxacin	None				0.016-0.12	0.03-0.12	0.008-0.25
Enrofloxacin	Swine	≤0.25	0.5	≥1	0.016-0.06	0.016-0.06	0.004-2
	Cattle	≤0.25	0.5	≥1			
Florfenicol	Swine	≤2	4	≥8	0.12-0.5	0.25-1	0.03-16
	Cattle	≤2	4	≥8			
Gamithromycin	Cattle	≤4	8	≥16	0.25-1	2-8	0.03-64
Gentamicin	Horses (GWG)	≤2	4	≥8	8-32	8-32	1-64
Marbofloxacin	None				0.016-0.12	0.016-0.06	0.004-0.5
Penicillin	Cattle (GWG)	≤0.25	0.5	≥1	0.016-0.06	0.12-1	0.002-2
Pradofloxacin	None				0.004-0.03	0.004-0.016	0.001-0.06
Spectinomycin	Cattle	≤32	64	≥128	N/A	N/A	1-256
Tetracycline	Swine (GWG)	≤0.5	1	≥2	0.12-1	0.25-2	0.03-4
Tiamulin	Swine	≤16		≤32	N/A	8-32	2-64
Tildipirosin	Swine	≤16			2-8	2-16	0.25-128
	Cattle	≤8	16	≤32			
Tilmicosin	Swine	≤16		≥32	2-16	4-32	0.5-128
Trimethoprim/ sulfamethoxazole	None				0.03/0.57- 0.125/2.38	0.016/0.28- 0.06/1.14	0.004-1
Tulathromycin	Swine	≤64			4-32	16-64	0.5-128
	Cattle	≤16	32	≥64			

# Data Analysis

- A random number was assigned to each participating laboratory for blinding purposes.
- Discrepant data were clarified and corrected, or excluded if correction was not apparent.
- Data summary included
  - Summary of each MHF-Y lot number for each QC organism and incubation condition,
  - Summary of MIC results for each QC organism for VFM and MHF-Y for each laboratory and for all laboratories for each incubation condition,
  - A graph was presented for each organism and antimicrobial for each media type.
  - A direct comparison of the VFM MIC data by QC organism isolate replicate to the corresponding MIC result in MHF-Y.
    - Data was excluded for isolate replicates for which there was no growth.
  - For antimicrobial/organism combinations where new QC ranges are proposed, Range Finder data is presented.

# Impression of MHF-Y (Prior to data analysis)

- Superior to VFM
  - Many had a very difficult time with very light, or no growth of VFM – Especially with *H. somni*, especially the QC organism.
- Often times two types of growth was present – typical button and diffuse growth, sometimes with edges folded over. Could be difficult to train how to read.
- Darker coloration of MHF-Y can make interpretation difficult at times. More of an issue for HS. AutoReaders may not work.
- Minimally noted very light growth for some antimicrobials



# Impression of MHF-Y (Prior to data analysis)

- How was reading of MHF-Y compared to VFM?
  - MHF-Y was easier to read than VFM.
  - Seemed to be a problem with VFM not supporting growth of HS adequately, or as well as previously experienced.
- How was your reading of the APP and HS isolate in an aerobic environment?
  - HS did not grow as well aerobically as compared to CO<sub>2</sub>, (QC strain).
    - Clinical isolates grew lighter aerobically, but grew adequately and much better than in VFM.
  - APP grew equally as well in both environments.
- Would you use MHF-Y for routine use?
  - Yes!

# CO<sub>2</sub> versus Aerobic?

MHF-Y MIC values >95% within current QC range for VFM for the indicated incubation conditions				
Antimicrobial	CO <sub>2</sub>		Aerobic	
	APP	HS	APP	HS
Ampicillin	No current range			
Cefovecin	Yes	Yes	Yes	No
Cefquinome	Yes	Yes	No	No
Ceftiofur	No	Yes	No	No
Danofloxacin	Yes	Yes	Yes	Yes
Enrofloxacin	Yes	Yes	Yes	Yes
Florfenicol	Yes	Yes	Yes	Yes
Gamithromycin	Yes	Yes	Yes	Yes
Gentamicin	Yes	Yes	No	No
Marbofloxacin	Yes	Yes	Yes	Yes
Penicillin	Yes	Yes	Yes	No
Pradofloxacin	Yes	Yes	Yes	Yes
Spectinomycin	No current range			
Tetracycline	Yes	Yes	Yes	Yes
Tiamulin	Yes	No current range		
Tildipirosin	Yes	Yes	No	No
Tilmicosin	Yes	Yes	Yes	Yes
Trimethoprim/ Sulfamethoxazole	Yes	Yes	Yes	Yes
Tulathromycin	No	Yes	No	No
	15/17 (88.2%)	16/16 (100%)	11/16 (68.8%)	9/16 (56.3%)



# CO<sub>2</sub> versus Aerobic?

Only CO<sub>2</sub> is presented for consideration

MHF-Y MIC values >95% within current QC range for VFM for the indicated incubation conditions

	CO <sub>2</sub>		Aerobic	
Antimicrobial	APP	HS	APP	HS
Ampicillin	No current range			
Cefovecin	Yes	Yes	Yes	No
Cefquinome	Yes	Yes	No	No
Ceftiofur	No	Yes	No	No
Danofloxacin	Yes	Yes	Yes	Yes
Enrofloxacin	Yes	Yes	Yes	Yes
Florfenicol	Yes	Yes	Yes	Yes
Gamithromycin	Yes	Yes	Yes	Yes
Gentamicin	Yes	Yes	No	No
Marbofloxacin	Yes	Yes	Yes	Yes
Penicillin	Yes	Yes	Yes	No
Pradofloxacin	Yes	Yes	Yes	Yes
Spectinomycin	No current range			
Tetracycline	Yes	Yes	Yes	Yes
Tiamulin	Yes	No current range		
Tildipirosin	Yes	Yes	No	No
Tilmicosin	Yes	Yes	Yes	Yes
Trimethoprim/ Sulfamethoxazole	Yes	Yes	Yes	Yes
Tulathromycin	No	Yes	No	No
	15/17 (88.2%)	16/16 (100%)	11/16 (68.8%)	9/16 (56.3%)

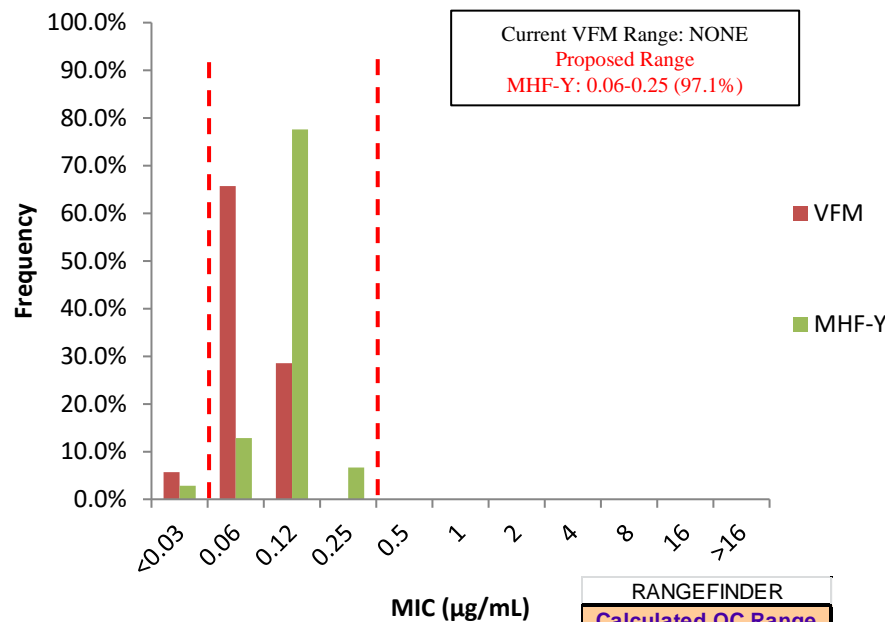
# Ampicillin – APP CO<sub>2</sub>

Table 1. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Ampicillin Broth Microdilution - CO2 incubation

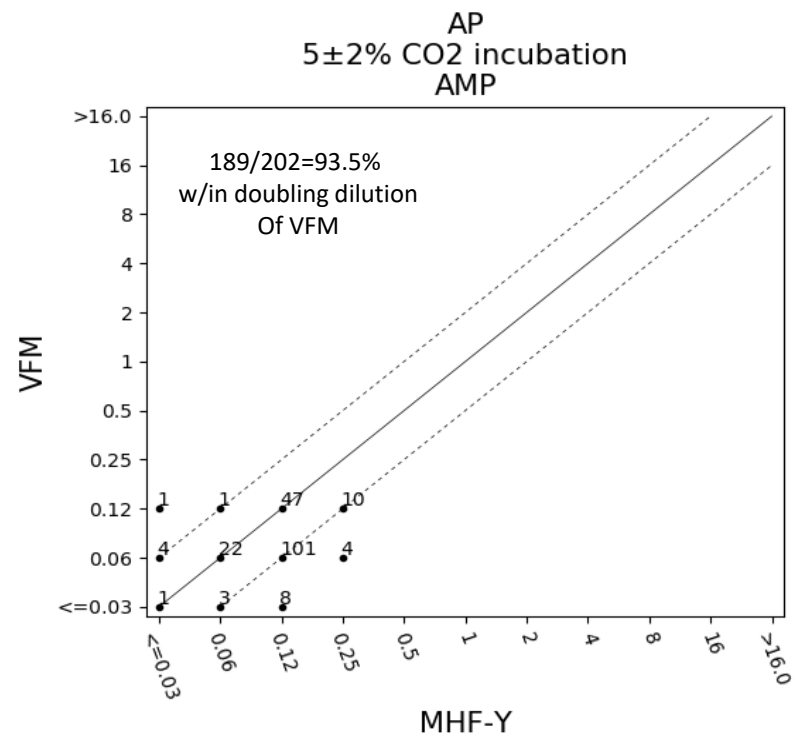
MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.03	2	3	1					1						1	6	2		4	6
0.06	11	14	2	8	1	4	1	7	2	9	6	5		5	3	8	14	46	27
0.12	51	49	63	2	26	6	26	2	28	1	21	5	27	4	20		15	20	163
0.25	6	4	4		3		3				3		3		1		1		14
0.5																			
1																			
2																			
4																			
8																			
16																			
>16																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	≤0.03	≤0.03	≤0.03	0.06	0.06	0.06	0.06	≤0.03	0.06	0.06	0.06	0.06	0.12	≤0.03	≤0.03	≤0.03	0.06	≤0.03	≤0.03
Max MIC	0.25	0.25	0.25	0.12	0.25	0.12	0.25	0.12	0.12	0.12	0.25	0.12	0.25	0.12	0.25	0.06	0.25	0.12	0.25
MIC <sub>mode</sub>	0.12	0.12	0.12	0.06	0.12	0.12	0.12	0.06	0.12	0.06	0.12	0.12	0.12	0.06	0.12	0.06	0.12	0.06	0.12

# Ampicillin – APP CO<sub>2</sub>

Figure 1. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Ampicillin Broth Microdilution - CO<sub>2</sub> incubation



RANGEFINDER	
Calculated QC Range	
0.063 to 0.25	
Dilution Range	
3	
% Obs. Captured	
97.1%	
Prob'ty Outside Range	
0.007	



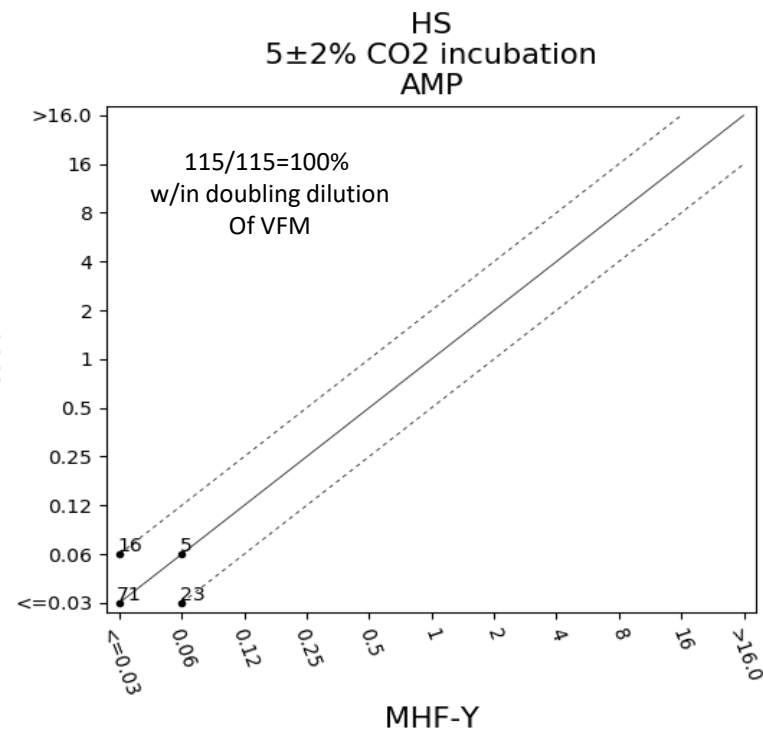
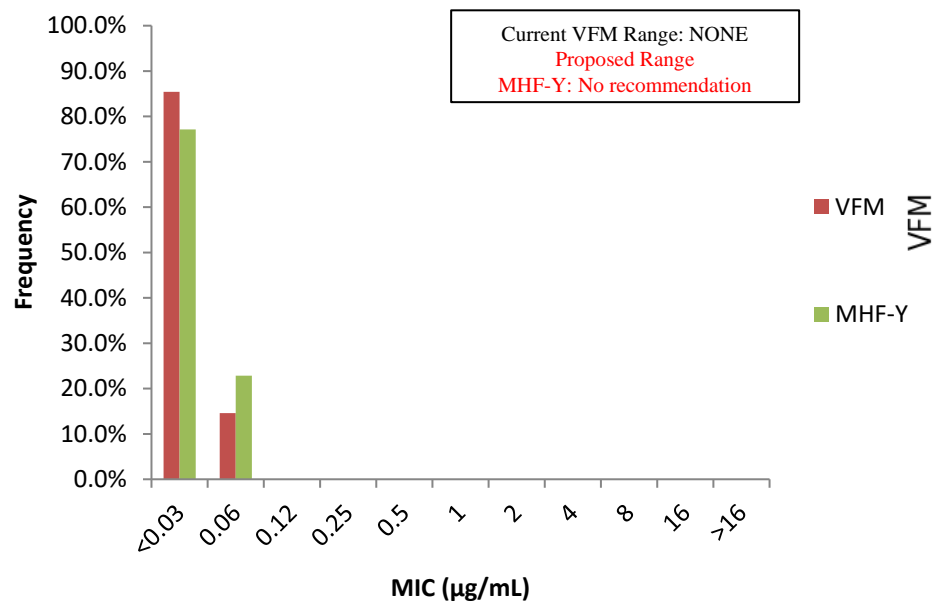
# Ampicillin – HS CO<sub>2</sub>

Table 3. *Histophilus somni* (ATCC 700025) vs Ampicillin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.03	66	43	53	7	24	8	28	9	13	4	29	3	23	10	21		24	41	162
0.06	4	27	17	3	9	2	2	1	17		1	1	6		9		4	7	48
0.12																			
0.25																			
0.5																			
1																			
2																			
4																			
8																			
16																			
>16																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth	1	1		1						6		6	1			10	2	23	3
Min MIC	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	N/A	≤0.03	≤0.03	≤0.03
Max MIC	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	N/A	0.06	0.06	0.06
MIC <sub>mode</sub>	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.06	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	N/A	≤0.03	≤0.03	≤0.03

# Ampicillin – HS CO<sub>2</sub>

Figure 3. *Histophilus somni* (ATCC 700025) vs Ampicillin Broth Microdilution - CO<sub>2</sub> incubation



# Ampicillin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Ampicillin				---	0.06–0.25

New Range for APP, none for HS

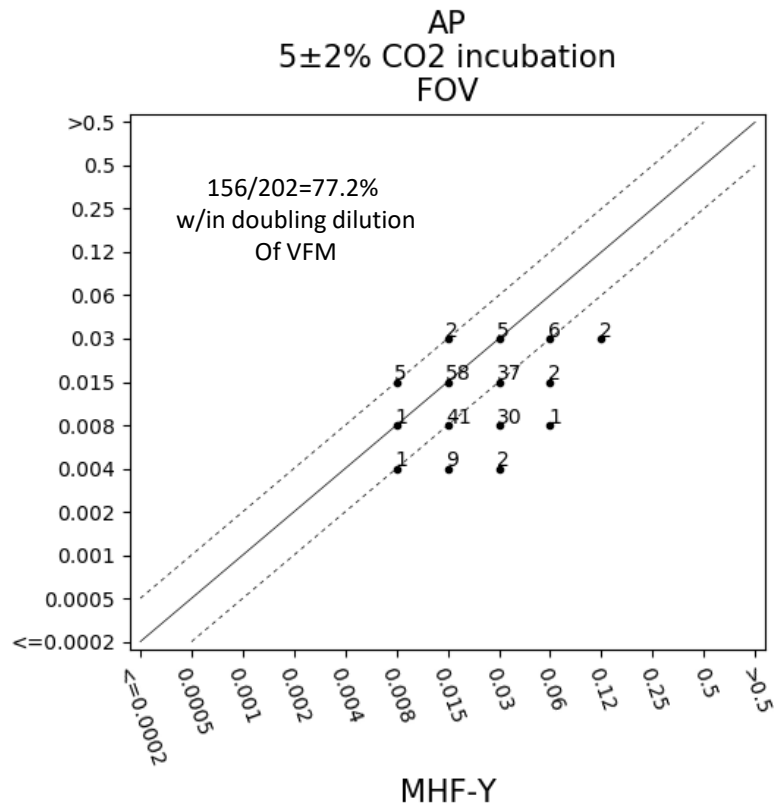
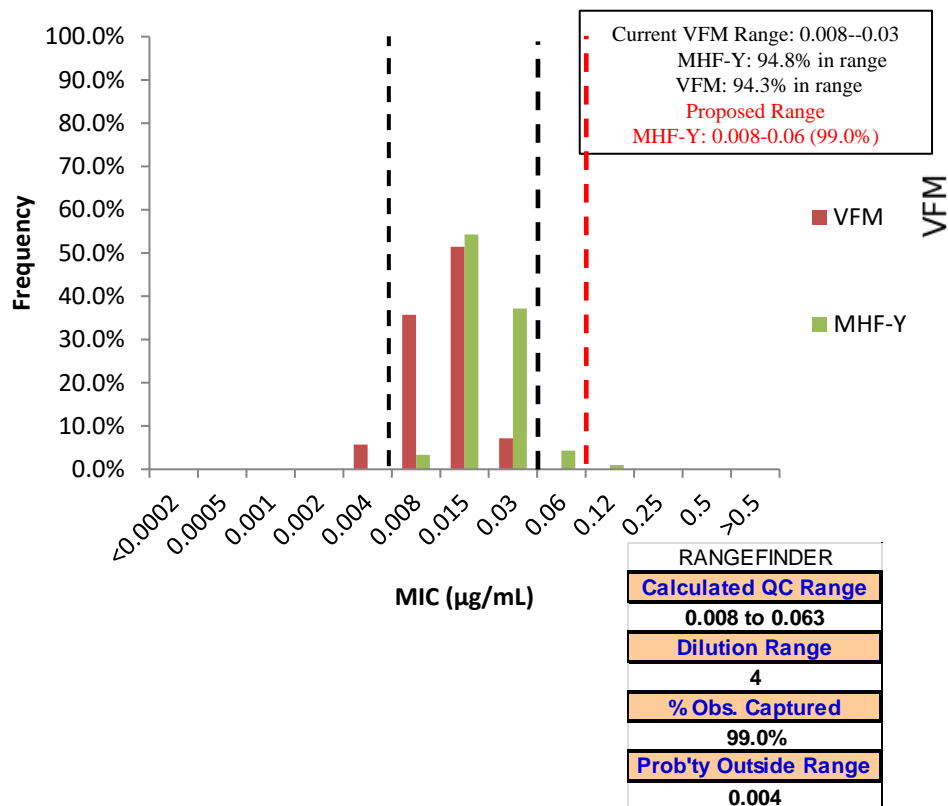


# Cefovecin – APP CO<sub>2</sub>

Table 5. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Cefovecin Broth Microdilution - CO2 incubation[illegible]

# Cefovecin – APP CO<sub>2</sub>

Figure 5. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Cefovecin  
Broth Microdilution - CO<sub>2</sub> incubation



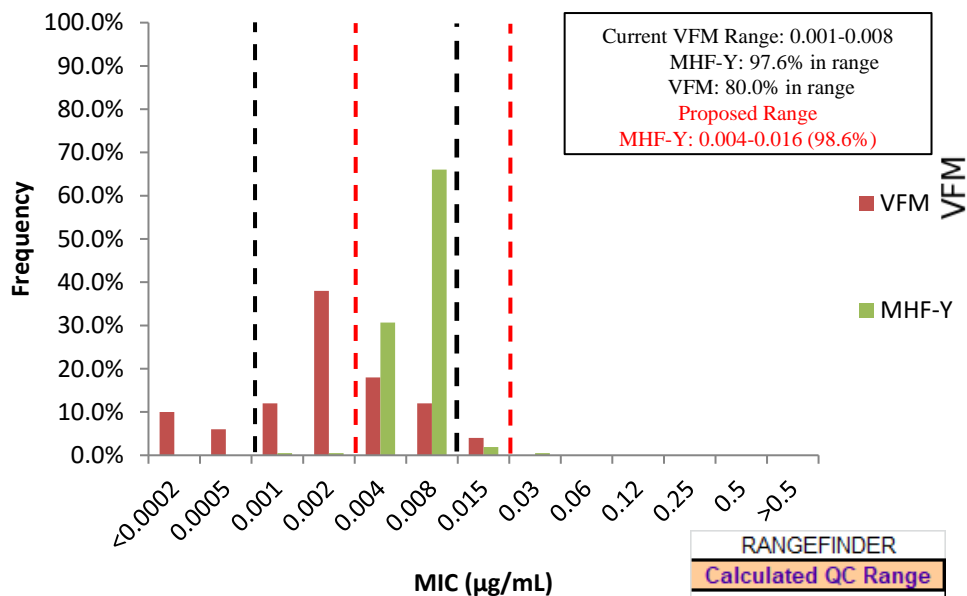
# Cefovecin – HS CO<sub>2</sub>

Table 7. *Histophilus somni* (ATCC 700025) vs Cefovecin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.0002				4						1								5	
0.0005				2						1								3	
0.001			1	3				2		1	1	1						6	1
0.002		1		1	1	7	0	8		2		1						19	1
0.004	17	27	21		15	2	2		2		21	2	25	5				9	65
0.008	52	40	48		17	1	27		28		8		4	5	26		30	6	140
0.015	2	1	1				1					2			3			2	4
0.03		1													1				1
0.06																			
0.12																			
0.25																			
0.5																			
>0.5																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		1		1						6		4	1			10		21	1
Min MIC	0.004	0.002	0.001	≤0.0002	0.002	0.002	0.004	0.001	0.004	≤0.0002	0.001	0.001	0.004	0.004	0.008	N/A	0.008	≤0.0002	0.001
Max MIC	0.015	0.03	0.015	0.002	0.008	0.008	0.015	0.002	0.008	0.002	0.008	0.015	0.008	0.008	0.03	N/A	0.008	0.015	0.03
MIC <sub>mode</sub>	0.008	0.008	0.008	≤0.0002	0.008	0.002	0.008	0.002	0.008	0.002	0.004	0.015	0.004	0.008	0.008	N/A	0.008	0.002	0.008

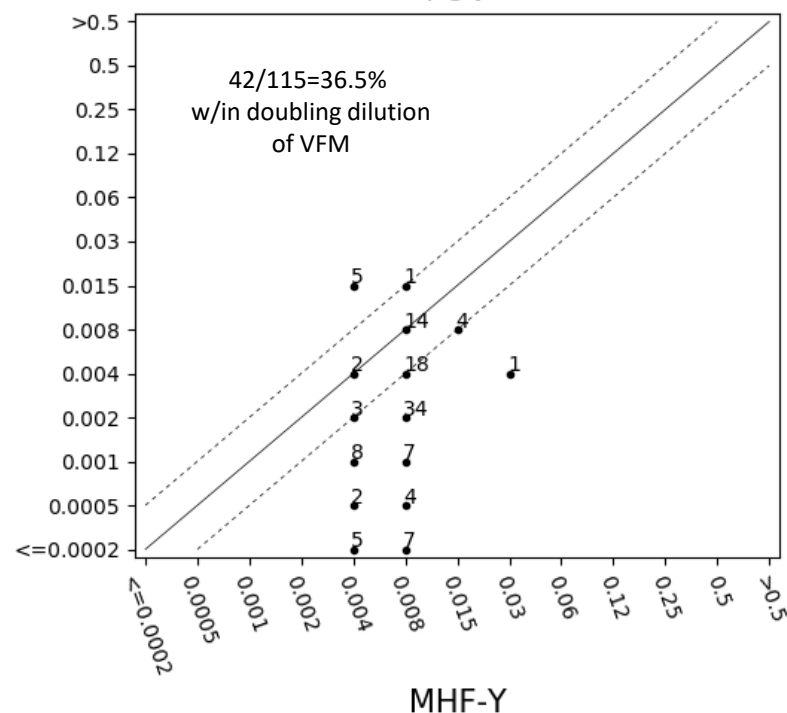
# Cefovecin – HS CO<sub>2</sub>

Figure 7. *Histophilus somni* (ATCC 700025) vs Cefovecin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGEFINDER
Calculated QC Range
0.004 to 0.016
Dilution Range
3
% Obs. Captured
98.6%
Prob'ly Outside Range
0.016

HS  
5±2% CO<sub>2</sub> incubation  
FOV



# Cefovecin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Cefovecin	30 µg	–	34–43 <sup>b</sup>	0.004-0.016	0.008–0.06

Change Ranges for HS and APP

# Cefquinome – APP CO<sub>2</sub>

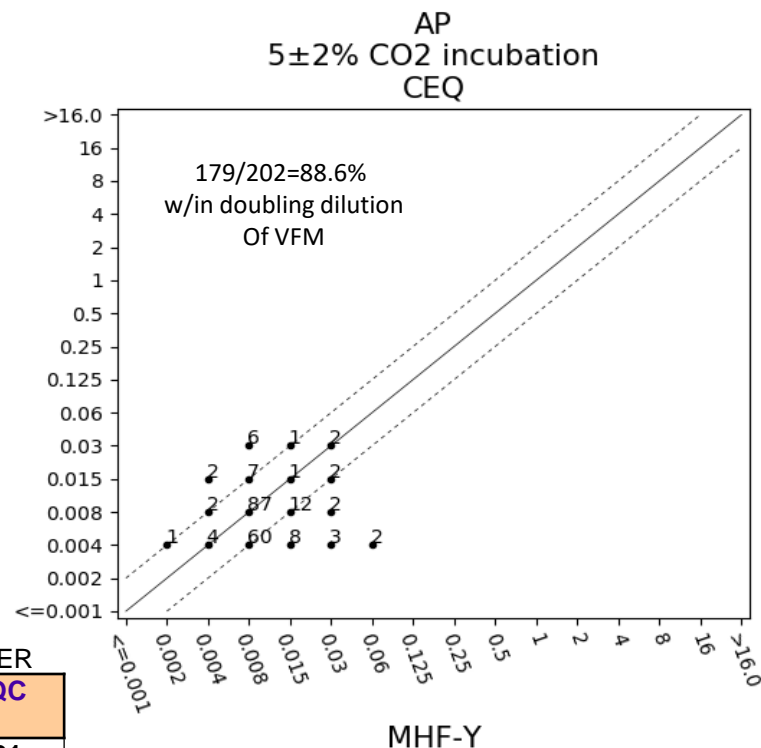
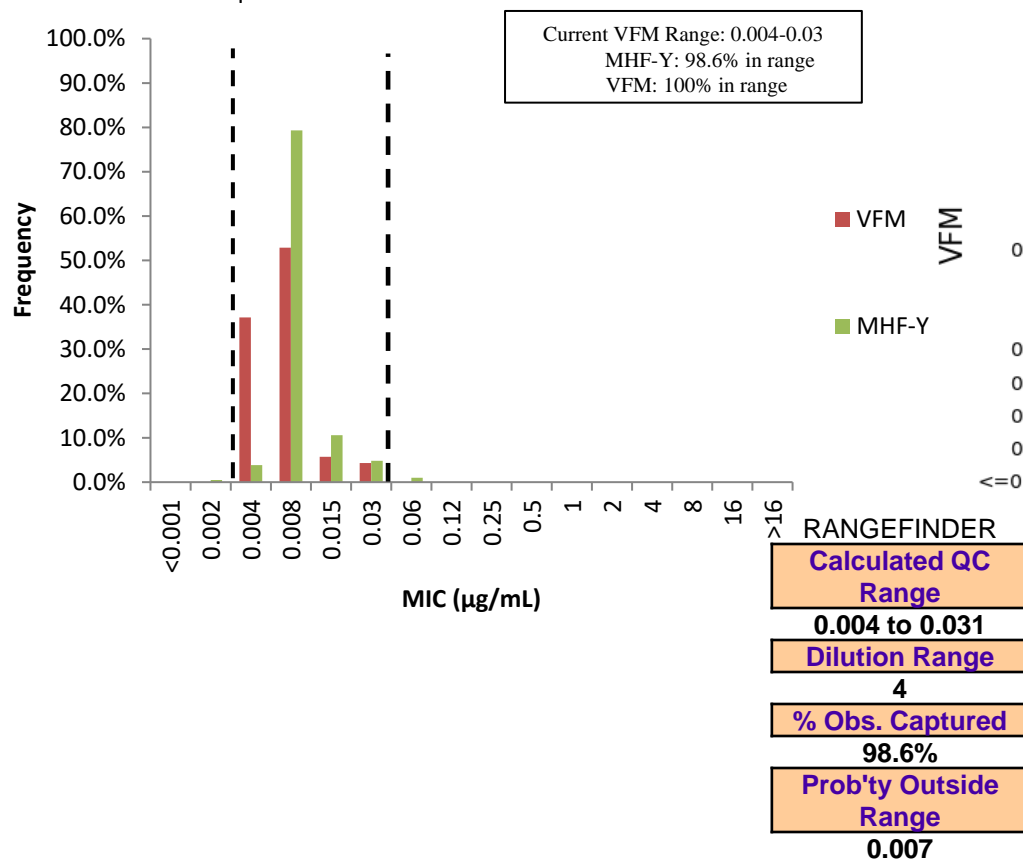
Table 9. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Cefquinome Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.001																			
0.002		1					1												1
0.004	6	1	1	4	1		1	3		8	4	2		3		6	2	26	8
0.008	50	57	58	5	25	9	24	7	30	1	19	6	15	7	29	2	23	37	165
0.015	9	5	8	1	1	1	4				3	1	13		1	1		4	22
0.03	4	5	1		3					1	2	1	2			1	3	3	10
0.06		1	1								2								2
0.12																			
0.25																			
0.5																			
1																			
2																			
4																			
8																			
16																			
>16																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth	1																2		2
Min MIC	0.004	0.002	0.004	0.004	0.004	0.008	0.002	0.004	0.008	0.004	0.004	0.004	0.008	0.004	0.008	0.004	0.004	0.004	0.002
Max MIC	0.03	0.06	0.06	0.015	0.03	0.015	0.015	0.008	0.008	0.03	0.06	0.03	0.03	0.008	0.015	0.030	0.03	0.03	0.06
MIC <sub>mode</sub>	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.004	0.008	0.008	0.008	0.008	0.008	0.004	0.008	0.008	0.008



# Cefquinome – APP CO<sub>2</sub>

Figure 9. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Cefquinome Broth Microdilution - CO<sub>2</sub> incubation



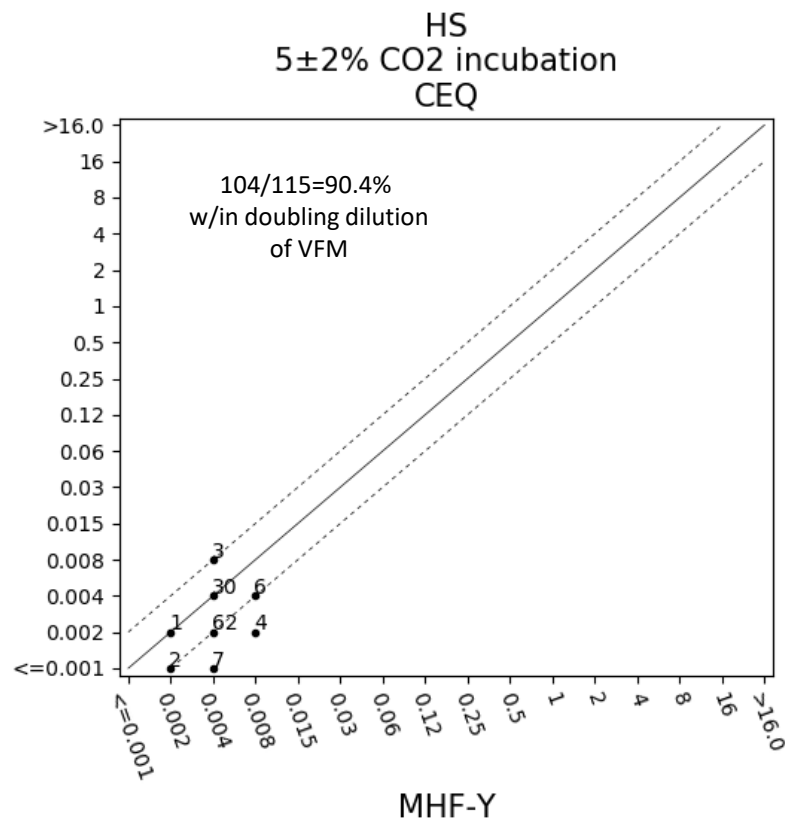
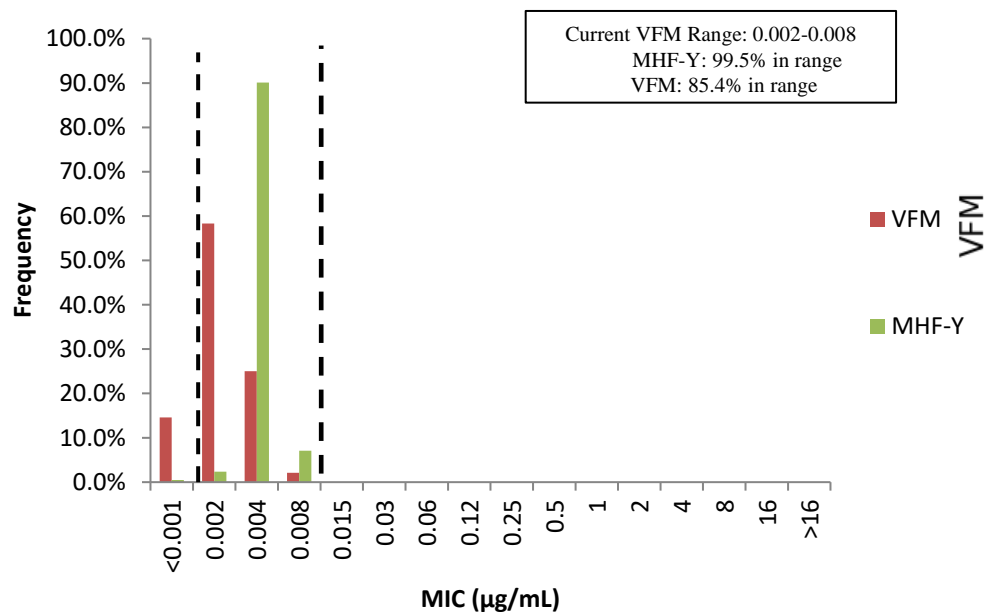
# Cefquinome – HS CO<sub>2</sub>

Table 11. *Histophilus somni* (ATCC 700025) vs Cefquinome Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.001			1	4				1		2	1							7	1
0.002	1	4		6	4	7		9		2		2	1	2				28	5
0.004	67	62	62		29	3	27		30		28	1	27	8	24		26	12	191
0.008	3	4	8				3				1	1	1		6		4	1	15
0.015																			
0.03																			
0.06																			
0.12																			
0.25																			
0.5																			
1																			
2																			
4																			
8																			
16																			
>16																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		1		1						6		6	1			10		23	1
Min MIC	0.002	0.002	≤0.001	≤0.001	0.002	0.002	0.004	≤0.001	0.004	≤0.001	≤0.001	0.002	0.002	0.002	0.004		0.004	≤0.001	≤0.001
Max MIC	0.008	0.008	0.008	0.002	0.004	0.004	0.008	0.002	0.004	0.002	0.008	0.008	0.008	0.004	0.008		0.008	0.008	0.008
MIC <sub>mode</sub>	0.004	0.004	0.004	0.002	0.004	0.002	0.004	0.002	0.004	0.002	0.004	0.002	0.004	0.004	0.008		0.004	0.002	0.004

# Cefquinome – HS CO<sub>2</sub>

Figure 11. *Histophilus somni* (ATCC 700025) vs Cefquinome Broth  
Microdilution - CO<sub>2</sub> incubation



# Cefquinome – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Cefquinome	30 µg	33–44	31–41	0.002–0.008	0.004–0.03

Keep Current Range for HS and APP

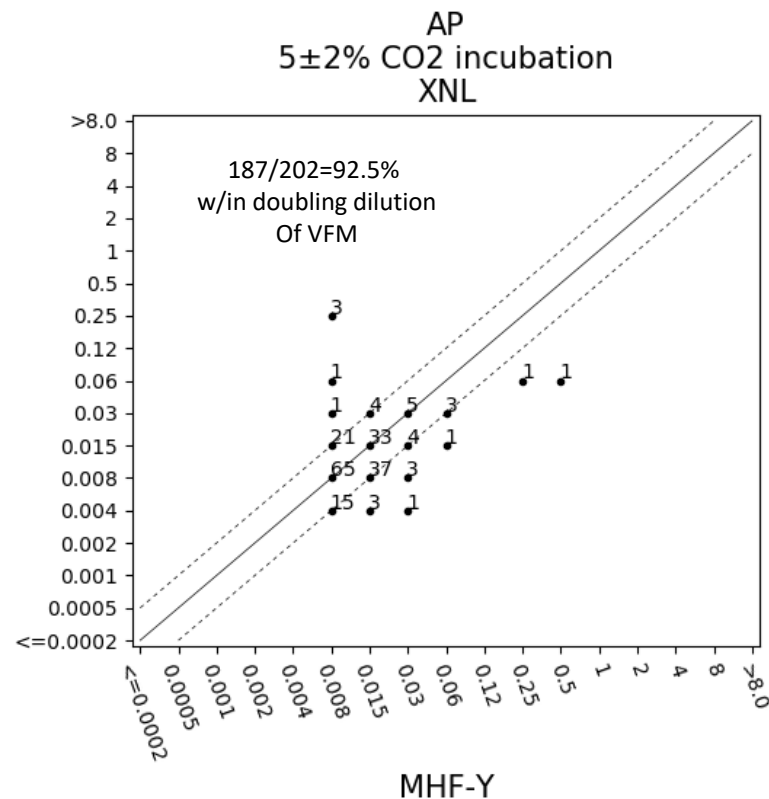
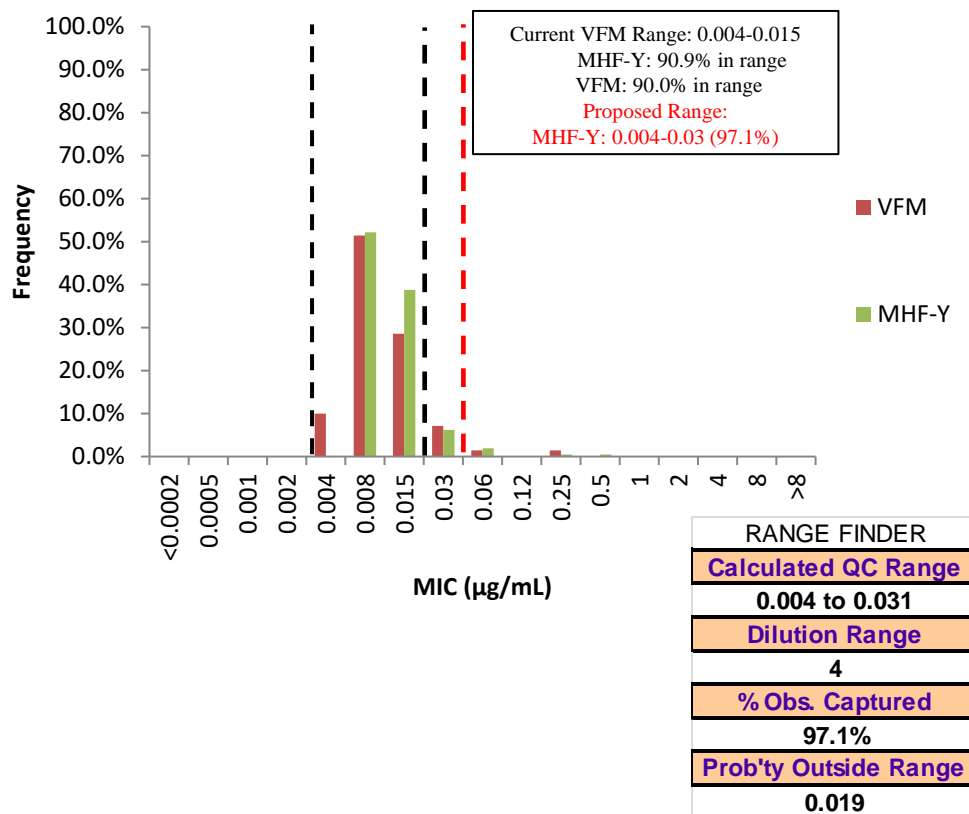
# Ceftiofur – APP CO<sub>2</sub>

Table 13. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Ceftiofur Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.0002																			
0.0005																			
0.001																			
0.002																			
0.004				1				1						1		4		7	
0.008	39	48	22	5	15	3	10	4	15	8	20	4	3	9	24	3	22	36	109
0.015	26	16	39	3	10	6	18	5	15	1	7	5	22		6		3	20	81
0.03	5	3	5	1	4	1	2			1	1	1	4			1	2	5	13
0.06		1	3		1						2	1	1			1		1	4
0.12																			
0.25			1													1	1	1	1
0.5		1															1		1
1																			
2																			
4																			
8																			
>8																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth		1															1		1
Min MIC	0.008	0.008	0.008	0.004	0.008	0.008	0.008	0.004	0.008	0.008	0.008	0.008	0.008	0.004	0.008	0.004	0.008	0.004	0.008
Max MIC	0.03	0.5	0.25	0.03	0.06	0.03	0.03	0.015	0.015	0.03	0.06	0.03	0.06	0.008	0.015	0.25	0.5	0.25	0.5
MIC <sub>mode</sub>	0.008	0.008	0.015	0.008	0.008	0.015	0.015	0.015	0.015	0.008	0.008	0.015	0.015	0.008	0.008	0.004	0.008	0.008	0.008

# Ceftiofur – APP CO<sub>2</sub>

Figure 13. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Ceftiofur  
Broth Microdilution - CO<sub>2</sub> incubation





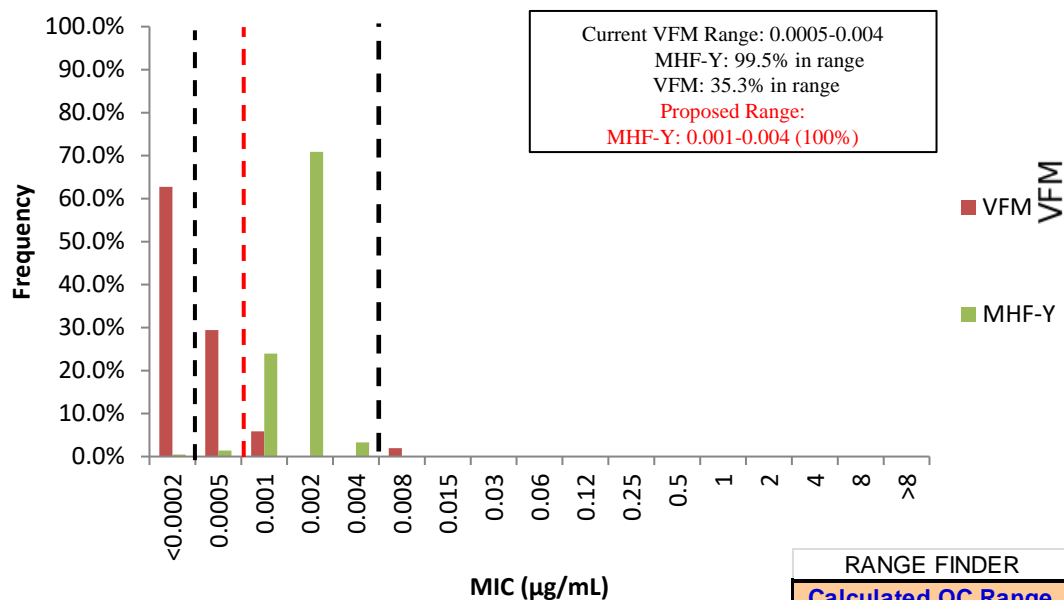
# Ceftiofur – HS CO<sub>2</sub>

Table 15. *Histophilus somni* (ATCC 700025) vs Ceftiofur Broth Microdilution - CO2 incubation

MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.0002	1			5		2		10		5		5		5			1	32	1
0.0005		2	1	5	2	7							1	3				15	3
0.001	20	16	15	1	7		4			24		15	2			1		3	51
0.002	49	50	52		24		25		29	6		13		27		27			151
0.004	1	3	3				1		1			1		3		1			7
0.008						1												1	
0.015																			
0.03																			
0.06																			
0.12																			
0.25																			
0.5																			
1																			
2																			
4																			
8																			
>8																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	≤0.0002	0.0005	0.0005	≤0.0002	0.0005	≤0.0002	0.001	≤0.0002	0.002	≤0.0002	0.001	≤0.0002	0.0005	≤0.0002	0.002	0	≤0.0002	≤0.0002	≤0.0002
Max MIC	0.004	0.004	0.004	0.001	0.002	0.008	0.004	≤0.0002	0.004	≤0.0002	0.002	≤0.0002	0.004	0.001	0.004	0	0.004	0.001	0.004
MIC <sub>mode</sub>	0.002	0.002	0.002	0.0005	0.002	0.0005	0.002	≤0.0002	0.002	≤0.0002	0.001	≤0.0002	0.001	≤0.0002	0.002	0	0.002	≤0.0002	0.002

# Ceftiofur – HS CO<sub>2</sub>

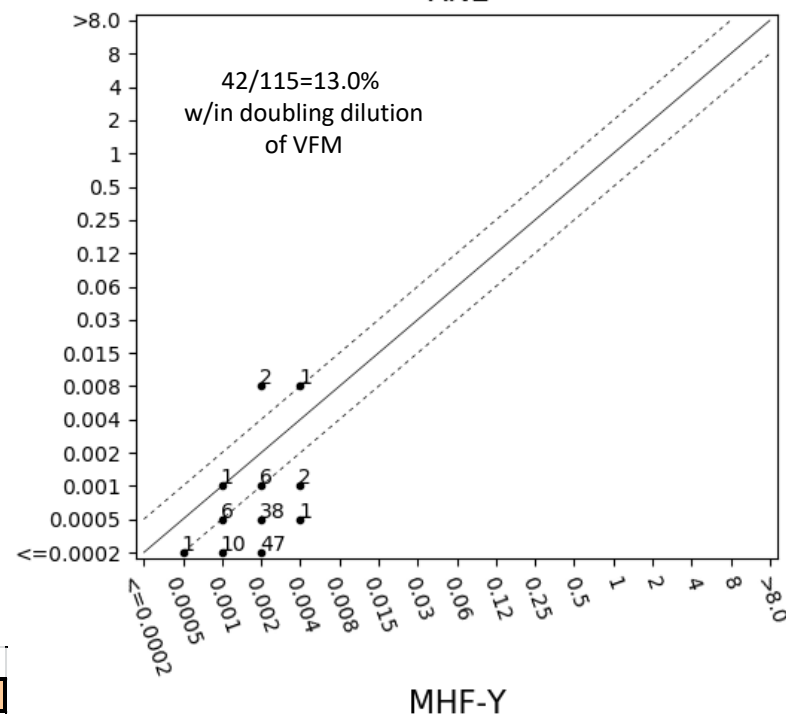
Figure 15. *Histophilus somni* (ATCC 700025) vs Ceftiofur Broth Microdilution - CO<sub>2</sub> incubation



Range Finder doesn't go below 0.001

RANGE FINDER
Calculated QC Range
0.001 to 0.004
Dilution Range
3
% Obs. Captured
100.0%
Prob'ty Outside Range
0.004

HS  
5±2% CO<sub>2</sub> incubation  
XNL



# Ceftiofur – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Ceftiofur	30 µg	36–46	34–42	0.001-0.004	0.004-0.03

Adjust Range for HS and APP

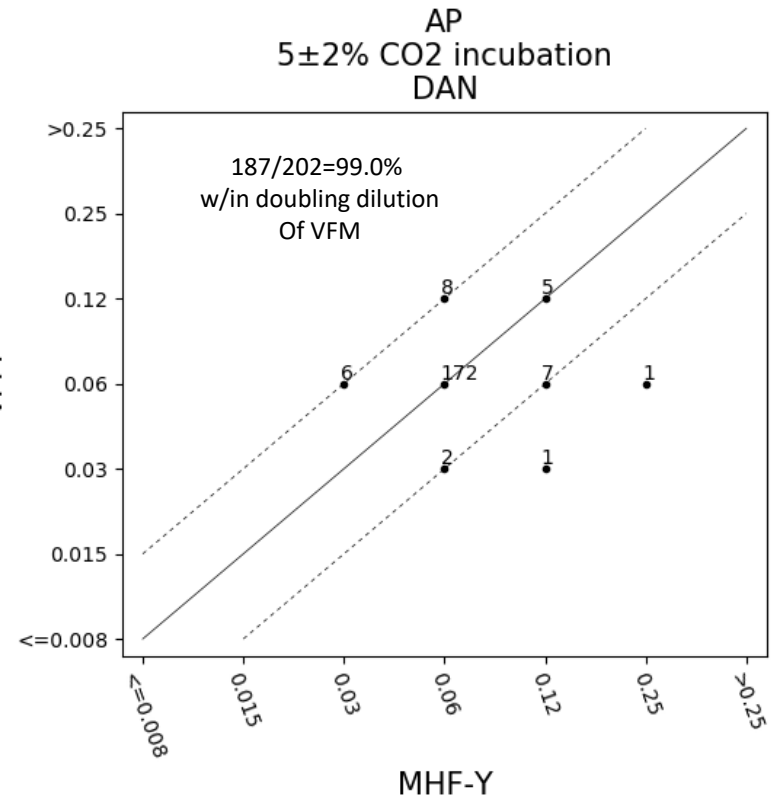
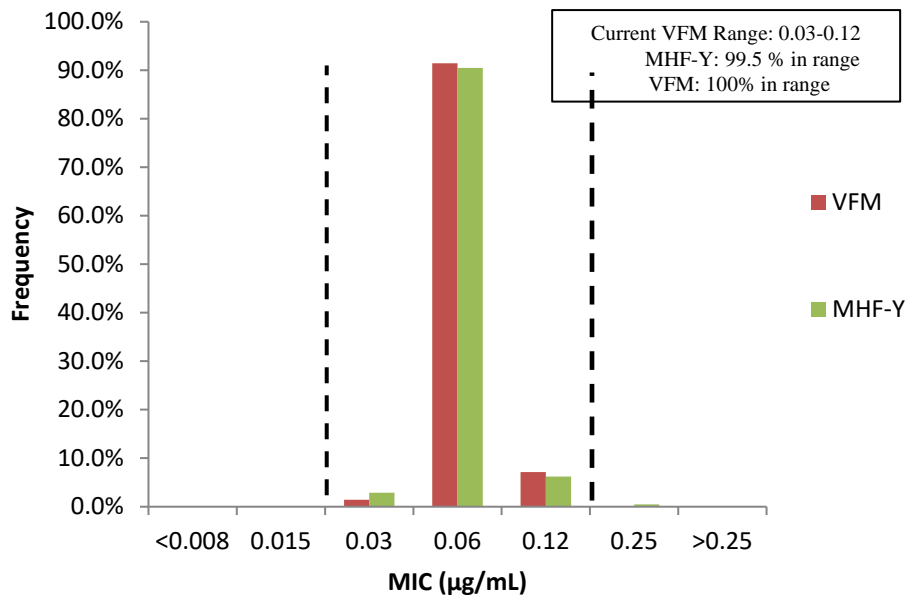
# Danofloxacin – APP CO<sub>2</sub>

Table 17. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Danofloxacin Broth Microdilution - CO2 incubation

[illegible]

# Danofloxacin – APP CO<sub>2</sub>

Figure 17. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs  
Danofloxacin Broth Microdilution - CO<sub>2</sub> incubation



# Danofloxacin – HS CO<sub>2</sub>

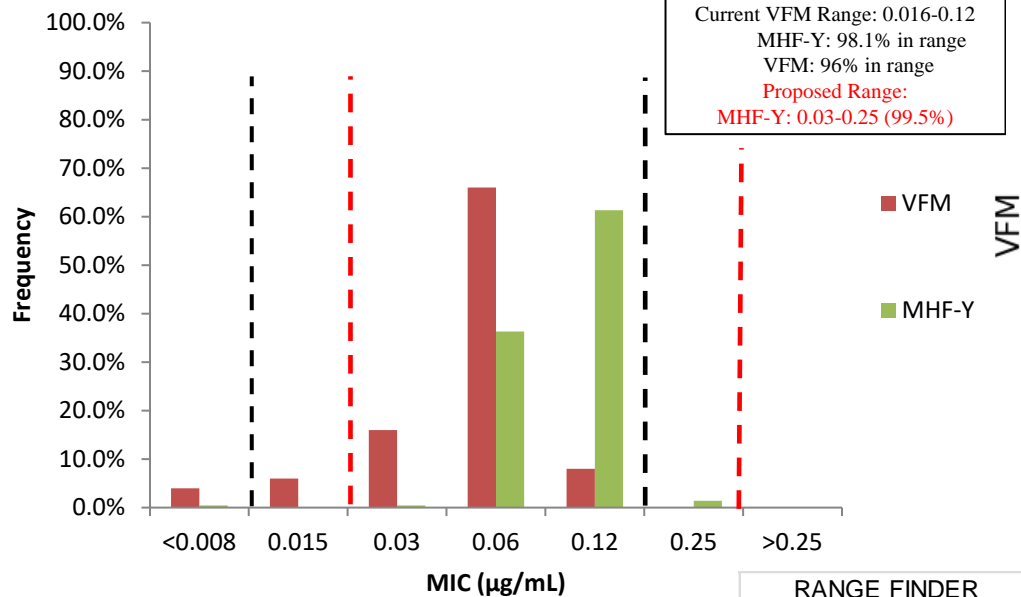
Table 19. *Histophilus somni* (ATCC 700025) vs Danofloxacin Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.008	0	0	1	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2	1
0.015	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
0.03	0	0	1	4	0	0	0	4	0	0	1	0	0	0	0	0	0	8	1
0.06	47	3	27	3	9	10	18	6	1	2	12	5	19	7	10	0	8	33	77
0.12	24	67	39	0	23	0	12	0	28	0	16	1	10	3	20	0	21	4	130
0.25	0	0	3	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	3
>0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		1		1						6		4	1			10		21	1
Min MIC	0.06	0.06	≤0.008	0.015	0.06	0.06	0.06	0.03	0.06	≤0.008	≤0.008	0.06	0.06	0.06	0.06	0	0.06	≤0.008	≤0.008
Max MIC	0.12	0.12	0.25	0.25	0.25	0.06	0.12	0.06	0.25	0.06	0.12	0.12	0.12	0.12	0.12	0	0.25	0.12	0.25
MIC <sub>mode</sub>	0.06	0.12	0.12	0.03	0.12	0.06	0.06	0.06	0.12	0.06	0.12	0.06	0.06	0.06	0.12	0	0.12	0.06	0.12

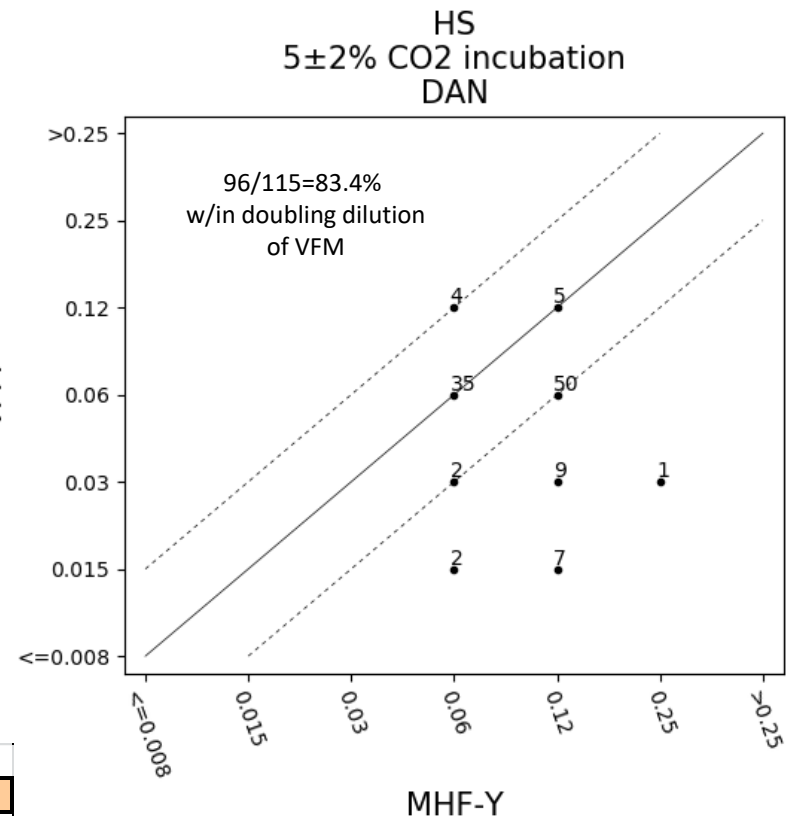


# Danofloxacin – HS CO<sub>2</sub>

Figure 19. *Histophilus somni* (ATCC 700025) vs Danofloxacin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
0.031 to 0.25
Dilution Range
4
% Obs. Captured
99.5%
Prob'ty Outside Range
0.001



# Danofloxacin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Danofloxacin	5 µg	26–36	27–36	0.03-0.25	0.03–0.12

Keep Current Range for APP and modify HS range

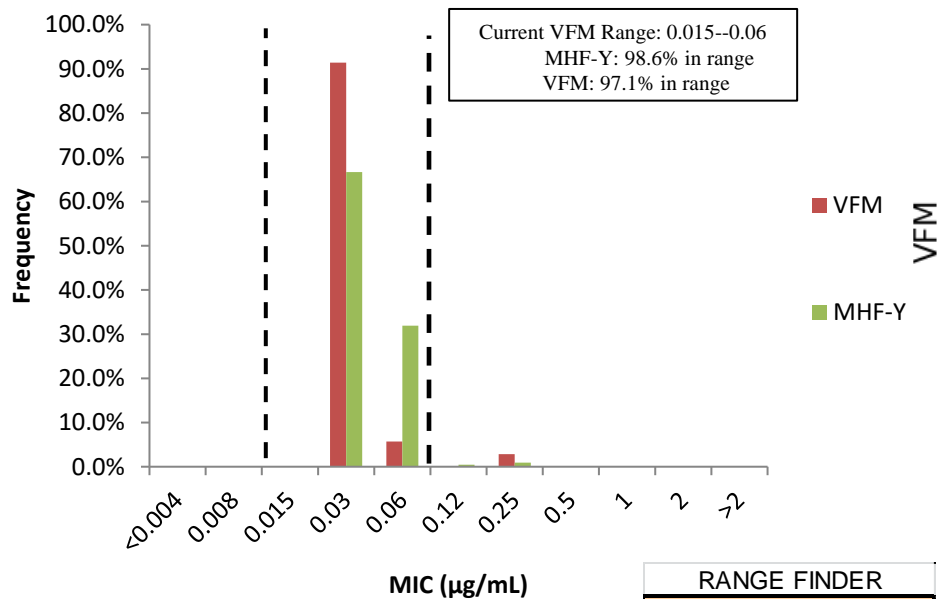
# Enrofloxacin – APP CO<sub>2</sub>

Table 21. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Enrofloxacin Broth Microdilution - CO<sub>2</sub> incubation

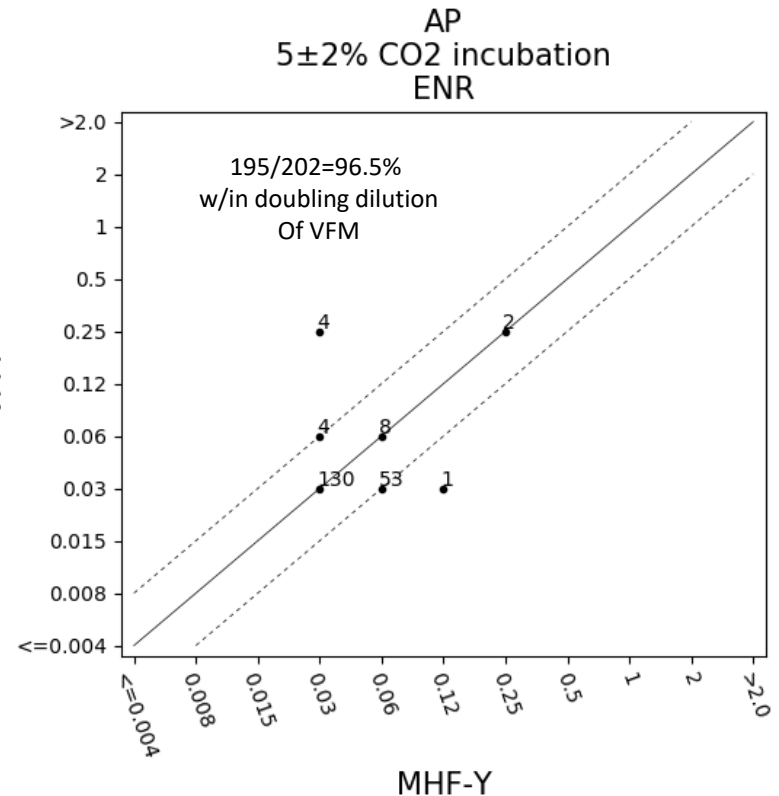
MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.004																			
0.008																			
0.015																			
0.03	64	35	41	10	20	9	14	10	18	10	28	8	13	9	27	8	20	64	140
0.06	6	33	28		9	1	16		12		2	2	17	1	3		8	4	67
0.12		1			1														1
0.25		1	1													2	2	2	2
0.5																			
1																			
2																			
>2																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Max MIC	0.06	0.25	0.25	0.03	0.12	0.06	0.06	0.03	0.06	0.03	0.06	0.06	0.06	0.06	0.06	0.25	0.25	0.25	0.25
MIC <sub>mode</sub>	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.25	0.03

# Enrofloxacin – APP CO<sub>2</sub>

Figure 21. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs  
Enrofloxacin Broth Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
0.016 to 0.063
Dilution Range
3
% Obs. Captured
98.6%
Prob'ty Outside Range
0.018



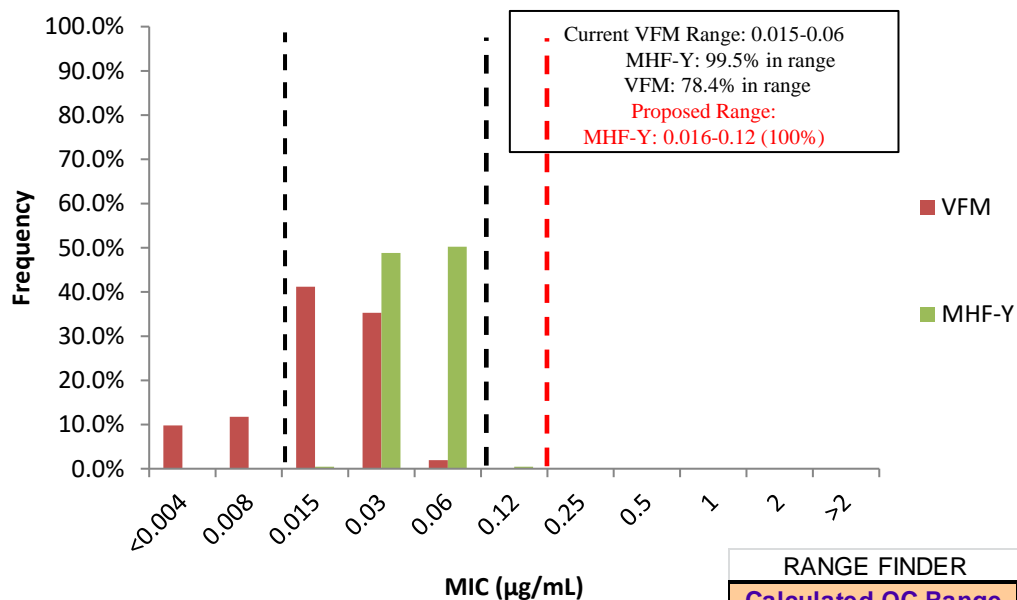
# Enrofloxacin – HS CO<sub>2</sub>

Table 23. *Histophilus somni* (ATCC 700025) vs Enrofloxacin Broth Microdilution - CO<sub>2</sub> incubation

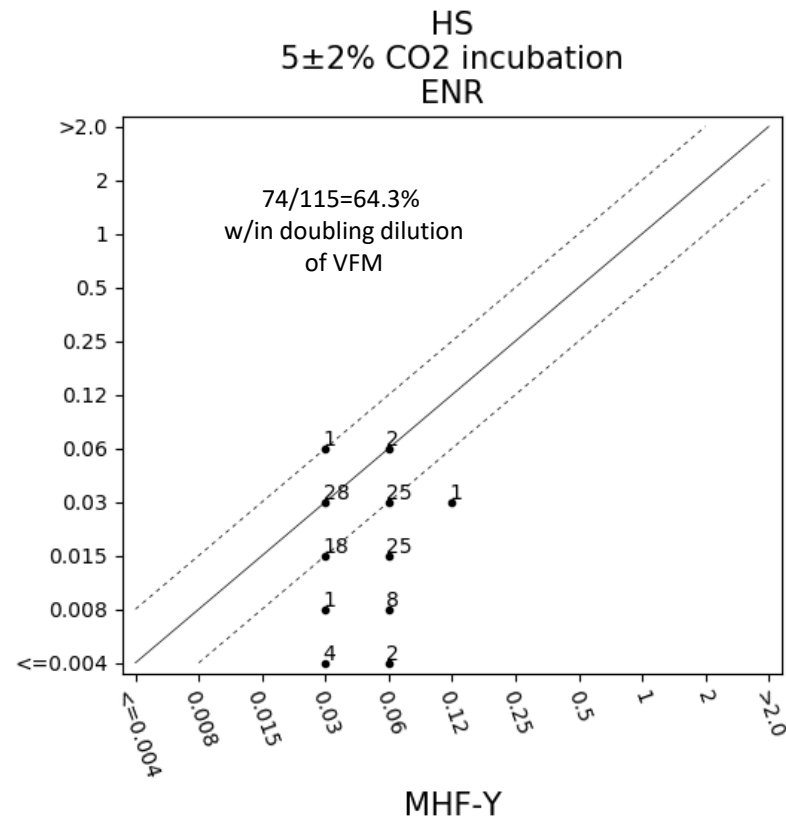
MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.004				1				1		3								5	
0.008				3				3										6	
0.015	1			7		5		6				3				1		21	1
0.03	59	5	40		12	5	21		9	2	20	2	18	9	11		13	18	104
0.06	11	65	31		21		9		21		10		12	1	18		16	1	107
0.12		1													1				1
0.25																			
0.5																			
1																			
2																			
>2																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	0.015	0.03	0.03	≤0.004	0.03	0.015	0.03	≤0.004	0.03	≤0.004	0.03	0.015	0.03	0.03	0.03	0	0.015	≤0.004	0.015
Max MIC	0.06	0.12	0.06	0.015	0.06	0.03	0.06	0.015	0.06	0.03	0.06	0.03	0.06	0.06	0.12	0	0.06	0.06	0.12
MIC <sub>mode</sub>	0.03	0.06	0.03	0.015	0.06	0.06	0.03	0.015	0.06	≤0.004	0.03	0.015	0.03	0.03	0.06	0	0.06	0.015	0.06

# Enrofloxacin – HS CO<sub>2</sub>

Figure 23. *Histophilus somni* (ATCC 700025) vs Enrofloxacin Broth Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
0.016 to 0.125
Dilution Range
4
% Obs. Captured
100.0%
Prob'ty Outside Range
0.000





# Enrofloxacin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Enrofloxacin	5 µg	32–38	31–38	0.016-0.12	0.016–0.06

Keep Current Range for APP and adjust HS

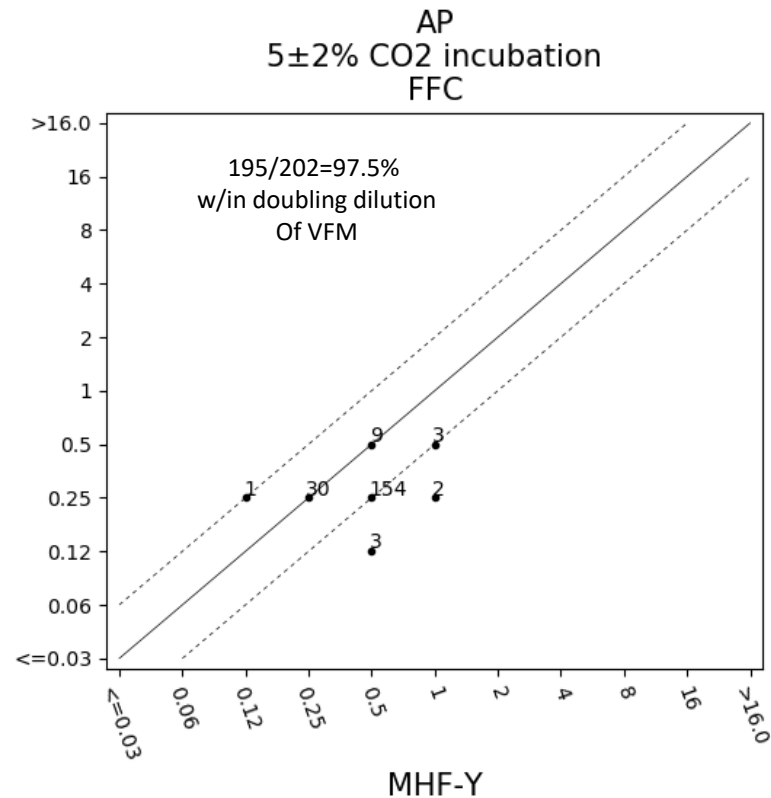
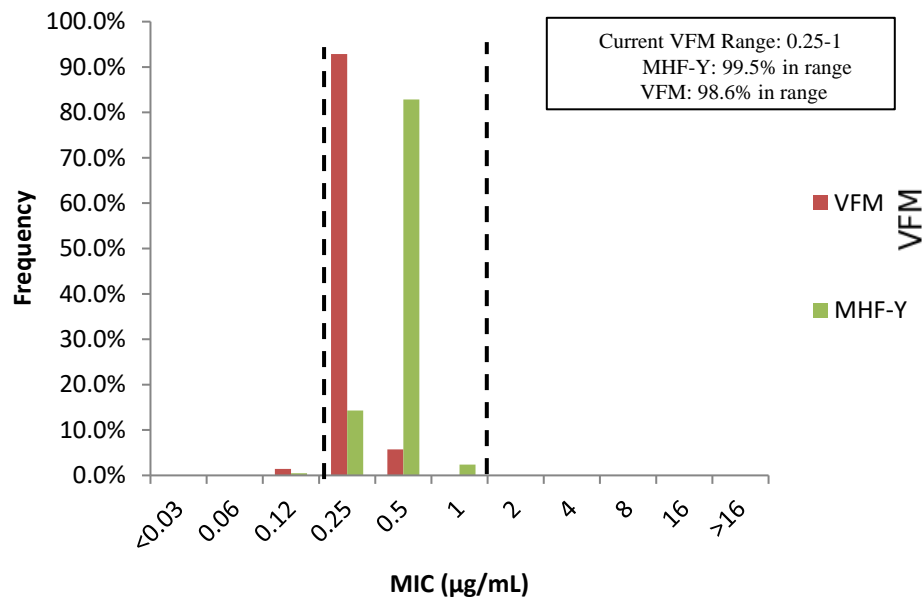
# Florfenicol – APP CO<sub>2</sub>

Table 25. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Florfenicol Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.03																			
0.06																			
0.12		1									1	1						1	1
0.25	10	17	3	10	1	9	1	10		10	20	9	2	9	2	8	4	65	30
0.5	59	50	65		29	1	26		30		9		28	1	28	2	24	4	174
1	1	2	2				3										2		5
2																			
4																			
8																			
16																			
>16																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	0.25	0.12	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.12	0.12	0.25	0.25	0.25	0.25	0.25	0.12	0.12
Max MIC	1	1	1	0.25	0.5	0.5	1	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.5	1	0.5	1
MIC <sub>mode</sub>	0.5	0.5	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.25	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5

# Florfenicol – APP CO<sub>2</sub>

Figure 25. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs  
Florfenicol Broth Microdilution - CO<sub>2</sub> incubation



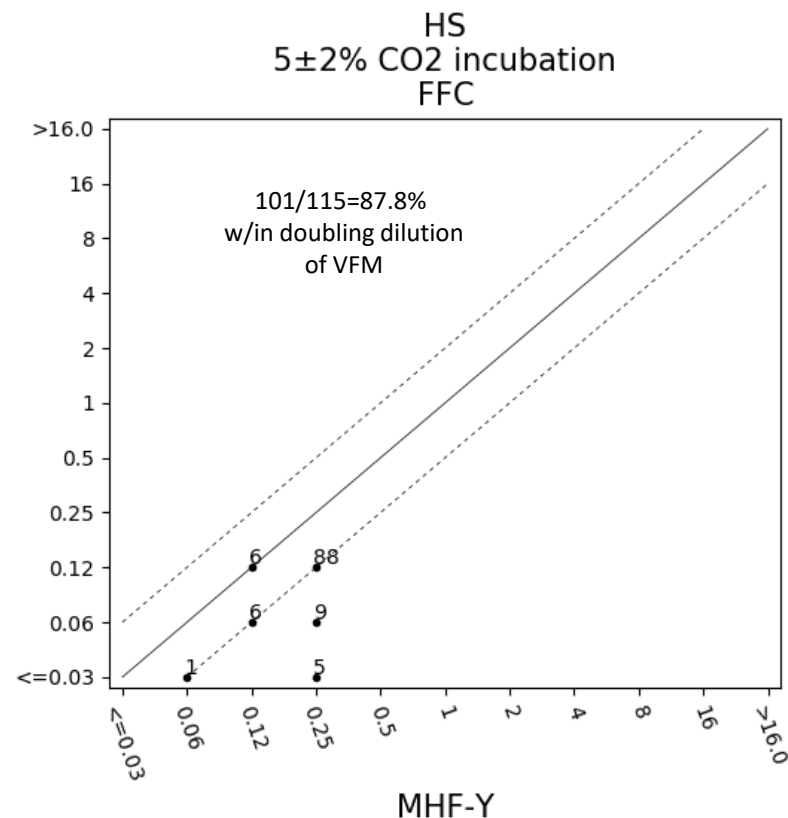
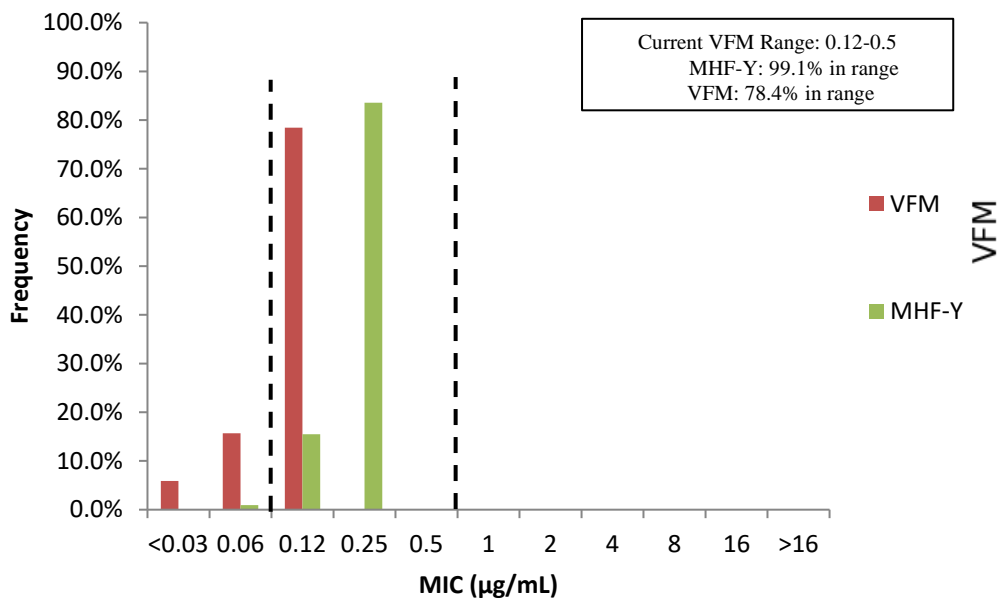
# Florfenicol – HS CO<sub>2</sub>

Table 27. *Histophilus somni* (ATCC 700025) vs Florfenicol Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.03				2						1								3	
0.06	1	1		3	2			2		3								8	2
0.12	14	12	7	6	4	10	1	8	2	1	25	5		10			1	40	33
0.25	56	58	64		27		29		28		5		30		30		29		178
0.5																			
1																			
2																			
4																			
8																			
16																			
>16																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	0.06	0.06	0.12	≤0.03	0.06	0.12	0.12	0.06	0.06	≤0.03	0.12	0.12	0.25	0.12	0.25		0.12	≤0.03	0.06
Max MIC	0.25	0.25	0.25	0.12	0.25	0.12	0.25	0.12	0.25	0.12	0.25	0.12	0.25	0.12	0.25		0.25	0.12	0.25
MIC <sub>mode</sub>	0.25	0.25	0.25	0.12	0.25	0.12	0.25	0.12	0.25	0.06	0.12	0.12	0.25	0.12	0.25		0.25	0.12	0.25

# Florfenicol – HS CO<sub>2</sub>

Figure 27. *Histophilus somni* (ATCC 700025) vs Florfenicol Broth  
Microdilution - CO<sub>2</sub> incubation



# Florfenicol – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Florfenicol	30 µg	34–44	31–40	0.12–0.5	0.25–1

Keep Current Range for HS and APP

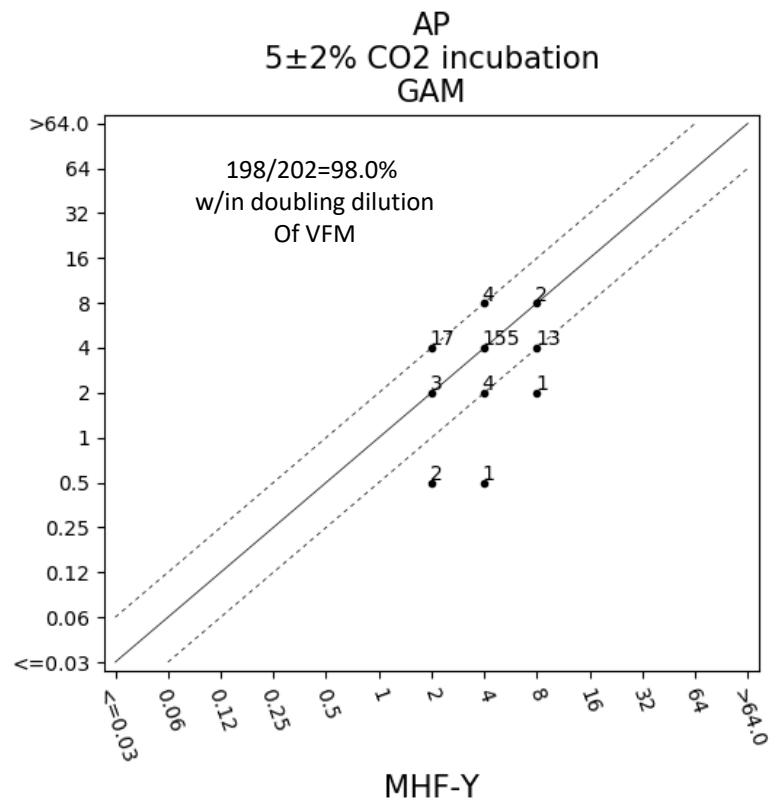
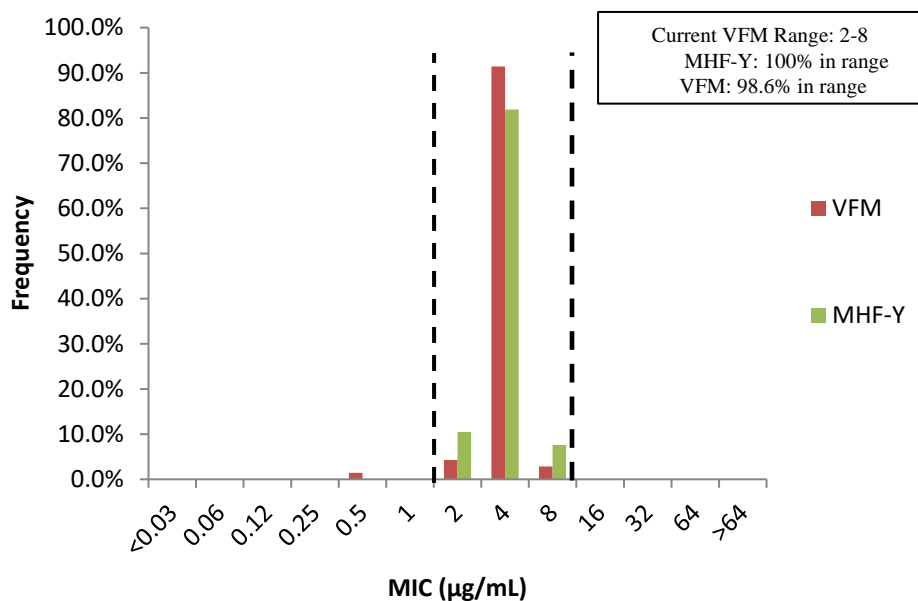


# Gamithromycin – APP CO<sub>2</sub>

Table 29. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Gamithromycin Broth Microdilution - CO2 incubation[illegible]

# Gamithromycin – APP CO<sub>2</sub>

Figure 29. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Gamithromycin Broth Microdilution - CO<sub>2</sub> incubation



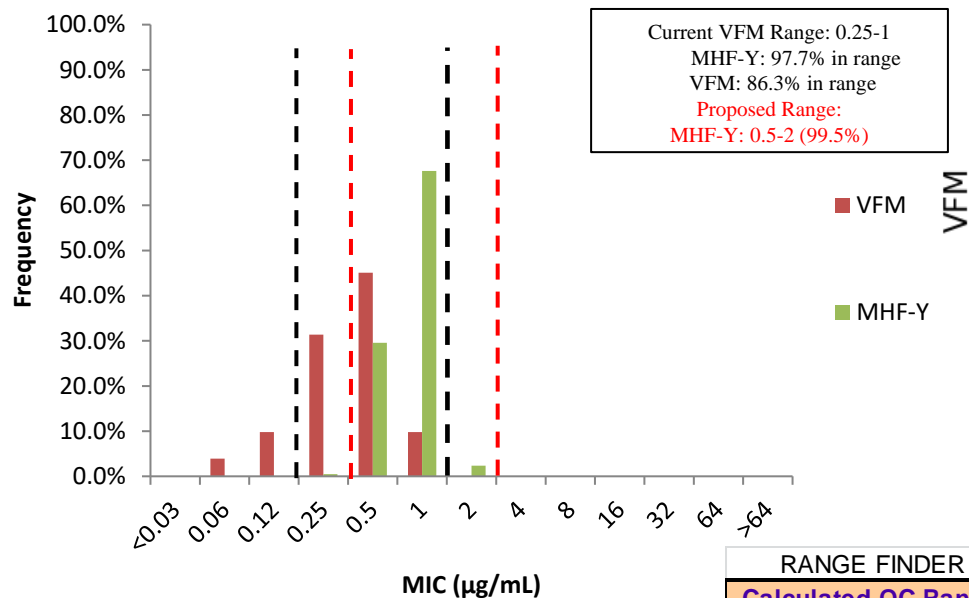
# Gamithromycin – HS CO<sub>2</sub>

Table 31. *Histophilus somni* (ATCC 700025) vs Gamithromycin Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.03																			
0.06				2														2	
0.12				2				1		2								5	
0.25	1			6				5		2				3			1	16	1
0.5	39	12	12	1	6	7	1	4	10	1	18	4	2	6	11		15	23	63
1	31	59	54		27	3	28		20		12	1	25	1	18		14	5	144
2			5				1						3		1				5
4																			
8																			
16																			
32																			
64																			
>64																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	0.25	0.5	0.5	0.06	0.5	0.5	0.5	0.12	0.5	0.12	0.5	0.5	0.5	0.25	0.5	0	0.25	0.06	0.25
Max MIC	1	1	2	0.5	1	1	2	0.5	1	0.5	1	1	2	1	2	0	1	1	2
MIC <sub>mode</sub>	0.5	1	1	0.25	1	0.5	1	0.25	1	0.25	0.5	0.5	1	0.5	1	0	0.5	0.5	1

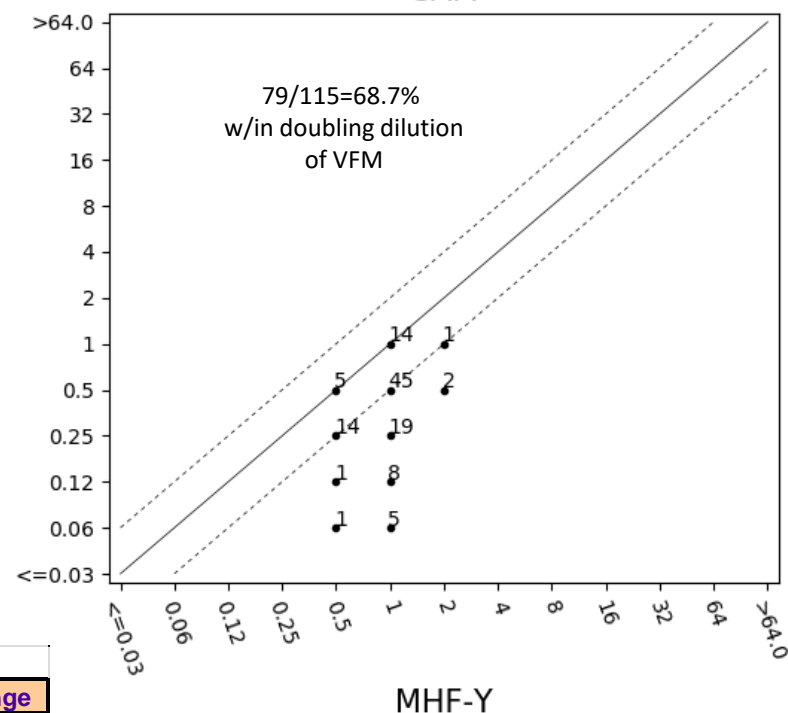
# Gamithromycin – HS CO<sub>2</sub>

Figure 31. *Histophilus somni* (ATCC 700025) vs Gamithromycin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGE FINDER	
Calculated QC Range	
0.5 to 2	
Dilution Range	
3	
% Obs. Captured	
99.5%	
Prob'ty Outside Range	
0.009	

HS  
5±2% CO<sub>2</sub> incubation  
GAM



# Gamithromycin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Gamithromycin	15 µg	18–29	14–19	0.5-2	2–8

Keep Current Range for APP or adjust HS

# Gentamicin – APP CO<sub>2</sub>

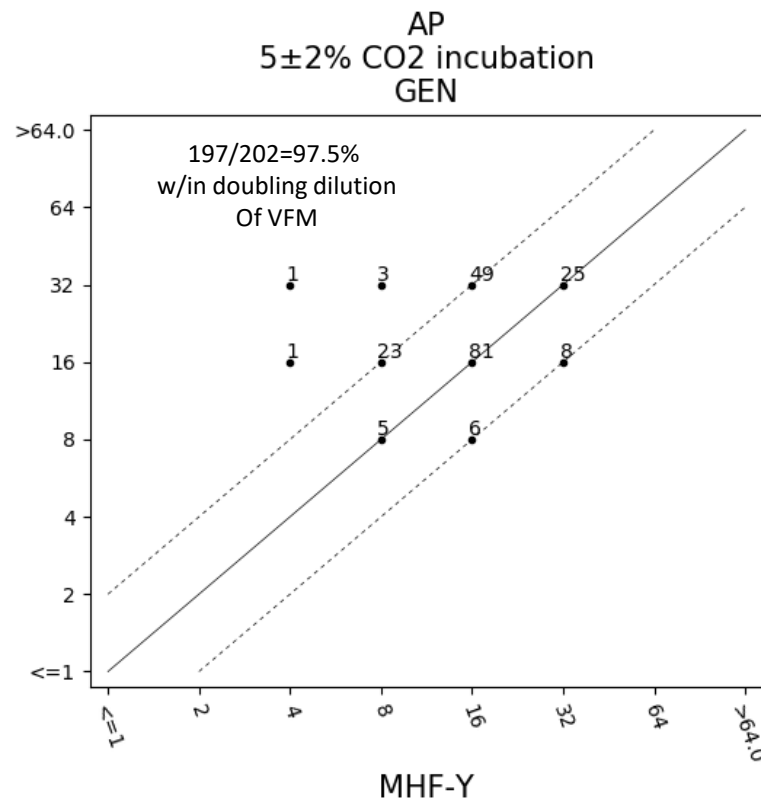
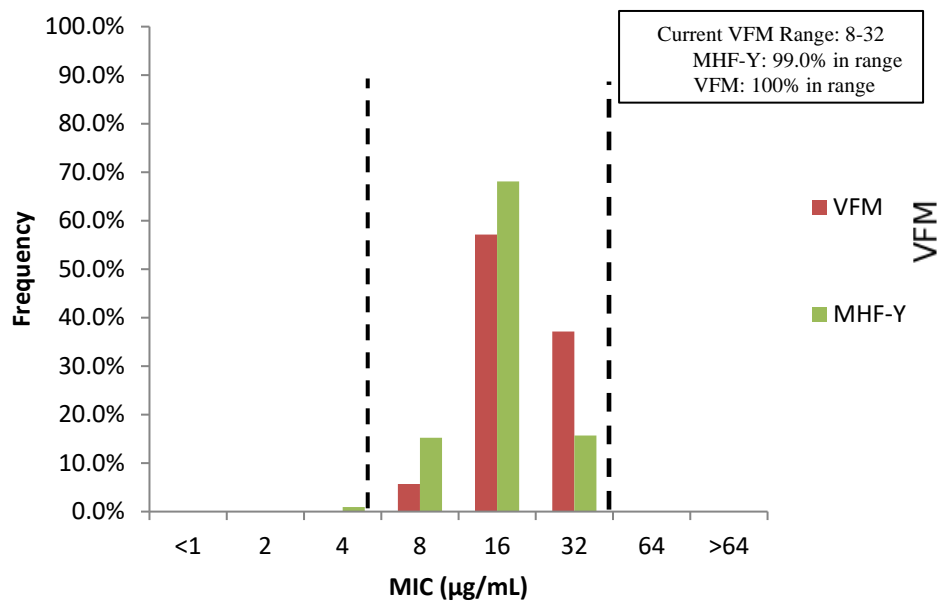
Table 33. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Gentamicin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤1																			
2																			
4	2									1				1				2	
8	16	16			1		7		3	1	3					3	18	4	32
16	46	51	46	7	21	5	18	10	27	2	22		15	9	28	7	12	40	143
32	6	3	24	3	8	5	5			7	4	10	15	1	1			26	33
64																			
>64																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	4	8	16	16	8	16	8	16	8	8	4	32	16	16	4	8	8	8	4
Max MIC	32	32	32	32	32	32	32	16	16	32	32	32	32	32	32	16	16	32	32
MIC <sub>mode</sub>	16	16	16	16	16	16	16	16	16	32	16	32	16	16	16	16	8	16	16



# Gentamicin – APP CO<sub>2</sub>

Figure 33. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs  
Gentamicin Broth Microdilution - CO<sub>2</sub> incubation



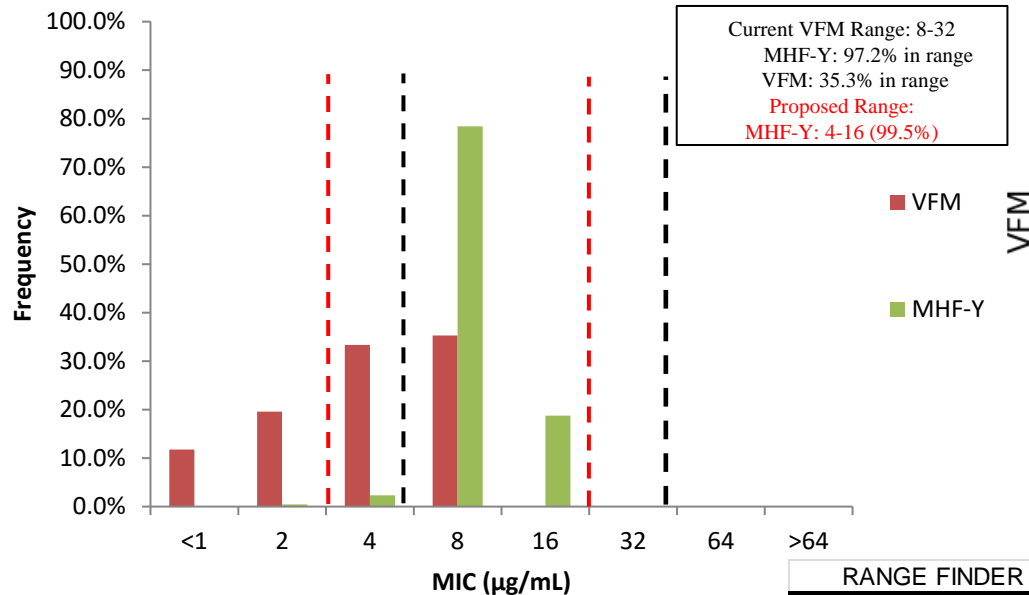
# Gentamicin – HS CO<sub>2</sub>

Table 35. *Histophilus somni* (ATCC 700025) vs Gentamicin Broth Microdilution - CO2 incubation

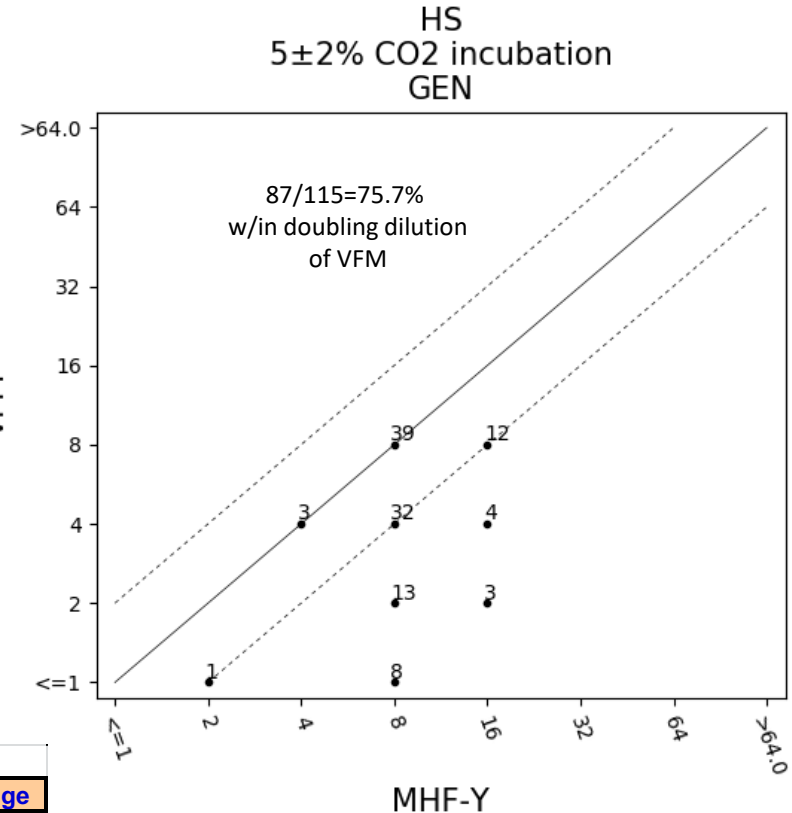
MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤1				4						2								6	
2	1			4	1			4		1		1						10	1
4	1	1	3	3	1	5	2	6		1		2				2		17	5
8	60	52	55		29	5	25		30	1	30	2	2	10	24		27	18	167
16	9	18	13		2		3						28		6		1		40
32																			
64																			
>64																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	2	4	4	≤1	2	4	4	2	8	≤1	8	2	8	8	8	0	4	≤1	2
Max MIC	16	16	16	4	16	8	16	4	8	8	8	8	16	8	16	0	16	8	16
MIC <sub>mode</sub>	8	8	8	4	8	8	8	4	8	≤1	8	8	16	8	8	0	8	8	8

# Gentamicin – HS CO<sub>2</sub>

Figure 35. *Histophilus somni* (ATCC 700025) vs Gentamicin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
4 to 16
Dilution Range
3
% Obs. Captured
99.5%
Prob'ty Outside Range
0.002



# Gentamicin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Gentamicin	10 µg	14–22	15–19	4–16	8–32

Keep Current Range for APP, change range for HS

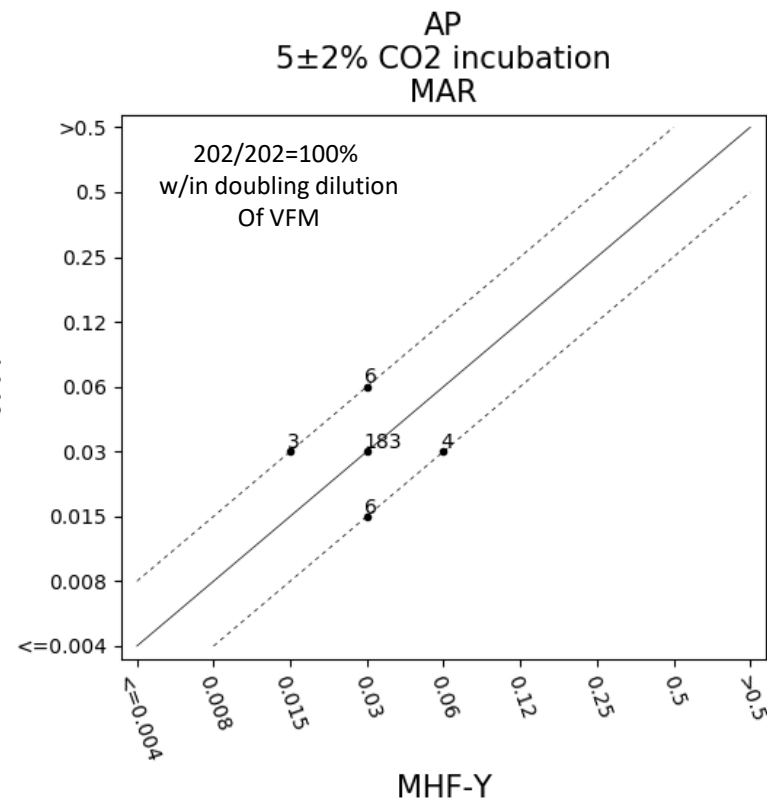
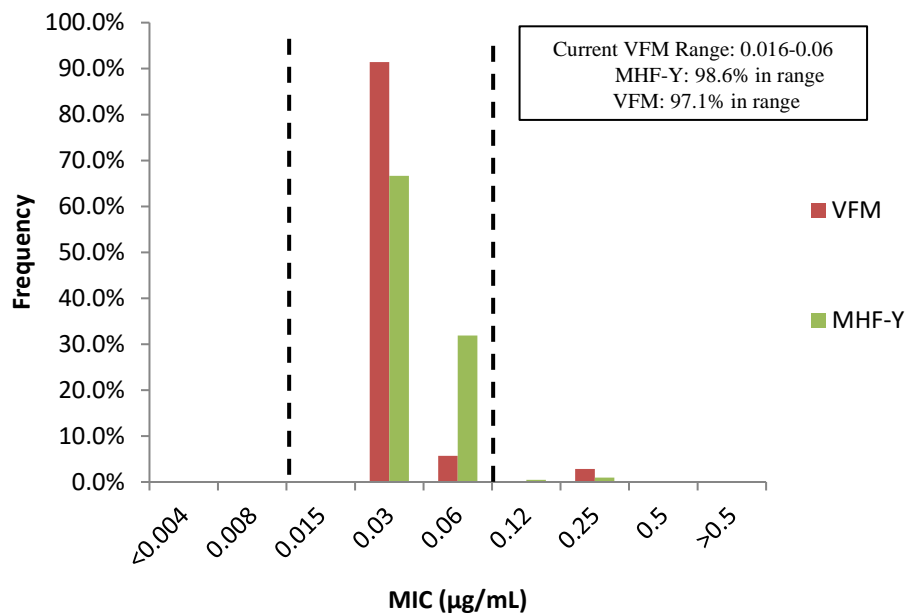
# Marbofloxacin – APP CO<sub>2</sub>

Table 37. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Marbofloxacin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.004																			
0.008																			
0.015																			
0.03	64	35	41	10	20	9	14	10	18	10	28	8	13	9	27	8	20	64	140
0.06	6	33	28		9	1	16		12		2	2	17	1	3		8	4	67
0.12		1			1														1
0.25		1	1													2	2	2	2
0.5																			
>0.5																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Max MIC	0.06	0.25	0.25	0.03	0.12	0.06	0.06	0.03	0.06	0.03	0.06	0.06	0.06	0.06	0.06	0.25	0.25	0.25	0.25
MIC <sub>mode</sub>	0.03	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.03	0.03

# Marbofloxacin – APP CO<sub>2</sub>

Figure 37. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Marbofloxacin Broth Microdilution - CO<sub>2</sub> incubation





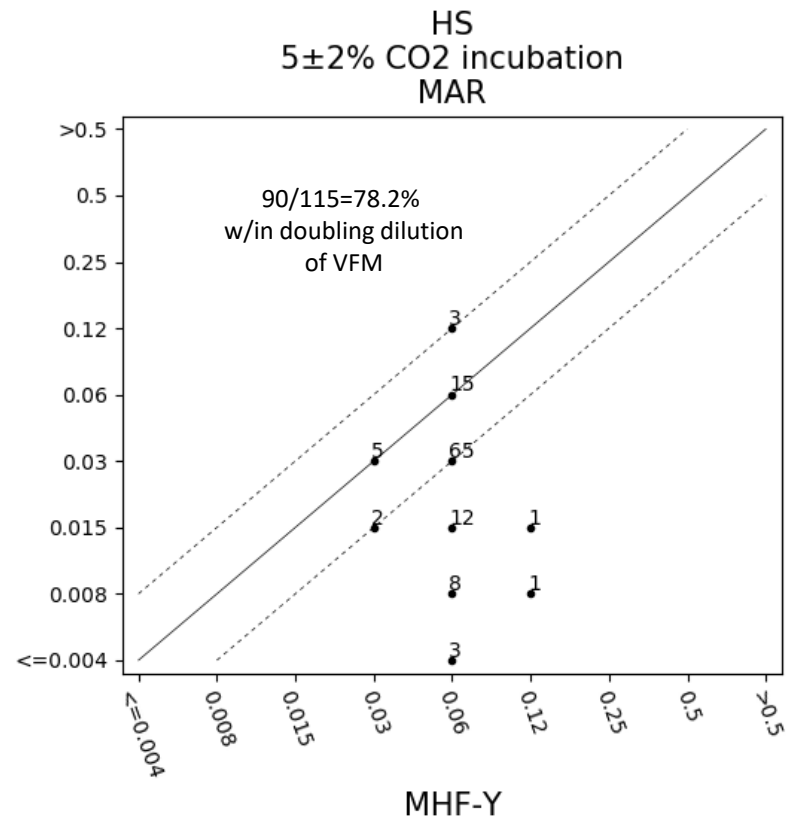
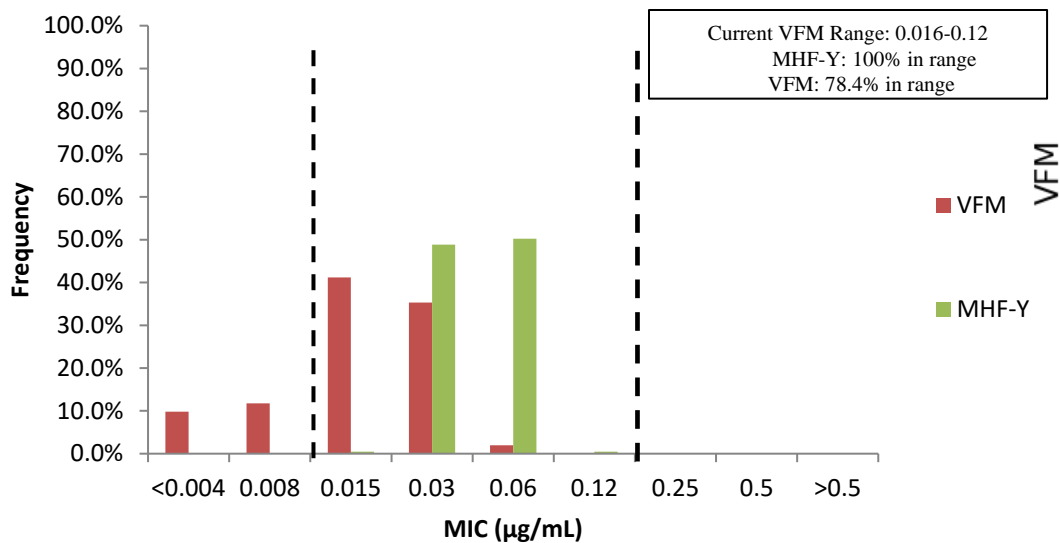
# Marbofloxacin – HS CO<sub>2</sub>

Table 39. *Histophilus somni* (ATCC 700025) vs Marbofloxacin Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.004				1				1		3								5	
0.008				3				3										6	
0.015	1			7		5		6				3					1	21	1
0.03	59	5	40		12	5	21		9	2	20	2	18	9	11		13	18	104
0.06	11	65	31		21		9		21		10		12	1	18		16	1	107
0.12		1													1				1
0.25																			
0.5																			
>0.5																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	0.015	0.03	0.03	≤0.004	0.03	0.015	0.03	≤0.004	0.03	≤0.004	0.03	0.015	0.03	0.03	0.03	0	0.015	≤0.004	0.015
Max MIC	0.06	0.12	0.06	0.015	0.03	0.03	0.06	0.015	0.06	0.03	0.06	0.03	0.06	0.06	0.12	0	0.06	0.06	0.12
MIC <sub>mode</sub>	0.03	0.06	0.03	0.015	0.06	0.03	0.03	0.015	0.06	0.03	0.03	0.015	0.03	0.03	0.06	0	0.06	0.015	0.06

# Marbofloxacin – HS CO<sub>2</sub>

Figure 39. *Histophilus somni* (ATCC 700025) vs Marbofloxacin Broth  
Microdilution - CO<sub>2</sub> incubation



# Marbofloxacin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Marbofloxacin	5 µg	30–41	30–40	0.016–0.12	0.016–0.06

Keep Current Range for HS and APP

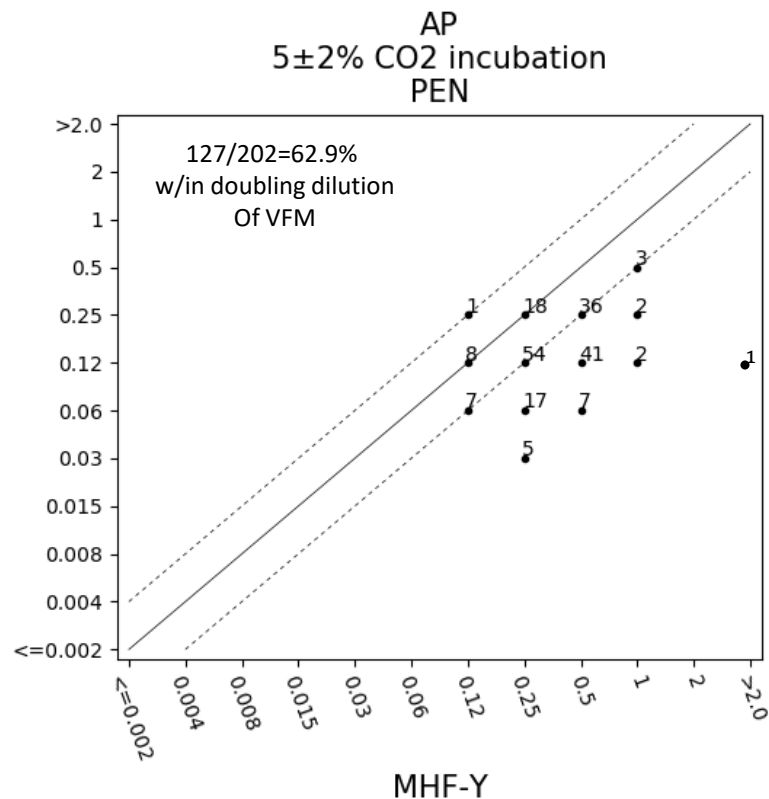
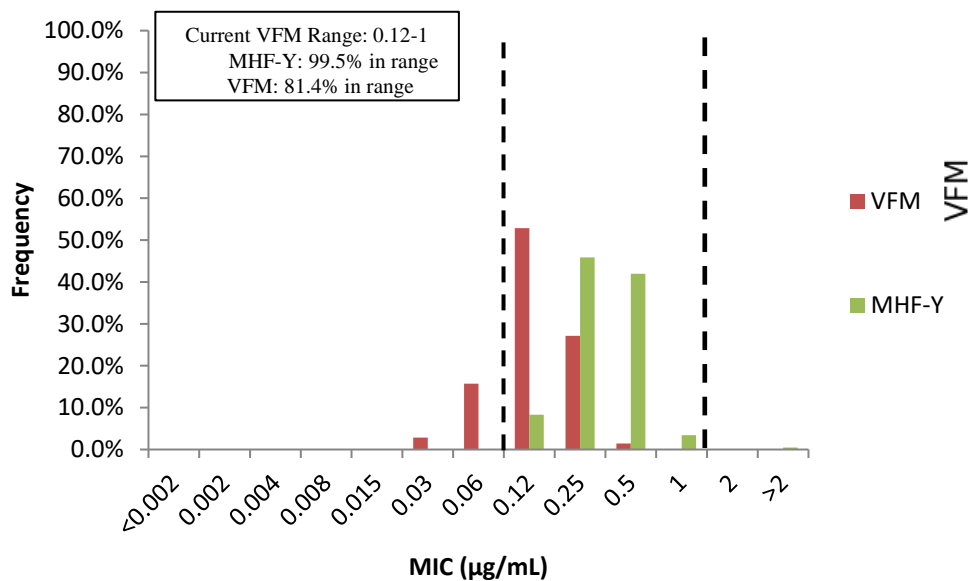
# Penicillin – APP CO<sub>2</sub>

Table 41. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Penicillin Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.002																			
0.002																			
0.004																			
0.008																			
0.015																			
0.03																2		2	
0.06				1				2		1						7		11	
0.12	6	7	4	4	8			8		9	3	7	1	8		1	5	37	17
0.25	27	38	29	5	9	9	9		11		19	3	14	2	12		20	19	94
0.5	33	21	32		13	1	16		14		6		14		18		5	1	86
1	3	1	3				5				2								7
2																			
>2	1											1							1
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth		3	2																5
Min MIC	0.12	0.12	0.12	0.06	0.12	0.25	0.25	0.06	0.25	0.06	0.12	0.12	0.12	0.12	0.25	0.03	0.12	0.03	0.12
Max MIC	>2	1	1	0.25	0.5	0.5	1	0.12	0.5	0.12	1	0.25	>2	0.25	0.5	0.12	0.5	0.5	>2
MIC <sub>mode</sub>	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.12	0.5	0.12	0.25	0.12	0.25	0.12	0.5	0.06	0.25	0.12	0.25

# Penicillin – APP CO<sub>2</sub>

Figure 41. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Penicillin  
Broth Microdilution - CO<sub>2</sub> incubation



# Penicillin – HS CO<sub>2</sub>

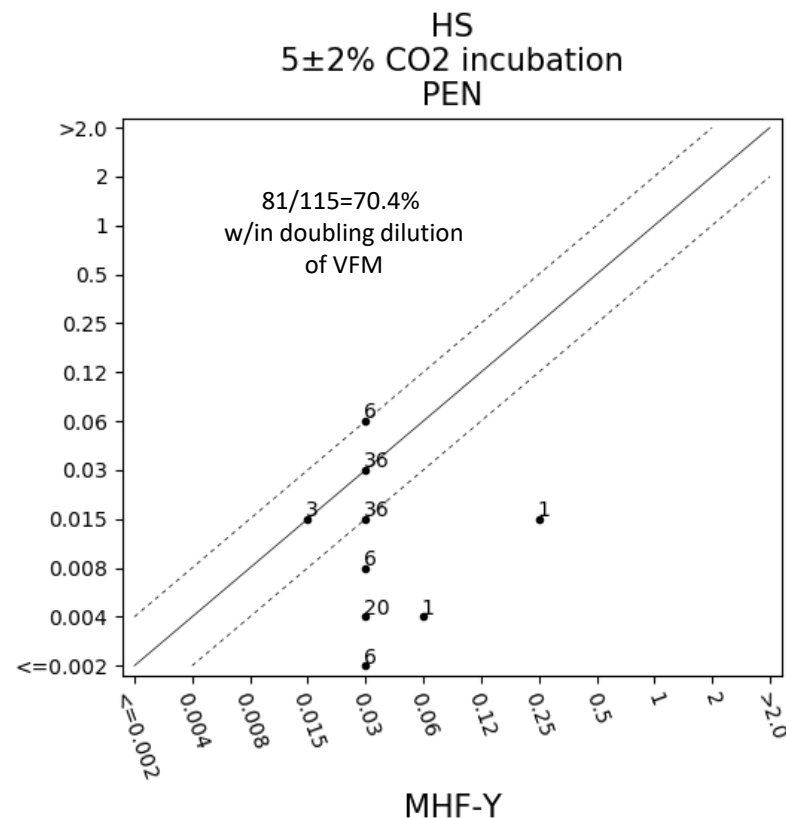
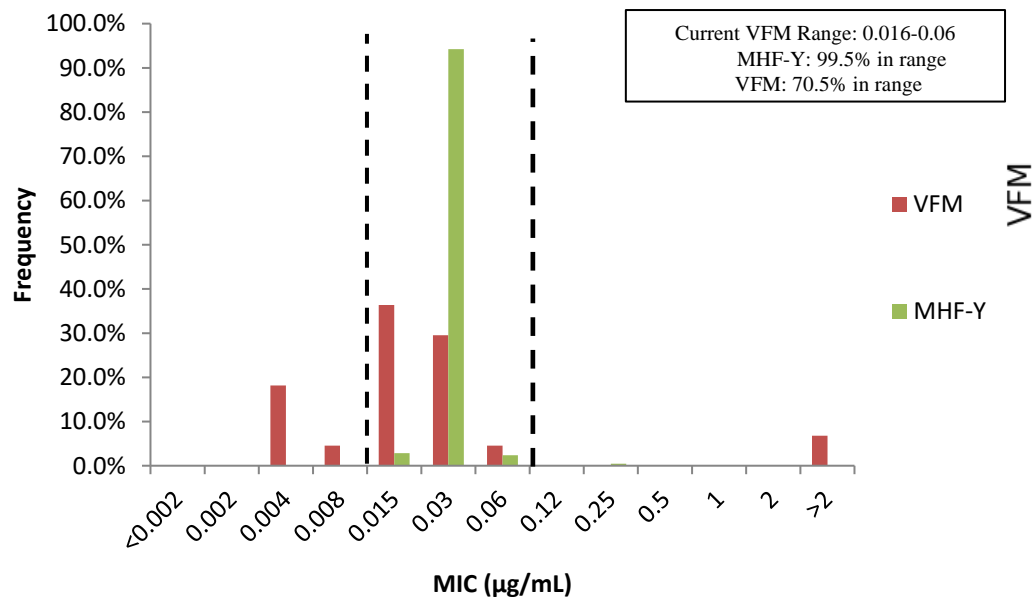
Table 43. *Histophilus somni* (ATCC 700025) vs Penicillin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.002																			
0.002																			
0.004																			
0.008								1		1								2	
0.015	2	3	1	1		6	3	4		3	5							16	6
0.03	67	63	67		33	4	27		26		26	1	28	8	30		27	13	197
0.06	2	1	2								1		1	2			3	2	5
0.12																			
0.25		1										1							1
0.5																			
1																			
2																			
>2				3														3	
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		3	1					5		8		4				10		27	4
Min MIC	0.015	0.015	0.015	0.004	0.03	0.015	0.015	0.008	0.03	0.004	0.015	0.02	0.03	0.03	0.03	0	0.03	0.004	0.015
Max MIC	0.06	0.25	0.06	>2	0.03	0.03	0.03	0.015	0.03	0.008	0.06	0.03	0.25	0.06	0.03	0	0.06	>2	0.25
MIC <sub>mode</sub>	0.03	0.03	0.03	0.004	0.03	0.015	0.03	0.015	0.03	0.004	0.03	0.015	0.03	0.03	0.03	0	0.03	0.015	0.03



# Penicillin – HS CO<sub>2</sub>

Figure 43. *Histophilus somni* (ATCC 70025) vs Penicillin Broth  
Microdilution - CO<sub>2</sub> incubation



# Penicillin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Penicillin	10 units	35–44	29–36	0.016–0.06	0.12–1

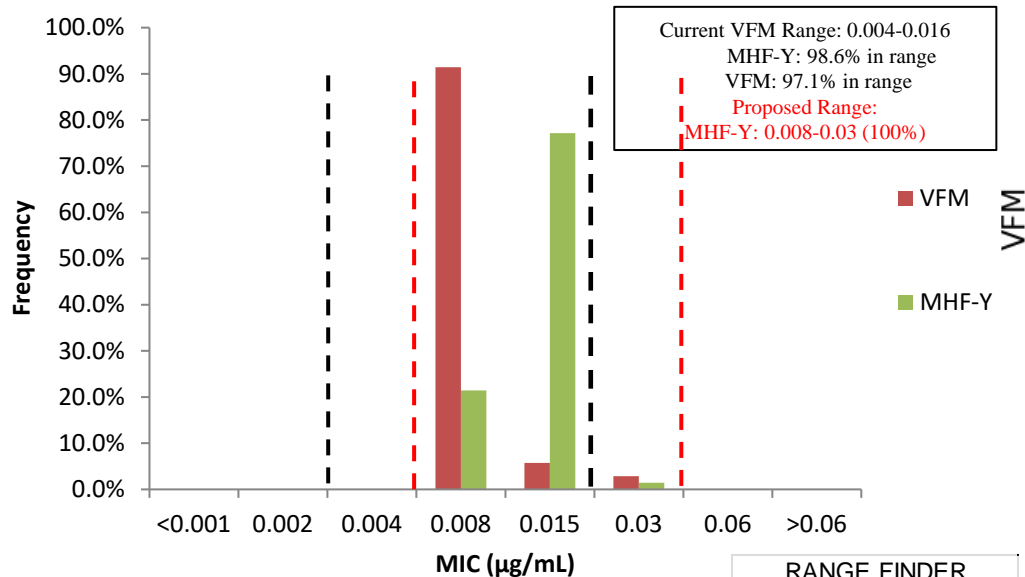
Keep Current Range for HS and APP

# Pradofloxacin – APP CO<sub>2</sub>

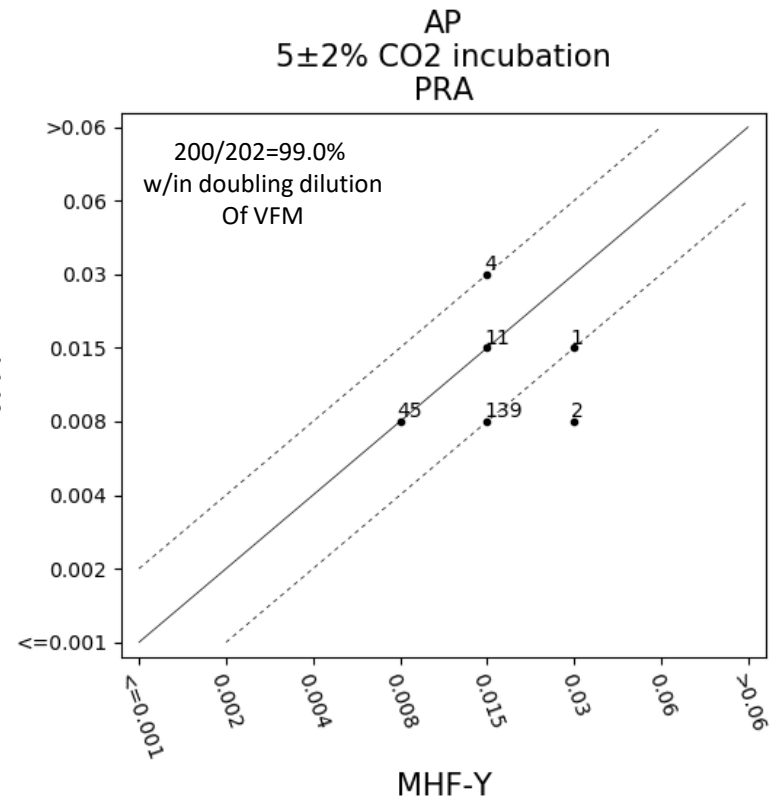
Table 45. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Pradofloxacin Broth Microdilution - CO2 incubation[illegible]

# Pradofloxacin – APP CO<sub>2</sub>

Figure 45. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Pradofloxacin  
Broth Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
0.008 to 0.031
Dilution Range
3
% Obs. Captured
100.0%
Prob'ty Outside Range
0.001



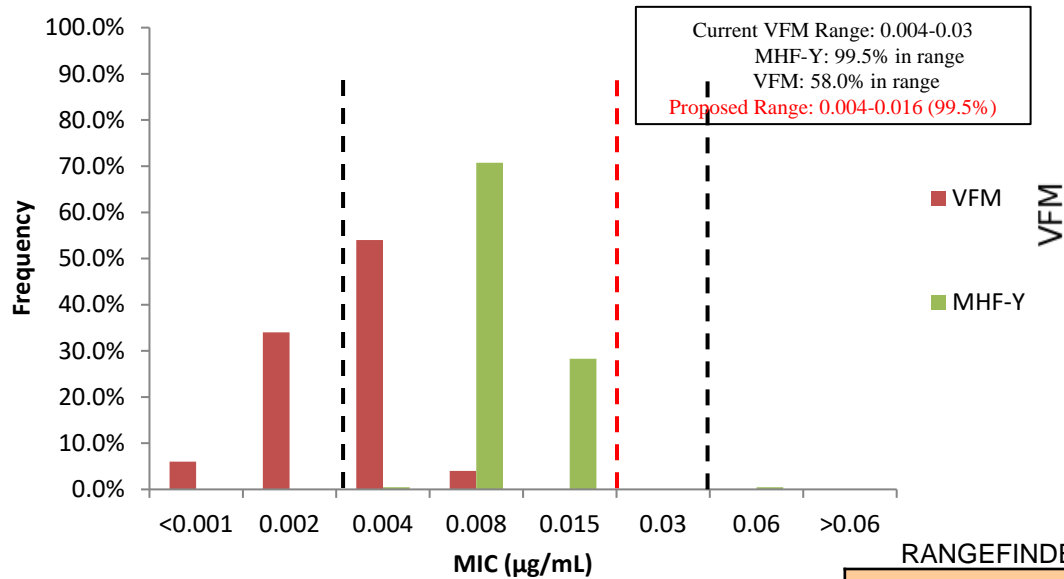
# Pradofloxacin – HS CO<sub>2</sub>

Table 47. *Histophilus somni* (ATCC 700025) vs Pradofloxacin Broth Microdilution - CO2 incubation

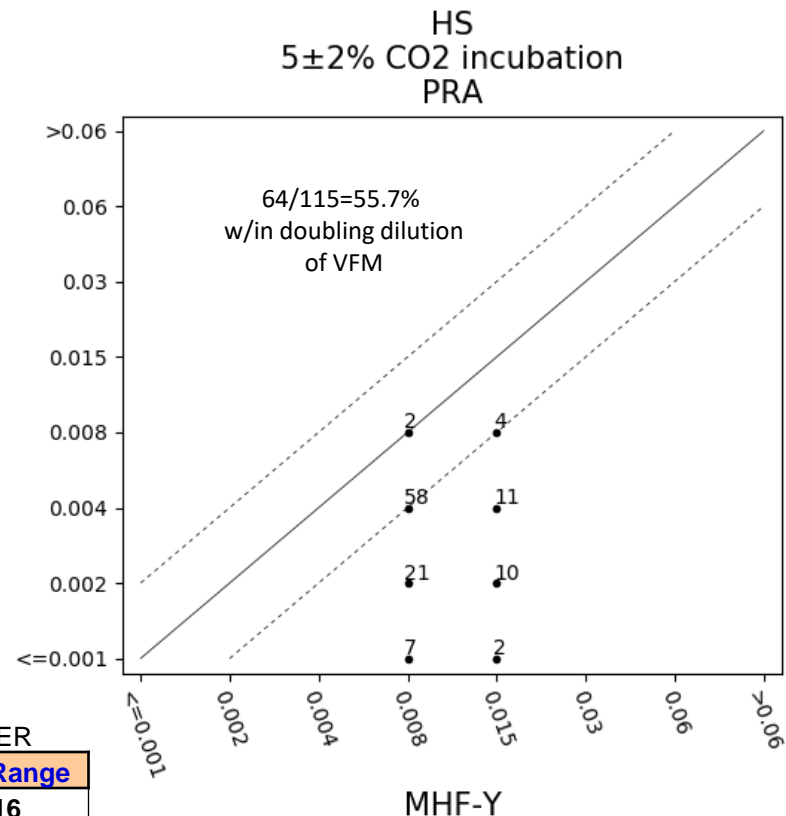
MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.001				3														3	
0.002				7		1		7		1		1						17	
0.004		1			1	9		3		2		5		8				27	1
0.008	65	35	50		23		28		14		25		28	2	18		14	2	150
0.015	6	34	20		9		2		16		4		1	0	12		16	0	60
0.03																			
0.06			1							1									1
>0.06																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		1		1						6		4	1			10		21	1
Min MIC	0.008	0.004	0.008	≤0.001	0.004	0.002	0.008	0.002	0.008	0.002	0.008	0.002	0.008	0.004	0.008	0	0.008	≤0.001	0.004
Max MIC	0.015	0.015	0.06	0.002	0.015	0.004	0.015	0.004	0.015	0.004	0.06	0.004	0.015	0.015	0.015	0	0.015	0.008	0.06
MIC <sub>mode</sub>	0.008	0.008	0.008	0.002	0.008	0.004	0.008	0.002	0.015	0.004	0.008	0.004	0.008	0.004	0.008	0	0.015	0.004	0.008

# Pradofloxacin – HS CO<sub>2</sub>

Figure 47. *Histophilus somni* (ATCC 700025) vs Pradofloxacin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGEFINDER	
Calculated QC Range	
0.004 to 0.016	
Dilution Range	
3	
% Obs. Captured	
99.5%	
Prob'ty Outside Range	
0.008	





# Pradofloxacin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Pradofloxacin	5 µg	—	32–41	0.004-0.016	0.008-0.03

Modify Current Ranges for HS and APP

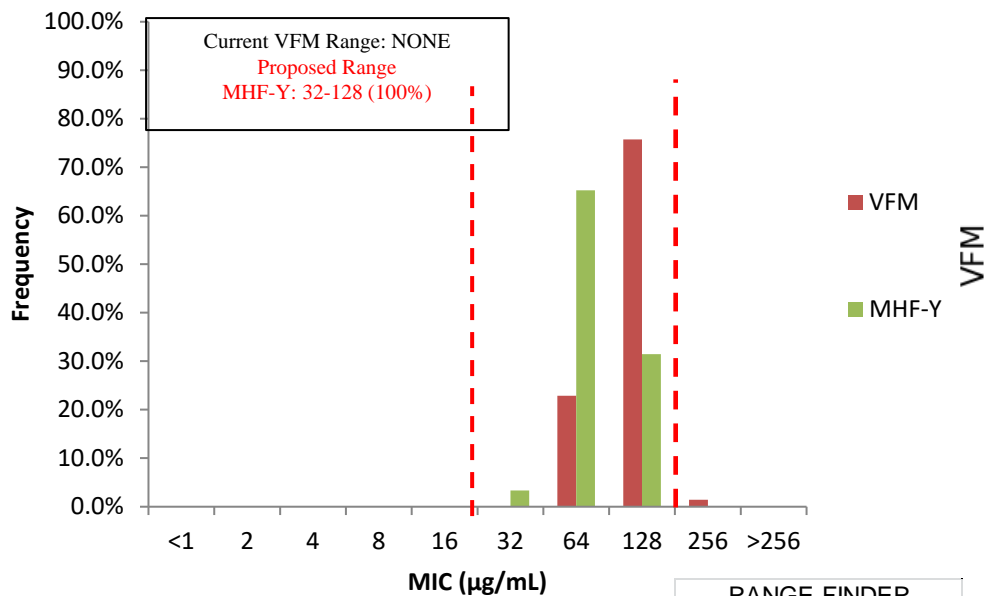
# Spectinomycin – APP CO<sub>2</sub>

Table 49. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Spectinomycin Broth Microdilution - CO<sub>2</sub> incubation

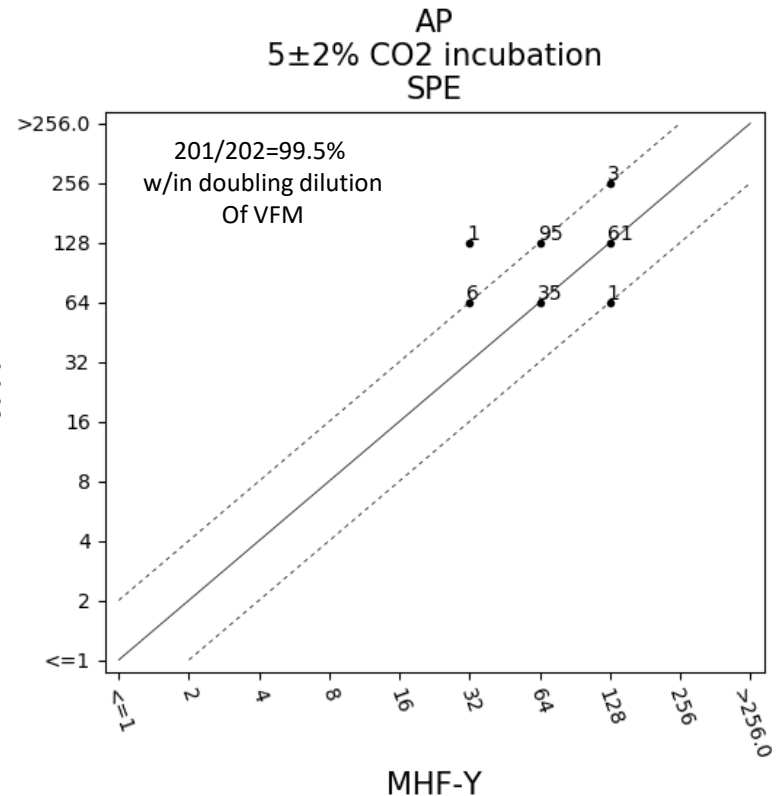
MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤1																			
2																			
4																			
8																			
16																			
32	5	1	1								1						6		7
64	54	49	34		13	1	22	5	28		25				25	10	24	16	137
128	11	20	35	10	17	9	8	5	2	10	4	9	30	10	5			53	66
256												1						1	
>256																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	32	32	32	128	64	64	64	64	64	128	32	128	128	128	64	64	32	64	32
Max MIC	128	128	128	128	128	128	128	128	128	128	128	256	128	128	128	64	64	256	128
MIC <sub>mode</sub>	64	64	128	128	128	128	64	128	64	128	64	128	128	128	64	64	64	128	64

# Spectinomycin – APP CO<sub>2</sub>

Figure 49. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Spectinomycin Broth Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
32 to 128
Dilution Range
3
% Obs. Captured
100.0%
Prob'ty Outside Range
0.010



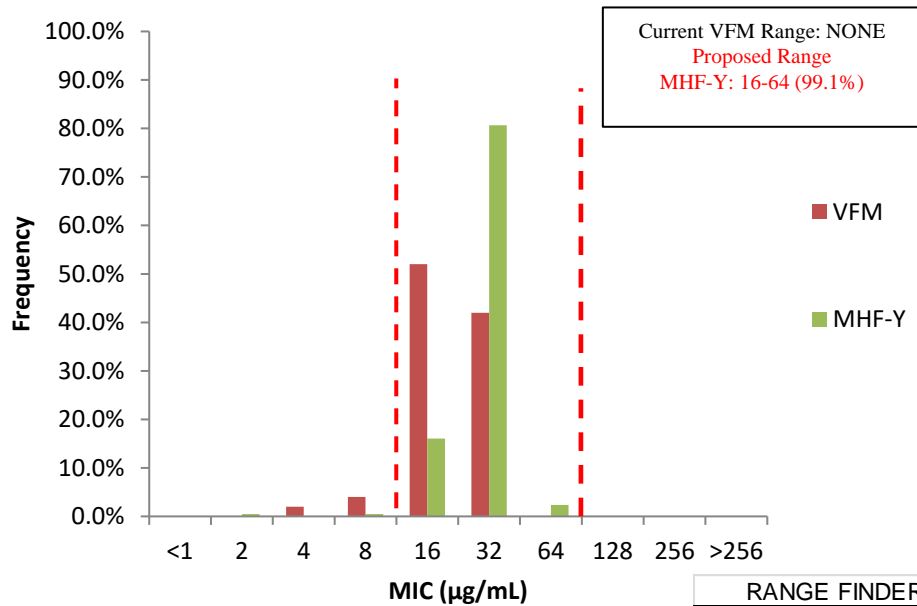
# Spectinomycin – HS CO<sub>2</sub>

Table 51. *Histophilus somni* (ATCC 700025) vs Spectinomycin Broth Microdilution - CO<sub>2</sub> incubation

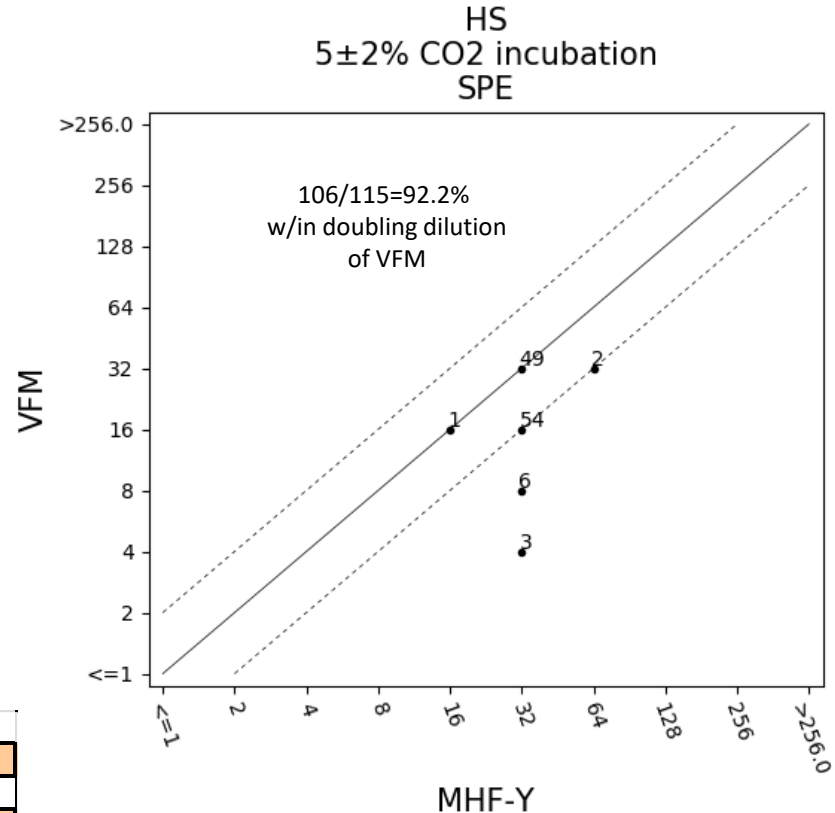
MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤1																			
2			1								1								1
4				1														1	
8			1	1				1									1	2	1
16	10	9	15	7	1	4		9	2	3	4			3			27	26	34
32	57	61	53	1	32	6	30		28	1	25	6	24	7	30		2	21	171
64	4		1										5						5
128																			
256																			
>256																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		1		1						6		4	1			10		21	1
Min MIC	16	16	8	8	16	16	32	8	16	16	16	32	32	16	32	0	8	8	8
Max MIC	64	32	64	32	32	32	32	16	32	32	32	32	64	32	32	0	32	32	64
MIC <sub>mode</sub>	32	32	32	16	32	32	32	16	32	16	32	32	32	32	32	0	16	16	32

# Spectinomycin – HS CO<sub>2</sub>

Figure 51. *Histophilus somni* (ATCC 700025) vs Spectinomycin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
16 to 64
Dilution Range
3
% Obs. Captured
99.1%
Prob'ty Outside Range
0.004



# Spectinomycin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Spectinomycin	—	—	—	16-64	32-128

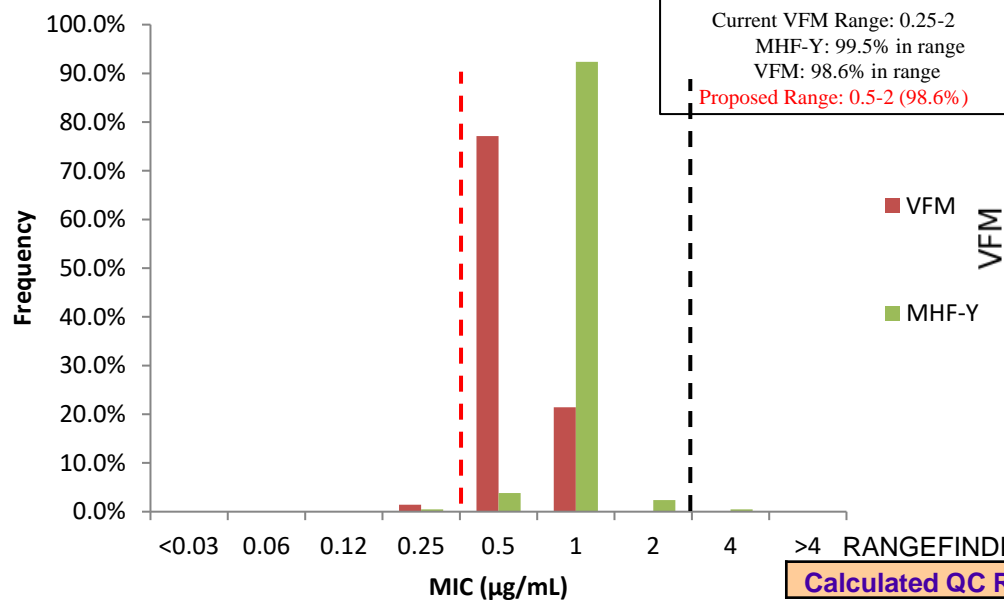


# Tetracycline – APP CO<sub>2</sub>

Table 53. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tetracycline Broth Microdilution - CO2 incubation[illegible]

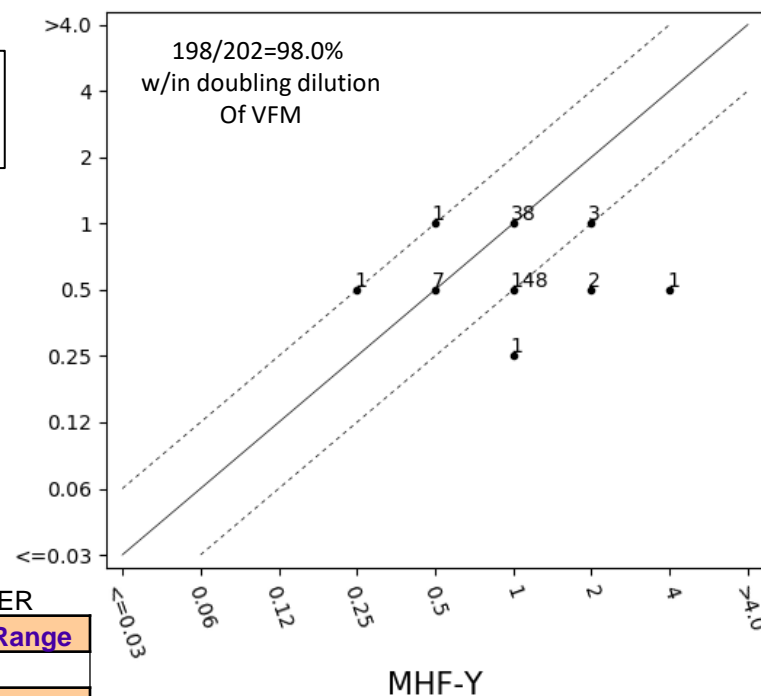
# Tetracycline – APP CO<sub>2</sub>

Figure 53. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tetracycline  
Broth Microdilution - CO<sub>2</sub> incubation



RANGEFINDER
Calculated QC Range
0.5 to 2
Dilution Range
3
% Obs. Captured
99.0%
Prob'ty Outside Range
0.000

AP  
5±2% CO<sub>2</sub> incubation  
TET



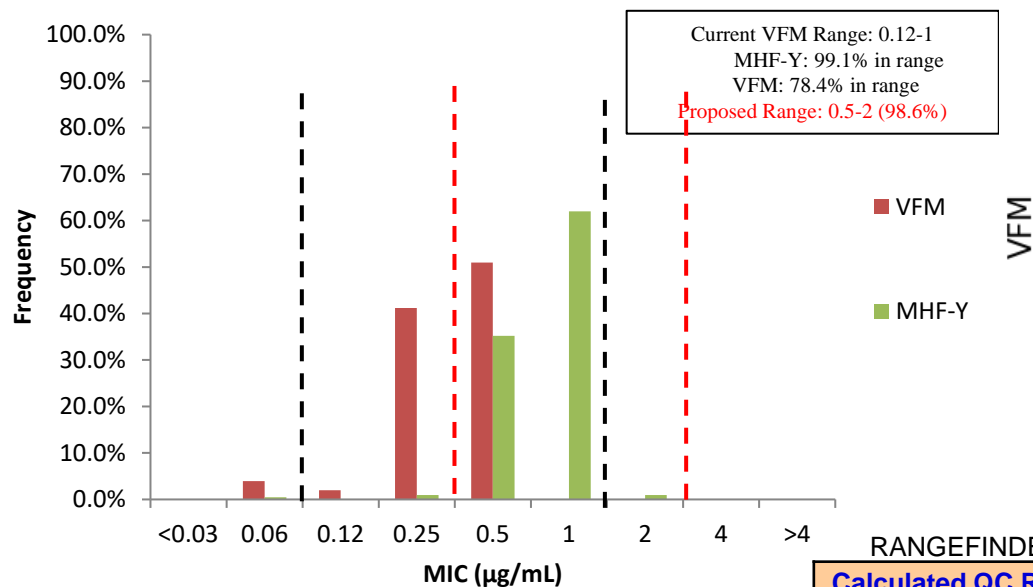
# Tetracycline – HS CO<sub>2</sub>

Table 55. *Histophilus somni* (ATCC 700025) vs Tetracycline Broth Microdilution - CO2 incubation

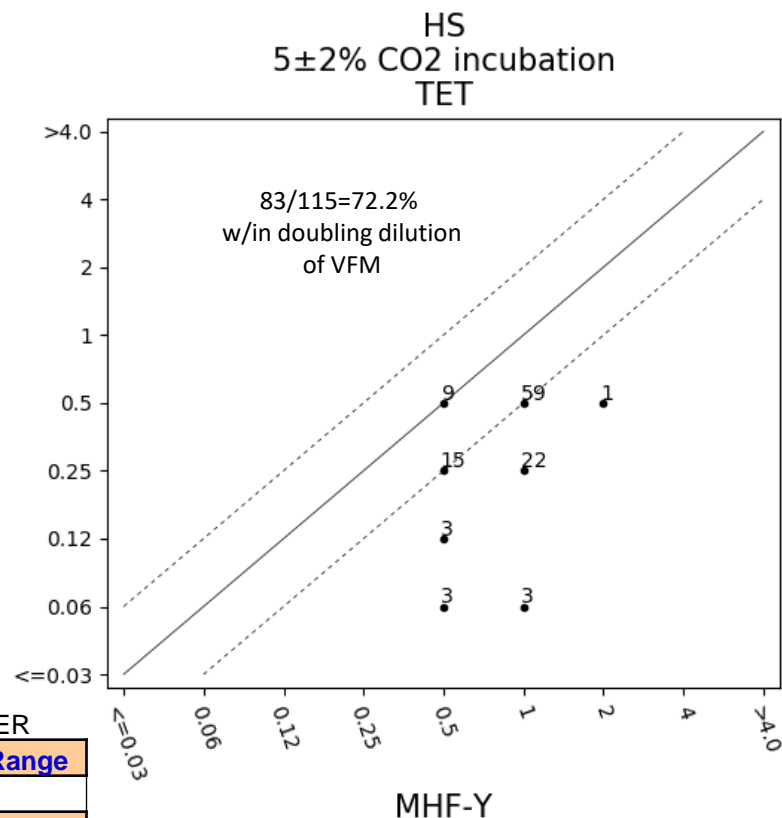
MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.03																			
0.06			1	2						1								2	1
0.12				1														1	
0.25	1	1		7		1		10		3	1		1					21	2
0.5	36	22	17		20	9	3		6	1	21	6	19	10		6		26	75
1	34	46	52		13		27		24		7		8		29		24		132
2		1	1										1		1				2
4																			
>4																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	0.25	0.25	0.5	0.06	0.5	0.25	0.5	0.25	0.5	0.25	0.06	0.5	0.25	0.5	1	0	0.5	0.06	0.25
Max MIC	1	2	2	0.25	1	0.5	1	0.25	1	0.5	1	0.5	2	0.5	2	0	1	0.5	2
MIC <sub>mode</sub>	0.5	1	1	0.25	0.5	0.5	1	0.25	1	0.25	0.5	0.5	0.5	0.5	1	0	1	0.5	1

# Tetracycline – HS CO<sub>2</sub>

Figure 55. *Histophilus somni* (ATCC 700025) vs Tetracycline Broth  
Microdilution - CO<sub>2</sub> incubation



RANGEFINDER	
Calculated QC Range	0.5 to 2
Dilution Range	3
% Obs. Captured	98.6%
Prob'ty Outside Range	0.024



# Tetracycline – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Tetracycline	30 µg	27–33	23–30	0.5-2	0.5-2

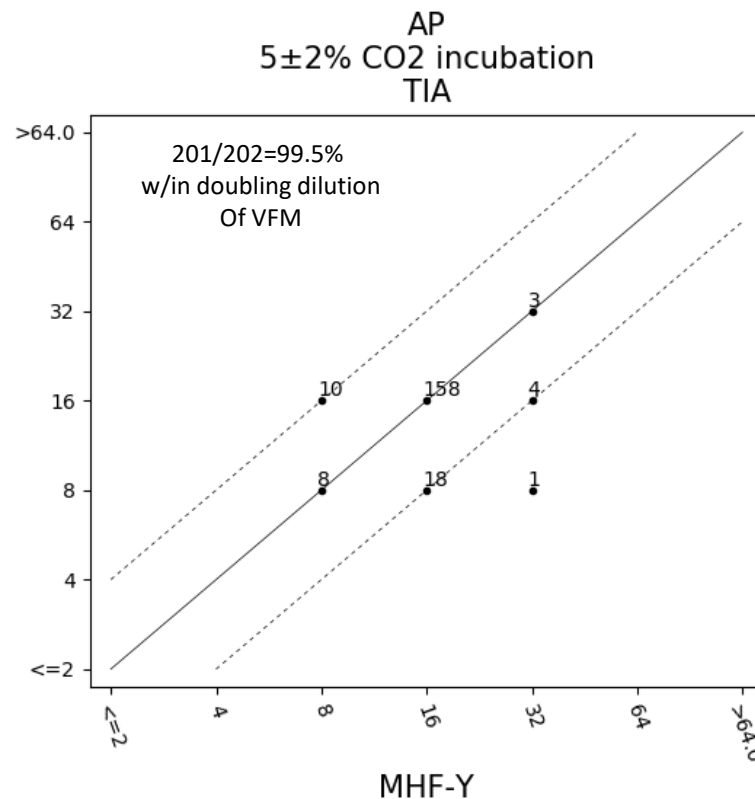
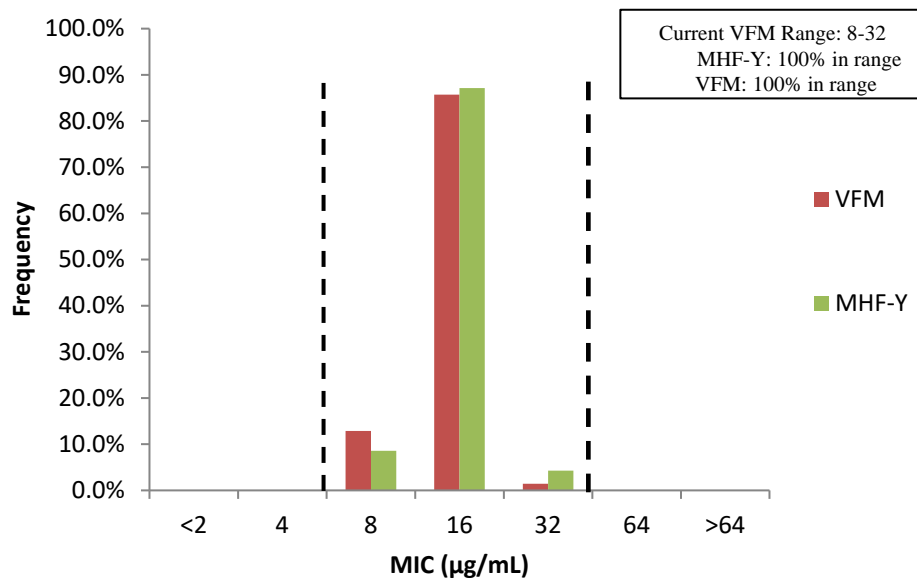
Modify Ranges for HS and APP

# Tiamulin – APP CO<sub>2</sub>

Table 57. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tiamulin Broth Microdilution - CO2 incubation[illegible]

# Tiamulin – APP CO<sub>2</sub>

Figure 57. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs  
Tiamulin Broth Microdilution - CO<sub>2</sub> incubation





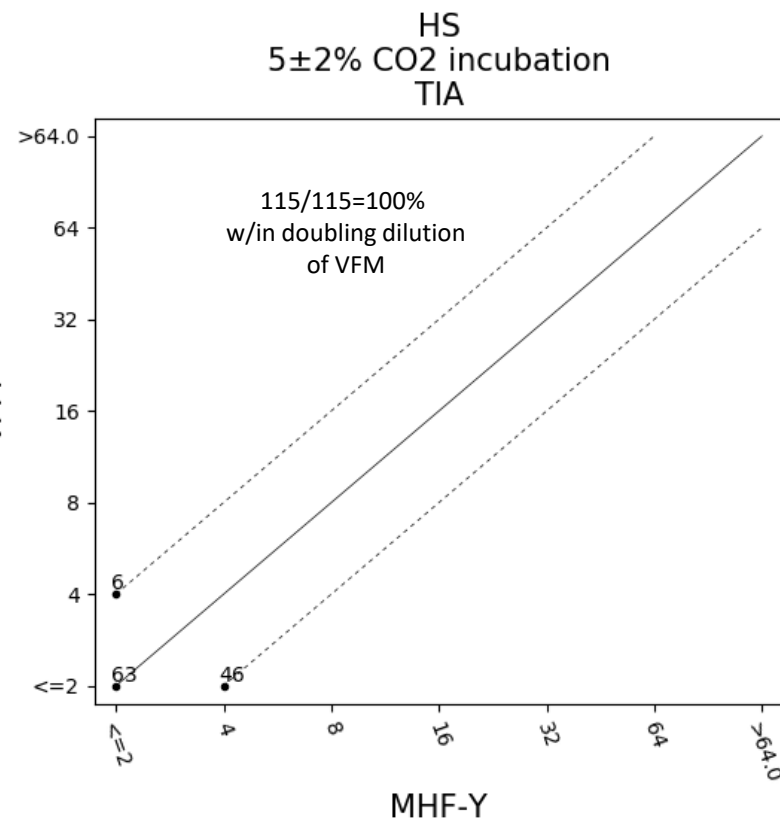
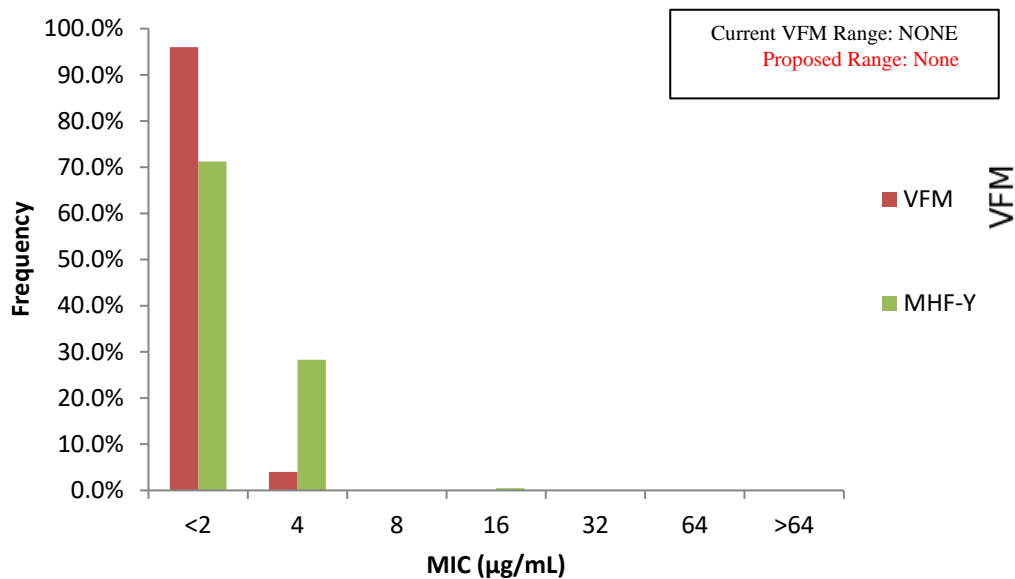
# Tiamulin – HS CO<sub>2</sub>

Table 59. *Histophilus somni* (ATCC 700025) vs Tiamulin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤2	51	48	52	10	28	10	15	10	15	3	26	6	25	9	12		30	48	151
4	20	22	18		5		15		15	1	3		4	1	18			2	60
8																			
16			1								1								1
32																			
64																			
>64																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth		1		1						6		4	1			10		21	1
Min MIC	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	0	≤2	≤2	≤2
Max MIC	4	4	4	≤2	4	≤2	4	≤2	4	4	4	≤2	4	4	4	0	≤2	4	4
MIC <sub>mode</sub>	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	4	0	≤2	≤2	≤2

# Tiamulin – HS CO<sub>2</sub>

Figure 59. *Histophilus somni* (ATCC 700025) vs Tiamulin Broth  
Microdilution - CO<sub>2</sub> incubation



# Tiamulin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Tiamulin	30 µg	–	12–19	–	8–32

Keep Current Range for APP, no range for HS

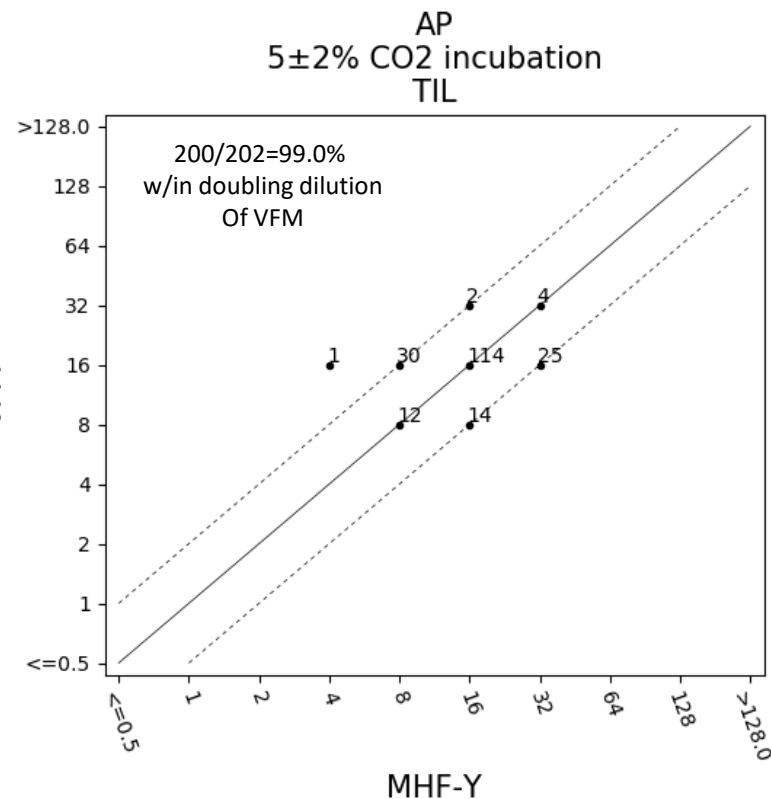
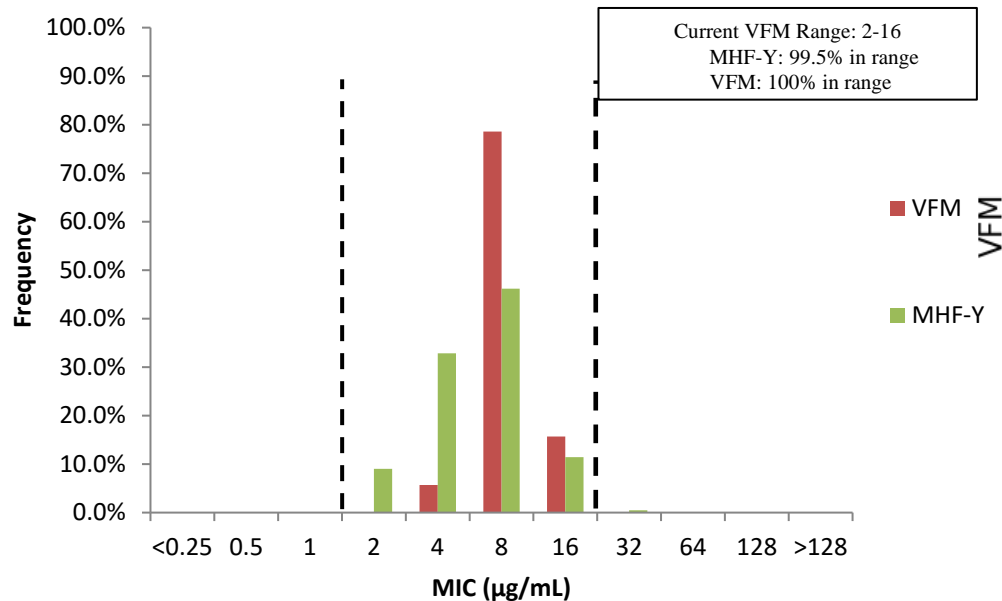
# Tildipirosin – APP CO<sub>2</sub>

Table X1. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tildipirosin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.25																			
0.5																			
1																			
2	19						3		2		1				3		10		19
4	38	17	14		10		12		11		9		1		8	4	18	4	69
8	11	42	44	10	19	9	15	10	17	10	20		8	10	17	6	1	55	97
16	2	10	12			1						10	21		2		1	11	24
32		1			1														1
64																			
128																			
>128																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	2	4	4	8	4	8	2	8	2	8	2	16	4	8	2	4	2	4	2
Max MIC	16	32	16	8	32	16	8	8	8	8	8	16	16	8	16	8	16	16	32
MIC <sub>mode</sub>	4	8	8	8	8	8	8	8	8	8	8	16	16	8	8	8	4	8	8

# Tildipirosin – APP CO<sub>2</sub>

Figure 61. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tildipirosin  
Broth Microdilution - CO<sub>2</sub> incubation



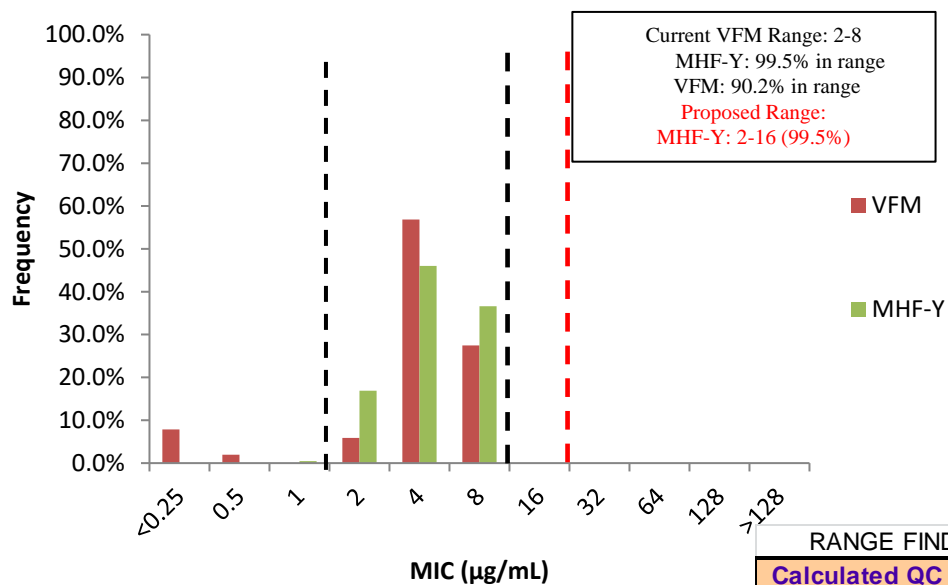
# Tildipirosin – HS CO<sub>2</sub>

Table X3. *Histophilus somni* (ATCC 700025) vs Tildipirosin Broth Microdilution - CO<sub>2</sub> incubation

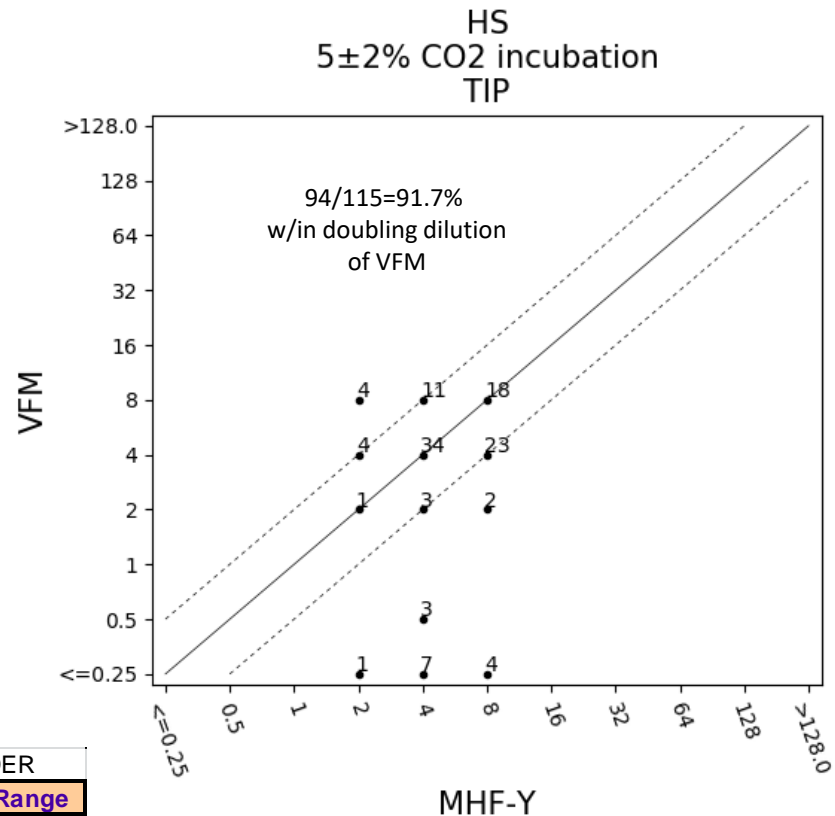
MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.25				4														4	
0.5				1														1	
1	1																1		1
2	22	8	6	2	2		5	1	4		8				2		15	3	36
4	38	36	24	4	16	5	15	8	19	3	14	1	3	8	19		12	29	98
8	10	27	41		15	5	10	1	7	2	8	4	27	2	9		2	14	78
16																			
32																			
64																			
128																			
>128																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	1	2	2	≤0.25	2	4	2	2	2	4	2	4	4	4	2	0	1	≤0.25	1
Max MIC	8	8	8	4	8	8	8	8	8	8	8	8	8	8	8	0	8	8	8
MIC <sub>mode</sub>	4	4	8	4	4	4	4	4	4	4	4	8	8	8	4	0	2	4	4

# Tildipirosin – HS CO<sub>2</sub>

Figure 63. *Histophilus somni* (ATCC 700025) vs Tildipirosin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
2 to 16
Dilution Range
4
% Obs. Captured
99.5%
Prob'ty Outside Range
0.010





# Tildipirosin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Tildipirosin	60 µg	15–24	15–23	2-16	2–16

Keep Current Range for APP and modify HS

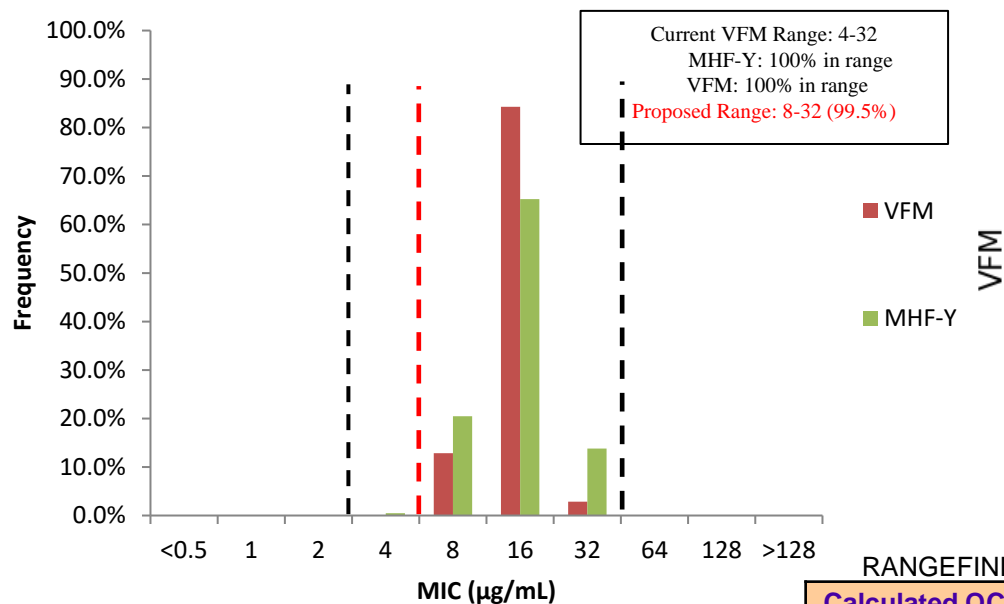
# Tilmicosin – APP CO<sub>2</sub>

Table X5. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tilmicosin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.5																			
1																			
2																			
4	1									1									1
8	38	4	1				5		7	5	11		1		7	4	12	9	43
16	28	57	52	10	25	8	21	10	23	5	18	10	9	10	23	6	18	59	137
32	3	9	17		5	2	4						20					2	29
64																			
128																			
>128																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	4	8	8	16	16	16	8	16	8	8	4	16	8	16	8	8	8	8	4
Max MIC	32	32	32	16	32	32	32	16	16	16	16	16	32	16	16	16	16	32	32
MIC <sub>mode</sub>	8	16	16	16	16	16	16	16	16	16	16	16	32	16	16	16	16	16	16

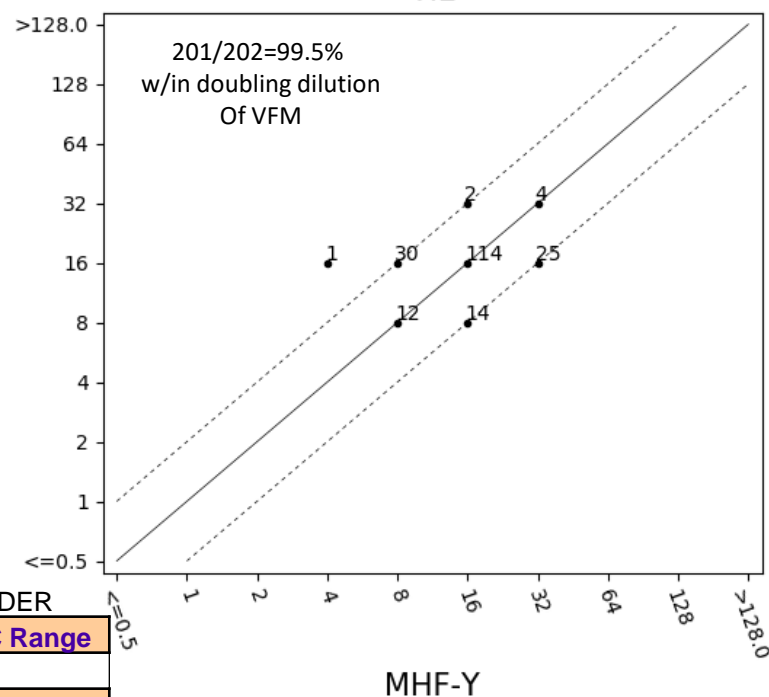
# Tilmicosin – APP CO<sub>2</sub>

Figure 65. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tilmicosin  
Broth Microdilution - CO<sub>2</sub> incubation



RANGEFINDER	
Calculated QC Range	8 to 32
Dilution Range	3
% Obs. Captured	99.5%
Prob'ty Outside Range	0.013

AP  
5±2% CO<sub>2</sub> incubation  
TIL



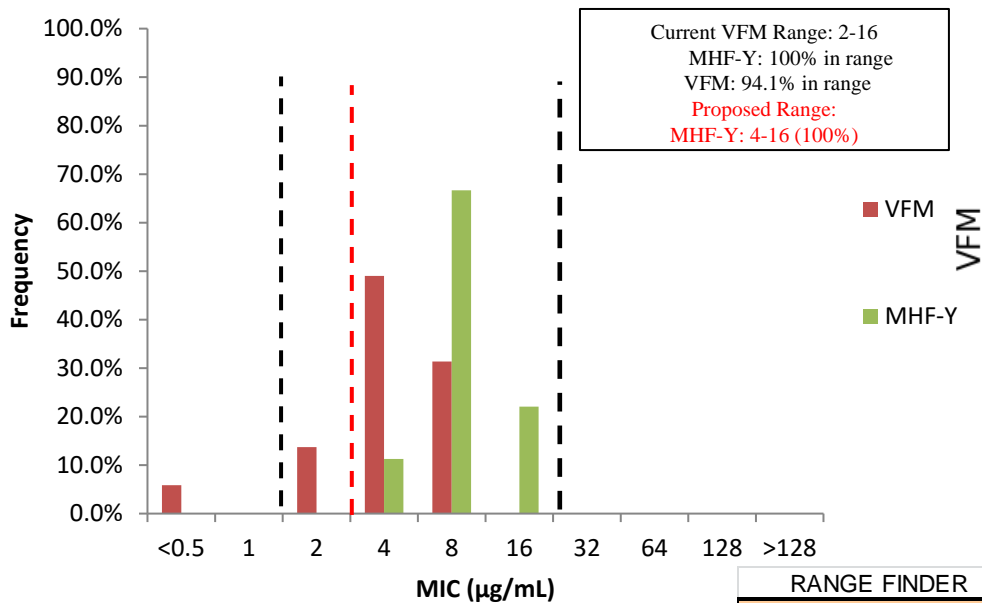
# Tilmicosin – HS CO<sub>2</sub>

Table X7. *Histophilus somni* (ATCC 700025) vs Tilmicosin Broth Microdilution - CO<sub>2</sub> incubation

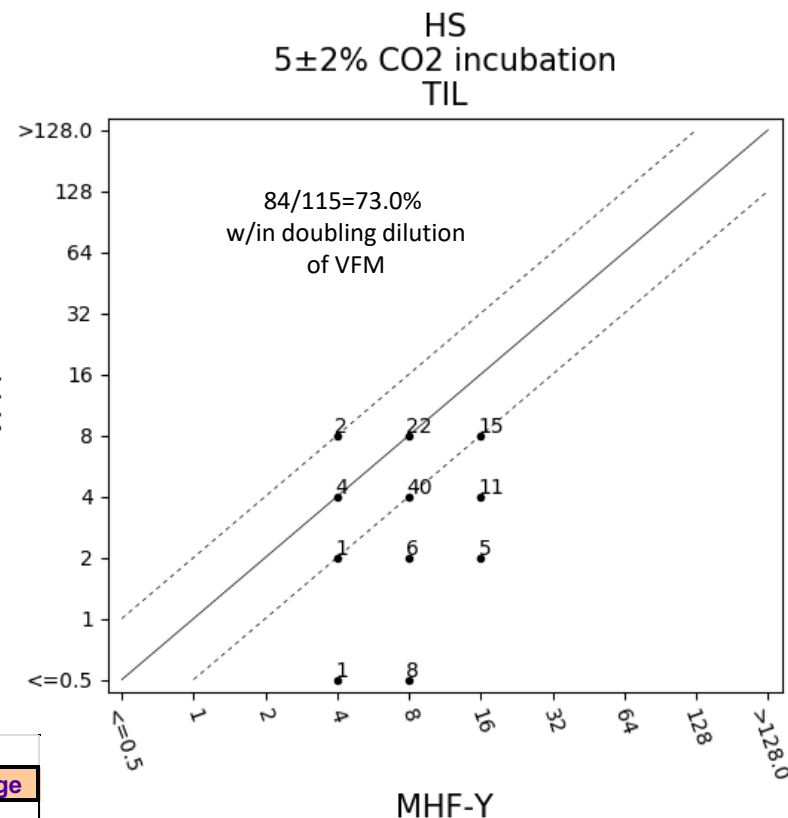
MIC	MHF-Y Lot 1	MHF-Y Lot 2	MHF-Y Lot 3	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
				VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.5				3														3	
1																			
2				4				2		1								7	
4	16	7	1	4	2	4	1	8	3	2	8			7	4		6	25	24
8	54	51	37		20	6	19		24	2	20	5	13	3	22		24	16	142
16	1	13	33		11		10		3		2		17		4				47
32																			
64																			
128																			
>128																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	4	4	4	≤0.5	4	4	4	2	4	2	4	8	8	4	4	0	4	≤0.5	4
Max MIC	16	16	16	4	16	8	16	4	16	8	16	8	16	8	16	0	8	8	16
MIC <sub>mode</sub>	8	8	8	4	8	8	8	4	8	8	8	8	16	4	8	0	8	4	8

# Tilmicosin – HS CO<sub>2</sub>

Figure 67. *Histophilus somni* (ATCC 700025) vs Tilmicosin Broth  
Microdilution - CO<sub>2</sub> incubation



RANGE FINDER
Calculated QC Range
4 to 16
Dilution Range
3
% Obs. Captured
100.0%
Prob'ty Outside Range
0.010



# Tilmicosin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Tilmicosin	15 µg	8–16	8–15	4-16	8-32

Adjust Current Range for HS and APP

# Trimethoprim/sulfamethoxazole – APP CO<sub>2</sub>

Table X9. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Trimethoprim/ Sulfamethoxazole Broth Microdilution - CO2 incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
<0.004																			
0.008			1				1												1
0.015	5	1	10			1	14							2				1	16
0.03	8	11	2			8	15							2	6			10	21
0.06		1				1								1	1			2	1
0.12	57	57	57	10	30			10	30	10	30	10	30	7	21	10	30	57	171
0.25																			
0.5																			
1																			
>1																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	0.015	0.015	0.008	0.12	0.12	0.015	0.008	0.12	0.12	0.12	0.12	0.12	0.12	0.03	0.015	0.12	0.12	0.015	0.008
Max MIC	0.12	0.12	0.12	0.12	0.12	0.06	0.03	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
MIC <sub>mode</sub>	0.12	0.12	0.12	0.12	0.12	0.03	0.03	0.12	0.12	0.12	0.12	0.12	0.12	0.03	0.12	0.12	0.12	0.12	0.12

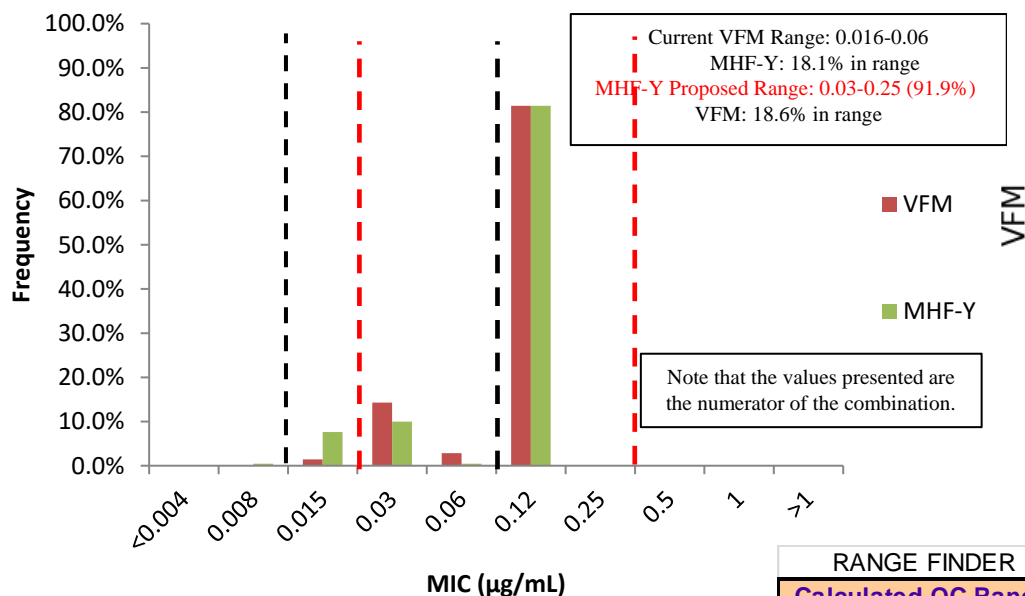
Note that the values presented are the numerator of the combination.



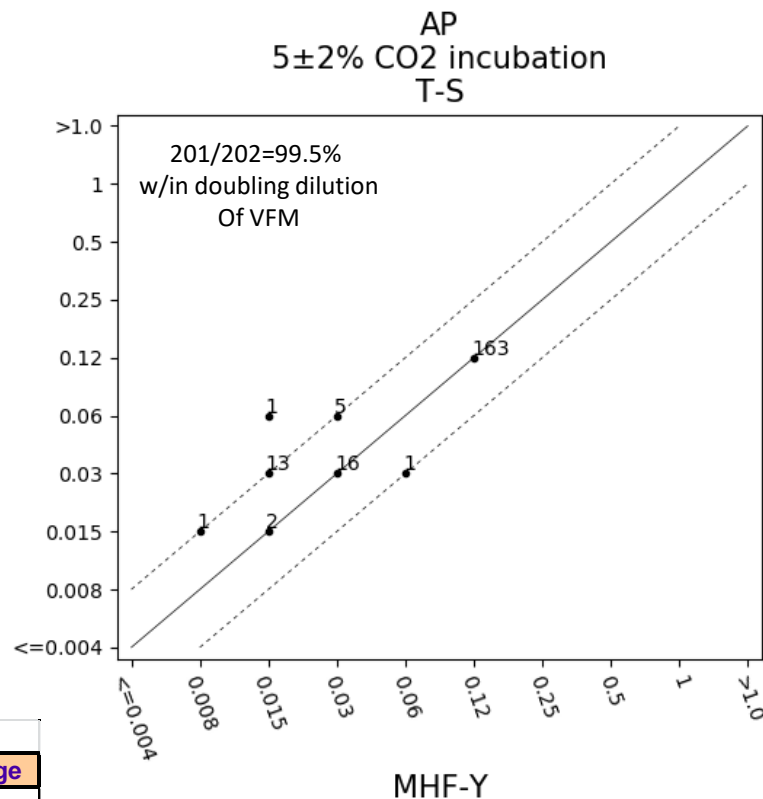
# Trimethoprim/sulfamethoxazole – APP CO<sub>2</sub>

## ALL LABS – 4 dilution range

Figure 69. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Trimethoprim/ Sulfamethoxazole Broth Microdilution - CO<sub>2</sub> incubation



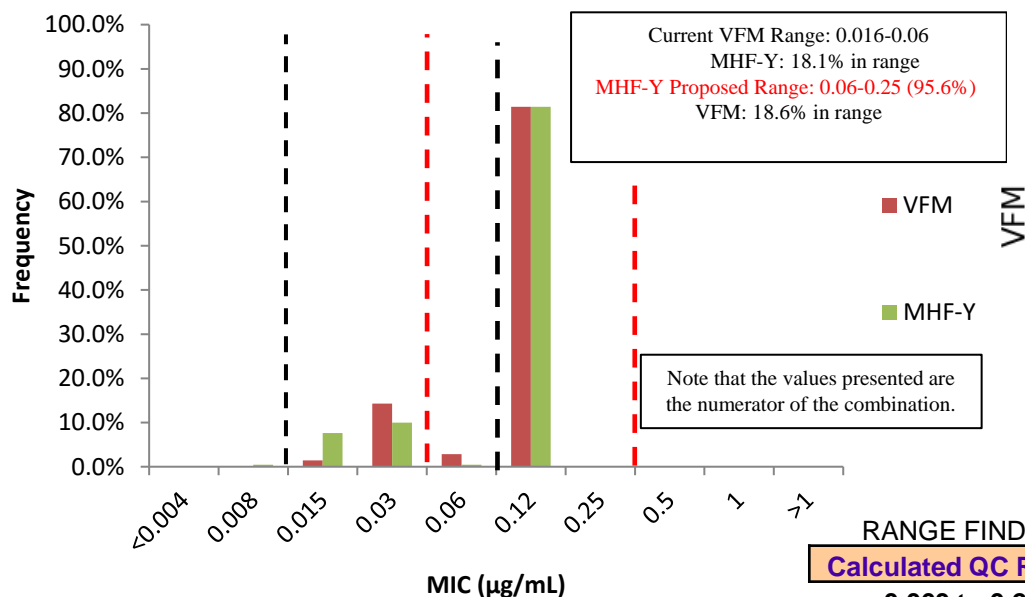
RANGE FINDER
Calculated QC Range
0.031 to 0.25
Dilution Range
4
% Obs. Captured
91.9%
Prob'ty Outside Range
0.042



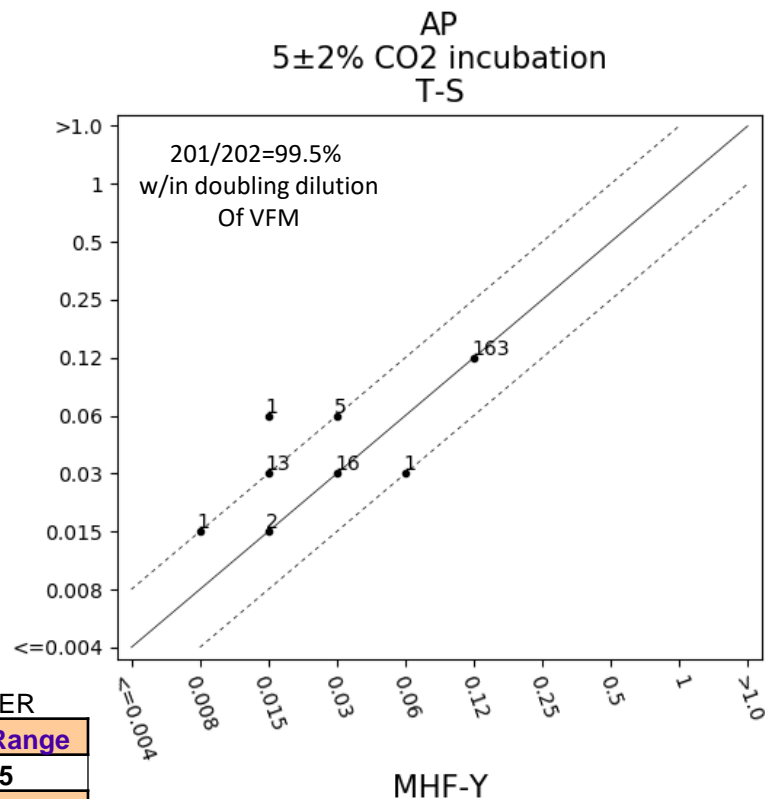
# Trimethoprim/sulfamethoxazole – APP CO<sub>2</sub>

EXCLUDING LAB 2 – 6 labs

Figure 69. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Trimethoprim/ Sulfamethoxazole Broth Microdilution - CO<sub>2</sub> incubation



RANGE FINDER	
Calculated QC Range	0.063 to 0.25
Dilution Range	3
% Obs. Captured	95.6%
Prob'ty Outside Range	0.002



# Trimethoprim/sulfamethoxazole – HS CO<sub>2</sub>

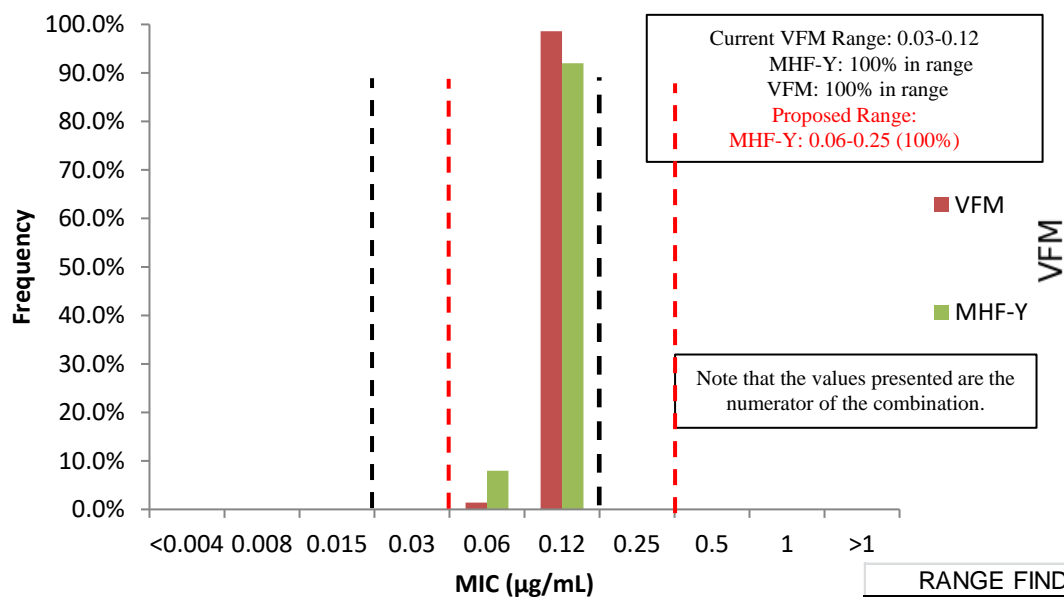
Table 71. *Histophilus somni* (ATCC 700025) vs Trimethoprim/ Sulfamethoxazole Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.004																			
0.008																			
0.015																			
0.03																			
0.06	1	5	11				14							1	3			1	17
0.12	70	66	60	11	33	10	16	10	30	10	30	10	30	9	27	10	30	70	196
0.25																			
0.5																			
1																			
>1																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth																			
Min MIC	0.06	0.06	0.06	0.12	0.12	0.12	0.06	0.12	0.12	0.12	0.12	0.12	0.12	0.06	0.06	0.12	0.12	0.06	0.06
Max MIC	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
MIC <sub>mode</sub>	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

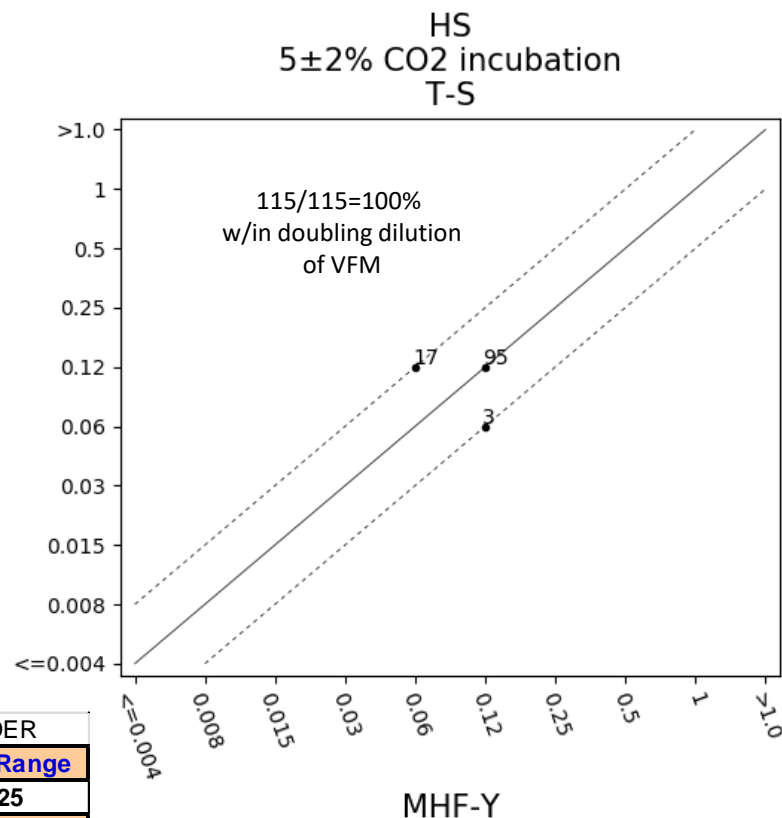
Note that the values presented are the numerator of the combination.

# Trimethoprim/sulfamethoxazole – HS CO<sub>2</sub>

Figure 71. *Histophilus somni* (ATCC 700025) vs Trimethoprim/ Sulfamethoxazole Broth Microdilution - CO2 incubation



RANGE FINDER	
Calculated QC Range	0.063 to 0.125
Dilution Range	2
% Obs. Captured	100.0%
Prob'ty Outside Range	0.016



# Trimethoprim/sulfamethoxazole – Proposed Range

**Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae***

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Trimethoprim-sulfamethoxazole (1/19)	1.25/23.75 µg	26–32	28–32	0.06/1.14– 0.25/4.75	0.06/1.14– 0.25/4.75

Adjust Current Range for HS and APP

# Tulathromycin – APP CO<sub>2</sub>

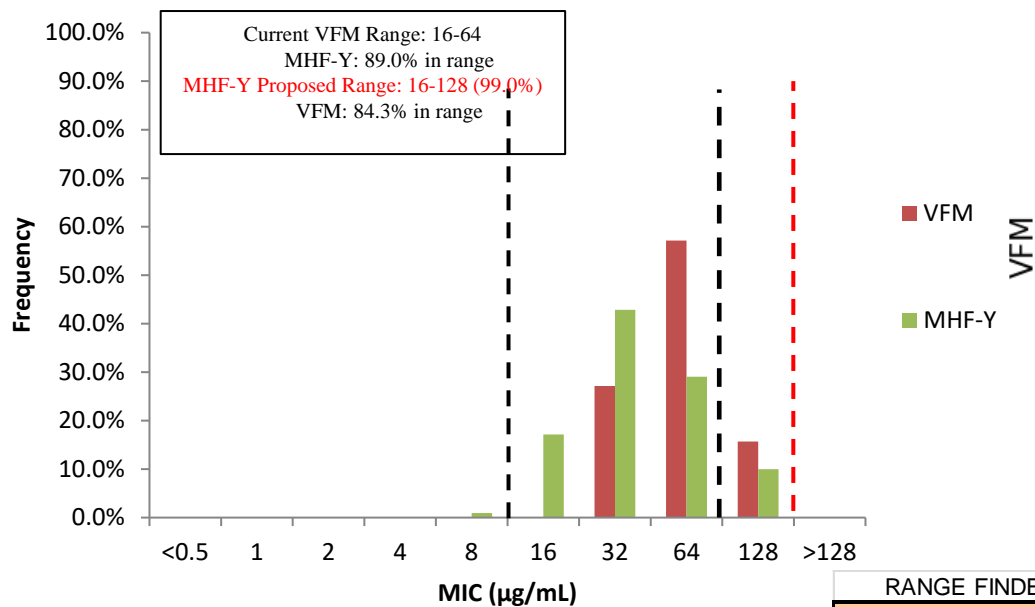
Table 73. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tulathromycin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.5																			
1																			
2																			
4																			
8	2															2		2	
16	34	1	1				7		9		2		1		9		8		36
32	24	30	36		10	1	16	2	13		9		2	6	20	10	20	19	90
64	9	30	22	10	18	8	7	8	8	10	19		8	4	1			40	61
128	1	9	11	2	1							10	19					11	21
>128																			
N	70	70	70	10	30	10	30	10	30	10	30	10	30	10	30	10	30	70	210
No Growth																			
Min MIC	8	16	16	64	32	32	16	32	16	64	16	128	16	32	16	32	8	32	8
Max MIC	128	128	128	64	128	128	64	64	64	64	64	128	128	64	64	32	32	128	128
MIC <sub>mode</sub>	16	64	32	64	64	64	32	64	32	64	64	128	128	32	32	32	32	64	32

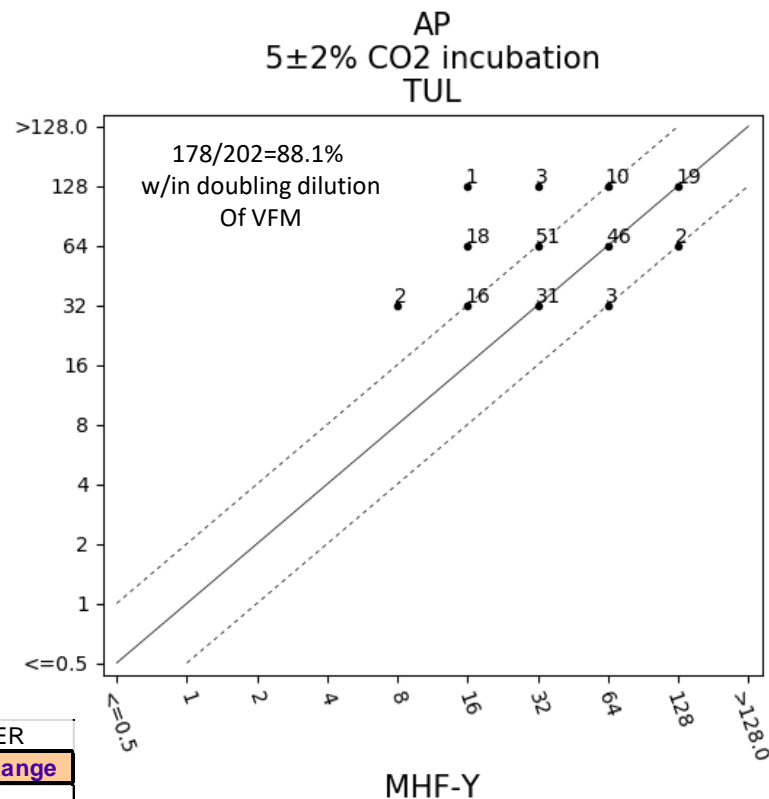


# Tulathromycin – APP CO<sub>2</sub>

Figure 73. *Actinobacillus pleuropneumoniae* (ATCC 27090) vs Tulathromycin  
Broth Microdilution - CO<sub>2</sub> incubation



RANGE FINDER	
Calculated QC Range	
16 to 128	
Dilution Range	
4	
% Obs. Captured	
99.0%	
Prob'ty Outside Range	
0.030	





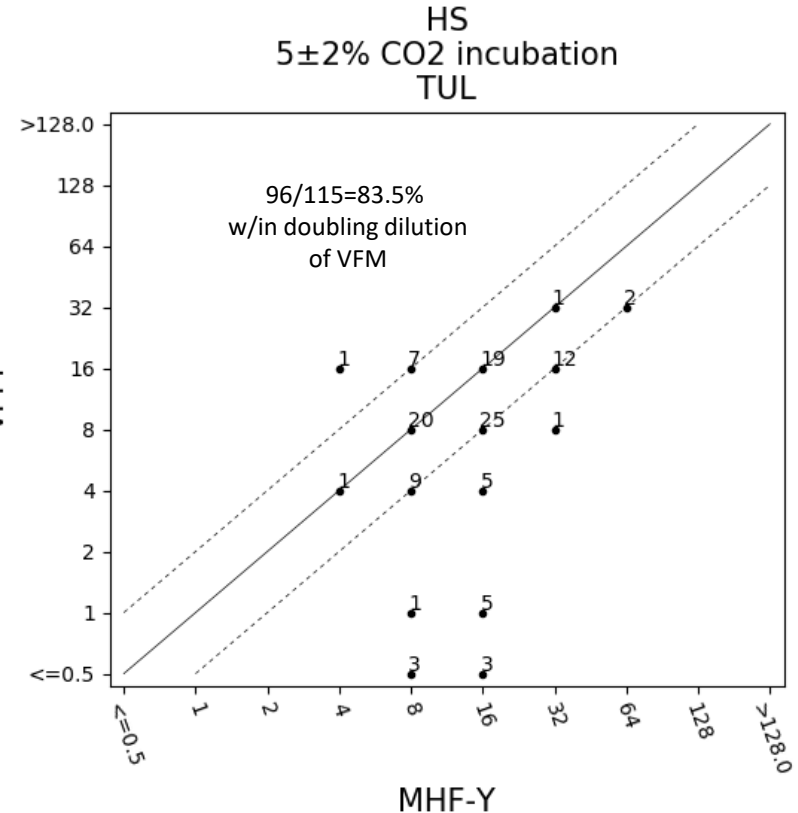
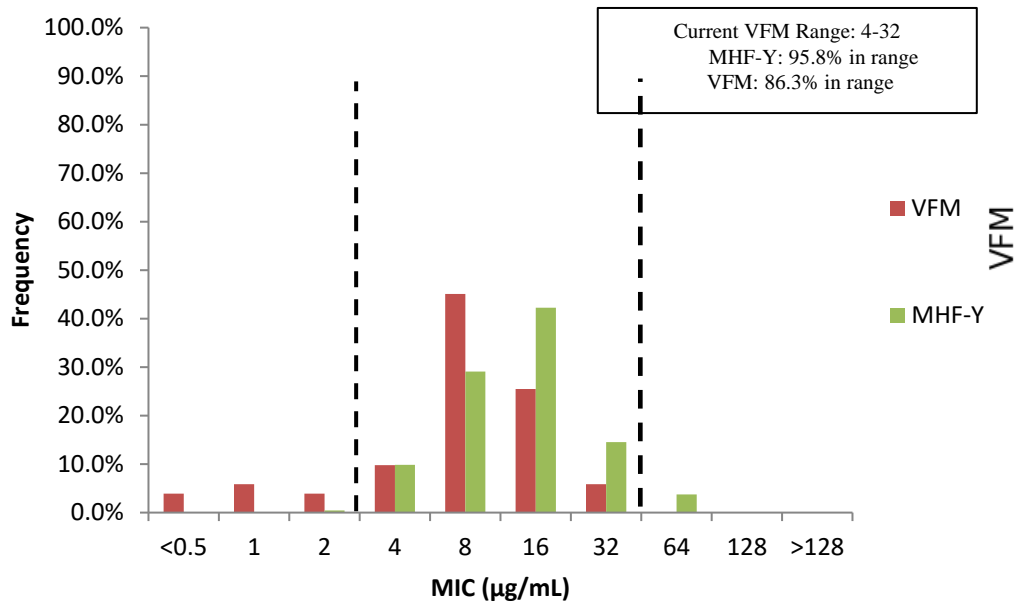
# Tulathromycin – HS CO<sub>2</sub>

Table 75. *Histophilus somni* (ATCC 700025) vs Tulathromycin Broth Microdilution - CO<sub>2</sub> incubation

MIC	MHF-Y	MHF-Y	MHF-Y	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		All Labs	
	Lot 1	Lot 2	Lot 3	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y	VFM	MHF-Y
≤0.5				2														2	
1				3														3	
2	1									2							1	2	1
4	11	6	4	2			1	1	1					2			19	5	21
8	30	21	11	4	9	2	9	8	13	1	3			8	18		10	23	62
16	20	33	37		22	8	16	1	15	2	23	2	2		12			13	90
32	9	7	15		2		4		1		4	3	20					3	31
64		4	4										8						8
128																			
>128																			
N	71	71	71	11	33	10	30	10	30	10	30	10	30	10	30	10	30	71	213
No Growth										5		5				10		20	
Min MIC	2	4	4	≤0.5	8	8	4	4	4	2	8	16	16	4	8	0	2	≤0.5	2
Max MIC	32	64	64	8	32	16	32	16	32	16	32	32	32	8	16	0	8	32	64
MIC <sub>mode</sub>	8	16	16	8	16	16	16	8	16	16	16	32	32	8	8	0	4	8	16

# Tulathromycin – HS CO<sub>2</sub>

Figure 75. *Histophilus somni* (ATCC 700025) vs Tulathromycin Broth  
Microdilution - CO<sub>2</sub> incubation



# Tulathromycin – Proposed Range

Table X. Acceptable Quality Control Ranges for *Histophilus somni* and *Actinobacillus pleuropneumoniae*

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC® <sup>a</sup> 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Tulathromycin	30 µg	16–26	8–18 <sup>c</sup>	4–32	16–128

Keep Current QC Range for HS and adjust APP

# ADJUSTMENT OF INTERPRETIVE CRITERIA

- No adjustments to breakpoints are proposed as only minor adjustments to QC ranges were required.
  - The AST group has set a precedent for this by adjusting QC ranges without adjustment of interpretive criteria
- If ANY adjustments are proposed by the VAST, the Working Group would need to contact any sponsor associated with the antibiotic or generic working group and allow them to coordinate such adjustment.

# Overall Proposal for Approval

Modifications proposed for next revision of VET01:

- Change all references of VFM to MHF-Y
- Replace VFM recipe in Appendix A3.5 with recipe for MHF-Y:

The steps for preparing 1 L MHF-Y for broth microdilution testing of *H. somni* and *A. pleuropneumoniae* are listed below.

Step	Action	Comments
1.	Prepare the MHB according to the manufacturer's instructions (see Appendix A3.1).	MHB (22.0 g) per L: <ul style="list-style-type: none"><li>• 3.0 g beef extract (from 300 g beef infusion)</li><li>• 17.5 g acid hydrolysis of casein</li><li>• 1.5 g starch</li></ul> Unless the MHB has the correct concentrations of divalent cations ( $\text{Ca}^{++}$ and $\text{Mg}^{++}$ ), add appropriate salts to provide 20 to 25 mg/L calcium and 10 to 12.5 mg/L magnesium (see A2.1).
2.	Mix the MHB and yeast extract (water-soluble portion of autolyzed yeast containing vitamin B complex) with 95.5% of total water volume and steam sterilize.	20.0 g yeast extract
3.	Cool to 8°C and add the LHB Add $\beta$ -NAD (filter sterilized 10 mg/mL in purified water)	100mL or 50mL LHB (see A2.2 for preparation of 50% water lysed horse blood, or use 50mL if use horse blood lysed by freeze/thaw without water dilution in water) 2.0mL $\beta$ -NAD

Abbreviations: LHB, lysed horse blood; MHB, Mueller-Hinton broth; pH, negative logarithm of hydrogen ion concentration;  
 $\beta$ -NAD, beta-nicotinamide adenine dinucleotide

- Modify Table X as indicated on the next slide

# Overall Proposal for Approval

**Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)**

Antimicrobial Agent	<i>Streptococcus pneumoniae</i> ATCC®a 49619	<i>Mannheimia haemolytica</i> ATCC® 33396	<i>Histophilus somni</i> ATCC® 700025 in MHF-Y	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090 in MHF-Y
Amoxicillin-clavulanate	0.03/0.015–0.12/0.06	–	–	–
<b>Ampicillin</b>	0.06–0.25	–	–	<b>0.06–0.25</b>
Cefovecin	0.12–0.5	–	<b>0.004–0.016</b>	<b>0.008–0.06</b>
Cefpodoxime	0.03–0.12	–	–	–
Cefquinome	0.015–0.06	–	0.002–0.008	0.004–0.03
Ceftiofur	0.12–0.5	–	<b>0.001–0.004</b>	<b>0.004–0.03</b>
Cephalothin	0.5–2	–	–	–
Chloramphenicol	2–8	–	–	–
Clindamycin	0.03–0.12	–	–	–
Danofloxacin	–	–	<b>0.03–0.25</b>	0.03–0.12
Doxycycline	0.016–0.12	–	–	–
Enrofloxacin	–	–	<b>0.016–0.12</b>	0.016–0.06
Erythromycin	0.03–0.12	–	–	–
Florfenicol	1–4	–	0.12–0.5	0.25–1
Gamithromycin	0.03–0.12	–	<b>0.5–2</b>	2–8
Gentamicin	–	–	<b>4–16</b>	8–32
Imipenem	0.03–0.12	–	–	–
Kanamycin-cephalexin	64/6.4	–	–	–
Marbofloxacin	–	–	0.016–0.12	0.016–0.06
Penicillin	0.25–1	–	0.016–0.06	0.12–1
Pradofloxacin	–	–	0.004–0.016	<b>0.008–0.03</b>
Rifampin	0.015–0.06	–	–	–
<b>Spectinomycin</b>	–	–	<b>16–64</b>	<b>32–128</b>
Tetracycline	0.06–0.5	–	<b>0.5–2</b>	<b>0.5–2</b>
Tiamulin	0.5–4	–	–	8–32
Tildipirosin	–	–	<b>2–16</b>	2–16
Tilmicosin	–	–	<b>4–16</b>	<b>8–32</b>
Trimethoprim-sulfamethoxazole	0.12/2.4–1/19	–	<b>0.06/1.14–0.25/4.75</b>	<b>0.03/0.57–0.25/4.75</b>
Tulathromycin	0.12–1	2–8	4–32	<b>16–128</b>
Vancomycin	0.12–0.5	–	–	–



# Overall Proposal for Approval

**Table 5B. (Continued)**

## MIC Testing Conditions for Clinical Isolates and Performance of QC

Organism	Method	Medium	Incubation	Comments
<i>Staphylococcus hyicus</i>	Broth microdilution	CAMHB + thymidine phosphorylase (0.2 IU/mL)	Ambient air; 18–24 hours; 35°C	For testing sulfonamides and trimethoprim only. <sup>1</sup>
<i>Streptococcus</i> spp.	Broth microdilution Agar dilution	CAMHB + LHB (2.5% to 5% v/v)  MHA + 5% defibrinated sheep blood	Ambient air; 20–24 hours; 35°C  5% CO <sub>2</sub> ; 20–24 hours; 35°C	
<i>Pasteurella multocida</i> and <i>M. haemolytica</i>	Broth microdilution Agar dilution	CAMHB <sup>b</sup>  MHA + 5% defibrinated sheep blood	18–24 hours; 35°C  18–24 hours; 35°C	Isolates tested by agar dilution that fail to grow in ambient air should be tested by broth microdilution.
<i>H. somni</i> and <i>A. pleuropneumoniae</i>	Broth microdilution	<b>MHF-Y</b>	<b>5% CO<sub>2</sub>; 20–24 hours; 35°C</b>	
	Agar dilution	Chocolate MHA	5% CO <sub>2</sub> ; 20–24 hours; 35°C	



# Next Steps

- Rapidly publish “supplement update” with this information (previous example of M100-S20-U)
  - Check with CLSI management to determine if a “supplement update” can be sent out due to the immediate need for this media.
  - The “supplement update” would aid laboratories in transitioning from VFM to MHF-Y in the interim cycle of VET01 being revised and published.
- Publish revised VET01 and VET08 and completely remove VFM media, methods, recommendations, etc.
  - Prepare project proposal for VET01 revision and the VAST SC members will review for approval in July
  - Submit project proposal by 1 August 2019 for Microbiology Expert Panel endorsement and Consensus Council for approval in September 2019

# Next Steps

- Disband VFM working group upon VET01 and VET08 revisions
  - Unless needed for further interpretive criteria adjustments.
  - Unless needed to address any user community comments, questions or other issues once the next edition is published.

# Supplement Update Proposal for Approval

Publish the alternative media and revised QC ranges for App and HS in a separate supplement earlier than the normal VET08 publication schedule with the following information:

- Revised Tables 2I and 2J with updates to:
  - Testing Conditions box (new media)
  - QC Recommendations box (new Table 5B)
- Revised Table 5B with new QC ranges and testing conditions
- New Appendix F with alternative media preparation

# Supplement Update Proposal for Approval

**Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)**

Antimicrobial Agent	<i>Streptococcus pneumoniae</i> ATCC®a 49619	<i>Mannheimia haemolytica</i> ATCC® 33396	<i>Histophilus somni</i> ATCC® 700025 in VFM	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090 in VFM	<i>Histophilus somni</i> ATCC® 700025 in MHF-Y	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090 in MHF-Y
Amoxicillin-clavulanate	0.03/0.015–0.12/0.06	–	–	–	–	–
Ampicillin	0.06–0.25	–	–	–	–	0.06–0.25
Cefovecin	0.12–0.5	–	0.001–0.008	0.008–0.03	0.004–0.016	0.008–0.06
Cefpodoxime	0.03–0.12	–	–	–	–	–
Cefquinome	0.015–0.06	–	0.002–0.008	0.004–0.03	0.002–0.008	0.004–0.03
Ceftiofur	0.12–0.5	–	0.0005–0.004	0.004–0.016	0.001–0.004	0.004–0.03
Cephalothin	0.5–2	–	–	–	–	–
Chloramphenicol	2–8	–	–	–	–	–
Clindamycin	0.03–0.12	–	–	–	–	–
Danofloxacin	–	–	0.016–0.12	0.03–0.12	0.03–0.25	0.03–0.12
<b>Doxycycline</b>	<b>0.016–0.12</b>	–	–	–	–	–
Enrofloxacin	–	–	0.016–0.06	0.016–0.06	0.016–0.12	0.016–0.06
Erythromycin	0.03–0.12	–	–	–	–	–
Florfenicol	1–4	–	0.12–0.5	0.25–1	0.12–0.5	0.25–1
Gamithromycin	0.03–0.12	–	0.25–1	2–8	0.5–2	2–8
Gentamicin	–	–	8–32	8–32	4–16	8–32
Imipenem	0.03–0.12	–	–	–	–	–
Kanamycin-cephalexin	64/6.4	–	–	–	–	–
Marbofloxacin	–	–	0.016–0.12	0.016–0.06	0.016–0.12	0.016–0.06
Penicillin	0.25–1	–	0.016–0.06	0.12–1	0.016–0.06	0.12–1
Pradofloxacin	–	–	0.004–0.03	0.004–0.016	0.004–0.016	0.008–0.03
Rifampin	0.015–0.06	–	–	–	–	–
<b>Spectinomycin</b>	–	–	–	–	16–64	32–128
Tetracycline	0.06–0.5	–	0.12–1	0.25–2	0.5–2	0.5–2
Tiamulin	0.5–4	–	–	8–32	–	8–32
Tildipirosin	–	–	2–8	2–16	2–16	2–16
Tilmicosin	–	–	2–16	4–32	4–16	8–32
Trimethoprim-sulfamethoxazole	0.12/2.4–1/19	–	0.03/0.57–0.125/2.38	0.016/0.28–0.06/1.14	0.06/1.14–0.25/4.75	0.03/0.57–0.25/4.75
Tulathromycin	0.12–1	2–8	4–32	16–64	4–32	16–128
Vancomycin	0.12–0.5	–	–	–	–	–

# Supplement Update Proposal for Approval

**Table 5B. (Continued)**

## MIC Testing Conditions for Clinical Isolates and Performance of QC

Organism	Method	Medium	Incubation	Comments
<i>Staphylococcus hyicus</i>	Broth microdilution	CAMHB + thymidine phosphorylase (0.2 IU/mL)	Ambient air; 18–24 hours; 35°C	For testing sulfonamides and trimethoprim only. <sup>1</sup>
<i>Streptococcus</i> spp.	Broth microdilution	CAMHB + LHB (2.5% to 5% v/v)	Ambient air; 20–24 hours; 35°C	
	Agar dilution	MHA + 5% defibrinated sheep blood	5% CO <sub>2</sub> ; 20–24 hours; 35°C	
<i>Pasteurella multocida</i> and <i>M. haemolytica</i>	Broth microdilution	CAMHB <sup>b</sup>	18–24 hours; 35°C	Isolates tested by agar dilution that fail to grow in ambient air should be tested by broth microdilution.
	Agar dilution	MHA + 5% defibrinated sheep blood	18–24 hours; 35°C	
<i>H. somni</i> and <i>A. pleuropneumoniae</i>	Broth microdilution	VFM	5% CO <sub>2</sub> ; 20–24 hours; 35°C	
		MHF-Y	5% CO <sub>2</sub> ; 20–24 hours; 35°C	
	Agar dilution	Chocolate MHA	5% CO <sub>2</sub> ; 20–24 hours; 35°C	

# Supplement Update Proposal for Approval

Add new media preparation:

Step	Action	Comments
1.	Prepare the MHB according to the manufacturer's instructions (see Appendix A3.1).	MHB (22.0 g) per L: <ul style="list-style-type: none"><li>• 3.0 g beef extract (from 300 g beef infusion)</li><li>• 17.5 g acid hydrolysis of casein</li><li>• 1.5 g starch</li></ul> Unless the MHB has the correct concentrations of divalent cations ( $\text{Ca}^{++}$ and $\text{Mg}^{++}$ ), add appropriate salts to provide 20 to 25 mg/L calcium and 10 to 12.5 mg/L magnesium (see A2.1).
2.	Mix the MHB and yeast extract (water-soluble portion of autolyzed yeast containing vitamin B complex) with 95.5% of total water volume and steam sterilize.	20.0 g yeast extract
3.	Cool to 8°C and add the LHB Add $\beta$ -NAD (filter sterilized 10 mg/mL in purified water)	100mL or 50mL LHB (see A2.2 for preparation of 50% water lysed horse blood, or use 50mL if use horse blood lysed by freeze/thaw without water dilution in water) 2.0mL $\beta$ -NAD



# Thank You - Participants

Donald J. Bade  
Chandra Machin  
Microbial Research, Inc. (MRI)

Cynthia C. Knapp  
Scott Killian  
Thermo Fisher Scientific

Dr. Sahin  
Carrie Thimmesch  
Iowa State University (ISU)

Maria M. Traczewski  
The Clinical Microbiology Institute  
(CMI)

Dana Dressel  
Meredith Hackel  
IHMA

Brian Lubbers  
Kansas State Veterinary Diagnostic  
Laboratory

Anil Thachil  
Melissa Sue Aprea  
Cornell University Animal Health Diagnostic Center



# **Thank You - Sponsors**

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**Without your Financial Support, we would not  
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