

7 August 2019

To: Recipients of VET08, 4th ed.

From: Jennifer K. Adams, MT(ASCP), MSHA
Vice President, Standards and Quality

Subject: Replacement Broth Medium for Veterinary Fastidious Medium and New
Actinobacillus pleuropneumoniae ATCC® 27090 and *Histophilus somni* ATCC®
700025 Minimal Inhibitory Concentration Quality Control Ranges

This notice is intended to inform users of a new broth medium approved for antimicrobial susceptibility testing (AST) of the veterinary fastidious pathogens *Actinobacillus pleuropneumoniae* and *Histophilus somni* and new minimal inhibitory concentration (MIC) quality control (QC) ranges for *A. pleuropneumoniae* ATCC® 27090 and *H. somni* ATCC® 700025. Mueller-Hinton fastidious broth medium with yeast extract (MHF-Y) may now be used in place of veterinary fastidious medium (VFM). This substitution applies to the usages of VFM as published in CLSI document VET01, *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*, 5th ed. and its supplement, CLSI document VET08, *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*, 4th ed. This memo has been provided to customers who have already received electronic and/or print copies of VET08 and will be included with all print versions going forward. This memo will also be posted on the CLSI website (“CLSI Document Corrections & Updates” page), included as an eCLIPSE™ Bulletin Board message, and linked in the VET08 listing in the CLSI Shop. The specific instances in which MHF-Y replaces VFM in the applicable methods are detailed in a separate VET01 memo. Revisions reflecting MHF-Y as an alternative to VFM and the new MIC QC ranges will be published in a forthcoming revised edition of VET08, 4th ed. (ie, the current edition). In VET08, 5th ed., the subcommittee plans to remove all mentions of VFM and include only MHF-Y.

In 2012, the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was notified that the vendor would no longer provide Supplement C™, an essential component of VFM, due to the discontinuation of a critical component by one of the vendor’s suppliers. Following discussions regarding the need for VFM, the component supplier and the vendor continued providing Supplement C™ and VFM through 2017. However, the product’s cost more than doubled, and the product was often back ordered. Additionally, there was no guarantee that the manufacturer would continue producing Supplement C™ beyond 2017. In response to these challenges, the subcommittee formed the Working Group (WG) on Veterinary Fastidious Medium. The WG’s objective was to develop a new medium that would replace VFM, not depend on the availability of Supplement C™, and adequately support the growth of *A. pleuropneumoniae* and *H. somni*. The WG’s other important goal was to evaluate the organisms’ MIC values on the two media such that breakpoints that were already approved by the Subcommittee on VAST for VFM would remain effective at predicting the antimicrobial agent susceptibilities to these important veterinary pathogens.

In May 2017, several batches of Supplement C™ were contaminated with bacteria and unusable, which resulted in extensive back ordering of VFM. The WG identified a suitable replacement media, MHF-Y, procured funding, and completed testing in April 2019. As of June 2019, Supplement C™ and VFM have been back ordered since December 2018, and their release is not expected until late August 2019.

During the 14-15 June 2019 meeting of the CLSI Subcommittee on VAST, data were presented that warranted the approval of MHF-Y to be used immediately as an alternative to VFM. For VET08, this decision included the addition of MIC QC ranges for *A. pleuropneumoniae* ATCC® 27090 and *H. somni* ATCC® 700025 using MHF-Y.

Excerpts from VET08 are included below for the following tables:

- Table 2I, Zone Diameter and MIC Breakpoints for *Actinobacillus pleuropneumoniae*
- Table 2J, Zone Diameter and MIC Breakpoints for *Histophilus somni*
- Table 5B, MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)

The first excerpt for each table shows the text as originally published in VET08, 4th ed. The second excerpt shows the revised text that will be included in a forthcoming revised edition of VET08, 4th ed. Updates to reflect these revisions will also be made in the Overview of Changes.

Table 2I. Zone Diameter and MIC Breakpoints for *Actinobacillus pleuropneumoniae* and Table 2J. Zone Diameter and MIC Breakpoints for *Histophilus somni* (as originally published in 4th ed.):

The testing conditions for *A. pleuropneumoniae* and *H. somni* as originally published in the 4th edition are shown in the identical Table 2I and Table 2J excerpt below.

<p>Testing Conditions</p> <p>Medium: Disk diffusion and agar dilution: Chocolate MHA (see VET01¹ for preparation of chocolate MHA) Broth dilution: VFM</p> <p>Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard using colonies from an overnight (18- to 24-hour) culture on a chocolate agar plate incubated in 5% CO₂</p> <p>Incubation: Disk diffusion and dilution methods: 35°C ± 2°C; 5% CO₂; 20-24 hours</p>	<p>Routine QC Recommendations (see Table 4B and 5B for acceptable QC ranges)</p> <p><i>A. pleuropneumoniae</i> ATCC® 27090 <i>Histophilus somni</i> ATCC® 700025</p> <p>When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.</p>
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Table 2I. Zone Diameter and MIC Breakpoints for *Actinobacillus pleuropneumoniae* and Table 2J. Zone Diameter and MIC Breakpoints for *Histophilus somni* (revised, to be published in forthcoming revised 4th ed.):

The revised testing conditions for *A. pleuropneumoniae* and *H. somni* are shown in the revised identical Table 2I and Table 2J excerpt below. All revisions are highlighted.

Testing Conditions	
Medium:	Disk diffusion and agar dilution: Chocolate MHA (see VET01 ¹ for preparation of chocolate MHA) Broth dilution: VFM (see VET01 ¹ for preparation of VFM) MHF-Y (see VET01 ¹ for preparation of MHF-Y)
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard using colonies from an overnight (18- to 24-hour) culture on a chocolate agar plate incubated in 5% CO ₂
Incubation:	Disk diffusion and dilution methods: 35 °C ± 2 °C; 5% CO ₂ ; 20-24 hours

Routine QC Recommendations (see Table 4B and 5B for acceptable QC ranges)
<i>A. pleuropneumoniae</i> ATCC ^{®a} 27090 <i>Histophilus somni</i> ATCC [®] 700025
When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods) (as originally published in 4th ed.):

The MIC QC ranges for *A. pleuropneumoniae* ATCC[®] 27090 and *H. somni* ATCC[®] 700025 and associated testing conditions as originally published in the 4th edition are shown in the Table 5B excerpt below.

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

Antimicrobial Agent	MIC QC Ranges, µg/mL		
		<i>Histophilus somni</i> ATCC [®] 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC [®] 27090
Amoxicillin-clavulanate		-	-
Ampicillin		-	-
Cefovecin		0.001-0.008	0.008-0.03
Cefpodoxime		-	-
Cefquinome		0.002-0.008	0.004-0.03
Ceftiofur		0.0005-0.004	0.004-0.016
Cephalothin		-	-
Chloramphenicol		-	-
Clindamycin		-	-
Danofloxacin		0.016-0.12	0.03-0.12
Doxycycline		-	-
Enrofloxacin		0.016-0.06	0.016-0.06
Erythromycin		-	-
Florfenicol		0.12-0.5	0.25-1
Gamithromycin		0.25-1	2-8
Gentamicin		8-32	8-32
Imipenem		-	-
Kanamycin-cephalexin		-	-
Marbofloxacin		0.016-0.12	0.016-0.06
Penicillin		0.016-0.06	0.12-1
Pradofloxacin		0.004-0.03	0.004-0.016
Rifampin		-	-
Tetracycline		0.12-1	0.25-2
Tiamulin		-	8-32
Tildipirosin		2-8	2-16
Tilmicosin		2-16	4-32
Trimethoprim-sulfamethoxazole		0.03/0.57-0.125/2.38	0.016/0.28-0.06/1.14
Tulathromycin		4-32	16-64
Vancomycin		-	-

MIC Testing Conditions for Clinical Isolates and Performance of QC

Organism	Method	Medium	Incubation	Comments
<i>H. somni</i> and <i>A. pleuropneumoniae</i>	Broth microdilution	VFM	5% CO ₂ ; 20-24 hours; 35 °C	
	Agar dilution	Chocolate MHA	5% CO ₂ ; 20-24 hours; 35 °C	

Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods) (revised, to be published in forthcoming revised 4th ed.):

The additional MIC QC ranges for *A. pleuropneumoniae* ATCC® 27090 and *H. somni* ATCC® 700025 and associated testing conditions are shown in the revised Table 5B excerpt below. All revisions are highlighted.

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

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	<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	-	-	-	-
Ampicillin	-	-	-	0.06-0.25
Cefovecin	0.001-0.008	0.008-0.03	0.004-0.016	0.008-0.06
Cefpodoxime	-	-	-	-
Cefquinome	0.002-0.008	0.004-0.03	0.002-0.008	0.004-0.03
Ceftiofur	0.0005-0.004	0.004-0.016	0.001-0.004	0.004-0.03
Cephalothin	-	-	-	-
Chloramphenicol	-	-	-	-
Clindamycin	-	-	-	-
Danofloxacin	0.016-0.12	0.03-0.12	0.03-0.25	0.03-0.12
Doxycycline	-	-	-	-
Enrofloxacin	0.016-0.06	0.016-0.06	0.016-0.12	0.016-0.06
Erythromycin	-	-	-	-
Florfenicol	0.12-0.5	0.25-1	0.12-0.5	0.25-1
Gamithromycin	0.25-1	2-8	0.5-2	2-8
Gentamicin	8-32	8-32	4-16	8-32
Imipenem	-	-	-	-
Kanamycin-cephalexin	-	-	-	-
Marbofloxacin	0.016-0.12	0.016-0.06	0.016-0.12	0.016-0.06
Penicillin	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	-	-	-	-
Spectinomycin	-	-	16-64	32-128
Tetracycline	0.12-1	0.25-2	0.25-2	0.5-2
Tiamulin	-	8-32	-	8-32
Tildipirosin	2-8	2-16	2-16	2-16
Tilmicosin	2-16	4-32	4-16	8-32
Trimethoprim-sulfamethoxazole	0.03/0.57-0.125/2.38	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	4-32	16-64	4-32	16-128
Vancomycin	-	-	-	-

MIC Testing Conditions for Clinical Isolates and Performance of QC

Organism	Method	Medium	Incubation	Comments
<i>H. somni</i> and <i>A. pleuropneumoniae</i>	Broth microdilution	VFM	5% CO ₂ ; 20-24 hours; 35 °C ± 2 °C	
		MHF-Y	5% CO ₂ ; 20-24 hours; 35 °C ± 2 °C	
	Agar dilution	Chocolate MHA	5% CO ₂ ; 20-24 hours; 35 °C	

If you require any additional clarification regarding these revisions, please contact CLSI Customer Service (customerservice@clsi.org).

We appreciate your commitment to CLSI and regret any inconvenience.