

7 August 2019

To: Recipients of VET01, 5th 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 VET01 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 VET01 listing in the CLSI Shop. The new MIC QC ranges are detailed in a separate VET08 memo. Revisions reflecting MHF-Y as an alternative to VFM will be published in a forthcoming revised edition of VET01, 5th ed. (ie, the current edition). In VET01, 6th 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.

Excerpts from VET01 are included below for the following sections:

- Subchapter 5.1.2.2, Broth Media for Testing Fastidious Organisms
- Subchapter 5.7, Special Considerations for Fastidious Organisms (including Table 2, Broth Dilution Testing Considerations for Fastidious Organisms)
- Appendix A, Preparation of Media, Supplements, and Reagents, section A3.5, Broth Media for Testing *H. somni* and *A. pleuropneumoniae*
- Appendix C, Conditions for Broth and Agar Dilution Antimicrobial Susceptibility Tests, Table C2, Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms

The first excerpt for each section shows the text as originally published in VET01, 5th ed. The second excerpt shows the revised text that will be included in a forthcoming revised edition of VET01, 5th ed. Updates to reflect these revisions will also be made in the Overview of Changes.

Subchapter 5.1.2.2. Broth Media for Testing Fastidious Organisms (as originally published in 5th ed.):

The text for broth media for testing fastidious organisms (*A. pleuropneumoniae* and *H. somni*) as originally published in the 5th edition is shown in the Subchapter 5.1.2.2 excerpt below.

5.1.2.2 Broth Media for Testing Fastidious Organisms

Media that can be used for testing fastidious organisms using methods described in this standard are:

- CAMHB + 2.5% to 5% lysed horse blood (LHB) (see Appendix A3.3)
- Veterinary fastidious medium (VFM) broth (see Appendix A3.5)

Instructions for preparing these media are provided in Appendix A.

Subchapter 5.1.2.2. Broth Media for Testing Fastidious Organisms (revised, to be published in forthcoming revised 5th ed.):

The revised text for broth media for testing fastidious organisms (*A. pleuropneumoniae* and *H. somni*) is shown in the revised Subchapter 5.1.2.2 excerpt below. All revisions are highlighted.

5.1.2.2 Broth Media for Testing Fastidious Organisms

Media that can be used for testing fastidious organisms using methods described in this standard are:

- CAMHB + 2.5% to 5% lysed horse blood (LHB) (see Appendix A3.3)
- Veterinary fastidious medium (VFM) broth (see Appendix A3.5.1)
- Mueller-Hinton fastidious broth medium with yeast extract (MHF-Y) (see Appendix A3.5.2)

Instructions for preparing these media are provided in Appendix A.

Subchapter 5.7. Special Considerations for Fastidious Organisms (as originally published in 5th ed.):

The recommended media for broth dilution testing of *A. pleuropneumoniae* and *H. somni* as originally published in the 5th edition is shown in the Subchapter 5.7 excerpt below.

5.7 Special Considerations for Fastidious Organisms

CAMHB, previously described for the rapidly growing aerobic pathogens, is not adequate for AST of fastidious organisms. If MIC tests are performed with fastidious organisms *P. multocida*, *M. haemolytica*, *A. pleuropneumoniae*, *H. somni*, *Streptococcus* spp. including *S. suis* or B-hemolytic and viridans group streptococci, the medium, QC procedures, breakpoints, and interpretive categories must be modified to fit each organism (see Table 2). Broth dilution testing of *A. pleuropneumoniae* and *H. somni* has been shown to be reliable when using VFM, as described in Table 2.

Table 2. Broth Dilution Testing Considerations for Fastidious Organisms

Step	Action	Organisms		
		<i>Streptococcus</i> spp. Including <i>S. suis</i> *	<i>P. multocida</i> and <i>M. haemolytica</i>	<i>A. pleuropneumoniae</i> and <i>H. somni</i>
1.	Using the colony suspension procedure (see Subchapter 5.3.2), prepare a suspension in MHB or saline from an organism grown on the source plates indicated and adjust with broth or saline to achieve turbidity equivalent to a 0.5 McFarland standard.	Inoculate the sheep blood agar plate and incubate for 18-20 hours in 5% CO ₂ .	Inoculate the sheep blood agar plate and incubate for 18-24 hours in ambient air or 5% CO ₂ .	Inoculate the nonselective blood or chocolate agar plate and incubate for 18-24 hours in 5% CO ₂ .
2.	Inoculate plates or trays prepared using the recommended medium within 15 minutes after adjusting the turbidity of the inoculum suspension (see Appendix A3 for medium preparation or obtain commercially).	Use CAMHB with LHB (2.5% to 5% v/v) (see Appendix A3.3).	Use CAMHB†.	Use VFM (see Appendix A3.5).

Subchapter 5.7. Special Considerations for Fastidious Organisms (revised, to be published in forthcoming revised 5th ed.):

The recommended media for broth dilution testing of *A. pleuropneumoniae* and *H. somni* is shown in the revised Subchapter 5.7 excerpt below. All revisions are highlighted.

5.7 Special Considerations for Fastidious Organisms

CAMHB, previously described for the rapidly growing aerobic pathogens, is not adequate for AST of fastidious organisms. If MIC tests are performed with fastidious organisms *P. multocida*, *M. haemolytica*, *A. pleuropneumoniae*, *H. somni*, *Streptococcus* spp. including *S. suis* or B-hemolytic and viridans group streptococci, the medium, QC procedures, breakpoints, and interpretive categories must be modified to fit each organism (see Table 2). Broth dilution testing of *A. pleuropneumoniae* and *H. somni* has been shown to be reliable when using VFM and MHF-Y, as described in Table 2.

Table 2. Broth Dilution Testing Considerations for Fastidious Organisms

Step	Action	Organisms		
		<i>Streptococcus</i> spp. Including <i>S. suis</i> *	<i>P. multocida</i> and <i>M. haemolytica</i>	<i>A. pleuropneumoniae</i> and <i>H. somni</i>
1.	Using the colony suspension procedure (see Subchapter 5.3.2), prepare a suspension in MHB or saline from an organism grown on the source plates indicated and adjust with broth or saline to achieve turbidity equivalent to a 0.5 McFarland standard.	Inoculate the sheep blood agar plate and incubate for 18-20 hours in 5% CO ₂ .	Inoculate the sheep blood agar plate and incubate for 18-24 hours in ambient air or 5% CO ₂ .	Inoculate the nonselective blood or chocolate agar plate and incubate for 18-24 hours in 5% CO ₂ .
2.	Inoculate plates or trays prepared using the recommended medium within 15 minutes after adjusting the turbidity of the inoculum suspension (see Appendix A3 for medium preparation or obtain commercially).	Use CAMHB with LHB (2.5% to 5% v/v) (see Appendix A3.3).	Use CAMHB [†] .	Use VFM (see Appendix A3.5.1) or MHF-Y (see Appendix A3.5.2).

Appendix A. Preparation of Media, Supplements, and Reagents (as originally published in 5th ed.):

The instructions for preparing VFM for broth microdilution testing of *A. pleuropneumoniae* and *H. somni* as originally published in the 5th edition are shown in the Appendix A excerpt below.

A3.5 Broth Media for Testing *H. somni* and *A. pleuropneumoniae*

The steps for preparing 1 L veterinary fastidious medium (VFM) 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"> • 3.0 g beef extract (from 300 g beef infusion) • 17.5 g acid hydrolysis of casein • 1.5 g starch <p>Unless the MHB has the correct concentrations of divalent cations (Ca⁺⁺ and Mg⁺⁺), add appropriate salts to provide 20 to 25 mg/L calcium and 10 to 12.5 mg/L magnesium (see A2.1).</p>
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 and nutritional supplement aseptically.	20.0 mL LHB (see A2.2 for preparation of LHB) 20.0 mL Supplement C™ (or the equivalent)

Appendix A. Preparation of Media, Supplements, and Reagents (revised, to be published in forthcoming revised 5th ed.):

The revised instructions for preparing VFM and MHF-Y for broth microdilution testing of *A. pleuropneumoniae* and *H. somni* are shown in the revised Appendix A excerpt below. All revisions are highlighted.

A3.5 Broth Media for Testing *H. somni* and *A. pleuropneumoniae*

A3.5.1 Veterinary Fastidious Medium

The steps for preparing 1 L veterinary fastidious medium (VFM) 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"> • 3.0 g beef extract (from 300 g beef infusion) • 17.5 g acid hydrolysis of casein • 1.5 g starch <p>Unless the MHB has the correct concentrations of divalent cations (Ca⁺⁺ and Mg⁺⁺), add appropriate salts to provide 20 to 25 mg/L calcium and 10 to 12.5 mg/L magnesium (see A2.1).</p>
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 and nutritional supplement aseptically.	20.0 mL LHB (see A2.2 for preparation of LHB) 20.0 mL Supplement C™ (or the equivalent)

A3.5.2 Mueller-Hinton Fastidious Medium With Yeast Extract

The steps for preparing 1 L Mueller-Hinton fastidious broth medium with yeast extract (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).	<p>The MHB contains 22.0 g/L of the following ingredients when prepared according to the manufacturer's instructions:</p> <ul style="list-style-type: none">• 3.0 g beef extract (from 300 g beef infusion)• 17.5 g acid hydrolysis of casein• 1.5 g starch <p>Unless the MHB has the correct concentrations of divalent cations (Ca⁺⁺ and Mg⁺⁺), add appropriate salts to provide 20 to 25 mg/L calcium and 10 to 12.5 mg/L magnesium (see A2.1).</p>
2.	Mix the MHB and yeast extract (water-soluble portion of autolyzed yeast containing vitamin B complex) with 95.5% of total water volume (when adding 50 mL LHB in step 3) or 95% of total water volume (when adding 100 mL LHB in step 3) and steam sterilize.	20.0 g yeast extract
3.	Cool to 2-8°C and add the LHB aseptically.	100 mL LHB (see A2.2 for preparation of 50% water LHB), or use 50 mL if using a freeze-thaw method for LHB aliquots without dilution in water
4.	Add 1.0 mL of 20 mg/mL β-NAD.	<p>Prepare 20 mg/mL β-NAD as follows:</p> <ul style="list-style-type: none">• Dissolve β-NAD in sterile deionized water to a concentration of 20 mg/mL.• Sterilize the solution through a 0.2-mm membrane filter.• Store aliquots of stock solution at -20°C to be defrosted as needed (do not refreeze unused solution).

Appendix C. Conditions for Broth and Agar Dilution Antimicrobial Susceptibility Tests, Table C2, Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms (as originally published in 5th ed.):

The testing conditions for *A. pleuropneumoniae* and *H. somni* as originally published in the 5th edition are shown in the Appendix C, Table C2 excerpt below.

Table C2. Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms

Organism/ Organism Group	Table	Medium	Incubation	Incubation Time	Minimal QC*
<i>Actinobacillus pleuropneumoniae</i> or <i>Histophilus somni</i>	VET08 ¹ Table 2I or 2J	Broth: VFM Agar: Chocolate MHA	Broth and agar: 35 °C ± 2 °C; 5% CO ₂	20-24 hours	<i>A. pleuropneumoniae</i> ATCC® 27090 or <i>H. somni</i> ATCC® 700025

Appendix C. Conditions for Broth and Agar Dilution Antimicrobial Susceptibility Tests, Table C2, Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms (revised, to be published in forthcoming revised 5th ed.):

The revised testing conditions for *A. pleuropneumoniae* and *H. somni* are shown in the revised Appendix C, Table C2 excerpt below. All revisions are highlighted.

Table C2. Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms

Organism/ Organism Group	Table	Medium	Incubation	Incubation Time	Minimal QC*
<i>Actinobacillus pleuropneumoniae</i> or <i>Histophilus somni</i>	VET08 ¹ Table 2I or 2J	Broth: VFM or MHF-Y Agar: Chocolate MHA	Broth and agar: 35 °C ± 2 °C; 5% CO ₂	20-24 hours	<i>A. pleuropneumoniae</i> ATCC® 27090 or <i>H. somni</i> ATCC® 700025

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.