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Instructions for Use

SHIBAM MEDIUM

Cat. no. A146	SHIBAM Medium, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

SHIBAM (STEC Heart Infusion washed Blood Agar with Mitomycin C) Medium is recommended for use as a selective and differential growth medium for the cultivation of Shiga toxin-producing *E. coli* (STEC) from food and other samples. This medium can be used as a screening method for non-O157 STEC as mentioned by the Food and Drug Administration in the Bacteriological Analytical Manual (FDA BAM).

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Escherichia coli is a diverse species of bacteria that includes non-pathogenic strains as well as strains possessing a wide variety of virulence factors that allows them to cause a heterogenous spectrum of disease in humans and animals. One such group of pathogenic *E. coli* are those that have acquired the ability to produce Shiga-like toxins 1 and/or 2 (Stx1 and/or Stx2). This group, collectively, is referred to by several names, including enterohemorrhagic *E. coli* (EHEC), verotoxin-producing *E. coli* (VTEC), or most commonly, Shiga toxin-producing *E. coli* (STEC).[1-2] Cattle appear to be the major reservoir for STEC, and outbreaks of disease are generally associated with the consumption of beef or other food products that have been in contact with contaminated beef.[3-4,11] STEC infections can cause gastroenteritis ranging from watery to bloody diarrhea, and severe infection can lead to hemolytic uremic syndrome (HUS). Treatment with antibiotics can paradoxically increase the severity of the disease by stimulating *E. coli* to release more toxin.

Historically, serotype O157 was the most commonly documented STEC serotype. However, not all STEC belong to this serotype. Over 250 serotypes have been identified as STEC, with over 100 of these being associated with human illness. Data suggests that 50% or more of human infections are caused by non-O157 STEC strains.[19] In the United States, O26, O45, O103, O111, O121, and O145 are considered to be the "top six" non-O157 STEC serotypes, though even this list does not account for all human cases.[5-6]

Research shows that production of enterohemolysin (not to be confused with alpha-hemolysin) by *E. coli* is highly correlated with Shiga toxin production. Colonies that express enterohemolysin produce beta-hemolytic colonies indicated by a clear zone when grown on media containing washed erythrocytes. In 2001, Sugiyama *et. al.* found that of 185 STEC strains tested--including O157--97% were beta-hemolytic on SHIBAM Medium.[10] Therefore, identification of hemolytic colonies on washed sheep blood agar plates has proven to be a useful screening method for STEC.[7]

SHIBAM Medium utilizes washed sheep blood cells in a blood agar base first described by Beutin *et. al.* and is appropriate as a screening tool for O157 and non-O157 STEC strains.[8] In addition, the medium includes improvements made by Kimura *et. al.* and Sugiyama *et. al.* in order to improve identification of STEC isolates.[9,10] Hardy Diagnostics SHIBAM Medium complies with the Food and Drug Administration, Bacteriological Analytical Manual (FDA BAM) formulation and the medium is listed in BAM as a screening method for non-O157 STEC.[18]

FORMULA

Ingredients per liter of deionized water:*

Agar	14.0 gm
Peptones	17.0 gm
Sodium Chloride	5.0 gm
Tryptose	5.0 gm
Yeast Extract	4.0 gm
Calcium Chloride	1.47 gm
Mitomycin C	0.5 mg
Washed Defibrinated Sheep Blood	40 ml

Final pH 7.2 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the sample should be inoculated onto an appropriate transport media such as modified Cary Blair (Cat. no. 280505) and refrigerated until inoculation. Consult listed references for information on sample collection.[12-18]

Method of Use: Allow plates to warm to room temperature prior to use. The agar surface should be dry prior to

inoculating. Inoculate the sample onto the medium as soon as possible after receipt in the laboratory. Incubate plates aerobically at 35-37ºC. for 16-24 hours. Observe plates for characteristic colony morphology and beta-hemolysis (clear zones).

INTERPRETATION OF RESULTS

STEC isolates should produce smooth, off-white colonies that are beta-hemolytic (produce a clear zone) on SHIBAM. Visualization of beta-hemolytic colonies can be facilitated by viewing plates through transmitted light or by using a light box (Cat. no. 378642000) to enhance clear zones. Most non-STEC *E. coli* will produce non-hemolytic or alpha-hemolytic (green zone) colonies. Species other than *E. coli* should be inhibited or produce non-hemolytic colonies (see Limitations for exceptions).

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Growth and/or hemolysis on SHIBAM Medium does not necessarily indicate the isolate produces Shiga-toxin, as some non-STEC strains may also produce enterohemolysin. Testing for confirmation of Shiga-toxin production should be carried out by approved methods.

E. coli ATCC 25922 may produce hemolysis on SHIBAM Medium (most likely due to production of alpha-hemolysin). *E. coli* strains carrying the alpha-hemolysin (α-hly) gene may demonstrate zones of hemolysis that are wider than strains that carry the enterohemolysin gene. alpha-hemolysin is most commonly found in uropathogenic *E. coli* and in certain ETEC, EPEC, and STEC strains. Thereofore, *E. coli* ATCC 8739 is recommended as the negative control for QC testing.[18]

Use of a light box (Cat. no. 378642000) will enhance visualization of clearing zones of beta-hemolysis.

Some strains of *Listeria* spp., *Staphylococcus aureus, Salmonella enterica, Serratia marcescens, Edwardsiella tarda,* and *Bacillus cereus* can produce beta-hemolytic growth on SHIBAM Medium. Selective pre-enrichment may be useful to limit the growth of these organisms. Gram staining, serological, and/or biochemical testing must be performed on all isolates for complete identification.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, transport media such as modified Cary Blair (Cat. no. 280505), applicator sticks, other culture media, incinerators, light box (Cat. no. 378642000), and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli O157 ATCC® 43889**	A	24hr	35°C	Aerobic	Growth; beta hemolysis

Escherichia coli ATCC® 8739	A	24hr	35°C	Aerobic	Growth; no hemolysis

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

SHIBAM Agar should appear opaque, and cherry red in color.

E. coli O45 STEC colonies growing on SHIBAM Agar (Cat. no. A146). Incubated aerobically for 24 hours at 35ºC. Note zones of clear beta-hemolysis as viewed through a light box. Image credit FDA BAM.

E. coli (ATCC® 8739) colonies growing on SHIBAM Agar (Cat. no. A146). Incubated aerobically for 24 hours at 35ºC. Note green alpha-hemolysis as viewed through a light box. Image credit FDA BAM.

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IFU-10734[A]

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HDQA 2207D [D]