IFU

Instructions for Use

TRYPTIC SOY BROTH WITH 6.5% SODIUM CHLORIDE

Cat. no. K126	Tryptic Soy Broth (TSB) with 6.5% Sodium Chloride, 13x100mm Tube, 5ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Tryptic Soy Broth (TSB) with 6.5% NaCl is a growth medium recommended for the differentiation of enterococci from the group D streptococci. This medium is also useful as an enrichment step for increasing sensitivity in the detection of MRSA in high risk patients.

SUMMARY

Historically, streptococci have been classified by phenotypic characteristics, such as hemolytic reactions, biochemical tests and Lancefield serological groups. Based on these criteria, enterococci were initially classified as group Dstreptococci. However, in the mid-1980s, molecular tests showed significantgenetic differences between these two groups. Consequently, enterococciare now placed in the genus of *Enterococcus* and the non-enterococcal group D streptococci remain classified as streptococci.[6-9]

The most clinically significant distinction between enterococci and group D streptococci is their difference in antimicrobial resistance. In general enterococci are more resistant to penicillins, cephalosporins, and aminoglycosides, permitting them to survive in hospital environments and cause nosocomial infections in patients receiving broad-spectrum antibiotics.[10,11] The last two decades have seen particularly virulent strains of *Enterococcus* resistant to vancomycin (commonly called Vancomycin-Resistant *Enterococcus* or VRE) emerge in nosocomial infections, especially in the U.S.[10,11]

Further studies on these microbes show differences in physiological characteristics. For example, most species of enterococci will grow in medium containing 6.5% NaCl. However, group D streptococci are inhibited when grown under conditions with high salt concentrations.[7,8]

Additionally, enrichment broths are commonly used to increase sensitivity testing for MRSA. Research shows an increase in isolation rates when using an enrichment broth prior to plating high-risk patient samples.[19] In this capacity, enrichment broths are primarily used as multibroths, where multiple swabs from the same patient are inoculated into a single broth. This method provides substantial savings in media costs and time needed to determine infection when compared to conventional testing methods.[20]

Hardy Diagnostics Tryptic Soy Broth (TSB) with 6.5% NaCl contains enzymatic digest of casein and enzymatic digest of soybean meal, which provide amino acids and complex nitrogenous compounds that promote microbial growth. Dextrose acts as a carbon energy source that facilitates growth. Dipotassium phosphate acts as a buffering agent. The addition of sodium chloride at a concentration of 6.5% permits the differentiation of salt-tolerant microorganisms from salt-intolerant species. At this strength, sodium chloride acts selectively by interfering with membrane permeability and osmotic and electrokinetic equilibria.[14]

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	65.0gm
Pancreatic Digest of Casein	17.0gm
Papaic Digest of Soybean Meal	3.0gm
Dextrose	2.5gm
Dipotassium Phosphate	2.5gm

Final pH 7.3 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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

Specimens appropriate for culture may be handled using various laboratory techniques. Refer to the appropriate reference(s) for specific procedures.[1,4,16,19,20]

Observe aseptic techniques and use standard laboratory precautions.

1. Inoculate tubes lightly with suspect bacterium.

    a. Using a single colony, lightly inoculate the side of the tube, just at the surface of the broth, so as not to

over-inoculate the sample. Alternatively, inoculate tubes using a 0.01ml loop with a 10^{-1} dilution of 18-24 hour primary cultures.

b. Swab specimens from high-risk patients may be directly inserted into the medium as in enrichment for MRSA.

2. Incubate tubes aerobically with loosed caps at 35 +/- 2ºC. for 18-24 hours.

3. Examine tubes at 18-24 hours and 42-48 hours for growth (turbidity). It may be necessary to compare tubes to an uninoculated control tube to determine if growth is present. Growth may be present with or without the formation of a precipitate.

INTERPRETATION OF RESULTS

Refer to the appropriate reference(s) for the accurate interpretation of test results.[1,4,16,19,20]

Growth in broth media indicates a positive reaction and is demonstrated by the presence of turbidity (cloudiness) when compared to an uninoculated control tube. A positive reaction may be present with or without the formation of a precipitate.

Lack of growth indicates a negative reaction when compared to an uninoculated control tube.

1. Most gram-positive enterococci will grow in TSB with 6.5% NaCl. Most strains will show turbidity within 24 hours.

2. Most gram-positive, non-enterococcal group D streptococci fail to grow in TSB with 6.5% NaCl after 48 hours.

3. This medium can be used as an enrichment broth for MRSA cultures from high-risk patients. Tubes will be positive for growth (turbid) after 24 hours. Subculture to an appropriate medium for further testing. Please consult appropriate references for more information.[4,16]

LIMITATIONS

Other gram-positive cocci besides enterococci and staphylococci (e.g. group B streptococci, aerococci and pedicocci) can grow in TSB with 6.5% NaCl broth. Therefore, it is recommended that biochemical and/or serological tests be performed on colonies from pure culture 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, applicator sticks, other culture media, incinerators, 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	
Enterococcus faecalis ATCC® 29212	A	24hr	35°C	Aerobic	Growth

Streptococcus bovis ATCC® 9809	B	24hr	35°C	Aerobic	Inhibited

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

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.

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