

Instructions for Use

TRIPLE SUGAR IRON (TSI) AGAR

Cat. no. L16	Triple Sugar Iron (TSI) Agar, 16x100mm Tube, 6ml Slant	20 tubes/box
Cat. no. L50	Triple Sugar Iron (TSI) Agar, 16x125mm Tube, 8ml Slant	20 tubes/box
Cat. no. R32	Triple Sugar Iron (TSI) Agar, 13x100mm Tube, 4ml Slant	20 tubes/box

INTENDED USE

Hardy Diagnostics Triple Sugar Iron (TSI) Agar is recommended for use in the differentiation of Enterobacteriaceae by their ability to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

SUMMARY

In 1917 Sulkin and Willett described a medium containing the carbohydrates glucose, lactose, and sucrose, and iron salts. ⁽⁸⁾ The medium showed fermentation of these carbohydrates, as well as hydrogen sulfide production. Hajna modified the medium in 1945 to contain phenol red as the pH indicator, and is the formulation still in use today. ⁽³⁾

Glucose is added to the medium since most enteric pathogens uniformly ferment this carbohydrate. Lactose and sucrose are added in ten times the amount of glucose, as most enteric pathogens do not ferment these sugars. As a result, non-pathogenic enterics which do ferment these sugars produce acid in the slant. Pathogenic enterics produce an initially acid slant from the low concentration of glucose, but as growth continues it changes to the alkaline reaction. Sodium thiosulfate is incorporated into the medium as a source of hydrogen sulfide. Ferrous ammonium sulfate serves as the indicator, which turns the butt black in the presence of free hydrogen sulfide gas. Enteric organisms that are capable of fermenting glucose will produce acid (a yellow butt and a red slant), a positive hydrogen sulfide result. Gas production may result and is seen as cracks and bubbles in the medium. If the slant and butt become alkaline, glucose has not been fermented. Organisms showing this reaction are defined as non-fermenters, and derive their nutrients from the peptones present in the medium.

TSI Agar is contained in a tube and is slanted to form a deep butt and short slant. Inoculation is performed with a straight needle by stabbing to the base of the butt, and streaking the slant when the needle is removed. The cap is replaced loosely to facilitate an aerobic atmosphere.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Lactose	10.0gm
Sucrose	10.0gm
Sodium Chloride	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Yeast Extract	3.0gm
Beef Extract	3.0gm
Dextrose	1.0gm
Ferric Ammonium Citrate	0.5gm
Sodium Thiosulfate	0.3gm
Phenol Red	0.024gm
Agar	12.0gm

Final pH 7.3 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or 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 dating 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 quality control incubation times.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Method of Use: Allow the TSI Agar to warm to room temperature before use. Using one isolated, pure colony inoculate, stab, and streak the specimen on the agar as soon as possible after collection. Incubate tubes aerobically at 35-37°C. for 18-24 hours. Examine reaction of medium.

INTERPRETATION OF RESULTS

An alkaline/acid (red slant/yellow butt) reaction is indicative of dextrose fermentation only. An acid/acid (yellow slant/yellow butt) reaction indicates the fermentation of dextrose, lactose and/or sucrose. The absence of carbohydrate fermentation results in an alkaline/alkaline (red slant, red butt) reaction. A blackening of the medium occurs in the presence of H₂S. Bubbles or cracks in the agar indicate the production of gas.

Consult listed references for the identification of colony morphology and further biochemical tests required for identification. ^(1,2,4,6)

LIMITATIONS

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

It is important to stab the butt of the medium. Failure to stab the butt invalidates this test. The integrity of the agar must be maintained when stabbing. Caps must be loosened during this test or erroneous results will occur.

TSI Agar must be read within the 18-24 hour stated incubation period. A false-positive reaction may be observed if read too early. A false-negative reaction may be observed if read later than 24 hours.

An organism that produces hydrogen sulfide may mask acid production in the butt of the medium. However, hydrogen sulfide production requires an acid environment, thus the butt portion should be considered acid.

TSI is not as sensitive in detecting hydrogen sulfide in comparison to other iron containing mediums, such as Sulfide Indole Motility (SIM) Medium (Cat. no. Q30). Thus, organisms that have weak hydrogen sulfide production may show only trace hydrogen sulfide activity, or none at all.

Certain species or strains may give delayed reactions or completely fail to ferment the carbohydrate in the stated manner. However, if the organism fails to ferment glucose within 48 hours, it most likely is not in the Enterobacteriaceae family.

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, slides, staining supplies, other culture media, microscope, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Salmonella enterica</i> ATCC® 14028	C	18-24hr	35°C	Aerobic	Growth; red slant, yellow butt, gas positive, black butt (H ₂ S produced)
<i>Escherichia coli</i> ATCC® 25922	C	18-24hr	35°C	Aerobic	Growth; yellow slant, yellow butt, gas positive, no H ₂ S produced
<i>Pseudomonas aeruginosa</i> ATCC® 27853	C	18-24hr	35°C	Aerobic	Growth; red slant, red butt, no gas, no H ₂ S produced
<i>Shigella sonnei</i> ATCC® 9290	C	18-24hr	35°C	Aerobic	Growth; red slant, yellow butt, no gas, no H ₂ S produced

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

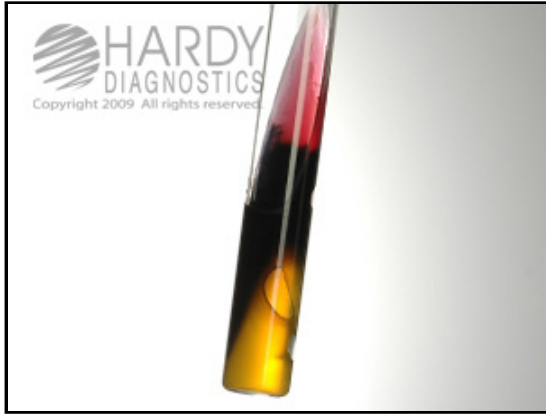
USER QUALITY CONTROL

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

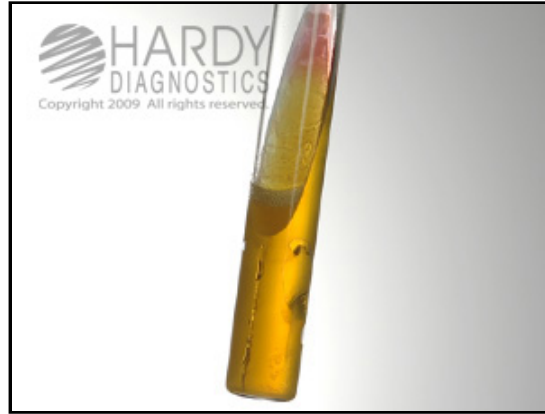
end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Triple Sugar Iron (TSI) Agar should appear slightly opalescent, with a possible slight precipitate, and red in color.



Salmonella enterica (ATCC[®] 14028) growing on TSI Agar (Cat. no. L50). Incubated aerobically for 24 hours at 35°C.



Escherichia coli (ATCC[®] 25922) growing on TSI Agar (Cat. no. L50). Incubated aerobically for 24 hours at 35°C.



Pseudomonas aeruginosa (ATCC[®] 27853) growing on TSI Agar (Cat. no. L50). Incubated aerobically for 24 hours at 35°C.



Uninoculated tube of TSI Agar (Cat. no. L50).

REFERENCES

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3. Hajna. 1945. *J. Bact.*; 49:516.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
6. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. Sulkin and Willett. 1917. *J. Med. Research*; 37:225.

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