IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

SS AGAR

Cat. no. G50	SS Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J139	HE Agar/SS Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics SS Agar is recommended for use as a selective and differential medium for the isolation of *Salmonella* and some *Shigella* species from clinical and non-clinical specimens.

SUMMARY

SS Agar was originally developed as a selective medium for the isolation of *Salmonella* and *Shigella* species. It was also developed to aid in the differentiation of lactose and non-lactose-fermenters from clinical specimens, suspected foods, and other such samples.

The basis for differentiation on SS Agar depends on the fermentation of lactose and the absorption of neutral red as the bile salts precipitate in the acidic condition. Neutral red turns red in the presence of an acidic pH, thus showing fermentation has occurred. The inclusion of bile salts, sodium citrate, and brilliant green serve to inhibit gram-positive and coliform organisms. *Salmonella*, *Shigella*, and other non-lactose-fermenting organisms appear as transparent or translucent colorless colonies on SS Agar. Sodium thiosulfate is added to the medium as a hydrogen sulfide source, and ferric citrate is added as an indicator for hydrogen sulfide production.

FORMULA

Ingredients per liter of deionized water:*

Lactose	10.0gm
Bile Salts No. 3	8.5gm
Sodium Citrate	8.5gm
Sodium Thiosulfate	8.5gm
Beef Extract	5.0gm
Proteose Peptone	5.0gm
Ferric Citrate	1.0gm
Brilliant Green	0.33mg
Neutral Red	0.025gm
Agar	13.5gm

Final pH 7.0 +/- 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), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat 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

Specimen Collection: Consult listed references for information on specimen collection.[1-3,5,7] 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 specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Method of Use: Allow the plates to warm to room temperature and the agar surface to dry before inoculating. Heavily inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37 degrees C. for 18-24 hours. Examine colonial morphology.

INTERPRETATION OF RESULTS

If lactose fermentation occurs, the medium will turn red due to the acidic pH. *Salmonella*, *Shigella*,and other non-lactose fermenters appear as transparent or translucent colorless colonies on SS Agar. Colonies of *Salmonella* spp. may appear with or without black centers.

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1-3,5,7]

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.

The incorporation of brilliant green into this medium makes it highly selective, and has been shown to inhibit the growth of some *Shigella* species. Thus, a nonselective but differential medium such as MacConkey Agar or HE Agar (Cat. nos. G35 or G63, respectively) should also be streaked to increase the recovery of fastidious, or low numbers of, gram-negative organisms.

The bile salts may crystallize over time. They appear as small spider-like puff balls within the medium and do not affect the performance of the medium.

Some strains of *Shigella*, such as *S. sonnei* and *S. dysenteriae* serovar 1, may ferment lactose relatively slowly, and colonies change to lactose-fermenting after cultivation for 2 or more days.[8]

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	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth; colorless colonies with or without black centers
Shigella flexneri ATCC® 12022	A	18-24hr	35°C	Aerobic	Growth; colorless colonies
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to complete inhibition; pink to rose red colonies with precipitate
Enterococcus faecalis ATCC® 29212	B	18-24hr	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.

PHYSICAL APPEARANCE

SS Agar should appear clear, and red-orange in color.

Salmonella enterica (ATCC® 14028) growing on SS Agar (Cat. no. G50). Incubated aerobically for 24 hours at 35ºC.

Shigella flexneri (ATCC® 12022) growing on SS Agar (Cat. no. G50). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) colonies growing on SS Agar (Cat. no. G50). Incubated aerobically for 24 hours at 35ºC.

Uninoculated plate of SS Agar (Cat. no. G50)

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

5. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

8. Ito et al. 1991. Possible Mechanisms Underlying the Slow Lactose Fermentation Phenotype in *Shigella* spp. *Appl. and Environ. Microbio.* 57(10): 2912-2917.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]