IFU

Instructions for Use

SIM (SULFIDE, INDOLE, MOTILITY) MEDIUM

Cat. no. Q30	SIM Medium, 16x100mm Tube, 8ml Deep	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics SIM Medium is recommended for the differentiation of gram-negative enteric bacilli on the basis of sulfide production, indole formation, and motility.

SUMMARY

The formulation of SIM Medium is designed to allow the detection of sulfide production, indole formation and motility.

The medium contains ferrous ammonium sulfate and sodium thiosulfate, which together serve as indicators for the production of hydrogen sulfide. Hydrogen sulfide production is detected when ferrous sulfide, a black precipitate, is produced as a result of ferrous ammonium sulfate reacting with H_2S gas.

Casein peptone, another component of SIM Medium, is rich in tryptophan. Organisms possessing the enzyme tryptophanase degrade tryptophan to indole. Indole is detected upon the addition of Kovacs Reagent (Cat. no. Z67) following incubation of the inoculated medium. Indole combines with p-dimethylaminobenzaldehyde and produces a red band at the top of the medium. A negative indole test produces no color change upon the addition of Kovacs Reagent.

The small amount of agar added to the medium provides a semi-solid structure allowing for the detection of bacterial motility. Motile organisms extend from the stab line and produce turbidity or cloudiness throughout the medium. Non-motile organisms grow only along the stab line and leave the surrounding medium clear.

SIM Medium also contains animal tissue which provides amino acids and nutrients necessary for bacterial growth.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	20.0gm
Peptic Digest of Animal Tissue	6.1gm
Ferrous Ammonium Sulfate	0.2gm
Sodium Thiosulfate	0.2gm
Agar	3.5gm

Final pH 7.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 15-30ºC. away from direct light. Media should not be used if there are any signs of contamination, deterioration 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

Specimen Collection: Specimen collection is not applicable since this medium is not intended for primary isolation from clinical specimens. As a general rule, 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. Consult listed references for information on specimen collection.[2-5]

Method of Use:

1. Using isolated colonies from an 18-24 hour culture on solid media, inoculate the SIM Medium by stabbing the center of the medium to a depth of 1/2 inch.

2. Incubate the inoculated medium aerobically at 35ºC. for 18-24 hours.

3. Observe for H_2S production and motility.

4. Once H_2S and motility reaction have been read and recorded, apply three drops of Kovacs Reagent (Cat. no. Z67) to the surface of the medium.

5. Observe for the development of a pink to red color.

INTERPRETATION OF RESULTS

A positive H_2S test is denoted by a blackening of the medium along the line of inoculation. A negative H_2S test is denoted by the absence of blackening.

A positive motility test is indicated by a diffuse zone of growth flaring from the line of inoculation.

A negative motility test is indicated by growth confined to the stab line.

A positive test for indole is denoted when a pink to red color band is formed at the top of the medium after addition of Kovacs Reagent. A yellow color denotes a negative indole test after addition of Kovacs Reagent.

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 inoculum should be taken from a solid medium. Use of an inoculum from a liquid or broth suspension will delay the initiation of growth and may result in erroneous results.

When inoculating semi-solid media, it is important that the inoculating needle be removed along the exact same line used to inoculate the medium. A fanning motion may result in growth along the stab line that may result in false-positive interpretation.

Motility and H_2S results must be interpreted prior to addition of Kovacs Reagent.

Weakly motile organisms or organisms that possess damaged flagella (due to heating, shaking, or other trauma) often result in false-negative motility tests. Motility results may be confirmed by performing a hanging drop motility test. Consult listed references for procedure.[2-4]

Some microorganisms, such as *Yersinia enterocolitica* , demonstrate motility best at 25ºC.

Organisms that require oxygen for growth, such as *Pseudomonas aeruginosa* , will produce a spreading film on the surface of the medium and will not extend from the line of inoculation where oxygen is depleted.

Erroneous results may occur if caps are not loose during incubation.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as inoculating loops, other culture media, Kovacs Reagent (Cat. no. Z67), swabs, applicator sticks, 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	
					Growth;

Salmonella enterica ATCC® 14028	D	18-24hr	35°C	Aerobic	Motility: positive, H_2S: positive (black color along stab line) Indole: negative
Escherichia coli ATCC® 25922	D	18-24hr	35°C	Aerobic	Growth; Motility: positive, H_2S: negative Indole: positive (Kovacs Reagent turns pink after adding three drops)

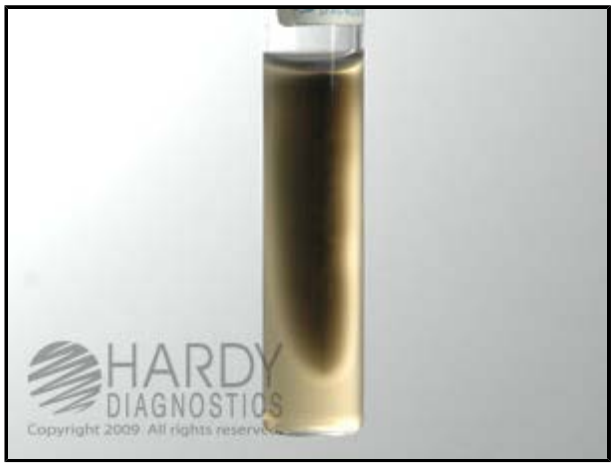
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

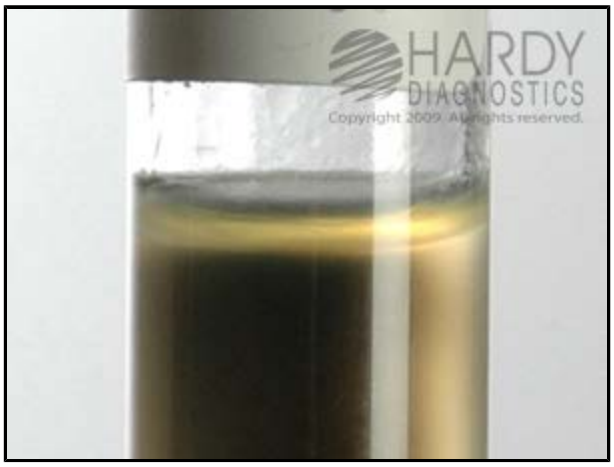
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

SIM Medium should appear semi-solid, slightly opalescent, and medium amber in color.



Salmonella enterica (ATCC® 14028) growing in SIM Medium (Cat. no. Q30). Incubated aerobically for 18 hours at 35ºC.



Three drops of Kovacs Reagent (Cat. no. Z67) were added to the tube with *S. enterica* . The abscence of a red color development was indicative of a negative indole test. Incubated aerobically for 18 hours at 35ºC.

Escherichia coli (ATCC® 25922) growing in SIM Medium (Cat. no. Q30). Incubated aerobically for 18 hours at 35ºC.

Three drops of Kovacs Reagent (Cat. no. Z67) were added to the tube with *E. coli* . The red color development was indicative of a positive indole test. Incubated aerobically for 18 hours at 35ºC.

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. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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

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

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

IFU-10735[A]

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality

management system is certified to ISO 13485.