Gillies Agar No. 2 (Sucrose Salicin Agar) M240

Intended Use:

Recommended for detection of motility, hydrogen sulphide and indole production, fermentation of sucrose and salicin during identification of *Salmonella* and *Shigella* species.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Tryptone	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate	0.250
Sucrose	10.000
Salicin	10.000
Bromothymol blue	0.010
Sodium thiosulphate	0.025
Agar	3.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 48.28 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Distribute in tubes and sterilize by autoclaving at 118° - 121°C for 15 minutes(12-15 lbs pressure). Allow the tubes to cool to 45-50°C in an upright position. Suspend Kovacs reagent strips and lead acetate papers from the cap or the cotton plug over the medium but not touching the surface of the medium.

Principle And Interpretation

Enterobacteriaceae genera consist of gram-negative bacilli and are widely distributed in nature. It includes pathogens such as *Salmonella, Shigella, Yersinia*, diarrheagenic *E.coli* and others. These bacteria cause multitude of diseases in humans and are frequently isolated from clinical specimens. Detection and identification of the bacteria are of importance both from clinical and epidemiological point of view. The other enterobacteria are essentially commensals or saprophytes (1). Gillies Agar No. 2 (2), a modification of Kohns Medium (5) is recommended for detection of motility, hydrogen sulphide, indole production and fermentation of sucrose and salicin. This medium is a reliable substitute for the conventional method of determining the biochemical identity of non-lactose fermenting colonies prior to confirmation by serological typing (1).

Fermentation of sucrose and salicin leads to acid production that causes the pH indicator dye, bromothymol blue, to change from blue to yellow. The accompanying gas production during fermentation causes bubbles to form, which appears in varying degrees from a slight splitting along the wire track to disruption of the medium. Non-motile organisms grow only along the line of inoculation whereas motile species show either a diffuse even growth spreading from the inoculum or more rarely localized outgrowths, which are usually fan shaped or occasionally nodular. Hydrogen sulphide production causes blackening of the lead acetate paper and the formation of indole gives a red colour in the Kovacs reagent strips. Peptone and tryptone serve as sources of essential nutrients for bacterial growth. Sodium chloride maintains the osmotic equilibrium of the medium. Sucrose and salicin are the fermentable carbohydrates with bromothymol blue as the pH indicator. Sodium thiosulphate aids in the production of hydrogen sulphide.

The specimen is inoculated into a preliminary enrichment medium such as Fluid Tetrathionate Broth Base (M032). After incubation at 35-37°C for 18-24 hours, this enriched culture is subcultured on a differential media such as Wilson and Blair Medium (M331) or MacConkey Agar (M081). Presumptive colonies are purified and pure cultures are used to inoculate the tubes of Gillies Agar No. 2.

Gillies Medium No. 2 is used by stab inoculating one half the depth of the medium using a straight needle. Kovacs reagent strips and lead acetate papers can be suspended from the cap or with the cotton plug over the medium but not touching the surface of the medium.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling:

The specimen is inoculated into a preliminary enrichment medium such as Fluid Tetrathionate Broth Base (M032).

After incubation at 35-37°C for 18-24 hours, this enriched culture is subcultured on a differential media such as Wilson and Blair Medium (M331) or MacConkey Agar (M081). Presumptive colonies are purified and pure cultures are used to inoculate the tubes of Gillies Agar No. 2.
Gillies Medium No. 2 is used by stab inoculating one half the depth of the medium using a straight needle. Kovacs reagent strips and lead acetate papers can be suspended from the cap or with the cotton plug over the medium but not touching the surface of the medium.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Additional biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder.
Gelling
Semisolid, comparable with 0.3% Agar gel.
Colour and Clarity of prepared medium
Green coloured, clear to slightly opalescent gel forms in tubes as butts.
Reaction
Reaction of 4.83% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH
7.20-7.60
Cultural Response
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	H2S	Indole	Motility	Sucrose/ Salicin
Proteus vulgaris ATCC 13315	50-100	luxuriant	weak reaction	weak reaction	positive, growth away from stabline causing turbidity	positive reaction,yellow colouration of the medium
Salmonella Typhi ATCC 6539	50-100	luxuriant	weak reaction	negative reaction,no colour development/ cloudy ring	positive, growth away from stabline causing turbidity	negative reaction
Shigella sonnei ATCC 25931	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring	negative growth along the stabline, surrounding medium remains clear	negative reaction

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Cruickshank R., Duguid J. P. Marmion B. P., Swain R. H. A., (Eds.), 1975, Medical Microbiology, 12th Edition, Vol. II, Churchill Livingstone.
2. Gillies R. R., 1956, J. Clin. Pathol., 9, 368.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Kohn J., 1953, J. Clin. Path., 6, 249.

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