# Technical Information

## Gillies Agar No. 2 (Sucrose Salicin Agar)

## Product Code: DM 1240

**Application: -** Gillies Agar No. 2 (Sucrose Salicin Agar) is recommended for detection of motility, hydrogen sulphide, indole production and fermentation of sucrose and salicin for identification of *Salmonella* and *Shigella* species.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Peptic digest of animal tissue | 10.000 |
| Casein enzymic hydrolysate | 10.000 |
| Sodium chloride | 5.000 |
| Disodium 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

## Principle & Interpretation

Enterobacteriaceae family consists of gram-negative bacilli widely distributed in nature. It includes pathogens such as Salmonella, Shigella, Yersinia, diarrheagenic E.coli and others. These bacteria cause large number 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 generally commensals or saprophytes [1]. Gillies Agar No. 2 [2], a modification of Kohns Medium [3] 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 for identification of non-lactose fermenting colonies biochemically prior to confirmation by serological typing [1].

Fermentation of sucrose and salicin leads to acid production indicated by change in colour from blue to yellow by pH indicator, bromothymol blue,. 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.

Peptic digest of animal tissue and casein enzymic hydrolysate 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 helps in the production of hydrogen sulphide.

The specimen is inoculated into a preliminary enrichment medium such as Fluid Tetrathionate Broth Base (DM1032). 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 (DM1331) or MacConkey Agar (DM1081). 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.

## Methodology

Suspend 48.28 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Distribute in tubes and sterilize by autoclaving at 118° - 12 1°C for 15 minutes. Allow the tubes to cool 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.

## Quality Control

**Physical 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 range** 7.20-7.60

**Cultural Response/ characteristices**
DM 1240: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

| Organism | Inoculum (CFU) | Growth | $H_2S$ | 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

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
**Prepared Media**: 2-8$^{o}$ in sealable plastic bags for 2-5 days.

## Further Reading

1. Cruickshank R., Duguid J. P. Marmion B. Churchill Livingstone.
2. Gillies R. R., 1956, J. Clin. Pathol., 9, 368. 3.Kohn J., 1953, J. Clin. Path., 6, 249.

## Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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