Specification

Differential and selective solid medium for the isolation of *Salmonella* and some *Shigella* species from clinical specimens, foods, etc.

Formula * in g/L

Meat extract	5,00000
Peptone	5,00000
Lactose	10,00000
Bile salts	5,60000
Sodium citrate	10,00000
Sodium thiosulfate	8,50000
Ferric citrate	1,00000
Brilliant green	0,00033
Neutral red	0,02500
Agar	15,00000

Final pH 6,90 ±0,2 at 25 ºC

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

Directions

Suspend 60.1 g of the powder in 1 L of distilled water. Bring to the boil, with frequent agitation and allow it to simmer gently dissolving the agar. Do not autoclave. Cool to 50°C, mix well and pour into sterile Petri dishes.

Description

SS Agar is a highly selective agar used for the isolation of *Salmonella* and *Shigella* species from very contaminated samples.
Selectivity is obtained by a high concentration of bile salts and brilliant green, which inhibits the growth of Gram positive bacteria. The growth of other Gram negative flora is highly repressed due to the presence of citrate and thiosulfate. Some coliforms may still grow on this medium. Differentiation between pathogenic species and coliforms is achieved by the colour change of the pH indicator (neutral red). Lactose fermenters produce a pink or red coloured medium and colonies, while non- fermenting species form colourless colonies and turn the medium yellow. Should any species produce H_2S, it is easily detected by the black precipitate of ferrous sulfide, which turn the colonies black.
The peptone and the meat extract are capable of inducing the growth of most pathogenic species, nevertheless some *Shigella* are very fastidious and may grow poorly.

Technique

If it is suspected that organisms might have been damaged and the viability of the microorganisms is poor i.e. (processed food, faeces from the patients under antibiotic treatment, etc.) it is advisable to proceed with a prior enrichment in Selenite-Cystine Broth Base or Tetrathionate Mueller Kauffman Broth Base. After enrichment, inoculate SS Agar plates heavily with the specimen and proceed in the same way as with other specimens on a less selective medium, such as Vert brilliant Agar or MacConkey Agar.
Incubate the inoculated plates à 37°C for 18-24 hours. The presumptive colonies should then be sub-cultured on differential media to be identified biochemically or serologically.

Appearance of the colonies after 24 hours on SS Agar:
- Shigella: Colourless, transparent and flat.
- Salmonella (Non H2S producers): Colourless, transparent and flat.
- Salmonella (H2S producers): Black or black centred, flat, with transparent borders.
- Proteus: Similar appearance as Salmonella colonies, but smaller in size.
- Escherichia coli: If they grow, they are small, convex and pink or red coloured.
- Coliforms (in general): Large, opaque, smooth and white or pink in colour.

Quality control

Incubation temperature: 37°C ±1,0 **Incubation time:** 21 ± 3 h

Inoculum: 10^{3}-10^{4} CFU (Productivity test qualitative)/ 10^{4}-10^{6} CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC® 29212	Partial inhibition	-
Escherichia coli ATCC® 25922	Total inhibition	-
Salmonella abony NCTC® 6017	Good	Colourless colonies with black center (H2S+)
Salmonella typhimurium ATCC® 14028	Good	Colourless colonies with black center (H2S+)
Salmonella enteritidis ATCC® 13076	Good	Colourless colonies with black center (H2S +)
Shigella flexneri ATCC® 12022	Good	Colourless colonies with transparent center (H2S-)

References

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· GRAY, L.D. (1995) Escherichia, Salmonella, Shigella and Yersinia. In Murray, Baron, Pfaller Tenover & Yolken (eds) Manual Clinical Microbiology. 6th ed. ASM Washington DC.
· HORWITZ, W.(2000) Official Methods of Analysis 17th ed. AOAC International. Gaithersburg. MD.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· LEIFSON, E. (1935) New culture media based on sodium deoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol., 40.581.
· WINN, W., S. ALLEN, W. JANDA, E. KONEMAN, G. PROCOP, P. SCHRECKENBERGER & G. WOODS (2006) Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins. Philadelphia.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).