



Wilson and Blair's BBS Agar Medium 10.(Triple Pack)

MM331

This medium is recommended for the selective subculture of *Salmonella* species in accordance with Indian Pharmacopoeia 2014.

Composition**

Ingredients	Gms / Litre
Nutrient Agar**	Part A
Peptone	10.000
Meat extract B #	10.000
Sodium chloride	5.000
Agar	20.000
Final pH (at 25°C)	7.4±0.2
Solution (i) Bismuth Sulphite Glucose Phosphate mixture	Part B (Gms/200ml)
Bismuth ammonio-citrate scales	6.000
Sodium sulphite	20.000
Disodium hydrogen phosphate	20.000
Glucose	10.000
Solution (ii) Iron citrate brilliant green mixture	Part C (Gms/45ml)
Ferric citrate, brown scales	0.400
Brilliant green	0.050

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 4.5 grams of Part A in 100 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Suspend 5.6 grams of Part B [Bismuth Sulphite Glucose Phosphate Mixture, solution (i)] in 20 ml sterile purified/ distilled water, boil the solution till all the ingredients are properly dissolved. Suspend 0.045 grams of Part C [Iron Citrate Brilliant Green Mixture, solution (ii)] in 4.5 ml sterile purified/ distilled water. Mix aseptically 20 ml of Part B and 4.5 ml of Part C with 100 ml of previously melted sterile Nutrient Agar (Part A) and cool to a temperature 60°C and pour into sterile Petri plates.

Note: Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this. User must ensure its compatibility with the latest edition.

Principle And Interpretation

Salmonella is a genus of gram-negative *Enterobacteriaceae* - commonly implicated in foodborne illness and the causative agent of typhoid and paratyphoid fever. *Salmonella* species have been isolated from humans and animals. More than 2000 serovars of *Salmonella* exists with each showing different host specificities. For example, humans are the only known natural reservoir for serotype *Salmonella Typhi* and serotypes *Salmonella Paratyphi* A, B and C (1). The organism can be transmitted by the faecal-oral route. It is excreted by humans in faeces and may be transmitted by contaminated water, food, or by person-to-person contact (with inadequate attention to personal hygiene).

Wilson and Blair's BBS Agar formulated by Wilson and Blair (2) is recommended for isolating *Salmonella* species especially *Salmonella Typhi* from clinical specimens. The selective reagent formulation is a modification of the bismuth sulphite reagent described by Hajna and Perry (3). This medium is particularly valuable for the isolation of *S. Typhi*. The medium is highly selective for Salmonellae, being inhibitory to coliforms, *Proteus*; occasional strains of coliforms grow to form dull green or brown colonies, but without a surrounding metallic sheen. This medium is recommended by Indian Pharmacopoeia for the selective subculture of *Salmonella* after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth (5). The

medium is also suitable for the isolation of lactose-fermenting strains of *Salmonellae* (which can not be differentiated on lactose containing differential media) since lactose is not the fermentable substrate used in this medium (4).

Peptone and meat extract B provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other growth nutrients. Sodium chloride maintains the osmotic balance. Brilliant green dye inhibits all gram positive bacteria.

Glucose is the fermentable carbohydrate. Bismuth is a heavy metal, which is inhibitory to most gram-negative enteric bacilli other than *Salmonella*. Ferric citrate is reduced by *Salmonella* species in presence of bismuth ammonium citrate and glucose to form iron sulphide, indicated by black coloured colonies. Disodium hydrogen phosphate buffers the medium well.

Quality Control

Appearance

Part A: Cream to yellow homogeneous free flowing powder Part B: White to offwhite homogeneous free flowing powder

Part C: Green homogeneous free flowing granules

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

After addition of Part B and Part C to Part A :Greenish yellow coloured, opaque gel forms in Petri plates.

Cultural Response

Growth Promotion is carried out in accordance with the method of IP. Cultural response was observed after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating ≤ 100 cfu (at 30-35°C for ≤ 24 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating ≤ 100 cfu (at 30-35°C for 24-48 hours).

Inhibitory properties

No growth of the test microorganism occurs at the specified temp for not less than longest period of time specified inoculating ≥ 100 cfu (at 30-35°C for ≥ 48 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
Growth Promoting + Indicative						
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	25 -100	≥ 50 %	Green colonies with black centre to black with metallic sheen	24 -48 hrs
<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	25 -100	≥ 50 %	Green colonies with black centre to black with metallic sheen	24 -48 hrs
Inhibitory properties						
<i>Escherichia coli</i> ATCC 8739 $\geq 10^3$		Inhibited	0	0 %		≥ 48 hours
<i>Shigella boydii</i> ATCC 8700 $\geq 10^3$		Inhibited	0	0 %		≥ 48 hours

Additional Microbiological

Testing

<i>Proteus mirabilis</i> ATCC 25933	50 -100	luxuriant	25 -100	≥ 50 %	green	24 -48 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	25 -100	≥ 50 %	black with sheen	24 -48 hrs
<i>Salmonella Typhi</i> ATCC 6539	50 -100	luxuriant	25 -100	≥ 50 %	black with sheen	24 -48 hrs
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	Inhibited	0	0%		≥ 48 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry period on the label.

Reference

- 1.Murray P.R., Baron J.H., Pfaller M.A., Jorgensen J.H. and Tenover F.C.,(Ed.), 2003, Manual of Clinical Microbiology, 8thEd., American Society for Microbiology, Washington, D.C.
- 2.Wilson W. J. and Blair E. M., 1926, J. Pathol. Bacteriol., 29 : 310.
- 3.Hajna A. A. and Perry C. A., 1938, J. Lab. Clin. Med., 23:1185.
- 4.Collee J.g., Fraser A.g., Marmion B.P., Simmons A., (Eds), Mackie and MC Cartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
- 5.The Indian Pharmacopoeia 2014,Government of India 2014, The Controller of Publication.

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