



## Wilson Blair Agar Base

M331

Wilson Blair Agar Base is recommended with the addition of selective reagent for the isolation of *Salmonellae* particularly *Salmonella Typhi*.

### Composition\*\*

Ingredients	Gms / Litre
Peptone, special	10.000
Beef extract	5.000
Dextrose	10.000
Sodium chloride	5.000
Agar	30.000
Final pH ( at 25°C)	7.3±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 60 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. To sterile melted base, aseptically add 4 ml of 1% brilliant green solution and 70 ml of selective reagent.

Selective Reagent

Solution 1 : 40 gm sodium sulphite in 100 ml distilled water.

Solution 2 : 21 gm dibasic sodium phosphate in 100 ml distilled water.

Solution 3 : 12.5 gm bismuth ammonium citrate in 100 ml distilled water.

Solution 4 : 0.96 gm ferrous sulphate in 20 ml distilled water with 2 drops of hydrochloric acid.

Prepare each solution separately and then combine. Boil the combined solution until a slate grey colour develops.

### 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 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* and *Shigellae*; occasional strains of coliforms grow to form dull green or brown colonies, but without a surrounding metallic sheen. 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 special and beef extract provide nitrogenous, carbonaceous compounds and other growth nutrients. Brilliant green dye inhibits all gram-positive bacteria. Dextrose is the fermentable carbohydrate. Ferrous sulphate aids in H<sub>2</sub>S production. Bismuth is a heavy metal, which is inhibitory to most gram-negative enteric bacilli other than *Salmonella*. Ferrous sulphate is reduced by *Salmonella* species in presence of bismuth sulphite and dextrose to form iron sulphide, indicated by black coloured colonies. Disodium hydrogen phosphate buffers the medium well. Sodium chloride balances the osmotic equilibrium.

Do not store the medium in refrigerator (4°C) for longer than 2 days, as the medium changes to green colour and reduces its selectivity.

## Quality Control

### Appearance

Cream to yellow homogeneous free flowing powder

### Gelling

Firm, comparable with 3.0% Agar gel.

### Colour and Clarity of prepared medium

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of the selective reagent and 1% Brilliant green, greenish yellow coloured, opaque gel forms in Petri plates.

### Reaction

Reaction of 6.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

### pH

7.10-7.50

### Cultural Response

Cultural characteristics observed with added 1% Brilliant green and selective reagents after an incubation at 35-37°C for 24-48 hours.

### Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<b>Cultural Response</b>				
<i>Escherichia coli</i> ATCC 25922	≥10 <sup>3</sup>	inhibited	0%	
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	≥50%	green
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant	≥50%	black with sheen
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	≥50%	black with sheen

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference

- Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- Wilson W. J. and Blair E. M., 1926, J. Pathol. Bacteriol., 29 : 310.
- Hajna A. A. and Perry C. A., 1938, J. Lab. Clin. Med., 23:1185.

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