



## Wilson Blair Agar Base

M331

### Intended Use:

With the addition of selective reagent recommended for the isolation of *Salmonella* Typhi.

### Composition\*\*

| Ingredients         | Gms / Litre |
|---------------------|-------------|
| Peptone special     | 10.000      |
| HM peptone B #      | 5.000       |
| Dextrose (Glucose)  | 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

# Equivalent to Beef extract

### Directions

Suspend 60.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. To sterile molten base, add 4 ml of 1% Brilliant green solution and 70 ml of Selective reagent.

Solution 1: 40 gms Sodium sulphite in 100 ml distilled water

Solution 2: 21 gms Dibasic sodium phosphate (Disodium hydrogen phosphate) in 100 ml distilled water

Solution 3: 12.5 gms Bismuth ammonium citrate in 100 ml distilled water

Solution 4: 0.96 gms Ferrous sulphate in 20 ml distilled water with 2 drops of Hydrochloric acid.

Prepare each solution separately and boil the combined solution until a slate grey colour develops. Mix well and pour into sterile Petri plates.

### 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 *Salmonella*, being inhibitory to coliforms, *Proteus* and *Shigella*; 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 *Salmonella* (which can not be differentiated on lactose containing differential media) since lactose is not the fermentable substrate used in this medium.

Peptone special and HM peptone B 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.

### Type of specimen

Clinical samples - faeces; Food and dairy samples; Water samples.

### Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7,8).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1. 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.

## 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

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.

| Organism  | Inoculum (CFU)   | Growth    | Recovery | Colour of colony |
|---|------------------|-----------|----------|------------------|
| <i>Escherichia coli</i> ATCC 25922 (00013*)       | ≥10 <sup>4</sup> | inhibited | 0%       |                  |
| <i>Proteus mirabilis</i> ATCC 25933               | 50-100           | luxuriant | ≥50%     | green            |
| <i>Salmonella</i> Typhi ATCC 6539                 | 50-100           | luxuriant | ≥50%     | black with sheen |
| <i>Salmonella</i> Typhimurium ATCC 14028 (00031*) | 50-100           | luxuriant | ≥50%     | black with sheen |

Key : (\*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

## Reference

1. 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.
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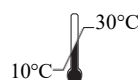
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