TM 489 – WILSON BLAIR AGAR BASE

INTENDED USE

For isolation and cultivation of *Salmonella Typhi* with addition of selective reagent.

PRODUCT SUMMARY AND EXPLANATION

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. 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 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. This medium is particularly valuable for the isolation of *S. Typhi*. The medium is highly selective for Salmonellae, 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 Salmonellae (which cannot be differentiated on lactose containing differential media) since lactose is not the fermentable substrate used in this medium.

COMPOSITION

Ingredients	Gms / Ltr
Peptone special	10.000
Beef extract	5.000
Dextrose	10.000
Sodium chloride	5.000
Agar	30.000

PRINCIPLE

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 H2S 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.

INSTRUCTION FOR USE

- Dissolve 60 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.

- Sterilize by autoclaving at 15 psi 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance 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.

pH (at 25°C) : 7.3 ± 0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Color of the colony	Incubation Temperature	Incubation Period
Proteus mirabilis	25933	50-100	Luxuriant	>=70%	Green	35-37°C	24-48 Hours
Escherichia coli	25922	>=10^3	Inhibited	0%	-	35-37°C	24-48 Hours
Salmonella Typhi	6539	50-100	Luxuriant	>=70%	Black with sheen	35-37°C	24-48 Hours
Salmonella Typhimurium	14028	50-100	Luxuriant	>=70%	Black with sheen	35-37°C	24-48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for

the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

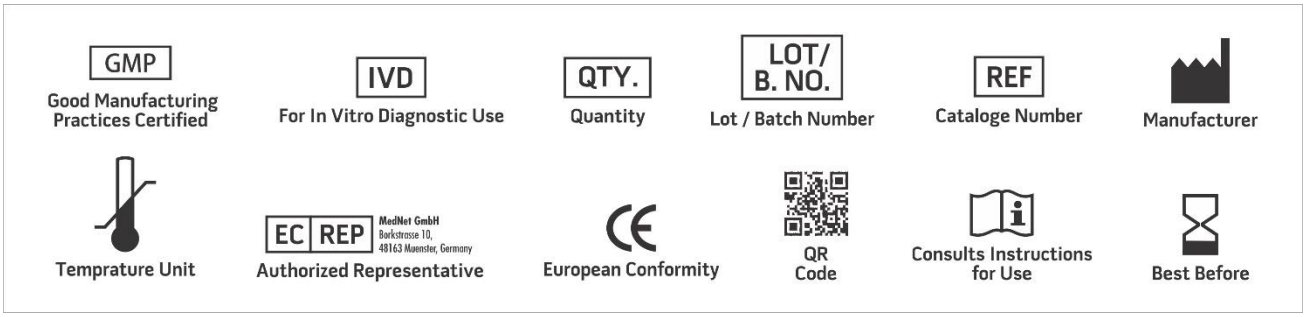
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., 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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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