# A MODIFICATION OF WILSON AND BLAIR'S BISMUTH SULPHITE AGAR (STABILIZED STOCK SOLUTIONS)

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Bismuth sulphite agar is by far the most efficient of all single media devised for the isolation of typhoid bacilli from stools. When tested in comparison with other media it was found not only to afford the highest percentage of positive results but also the maximum inhibition of saprophytic growth. Its remarkable selectivity is demonstrated by the fact that negative plates are often completely sterile, and positive ones often consist exclusively of typhoid colonies (Loureiro & Matos, unpublished observations).

It may, however, be objected against the medium that the regularity of its performance is disturbed by a series of difficulties arising in the preparation of plates with the correct composition. As Wilson & Blair (1931) themselves state, many authors, with experience with the medium, have complained about such difficulties. We experienced trouble when testing the modification called *Stock bismuth sulphite mixture*. This suspension gave good results when fresh, but it was so altered after one or two weeks of standing that in plates prepared with it the growth of *Bact. typhosum* was totally inhibited.

The difficulties were to some extent overcome by the introduction of Difco powdered medium, which gave consistently good results, but its high price and possible difficulties of supply, still make it worth while to prepare a medium from the original ingredients. These facts justify the publication of a further modification of the *Stock bismuth sulphite mixture* which entirely removes the inconvenience of working with unstable solutions, and moreover, does not necessitate frequent weighing as the prescriptions called by Wilson & Blair *Old standard medium* and *New standard medium*.

Alteration of the bismuth sulphite suspension is probably due to oxidative changes, which are accelerated by the presence of large amounts of precipitate. After trying all sorts of combinations with the basic ingredients it was found that oxidation may be avoided by making, instead of a single solution containing precipitate, three separate clear solutions which are mixed extemporaneously. This new prescription, which we suggest calling *A.B.C. bismuth sulphite agar*, is made up in the following manner.

## Agar base

The agar base, as in Wilson & Blair's original formula, is a 3·0% extract agar.

Divide the medium in 100 ml. portions, or multiples, sterilize, and add

while hot 0·4 ml. per 100 ml. of a 1% solution of *Bacto*-brilliant green. This dye-agar can be stored for months in a dark place.

*Solution A. Bismuth*

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|---|---|
| Bismuth citrate ... ... | 12·0 g. |
| Water ... ... ... ... | 10 ml. |
| Ammonia (sp. gr. = 0·880) ... | 6·4 ml. |

Make a paste with bismuth and water, slowly stirring until the solution is clear. Then add water to 100 ml.

This solution keeps indefinitely. Any kind of bismuth citrate of good pharmaceutical grade will be found satisfactory.

*Solution* B. *Sulphite-phosphate*

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|---|---|
| $Na_2SO_3$, anhydrous ... ... | 10 g. |
| $Na_2HPO_4$, anhydrous ... ... | 5·2 g. |
| Water ... ... ... ... | 46 ml. |

This solution keeps indefinitely in well stoppered bottles. It may crystallize in cold weather, but the crystals melt easily after a short stay in the thermostat.

*Solution* C. *Glucose-iron*

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|---|---|
| Glucose ... ... ... ... | 5·0 g. |
| Water ... ... ... ... | 25 ml. |

Dissolve under moderate heating. When dissolved add

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|---|---|
| $FeSO_4,7H_2O$ ... ... ... | 0·4 g. |

This solution is the only one not entirely stable. The high concentration of glucose makes it, however, much less oxidizable than a plain ferrous sulphate solution.

In well-stoppered small bottles it keeps unaltered for many months. After a bottle is started a slight precipitate of ferric oxide slowly develops. Even so, after eight months of intermittent use the contents of a small bottle still gave the same satisfactory result as the fresh solution.

*Preparation of the medium*

The medium is prepared by adding to 100 ml. of the melted agar base 5 ml. of solution B (phosphate-sulphite) and 2·5 ml. each of solution A (bismuth) and solution C (glucose-iron).

It is indifferent whether the solutions are added separately to the agar base or have been mixed together previously. Heating the mixture to the boiling point, as Wilson & Blair suggest, causes neither deterioration nor improvement. This is a further proof that of all subtle factors that may affect the bacteriostatic effectiveness of the bismuth sulphite mixture only one is

dominant, namely that the suspension must be free of oxidation products. We suggest making the mixture of the solutions in a test-tube and pouring it after a few minutes into melted agar, of which 100 ml. are distributed in six Petri dishes.

The solidified plates may be preserved for two or three days in the ice-box. If stored longer the characteristic greyish yellow colour changes to green and the growth of *Bact. typhosum* becomes scanty or is inhibited.

Fresh plates invariably yield a result similar to that of a 'good' Wilson & Blair medium. *Bact. typhosum* grows in the form of characteristic large, black, glistening colonies, surrounded by a dark, glistening halo. *Bact. coli* is completely, or almost completely, inhibited.

## REFERENCE

WILSON, W. J. & BLAIR, E. M. McV. (1931). Further experience of the bismuth sulphite media in the isolation of *Bacillus typhosus* and *B. paratyphosus* B from faeces, sewage and water. *J. Hyg., Camb.*, **31**, 138–61.