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THE RELIABILITY OF BROMTHYMOL-BLUE LACTOSE AGAR AND BACTO PHENOL-RED MANNITOL AGAR FOR THE ISOLATION OF PATHOGENIC STAPHYLOCOCCI

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Since 1937 more than 7,000 samples have been tested routinely in this laboratory on Bacto bromthymol-blue lactose agar (Chapman *et al.*, 1937) and Bacto phenol-red mannitol agar (Chapman, Lieb, and Curcio, 1938) for the isolation of plasma-coagulating staphylococci. Although our impressions of the results obtained have been favorable, the publication of an adverse (but unsubstantiated) opinion by an investigator of recognized ability (Blair, 1939) made it imperative to undertake a critical and extensive analysis of the methods advocated.

The efficiency of an isolation method is determined by (1) the proportion of samples that give positive results, (2) the relative numbers of colonies of the organism in different samples, and (3) the proportion of transplants that prove to be pure cultures of the organism sought.

Using bromthymol-blue lactose agar prepared in this laboratory, we (Chapman *et al.*, 1937) found that 6.2 per cent of the transplants were nonpathogenic staphylococci and that 1.5 per cent of the pathogens had failed to grow on the medium. The Difco product has proved satisfactory also, as the following analysis will show.

In making this analysis it must be remembered that, although they are highly efficient, neither bromthymol-blue lactose agar nor Bacto phenol-red mannitol agar alone provides maximum recovery of pathogenic staphylococci in every instance but that best results are obtained by plating the sample on both media. Moreover, the incubation period is important—20 to 24 hours for Bacto phenol-red mannitol agar, and 40 to 48 hours for bromthymol-blue lactose agar.

The results to be reported were obtained with the Difco products. Other formulae and other brands of ingredients may give rather different results, and their performance should be checked before they are used routinely. Bromthymol-blue is the more variable chemical.

PROPORTION OF SAMPLES THAT SHOW PLASMA-COAGULATING STAPHYLOCOCCI

That there is maximum recovery of plasma-coagulating staphylococci can best be demonstrated by the number of such organisms in samples considered negative by the combined methods. No such organisms were recovered in 400 samples from the nose, throat, and other parts of the body. A few samples showed plasma-coagulating staphylococci on one medium only, and this is one

reason why both media should be used. It is estimated that about 1 sample in 1,000 may give falsely negative results.

RELATIVE NUMBER OF PLASMA-COAGULATING STAPHYLOCOCCI IN DIFFERENT SAMPLES

It was found (Chapman, Lieb, and Curcio, 1938) that 14.8 per cent of pathogenic staphylococci were markedly inhibited and that 37.7 per cent were slightly inhibited on bromthymol-blue lactose agar. Since Bacto phenol-red mannitol agar does not inhibit staphylococci (Chapman, Lieb, and Curcio, 1938) it affords an excellent test of the inhibiting properties of bromthymol-blue lactose agar.

The two methods were compared in a series of 357 tests. The number of plasma-coagulating staphylococci was slightly higher on phenol-red mannitol agar in 9 tests (2.5 per cent), moderately higher in 5 (1.4 per cent), and considerably higher in 2 (0.6 per cent). However, bromthymol-blue lactose agar has its advantages. The number of plasma-coagulating colonies on it was considerably larger than on Bacto phenol-red mannitol agar in 10 tests (2.8 per cent), moderately larger in 11 (3.1 per cent), and slightly larger in 20 (5.6 per cent). In another 10 samples the phenol-red mannitol agar plates were so overgrown that it was impossible to study any staphylococci that might have been present.

PROPORTION OF TRANSPLANTS THAT WERE PLASMA-COAGULATING STAPHYLOCOCCI

Of 3,606 cultures that have been isolated by these methods, 77.4 per cent clotted plasma and only 37 (1.0 per cent) were not staphylococci. By previous methods only 43.9 per cent of the cultures clotted plasma.

DISCUSSION

One of the theoretical objections to the methods advocated for the selective isolation of plasma-coagulating staphylococci is based on the findings of a large number of investigators that mannitol is fermented by a large proportion of nonpathogenic staphylococci. Some authors (e.g., Winslow, Rothberg, and Parsons, 1920; Hoffstadt and Youmans, 1934; and Cruikshank, 1937) observed this property in about one half of the nonpathogenic staphylococci they studied. It was shown (Chapman and Stiles, 1940) that, by using a short incubation period, a more nutritive medium, and a more suitable indicator, acid production from mannitol was limited almost entirely to strains that coagulated plasma.

In the present series, 7.1 per cent of the cultures produced acid from mannitol but did not clot plasma. On the contrary, 1.5 per cent clotted plasma but failed to produce acid from mannitol by the method used.

A further criticism is based on the fact that bromthymol-blue lactose agar does not entirely inhibit other bacteria. It does, however, inhibit "most other bacteria" (Chapman *et al.*, 1937). Moreover, it inhibits most nonpathogenic staphylococci also. It was pointed out (Chapman, Lieb, and Curcio, 1938)

that yeasts, enterococci, and coliforms also grow on the medium, but there is little chance of confusing them with colonies of pathogenic staphylococci because the latter are so characteristic. The coliforms may be almost entirely eliminated by adding potassium tellurite to the medium (Chapman, 1944). A heavy suspension should not be used, and the inoculum should be sufficiently light to give discrete colonies.

Bacto phenol-red mannitol agar was not claimed to inhibit other bacteria. Its value lies in the appearance of the colonies, which is so characteriatic that they may be recognized in all but a few instances where there is excessive contamination or an excess of bacteria (e.g., *Neisseria catarrhalis*) that produce an alkaline reaction which obscures the acid products of the staphylococci.

CONCLUSIONS

Plasma-coagulating staphylococci may be isolated by plating the sample on bromthymol-blue lactose agar and on Bacto phenol-red mannitol agar and by selecting typical or suspicious colonies after incubation for the specified times.

By these methods there is little chance that any plasma-coagulating staphylococci may fail to be recovered, and 77 per cent of the transplants prove to be plasma-coagulating staphylococci.

REFERENCES

BLAIR, J. E. 1939 The pathogenic staphylococci. Bact. Revs. **3**, 97–146.

CHAPMAN, G. H. 1944 The isolation of pathogenic staphylococci from feces. J. Bact., **47**, 211.

CHAPMAN, G. H., LIEB, C. W., BERENS, C., AND CURCIO, L. 1937 The isolation of probable pathogenic staphylococci. J. Bact., **33**, 533–543.

CHAPMAN, G. H., LIEB, C. W., AND CURCIO, L. G. 1938 The use of bromthymol blue agar and phenol red mannitol agar for the isolation of pathogenic types of staphylococci. Am. J. Clin. Path., Tech. Suppl., **2**, 3–11.

CHAPMAN, G. H., AND STILES, M. H. 1940 Carbohydrate fermentation reactions of staphylococci. Am. J. Clin. Path., **10**, 380–386.

CRUIKSHANK, R. 1937 Staphylocoagulase. J. Path. Bact., **45**, 295–303.

HOFFSTADT, R. E., AND YOUMANS, G. P. 1934 The genetic significance of the dissociants of *Staphylococcus aureus*. J. Bact., **27**, 551–560.

WINSLOW, C.-E. A., ROTHBERG, W., AND PARSONS, E. I. 1920 Notes on the classification of orange and white staphylococci. J. Bact., **5**, 145–167.