DEHYDRATED MEDIUM VS. FRESH T-H BROTH  

A Dehydrated Medium for the Preparation of Type Specific Extracts of Group A Streptococci

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Received for publication November 20, 1953

In studies involving the serological typing of Group A beta hemolytic streptococci, it is essential to use a medium which favors the development and maintenance of the type specific M protein. The standard medium recommended for this purpose is a modified Todd-Hewitt (T-H) broth (Swift, in Dubos, 1948), prepared with a fresh infusion of beef heart. The present study was undertaken to determine whether a dehydrated medium could be substituted for fresh T-H broth.

MATERIALS AND METHODS

Streptococcus strains used in the study included both stock cultures and recently isolated cultures of various types, the majority being strains of types 1, 3, 5, 6, 12, and 18.

The fresh T-H broth which served as the control medium was prepared in the media unit of the Communicable Disease Center according to the recommended modified formula (Swift in Dubos, 1948) except for sterilization in an autoclave (121 C for 20 minutes) rather than in an Arnold steam sterilizer.

The following commercially available dehydrated media were studied: Difco Brain Heart Infusion Broth; Difco A-C Medium; Difco Tryptose Phosphate Broth; and BBL Trypticase Soy Broth. In addition, the Difco Laboratories supplied an experimental dehydrated medium prepared to conform in all respects to the modified T-H formula.

The streptococcal cultures studied were grown simultaneously in fresh T-H broth and in one or more of the test media. Extracts were prepared and tested according to Lancefield's procedures (Swift, Wilson and Lancefield, 1943), that is, acid-heat extraction and capillary tube precipitin test. Comparisons were made...
TABLE 1. Comparison of dehydrated media with fresh Todd-Hewitt broth for typing group A beta hemolytic streptococci

<table>
<thead>
<tr>
<th>MEDIA</th>
<th>TEST MEDIUM</th>
<th>FRESH TODD-HEWITT</th>
<th>NO. TOTAL STRAINS TYPED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. strains typed</td>
<td>No. strains not typed</td>
<td>No. strains typed</td>
</tr>
<tr>
<td>Difco Tryptose Phosphate vs. Fresh Todd-Hewitt</td>
<td>75</td>
<td>29* (72%)</td>
<td>100</td>
</tr>
<tr>
<td>BBL Trypticase Soy vs. Fresh Todd-Hewitt</td>
<td>50</td>
<td>23* (72%)</td>
<td>81</td>
</tr>
<tr>
<td>Dehydrated Todd-Hewitt vs. Fresh Todd-Hewitt</td>
<td>105</td>
<td>5* (95%)</td>
<td>106</td>
</tr>
</tbody>
</table>

* Typed only from fresh T-H.
** Typed only from test medium.

only between cultures which had been inoculated from a common source, extracted in parallel, and tested at the same time with antiserum from a single vial.

The precipitin tests were read as follows: 1) Weak; precipitate throughout the column just visible with the naked eye after 2 hours incubation, settling to less than 0.5 mm at the base of the column after overnight refrigeration. 2) Moderate; larger and/or more numerous precipitate particles settling to 0.5 to 1.5 mm. 3) Strong; particles settling to 1.5 mm or more. In keeping with the standard practice in this laboratory, weak reactions were considered unsatisfactory for type designation of the test cultures.

RESULTS

Work with the Difco Brain Heart Infusion and A-C broths was discontinued early in the study after observation that they gave markedly inferior serological results.

Results with the other media are summarized in Table 1. In every case a few cultures were typed from the test medium and not from the control T-H broth. However, with both the tryptose phosphate and trypticase soy broths this slight advantage was definitely outweighed by the large number of strains typed only from T-H broth. On the other hand, the dehydrated T-H broth gave essentially the same net result as the fresh T-H broth since the five strains typed only from the fresh infusion were balanced by four strains typed only from the dehydrated broth.

DISCUSSION

The results demonstrate that the standard dehydrated media included in the study are inferior to T-H broth for the growth of group A streptococci which are to be typed serologically. That this inferiority is not due to the dehydration process per se is evidenced by the production of a dehydrated medium which is fully as satisfactory as fresh T-H broth. No attempt was made to determine what factor or factors might be responsible for the superiority of the T-H formula. Others workers have emphasized the importance of a final pH of 7.6 to 8.0 (Lancefield, 1949) and the presence of neopeptone (Elliot, 1945) in media used for streptococcus serological studies. In the media tested, these criteria were met only by the T-H broths.

ACKNOWLEDGEMENTS

We are indebted to the Difco Laboratories and the Baltimore Biological Laboratories for their cooperation in supplying the dehydrated media used in this study.

SUMMARY

A study was undertaken to determine whether a dehydrated medium could be substituted for freshly prepared Todd-Hewitt broth for growing group A beta hemolytic streptococci for serological typing. Four standard dehydrated media were studied. Although all four supported growth of the streptococci, they were definitely inferior to the fresh T-H broth in maintaining type specificity. A dehydrated medium, prepared to conform in all respects to the T-H formula, gave results entirely comparable to those obtained with the freshly prepared product. The study confirms previous reports regarding the superiority of T-H broth for this specialized work. In addition, it has been demonstrated that the preparation of a dehydrated T-H medium is entirely feasible and practicable.

REFERENCES

Swift, H. F. The streptococci (286).
Lancefield, R. C. 1949 Personal communications.