Technical method

A modified Sandiford’s counter stain for smears of blood cultures

MD WILLIAMS AND A BORNEMISZA Department of Microbiology, Frenchay Hospital, Bristol, UK

The early detection of bacteria in blood cultures is an important function in clinical microbiology and is needed to provide useful information to the clinician. Several workers recommend routine Gram stained smears at each subculture, and this is now common practice. The organisms most frequently found in hospital-acquired bacteraemia are Gram-negative, and unfortunately these are difficult to distinguish from background debris using Gram’s stain and its modification. An improved staining technique would therefore be very desirable.

Consequently, we have made a comparison between Gram’s stain and Sandiford’s stain on smears prepared at subculture of routine blood cultures (5 ml patient’s blood + 100 ml Brewer’s thioglycollate medium). Our initial experiences with Sandiford’s showed poor Gram-negative differentiation, which led us to modify the proportions of pyronin and to use a phosphate buffer, pH 7.3, as the solute. This gave better differentiation and stability to the stain. Both of these factors were controlled by using smears prepared from blood cultures known to contain Escherichia coli.

Received for publication 4 March 1980

Comparison of Sandiford and Gram stained smears of 56 positive blood cultures

<table>
<thead>
<tr>
<th>Organisms seen</th>
<th>Positive culture</th>
<th>Organisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Sandiford</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>2</td>
<td>*Staphylococcus epidermidis (2)</td>
</tr>
<tr>
<td>+</td>
<td>1</td>
<td>*Staphylococcus epidermidis (1)</td>
</tr>
<tr>
<td>—</td>
<td>5</td>
<td>Escherichia coli (1)</td>
</tr>
<tr>
<td>+</td>
<td>48</td>
<td>Staphylococcus epidermidis (1)</td>
</tr>
</tbody>
</table>

*Obtainable from Difco Laboratories, PO Box 148, Central Avenue, West Molesey, Surrey, UK.

+ organisms seen; — no organisms seen; * scanty growth; ( ) numbers of each type of organisms isolated.
(Any discrepancy between the stains was rechecked to rule out any observer bias.)
Technical method

growth of Staph. epidermidis. The ease of distinguishing Gram-negative organisms against the background debris found in the smears, and the increased numbers of organisms seen, cannot be overemphasized (see Figure). This was especially so with small Gram-negative organisms, such as Bacteroides sp.

Discussion

Similarity in staining of Gram-negative organisms and the background debris makes recognition difficult and delays the information reaching the clinician. Some laboratories solve the problem by using May-Grünwald or Giemsa stains, which satisfactorily demonstrate the presence of all types of organisms but do not determine their Gram reaction. A Gram stain in addition is therefore necessary and takes more time. Alternative techniques8-9 distinguish Gram-negative bacteria (red) from the background (yellow) but are relatively time-consuming, and in our hands these methods give poor contrast. Recently, Sandiford's stain10 was described for use in histological sections,11 where Gram-positive (blue/black) and Gram-negative (red) organisms are contrasted well against a blue/green background.

Using the modified Sandiford's stain described here, a good contrast is obtained even in the thick part of the smear, a greater number of organisms are visible, and these are more easily seen. These factors, together with a long shelf-life (several months) and a reduced variability in staining, lead us to recommend its use where the stained smear is the method used for early detection of growth in a blood culture.

We thank Dr KJ Harrison for her help and encouragement, Mr K Dance for the photomicrographs, and Difco Laboratories for financial help.

References

1 Blazevic DJ, Stamper JE, Matsen JM. Comparison of macroscopic examination, routine Gram stains, and routine subcultures in the initial detection of positive blood cultures. Applied Microbiology 1974;27:537-9.

Requests for reprints to: Mr MD Williams, Chief MLSO, Microbiology Department, Royal Hampshire County Hospital, Romsey Road, Winchester, Hants, UK.