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 subcultureable 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.

Method

1. Stain with 1% aqueous methyl violet 1 min
2. Rinse in tap water
3. Stain with Lugol's iodine 2 min
4. Rinse in tap water
5. Decolorize with acetone
6. Rinse in tap water and blot dry
7. Counterstain with Sandiford's stain 2 min
8. Rinse in tap water (do not wash) and blot dry.

Gram-positive organisms — blue/black
Gram-negative organisms — red
Background and cellular debris — blue/green.

Altogether 414 blood culture smears were examined and 358 were smear negative (Gram and Sandiford's) and yielded no growth; 56 yielded growth on subculture, and a comparison of the smears is listed in the Table. There was a marginal increase in the number of positive smears with Sandiford stain, and the three Sandiford-negative smear yields only scanty

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

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</td>
<td>Sandiford</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>+</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>—</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>48</td>
</tr>
</tbody>
</table>

*Staphylococcus epidermidis (2)
*Staphylococcus epidermidis (1)
Acinetobacter sp. (1)
Escherichia coli (1)
Staphylococcus epidermidis (1)
Acinetobacter sp. (2)
Bacillus sp. (1)
Candida sp. (1)
Escherichia coli (10)
Pseudomonas aeruginosa (1)
Staphylococcus pyogenes (12)
Streptococcus pneumoniae (1)

Bacteroides sp. (1)
Micrococcus sp. (1)
Anaerobic streptococcus (1)
Bacteroides sp. (1)
Diphtheroid sp. (1)
Proteus sp. (1)
Staphylococcus epidermidis (9)
Streptococcus faecalis (7)

+ 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.)
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Some clinician. Increased numbers of organisms seen, cannot be over-emphasised (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 techniques 8-9 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 stain 10 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.

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References


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