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[1] 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,[2] 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*.

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Modified Sandiford's stain

Malachite Green	0·05 g
Pyronin Y*	0·30 g
Dulbecco A phosphate buffer pH 7·3	100 ml

Store in a dark bottle.

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	Decolorise 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.	

Result

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

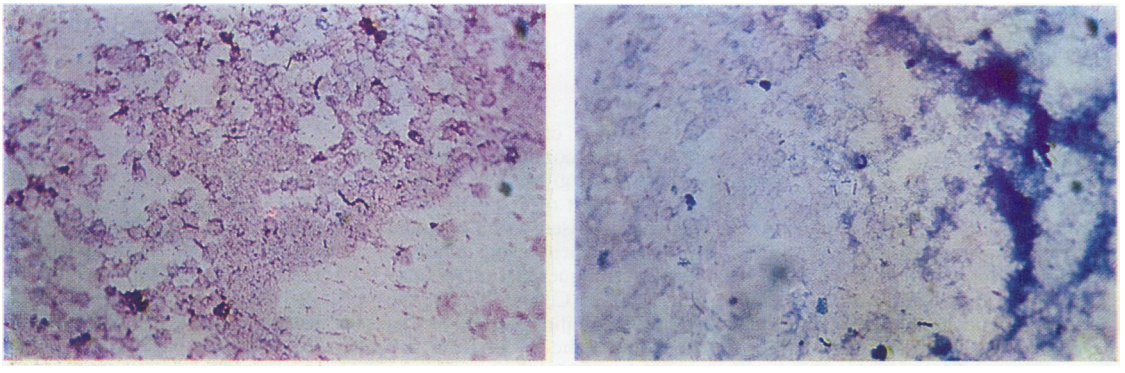
Altogether 414 blood culture smears were examined and 358 were smear negative (Gram and Sandiford's) and yielded no growth; 56 yielded growth on subculture, and a comparison of the smears is listed in the Table. There was a marginal increase in the number of positive smears with Sandiford stain, and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

Organisms seen		*Positive culture*	*Organisms isolated*	
Gram	*Sandiford*			
—	—	2	**Staphylococcus epidermidis* (2)	
+	—	1	**Staphylococcus epidermidis* (1)	
—	+	5	*Acinetobacter* sp. (1)	*Bacteroides* sp. (1)
			Escherichia coli (1)	*Micrococcus* sp. (1)
			Staphylococcus epidermidis (1)	
+	+	48	*Acinetobacter* sp. (2)	*Anaerobic streptococcus* (1)
			Bacillus sp. (1)	*Bacteroides* sp. (1)
			Candida sp. (1)	*Diphtheroid* sp. (1)
			Escherichia coli (10)	*Proteus* sp. (1)
			Pseudomonas aeruginosa (1)	*Staphylococcus epidermidis* (9)
			Staphylococcus pyogenes (12)	*Streptococcus faecalis* (7)
			Streptococcus pneumoniae (1)	

+ 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.)



Smears from a blood culture yielding E. coli *and* Streptococcus faecalis (*magnification* × *500*): (left) *Gram* + *0·1% carbol fuchsin counterstain;* (right) *Gram* + *modified Sandiford's counterstain.*

growths 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 overemphasised (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[3–9] 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

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.
2. Davis BD, Dulbecco R, Eisen HN, Ginsberg HS, Wood WB. *Microbiology* 2nd ed. New York and London: Harper and Row, 1973;767.
3. Cook HC. *Manual of Histological Demonstration Techniques*. London: Butterworths, 1974;122.
4. Sowter C, McGee ZA. Evaluation of a new technique for the demonstration of gonococci and other microorganisms in host cells. *J Clin Pathol* 1976;**29**:433-7.
5. Humberstone FD. A Gram-type stain for the simultaneous demonstration of gram-positive and gram-negative micro-organisms in paraffin sections. *J Med Lab Technol* 1963;**20**:153-6.
6. Brown JH, Brenn L. A stain for bacteria in tissue. *Bull Johns Hopkins Hosp* 1941;**48**:69.
7. Brown RC, Hopps HC. Staining of bacteria in sections. *Am J Clin Pathol* 1973;**60**:234-40.
8. Davis JC. A gram stain for blood cultures, body fluids and tissues. *Am J Med Technol* 1976;**42**:417-23.
9. Taylor RD. Modification of Brown and Brenn's Gram stain for differential staining of gram-positive and gram-negative bacteria in tissue sections. *Am J Clin Pathol* 1966;**46**:472-4.
10. Cruickshank R, Duguid JP, Marmion BP, Swain RHA. *Medical Microbiology* 12th ed, vol 2. London and Edinburgh: Churchill-Livingstone, 1975;36.
11. Leaver RE, Evans BJ, Corrin B. Identification of Gram-negative bacteria in histological sections using Sandiford's counterstain. *J Clin Pathol* 1977;**30**:290-1.

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