

Evaluation of Three Media for Selective Isolation of Gram-Positive Bacteria from Burn Wounds

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Three media, phenylethyl alcohol blood agar, esculin-mannitol agar, and Columbia CN blood agar, were studied for the selective isolation of gram-positive bacteria from swab cultures of burn wounds.

Bacteriological culturing of burn wounds frequently yields a preponderance of gram-positive and gram-negative bacteria that are not indigenous to normal skin (4). The continual need to monitor burn areas of patients as one means of infection control requires handling multiple weekly burn cultures on each patient and is usually limited to surface cultures taken with swabs (2, 3, 5, 6). Laboratories may be faced with a problem of processing swab cultures from burns because the swab must be streaked on a battery of plate media to anticipate several different kinds of organisms which might be present. Use of a medium selective for gram-positive bacteria is particularly important where specimens containing gram-negative bacilli may obscure the growth of gram-positive bacteria on nonselective blood agar. Three media were compared to determine their uses and limitations in the selective isolation of gram-positive bacteria from burns.

Cultures of burns were taken with moistened swabs (culturettes, Scientific Products, Houston, Tex.). Swabs were delivered to the laboratory and inoculated on media within 30 min. Each swab was rolled over the surface of one quadrant of each agar medium, and loops were used to streak the remainder of each plate. The various plate media were stacked randomly so that no one medium was inoculated first each time. All media were incubated aerobically at 37 C and observed daily for a period of 72 h.

Gram-negative bacilli were identified by the methods of Douglas and Washington (1). Gram-positive organisms were identified by procedures previously reported (7, 8).

Selective media for gram-positive bacteria consisted of phenylethyl alcohol blood agar (PEA), Columbia CN blood agar (CNA), and esculin-mannitol (EM) agar (P. A. Granato and P. D. Ellner, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 99, 1973). The EM agar was pre-

pared with Columbia CN agar base to which were added per liter: mannitol, 10 g; esculin, 1 g; ferric ammonium citrate, 0.5 g; and sodium phenol red, 25 mg (Paul A. Granato, personal communication). This medium was later modified in one experiment by reducing the amount of mannitol present. Gram-negative bacteria were isolated on MacConkey agar and yeasts were isolated on Snyder agar. Five-percent human blood agar for general cultivation of bacteria was made with Columbia agar base. All media were obtained from BBL.

In one experiment, various types of streptococci isolated from burns or from other sources of patients were grown in brain heart infusion broth (BBL), diluted to the Kirby-Bauer antibiotic turbidity standard, and streaked on various media to compare their relative growth rates and colonial morphology.

Isolation of gram-positive bacteria from burns on selective media was studied in two parts. Group A consisted of 92 burn cultures in which PEA agar was compared with CNA agar and regular Columbia blood agar. In Group B, 87 burn wound cultures were used, and PEA agar was compared to EM agar and Columbia blood agar. The EM-CNA media were not compared because both were prepared from the same selective agar base.

Table 1 summarizes the ability of each of the three gram-positive selective media to inhibit the growth of gram-negative bacilli. In the combined total of 179 burn cultures (groups A and B), 48 (26.8%) of the cultures contained one or more gram-negative bacilli totaling 64 strains. The EM-CNA media completely inhibited all gram-negative bacilli from growing after 72 h of incubation, but more than half of the *Pseudomonas aeruginosa* strains grew on PEA agar in 24 h. This occurred when PEA agar was used within 8 h of preparation. One strain each of *Escherichia coli* and *Proteus mirabilis* also

grew on PEA agar after 72 h of incubation.

A total of 58 (32.4%) of the cultures in groups A and B contained one or more gram-positive bacteria or yeasts (Table 2). There were 17 occasions when the growth of *P. aeruginosa* on PEA or regular blood agar obscured recognition or isolation of the gram-positive bacteria or yeasts. Yeasts, corynebacteria, and group A streptococci grew poorly or not at all on PEA agar and generally required 48 h of incubation before colonies appeared on EM agar. *Staphylococcus aureus* grew rapidly on EM agar and was presumptively recognized by mannitol fermentation. Five strains of *Streptococcus faecalis* were isolated in group B, which fermented mannitol on EM agar without esculin hydrolysis.

TABLE 1. Comparative selectivity of media in inhibiting the growth of gram-negative bacilli isolated from burns

Group	Total cultures	Medium	Bacteria growing on various media	
			<i>P. aeruginosa</i>	<i>Enterobacteriaceae</i>
A	92	PEA	13/20 ^a	1/11
		CNA	0/20	0/11
B	87	PEA	11/22	1/11
		EM	0/22	0/11

^a Number of strains of gram-negative bacilli growing on gram-positive selective media/total gram-negative bacilli isolated. Total strains based on recovery from blood and/or MacConkey agar.

ysis. These strains were streaked on EM agar containing 1.0, 0.5, 0.25, and 0.125% mannitol. The strains preferentially attacked mannitol when the carbohydrate was used in concentrations of 0.5% or more. When the mannitol concentration was kept below 0.5%, the mannitol was fermented and esculin was also hydrolyzed by the five enterococcal strains.

The recognition of *Streptococcus pyogenes* in burns is of primary importance in any acutely burned patient (8). None of these organisms was isolated in the group B study. Therefore, five strains each of *S. pyogenes*, *S. agalactiae*, and oral viridans streptococci were streaked on PEA, EM, CNA, and regular blood agars for comparative growth. These streptococci did not grow on PEA in 24 h, but appeared at 48 h of incubation as minute colonies with poor hemolytic zones. The strains required 48 h to grow on EM agar, but colonies were less than 1 mm in diameter and undifferentiated. All of the strains grew in 24 h on CNA and regular blood agars producing characteristic types of alpha and beta hemolysis.

The results of this study could have been affected by poor distribution of organisms collected on a swab and their inoculation on a series of media. This is an inherent problem with all burn swab culture techniques, particularly when the size of a mixed inoculum cannot be predicted. There were, nevertheless, some definite indications as to the uses or limitations of three selective media for the isolation of gram-positive bacteria from burns. The least

TABLE 2. Comparative isolation of gram-positive bacteria and yeasts from burns on various media

Group organisms	Total strains isolated ^a	Media				
		CNA	EM	PEA	Blood	Snyder
A						
<i>S. aureus</i>	27	25		22	22	0
<i>S. epidermidis</i>	3	3		3	1	0
Yeasts	3	3		0	2	3
Corynebacteria	2	2		1	2	0
Enterococci	5	5		4	3	0
Group A streptococci	3	3		1	3	0
Total	43	41		31	33	3
B						
<i>S. aureus</i>	23		23	20	20	0
<i>S. epidermidis</i>	7		7	7	7	0
Yeasts	5		5	0	4	5
Corynebacteria	6		6	0	6	0
Enterococci	11		11	8	6	0
Total	51		52	35	43	5

^a Based on isolation of organisms from each specimen counting all media combined.

satisfactory medium was PEA blood agar, due to the ability of *P. aeruginosa* to grow rapidly on the medium and often obscure the growth of gram-positive bacteria. EM agar was an excellent medium for prompt isolation and presumptive recognition of *S. aureus* and *S. faecalis*, although some strains of the latter bacterium fermented mannitol without esculin hydrolysis which caused some confusion. A catalase test done on colonies of these organisms on EM agar would aid in their identification. *Corynebacteria*, *S. epidermidis*, and yeasts grew on EM agar, but 48 to 72 h of incubation was necessary for recognition. Hemolytic streptococci other than the enterococci grew poorly on EM agar and did not produce colonies with notable characteristics helpful in identification. With the exception of these streptococci, however, EM agar was considered an excellent selective and differential medium for burns. Columbia CN blood agar, however, permitted the most rapid growth of the greatest variety of gram-positive bacteria isolated from burn wounds.

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LITERATURE CITED

1. Douglas, G. W., and J. A. Washington. 1970. Identification of the *Enterobacteriaceae* in the clinical laboratory. Center for Disease Control, Atlanta, Ga.
2. Georgiade, N. G., M. C. Lucas, and S. Osterhout. 1970. A comparison of methods for the quantitation of bacteria in burn wounds. II. Clinical evaluation. *J. Clin. Pathol.* **53**:40-42.
3. Hoopes, J. E., H. R. Butcher, H. W. Margraf, and D. L. Gravens. 1971. Silver lactate burn cream. *Surgery* **70**:29-37.
4. Johnson, J. E. III. 1971. Wound infections. *Postgrad. Med.* **50**:126-135.
5. Ollstein, R. N., F. C. Symonds, G. F. Crikelair, and L. Pelle. 1971. Alternate case study of topical sulfamylon and silver sulfadiazine in burns. *Plast. Reconstructr. Surg.* **48**:311-317.
6. Shuck, J. M., L. E. Einfeldt, and M. P. Trainor. 1972. Sulfamylon solution dressings in the management of burn wounds: preliminary clinical report. *J. Trauma* **12**:999-1002.
7. Smith, R. F., C. L. Bettge, S. L. Dayton, and J. J. Jorgensen. 1973. Characterization of *Staphylococcus aureus* in a pediatric burn unit. *Appl. Microbiol.* **25**:15-20.
8. Smith, R. F., S. L. Dayton, and D. D. Chipps. 1973. Autograft rejection in acute burn patients: relation to colonization by *Streptococcus agalactiae*. *Appl. Microbiol.* **25**:493-495.