

## Efficacy of Direct Gram Stain in Differentiating Staphylococci from Streptococci in Blood Cultures Positive for Gram-Positive Cocci

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Received for publication 20 October 1977

A preponderance of clusters seen on direct Gram stain of blood cultures positive for gram-positive cocci was 98% sensitive and 100% specific for identification of staphylococcal species or of *Peptococcus*. A preponderance of chains, pairs, or both was 100% sensitive and 98% specific for identifying streptococci. Further presumptive identification of either staphylococci or streptococci based on microscopic morphology was unreliable. The direct Gram stain is highly reliable for differentiating staphylococci from streptococci and should be of considerable value to clinicians selecting initial antimicrobial therapy.

In most clinical laboratories, positive blood cultures are first detected by encountering macroscopic evidence of growth or by routine direct Gram-stained smears (2). The initial report to a physician usually consists of the microscopic morphology of the organism based on the direct Gram stain (1). Approximately 12% of the time, subculture on solid media will provide the first evidence of a positive culture (2), in which case considerably more initial characterization of the organism beyond microscopic morphology is then possible.

Because morphology based on the direct Gram stain is largely the sole initial information pertaining to the identity of a blood isolate, we questioned the reliability of the smear in differentiating staphylococci from streptococci in blood cultures positive for gram-positive cocci. In a survey of neighboring hospital laboratories, we found that up to one-half of the time the laboratory reported blood cultures positive for gram-positive cocci simply as "gram-positive cocci," without further noting whether clusters, chains, or pairs were in preponderance. If staphylococci could be reliably distinguished from streptococci on the direct smear, physicians might be able to select more appropriate initial antimicrobial therapy while awaiting complete microbiological identification of the organisms and results of susceptibility testing. We report the results of two studies: (i) a retrospective review of a 33-month experience in the laboratory of our hospital and (ii) a 14-month prospective study.

### MATERIALS AND METHODS

**Blood culture techniques.** Five milliliters of blood was aseptically drawn from the patient and transferred

to a closed 50-ml bottle containing tryptic soy broth with 0.025% sodium polyanethol sulfonate added (Difco Laboratories). Bottles were not vented unless yeasts or pseudomonads were suspected and were incubated at 35°C. At 6 to 12 h and daily thereafter, bottles were inspected for macroscopic evidence of growth. Direct Gram-stained smears and subcultures in thioglycolate broth and on sheep blood agar and chocolate agar plates were routinely performed at 12 to 24 h, 5 days, and 14 days and whenever bottles showed macroscopic evidence of growth. Anaerobic subcultures were performed when aerobic subcultures of a culture positive on direct Gram stain showed no growth at 24 h. Isolates were identified by using standard criteria and techniques.

During the periods reviewed, five to seven technicians working on a rotating basis processed approximately 500 blood cultures monthly. Between 12 and 15% were positive, approximately one-half of the time for gram-positive cocci.

**Retrospective study.** We reviewed the results of the first positive direct Gram stain and final characterization of all blood cultures drawn between 16 July 1973 and 7 April 1976 yielding gram-positive cocci and in which the direct Gram stain was recorded as positive.

**Prospective study.** Between 7 April 1976 and 7 June 1977, we undertook a prospective study to determine whether we could further improve this initial morphological categorization, especially the differentiation of staphylococci from streptococci. Technicians were requested to note and record as much morphological detail as possible on review of direct Gram stains. They were specifically requested to try to routinely determine whether clusters, chains or paired forms, or both were predominant. The categorization "gram-positive cocci" was to be employed only when further morphological characterization was not possible.

**Parameters determined.** Sensitivity and specificity of diagnostic tests have been defined and comprehensively discussed by Galen and Gambino (3). A

preponderance of clusters on direct Gram stain was considered a true-positive test for staphylococci and *Peptococcus*, and a preponderance of pairs, chains, or both was considered a true-positive test for streptococci; conversely, clusters predominating in a culture yielding streptococci and pairs, chains, or both in a culture containing staphylococci were regarded as false-positive tests.

## RESULTS

**Retrospective study.** Details of the Gram stain morphology based on initial direct smears were recorded for 443 blood cultures yielding gram-positive coccal species (Table 1). In 155 instances (34.9%), the initial microscopic description contained no information further than gram-positive cocci. However, a preponderance of clusters was 100.0% sensitive (186/186) and 93.1% specific (95/102) for staphylococci or *Peptococcus*. A preponderance of chains strongly suggested streptococci other than *Streptococcus pneumoniae* or enterococci; a predominance of pairs or chains was 93.1% sensitive and 100.0% specific for streptococcal species.

**Prospective study.** It was apparent that with effort, gram-positive cocci seen on a direct Gram-stained smear could almost uniformly be categorized as gram-positive cocci, primarily in clusters, or gram-positive cocci, predominantly in pairs, in chains, or in pairs and chains (Table 2). A predominance of clusters was 97% sensitive (77/79) and 100% specific (46/46) for *Staphylococcus aureus*, coagulase-negative staphylococci, or *Peptococcus*; mainly pairs or chains

TABLE 1. Retrospective review of direct Gram stains of blood cultures positive for gram-positive cocci<sup>a</sup>

Final identification	Gram-positive cocci	Preponderance of:		
		Clusters	Pairs	Chains
Group A streptococci	4			11
Group D <i>Streptococcus</i>	24	4	2	27
Groupable streptococci, other than A or D	9	2		13
Viridans streptococci	10	1		25
<i>S. pneumoniae</i>	6		9	3
<i>Peptostreptococcus</i> spp.	3			5
<i>S. aureus</i>	49	85		
Coagulase-negative staphylococci	50	95		
<i>Peptococcus</i>		6		

<sup>a</sup> Technician's description of bacterial morphology. One culture yielding both staphylococci and streptococci is excluded.

TABLE 2. Prospective study—efficacy of direct Gram stain in differentiating staphylococci from streptococci in blood cultures positive for gram-positive cocci<sup>a</sup>

Final identification	Gram-positive cocci	Preponderance of:	
		Clusters	Pairs, chains, or both
Streptococcal spp. <sup>b</sup>	1		46
Staphylococcal spp. <sup>c</sup>		74	
<i>Peptococcus</i> spp.		3	2

<sup>a</sup> Technician's description of morphology. Two cultures, one yielding both staphylococci and streptococci and one showing gram-positive cocci on smear but negative on culture, are excluded.

<sup>b</sup> Group D streptococci (18 isolates), other groupable streptococci (17 isolates), *S. pneumoniae* (11 isolates), *Peptostreptococcus* spp. (1 isolate).

<sup>c</sup> *S. aureus* (34 isolates), coagulase-negative staphylococci (40 isolates).

was commensurately 100% sensitive and 97% specific for streptococci. No staphylococcal isolate failed to show a preponderance of clusters or, conversely, showed mainly pairs or chains. Two out of five *Peptococcus* isolates showed mainly pairs on direct smear.

It was not possible to differentiate *S. aureus* from *Staphylococcus epidermidis* and other coagulase-negative members of the family *Micrococcus* based on Gram stain morphology. Various streptococci, including *S. pneumoniae*, were equally divided between a preponderance of chains and of pairs. *S. pneumoniae* could occasionally be identified presumptively based on the presence of larger lancet-shaped diplococci; however, overall, attempts to reliably distinguish streptococcal species on the basis of their microscopic morphology were not successful.

## DISCUSSION

This study demonstrates that, by making a concerted effort to note accurately all details of microscopic morphology in the initial Gram-stained smear of a blood culture positive for gram-positive cocci, staphylococci can be distinguished from streptococci with nearly 100% specificity. A preponderance of clusters is virtually pathognomonic of staphylococcal species and chains, pairs, or both of streptococci. Further identification of staphylococci or streptococci based on microscopic morphology is unreliable.

This information should be of considerable value to clinicians in making therapeutic decisions pending full characterization of the isolate and antimicrobial susceptibility testing. If clusters are present, initial antimicrobial therapy

should be selected for efficacy against staphylococci, e.g., a semisynthetic penicillinase-resistant penicillin, a cephalosporin, clindamycin, or vancomycin. If chains or pairs predominate, aqueous penicillin G or ampicillin would seem most appropriate, assuming the patient is not allergic to penicillin. Whether to use ampicillin or penicillin combined with an aminoglycoside, e.g., for presumptive enterococcal bacteremia, can usually be determined by the clinical situation. In both instances in which a nonstreptococcal species showed chains rather than clusters, *Peptococcus* was involved. However, peptococci are almost uniformly sensitive to penicillins (4), and thus false characterization as staphylococci would be unlikely to have adverse therapeutic consequences.

If a preponderance of clusters is seen, suggesting staphylococci, direct tests can also be set up for elaboration of coagulase and beta-lactamase (John A. Washington II, personal communication).

Hospital laboratories should strive to note maximal morphological detail of gram-positive cocci seen on direct Gram stains of positive blood cultures and transmit this information and the

accompanying presumptive identification to the clinician: i.e., gram-positive cocci in clusters, presumptive staphylococcal species; gram-positive cocci in pairs and chains, presumptive streptococcal species.

#### ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI-00432 from the National Institute of Allergy and Infectious Diseases.

We acknowledge and appreciate the cooperation and assistance of the staff members of the Clinical Microbiology Laboratory, University of Wisconsin Hospitals (Julie Franz, head microbiologist; Vadakepat Ramgopal, director).

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