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Microscopy of Stained Urine Smears to Determine the Need for Quantitative Culture

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Consecutive specimens (2,564) of urine were cultured quantitatively, and Gram-stained smears were prepared from centrifugates as well as from the uncentrifuged specimens. About half of the specimens harbored organisms, and the quantity seen in smears of the centrifugates correlated reasonably well with the numbers of viable organisms cultured from the specimens. For example, smears of 900 centrifugates had one or more organisms per oil immersion field; 712 of their respective specimens proved to have colony counts of 10^5 or greater, and 120 had 10^4 to $<10^5$. For 188 specimens, however, the smears falsely predicted $\geq 10^5$ colony-forming units (many of these fell into the group that had 10^4 to $<10^5$) but failed to predict clinically significant concentration of 10^5 or greater in only 31. Predictive values are presented also for lesser quantities of organisms seen in such smears. With rare exception, smears of centrifugates were superior to those of whole specimens as predictors of the concentration of viable organisms. Evaluation of smears proved not to be biased by the operator; values did not deviate significantly depending upon whether one or several microbiologists performed the evaluations.

Current recommendations (1) for microbiological examination of urine include a Gram-stained smear of uncentrifuged urine as well as quantitative culture to estimate the number of organisms and for quality control. Both procedures require skilled personnel, however, and are time consuming. A study was undertaken to evaluate the efficacy of direct microscopic examination of stained smears of specimens to determine if a specimen should be cultured or if that specimen could be reported, instead, as not having a clinically significant number of organisms, without determination of how many were viable.

MATERIALS AND METHODS

A total of 2,564 consecutive specimens, either fresh or refrigerated, were processed. Well-mixed urine was inoculated onto an agar plate containing 5% sheep blood agar and Tergitol 7 (Difco Laboratories, Detroit, Mich.), using a Jorgenson platinum loop calibrated to deliver approximately 0.001 ml (no. N2075-2; Scientific Products, Inc., Evanston, Ill.), and streaked to provide maximum separation of colonies; one drop was deposited onto a clean slide, allowed to spread and dry, fixed, and Gram stained; and 5 ml was centrifuged for 10 min at $360 \times g$. One drop of the centrifugate was deposited onto a clean slide, dried, fixed, and stained as before. In the first phase of the study, which involved 2,103 specimens, all stained smears were evaluated by one individual. The smears of 461 additional consecutive urines were examined by various unselected technologists in the bacteriology section. All smears were examined for 30 s with an oil immersion lens, and the organisms were quantitated as "rare" when up to a total of four organisms were noted, "occasional" when having more than a total of four organisms during the entire examination but not one in each field, or "multiple" when one or more organisms per field were noted. To qualify for the latter category, at least one organism had to be seen in each field. Beyond these categories, quantitation proved unnecessary; even with a smear such as one that had an average of one per field, with multiple organisms in some fields and none in others (which would be rated occasional), the number of colony-forming units (CFU) was never as high as 10^5.

RESULTS

Data from the entire 2,564 specimens were compiled irrespective of whether one or more operators had been involved and analyzed in terms of clinically significant or insignificant errors. An insignificant error represented an instance when the quantity of organisms seen in the direct smear predicted a clinically significant number of viable organisms to be in that specimen but for which, in fact, culture proved this not the case; i.e., the specimen would have been cultured unnecessarily. A clinically significant error, on the other hand, was one in which the smear falsely indicated that quantitative culture would be unneccessary, for ac-

tual culture showed, to the contrary, a clinically significant number of CFU.

Evaluation of the data (Table 1) indicated that any smear having multiple organisms in the centrifugate would have a colony count of 10^5 CFU or higher. When $\geq 10^5$ CFU was used as the criterion of urinary infection, we found an insignificant error in 188 cases (7%) and significant error in 31 cases (1%). When the clinically significant level was lowered to $\geq 10^4$, an additional 47 (2%) significant errors were noted, giving a maximum significant error rate of 3%.

We evaluated our data to determine also how many more clinically significant colony counts would have been detected if specimens rated less than multiple had been cultured quantitatively. Culturing of specimens having smears judged rare or occasional revealed an additional 47 (2%) in the category of 10^4 to $<10^5$ CFU and 31 (1%) with $\geq 10^5$ CFU, but 307 (12%) of such specimens had $<10^4$ CFU and thus would have been cultured needlessly. Assuming that there must be at least 10^5 CFU to be indicative of disease, there would have been, in all, 354 unnecessary cultures, and only 31 additional clinically significant counts would have been uncovered.

Smears of uncentrifuged urines that were rated multiple usually had colony counts $\geq 10^5$, although a few such counts would have been missed had the centrifugate not been examined also. Frequently a centrifugate was scored multiple when only rare or occasional, or even no, organisms were seen in the smear of the unspun specimen. There were times, however, when the smear of a centrifugate was unsatisfactory, viz. when excessive amounts of particulate material obscured the presence of organisms. In the latter instances, in which resorting to use of uncentrifuged specimen was necessary and when organisms were seen in the direct smear of such and graded as multiple, their presence proved clinically significant.

TABLE 1. *Relationship between the quantity of organisms seen in stained smears of centrifugates and the number of CFU in 2,564 urine specimens*

Organisms seen	Colony count per ml of uncentrifuged specimen			
	$\geq 10^5$[a]	10^4 to $<10^5$	$<10^4$	0
None	0	0	0	1,279
Rare or occasional[b]	31	47	307	0
Multiple[b]	712	120	68	0

[a] CFU.
[b] See text.

Whether or not smears were evaluated by a single individual or by various individuals proved inconsequential. Of 461 of the above specimens, examined by unselected individuals, there was a total of 44 (10%) insignificant errors and only 10 (2%) and 17 (4%) cumulative, significant errors when $\geq 10^5$ and $\geq 10^4$ CFU, respectively, were accepted as clinically significant.

DISCUSSION

In the text on urinary tract infections by Kunin (3) it is stated that a number of workers have reported that stain methods correlate with quantitative culture about 80 to 90%. McLin and Tavel (4), however, found that 25% of infections would be missed by examination of smears alone, even of centrifugates, but infection was defined as 1,500 or more colonies per ml of urine. Unfortunately, the basis for this presumed significance was not well documented. Furthermore, it was not clear who examined the smears, and in our experience training in microbiology is indispensable. Jorgensen and Jones (2) also compared Gram stains of centrifugates as well as Limulus assay with culture methods for detection of significant bacteriuria. Their criteria for positivity of a smear was at least one organism per oil immersion field and at least 10^5 viable organisms per ml. Overall, the smears agreed with cultures 89% of the time compared with our 99% when we used the same criteria. These latter investigators pointed out, however, that their percent agreement was lower than in previous studies. Robins et al. (5) studied uncentrifuged specimens from asymptomatic patients and concluded that correlation between microscopic bacteriuria and quantitative culture was sufficiently reliable so that a culture need not be done when microscopy was essentially normal, i.e., during the absence of bacteria and leukocytes.

In our opinion, based on our experiences, there are certain circumstances when a properly evaluated Gram stain of centrifuged urine can be utilized as a screening procedure to determine whether or not a specimen should be cultured quantitatively. These circumstances are when a patient is: (i) asymptomatic, (ii) not receiving antimicrobial therapy, or (iii) not being seen for recurrent urinary tract infections. The advantages of the smear should be weighed, however, against the amount of valuable time that will be expended by skilled laboratory personnel. In laboratories where stained smears are a part of the routine microbiological examination of urine specimens, the time nec-

essary to centrifuge a specimen is relatively slight for the return of eliminating a large number of culture procedures.

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