Simple Disposable Method for Quantitative Cultures of Urine

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A disposable kit was tested as a means of detecting significant bacteriuria by quantitative culture of urine. The total error in 3,563 specimens tested by five investigators was less than 1%. The method was very effective in differentiating significant bacteriuria, i.e., more than 100,000 bacteria per ml of urine from uninfected urine. In specimens from patients with urinary tract abnormalities who had mixed bacterial flora, the absolute numbers obtained with the dip-inoculum method had a 10% variation when compared to results obtained by calibrated loop or dilution pour plate methods. Therefore, the main utility of the kit is for screening and following patients after therapy. A significant delay in time between inoculation of the medium in the kit with the freshly voided urine and incubation of the kit to promote growth did not affect the reliability of the kit as a method of doing quantitative urine cultures to detect bacteriuria.

The problems of collection of urine, refrigeration of the urine prior to its quantitative culture, and transport of urine to the site of culturing have made it difficult to obtain reliable urine cultures and colony counts from patients. Mackey and Sandys developed a reusable two-piece plastic spoon which could be autoclaved and filled with medium by the physicians using it in an effort to overcome some of these limitations (5). Their dip-inoculum method made it possible to obtain reliable colony counts and prompted others to develop the dip-slide-inoculum method (2). Various kits for quantitative urine culture are now available, but, because of complexity in preparing and using these methods, expense, or unreliable results, none has gained widespread acceptance.

The dip-inoculum method has been modified to provide a kit that is reliable and sensitive in detecting bacteriuria at low cost. Data are presented to show that it is an accurate way to quantitate the number of bacteria in urine and that it overcomes the delay between collection of the urine and inoculation of the medium for the colony count. It has provided sensitive, reliable, quantitative cultures of urine in the hands of several groups of laboratory personnel as described in this communication.

MATERIALS AND METHODS

Kit. It was necessary to design a plastic spoon which could be easily mass-produced in a simple injection molder (Fig. 1). To prevent dehydration of the medium due to air leakage, a special bottle and cap were designed which provided a tight permanent seal, yet the lid could easily be removed and replaced by any person using the kit. Each spoon is filled with an exact amount of a cysteine-lactose-electrolyte-deficient medium (CLED) available from Oxoid. This medium was chosen because it readily supports the growth of the usual urinary tract pathogens, prevents the spreading of Proteus species, and also allows easy detection of contamination of the urine specimen by supporting growth of the usual contaminants (3, 5, 6).

The surface of the medium is inoculated by dipping it into urine sufficient for all of the medium to be contacted by the urine, and then removing it and replacing it in its carrying bottle. Simple immersion of the medium into the urine is all that is required. If there is a very small volume of urine, it is possible to pour the urine over the surface of the medium, allowing the excess to run off. Contact is the only requisite to adequate inoculation. The most important consideration in inoculation is to expose the cultural medium to the urine as soon after it is collected as possible. Early inoculation is recommended to prevent changes in bacterial numbers present in the urine at the time of voiding. After the plastic spoon has been returned to its carrying bottle, it is transported to the laboratory.

Incubation at 35 to 37 C for 18 to 24 hr will allow colonies to develop on the medium. Previous work by Mackey and Sandys (5) showed that a controlled surface area exposed to the urine could be standardized so that, when there are more than 10^5 bacteria per ml of urine, confluent or semiconfluent growth of bacteria will occur. This is demonstrated in Fig. 2 which shows the confluent growth. This was further con-
firmed by comparing the 500 specimens with colony counts of greater than $10^6$ per ml of urine as determined by duplicate-dilution pour-plate methods. On all of these specimens the kit gave confluent growth. When discreet colonies are present, they are counted and multiplied by the factor 0.6 (4). The result is the number of bacteria, in thousands, per milliliter of urine; that is, when 100 colonies are present, multiply 100 X 0.6. This means that there were 60,000 bacteria per ml of urine. Contaminant bacteria are easily recognized because of low counts with colonies which have variable shapes, colors, and sizes. The easy differentiation between no growth, minimal contamination with mixed kinds of bacteria, and significant bacteriuria is demonstrated in Fig. 3, 4, and 5.

Reliability in establishing colony counts. A total of 3,563 urine specimens were tested simultaneously with calibrated-loop or duplicate-dilution pour-plate methods and the dip-inoculum method. Of these, 2,000 specimens were tested by the following investigators: F. H. Kayser, Institute for Medical Microbiology, Zurich; Jack S. Remington, Stanford University School of Medicine and the Palo Alto Research Foundation, Palo Alto, Calif.; Sydney Finegold, Wadsworth Veterans Administration Hospital, and the UCLA School of Medicine, Los Angeles, Calif.; and Michael Miller, University of Oregon Medical School, Portland. The other 1,563 specimens were from adult patients in the hospitals and clinics of the University of Oregon Medical School, Portland, or adult and pediatric patients at the Sacramento Medical Center, Sacramento, Calif.

Tests of accuracy of colony counts with a delay between inoculation of the kit and incubation. Clean-catch or catheter-collected urine specimens from 607 patients were tested. Urine was collected in a sterile glass bottle and taken immediately to the laboratory. Duplicate-dilution pour plates in nutrient agar were prepared. The surface of a plate containing blood-agar and the surface of a plate containing MacConkey agar were each inoculated by a standard calibrated-loop method. At the same time, one of the kits from the dip-inoculum method was inoculated. The pour plates, the blood-agar, and MacConkey plates were incubated immediately, and colony counts were determined after overnight incubation at 37 C. The inoculated kit was kept at room temperature for 72 hr and incubated overnight at 37 C; a colony count was performed by an independent observer.

FIG. 1. Dip-inoculum kit consisting of the storage and transport bottle and the plastic spoon with its easily gripped handle filled with the cysteine-lactose-electrolyte-deficient medium.

FIG. 2. Close-up view of confluent growth of Escherichia coli on the surface of the CLED medium after overnight incubation at 36 C.

FIG. 3. Close-up of the surface of the medium of a kit that had no growth after inoculation and incubation. Compare with Fig. 2.

FIG. 4. Close-up of colonies which appeared on the surface of the medium in the kit when a contaminated urine specimen was used for inoculation. Note the variation in colony size and density. These would have various colors in addition. This is easily identified as a contaminated specimen.
RESULTS

There were 18 false-positive tests (0.6%) with the kit in 2,893 specimens which had no growth or less than 10,000 bacteria per ml of urine as determined by the standard methods (Fig. 6). Calibrated-loop and pour-plate methods detected 528 specimens containing more than 100,000 bacteria per ml of urine. The kit method agreed in 99% of the specimens. The few false-negative errors could not be explained since the patients' urine contained more than 100,000 bacteria per ml when retested with the kit at a later date.

There were 142 specimens which contained between 10,000 and 90,000 bacteria per ml of urine as measured by calibrated-loop or dilution pour-plate methods. There were 10 false negatives and 5 false positives in these 142 specimens with the kit method. When these 15 patients were retested, 14 had no growth and were obviously contaminated on the first test. This was suspected by the kit method because of mixed flora on the first specimen.

The total error was 1.1% in the 3,563 specimens tested by the five laboratories. This low false-positive error greatly decreased the need for repeat quantitative cultures, and the low false-negative error confirmed that this was a sensitive, reliable method for detecting bacteriuria.

Delay between inoculation and incubation of the kit to promote bacterial growth. A 72-hr delay between inoculation of the urine onto the medium in the kit and incubation of the kit to promote bacterial growth had no effect on colony counts obtained from 607 specimens (Fig. 7). There was a 1% total false-positive and false-negative error rate in these 607 specimens. This compared favorably with the overall 1.1% error rate obtained with the 3,563 specimens tested without a delay between inoculation and incubation.

DISCUSSION

This method permitted reliable quantitative urine cultures even when there was necessarily a time delay of several hours between inoculation of the urine and incubation of the kit. Although it has been assumed that the time between collection of the urine and inoculation would be an important variable, this was not specifically tested. We have repeatedly stressed the importance of inoculating the medium in the plastic spoon as soon as the urine is collected. We feel
This is possible because the kits can be stored in the offices or on any ward in the hospital and eliminates the problem of refrigeration and transportation of the urine to a central facility where the urine could be inoculated. The method of inoculating seems to be unimportant since there was adequate detection of bacteriuria in those specimens where the duplicate-dilution pour-plate method or the calibrated-loop method showed bacteriuria and the kit was inoculated by either pouring the urine over the surface of the medium or by dipping the medium into the urine. There is no time factor required for exposing the medium to the urine for a given length of time. This makes it a rapid, easy, method of inoculating many urines at one time.

Because it is possible to mass-produce the kits, they can be made available for office and hospital use at low expense. This factor has frequently inhibited physicians from doing quantitative urine cultures in patients with symptoms of urinary-tract infection and as a screening procedure to detect asymptomatic bacteriuria.

It can no longer be argued that screening for bacteriuria is an unnecessary test. Infection of the urinary tract with associated bacterial infection of the kidneys is felt to be one of the preceding events leading to "pyelonephritis" which is probably the most common cause of renal failure. It has been proven that a single attack of acute infection may be succeeded by a measurable shrinkage of the kidney and, in children, resistant infection in the urinary tract may lead to scar formation and failure of growth of the kidney. In addition, studies with large numbers of patients free of symptoms indicate that pregnant and nonpregnant women have a high frequency of asymptomatic bacteriuria (5). Recent studies on American school children and on children in England confirm that between 1 and 2% of school-age girls free of symptoms have significant numbers of bacteria in the urine (3; D. G. Kelly, S. F. Cohalane, and F. A. Duff, unpublished data). Furthermore, investigation revealed that a majority of these children with bacteriuria had some structural abnormality of their urinary tract. In addition, in adults, those with bacteriuria often were found to have renal scarring, hypertension, and, in pregnancy, a high risk of complications to the mother and baby (1).

The availability of a dip-inoculum transport kit that will provide a sensitive, accurate, inexpensive method of detecting bacteriuria will make it possible to adequately identify large numbers of patients with asymptomatic bacteriuria. Only when this is done will it be possible to develop adequate plans for long-term management of these patients to guarantee eradication of the bacteriuria. It must be realized that use of this method is recommended to detect bacteriuria. Once the presence of more than 100,000 colonies of bacteria per ml of urine is confirmed for a patient, it is now necessary to isolate the infecting organism. When pure bacterial growth is present on the surface of the medium in the spoon, it is simple to subculture to routinely used bacterial media and identify the bacteria by traditional methods. This also allows antibiotic susceptibility testing. It is possible to obtain a positive culture, identify the organism by subculture, perform susceptibility tests, and proceed to treat those patients with infection. It is also possible that organisms can be identified directly from the surface of the CLED medium in the spoon by a series of rapid biochemical tests, which is the subject of a current investigational effort. Since screening results with the disposable kit described were comparable to cultural results obtained by the duplicate-dilution and calibrated-loop methods, it is now possible to do quantitative urine cultures whenever and wherever urine is collected, either in the office or in massive screening projects.

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