Slide Method for Detection of Antibody-Coated Bacteria in Urine Sediments

ROSEMARIE R. MATUSCAK,† A. WILLIAM PASCULLE,* LOUISA BARNHART,† R. WAYNE ATCHISON,† AND ROBERT B. YEE†

Department of Microbiology, Graduate School of Public Health,† and Department of Pathology, School of Medicine,* University of Pittsburgh, Pittsburgh, Pennsylvania 15261

An immunofluorescent slide method incorporating 0.1% (wt/vol) Evans blue as a counterstain was developed and compared with a recently described tube method. Seventy-one urine specimens were tested concurrently by both methods. Results of the two methods agreed in 70 specimens and disagreed in only 1. We found the slide method to be less costly and time consuming than the tube method.

The proper management of urinary tract infection depends on the accurate diagnosis of infection, including determination of upper or lower urinary tract involvement. The most reliable methods for determining the site of infection, ureteral catheterization and the bladder washout technique, are time consuming and likely to introduce bacteria into the urinary tract (1, 19).

Thomas et al. (19) have described a direct immunofluorescence test as an aid in determining the site of urinary tract infection. They demonstrated a close correlation between the presence of antibody-coated bacteria in urine sediment and renal involvement. These observations were confirmed by Jones et al. (13), who correlated the results of the immunofluorescence test with the bladder washout technique. Immunofluorescence tests have been used successfully by other investigators in identifying the site of urinary tract infection (4, 5, 8, 14, 16).

Antibody-coated bacteria have also been found in the urine sediments of patients with bacterial prostatitis (10, 19). The immunofluorescence technique is not useful in determining the site of involvement in urinary tract infections caused by yeast (3, 7).

This study was designed to develop a direct slide immunofluorescence method for detecting antibody-coated bacteria. Such a method would be reliable and noninvasive, but more convenient and less costly than the tube method of Thomas and co-workers (19).

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† Present address: Department of Medical Technology, School of Health Related Professions, University of Pittsburgh, Pittsburgh, PA 15261.

MATERIALS AND METHODS

Specimens. In-patient urine specimens (71) submitted to the clinical microbiology laboratory at Presbyterian-University Hospital, Pittsburgh, Pa. were examined for the presence of antibody-coated bacteria concurrently with the slide and tube methods. The specimens were clean-catch midstream urines or those obtained by either small-bore catheterization or by aseptic aspiration from indwelling catheters. To avoid analysis of potentially contaminated specimens, only those containing 10^5 colony-forming units per ml of one or two bacterial species were selected for analysis. With the preceding exception, the specimens were selected at random. The urine specimens were stored at 4°C for 24 to 48 h before testing for antibody-coated bacteria.

Antiserum. Fluorescein isothiocyanate-conjugated anti-human globulin and anti-human albumin of goat origin were obtained from Meloy Laboratories, Springfield, Va.

Tube method. The tube method was adapted from the method of Thomas et al. (18). Two 0.5-ml portions of each specimen were centrifuged at 1,500 × g for 10 min at room temperature in a Sorvall RC-2B centrifuge fitted with an SS-34 rotor. The supernatant fluids were discarded, and the sediments were washed twice with 0.5-ml portions of 0.01 M phosphate-buffered saline, pH 7.2. After discarding the supernatants from the second washing, 0.2 ml of conjugated anti-human globulin was added to one of the tubes. To the other tube, which served as a control to detect nonspecific staining, 0.2 ml of conjugated anti-human albumin was added. After incubation at 37°C for 30 min, both tubes were centrifuged as described above, and the sediments were washed twice in phosphate-buffered saline. One drop (ca. 0.05 ml) of each sediment was placed in each of two 10-mm pre-etched circles on a microscope slide (Clay-Adams, Parsippany, N.J.) and air dried. Glass cover slips were mounted on each smear using a semipermanent mounting fluid (17).

Slide method. For the slide method, 5 ml of urine was centrifuged as described above, the supernatant was discarded, and the sediment was washed once with 5 ml of phosphate-buffered saline. After the wash
ing the supernatant was discarded, and the sediment was suspended in 0.1 ml of phosphate-buffered saline. Smears of the sediment were prepared in duplicate on a pre-etched loop. The smears were then air dried and fixed in acetone at room temperature for 5 min. The test smear was covered with 1 drop of conjugated anti-human globulin, whereas the control smear was treated with 1 drop of conjugated anti-human albumin. After incubation at 37°C for 30 min in a moist chamber, the slides were rinsed with phosphate-buffered saline and then soaked in two changes of buffer for 5 min each. The smears were covered with Evans blue (0.1%, wt/vol), incubated for 20 min at room temperature, and then rinsed and soaked in phosphate-buffered saline for 5 min. Finally, the slides were rinsed quickly in distilled water and air dried. Cover slips were mounted on the slides as described above.

**Reading slides.** Slides were read for intensity of fluorescence by using an American Optical series 10 microscope with a vertical illuminator (American Optical Corp., Buffalo, N.Y.). A BG12 + KV418 exciter filter and an OG515 barrier filter was used. Each slide was viewed for at least 10 min using a 10x ocular and a 97x objective. Intensity of fluorescence was graded as 1+ to 4+. Specimens containing at least two fluorescing bacterial cells per slide were recorded as positive. Lack of fluorescence or minimally visible fluorescence was recorded as negative. Fluorescence of each specimen was considered specific when the albumin control was negative.

**RESULTS**

Of the 71 urine specimens tested concurrently, 23 specimens (32%) were positive by both methods, 47 (66%) were negative by both methods, and 1 (2%) gave discrepant results (positive by the slide test and negative by the tube test). Ten (42%) of the positive specimens contained *Escherichia coli*, whereas six (25%) contained *Klebsiella pneumoniae*. The eight remaining positive specimens yielded *Proteus* sp. (2 isolates), enterococci (2 isolates), *Staphylococcus aureus* (2 isolates), and one isolate each of *Enterobacter aerogenes* and *Acinetobacter calcoaceticus* var. *anitratus*. A similar distribution of organisms was found among the specimens which were negative for antibody-coated bacteria. In the case of the specimen which gave discrepant results, culture of the specimen revealed $2 \times 10^6$ *Streptococcus faecalis* and $>1 \times 10^6$ *E. coli* cells. Only the streptococci in this sample fluoresced. The fluorescent cocci were observed with the slide method, but could not be detected with the tube method. Similar results were obtained upon repeat examination.

Albumin controls were negative in all cases except one. This specimen was fluorescence positive with both the tube and slide methods.

All positive specimens contained two or more fluorescing cells per smear, with the following exception. A single fluorescing cell was observed in one specimen tested with the tube method, whereas several fluorescing cells were detected with the slide method.

Smears of urine sediment containing antibody-coated bacteria were held at $-70^\circ$C to determine the effect of storage at this temperature. The specimens which were stored at $-70^\circ$C for 3 weeks showed no decrease in the intensity of fluorescence. However, a decrease in fluorescence was noted in 4 of the 7 specimens held longer than 3 weeks.

**DISCUSSION**

In our laboratory, the slide immunofluorescence method correlated closely with the tube method described by Thomas et al. (19). Of the 71 urine specimens tested in the comparative study, only 1 gave discrepant results. This discrepancy may have been due to the difference in volume of urine used in the two methods. Greater numbers of bacteria can be recovered from a specimen with the slide test since the initial volume (5 ml) of urine used in this method is larger than the initial volume (0.5 ml) used in the tube test. Detection of low numbers of antibody-coated bacteria with the slide method is therefore more likely than with the tube method. Since the fluorescing enterococci were present in low numbers ($2 \times 10^7$/ml) in the original specimen from this female patient, it is possible that they represent contamination of the specimen with urethral flora which may also be antibody coated (12).

The work of Nichols and McComb (15) and Closs and Aarli (2) formed the basis for incorporating counterstain in the immunofluorescent slide test for detecting antibody-coated bacteria. The incorporation of 0.1% (wt/vol) Evans blue counterstain proved effective in reducing non-specific background fluorescence and made interpretation of the slides easier.

In our laboratory, the immunofluorescent slide method has several advantages. The test requires only small amounts of conjugated globulin. Using albumin controls obviates the necessity of confirming each positive test with a fluorescence inhibition test. Minimal time is spent in centrifuging specimens. The counterstain makes interpretation of the results easier. Finally, smears of urine sediment can be stored at $-70^\circ$C for at least 3 weeks and used as controls.

Further evaluation is necessary, however, to establish the usefulness of the immunofluorescence method in the clinical laboratory. In studies conducted on children with urinary tract infection, Hellerstein and co-workers (9) found a poor correlation between the presence of an-
tibody-coated bacteria in urine sediment and upper urinary tract infection.

Harding et al. (6) demonstrated a correlation between the presence of antibody-coated bacteria and upper urinary tract infection, but that the absence of antibody-coated bacteria did not rule out kidney involvement.

These discrepancies may be attributed to the use of different criteria in defining a positive test. Hellerstein and co-workers (9) considered only strongly fluorescent bacteria to be antibody-coated. Harding et al. (6) defined a positive specimen as one containing at least five uniformly fluorescing cells. A positive test should be clearly defined both in terms of the minimum number of fluorescing bacteria and the intensity of fluorescence required.

The definition of a positive test used in our laboratory was based on the findings of Jones (11). One or two fluorescing cells on slides prepared using the tube method were considered significant in our study. As explained previously, all except one positive specimen screened by the tube method contained more than two fluorescing cells. Most of the positive specimens in our study contained moderately or weakly fluorescing cells.

Harding and co-workers (6) recently modified the tube method for use as a slide technique. Although we made no attempt to correlate a positive test with upper urinary tract involvement, these investigators determined the site of infection by using the bladder washout technique and found that the presence of antibody-coated bacteria in urine sediment correlated with upper urinary tract involvement. The results of the present study and those of Harding et al. (6) indicate that the slide method is as reliable as the tube method in detecting antibody-coated bacteria in urine sediments.

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LITERATURE CITED


