Reproducibility of Interpretation of the Test for Antibody-Coated Bacteria in Urinary Sediment

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The direct immunofluorescence technique for detecting antibody-coated bacteria in urinary sediment is felt to be useful in distinguishing infection of the kidney from infection of the bladder. An independent, blind multiple-reading system was used to measure interobserver variability in the evaluation of slides of urinary sediments for antibody-coated bacteria. Three independent observers agreed unanimously on first reading in 88% of 253 specimens. When compared with the majority opinion, the sensitivity and specificity of an individual reading were 91 and 95%, respectively.

The use of direct immunofluorescence as a method of detecting antibody-coated bacteria (ACB) in the urine of patients with urinary tract infections was reported by Thomas et al. (11), who noted a strong correlation between the presence of ACB and the clinical diagnosis of upper urinary tract infection. This correlation was supported by Jones et al. (8), who found that presence of ACB was closely correlated with renal bacteriuria as determined by the bladder wash-out technique of Fairley et al. (2). Subsequent studies have continued to support this correlation (4, 12), although bacteria in urine from males with prostatic infections have been shown to give a positive test for ACB (6), and fungus in urine also cannot be satisfactorily evaluated (1, 5).

The investigations of Thomas, Jones, and others (4, 8, 11, 12) have been directed at establishing the external validity of this test by comparing the test to either clinical or accepted laboratory methods for localization of bacteriuria. A recent report by Jones et al. (6) examined whether the results of the test were consistent when multiple specimens were obtained from the same patient. However, the ACB test involves subjective interpretation of a slide as positive or negative. Because subjective interpretation cannot be calibrated or quantified, we felt it was necessary to examine the extent to which differing interpretations could occur in the evaluation of a given specimen. We therefore designed a multiple-reading system to determine the internal validity of this test.

MATERIALS AND METHODS

Urine specimens examined for ACB were obtained from hospitalized patients. A portion of urine was

frozen at the time of collection, and the specimen was then cultured by the quantitative loop technique. Criteria for positive urine cultures were the presence of $>10^5$ organisms of a single species per ml of urine for clean-catch specimens and the presence of $>10^4$ organisms of any number of species per ml for syringe aspirate specimens obtained from catheterized patients.

The urine specimens positive by culture were tested for the presence of ACB by direct immunofluorescence with fluorescein-conjugated horse antiserum to human globulin (Robox Surgical Instrument Co., Washington, D.C.) by the method described by Thomas et al. (11). Each slide was read independently by three observers (D.R.S., P.M.T., and J.E.M.). A slide was defined as positive if two or more ACB-positive cells were found on the slide and negative if no ACB were found after approximately 5 min of observation. When the three observers agreed on the initial evaluation, the slide was classed accordingly and not reread. If observers gave differing interpretations, specimens were recoded and reread independently by each of the three observers. Slides were classed as "indeterminate" when less than five of the six readings did not concur.

RESULTS

During the study, 253 specimens meeting the criteria for positive urine cultures were evaluated for ACB. The results of classification of these specimens are shown in Table 1. Of the 253 specimens, 223 were classified definitively by unanimous agreement of the three independent readers in the first round of reading. Of these 223, 53 were called ACB positive and 170 were called ACB negative. As measured by the method of Fleiss (3), the overall extent of agreement among the three independent observers in the first round of reading was 0.90.

In the first round of reading of the 253 speci-

mens, disagreements between the three independent observers occurred for 30 specimens. When these 30 specimens were read in a second round by the three independent observers, unanimous agreement occurred in 20 specimens; 9 were unanimously called ACB positive and 11 were unanimously called ACB negative. For the 10 specimens with no unanimous consensus in either round, the ACB result was classified indeterminate.

Since each of these 30 specimens was read independently twice by each of the three observers, these 30 specimens underwent six independent readings. Of the 10 specimens classified as indeterminate (i.e., disagreement in both rounds), 6 specimens produced the same unequal split of opinions (viz., two observers saying ACB positive and four saying ACB negative), and 4 specimens produced the equal split (viz., three observers saying ACB positive and three saying ACB negative.)

If one presumes that the majority opinion is correct, the individual opinions can be compared with this presumptive standard to calculate conservative values for sensitivity and specificity of evaluating urine preparations for ACB. Table 2 classifies the 253 specimens according to this presupposition. There were 223 specimens in which all three observers agreed (unanimous in the first round) with the presumed standard and

none disagreed; of these, the majority opinion indicated ACB positive in 53 and ACB negative in 170. For 20 specimens, five of the independent observations agreed with the presumed standard and one disagreed; of these, majority opinion indicated ACB positive in 9 and ACB negative in 11. For six specimens, four of the independent observations agreed with the presumed standard and two disagreed; all six of these specimens were ACB negative by the majority opinion. Four specimens had three observations saying ACB positive and three saying ACB negative; in these cases there was no majority opinion, and no correct answer was presumed. For purposes of sensitivity and specificity calculations, these four specimens were included both as presumed ACB positive and as presumed ACB negative.

Counting each observation separately, there were 849 independent observations. There were 237 observations where the presumed correct interpretation was ACB positive, and the observers read 216 correctly ACB positive, making the sensitivity of reading the specimens 91%. On the other hand, if the four specimens with equal split in interpretation were all assumed to be "truly" negative, then the sensitivity would be 96%. Specificity calculations can be made in a similar fashion. When the 4 specimens with evenly split evaluations are all assumed to be truly negative, of the 636 observations of presumed ACB-negative slides, 601 were correctly read as negative, making the specificity 95%. If the same four specimens are assumed positive, then specificity becomes 96%.

DISCUSSION

The examination of urinary sediment for ACB as a method for localizing the site of urinary tract infection has several advantages over previous methods. It is readily adapted to the routine diagnostic laboratory, and it is noninvasive. The test has been found useful in predicting the

TABLE 1. *Results of evaluation of urinary sediments for ACB by three independent observers*

Reading	No. of specimens		
	Total	With unanimous agreement ^a	Without unanimous agreement
1	253	223 (88)	30 ^b
2	30 ^b	20 (66)	10

^a Percentage of total in parentheses.

^b One interpretation differed from the other two on the first reading, and specimens were recorded and reread without knowledge of previous results.

TABLE 2. *Comparison of observer opinions with majority opinion as presumed standard for calculating sensitivity and specificity of reading ACB preparations*

No. agreeing/no. disagreeing with majority opinion	Majority opinion					
	ACB positive			ACB negative		
	No. of specimens	No. of observations		No. of specimens	No. of observations	
		Agree	Disagree		Agree	Disagree
3/0	53	159	0	170	510	0
5/1	9	45	9	11	55	11
4/2	0	0	0	6	24	12
3/3 ^a	4	12	12	4	12	12

^a Calculations of sensitivity and specificity performed twice, placing these four cases in each majority opinion category.

need for prolonged antibiotic therapy in some patients (9), but differing opinions about the test have occurred (10).

One of the potential explanations for differences might be interobserver variability in the subjective evaluation of the slides. Our results suggest that this is not a major factor, using the criteria expressed for a positive culture and a positive slide. Total agreement in the initial assessment was 88%, and sensitivity and specificity were at least 91 and 94%, respectively, for any single evaluation. Further experience will provide more data on errors in classification due to biological or other factors, but reproducibility of interpretation of the test in this study was high.

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