

Comparison of the Streptozyme Test with the Antistreptolysin O, Antideoxyribonuclease B, and Antihyaluronidase Tests

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The streptozyme test was compared with the antistreptolysin O (ASO), antideoxyribonuclease B (ADN-B), and antihyaluronidase (AH) tests. One hundred and twenty-seven human serum specimens were tested by each of the four tests. The streptozyme test detected more specimens with elevated titers than any single test; however, it was not as effective as the combination of the ASO and ADN-B tests. The streptozyme test appears to be particularly useful for laboratories which rely solely on the ASO test for serological evidence of a streptococcal infection. The test can be used in these laboratories to screen all specimens with low ASO titers (<170) for the detection of ADN, AH, antinicotinamide adenine dinucleotidase, and anti-streptokinase.

The antistreptolysin O (ASO) test is the most widely used serological test for the detection of group A streptococcus infections. Elevated ASO titers (>200 units) are found in approximately 80% of patients with clinical manifestations of acute rheumatic fever. Stollerman et al. (5) found that, when the antihyaluronidase (AH) test is performed simultaneously with the ASO test, the number of sera with elevated titers in at least one of the tests increases to 90%. Furthermore, performing the anti-streptokinase (ASK) test, in addition to the other tests, increased the number of patients whose serum showed an elevation of at least one of the three antibodies (ASO, AH, ASK) to 95%. In a similar study, Ayoub and Wannamaker (1) found that the simultaneous performance of the ASO, antideoxyribonuclease B (ADN-B), and antinicotinamide adenine dinucleotidase (ANAD) tests will show an elevation of at least one antibody in approximately 95% of rheumatic fever patients, as compared with an elevation in 80% of the patients when only one of the tests is performed. Thus it appears that using a combination of antibody tests will increase the number of sera with elevated titers detected in patients with streptococcal infections. The streptozyme test (Princeton Laboratories, Inc.) is designed to detect all five antibodies (ASO, ADN-B, AH, ANAD, and ASK) in a single test. This is accomplished by using as the streptozyme reagent a standardized suspension of aldehyde-fixed sheep red

blood cells sensitized with streptolysin, deoxyribonuclease, hyaluronidase, nicotinamide adenine dinucleotidase, and streptokinase. This report compares the results of the streptozyme test with titers obtained with three of the antibody tests, ASO, ADN-B, and AH.

MATERIALS AND METHODS

One hundred and twenty-seven human serum specimens were tested by the streptozyme, micro ASO hemolytic, micro ADN-B, and AH tests.

The streptozyme test is performed by adding a drop of streptozyme reagent to a drop of a 1:100 dilution of serum on a glass slide, mixing the two with a toothpick, and slowly tilting the slide for 2 min. Positive sera show visible agglutination, whereas negative sera are uniformly turbid or slightly granular.

Details of the ASO and ADN-B tests have been described previously (3, 4). Both are microtitration techniques using serum dilutions of 1:60, 1:85, 1:120, 1:170, 1:240, 1:340, 1:480, 1:680, 1:960, 1:1,360, 1:1,920, and 1:2,720.

The AH test procedure was the one supplied with the commercial AH reagents and is a tube test similar to that of Harris and Harris (2). The serum dilutions are twofold from 1:32 through 1:2,048.

Instructions received with the streptozyme reagent state that the test is usually positive with sera with an ASO titer of ≥ 166 ; therefore, in the present study an ASO titer of ≥ 170 is considered elevated. Since we have no information on the relationship between the streptozyme test and ADN-B or AH titers, these latter two are considered elevated if they are ≥ 170 and ≥ 256 , respectively.

RESULTS

The results of the streptozyme test are compared with results of the ASO, ADN-B, and AH tests individually in Table 1 and with the results of combinations of the ASO, ADN-B, and AH tests in Table 2. When we compare elevated ASO, ADN-B, and AH titers with positive streptozyme tests, we find the best correlation between the ASO test and the streptozyme test. Of the 70 sera with an elevated ASO titer, 69 (98.6%) were positive when tested by the streptozyme test. In the absence of the ASK and the ANAD tests, it can be argued that the one failure may be due to a "false-positive" ASO test; however, it is unlikely that this is the case because the ADN-B titer of this specimen was also elevated. The poorest agreement was between the streptozyme test and the ADN-B test. Sixty-eight (85.0%) of the 80 specimens with elevated ADN-B titers were positive by the streptozyme

test. On the other hand, there were 12 specimens with elevated ADN-B titers but negative streptozyme test results. Five of these 12 sera also had an elevated ASO or AH titer, or both, in addition to the elevated ADN-B titer and are thus eliminated from the category of possible "false-positive" ADN-B tests. In other words, the streptozyme test is "falsely negative" with these five specimens. The remaining seven specimens negative by the streptozyme test may or may not represent "false-positive" ADN-B tests. ANAD and ASK tests on these specimens would have been helpful for determining their status. Agreement was good between the AH test and the streptozyme test: 60 (93.7%) of the 64 specimens with elevated AH titers were positive by the streptozyme test. It is unlikely that the four specimens missed by the streptozyme test represent "false positive" AH tests because their titers were shown to be elevated by the ADN-B test as well as the AH test.

TABLE 1. Comparison of streptozyme test results with antistreptolysin O (ASO), antideoxyribonuclease B (ADN-B), and antihyaluronidase (AH) test results

Determination	ASO titer		ADN-B titer		AH titer	
	<170	≥170	<170	≥170	<256	≥256
Streptozyme positive (87) ^a	18	69	19	68	27	60
Streptozyme negative (39)	38	1	27	12	35	4
Streptozyme doubtful (1)	1	0	1	0	1	0
Total	57	70	47	80	63	64

^a Numbers in parentheses represent number of sera tested.

TABLE 2. Comparison of the streptozyme test with combinations of the antistreptolysin O (ASO), antideoxyribonuclease B (ADN-B), and antihyaluronidase (AH) tests^a

Determination	ASO and/or ADN +	ASO and/or AH +	ADN and/or AH +	ASO, AH, and/or ADN +	ASO, AH, and/or ADN -
Streptozyme + (87)	83	75	78	84	3
Streptozyme - (39)	12	5	12	12	27
Streptozyme ± (1)					1
Total	95	80	90	96	31

^a ASO + and ADN + refers to a titer ≥ 170. AH + refers to a titer ≥ 256.

Comparing the number of specimens with positive streptozyme tests but low ASO, ADN-B, or AH tests, we find the following: 18 specimens with an ASO titer <170, 19 specimens with an ADN-B titer <170, and 27 specimens with an AH titer <256. Three of the specimens in this group are considered possible "false-positive" streptozyme tests because they have low titers to the other three tests (ASO, ADN-B, and AH). However, they may have elevated ANAD or ASK titers, but we do not know because these tests were not done. Disregarding these three, there were 15 specimens with an ASO titer <170, 16 specimens with an ADN-B titer <170, and 24 specimens with an AH titer <256. In other words, the streptozyme test detected 15 specimens that were positive by the ADN or the AH test, or by both tests, that otherwise would have been missed if the ASO test had been the only test performed. Although the streptozyme test also detected 16 specimens missed by the ADN-B test and 24 specimens missed by the AH test, these findings are not as significant because rarely, if ever, is either of these tests the only one performed in a laboratory as is the case with the ASO test. In fact, the majority of laboratories do not perform the ADN-B or AH tests. Of the 16 specimens with low ADN-B titers, eight had elevated titers by both the ASO and AH tests, seven had elevated titers by the ASO test only, and one had an elevated titer by the AH test only. Thus, 15 of these 16 specimens had elevated ASO titers and would have been detected if the ASO test had been the only test performed. On the other hand, 9 of the 24 specimens with low AH titers would have been missed if the ASO test had been the only test used.

The streptozyme test detected a greater number of specimens with elevated titers than did the ASO, ADN-B, or AH tests alone, which is not surprising since it contains multiple antigens. It detected 17 more specimens with elevated titers than the ASO test alone; 7 more than the ADN-B test alone; and 23 more than the AH test alone. On the other hand, when the ASO and ADN-B test were used together, they detected eight specimens with elevated titers (in at least one of the tests) which were negative by the streptozyme test. A combination of all three tests (ASO, ADN-B, and AH) detected nine specimens with elevated titers (in one or more of the tests) which were negative by the streptozyme test.

DISCUSSION

An evaluation of the streptozyme test on the basis of sensitivity and specificity is complicated by the fact that it contains multiple antigens. Sensitivity cannot be determined by comparing positive streptozyme tests with positive conventional tests such as the ASO test, because there is no way of telling how many of the streptozyme positives are due to ASO antibodies and how many are due to one of the other antibodies. However, the streptozyme test appears to be less sensitive for detecting ADN than ASO or AH antibodies as evidenced by the number of specimens with negative streptozyme tests but with elevated ADN-B titers. Also, the streptozyme test is less

sensitive for detecting multiple antibodies (ASO, ADN-B, and AH) than a combination of the conventional ASO, ADN-B, and AH tests. We are not able to properly judge specificity of the streptozyme test because we evaluated it against only three of the five antibodies it is designed to detect.

Notwithstanding the unimpressive sensitivity level of the streptozyme test in the present study, we believe that it does have value as a screening test. The fact that it can detect multiple antibodies and requires only a few minutes to complete makes it particularly useful for laboratories which perform only the ASO test. It can be used in these laboratories to screen specimens with low ASO titers (<170) for the presence of ADN, AH, ANAD, and ASK.

LITERATURE CITED

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