Quantitative Evaluation of Variation in Composition of the Streptozyme Agglutination Reagent for Detection of Antibodies to Group A Streptococcal Extracellular Antigens

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The antibody titers of 90 acute- and convalescent-phase sera from 30 patients (mean age, 15.0 years) were studied with five different lots of Streptozyme reagent to determine whether variation exists among lots of reagent and whether this can affect clinical interpretation of antibody titers. Each serum was tested simultaneously with each of the five lots of reagent. Lot-to-lot variation in the reagent resulted in a significant difference (≥ 2 dilution increments) in antibody titer for 61 (68%) of the 90 sera tested. In addition, differences among the five lots also resulted in variation in determining whether a significant rise in titer occurred from the acute-phase to the convalescent-phase serum for a given patient. These data indicate a need for precise identification and quantitation of the streptococcal antigens coated onto erythrocytes used for the Streptozyme test reagent.

The Streptozyme test is a slide agglutination test for the detection of antibodies to several group A streptococcal extracellular antigens (3). It is a relatively simple-to-perform slide agglutination test, and since its introduction in the early 1970s, it has become one of the most widely used tests in clinical and hospital laboratories for the determination of group A streptococcal antibodies. This agglutination test has been compared with the more conventional neutralization tests for streptococcal antibodies such as the anti-streptolysin O and the anti-deoxyribonuclease B tests (2, 5, 7, 9); yet because the Streptozyme reagent contains multiple nonpurified streptococcal antigens coated onto ovine erythrocytes, the ability to standardize the reagent has been questioned (5, 6, 8). Preliminary work from this laboratory has suggested reagent variation from lot to lot (5, 6). This has raised important questions about clinical interpretation of this test because the manufacturer's recommendations indicate a specific level (agglutination at a 1:100 serum dilution) at which the test should be considered "positive." Thus, quantitative or qualitative variation in one or more of the streptococcal extracellular antigens on the erythrocytes could adversely affect the titer and would be clinically undesirable.

This report presents results of studies of variation in the strength of five different lots of Streptozyme reagent with 90 acute- and convalescent-phase sera from 30 patients. In addition, we determined whether variation in Strepto-

zyme reagent strength affects the ability of this test to demonstrate a significant rise in antibody titer from acute-phase to convalescent-phase sera.

MATERIALS AND METHODS

All sera were obtained during an intensive prospective study of streptococcal upper respiratory tract infections and the carrier state. The protocol has been presented in detail elsewhere (4). Briefly, all individuals were seen initially for history, physical examination, and cultures of the anterior nares, throat, and saliva. In addition, acute-phase serum was obtained for streptococcal antibody titers. All individuals were seen again at 3 and 8 weeks after the initial visit; the same laboratory studies and a physical examination were performed at each of these convalescent clinic visits, and a history was obtained. All patients from whom group A streptococci were recovered were treated with antibiotics according to the current recommendations of the American Heart Association (1).

Streptozyme-measured antibodies (SZMA) were determined according to the manufacturer's instructions (Wampole Laboratories, Cranbury, N.J.) except that the dilution scheme was modified as previously described (5) to make it similar to the 0.1-log dilution increments used in the conventional neutralization tests for streptococcal antibodies. Instead of the manufacturer's dilution scheme of 1:100, 1:200, 1:400, and 1:600, we used a dilution scheme of 1:60, 1:120, 1:180, 1:240, 1:360, 1:480, 1:600, 1:720, 1:840, 1:1,080, etc.

Five different lots of Streptozyme reagent were available for these comparison studies: OE 7204, OE 7209, OD 7211, OC 7202, and OF 7204.

Acute- and convalescent-phase (3 and 8 weeks after the initial visit) sera were selected from patients be-

longing to three previously determined groups of individuals. Three sera (one acute phase and two convalescent phase) were available from all patients. Individuals whose sera were studied were chosen randomly, knowing only which of the groups described below the patient represented and whether an adequate amount of all three sera (frozen at -10°C) was available. The groups were as follows. Group 1 consisted of individuals from whom no beta-hemolytic streptococci were recovered at any of the three visits. Group 2 consisted of individuals found to have group A beta-hemolytic streptococci at the initial visit. Included in this group were 5 individuals who were treated initially and in whom the organism was eradicated and 10 individuals who required additional courses of antibiotics because of persistence of the homologous serotype despite therapy. Group 3 consisted of individuals who had only non-group A beta-hemolytic streptococci (primarily groups C, G, and F) recovered at the initial visit but who did not harbor group A streptococci at any of the subsequent clinic visits.

Each serum was simultaneously tested with the five lots of Streptozyme reagent. The acute- and convalescent-phase sera from each individual patient were tested on the same day along with appropriate controls (human control sera were tested on each occasion).

RESULTS

A total of 90 sera (30 individuals, each with 3 sera; mean age, 15 years) were tested with each of the five lots of Streptozyme reagent. The variation in titer (i.e., the difference in SZMA titer for a given serum as determined by the number of dilution increments) for each serum was evaluated (Table 1). If there was no variation in the SZMA titer of a single serum with the five lots, the results for that serum were recorded in the "0" column in Table 1. If there was a one-dilution-increment variation in titer for the serum, it was recorded in the "1" column, etc. A two-dilution-increment difference or greater (ca. ≥ 0.2 log) for a given serum was considered a significant variation among the five lots. There was a significant difference in SZMA titer for the majority (61 sera, 68%) of the 90 sera tested (Table 1). This variation in titer

indicates lot-to-lot differences in the Streptozyme reagent.

In clinical practice, streptococcal antibody titers are very frequently determined on only a single submitted serum from a patient. However, the most reliable use of a streptococcal antibody test is to determine whether a rise in titer has occurred from the acute-phase to the convalescent-phase serum by simultaneously testing both sera. For this reason, we determined whether variation in the composition of the Streptozyme reagent might influence the magnitude of a rise in SZMA titer from acute- to convalescent-phase sera. A two-dilution-increment or greater rise from acute-phase to convalescent-phase serum was considered significant. If a two-dilution-increment or greater rise could be demonstrated with at least one of the five lots and if at least one of the remaining lots did not result in this magnitude of rise, this was defined as a significant discrepancy between lots of Streptozyme reagent for that individual tested.

By this criterion, for 11 (37%) of the 30 individuals studied, a discrepancy was noted in the ability to show a significant SZMA titer rise by the five lots of Streptozyme reagent; however, for the majority (19 individuals, 63%), no discrepancy was demonstrable.

DISCUSSION

These data document variation among the tested lots of Streptozyme agglutination reagent. This is especially evident when evaluating the results obtained from testing single sera which is what usually occurs in the clinical laboratory. Furthermore, these data suggest that even if both acute- and convalescent-phase sera were tested, unless the testing of all sera were performed simultaneously with the same lot of reagent, erroneous interpretation might result.

For 68% of the 90 single sera tested, there was a two-dilution-increment or greater difference when comparing results with the five lots of Streptozyme reagent. Although this percentage was somewhat greater for the 15 sera tested from individuals without isolation of group A streptococci (group 1, 87%), the variation in the percentages among the three groups (1, 2, and 3) was not remarkably different.

We evaluated whether individual lots of Streptozyme reagent can significantly influence a demonstrable titer rise from acute-phase to convalescent-phase serum. Significant differences in the magnitude of SZMA titer rise were least likely to be found for the 15 individuals from whom group A streptococci were isolated (2 of 15 [7%] with a significant difference). In contrast, for those without isolation of beta-he-

TABLE 1. Variation in SZMA titers with five different lots of Streptozyme reagent

Group	No. of sera tested	No. (%) of sera with variation (in dilution increments) detected among 5 lots of reagent			
		0	1	2	≥ 3
1	15	2 (13)	0	11 (74)	2 (13)
2	45	4 (9)	12 (27)	14 (31)	15 (33)
3	30	2 (7)	9 (30)	12 (40)	7 (23)
Totals	90	8 (9)	21 (23)	37 (41)	24 (27)

molytic streptococci (group 1) and for those patients with isolation of non-group A beta-hemolytic streptococci (group 3), a significant difference in the magnitude of the SZMA titer rise between acute and convalescent-phase sera was found for 60% of the individuals when comparing results with the five lots of Streptozyme reagent.

To confirm that these important differences are attributable to different lots of reagent, observer variation with multiple determinations for a single serum with the same lot of reagent must also be considered. Previously published data (5) and subsequent laboratory experience (E. L. Kaplan and C. Kunde, unpublished data), indicate that, at most, a one-dilution-increment variation can be found with multiple determinations with the same reagent lot. However, even this degree of variation has been uncommon in my experience. Therefore, it is unlikely that observer variation has influenced these results.

This study indicates that significant variation in strength does occur among manufactured lots of the Streptozyme reagent. As indicated by the data, this amount of variation can result in a clinically important difference in the SZMA titer of a single serum. This is not surprising in view of the preparation of the reagent with nonpurified streptococcal extracellular antigens (3).

This simple slide agglutination test has several potentially important advantages for determining streptococcal antibodies (3, 5). However, it appears that significant error can be introduced due to the variation in lots of reagent. Previous studies from our laboratory have indicated problems with the specificity of the Streptozyme reagent for documenting group A streptococcal infections (5). This present study further documents a need for precise identification and quantitation of the streptococcal antigens coated onto

the erythrocytes used in the Streptozyme reagent.

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