Comparison of Broth and Human Serum as the Diluent in the Serum Bactericidal Test

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The use of serum rather than broth as the diluent in the serum bactericidal test results in a significant decrease in the test level among patients receiving highly protein-bound semisynthetic penicillins.

A large degree of variation in the performance of the serum bactericidal test (SBT) was noted in our recent survey of 26 diagnostic microbiology laboratories (4). Only two of these laboratories used human serum as a diluent. The importance of this variable is suggested by Kunin's (3) finding that the minimal inhibitory concentration in the broth dilution susceptibility test, with antibiotics with a high degree of protein binding, was significantly elevated when serum rather than broth was used as the diluent. The relevance of this observation to the SBT has not been studied systematically. This report presents the results of such a comparison among clinical specimens studied in our laboratory.

The SBT was performed on duplicate portions of 42 clinical specimens using either brain heart infusion (BHI) broth or human serum as diluents. A pool of 500 ml of serum was obtained from two donors, heat inactivated at 56 C for 30 min, and frozen in 10-ml portions. In the test procedure, 0.5 ml of the patient's serum was added to each of the first two tubes. Five-tenths milliliter of diluent (BHI or serum) was added to tubes 2 to 12. Serial twofold dilutions were made with a final volume of 0.5 ml. Five-tenths milliliter of a 10^{-8} dilution of an 18 to 24-h culture of the patient's infecting organism in BHI was added to each tube giving a final volume of 1 ml, and resulting in a 1:2 to 1:4096 range of dilutions of the patient's serum. After 24 h of incubation, 0.02 ml of the mixture from the clear tubes was spread on sections of a blood agar plate, and the plate was incubated for 24 h. A growth of 10 or less colonies on the subculture was considered "bactericidal" and the last tube showing such a result was recorded as the SBT level.

A comparison of SBT levels obtained with BHI broth or human serum among patients receiving a single antibiotic is presented in Fig.

1. In most sera, the levels were one- to threefold higher in broth than in serum. Those semisynthetic penicillins with a protein binding of 90% or greater (dicloxacillin, cloxacillin, nafcillin, and oxacillin) were affected most commonly with all seven tests with a detectable level showing two- to threefold changes. An unexplained exception was cephazolin which is 86% protein bound (2), but which gave similar results with both diluents.

The influence of the two diluents on SBT levels among eight patients receiving combinations of antibiotics is shown in Fig. 2. Although each combination was different, the level was two tubes higher in three tests when broth rather than serum was used as the diluent.

The results of the present study suggest that human serum should be used as the standard

FIG. 1. A comparison of SBT levels obtained with BHI broth or human serum among patients receiving a single antibiotic.

FIG. 2. A comparison of SBT levels obtained with BHI broth or human serum among patients receiving two or more antibiotics.

diluent in the SBT. Serum simulates more closely the situation in the patient and may be particularly important in assessing the SBT

level among patients receiving semisynthetic penicillins which are highly protein bound. However, the ultimate confirmation of the importance of using serum as the diluent in this procedure must come from a comparison of the results of this *in vitro* test with the patient's clinical response to a given infection. A reassessment of such a comparison seems particularly important since the clinical effectiveness of a level of $\geq 1:10$ was obtained with broth as the diluent and before the wide use of semisynthetic penicillins (1).

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