# Method of Stabilizing Antigen Emulsion Used In VDRL Syphilis Tests

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ONE of the most important factors contributing to reproducibility of flocculation tests for syphilis is the ability to prepare antigen emulsions of uniform reactivity. Deviation from prescribed methods, use of reagents of poor quality, unclean glassware, and temperature variants may result in antigen emulsions of substandard reactivity.

The most widely used microflocculation tests employing cardiolipin antigen, described in the 1955 Manual of Serologic Tests for Syphilis (*1*), limit the use of antigen suspensions to the day on which they are prepared, or, as in the Kline standard test, to 48 hours.

A method whereby antigen emulsions of standard reactivity might be preserved for use in these tests would appear to be a valuable aid in promoting uniformity of test performance by eliminating the necessity of preparing them each day tests are performed, thus permitting their storage, shipment, and use over relatively long periods of time.

Antigen emulsions preserved for varying periods of time by the addition of preservatives or by storage at low temperatures have been used in other procedures. Hinton glycerinated indicator (*1*) can be used for at least 3 weeks when stored in the refrigerator. Chediak (*2*) added benzoic acid or beef albumin to cholesterolized alcoholic extracts of beef heart used to preserve emulsions in a microflocculation test on whole blood. Kurtz and Hill (*3*) extended the use of a Kahn antigen emulsion to 36 hours by centrifugation and resuspension of the sediment in 0.9 percent saline. Modified antigen emulsions used by Rappaport and Eichhorn (*4*, *5*) for several microflocculation tests were satisfactory for use for 2 weeks when stored on ice, and Portnoy and co-workers (*6*) modified VDRL antigen emulsion by centrifugation and the addition of choline chloride for use in a screening procedure and found that it could be used for 1 week if 0.01 percent merthiolate was added.

Investigative studies were undertaken at the Venereal Disease Research Laboratory to determine whether some of these reagents or procedures could be applied to the stabilization of antigen emulsions for the most widely used microflocculation procedures, such as the VDRL, Kline, and Mazzini tests, without affecting standard test reactivity level.

This report describes a method employing benzoic acid in the preparation of a stable antigen emulsion of standard reactivity for use in the VDRL slide, tube, and spinal fluid tests. Stabilized antigen emulsions prepared in this manner retained their standard level of reactivity during shipment and long storage periods, thus eliminating the necessity of preparing fresh antigen emulsions each day tests were performed.

## Materials and Method

A stock 1.0 percent alcoholic solution of benzoic acid was prepared by dissolving 1 gm. of benzoic acid, reagent grade, in 100 ml. of absolute ethyl alcohol. This solution was stored in a glass-stoppered flask at 6°–10° C., and was satisfactory for use indefinitely.

VDRL antigen emulsion was prepared in 10.0 ml. volumes as described in the published method (*1*). To each 10.0 ml. volume of freshly prepared material, 0.1 ml. of stock 1.0 percent benzoic acid solution was added so that the final concentration of stabilizing substance was 0.01 percent. Each aliquot of this stabilized antigen emulsion was tested with control serums, and all those of standard reactivity were pooled. The pool was dispensed in convenient volumes of 5.0 ml. or 10.0 ml. into screw-capped vials.

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### In Routine Use

Stabilized antigen emulsion is now used at the Venereal Disease Research Laboratory in the routine performance of the VDRL slide, tube, and spinal fluid tests. A volume of antigen emulsion sufficient for approximately 1 week's testing is prepared and stored in the refrigerator at 6°–10° C. An aliquot sufficient for 1 day's testing is removed from the stock bottle, warmed to room temperature, and then checked with control serums for standard reactivity before use in testing individual serums. A new aliquot from the refrigerator is used each day.

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Four of these vials were stored at 37° C., and one vial was tested each week for 4 weeks. The remaining vials were divided into two groups. One group was stored at room temperature (23°–29° C.); the other was stored in the refrigerator at 6°–10° C. Samples of antigen from each group were tested periodically in comparison with an emulsion of standard reactivity, freshly prepared on the day of testing. A vial of stabilized antigen emulsion was used for at least 1 week if it retained its standard level of reactivity for that period of time. Antigen emulsions stored at 6°–10° C. were allowed to stand at room temperature for at least 30 minutes before use. When testing was completed they were returned to the refrigerator.

Samples of stabilized emulsions also were sent through the mail and, after 3 days in transit, were returned to the laboratory and tested for a period of 7 consecutive days when stored at 6°–10° C.

## Results

The keeping qualities of the stabilized antigen emulsions prepared and handled as described are summarized in the table.

The stabilized antigen emulsions were satisfactory for use for 7 consecutive days after storage at 6°–10° C. for periods of 4 to 6 weeks. In one instance, an unopened vial was refrigerated for 4 months and found to be of standard reactivity when tested.

The stabilized antigen emulsions stored continuously at room temperature also retained their standard level of reactivity for relatively long periods, although storage at 23°–29° C. was not as satisfactory as refrigerated storage. Emulsions were satisfactory for use for periods varying between 2 and 4 weeks. Deterioration tended to occur more rapidly as temperatures exceeded these limits.

Emulsions were stored at 37° C. in order to determine the effect of the highest probable temperatures to which they would be exposed during shipment. When stored at this temperature in the incubator, they were found to be of standard reactivity after 2 weeks; however, slight but definite loss in reactivity was noted after 4 weeks' incubation.

The mailed specimens showed no change in reactivity during a period of 7 consecutive days of testing.

Comparative VDRL slide tests were also performed with stabilized and freshly prepared antigen emulsions (duplicates of each) on 570 serums selected at random from specimens submitted to this laboratory for testing. Stabilized emulsions were stored from 1 to 6 weeks at 6°–10° C. before use. Discrepancies in results obtained between the stabilized and freshly prepared antigen emulsions were not more numerous than those obtained with duplicate, freshly prepared antigen emulsions. In all instances, disagreements represented critical, borderline reactions, where the results might have been read either as rough nonreactors or as minimal weak reactors. Stabilized

**Effect of storage time and temperature on reactivity of stabilized VDRL emulsions**

| Storage method | Period of storage (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Refrigerator (6°–10° C.) | SR | SR | SR | SR | SR | SR |
| Room temperature (23°–29° C.) | SR | SR | SR | SR | LR | ---- |
| Incubator (37° C.) | ---- | SR | ---- | LR | ---- | ---- |
| Mailed (3 days in transit) | [1] SR | ---- | ---- | ---- | ---- | ---- |

[1] Not tested beyond 7 consecutive days.

SR—standard reactivity; LR—less reactive than standard.

emulsions were found to be equally satisfactory for use in the VDRL tube flocculation tests with serums and the VDRL tests with spinal fluid.

The use of benzoic acid as a stabilizing substance therefore did not appear to affect the reactivity level of the antigen emulsion for any of these three tests.

## Discussion

During the course of these studies, other preserving substances and factors which might affect the relative stability of antigen suspensions were investigated. The presence of alcohol in these suspensions and its possible effect on their stability were considered, since it has been observed that in some instances emulsions containing no alcohol deteriorate less rapidly than those containing alcohol. Alcohol was removed from VDRL antigen emulsions by dialysis, or by centrifugation at 20,000 × g. and resuspension of the sediment in VDRL buffered saline. Both methods, however, resulted in preparations that were less reactive than standard.

The use of proteins as stabilizing substances for colloids and oil-in-water emulsions is well known. Bovine albumin in varying concentrations was added to VDRL emulsions, but in all instances these preparations were undersensitive and showed evidence of contamination after 1 week's storage at 6°–10° C.

Glycerin, too, is a substance commonly used to stabilize emulsions by decreasing surface tension. In addition, as previously indicated, it is used in the preparation of Hinton glycerinated indicator which can be used for 3 weeks. Addition of glycerin to VDRL emulsions, however, either affected their standard reactivity level, or, when used in low concentration, did not preserve them for any significant period of time.

The use of various salts of the fatty acids, such as the stearates, oleates, and palmitates, was also considered. None of these substances, however, was available as a reagent grade chemical readily soluble in either water or alcohol; they were therefore not used.

Of all the substances tested, benzoic acid proved to be the most satisfactory for stabilizing VDRL antigen emulsions. It was readily available as a reagent grade chemical, a 1.0 percent stock solution was stored and used for long periods of time without deterioration, and an extremely low concentration (0.01 percent) was effective in preserving the standard reactivity level of the emulsions for long periods of time.

Preliminary studies have also been carried out using benzoic acid as a stabilizing substance for preserving the standard reactivity of antigen suspensions used in the Kline standard and Mazzini microflocculation tests. Results obtained with both these procedures will be reported later.

## Summary

A procedure employing benzoic acid as a stabilizing substance for preserving the reactivity level of antigen emulsions for use in the VDRL tests is described.

The reactivity of the stabilized emulsions varied according to the temperature at which they were stored and the length of time they were kept.

Comparative results obtained on 570 serums tested with freshly prepared and with stabilized emulsions in the VDRL slide test indicated that benzoic acid does not affect reactivity, levels.

Of several stabilizing substances tested, benzoic acid was the most satisfactory.

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