comprehensive enough to delineate clearly the relative efficiencies of the tests examined and are presented only as a preliminary report. Further study of the Chediak tests will be the subject of a later report.

NOTE: Since this study was completed, further trials have indicated that the reactivity coverage of the Chediak-VDRL test may be expanded by using both a diluted and an undiluted antigen emulsion.

Summary

1. Results obtained with the Chediak and Chediak-VDRL tests on dried blood specimens stored for 24 and 72 hours at room temperature are presented.

2. The results of the Chediak, Chediak-VDRL, and quantitative VDRL slide tests on blood specimens from 196 donors are presented.

3. A modified Chediak test technique (Chediak-VDRL) is described.

4. Advantages and disadvantages of Chediak-type tests are discussed.

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A Micromodification Of the VDRL Slide Test

By GEORGE R. CANNEFAX, B.S.
HAROLD R. BEYER
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Except for the lack of an authoritative sensitivity and specificity evaluation, the micromodification of the VDRL slide test apparently provides a relatively simple and satisfactory method of collecting and testing small amounts of blood from infants, young children, and adults who present a problem in the collection of blood by venipuncture.

The results of testing 1,388 simultaneously collected specimens by the regular and the micro-VDRL test techniques are presented in this report. A request has been made that this modification of the VDRL slide test be included in the next National Evaluation of Serodiagnostic Tests for Syphilis.

Materials

Melting point capillary tubes (Kimble Glass Co. item 34500): Outside diameter, 1.5 to 2.0 mm.; length, 100 mm.; open at both ends. One hundred pieces are supplied in a corked glass vial. Prior to use the tubes are washed with Orvis detergent, rinsed with tap water followed by distilled water, and dried in a hot-air sterilizer. These tubes are used for the collection of blood specimens.

Glass tubing (Kimble Glass Co. item 46470): Glass tubing with an outside diameter of 4 mm., purchased in 4-foot lengths, and cut into 105-mm. lengths. The ends should be fire-polished. These tubes, when fitted with a rubber cap on each end, serve as carrying or protecting containers for the collection tubes.

Rubber caps for closing both ends of the protection tube: Micro rubber policemen as used with A. H. Thomas item 8804 or any cap that will fit 4-mm. glass tubing.

Ungraduated micropipettes: Drawn from 4-mm. outside diameter glass tubing. The pipette should have a

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length of 80 mm. and an orifice approximately 0.5 mm. in diameter.

Wax ring maker: Similar to A. H. Thomas item 3619–A. The ring maker should be wrapped with thread to make a ring 8- to 10-mm. inside diameter when dipped in molten wax at a temperature of approximately 120° C.

Automatic micropipette (A. H. Thomas item 8212–E): The rubber bulb supplied with this instrument must be modified for use with 4-mm. glass tubing. A rubber plug, with a hole large enough to hold the glass micropipette securely, is fitted snugly into the rubber bulb of the automatic pipette.

Test tubes 50 by 6 mm.: For receiving serum.

Rubber bulb (A. H. Thomas item 8773–L): For transferring serum from collection tubes to 50- by 6-mm. test tubes.

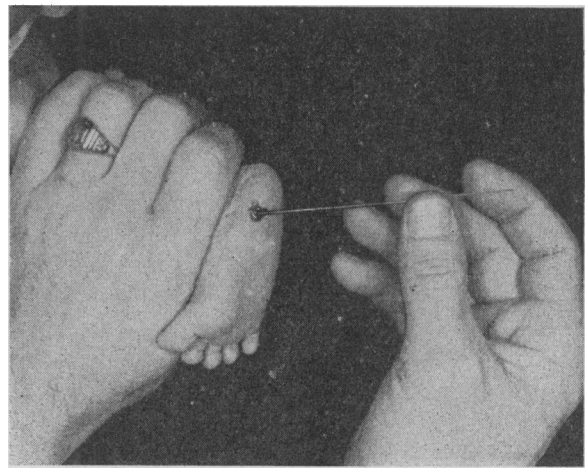

Figure 1. Collecting a specimen of blood from an infant.

Methods

Collection of Specimens

A finger or heel is punctured so that there is a free flow of capillary blood, more profuse than the bleeding produced by the usual puncture for blood count and hemoglobin estimations. A No. 11 Bard-Parker or similar knife blade run through a No. 3 cork stopper may be used. The point of the blade should protrude from the cork approximately one-fourth inch. The blade and stopper are inexpensive and provide a control on the depth of the incision. If the cork is quickly and firmly pressed against the skin, an adequate flow of blood will usually be obtained. The lateral surface of the finger or heel bleeds more freely than the midline palmar or plantar surface.

Although the tube is called a "capillary tube" it will not fill by capillary attraction if it is held perpendicularly. The blood is collected by holding the tube nearly horizontal to the incision. In that position the blood will flow rapidly into the tube. If the incision requires massaging to well up more blood, the end of the tube is temporarily closed, or the tube is held horizontally so that the column of blood does not move along the tube and result in an air space when more blood is collected. The presence of air spaces in the column of blood may result in insufficient serum for testing.

When the column of blood is within approximately 10 mm. of the upper end of the tube, one end is plugged by forcing it into a ¼-inch pad of nonhardening modeling clay. The other end does not require a plug. The collection tube is placed in the protecting or carrying tube and the rubber caps applied. The protecting tube, securely wrapped with a serologic test request form or other identification, is placed in a suitable mailing container and forwarded to the laboratory.

Calibration of Automatic Micropipette

The automatic pipette is calibrated to hold 0.015 ml. of serum by adjusting the knurled locking nut so that this amount is drawn into a 0.1- or 0.2-ml. pipette not more than 80 mm. in length that is calibrated to the tip. If the pipette has a diameter greater than 4 mm., it must be heated and drawn so that a constricted area, about 80 mm. from the tip of the pipette, measures approximately 4 mm. The pipette is scored with a file in the center of the constricted area, broken at that point, and the end fire-polished. The automatic pipette should be checked with this pipette each day before use to determine if 0.015 ml. is drawn into the ungraduated pipettes used for measuring serum.

Preparation of Wax Rings

Wax rings with a diameter of 8 to 10 mm., preferably nearer 8 mm., may be made of paraffin or other wax mixtures in common use. A single ring maker, not commercially available at this time, similar to A. H. Thomas item 3619–A, may be used for making the rings. The ring maker may be shaped from a paper clip bent to a diameter of 10 mm. and closed with a drop of solder. When wrapped with thread and dipped in paraffin at a temperature

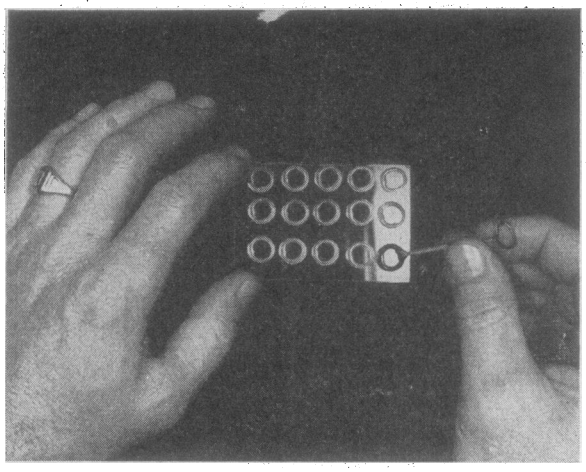

Figure 2. Making wax rings for the micro-VDRL test.

of 120° C., the paper-clip ring maker will produce rings with an internal diameter of approximately 8 mm.

Preparation of Specimen for Testing

When the blood is received in the laboratory, the request slip accompanying the specimen is numbered and the melting-point tube is placed in a 100- by 13-mm. test tube similarly numbered. Before centrifuging, the clot is freed from the side of the melting-point tube by means of a wire stylet such as those supplied with 19-gauge hypodermic needles, or by a similar small wire. The specimen is centrifuged in the numbered test tube for 15 minutes at about one-half the revolutions per minute used with venous specimens. Inactivation is accomplished by placing the melting-point tube in a numbered 100- by 13-mm. test tube which is filled with water at 56° C. This tube has a small amount of cotton in the bottom sufficient to raise the end of the melting-point tube above the lip of the water-filled test tube. The inactivation temperature is maintained by placing the water-filled test tube in a serologic water bath commonly used for inactivation. After inactivation the melting-point tube is placed in the numbered test tube that was used to hold the specimen during centrifugation.

A small rubber bulb similar to that used with tubes of smallpox vaccine is placed over the serum end of the melting-point tube and an ampule file is used to score the tube just above the level of the clot. The tube, held horizontally, is broken at that point and the serum is forced by means of the rubber bulb into a 50- by 6-mm. test tube, which is numbered with a wax pencil and/or placed in a wooden block with numbered holes.

Qualitative Testing

Serum is measured into the center of a wax ring by means of the automatic pipette and the ungraduated glass micropipette. The slide on the actuating arm of the automatic pipette is placed in the lower position. (The arm is actuated and the slide moved up and down with the thumb.) The tip of the glass pipette is placed in the serum and the arm depressed to force out serum that may have entered the glass tip by capillary attraction, the arm released, and the serum drawn into the glass pipette. The serum is discharged from the pipette into the center of a wax ring by raising the slide and depressing the arm. VDRL slide test (*1*) antigen is dropped onto the serum by means of a 25-gauge hypodermic needle attached to a 2-ml. syringe. The needle and syringe must be held vertically and deliver approximately 180 drops per milliliter. The serum-antigen mixture is rotated, read, and reported as described in the VDRL slide test procedure.

Quantitative Testing

Using an automatic pipette, 0.015 ml. of 0.9-percent sodium chloride solution is placed in each of 10 wax rings. Serum is drawn into the glass pipette attached to the automatic pipette as in qualitative testing. The slide of the automatic pipette is raised to the upper position and the 0.015 ml. of serum expelled into the first ring. With the slide held in the upper position the arm is repeatedly depressed and released (at least three times) so that the serum and saline are drawn into and forced out of the glass pipette to insure thorough mixing. When the mixture is expelled the last time, the slide is allowed to fall into the lower position. The tip of the glass pipette is placed in the serum dilution; the arm is depressed to force out any fluid that may have been drawn into the tip by capillary attraction, and released. The serum dilution thus obtained in the glass pipette is transferred to the second ring containing saline, and mixing manipulation is repeated. VDRL slide test antigen is added to each serum dilu-

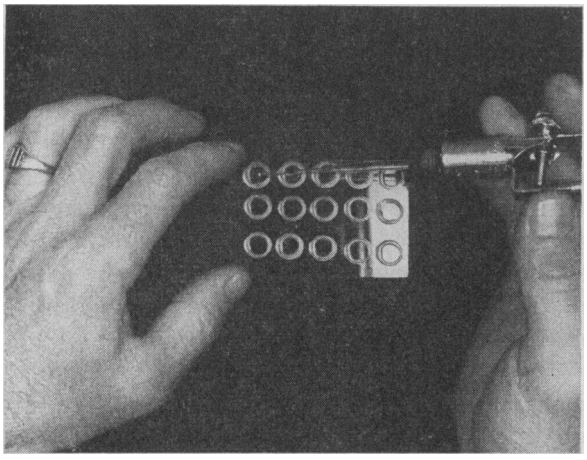

Figure 3. Placing 0.015 ml. of serum in wax rings.

tion as described for qualitative testing. The serum dilutions are rotated, read, and reported as described in the VDRL slide test procedure.

Results

Qualitative Testing

The results of qualitative testing, in which 1,388 specimens were tested with the regular and micro-VDRL procedures, are shown in table 1. In this series the micro procedure was 1.8 percent more positive than the regular VDRL test when positive results were considered. When positive and weakly positive results were combined the micro procedure was 1.7 percent more positive. These differences in sensitivity are not statistically significant.

Table 2 is a presentation of comparative results of the two tests with respect to agreement or disagreement. The number of specimens found to be positive by both the VDRL slide and micro-VDRL slide tests is 641. Similarly, 510 specimens are identified by both tests as being negative. The total agreement between the two tests on the same specimens is 84.08 percent. However, if only two classifications of test results are used (positive-weakly positive and negative-rough negative) the total agreement of the two tests is 91.28 percent.

Quantitative Testing

Results of quantitative testing with 238 specimens are shown in table 3. It will be seen that 217, or 91.2 percent, of the entire series did not vary by more than one dilution between the regular and micro-VDRL slide tests. These variations and those of greater magnitude probably are due to technical errors. It is assumed that variations of this type will decrease as technical skill with small quantities increases.

Field Collection of Specimens

Field collectors were supplied with materials for collection of blood and were advised to procure specimens for the micro test in all instances in which venipuncture had failed or did not appear feasible. These workers received a demonstration of the collecting technique but were otherwise inexperienced in this type of specimen collection. Specimens of blood for the micro test were collected by the Arkansas State Board of Health in 1,451 cases in which venipuncture was not feasible, and the specimens forwarded to the Medical Center for testing.

Of these specimens 1,334 (91.9 percent), were satisfactory for qualitative testing. One hundred and seventeen (8.1 percent) were unsatisfactory for testing because of (1) insufficient quantity (93, 6.4 percent), (2) hemolysis (15, 1 percent), and (3) broken collection tubes (9, 0.6 percent). The results of the 1,334 specimens that were tested with the micro-VDRL test are as follows: 154 (10.6 percent) specimens were found to be positive; 1,180 (81.3 percent) specimens, negative.

Table 1. Results of 1,388 qualitative tests. VDRL slide test and micro-VDRL slide test

Test procedure	Positive		Weakly positive		Rough negative		Negative	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
VDRL slide test	692	49. 9	65	4. 7	60	4. 3	571	41. 1
Micro-VDRL slide test	717	51. 7	63	4. 5	45	3. 2	563	40. 6

Table 2. Comparative testing (regular and micro-VDRL slide tests) of 1,388 simultaneously obtained specimens

Micro-VDRL slide test results	VDRL slide test results				
	Positive	Weakly positive	Rough negative	Negative	Total
Positive	641	34	16	26	717
Weakly positive	25	8	10	20	63
Rough negative	13	9	8	15	45
Negative	13	14	26	510	563
Total	692	65	60	571	1, 388

Table 3. Results of 238 quantitative tests: micro-VDRL slide test and VDRL slide test

Micro-VDRL more positive				Both techniques same titer	VDRL more positive			
Difference in titer [1]					Difference in titer [1]			
4	3	2	1		1	2	3	4
2	2	2	32	96	89	15	0	0

[1] Expressed as an increase in terms of serial dilutions.

Approximately one-third of the specimens which had quantities insufficient for testing came from the group of patients under 3 years of age. Thirty-eight of the positive specimens did not have sufficient serum for quantitation, and 181 of the negative specimens would have lacked sufficient specimen material if quantitation had been required.

Age was reported on the request form for 1,115 of the patients from whom blood specimens were collected in the field. Of these 1,115 patients, 167 (15 percent) were less than 1 year old; 189 (17.0 percent) were between 1 and 3 years; 287 (25.7 percent) were between 3 and 10 years; and 472 (42.3 percent) were between 10 and 60 years of age.

Summary

The micromodification of the VDRL slide test permits qualitative and quantitative testing with a specimen of 0.1 to 0.15 ml. (2 to 3 drops) of capillary blood.

The qualitative and quantitative modifications of the VDRL slide test herein described consist of the performance of that test with one-third amounts of serum, antigen, and surface area.

Specimen material is obtained by finger, toe, or heel puncture and collection in "capillary" glass tubes.

An automatic pipette, fitted with an inexpensive ungraduated glass pipette, is employed for serum measurement. Calibration of the automatic pipette and fabrication of the ungraduated glass pipettes are described.

It was found that 6.4 percent of all the specimens received were of insufficient quantity for qualitative testing. The collection of specimens in "capillary" glass tubes would be greatly facilitated if an automatic lancet with a thin blade 4 mm. wide set to protrude 2 mm. were available. Such a lancet has been fashioned from the conventional type, and its use at the medical center has resulted in 100-percent collection of sufficient specimen material.

Sensitivity and specificity ratings have not been determined for this modification of the VDRL slide test. However, since the series of tests reported here showed the micro procedure to yield 1.8 percent more positive results, it may be assumed, for the present, that the procedure may be a little more sensitive. It does not appear probable that this percentage increase in test sensitivity is sufficient to reduce the specificity of this modification below acceptable limits since the difference as shown with these data is not statistically significant.

Quantitative testing is accomplished by preparing serum dilutions within the wax rings of the slide in place of test tubes. Comparative quantitative testing of the micro technique and the regular VDRL slide test has shown 91.2 percent of the tests to vary by no more than one dilution. It is assumed that variations greater than one dilution have been due to faulty technique and that the incidence and magnitude of variations will decrease as experience with the test procedure increases.

Most of the patients from whom specimens were obtained in the field probably would not have had the benefit of a serologic test for syphilis if this new collecting technique had not been available to the field worker. As previously indicated, analysis of 1,115 patients for whom age was reported showed that 32 percent

of the patients were less than 3 years of age, 57.7 percent were under age 10, and 42.3 percent over age 10.

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A Statistical Evaluation Of the FPM Test

By AD HARRIS
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HENRY MILLER, B.S.

A further evaluation of the efficiency of the filter paper microscopic (FPM) test (*1*) as a "detector" test for syphilitic infection, based on specimens from 276 donors, including the specimens from the 266 donors analyzed in the preliminary report (*2*) is presented in this paper. As noted in that report, collection and distribution of blood specimens were carried out by the Eastern Medical Center, Durham, N. C., and by the staff of the Venereal Disease Research Laboratory at the Alto Medical Center, Alto, Ga.

Each Tuesday, blood specimens from 10 to 30 donors were collected and five filter paper strips and five tubes of whole blood were prepared from each specimen. One filter paper strip and one tube of whole blood from each donor were sent to each of the following laboratories: Dr. Kahn, Dr. Kline, Mr. Mazzini, the Eastern Medical Center, and the Venereal Disease Research Laboratory. On the following Friday each laboratory performed the FPM test on the filter paper in accordance with the test protocol and, in addition, any modification of the FPM test that they might devise. The tube of whole blood was used to perform tests commonly used in the various laboratories, hereafter referred to as standard tests. Antigen used for the FPM and VDRL tests, if performed, was distributed by the Venereal Disease Research Laboratory.

At the time of collection and distribution of specimens, the Eastern Medical Center and the Alto Medical Center established a diagnosis by clinical and serologic findings of all donors from whom the specimens for this study were taken.

For purposes of evaluation, two methods of comparison of the FPM test are presented. The first method discussed is that of comparing the test results of each participating laboratory with established diagnostic results. The second method disregards diagnostic findings and compares the results of the FPM test findings in a particular laboratory with the other tests performed in that laboratory. The second method of evaluation has been used for the following reasons: (*a*) A diagnosis by clinical means is not always obtainable in actual practice; (*b*) an error in diagnosis is possible. Certain specimens collected from donors diagnosed as positive or doubtful have been found to be negative by the standard tests. The agreement by all tests in a laboratory as to the negativity of these particular (diagnosed positive) specimens ranges from 2 specimens in the Venereal Disease Research Laboratory to 18 specimens in the Kahn laboratory. This type of disagreement (diagnosed positive, tested negative) may be due to laboratory technique or to an error in diagnosis. In either case a comparison of tests within laboratories seems justifiable.

In both methods, comparison of specimens as to agreement or disagreement is made only when tests have been performed. Tests giving doubtful reactions are considered positive, since it is not the purpose of this study to analyze the quantitative results produced by the FPM test.

Method 1

The various diagnoses of syphilis, based on clinical and serologic findings, established by the Eastern and Alto Medical Centers have been classified as follows:

Mr. Harris is a serologist and the assistant director of the Venereal Disease Research Laboratory; Dr. Olansky is the director; Mr. Miller is a statistician in the Division of Venereal Disease, Public Health Service.