Small-Quantity Blood Tests for Syphilis

The procedures reported in the five papers that follow have been developed in response to a need for methods of examining the blood of infants for syphilis when it is impracticable to draw the quantities required for standard serologic tests. Also, a technique is needed to mail samples long distances under particularly adverse conditions.

The techniques described present difficulties in securing suitable samples, and in comparative tests all have shown a lower sensitivity than the conventional serologic tests. However, with these techniques, serologic tests for syphilis may be performed on certain patients and under conditions which would completely preclude testing by conventional means. The results suggest the need for further study to determine whether, under special circumstances, the tests may be useful or whether the techniques can be improved.

A Comparison Of Serologic Tests

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At the direction of the office of the chief, Division of Venereal Disease, Public Health Service, a study to determine the relative efficiency of tests for syphilis requiring small amounts of blood, such as could be collected by finger puncture, was organized. Results of an evaluation of the FPM tests (*1*) and a preliminary study of the Chediak test (*2*) have been reported. A modification of the Chediak test using cardiolipin-lecithin antigen is described in the latter report (page 572 of this issue of *Public Health Reports*).

Study of the Chediak tests has been extended to include several testing centers. The purpose of this article is to present and discuss data and information obtained during this study as they relate to the relative efficiency of: (*a*) the Chediak test, (*b*) several modifications of this method for testing dried whole blood, and (*c*) a micromodification of the VDRL slide test by Cannefax and Johnwick (*3*) compared with other serologic tests for syphilis performed on heated serum. The laboratories of Dr. R. L. Kahn; Dr. B. S. Kline; Mr. L. Mazzini; the Medical Center, Public Health Service, Hot Springs National Park, Ark.; and the Venereal Disease Research Laboratory, Public Health Service, Chamblee, Ga., participated in this investigation.

Dr. Olansky is director of the Venereal Disease Research Laboratory, Venereal Disease Division, Public Health Service, Chamblee, Ga.; Mr. Harris, assistant director, is in charge of the serology section; Miss Vinson, Mr. Bossak, and Mr. Portnoy are bacteriologists in the serology section—Miss Vinson in the research unit; Mr. Bossak, assistant chief; and Mr. Portnoy, head of the testing unit.

Method

Blood specimens were collected from 360 donors and distributed to the five participating laboratories during the period of the study. This was accomplished by collecting blood from 20 donors (17 to 18 syphilic patients and 2 or more presumably nonsyphilitic individuals) at the medical centers in Hot Springs, Ark., and Alto, Ga., on Monday of each week and mailing

the specimens to the laboratories. Testing was performed on Thursday of each week in all laboratories so that approximately 72 hours elapsed between collection and testing, even in those instances when the specimens reached the laboratory earlier.

Six vacutainers (10 ml.) of blood were collected from each donor. Blood was immediately removed from the last vacutainer and used to prepare 10 slides, each containing 2 drops (0.05 ml.) of blood, for the Chediak tests, and two capillary tubes for the micro-VDRL slide test. One vacutainer of blood and two slides containing dried blood, from each patient, were sent to each participating laboratory on the day bloods were collected. Capillary tubes of blood were distributed only to the two Public Health Service laboratories.

Each of the five laboratories performed the Chediak test, a modification of the Chediak test using VDRL antigen, and any other modification of the Chediak technique that they might select on the specimens supplied as dried blood on the two glass slides. The tubes of blood supplied serum that was tested quantitatively by any standard method in use at the laboratory. The Medical Center, Hot Springs National Park, Ark., and the Venereal Disease Research Laboratory, Chamblee, Ga., each performed quantitative microtests on the capillary tube specimens, using the micromodification of the VDRL slide test (*3*).

Antigens for the Chediak test and for those tests employing VDRL antigen were distributed by the Venereal Disease Research Laboratory from common lots. Antigen for the Chediak test had been prepared and was supplied for this study by Dr. Chediak.

Before the survey started, at least one technical worker from each of the testing laboratories was sent to the Venereal Disease Research Laboratory for training in the Chediak and the Chediak-VDRL test techniques. Mr. Cannefax visited the Venereal Disease Research Laboratory to demonstrate the micro-VDRL slide test.

The results of all tests were recorded on report forms provided for this purpose and returned to the Venereal Disease Research Laboratory for review and compilation. Final tabulation and statistical analysis of these findings were made in Washington by the Division of Venereal Disease, Public Health Service.

CHEDIAK TEST

(As described and demonstrated by Dr. A. Chediak)

Reagents:

1. Chediak antigen.
2. 3.5-percent sodium chloride solution.
3. 1-percent sodium carbonate solution.

Equipment:

1. Chediak 3-piece slide holders.
2. ¼-inch steel bearings.
3. Electromagnet or forceps.
4. Microscope with 60× magnification.

Preparation of Antigen Emulsion:

1. Prepare alkaline saline solution by adding 0.12 ml. of 1-percent sodium carbonate solution to 10 ml. of 3.5-percent sodium chloride solution. Mix well.
2. In one tube (15 x 85 mm.) place 1 ml. of alkaline saline solution.
3. In second tube, place 0.1 ml. of Chediak antigen.
4. Heat both tubes in 56° C. water bath for 5 minutes.
5. Mix by pouring saline into the antigen, and back and forth three times.
6. Place tube containing emulsion in 56° C. water bath for 2 minutes.
7. Check emulsion by examining a drop at 50× to 60× magnification. Particles should be evenly dispersed with no clumping. This emulsion should be used within 5 minutes.

Technique:

1. Place slides on holder, fastening top to make a well around each specimen.
2. Add two ¼-inch ball bearings to each specimen.
3. Add 0.03 ml. of 3.5-percent sodium chloride solution to each specimen. This may be accomplished by delivering the salt solution from a 0.2-ml. pipette (graduated in 1/100 ml.) or by dropping from a syringe fitted with a 15-gauge needle held in a vertical position. The needle should be tested for delivery of 0.03 ml. of 3.5-percent sodium chloride solution on the day of use.
4. Shake slide holders with irregular motion for 1 minute or until dried blood is resuspended in saline.
5. Add 0.03 ml. of Chediak antigen emulsion with a 0.1- or 0.2-ml. pipette graduated in 0.01 ml.
6. Rotate at 180 rpm for 3 minutes.
7. Remove ball bearings with electromagnet or forceps.
8. Place cover on slide holder and let stand for 20 minutes.
9. Read, using microscope with 60× magnification. Tests should be read within 30 minutes but not prior to 20 minutes after rotation.
10. Report as follows:

Negative	No clumping.
Doubtful	Small clumps.
Positive	Moderate and large clumps.

CHEDIAK-VDRL TEST

Reagents:

1. VDRL flocculation antigen.
2. VDRL buffered saline solution.
3. 3.5-percent sodium chloride solution.

Equipment:

1. Chediak 3-piece slide holders.
2. ¼-inch steel ball bearings.
3. Electromagnet or forceps.

Preparation of Antigen Emulsion:

1. Prepare and check VDRL antigen emulsion as directed in the Manual of Serologic Tests for Syphilis (*4*).

2. Prepare a diluted VDRL antigen emulsion by adding one part of VDRL buffered saline solution to one part of VDRL antigen emulsion. The diluted emulsion should be allowed to stand 10 minutes before use and should be used within an hour.

Technique:

(Two dried-blood specimens from the same donor are tested simultaneously.)

1. Place slides on holder, fastening top to make a well around each specimen.

2. Add two ¼-inch ball bearings to each specimen.

3. Add 0.03 ml. of 3.5-percent sodium chloride solution to each specimen. This may be accomplished by delivering the salt solution from a 0.2-ml. pipette (graduated in 1/100 ml.) or by dropping the solution from a syringe fitted with a 15-gauge needle held in a vertical position. On the day of use, the needle should be tested for delivery of 0.03 ml. of 3.5-percent sodium chloride solution.

4. Shake slide holders with irregular motion for 1 minute or until dried blood is resuspended in saline.

5. To one specimen, add 0.03 ml. of VDRL antigen emulsion. To the second specimen, add 0.03 ml. of diluted VDRL antigen emulsion. Emulsions are added with a 0.2-ml. pipette graduated in 0.01 ml.

6. Rotate at 180 rpm for 3 minutes.

7. Remove ball bearings with electromagnet or forceps.

8. Read tests immediately, using microscope with 60× magnification.

9. Report as follows:

Reactive (R)------ Definite clumping of antigen particles.

Nonreactive (N)-- No clumping of antigen particles, or very slight roughness.

NOTE: A test report is the composite of results obtained with diluted and undiluted antigen emulsions. When either result is reactive (although the other may be nonreactive), the report shall be "reactive." When both results are nonreactive, report shall be "nonreactive."

CHEDIAK-KLINE TEST

Reagents:

1. Standard Kline antigen emulsion (cardiolipin-lecithin antigen). Prepare antigen emulsion as directed in Manual of Serologic Tests for Syphilis (*4a*).

2. 2.0-percent sodium chloride solution.

Equipment:

1. Chediak 3-piece slide holders.
2. ¼-inch steel ball bearings.
3. Electromagnet or forceps.

Technique:

1. Place slides on holder, fastening top to make a well around each specimen.

2. Add two ¼-inch ball bearings to each specimen.

3. Add 0.06 cc. of 2.0-percent sodium chloride solution to each specimen. This may be accomplished by delivering the salt solution from a 0.2-cc. pipette (graduated in 1/100 cc.) or by dropping two drops from a syringe fitted with a 15-gauge needle held in a vertical position. The needle should be tested for delivery of 0.03 cc. of 2-percent sodium chloride solution on the day of use.

4. Shake slide holders with irregular motion for 1 minute or until dried blood is resuspended in the salt solution.

5. Remove ball bearings with electromagnet or forceps.

6. To each specimen add 1 drop of standard Kline antigen emulsion (0.008 cc.).

7. Rotate at 180 rpm for 4 minutes.

8. Read tests immediately using a microscope with 100× magnification.

9. Report results as with the standard Kline test (Manual of Serologic Tests for Syphilis (*4b*)).

CHEDIAK-MAZZINI TEST

Reagents:

1. Mazzini-cardiolipin antigen (*7*).
2. Mazzini buffered saline solution.
3. 0.9-percent sodium chloride solution.

Equipment:

1. Chediak 3-piece slide holders.
2. ¼-inch steel ball bearings.
3. Electromagnet or forceps.

Preparation of Antigen Emulsion (*5*):

1. Pipette 0.4 ml. of the buffered saline solution to the bottom of a 30-ml. round bottle.

2. With a 1-ml. pipette, measure 0.4 ml. of the cholesterolized antigen (measurement is made from the tip of the pipette). Hold the bottle in the left hand and, imparting a rapid and constant rotating motion to the bottle, add the antigen directly and at once, blowing out whatever antigen is left in the pipette. Draw the emulsion into and out of the pipette exactly six times, returning all the emulsion left in the pipette on the last mixture.

3. Add 2.6 ml. of the buffered saline solution. Cork the bottle with a paraffin-coated cork and shake from bottom of the bottle to cork and back 50 times in 15 seconds.

Technique:

1. Place slides on holder, fastening top to make a well around each specimen.

2. Add two ¼-inch ball bearings to each specimen.

3. Add 0.03 ml. of 3.5-percent sodium chloride solution to each specimen. This may be accomplished by delivering the salt solution from a 0.2-ml. pipette (graduated in 1/100 ml.) or by dropping the solution from a syringe fitted with a 15-gauge needle held in a vertical position. On the day of use, the needle should be tested for delivery of 0.03 ml. of 3.5-percent sodium chloride solution.

4. Shake slide holders with irregular motion for 1 minute or until dried blood is resuspended in saline.

5. Add Mazzini cardiolipin antigen emulsion from observation tube fitted with 25-gauge needle held at approximately a 45° angle.

6. Rotate at 180 rpm for 4 minutes.

7. Remove ball bearings.

8. Add one drop of 0.9-percent sodium chloride solution from a medicine dropper.

9. Rerotate at approximately 100 rpm for 4 minutes.

10. Read tests immediately.

11. Report as:

Negative	No clumping.
Weakly positive	Slight to moderate clumping.
Positive	Definite clumping.

CHEDIAK-KAHN TEST

Reagents:

1. Kahn standard antigen (lot 140B).
2. 0.9-percent sodium chloride solution.

Equipment:

1. Chediak 3-piece slide holders.
2. ¼-inch steel ball bearings.
3. Electromagnet or forceps.

Preparation of Antigen Suspension:

1. Same as for standard Kahn test. Prepare antigen emulsion as directed in Manual of Serologic Tests for Syphilis (*4c*).

Technique:

1. Place slides on holder, fastening top to make a well around each specimen.
2. Add two ¼-inch ball bearings to each specimen.
3. Add 0.05 ml. of 3.5-percent sodium chloride solution to each specimen. This may be accomplished by delivering the salt solution from a 0.2-ml. pipette (graduated in 1/100 ml.).
4. Shake slide holders with irregular motion for 1 minute or until dried blood is resuspended in saline.
5. Add 0.008 ml. of Kahn antigen suspension with a 0.1-ml. pipette graduated in 0.001.
6. Rotate at 180 rpm for 3 minutes.
7. Remove ball bearings with electromagnet or forceps.
8. Read tests immediately, using microscope with 60× magnification.
9. Report as follows:

Negative	No clumping.
Doubtful	Small clumps.
Positive	Moderate and large clumps.

MICRO-VDRL SLIDE TEST (CANNEFAX)

This test is described in detail in "A Micromodification of the VDRL Slide Test," by Cannefax, Beyer, and Johnwick, on page 576 of this issue of *Public Health Reports*.

Results

Only qualitative test results obtained in the five laboratories with each test procedure are recorded in tables 1–5 since quantitative results are not obtained by any of the Chediak procedures. Qualitative test findings offer a basis for comparison of testing efficiency if only the ability of a test to react in a weakly or strongly positive manner with specimens from syphilitic donors is considered. This ability to "detect" serologically positive blood specimens is important if the tests requiring only small-volume

Table 1. Results obtained on whole blood and on dried blood specimens tested in the Venereal Disease Research Laboratory, Chamblee, Ga.

Tests	307 syphilitic donors					45 presumably nonsyphilitic donors				
	Positive	Weakly positive or doubtful	Negative	Not tested	Percent reactive	Positive	Weakly positive or doubtful	Negative	Not tested	Percent negative
On serum:										
Kahn standard	284	17	6	-------	98	0	2	43	-------	95.6
VDRL slide	286	14	7	-------	97.7	0	0	45	-------	100
Micro-VDRL slide	228	34	21	24	92.6	2	7	33	3	78.6
On dried blood:										
Chediak	64	135	108	-------	64.8	0	12	33	-------	73.3
Chediak-VDRL	Reactive 266		41	-------	86.6	Reactive 3		42	-------	93.3

blood collection, such as the Chediak test, are used for screening child or baby groups for congenital or acquired infections.

The results of tests on specimens from eight of the presumably nonsyphilitic blood donors used in this study were omitted from final tabulation because other than negative reactions were obtained on the whole-blood sample tested by one or more author serologists, and adequate information regarding the clinical status of these individuals could not be obtained. Only the author's test, as performed in his laboratory, was considered in this regard. Positive or weakly positive (doubtful) reactions were produced by five of these specimens in the Mazzini test, six in the VDRL slide test, three in the Kline test, and two in the Kahn test.

Results of the Chediak and Chediak-VDRL tests, as reported by the five laboratories, are compared with the quantitative VDRL slide test findings in tables 6 and 7. These tables present the zones of relative agreement between the tests on dried blood specimens and the VDRL slide test in terms of quantitation. The VDRL slide test results used in these tables were those reported by the Venereal Disease Research Laboratory.

Reports of the Chediak and Chediak-VDRL test results from the five laboratories on dried blood specimens from 45 presumably nonsyph-

Table 2. Results obtained on whole blood and on dried blood specimens tested in the laboratory of the Public Health Service Medical Center, Hot Springs National Park, Ark.

Tests	307 syphilitic donors					45 presumably nonsyphilitic donors				
	Positive	Weakly positive or doubtful	Negative	Not tested	Percent reactive	Positive	Weakly positive or doubtful	Negative	Not tested	Percent negative
On serum:										
Kahn standard	275	9	23	-------	92.5	0	0	45	-------	100
Kolmer complement-fixation	259	6	38	4	87.5	0	0	44	1	100
VDRL slide	270	11	26	-------	91.5	0	0	45	-------	100
Micro-VDRL slide	273	11	23	-------	92.5	1	0	44	-------	97.8
On dried blood:										
Chediak	145	88	74	-------	75.9	3	4	38	-------	84.4
Chediak-VDRL	Reactive 272		35	-------	88.6	Reactive 4		41	-------	91.1

Table 3. Results obtained on whole blood and on dried blood specimens tested in Dr. Kahn's laboratory

Tests	307 syphilitic donors					45 presumably nonsyphilitic donors				
	Positive	Weakly positive or doubtful	Negative	Not tested	Percent reactive	Positive	Weakly positive or doubtful	Negative	Not tested	Percent negative
On serum:										
Kahn standard	276	7	24	-------	92.2	0	0	45	-------	100
Kahn presumptive	298	1	7	1	97.7	2	1	42	-------	93.3
On dried blood:										
Chediak	203	36	68	-------	77.9	10	9	25	1	56.8
Chediak-Kahn	229	13	31	34	88.6	20	5	14	6	35.9
Chediak-VDRL	Reactive 278		23	6	92.4	Reactive 29		14	2	32.6

ilitic donors are listed in table 8. Specific disagreements are noted in the footnotes to this table.

Discussion

The Chediak test as performed in the Venereal Disease Research Laboratory (table 1) was appreciably less sensitive than the other tests for syphilis, producing positive or doubtful reactions in approximately two-thirds of the specimens from syphilitic donors that gave those reactions in the other tests. The relative percentage reactivity of the Chediak test on specimens from syphilitic donors was not the same in each laboratory. The percentages ranged from 60.8 percent (Kline laboratory, table 4) to 77.9 percent (Kahn laboratory, table 3) as compared with the standard flocculation test results on serum which ranged between 91.5 percent (VDRL slide test, table 2) and 98.7 percent (Mazzini-cardiolipin test, table 5) and the Kolmer complement-fixation test result of 87.5 percent (table 2). These findings indicate that the Chediak test detected 70 to 80 percent of the syphilitic donors in this study whose

Table 4. Results obtained on whole blood and on dried blood specimens tested in Dr. Kline's laboratory

Tests	307 syphilitic donors					45 presumably nonsyphilitic donors				
	Positive	Weakly positive or doubtful	Negative	Not tested	Percent reactive	Positive	Weakly positive or doubtful	Negative	Not tested	Percent negative
On serum:										
VDRL slide flocculation	281	11	15	-------	95.1	0	0	45	-------	100
Kline standard	286	12	9	-------	97.1	0	0	45	-------	100
Kline diagnostic	269	18	20	-------	93.5	0	0	45	-------	100
Kline exclusion	293	6	8	-------	97.4	0	1	44	-------	97.8
On dried blood:										
Chediak	98	77	113	19	60.8	1	6	37	1	84.1
Chediak-Kline	226	30	32	19	88.8	0	0	44	1	100
Chediak-VDRL	Reactive 242		46	19	84.3	Reactive 0		44	1	100

Table 5. Results obtained on whole blood and on dried blood specimens tested in Mr. Mazzini's laboratory

Tests	307 syphilitic donors					45 presumably nonsyphilitic donors				
	Positive	Weakly positive or doubtful	Negative	Not tested	Percent reactive	Positive	Weakly positive or doubtful	Negative	Not tested	Percent negative
On serum:										
VDRL slide	273	25	9	-------	97.1	0	1	43	1	97.8
Mazzini (cardiolipin)	285	18	4	-------	98.7	0	0	44	1	100
Mazzini (lipoidal)	251	15	6	35	97.8	0	2	37	6	94.9
On dried blood:										
Chediak	134	96	71	6	76.4	5	7	33	-------	73.3
Chediak-Mazzini	228	38	35	6	88.4	0	4	41	-------	91.1
Chediak-VDRL	Reactive 275		29	3	90.5	Reactive 7		38	-------	84.4

blood gave positive or doubtful reactions in standard tests for syphilis using serum.

The modified Chediak test using VDRL test antigen, and referred to as the Chediak-VDRL test, was the only modification of the Chediak test performed by all five participating laboratories. This technique called for reporting results as "reactive" and "nonreactive" so that all reactions equivalent to positive and doubtful or weakly positive are included under the "reactive" heading. In each laboratory, this test was more reactive on specimens from syphilitic donors than was the Chediak test. The Chediak-VDRL test showed reactivity percentages of 86.6, 88.6, 92.4, 84.3, and 90.5, respectively, and a reactivity percentage of 88.5 percent for all laboratories. These figures show a closer relationship with test results obtained by serum tests since approximately 90 percent of the reactors in the specimens from

Table 6. Results obtained by five laboratories with the Chediak test compared with quantitative VDRL slide test findings on specimens from 307 syphilitic donors

Chediak test	Quantitative VDRL slide test (dils)												
	Negative	<1	1	2	4	8	16	32	64	128	256	512	Total
Reactive in:													
5 laboratories	1	----	1	12	12	19	17	12	11	3	1	----	89
4 laboratories	----	4	4	6	7	12	16	10	6	2	1	1	69
3 laboratories	2	2	4	7	4	9	5	12	8	3	----	----	56
2 laboratories	3	3	4	----	6	3	7	5	4	4	2	1	42
1 laboratories	1	4	1	3	----	----	1	7	1	3	2	----	23
Negative in all 5 laboratories	----	----	1	----	1	----	----	1	----	----	----	----	3
Total	7	13	15	28	30	43	46	47	30	15	6	2	282
Not tested in all 5 laboratories	----	1	6	----	3	4	7	1	2	----	1	----	25
Grand total	7	14	21	28	33	47	53	48	32	15	7	2	307

NOTE: Agreement in 5 laboratories ..... 92 (32.62 percent)
Partial agreement (agreement in 4 laboratories; disagreement in 1) ..... 92 (32.62 percent)
Partial disagreement (disagreement in 3 laboratories; agreement in 2) ..... 98 (34.75 percent)

Total specimens tested in all 5 laboratories 282

Table 7. Results obtained by five laboratories with the Chediak-VDRL test compared with quantitative VDRL slide test findings on specimens from 307 syphilitic donors

Chediak-VDRL test results	Quantitative VDRL slide test (dils)												
	Negative	<1	1	2	4	8	16	32	64	128	256	512	Total
Reactive in:													
All 5 laboratories	----	----	1	15	22	41	41	43	28	7	4	1	203
4 laboratories	----	3	5	8	7	3	2	2	1	3	----	----	34
3 laboratories	1	2	3	2	----	----	2	----	----	3	1	----	14
2 laboratories	2	3	3	1	1	----	----	----	----	----	1	----	11
1 laboratory	3	3	3	----	----	----	1	----	----	2	----	----	12
Negative in all 5 laboratories	1	2	1	----	----	----	----	----	----	----	----	1	5
Total test	7	13	16	26	30	44	46	45	29	15	6	2	279
Not tested in all 5 laboratories	----	1	5	2	3	3	7	3	3	----	1	----	28
Grand total	7	14	21	28	33	47	53	48	32	15	7	2	307

NOTE: Total agreement (5 laboratories) ..... 208 (74.55 percent)
Partial agreement (agreement in 4 laboratories; disagreement in 1) ..... 46 (16.49 percent)
Partial disagreement (disagreement in 3 laboratories; agreement in 2) ..... 25 (8.94 percent)

Total specimens tested in all 5 laboratories 279

Table 8. Results obtained by five laboratories with the Chediak and Chediak-VDRL tests on specimens from 45 presumably nonsyphilitic donors

Results	Chediak test	Chediak-VDRL test
	Number of specimens	Number of specimens
Negative in:		
All 5 laboratories	13	12
4 laboratories	[1] 16	[2] 22
3 laboratories	[3] 7	[4] 7
2 laboratories	[5] 4	0
1 laboratory	[6] 2	[7] 1
No laboratory	1	0
Not tested in 1 or more laboratories	2	3
Total tested in all 5 laboratories	43	42

NOTE: Number of reactors in each laboratory was:
[1] Kahn, 7; Mazzini, 5; Hot Springs, 1; Venereal Disease Research Laboratory, 3.
[2] Kahn, 20; Hot Springs, 2.
[3] Kahn, 6; Kline, 2; Hot Springs, 2; Venereal Disease Research Laboratory, 2.
[4] Kahn, 7; Mazzini, 6; Venereal Disease Research Laboratory, 1.
[5] Kahn, 3; Mazzini, 3; Venereal Disease Research Laboratory, 3; Kline, 2; Hot Springs, 1.
[6] Kahn, 2; Kline, 2; Venereal Disease Research Laboratory, 2; Hot Springs, 1; Mazzini, 1.
[7] All laboratories except Kline, 1.

the syphilitic donor group, with all tests, were detected by this method. Inspection of the reactivity percentage figures for each test (tables 1–5) shows that an even closer agreement exists between the Chediak-VDRL test and the selected single testing procedures.

The third group of tests performed on dried blood samples included the Chediak-Kahn, Chediak-Kline, and Chediak-Mazzini tests using the respective antigens designated by the latter names. These tests showed reactivity ratings of 88.6 percent, 88.8 percent, and 88.4 percent, respectively, so the ability of these tests to produce positive or doubtful reactions on the specimens from syphilitic donors appears to be about the same as the Chediak-VDRL test.

The relative specificity of these tests on dried blood is not so clear from the reported findings. The number of positive plus doubtful reactions obtained by the Chediak method on the dried blood samples from presumably nonsyphilitic donors as recorded in tables 1–5 are 12, 7, 19, 7, 12, with an average of 11.4, yielding an over-all specificity rating of approximately 75 percent. However, it is noted that the largest number of these reactions were obtained in one laboratory (table 3) that also reported only 14 negative reactions on this group of specimens from presumably nonsyphilitic donors using the Chediak-Kahn and Chediak-VDRL procedures. This may indicate that the dried blood samples tested by this laboratory were either not similar, at the time of testing, to those tested in the other laboratories or that technical difficulties prevented the obtaining of clearly negative reactions at this testing station.

The Chediak-VDRL modification, as performed in the five laboratories, failed to give negative findings in 3, 4, 29, 0, and 7 instances, respectively, in the "negative" (presumably nonsyphilitic) donor group as recorded in tables 1–5. The lack of agreement between laboratories is greatest in this group of reports, so an average of findings under these circumstances probably would have little significance. The major disagreement in this regard was also from a single laboratory (table 3).

The results recorded in tables 1, 2, 4, and 5 show, in each instance, that the modifications of the Chediak test (Chediak-VDRL, Chediak-Kline, Chediak-Mazzini) had better sensitivity and specificity ratings than the original Chediak test performed at the same time in the four laboratories. These four tests employed cardiolipin-lecithin antigens. In the fifth instance (table 3), the two modified Chediak tests (Chediak-Kahn and Chediak-VDRL) were more reactive than the original Chediak test. However, all three of these tests had very poor specificity ratings. Findings reported by all five laboratories indicate that the Chediak test, modified to use cardiolipin-lecithin antigens, may be operated at a more efficient level than the original Chediak test as a "detector test" for syphilis. Evidence acquired during this study shows no definite preference for any one of the cardiolipin antigens used (Kline, Mazzini, VDRL).

Comparative reproducibility of the Chediak and Chediak-VDRL tests as portrayed in tables 6 and 7 favors the latter test. Complete agreement between results obtained in all five laboratories is more than twice as great with the Chediak-VDRL test (74 percent as opposed to

32 percent) and approximately 90-percent agreement was obtained by four of the five laboratories using this test. This indicates that a favorable percentage of agreement may be expected from laboratories performing the Chediak-VDRL test without lengthy technician training periods. However, these findings also may reflect less variability in antigen emulsions used from time to time in the several laboratories rather than a direct human variable such as ability to conduct tests or read results. The VDRL antigen emulsion is more stable and may be used for a longer time after being prepared than the Chediak antigen emulsion.

The micro-VDRL slide test results reported by two laboratories (tables 1 and 2) were in close agreement as to reactivity on specimens from syphilitic donors showing that 92.6 percent and 92.5 percent, respectively, of the specimens tested gave positive or weakly positive findings. However, 9 of 42 specimens from the nonsyphilitic donor group were reported by the Venereal Disease Research Laboratory as positive or weakly positive with the micro-VDRL test and only one positive reaction was reported by the Medical Center laboratory on 45 specimens from the same group.

It was also noted that 27 (8 percent) of the 352 specimens (307 from syphilitic donors, and 45 from presumably nonsyphilitic donors) submitted in capillary tubes for the micro-VDRL test were not tested at the Venereal Disease Research Laboratory while reports of microtest results were issued on all 352 such specimens by the Medical Center laboratory. The 27 specimens listed under the "not tested" heading for the micro-VDRL test by the Venereal Disease Research Laboratory were untestable due to loss of serum either in transit or in the centrifuge, or due to breakage of the capillary tube in the centrifuge. These factors are not evident in the reports of this test by the Medical Center laboratory because serum from the vacutainer tubes was used for testing whenever the capillary tube specimen was lost through leakage or breakage. The number of these losses that occurred is not recorded.

The relative efficiency of a testing procedure is based not only on test specificity and sensitivity but also on the effectiveness with which an adequate specimen can be obtained and delivered to the laboratory. Loss of serum by breakage or leakage of tube in transit or through normal handling in the laboratory weighs against the micro-VDRL slide test procedure if the experience of the Venereal Disease Research Laboratory in this study indicates the average expectancy for adequate specimens to be received in the laboratory. A loss of 8 percent of the specimens submitted reduces collection rates to at least 92 percent, if an adequate specimen could be obtained from every donor. However, since the capillary tube is essentially similar to the large blood tubes, it is probable that deterioration of the blood sample in the capillary tube would not be more rapid than if collected in a larger tube.

The micro-VDRL slide test provides for quantitation if an adequate blood sample is collected. This would require approximately 0.15 ml. of blood, an advantage over the tests on dried blood specimens that do not provide for quantitation.

Findings reported in this study indicate that the Chediak test modifications using cardiolipin-antigens and the micro-VDRL slide test would be approximately equally effective as "detector tests" for syphilis. The modified Chediak tests detected approximately 90 percent of the specimens that gave positive reactions in other tests when performed on 72-hour-old blood samples. Previous studies have shown that dried blood samples are more reactive when stored for shorter periods of time. The 8-percent loss of capillary blood specimens for the micro-VDRL slide test placed this test in a comparable position with the Chediak modifications.

A field study of these two types of collection and testing procedures would be needed to determine the method of choice. Several factors that may influence this selection are (*a*) type of donor group, whether adult, child, or infant, (*b*) time interval between blood collection and testing, and (*c*) capability of the laboratory to perform either test efficiently.

Summary

1. Results obtained in five laboratories with the Chediak test and its modifications on dried

blood specimens plus several other tests on heated serum are presented.

2. The relative reproducibility of the Chediak and Chediak-VDRL tests among the five participating laboratories is shown in tabular form and is discussed.

3. Relative efficiency of the tests on dried blood specimens, as compared to tests on heated serum as "detector" tests for syphilis is discussed.

4. The micro-VDRL slide test findings, as reported by two laboratories, are presented and compared with results of other testing procedures.

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The Chediak Test— A Preliminary Report

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The development of a test for syphilis requiring only a small amount of blood that could be collected with a minimum of equipment and difficulty by relatively untrained personnel has been the object of several investigative studies (*1–9*). Such a test would aid considerably in the detection of cases of syphilis from which collection of the amounts of blood necessary for the standard testing procedures, using serum, is difficult or impractical due to lack of either adequate facilities or adequately trained workers.

In 1932 Dr. Alejandro Chediak of Havana, Cuba, published a technique for the serodiagnosis of syphilis requiring the collection of only a single drop of blood. The Venereal Disease Research Laboratory has recently studied this method as it was demonstrated by Dr. Chediak and explained in a personal communication from him. The purpose of this presentation is to report results obtained with the Chediak test and modifications of this technique using cardiolipin-lecithin antigens, under specified conditions.

CHEDIAK TEST

The mechanics of the Chediak test were retained with only minor changes throughout this study, using equipment and antigen supplied by Dr. Chediak. A brief summary of this method as demonstrated by Dr. Chediak during a visit to the Venereal Disease Research Laboratory, follows:

1. A drop of dried, "homogenized" blood, collected on a glass slide, is resuspended in 0.03 ml. of 3.5-percent sodium chloride solution. This is accomplished by placing the slide in a slide holder that forms a well above the blood sample so that two ¼-inch steel balls may be put into each blood-saline mixture. The blood is then dissolved or resuspended by rotating the slide holder for approximately 1 minute.

2. After 0.03 ml. of antigen emulsion is added to each specimen, the specimens are rerotated on a flat-bed rotator for 3 minutes at 180 rpm.

3. Steel balls are removed, glass covers are placed into slide holders to prevent drying, and specimens are allowed to stand 20 to 30 minutes before being examined.

4. Slide holder covers are removed and specimens are read with a microscope at 60× maginfication. Small clumps of antigen particles are interpreted as a doubtful reaction, large clumps indicate a positive reaction, and no clumping of antigen particles is read as a negative reaction.

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