

A Comparative Study of the Results Obtained from Flocculation and Complement Fixation Tests Carried out among 3,994 Selectees and Volunteers in 1941¹

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WITH THE EXTENSION of the Selective Service Act to Puerto Rico during 1941, a large number (19,395) of blood specimens, taken principally from men between the ages of twenty and thirty-five, were sent for serodiagnostics examinations to the seven public health laboratories of the Department of Health of Puerto Rico. In most instances, the men reported to the health units of their respective communities, where the specimen was obtained and from where it was forwarded to the corresponding laboratory. An analysis of these tests was reported by the authors during 1942.²

Four thousand six hundred and seventy-two (4,672) specimens were sent to the central laboratory at San Juan, where 3,994 were tested simultaneously by four different standard techniques: by three flocculation tests (Kahn, Kline, and Hinton) and by Kolmer's complement-fixation test (simplified, with one dose of serum). These specimens were not selected, but enough serum was obtained from them to perform the four tests mentioned above. A comparative analysis of the results forms the basis for the present study.

No attempt has been made to correlate the results with the clinical histories or physical examinations of these individuals.

STATISTICAL DATA

All the data collected are tabulated in the two charts and eight tables appended to the text.

Chart 1 shows the number and percentage of positive, negative, and doubtful reactions obtained from each individual test and from all four tests.

Chart 2 presents the results in percent of the total examinations

1. Received for publication March, 1943.

2. O. Costa Mandry and J. L. Janer, "Studies of Syphilis in Puerto Rico. III. Survey Based on the Results of Flocculation Tests among 19,935 Selectees and Volunteers during 1941," *P.R.J. Pub. Health & Trop. Med.*, 19:483-492, 1944.

made, wherein there was found agreement in all, or in some of the techniques employed.

Table 1 shows the percentage of positive, negative, and doubtful reactions obtained from each of the four tests employed when examining the 3,994 specimens. This table does not show the relation between the tests wherein the results varied.

Table 2 presents the percentage of distribution by results obtained in the 3,644 specimens, in which these results were the same with all four techniques: positive, 9.6 percent, negative, 90.4 percent, doubtful, 0 percent. Out of 3,994 tests, 82.4 percent (3,293) coincided in their negative results and 8.8 percent (351) in their positive results, or a total of 91.2 percent (3,644). Three hundred and fifty specimens (8.8 percent) did not coincide in the results obtained from all four procedures.

Table 3 shows the agreement in the results obtained when combinations of two techniques were utilized in testing the 3,994 specimens.

Table 4 shows a comparison of the results obtained, in number and percentage, among 350 specimens wherein the results differed in one or more tests, when combinations of two techniques were employed.

Table 5 presents the same results from combinations of three techniques.

Table 6 shows the number and percentage of positive, negative, and doubtful reactions obtained among 350 specimens, wherein the results differed in one or more of the four techniques employed.

Table 7 summarizes the varying results obtained from one or more of the techniques employed.

Table 8 summarizes the results from four different techniques used in the 3,994 specimens.

The following detailed explanation serves to interpret the different results charted in Master Tables 7 and 8.

When one technique is used, any specimen will give one of three possible results—negative, positive, or doubtful. When two techniques are utilized simultaneously, the results obtained with the first technique will combine in three ways with the results obtained from the second, irrespective of order. As there are three possible results to be obtained from one technique, each of which can combine in three ways with the results obtained from a second technique, we find that the number of combinations of results that can be obtained by using two techniques simultaneously is 3×3 or 9, or $(-)$, $(+)$, $(-D)$, $(+-)$, $(++)$, $(+D)$, $(D-)$, $(D+)$, (DD) .

If a third technique is used simultaneously with the first two on each blood specimen, each of the nine different combinations of possible results, when using the two techniques as described above, can combine in three ways with the results obtained from the third technique. Thus, using a third technique increases the number of possible combinations of results to 3×9 or 27, $(---)$, $(-+)$, $(-D)$, and so forth. By following the same line of reasoning, if a fourth technique is added, each of the 27 possible combinations of results from three techniques can combine with either a negative, positive, or doubtful result from the fourth technique, and the total number of possible combinations would be increased to 81 (27×3 equals 81).

Under the heading "Kahn," Table 8 has four groups of columns that read from left to right, as follows: Negative, Positive, Doubtful, and Total. Each group of columns is subdivided according to the results obtained in the Kline test, that is, under the Kahn Negative, we have Kline $-$, $+$, D , T ; the same is found under Kahn Negative, Positive, Doubtful, and Total. On the left side of the table, we have the heading "Kolmer" and next to it, to the right, we read from top to bottom: Negative, Positive, Doubtful, Total. Each of these Kolmer results is subdivided into 4 rows that read from top to bottom: Hinton $-$, $+$, D , T .

The intersection of the rows and columns that make up the table forms 256 cells, intersectionals or subdivisions, inside each of which a number is written. Of these 256 cells there are 81, each of which belongs to one of the possible combinations of results of the four techniques employed on each specimen: Kahn, Kline, Kolmer, and Hinton. The rest of them, 175 cells, gives the totals (partial and grand).

To see how the table works, let us try to interpret some of the cells of which it is made. First, we shall refer to the cell on the upper left hand corner. It reads 3,293 and belongs to one of the columns that make up the group of Kahn negatives. To be more specific, it belongs to the column in the group that tells about the Kahn negatives that were also Kline negatives. It also belongs to one of the rows that make up the groups of Kolmer negatives, and more specifically, to the row of Kolmer and Hinton negatives. Therefore, this cell tells us the number of samples that gave a negative result by all four techniques.

Suppose we now take the next cell to the right, which reads 6. Although it is in the same group of Kahn negatives, it belongs to the column of Kline positives. With respect to the Kolmer and Hinton tests, it lies in the row of the Kolmer negative group that also gave a negative Hinton reaction. Therefore this cell tells us that 6 blood specimens were negative in the Kahn, Kolmer, and Hinton tests, but were positive in the Kline. Starting with the first cell discussed and, reading from top to bottom in that same column, we find that the fourth cell has the number 3,386. By using the above reasoning, we can see that this cell stands for the number of samples that were negative by the Kahn, Kline, and Kolmer techniques, disregard-

ing the Hinton, for it is in the row that belongs to the total Hinton tested specimens that were negative in all the other three techniques.

Thus, by finding the position of each cell (column and row to which it belongs) with respect to the result of each test, we can determine the combination of results to which the cell belongs. The number within the cells tells us the frequency with which the combination was observed.

DISCUSSION

A total of 3,994 blood specimens was examined by each of four different standard techniques. The results obtained from all four techniques coincided in 3,644 (91.2 percent) specimens. In 350 (8.8 percent) samples the results differed in one or more of the techniques. Of the latter samples, 273 (78 percent) coincided in their results in three of the techniques, and 77 (22 percent) in two only.

Positive Results. Three hundred and fifty-one (8.8 percent) specimens gave positive results by all four tests. Five hundred and five specimens (505) were positive in the Kahn test with 69.5 percent of these giving the same results when the other three techniques were utilized. Five hundred and fifteen (515) were positive in the Kline; of these, 68.2 percent gave similar results in the other three tests. Four hundred and ninety-four (494) were positive in the Hinton test, with 71 percent of these showing positive results in the other three. In the Kolmer test, 424 specimens were positive; of these 82.8 percent were also positive when the three other tests were used.

Seventy-three of the 350 specimens, the results of which did not agree, were positive Kolmers; similar results were obtained for 17, 19, and 9 of these in the Kahn, Kline, and Hinton tests, respectively. One hundred and fifty-four (154) were positive Kahns; identical results were likewise obtained in 17, 151, and 88 of these in the Kolmer, Kline, and Hinton tests, respectively. One hundred and sixty-four were positive Klines, while 19, 151, and 93 of these were also positive Kolmers, Kahns, and Hinton tests, respectively. One hundred and forty-three (143) were positive in the Hinton test; 9, 88, and 93 of these were also positive in the Kolmer, Kahn, and Kline tests, respectively.

Negative Results. A negative result was obtained in all four of these tests for 3,293 (82.4 percent) of the specimens. Of the total 3,994 samples examined, 3,489 (87.4 percent) gave negative results in the Kahn test; 3,293 (94.4 percent) were also negative by all four tests. There were 3,477 negative Klines, of which 3,293 (94.7 per-

cent) were also negative by the other three tests. Three thousand four hundred and ten (3,410) specimens gave negative Hinton reactions; of these 3,293 (96.6 percent) were also negative by the other tests. Three thousand five hundred and fifteen (3,515) specimens gave negative Kolmer reactions and 3,293 (93.7 percent) of these were also negative when the other three techniques were utilized.

Two hundred and twenty-two (222) of the 350 specimens, the results of which did not coincide, were Kolmer negatives. Of these 105, 96, and 43 gave negative Kahn, Kline, and Hinton reactions, respectively. One hundred and ninety-six (196) were negative Kahns; of these 105, 181, and 73 were also negative Kolmers, Klines, and Hinton tests, respectively. One hundred and eighty-four (184) were negative Klines, similar results being obtained for 96, 181, and 66 when the Kolmer, Kahn, and Hinton techniques, respectively, were utilized. One hundred and seventeen (117) were negative Hinton tests, of which 43, 73, and 66 were likewise negative by the Kolmer, Kahn, and Kline tests, respectively.

Doubtful Results. It is interesting to note that there was no single specimen that gave a doubtful reaction in all four tests. The highest percentage of doubtfuls was found in the Hinton test (2.2 percent), the lowest in the Kahn (0 percent). The Kolmer reactions showed 1.4 percent while the Kline, only 0.1 percent.

The two doubtful Kline reactions (0.1 percent) were negative when the Kolmer, Kahn, and Hinton tests were utilized.

Of the 90 tests giving doubtful Hinton reactions, 68 were negative, 22 positive, and none doubtful by the Kahn technique; 68 were negative and 22 positive by the Kline; 62 were negative, 20 positive, and 8 doubtful by the Kolmer technique. Of the 55 specimens showing doubtful Kolmer reactions, 35 were negative and 20 positive by the Kahn technique; 34 were negative and 21 positive by the Kline, while 30 were negative, 17 positive, and 8 doubtful by the Hinton.

SUMMARY

1. Three thousand nine hundred and ninety-four (3,994) blood samples from volunteers and selectees were examined simultaneously by four standard techniques: by the Kahn, Kline, and Hinton flocculation tests and by a complement-fixation test—Kolmer's simplified technique.

2. The results in 3,644 (91.2 percent) specimens coincided in all four tests. Of these 90.4 percent (3,293) coincided in their negative

results, 9.6 percent in their positive results, and none in their doubtful results.

3. Three hundred and fifty specimens (8.8 percent) gave different results; 273 (78 percent) coincided in three of the four tests, 77 (22 percent) in only two of them.

4. The same result was never obtained with any of the doubtful reactions by any one individual test, when all four procedures were utilized simultaneously.

5. Of the total 3,994 specimens, the negative and positive results were as follows: Kahn, 87.4 percent and 12.6 percent, respectively; Kline, 87 percent and 12.9 percent, respectively; Hinton, 85.4 percent and 12.4 percent, respectively; Kolmer, 88 percent and 10.6 percent, respectively.

6. Doubtful reactions were 0 percent for Kahn, 0.1 percent for Kline, 1.4 percent for Kolmer, and 2.2 percent for Hinton.

7. No attempt was made to evaluate the results of the positive tests with the findings of the physical examinations to which these men were subjected.

8. The results of this limited study tend to emphasize the necessity of carrying out two different tests on every blood sample. The range of coincidence in any two individual tests for the 3,994 specimens, when examined simultaneously by the four techniques mentioned, was between 92.7 and 99.5 percent.

TABLE 1

Test	Negative		Positive		Doubtful	
	Number	Percent	Number	Percent	Number	Percent
Kahn	3,489	87.4	505	12.6	0	0.0
Kline	3,477	87.0	515	12.9	2	0.1
Hinton	3,410	85.4	494	12.4	90	2.2
Kolmer	3,515	88.0	424	10.6	55	1.4

TABLE 2

Results	Number	Percent	Percentage of Total That Group Represents
Negative	3,293	90.4	82.4
Positive	351	9.6	8.8
Doubtful	0	0.0	0.0
Total	3,644	100	91.2

TABLE 3

	Number	Percent
Kahn-Kline	3,976	99.5
Kahn-Kolmer	3,766	94.3
Kahn-Hinton	3,805	95.3
Kline-Kolmer	3,759	94.1
Kline-Hinton	3,803	95.2
Hinton-Kolmer	3,704	92.7

TABLE 4

Results	Kahn-Kline		Kahn-Kolmer		Kahn-Hinton		Kline-Kolmer		Kline-Hinton		Hinton-Kolmer	
	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
Negative	181	51.7	105	30.0	73	20.9	96	27.4	66	18.8	43	12.3
Positive	151	43.2	17	4.8	88	25.1	19	5.4	93	26.6	9	2.6
Doubtful	0	0	2	0.6	4	1.1	0	0	0	0	8	2.3
Total	332	94.9	124	35.4	165	47.1	115	32.8	159	45.4	60	17.2
Did not coincide	18	5.1	226	64.6	185	52.9	235	67.2	191	54.6	290	82.8
Grand Total	350	100.0	350	100.0	350	100.0	350	100.0	350	100.0	350	100.0

TABLE 5

Results	Kahn-Kline-Kolmer		Kahn-Kline-Hinton		Kahn-Hinton-Kolmer		Kline-Kolmer-Hinton		Any Combination of Three Techniques	
	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
Negative	93	26.6	64	18.3	8	2.3	2	0.6	167	47.7
Positive	17	4.8	88	25.1	0	0	1	0.3	106	30.3
Doubtful	0	0	0	0	0	0	0	0	0	0.0
Total	110	31.4	152	43.4	8	2.3	3	0.9	273	78.0
Did not coincide	240	68.6	198	56.6	342	97.7	347	99.1	77	22.0
Grand Total	350	100.0	350	100.0	350	100.0	350	100.0	350	100.0

TABLE 6

Test	Negative		Positive		Doubtful	
	Number	Percent	Number	Percent	Number	Percent
Kahn	196	56.0	154	44.0	0	0.0
Kline	184	52.6	164	46.8	2	0.6
Kolmer	222	63.4	73	20.9	55	15.7
Hinton	117	33.4	143	40.9	90	25.7

TABLE 7

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TABLE 8

	Kahn											
	Negative				Positive				Doubtful			
	Kline				Kline				Kline			
	—	†	D	T	—	†	D	T	—	†	D	T
Hinton	3,293	6	2	3,301	2	33	0	35	0	0	0	0
	41	3	0	44	0	73	0	73	0	0	0	0
	52	1	0	53	1	8	0	9	0	0	0	0
	3,386	10	2	3,398	3	114	0	117	0	0	0	0
Kolmer	36	1	0	37	0	7	0	7	0	0	0	0
	8	1	0	9	0	351	0	351	0	0	0	0
	10	0	0	10	0	10	0	10	0	0	0	0
	54	2	0	56	0	368	0	368	0	0	0	0
Total	28	0	0	28	0	2	0	2	0	0	0	0
	1	1	0	2	0	15	0	15	0	0	0	0
	5	0	0	5	0	3	0	3	0	0	0	0
	34	1	0	35	0	20	0	20	0	0	0	0
Total	3,357	7	2	3,366	2	42	0	44	0	0	0	0
	50	5	0	55	0	439	0	439	0	0	0	0
	67	1	0	68	0	21	0	22	0	0	0	0
	3,474	13	2	3,489	3	502	0	505	0	0	0	0

† = Positive
 — = Negative
 D = Doubtful
 T = Total

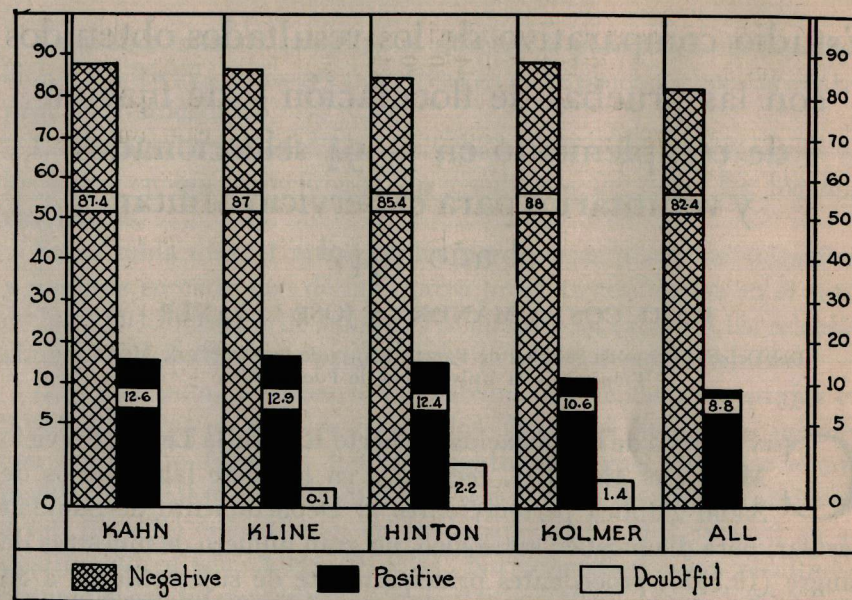


CHART 1

Results in percent of total examinations among 3994 blood specimens tested simultaneously by four standard techniques.

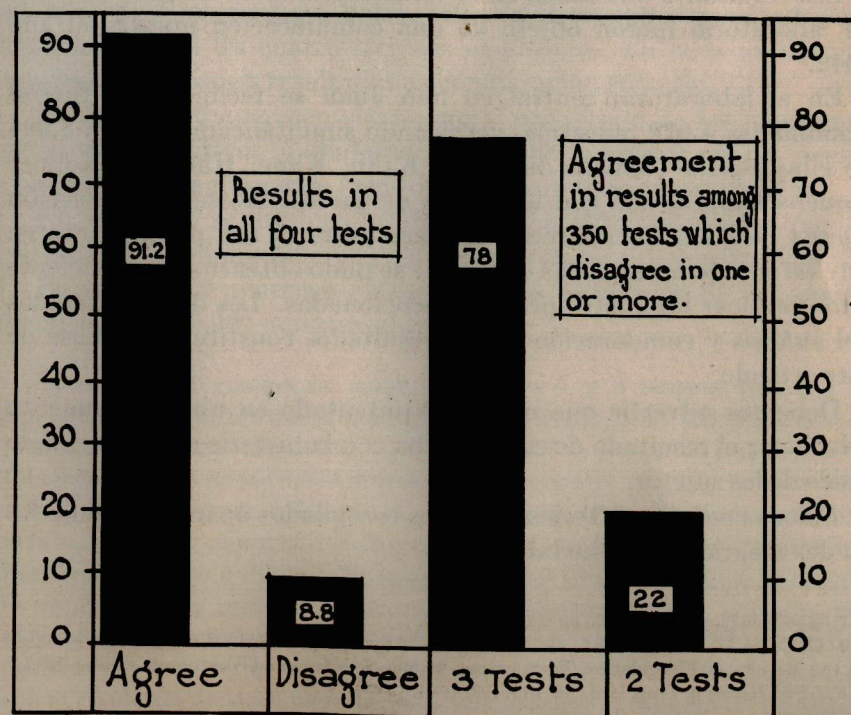


CHART 2