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For Pullorum Disease**

By **H. Van Roekel and M. K. Clarke**

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The whole blood test was introduced several years ago in response to a demand for a method of testing less expensive than the standard tube test. This newer test has not proved entirely satisfactory, and the work here described was undertaken to gain more knowledge of the factors which influence its effectiveness and reliability in eliminating pullorum infection from poultry flocks.

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THE WHOLE BLOOD AGGLUTINATION TEST FOR PULLORUM DISEASE

By H. Van Roekel, Chief of Laboratory, and M. K. Clarke, Laboratory Assistant,
Poultry Disease Control¹

In the control and eradication of pullorum disease among domestic fowl the macroscopic agglutination (tube) test is regarded as a reliable and indispensable means of detecting infected birds. The application of the test along with effective control and eradication measures has led to the establishment of flocks which are free from the disease. This fact has been clearly demonstrated in certain sections which at one time suffered heavy losses from the disease but later enjoyed the benefits derived from pullorum-disease-clean flocks obtained through a supervised and effective testing program.

The testing of flocks for pullorum disease has not received equal encouragement in all sections of this country. This may be attributed to various factors. The cost of the tube method and its inadaptability to the poultry industry in certain sections have been considered to be responsible for the lack of interest and initiative taken in pullorum-disease eradication. Consequently, with a demand for a less expensive method of testing, a field test was introduced by Schaffer, MacDonald, Hall and Bunyea (7) and Coburn and Stafseth (2).

While some investigators and regulatory testing agencies would lead one to believe that this field test is effective in the control and eradication of pullorum disease, others do not accept this view. It is clearly evident, as judged from the investigations reported, that further research is essential to perfect and standardize the technique of the whole blood test in order to increase its efficiency. The purpose of this investigation was to gain a more thorough insight into the factors which influence the effectiveness and reliability of the whole blood test in eliminating pullorum infection from poultry flocks.

HISTORICAL RESUME

Schaffer, MacDonald, Hall and Bunyea (7) report the use of a stained antigen and fresh whole blood in the rapid whole blood test for pullorum disease. The antigen and whole blood are placed and mixed on a glass plate, in the approximate dilution of one part of blood to two or three parts of antigen. A pipette which delivers a drop of antigen, approximately .05 cc., and a wire loop measuring .02 cc. of blood were used. Rotating of the plate was found unnecessary to hasten or bring about agglutination. Reactions which occurred within one minute were regarded as definitely positive, while slower reactions were considered suspicious, and slight reactions appearing after two minutes were disregarded. It was observed that the greater the agglutinating power of the blood, the more rapid the clumping and the larger the clumps. A very fine granulation which is barely visible to the naked eye should be regarded as negative.

Coburn and Stafseth (2) described a whole blood test in which they used an antigen that differs slightly from that employed by Schaffer et al. Equal amounts of antigen and blood measured with standardized pipettes were placed and

¹The authors wish to extend their appreciation to Prof. O. S. Flint for taking the photographs.

mixed on porcelain plates. Readings were made within one to three minutes. Thirty birds were tested with the whole blood and tube methods. The latter method detected 15 negatives, 9 positives, and 6 doubtfuls, while the whole blood test detected 18 negatives and 12 positives.

Welch (10) observed with the whole blood test that in using dry blood smears a great variation in thickness of the smears existed and the amount of blood tested was difficult to determine. The author calls attention to the fact that factors exist which lead to errors. It was observed that birds with low agglutinating titres might not react to the whole blood method. The author concludes that the whole blood test, in spite of its deficiencies, has a place in certain areas when the use of the tube test seems impractical.

Durant (3) in a comparative study of the tube, rapid serum, and whole blood tests found that, among 259 birds tested, 94 were positive to the tube method and 63 were positive to the whole blood test. In a group of 23 birds that were positive to the tube method alone, 18 exhibited lesions of pullorum disease and 13 yielded *S. pullorum*. Among 6 birds that were positive to the whole blood test alone, 4 showed gross lesions and *S. pullorum* was isolated from one. The author suggests that until the rapid test has proved as accurate and dependable as the tube agglutination test, it should not be substituted in campaigns for the eradication of pullorum disease.

Van Roekel, Bullis, Flint, and Clarke (9) report that the tube test was more sensitive in detecting infected birds than the whole blood test. *S. pullorum* was isolated from birds that had not reacted at any time to the whole blood test. Attention is called to factors which exert an influence on the efficiency of the whole blood test. It is emphasized that the whole blood test should be perfected by using a dilution which will be effective and constant in detecting infected birds with low or fluctuating titres.

Barger and Torrey (1) report as follows:—"Using the tube test as an arbitrary standard of perfection in the detection of reactor fowls the whole blood agglutination test has approached an efficiency of nearly 100 percent in the routine testing of farm flocks." Factors such as constant temperature, proper light, suitable equipment to keep out dust, and skill of the operator were regarded as influencing the accuracy of the test. Equal amounts of blood and antigen were placed on a microscopic slide and mixed with a toothpick. The slide was then placed in a specially devised incubator heated by water to maintain a temperature of 25° C. High temperatures were reported to cause rapid evaporation. Frequent rotation of the slides is recommended to hasten agglutination and give a more definite reaction. It is emphasized that partial, flaky, or pseudo-reactions must not be confused with true agglutination.

Graham, Thorp, and Torrey (4) tested 1369 blood specimens with the whole blood and tube methods. The index of agreement of positives and negatives between the standard tube and the rapid whole blood method was 0.848. The interpretations of the 1369 tests were classified as follows:—

<i>Interpretation</i>	<i>Whole Blood</i>	
	<i>Tube Test</i>	<i>Test</i>
Positive.....	486	520
Positive and ropy.....	88	—
Partial.....	20	22
Trace.....	3	1
Negative.....	772	826

It is apparent that the tube test is more sensitive than the whole blood test. However, the authors state that a higher degree of correlation (98 percent) was obtained between the standard tube, rapid serum, and whole blood tests in several flocks subjected to repeated testing. They conclude that these observations indicate that the whole blood test, in properly trained hands, constitutes the simplest and most practical diagnostic procedure yet described for diagnosis of pullorum disease.

Torrey and Graham (8) investigated the problem of doubtful or partial reactors to the whole blood test from the standpoint of pullorum-disease control. It was observed that doubtful reactors were encountered more frequently in heavily infected flocks. In repeated testing of 25 doubtful reactors with the whole blood and tube methods, two types of reactors ("consistent" and "inconsistent") were observed. Among the 25 birds tested, 21 (84 percent) were classified as inconsistent with the whole blood test, and 19 (76 percent) with the tube test. Necropsies of these birds revealed no gross characteristic lesions of the disease. *S. pullorum* was isolated from one bird which was positive to the tube test, except on one test which was doubtful. With the whole blood test, this bird was negative on three tests, doubtful once, and positive twice. These results suggest that the tube test is apparently more sensitive than the whole blood test.

Higgins and Schroeder (5) tested 108 pullets with the tube test (diagnostic dilution not stated) and with five stained antigens for the whole blood test. The results show that the tube test was more sensitive than the whole blood test, yet the authors conclude that very satisfactory agreement in the comparative results was obtained, and that the whole blood test was equal in diagnostic value to the tube agglutination method for detecting infected birds.

An extensive investigation relative to the efficiency of the whole blood test was made by Lubbehusen and Beach (6). Equal amounts of blood and antigen were employed. It was observed that more satisfactory results were obtained by mixing the blood and antigen without subsequent rotating of the plate; incubating the tests for at least six to eight minutes at a constant temperature of 80° C.; and employing adequate light in order to insure accurate interpretation of the tests.

Fourteen groups of birds on five farms were tested, representing a total of 91,449 test birds of which 42,214 were also tested with the tube test. It was emphasized that the efficiency of the whole blood test should not be based on decrease in percentage of reactors, but instead upon comparative results with the tube test and bacteriological findings in birds necropsied. A total of 405 reactors was detected by both methods, of which 217 yielded *S. pullorum* and 188 were negative. The routine whole blood test detected 84 of the 217 infected birds, giving a test efficiency of 38.71 percent. With the whole blood test conducted under laboratory conditions, a test efficiency of 76.5 percent was obtained. The routine tube test (1:25 dilution) detected 216 of the 217 infected birds with a test efficiency of 99.53 percent. The routine tube test failed to detect one infected bird which had given a strong positive reaction with the whole blood test.

The authors state that repeated testing by the whole blood test, although rendering the flock completely negative to that test, failed in each instance to detect all infected fowls or all that would react to the tube test. The whole blood test was more efficient when conducted in the laboratory than when conducted in the field. The number of infected birds in the various flocks was reduced to 1 percent or less by repeated testing with the whole blood method.

It is mentioned that factors peculiar to field conditions apparently are operating which influence the efficiency of the whole blood method. The authors conclude that the efficiency of the whole blood (stained antigen) test will not permit of its exclusive use in a program of pullorum-disease eradication, and that official recognition should be reserved to the tube test results until the whole blood test has been perfected to the point where its efficiency equals that of the tube test.

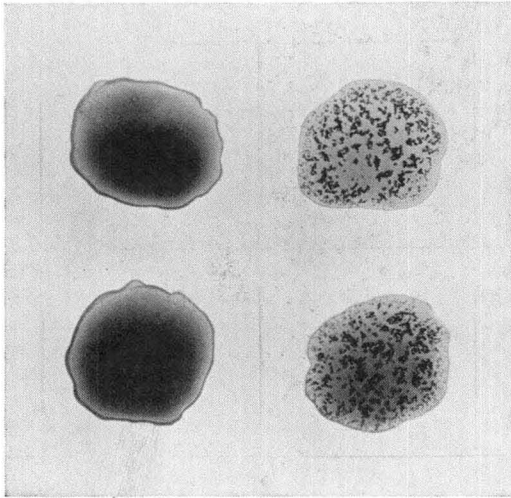


Figure 1. The Whole Blood Agglutination Test.

The mixtures in the squares on the left show a negative reaction; the mixtures on the right show a positive reaction.

TECHNIQUE OF TESTING METHODS USED

Whole Blood Method. The antigen employed was prepared according to the procedure recommended by U. S. B. A. I., U. S. Patent No. 1,816,026, with a slight modification. The organisms were cultivated on nutrient meat extract agar instead of meat infusion agar. The pullorum antigen strains selected by the Antigen Committee of the Pullorum Conference of the Northeastern States were employed. An improvised testing apparatus was used, consisting of a wooden box, insulated with Celotex and lined with metal. The antigen and blood were placed on a glass slide which fitted into a removable frame covered with glass to serve as a protection against dust and to retain the heat. A temperature between 85° and 95° F. was maintained by two (paint coated) electric bulbs, controlled by a thermostat. An electric bulb provided a sufficient and constant supply of light. The antigen was placed on the glass slide with a special pipette, which delivered approximately .045 cc. The blood was measured with a wire loop which conveyed approximately 0.35 to .04 cc. The blood was added to the antigen and mixed with the wire loop. At definite intervals the frame holding the test plate was rotated. The results of the tests were recorded following three minutes' incubation.

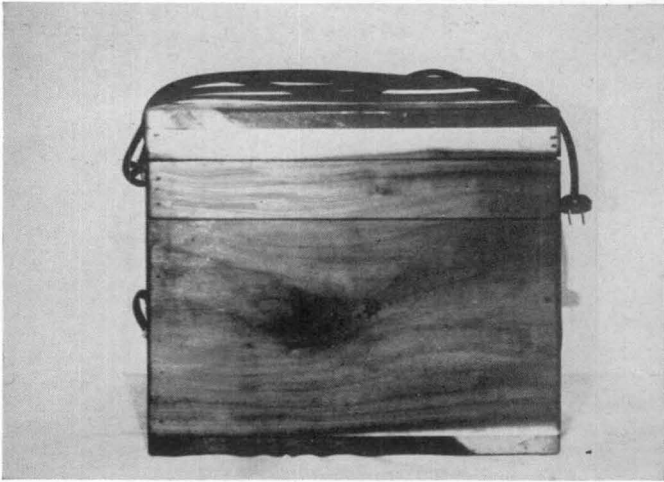


Figure 2. An Electrically Heated and Lighted Apparatus Used for the Whole Blood Agglutination Test.

Size 15 by 15 inches, by 14 inches deep. Construction consists of a wooden frame lined with Celotex and metal.

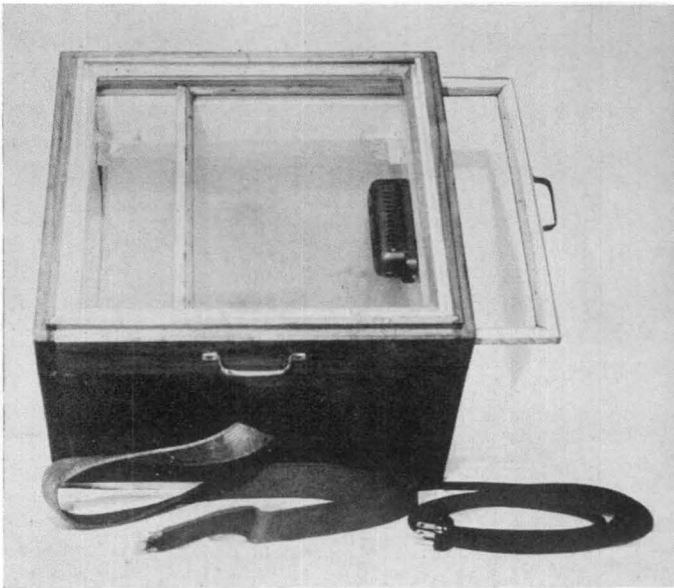


Figure 3. Same Apparatus with Cover Removed.

The test plate (size 12 by 12 inches) slides into a removable frame which is covered with glass.

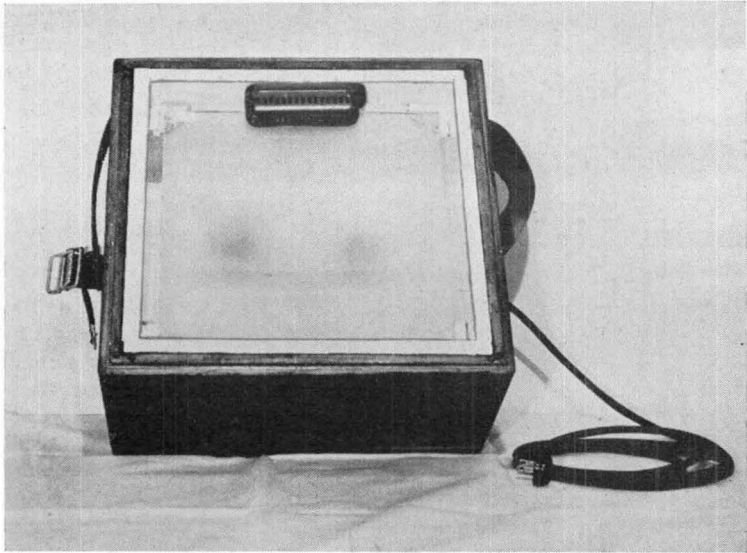


Figure 4. Same Apparatus as in Figure 3 with the Test Plate Holder Removed. The thermostat and frosted glass plate which rests on shoulders are exposed. The frosted glass plate permits uniform heat distribution.

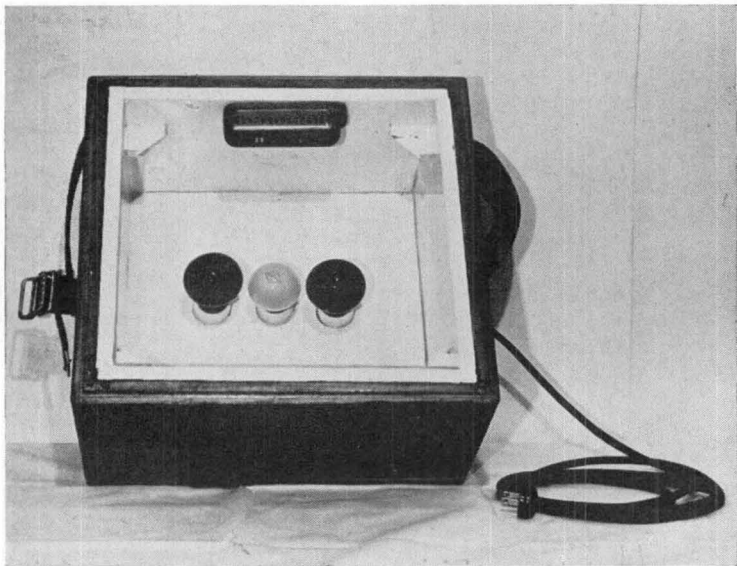


Figure 5. Same Apparatus as in Figure 4 with the Frosted Glass Plate Removed, Exposing the Two Dark Heat Bulbs and the White Light Bulb.

At the outset of the investigation a testing apparatus was employed which was heated with tempered water. The temperature of the test plate was maintained between 80° and 95° F. The apparatus was not equipped with artificial light.

Tube Method. The technique of the tube agglutination method was identical with that described in the Standard Methods (11) of Diagnosis for Pullorum Disease. A diagnostic dilution of 1:25 and an incubation period of 24 hours at 37° C. were employed.

MISCELLANEOUS EXPERIMENTS

Since standardization of the tube test has brought forth more uniform and reliable results in pullorum-disease testing, it is only logical to assume that the same would hold true if the technique of the whole blood test were perfected and standardized. It is apparent that there is a great lack of uniformity in the manner in which the whole blood test is conducted at the present time. Investigations have revealed several factors that appear to play an important role in the accuracy of the test. The need for further investigation of these influencing factors is apparent, since various investigators disagree on certain phases of the whole blood test. Increased efficiency of this method cannot be expected until each problem has been analyzed and solved. This naturally requires a scientific approach in order to determine the true significance of the different factors influencing the accuracy of the test.

In the development and standardization of the tube test, the serum-antigen dilution factor was found to play an important role in the successful elimination of infected birds. At first the fact was not appreciated that some infected birds may exhibit low titres or intermittent reactions. Such birds would be overlooked with a serum-antigen dilution above the serum agglutination titre. It follows that with the use of the whole blood test the blood-antigen dilution should receive further study. Measurements of blood and antigen have been lacking in uniformity and constancy in the methods described.

In a few miscellaneous experiments an effort was made to determine what variation in the quantities of blood and antigen might exist in the whole blood test and its influence on the agglutination reaction.

Antigen Measurements. A special pipette, simulating a medicine dropper, was used for measuring the antigen by drops. Realizing that the size of the drops might vary, a series of measurements was made to determine the amount of antigen and its variation in the drops. Table 1 shows that in a total of 100 drops the quantity of antigen varied from .042 to .055 cc. The majority of the drops ranged from .044 to .047 cc., and the average quantity for the 100 drops was .04709 cc. While these measurements were made in the laboratory under most favorable conditions, it is likely that the variation might be even greater under field conditions, when several operations are carried on simultaneously and at greater speed.

TABLE 1.--VARIATION IN MEASUREMENTS OF ANTIGEN.

<i>Quantities of Antigen</i> (cc. per drop)	<i>Number of Drops</i>
.042	2
.043	4
.044	17
.045	19
.046	10
.047	16
.048	3
.049	6
.05	4
.051	6
.052	4
.053	2
.054	5
.055	2
	<hr/>
	100

Whole Blood Measurements. The whole blood was measured with a wire loop having a 6 mm. diameter. With the wire loop the blood was collected from the wing vein puncture and measured with a graduated pipette. It was impossible to obtain uniform loopfuls of blood from each bird, especially when the blood did not escape freely or did not accumulate to such an extent that the loop would fill and withdraw the proper amount of blood. Table 2 shows that greater variation was found in 100 blood measurements than in the antigen measurements. While the majority of the measurements ranged from .03 to .045 cc., the minimum and maximum amounts were .022 and .05 cc., respectively, a range of .028 cc. The average for the 100 measurements was .03541 cc. It is reasonable to suspect that such a variation would influence the degree of agglutination reaction, especially in low-titre birds.

TABLE 2.--VARIATION IN MEASUREMENTS OF FRESH WHOLE BLOOD.

<i>Quantities of Whole Blood</i> (cc. per wire loop)	<i>Number of Loopfuls</i>
.02 — .024	2
.025 — .029	7
.03 — .034	28
.035 — .039	31
.04 — .044	30
.045 — .05	2
	<hr/>
	100

Influence of Blood-Antigen Dilution on Degree of Agglutination. In this experiment a number of positive-reacting birds were employed. The blood samples were collected into tubes containing 10 percent sodium citrate solution in amounts (1 part sodium citrate and 14 parts blood) to prohibit coagulation. The drop method was used for measuring the antigen, while a graduated pipette was employed to measure the citrated whole blood. The amounts of blood tested were

.04, .02, .01, .005, and .001 cc. The tests were made at a temperature of 78° to 80° F., and readings were recorded after a 3-minute incubation period. Table 3 shows the results of testing 10 samples with the whole blood test. The results with the tube test represent the serum titre of the birds four days prior to the time the samples were collected for the whole blood test. Six of the ten samples were tested twice. On second test the reaction did not appear as strong as the first. It may be seen that the degree of reaction with the whole blood test correlates in a measure with the degree of reaction and titre observed with the tube method. While a reaction was obtained in all samples tested, it was extremely weak in two cases and might have been overlooked under field conditions. It is of interest to note that an amount of blood as small as .001 cc. is able to produce a reaction.

TABLE 3.--INFLUENCE OF BLOOD-ANTIGEN DILUTION
ON DEGREE OF AGGLUTINATION.

Bird No.	Trial	Whole Blood Test					Serum Agglutination Titre (Tube Method)									
		.04	.02	.01	.005	.001	10	20	40	80	160	320	640	1280	2560	
92002	1st	2	1	0	0	—										
	2nd	1	0	0	0	—	3	2	1	0						
53747	1st	4	4	4	4	1										
	2nd	4	3	4	3	1	4	4	4	4	3	2	1	1	0	
91290	1st	4	2	1	0	—										
	2nd	3	1	1	0	—	3	2	1	0						
91932	1st	3	1	1	0	—										
	2nd	3	1	0	0	—	4	3	2	1	0					
90993	1st	4	4	3	2	1										
	2nd	4	4	3	1	0	4	4	3	2	1	0				
91920	1st	2	1	1	0	—										
	2nd	3	1	0	—	—	4	3	2	1	0					
91040	1st	1	1	0	0	—	3	2	1	0						
91822	1st	4	4	4	4	2	4	4	4	4	3	2	1	0		
91963	1st	4	3	2	1	0	4	3	2	1	0					
51007	1st	3	1	0	—	—	4	3	2	1	0					

Legend

Tube Method: 4—complete agglutination
3—incomplete agglutination
2—partial agglutination
1—slight agglutination
0—no agglutination

Whole Blood: 4—numerous and large clumps developing rapidly
3—numerous and medium clumps developing quite rapidly
2—fine clumps and less numerous developing rapidly or slowly
1—very fine clumps and few in number
0—no agglutination

In a second trial, five positive birds were tested with both the whole blood and the tube methods, as shown in Table 4. Each sample was tested twice with the whole blood test with practically the same results. The citrated as well as the uncitrated blood serum was tested with the tube method, with no significant differences in titre. Three birds in this group (91040, 91963, and 90993) were

TABLE 4.--INFLUENCE OF BLOOD-ANTIGEN DILUTION ON DEGREE OF AGGLUTINATION.*

Bird No.	Trial	Whole Blood Test					Serum Agglutination Titre (Tube Method)								
		.04	.02	.01	Blood (cc.) .005	.001	10	20	40	80	160	320	640	1280	2560
91040	1st	1	1	0	—	—									
	2nd	1	1	0	—	—	4	3	2	1	0				
91963	1st	4	4	2	1	0									
	2nd	4	4	2	1	0	4	4	4	4	3	1	0		
90993	1st	4	4	3	2	0									
	2nd	4	4	3	1	0	4	4	4	4	4	2	1	0	
90969	1st	1	0	0	—	—									
	2nd	1	0	0	—	—	4	3	2	1	0				
91941	1st	2	0	0	—	—									
	2nd	1	0	0	—	—	4	4	2	0					

*Refer to legend of Table 3.

tested two weeks previously, and the results are listed in Table 3. It may be seen that the reactions obtained at both trials agree very closely.

EXPERIMENTAL TESTING IN COMMERCIAL FLOCKS

In determining the true value of the whole blood test as a diagnostic means of detecting "carriers" of pullorum infection, it was deemed expedient to select infected flocks of a desirable size which could be tested with the whole blood test at frequent intervals until negative. After no more reactors could be detected with the whole blood test, the flock was to be check-tested with the standard tube method. All birds that showed reactions of interest from a diagnostic point of view were either retained in quarantine on the premises or taken to the laboratory for further observation.

The scope of this investigation also included a determination of the comparative efficiency of the whole blood and standard tube methods, by using both methods simultaneously in eliminating pullorum-disease "carriers" from an infected flock through repeated testing. Likewise in this phase of the investigation, birds which gave reactions of diagnostic interest were subjected to further study.

Results

Two known infected flocks (A and B) were selected and tested with the whole blood test to determine its efficiency as a diagnostic means of detecting pullorum-disease-reacting birds. The original plan was to test the flocks at approximately monthly intervals until no reactors appeared and then to check test them with the standard tube test. Unfortunately this plan could not be followed, due to the fact that reactors continued to appear on retest, which threatened to delay the hatching season; hence the flock owners requested that the whole blood test be supplemented with the standard tube test in order to hasten eradication of the infected birds in the flock. At the completion of the test all reactors were check-

tested with the whole blood test, and blood samples were collected for the standard tube test.

As mentioned previously birds which gave agglutination reactions but could not be classified as definite positive reactors, were either retained in quarantine on the premises or submitted to the laboratory for further study. All other reactors were eliminated immediately for slaughter. The pens were cleaned and disinfected after each test if conditions permitted.

Flock A. This flock consisted of 1187 birds which were tested in October, 1934. The testing apparatus heated by tempered water was used in this test. Fourteen reactors were detected on the first test. The testing results of both methods and the disposal of the reactors are presented in Table 5.

TABLE 5.--TESTING RESULTS WITH REACTORS IN FLOCK A.

Bird No.	Reaction with W. B. T.	Routine Tube Test 1:25	Reaction with Tube Method Dilution							Disposal
			10	20	40	80	160	320	640	
93171	1	2	3	2	1	1	0	0	0	} Quarantine
93177	1	0	2	1	1	0	0	0	0	
93247	1	1	2	2	1	1	0	0	0	
93293	1	0	1	0	0	0	0	0	0	
93372	1	1	Unsatisfactory							} Laboratory
93394	1	1	3	2	1	1	0	0	0	
93527	1	4	4	4	4	3	0	0	0	
93550	1	4	4	4	2	0	0	0	0	
93523	2	4	4	4	4	3	0	0	0	
93507	3	4	4	4	3	1	0	0	0	} Slaughter
93522	3	4	4	4	4	4	1	0	0	
93529	4	4	4	4	4	4	4	1	0	
93539	4	4	4	4	4	4	4	1	0	
93547	4	4	4	4	4	4	4	2	0	

The blood of three birds produced delayed clumping or agglutination which was impossible to distinguish from a true partial reaction. On the strength of the tardiness of the reaction the birds were not removed from the flock.

The second test was conducted in November 1934, and revealed two strong reactors among 1157 birds. Of the four doubtful reactors detected on the first test and held in quarantine, three gave slight reactions on the second test and one was negative. These birds were sold before the next test was conducted.

In view of the fact that the infection apparently was limited to one pen of birds, only this group of birds was tested on December 13, 1934. The testing apparatus electrically heated and lighted was used in this and the subsequent test. Among the 37 birds one strong reactor was detected.

In January the entire flock (1067 birds) was tested with both the whole blood and tube methods in order to expedite eradication. Two birds reacted with both methods and seven reacted suspiciously only with the tube test. One of the two

reactors (93546) gave a partial reaction with the whole blood test; but in two check tests with this method, slightly suspicious and negative reactions, respectively, were obtained. With the tube test the serum of this bird gave a "4" reaction in the 1:25 dilution and on dilution test revealed a titre of 1:320. This bird was submitted to the laboratory, while the seven reactors to the tube test were placed in quarantine. At the completion of this test the owner was advised to dispose of the small group of birds which had been maintained in quarantine and continued to reveal positive reactors.

In February the entire flock was retested with the tube test and no reactors were detected. This group of birds was not further tested since it had passed two consecutive negative tests with the tube method. Of the seven suspicious birds detected on the previous test two had been sold and the remaining five were negative.

TABLE 6.--SUMMARY OF TESTS--FLOCK A.

<i>Date</i>	<i>Testing Method</i>	<i>Number of Birds</i>	<i>Number of Reactors</i>
October	Whole Blood	1187	14
November	Whole Blood	1157	2
December	Whole Blood	37	1
January	Whole Blood and Tube	1067	9
February	Tube	1000	0
Total		4448	26

Six of the 26 reactors were submitted to the laboratory for further study. The observations for each bird are presented in Table 7.

In summarizing the testing results for Flock A, it may be stated that the whole blood test detected the majority of infected birds on the first test, but positive reactors continued to appear by the use of this method for four consecutive tests. Whether the whole blood test would have been capable of eliminating all infected birds from the flock by further testing the unit of birds in which reactors continued to appear, remains a question. To what extent the persistence of infection was influenced by the degree of sensitivity of the whole blood test is impossible to determine from the results. Delayed weak reactions, as well as doubtful reactions occurring within the reading time were observed. As may be seen from the agglutination and necropsy results, the reaction with the whole blood test might be very weak or even negative, whereas with the tube test a complete reaction was exhibited in the 1:25 dilution. Bacteriological examination of such reactors would yield *S. pullorum* in some instances. Weak or doubtful reactions appeared identical for birds that proved to be either negative or positive on bacteriological examination. Birds exhibiting a low titre with the tube test generally gave a negative, weak or doubtful reaction with the whole blood test. As the serum titre fluctuated within a definite range, the reaction of the latter method might also fluctuate. Birds with high titres usually produced rapid and distinct reactions with the whole blood method, although in one instance bird 93546 reacted differently but its serum titre was as great as that of some birds which gave consistent strong reactions. The failure to isolate *S. pullorum* from this bird may be attributed to the examination or to the fact that the infection was of recent origin, and no definite gross foci had been established.

TABLE 7.--FURTHER OBSERVATIONS ON SIX REACTORS IN FLOCK A.

Bird No.	Date Detected	Routine Tube Test 1:25	Date of Test	Tube Test						Whole Blood Test	Date of Necropsy	S. pullorum Isolated	
				10	20	40	Dilution 80	160	320				640
93372	10/8/34	1	10/8/34	U	U	U	1	1	0	0	1	10/30/34	Negative
			10/17/34	0	0	0	0	0	0	0	1		
			10/23/34	0	0	0	0	0	0	0	0		
			10/27/34	NT							0		
			10/30/34	0	0	0	0	0	0	0			
93394	10/8/34	1	10/8/34	3	2	1	1	0	0	0	1	10/30/34	Negative
			10/17/34	1	0	0	0	0	0	0	0		
			10/23/34	1	0	0	0	0	0	0	0		
			10/27/34	NT							0		
			10/30/34	2	1	1	0	0	0	0			
93523	10/9/34	4	10/9/34	4	4	4	3	0	0	0	2	10/30/34	Positive
			10/17/34	4	4	3	1	0	0	0	2		
			10/23/34	4	4	4	1	0	0	0	3		
			10/27/34	NT							0		
			10/30/34	4	4	2	1	0	0	1			
93527	10/9/34	4	10/9/34	4	4	4	3	0	0	0	1	10/30/34	Positive
			10/17/34	4	4	3	2	0	0	0	2		
			10/23/34	4	4	4	1	0	0	0	1		
			10/27/34	NT							1		
			10/30/34	4	4	2	1	0	0	2			
93550	10/9/34	4	10/9/34	4	4	2	0	0	0	0	1	12/21/34	Positive
			10/17/34	3	2	1	0	0	0	0	1		
			10/23/34	3	2	1	0	0	0	0	2		
			10/27/34	4	4	4	2	0	0	0	2		
			10/30/34	4	4	4	1	0	0	0	3		
			11/7/34	4	4	3	1	0	0	0	4		
			11/10/34	4	3	1	0	0	0	0	4		
			11/15/34	4	4	3	2	0	0	0	4		
			11/20/34	4	4	3	1	0	0	0	4		
			11/30/34	4	4	4	4	3	0	0	1		
12/13/34	4	4	4	4	3	2	0	4					
12/19/34	4	4	4	4	3	1	0	4					
12/21/34	4	4	4	4	3	2	0	NT					
93546	1/4/35	4	1/4/35	4	4	4	3	1	1	0	3	1/9/35	Negative
			1/9/35	4	4	4	3	1	1	0	2		

U—unsatisfactory test
NT—not tested

Flock B. On October 2 and 3, 1934, 826 birds were tested with the whole blood test, using the apparatus equipped with the water heater. The testing operations were conducted in the same manner as described for Flock A. The first test represents only a partial flock test since not all the birds had been taken in from range. Among the 826 birds tested, 29 reactors were detected. The blood of one bird (92237) exhibited a delayed reaction which was observed only after the bird had been returned to the flock. This bird was not regarded as a reactor and will be mentioned again in the discussion of subsequent tests. Of the 29 reactors, six were submitted to the laboratory for further study and the remainder were slaughtered.

On October 31 and November 2, 1934, 1120 birds (349 first test and 771 second test) were tested by the whole blood method. Eleven reactors were detected among the 349 first-test birds. Again one bird (99017) gave a delayed reaction but was not removed from the flock until a later test. Of the eleven reactors, five were submitted to the laboratory and the remainder were slaughtered. Among the 771 birds tested for the second time, two reactors were detected and removed for slaughter.

On December 10 and 11, 1934, 976 birds were tested and revealed ten reactors. The electrically equipped testing apparatus was used on this and subsequent tests. The blood of two birds (99100 and 92237) gave delayed reactions. One, 92237, reacted in a similar manner on the first test, but did not react on the second test. Three reactors (99255, 99017 and 99087) were submitted to the laboratory. Bird number 99017 gave a delayed reaction with the whole blood method on the second test. A small group of birds, approximately 80 in number, which had passed two negative tests and were confined in a separate building were not tested at this time.

On January 14 and 15, 1935, the entire flock, consisting of 989 birds, was tested and revealed four reactors (99052, 99130, 99239 and 99302). Four birds (99039, 99097, 99100 and 92297) gave delayed reactions. Bird 99100 reacted in a similar manner on the previous test, but was permitted to remain in the flock on the strength of the type of reaction. One (99130) of the four reactors was submitted to the laboratory.

Since reactors continued to appear in two pens and the owner was desirous of eliminating the infection in the shortest possible time, the birds in these two pens were retested at two-week intervals and the whole blood test was supplemented with the standard tube test.

On January 28, 1935, a total of 292 birds was tested. Nine birds were detected as reactors: two (99100 and 99186) reacting to both methods, one (99272) to the whole blood test, and six (91923, 99039, 99077, 99153, 99181 and 99250) to the tube test. Seven of the nine reactors were submitted to the laboratory. Of the other two reactors, one (99077) died shortly after the test and the other (91923) remained on the premises but gave a negative reaction to both methods on the next test.

On February 11, 1935, the entire flock was tested with both methods and no reactors were detected. Nine delayed reactions were observed with the whole blood test in one pen of birds which had never revealed reactors. On subsequent test these delayed reacting birds were negative.

On February 25, 1935, no reactors were detected among birds tested on January 28. Approximately two weeks later (March 11 and 12) the entire flock was tested with negative results. No further testing was conducted, since the entire flock had passed two consecutive negative tests at least one month apart.

TABLE 8.--SUMMARY OF TESTS--FLOCK B.

<i>Date of Test</i>	<i>Number of Birds</i>	<i>Number of Reactors</i>
October 2 and 3, 1934	826	29
October 31 and November 2	1120	13
December 10 and 11	976	10
January 14 and 15, 1935	989	4
January 28	292	9
February 11 and 12	851	0
February 25	204	0
March 11	795	0
Totals	6053	65

{ 1 W. B. T.
2 both tests
6 tube test

The agglutination studies and necropsy results on the 22 reactors submitted to the laboratory are shown in Table 9.

TABLE 9.--FURTHER OBSERVATIONS ON TWENTY-TWO REACTORS IN FLOCK B.

Bird No.	Date Detected	Routine Tube Test 1-25	Date of Test	Tube Test								Whole Blood Test	Date of Necropsy	S. pullorum Isolated
				10	20	40	80	160	320	640	1280			
92043	10/2/34	3	10/2/34	4	4	3	1	0	0	0	0	3	10/22/34	Positive
			10/13/34	4	4	4	2	1	0	0	0	3		
			10/17/34	4	4	3	2	0	0	0	0	3		
			10/22/34	4	3	3	2	0	0	0	0	NT		
92100	10/2/34	4	10/2/34	4	3	2	0	0	0	0	4	10/22/34	Positive	
			10/13/34	4	4	4	4	3	1	0	0			4
			10/17/34	4	4	4	4	3	1	0	0			4
			10/22/34	4	4	4	3	2	1	0	0			4
92308	10/2/34	4	10/2/34	4	4	3	1	0	0	0	2	10/22/34	Positive	
			10/13/34	4	4	4	4	3	2	1	0			4
			10/17/34	4	4	4	4	3	1	0	0			4
			10/22/34	4	4	4	3	2	1	1	0			4
92364	10/2/34	4	10/2/34	3	2	1	0	0	0	0	4	10/22/34	Positive	
			10/13/34	4	4	4	4	3	1	0	0			4
			10/17/34	4	4	4	4	3	1	0	0			4
			10/22/34	4	4	4	3	2	0	0	0			4
92368	10/2/34	4	10/2/34	4	4	3	1	0	0	0	0	1	12/26/34	Positive
			10/13/34	4	4	3	1	0	0	0	0	2		
			10/17/34	4	4	2	1	0	0	0	0	2		
			10/23/34	3	2	1	1	0	0	0	0	1		
			10/27/34	3	2	1	0	0	0	0	0	0		
			10/30/34	4	3	2	1	0	0	0	0	0		
			11/7/34	4	3	3	2	1	0	0	0	4		
			11/10/34	4	3	2	1	0	0	0	0	4		
			11/15/34	4	4	3	2	1	0	0	0	4		
			11/20/34	4	4	4	4	2	0	0	0	3		
			11/30/34	4	4	3	2	1	0	0	0	2		
			12/13/34	4	4	4	2	0	0	0	0	2		
			12/19/34	4	4	4	3	2	0	0	0	4		
12/26/34	4	4	4	4	2	0	0	0	4					
92517	10/2/34	2	10/2/34	4	4	2	0	0	0	0	0	1	12/26/34	Positive
			10/13/34	4	4	2	1	0	0	0	0	2		
			10/17/34	4	4	4	1	0	0	0	0	1		
			10/23/34	4	3	2	1	0	0	0	0	3		
			10/27/34	4	4	4	2	0	0	0	0	2		
			10/30/34	4	4	1	1	0	0	0	0	0		
			11/7/34	4	3	2	0	0	0	0	0	1		
			11/10/34	3	2	1	0	0	0	0	0	0		
			11/15/34	4	3	2	1	0	0	0	0	2		
			11/20/34	4	3	2	0	0	0	0	0	1		
			11/30/34	4	4	1	0	0	0	0	0	1		
			12/13/34	4	4	3	0	0	0	0	0	1		
12/19/34	4	4	4	2	0	0	0	0	2					
12/26/34	4	4	4	3	0	0	0	0	2					
99017	12/11/34	NT	12/11/34	4	4	4	2	1	0	0	0	1	12/31/34	Positive
			12/19/34	4	4	4	4	3	1	0	0	4		
			12/26/34	4	4	4	4	3	1	0	0	2		
			12/31/34	4	4	4	3	2	0	0	0	2		
99039	1/28/35	2	1/28/35	NT							0	3/2/35	Positive	
			2/5/35	4	4	4	1	0	0	0	0			0
			2/15/35	4	4	4	4	3	0	0	0			1
			2/20/35	4	4	4	3	1	0	0	0			1
			2/28/35	4	4	4	1	0	0	0	0			1
99072	10/31/34	4	10/31/34	4	4	4	3	1	0	0	0	3	12/27/34	Positive
			11/10/34	4	4	4	2	2	1	1	0	4		
			11/15/34	4	4	4	4	3	3	2	1	4		
			11/20/34	4	4	4	4	4	2	1	0	4		
			11/30/34	4	4	4	4	4	2	1	0	4		
			12/13/34	4	4	4	4	3	2	1	0	4		
			12/19/34	4	4	4	4	4	3	2	0	4		
12/26/34	4	4	4	4	4	4	2	0	3					
99087	12/11/34	NT	12/11/34	4	3	3	1	0	0	0	0	2	12/31/34	Positive
			12/19/34	4	4	4	2	0	0	0	0	3		
			12/26/34	4	4	4	4	3	0	0	0	4		
			12/31/34	4	4	4	3	1	0	0	0	4		
99100	1/28/35	4	1/28/35	NT							3	3/2/35	Positive	
			2/5/35	4	4	4	4	3	1	0	0			4
			2/15/35	4	4	4	3	2	1	0	0			2
			2/20/35	4	4	4	3	2	0	0	0			2
			2/28/35	4	4	4	2	1	0	0	0			2

TABLE 9.--CONTINUED.

Bird No.	Date Detected	Routine Tube Test 1:25	Date of Test	Tube Test								Whole Blood Test	Date of Necropsy	S. pullorum Isolated	
				Dilution											
				10	20	40	80	160	320	640	1280				
99107	10/31/34	4	10/21/34	4	4	3	2	0	0	0	0	4	2/5/35	Positive	
			11/10/34	4	2	1	0	0	0	0	0	0			4
			11/15/34	4	3	2	1	0	0	0	0	0			3
			11/20/34	3	3	2	1	1	0	0	0	0			2
			11/30/34	4	4	4	3	3	0	0	0	0			4
			12/13/34	4	4	4	4	4	3	3	1	4			4
			12/19/34	4	4	4	4	3	3	3	2	4			4
			12/26/34	4	4	4	4	4	4	3	2	4			4
			12/31/34	4	4	4	4	3	3	3	3	4			4
			1/8/35	4	4	4	4	3	3	3	3	4			4
			1/16/35	4	4	4	4	4	4	4	4	4			4
			1/23/35	4	4	4	4	4	4	4	4	4			4
			1/31/35	4	4	4	3	3	3	2	2	4			4
2/5/35	4	4	4	4	4	4	3	2	4	4					
99130	1/15/35	NT	1/15/35	NT								2	2/5/35	Positive	
			1/16/35	4	4	3	2	0	0	0	0	2			
			1/23/35	4	4	4	2	0	0	0	0	3			
			1/31/35	4	4	4	3	1	0	0	0	4			
			2/5/35	4	4	3	1	0	0	0	0	3			
99153	1/28/35	1	1/28/35	NT								6	3/2/35	Negative	
			2/5/35	1	1	0	0	0	0	0	0	0			
			2/15/35	0	0	0	0	0	0	0	0	0			
			2/20/35	0	0	0	0	0	0	0	0	0			
2/28/35	0	0	0	0	0	0	0	0	0						
99181	1/28/35	4	1/28/35	NT								0	3/1/35	Positive	
			2/5/35	4	4	4	1	0	0	0	0	0			
			2/15/35	4	4	3	2	0	0	0	0	1			
			2/20/35	4	4	4	2	0	0	0	0	2			
2/28/35	4	4	4	4	2	0	0	0	0	2					
99186	1/28/35	4	1/28/35	NT								2	2/5/35	Positive	
			1/31/35	4	4	4	3	1	0	0	0	2			
			2/5/35	4	4	4	4	2	0	0	0	4			
99188	10/31/34	4	10/31/34	4	4	4	3	1	0	0	0	3	12/13/34	Positive	
			11/10/34	4	4	3	2	1	0	0	0	3			
			11/15/34	4	4	4	3	1	0	0	0	4			
			11/20/34	4	4	4	3	2	0	0	0	3			
			11/30/34	4	4	4	4	1	0	0	0	4			
			12/13/34	4	4	4	3	1	0	0	0	3			
99198	10/31/34	4	10/31/34	4	4	4	3	1	0	0	0	2	2/5/35	Positive	
			11/10/34	4	3	2	1	0	0	0	0	2			
			11/15/34	4	3	2	1	0	0	0	0	2			
			11/20/34	4	4	4	1	0	0	0	0	0			
			11/30/34	4	4	4	2	0	0	0	0	0			
			12/13/34	4	4	4	3	1	0	0	0	2			
			12/19/34	4	4	4	3	2	0	0	0	4			
			12/26/34	4	4	4	4	3	1	0	0	3			
			12/31/34	4	4	4	4	3	1	1	0	3			
			1/8/35	4	4	4	4	4	3	1	0	4			
			1/16/35	4	4	4	4	4	3	2	1	4			
			1/23/35	4	4	4	4	4	4	4	4	4			
			1/31/35	4	4	4	4	4	3	3	2	4			
2/5/35	4	4	4	4	4	3	2	1	4						
99226	10/31/34	4	10/31/34	4	4	4	3	1	0	0	0	1	12/13/34	Positive	
			11/10/34	4	3	2	1	0	0	0	0	2			
			11/15/34	4	4	3	2	1	0	0	0	2			
			11/20/34	4	4	4	4	3	0	0	0	4			
			11/30/34	4	4	4	4	4	2	1	0	4			
			12/13/34	4	4	4	4	4	3	2	2	4			
99250	1/28/35	1	1/28/35	NT								0	3/1/35	Negative	
			2/5/35	4	2	1	0	0	0	0	0	0			
			2/15/35	3	2	1	0	0	0	0	0	0			
			2/20/35	3	1	0	0	0	0	0	0	0			
			2/28/35	4	3	1	0	0	0	0	0	0			
99255	12/10/34	NT	12/10/34	4	4	3	1	0	0	0	0	3	12/31/34	Positive	
			12/19/34	4	4	3	1	0	0	0	0	3			
			12/26/34	4	4	2	0	0	0	0	0	0			
			12/31/34	4	3	1	0	0	0	0	0	NT			
99272	1/28/35	0	1/28/35	0	0	0	0	0	0	0	0	2	2/22/35	Negative	
			1/31/35	0	0	0	0	0	0	0	0	0			
			2/5/35	0	0	0	0	0	0	0	0	0			
			2/15/35	0	0	0	0	0	0	0	0	0			
			2/20/35	0	0	0	0	0	0	0	0	0			

NT—not tested.

The results show that no reactors appeared after all the birds had been subjected to four tests with the whole blood method, although the birds tested on January 28 (fourth test) were also tested with the standard tube method which detected six additional reactors, two of which proved to be infected. The results obtained in Flock B are of very great interest in that delayed reacting birds are of significance in eradicating the disease from a flock. Some birds exhibited delayed reactions consistently or intermittently on subsequent tests, whereas others became either distinctly negative or positive. Delayed reactions were exhibited by 15 birds during the testing of this flock: two (99017 and 99100) were later detected as positive reactors, whereas the remaining 13 became negative to the whole blood test. Of this latter group one (99039) was later detected with the tube test and found infected on necropsy. (*S. pullorum* was isolated from pericardial fluid, liver, spleen, peritoneum, ovary, and abdominal cyst). Bird 99181 was also detected on the fourth test with the tube method but did not react with the whole blood test. This bird gave a doubtful reaction with the whole blood method at a later date and at necropsy *S. pullorum* was isolated from the pericardial fluid, liver, spleen, peritoneum and ovary. Among the 22 reactors examined at the laboratory, *S. pullorum* was recovered from 19. Bacteriological examination of two of the delayed reacting birds revealed that *S. pullorum* was isolated from the ovary and abdominal cyst in one (99017), and from the pericardium, liver, peritoneum, ovary, and abdominal cyst in the other (99100). A great fluctuation in the degree of reaction with the whole blood test was observed in some birds that were tested at short intervals. Infrequently this fluctuation could be correlated with the variation in serum titre. One bird (99272), reacting to the whole blood test only when submitted to the laboratory, did not react to either method on subsequent tests, and *S. pullorum* was not isolated. Two birds reacting only to the tube test did not reveal the organism on bacteriological examination.

Flock C. This flock had not revealed reactors for two consecutive years. However, on initial test with the standard tube method 17 reactors were detected among 445 birds tested. This flock appeared satisfactory for the purpose of comparative testing with the whole blood and standard tube methods. Upon recognition of infection in the flock, the owner was requested not to dispose of reactors until after the first comparative test.

On October 24 and 25, 1934, the entire flock was tested with the whole blood test and standard tube method, using the testing apparatus with the improvised water heating system. A total of 1053 birds was tested and revealed 40 reactors—19 with the tube method, 2 with the whole blood test, and 19 with both methods. The reactors were treated in the following manner:—

	<i>Slaughter</i>	<i>Quarantine</i>	<i>Laboratory</i>	Total
Birds reacting to tube test only . . .	0	16	3	19
Birds reacting to W. B. T. only . . .	0	0	2	2
Birds reacting to both methods . . .	10	4	5	19
Total	10	20	10	40

On November 28, 1934, a total of 732 birds was tested with both methods and only three birds gave a doubtful reaction with the tube method. These birds (95825, 95829 and 96027) were submitted to the laboratory for further study. A part of the flock, representing old hens which had been non-reacting for two seasons, was not tested. Among the 20 reacting birds held in quarantine, 18 survived the second test and revealed three reactors (one with both methods

and two with the tube test). Unfortunately the owner failed to send these birds to the laboratory.

The third test was conducted on January 7, 1935, when the entire flock (894 birds) was tested with the tube test only and no reactors were detected at this time. No further testing was conducted in this flock.

TABLE 10--SUMMARY OF TESTS--FLOCK C.

<i>Date of Test</i>	<i>Number of Birds</i>	<i>Number of Reactors</i>
October 24 and 25, 1934.....	1053	40 { 19 tube test 2 W. B. T. 19 both tests
November 28.....	732	3 tube test
	18 (reactors in quarantine)	3 { 2 tube test 1 both tests
January 7, 1935.....	894	0
Total.....	2697	46

The results of the agglutination tests and necropsies of the 13 reacting birds submitted to the laboratory are shown in Table 11.

S. pullorum was isolated from 5 of the 13 reactors submitted to the laboratory. Four birds (92817, 92842, 95928, and 95842) gave delayed weak reactions with the whole blood test. Birds 92817 and 92842 did not react with the tube method on initial test, while the sera of birds 95928 and 95842 gave a strong reaction in the 1:25 dilution. *S. pullorum* was isolated from the latter two birds. Bird 96027 gave a partial reaction with the tube method but failed to react with the whole blood test, and on necropsy *S. pullorum* was recovered from the pericardium, liver, spleen, peritoneum, ovary and abdominal cyst. The serum of bird 95824 gave complete agglutination on initial test with the tube method, but subsequent tests were either negative or slightly suspicious. This bird did not react to the whole blood test and *S. pullorum* was not isolated. The observations concerning bird 95842 are of interest in that its titre gradually decreased below the 1:10 dilution and on necropsy *S. pullorum* was recovered from pericardial exudate. It is quite possible that complete recovery from infection might have followed since the organism appeared to be confined to the pathological process of the heart. Low-titre birds were either negative or gave inconsistent positive reactions with the whole blood test. On the initial test of the flock the tube test detected 19 reactors which were negative to the whole blood method. On second test 15 of these reactors (three submitted to the laboratory and one died) were tested and three continued to react. One also reacted to the whole blood test.

Flock D. This flock had not revealed pullorum-disease reactors for a period of 8 consecutive years. It was selected as a negative flock to determine the accuracy of the whole blood test as compared with the tube test in ascertaining the true status of the birds. The technique for the testing methods was the same as that employed in the first test of Flock A. A total of 906 birds was tested. Sixteen birds gave reactions that were slightly suspicious. Two of these reactions were delayed. With the tube test the sera of three birds gave a slightly suspicious reaction. None of the reacting birds were requested for necropsy since the degree of reaction observed with either test and the history of the flock did not suggest the presence of pullorum infection.

TABLE 11.--FURTHER OBSERVATIONS ON THIRTEEN REACTORS IN FLOCK C.

Bird No.	Date Detected	Routine Tube Test 1:25	Date of Test	Tube Test						Whole Blood Test	Date of Necropsy	S. pullorum Isolated		
				Dilution										
				10	20	40	80	160	320	640	1280			
92817	10/25/34	0	10/25/34	NT								1*	12/29/34	Negative
			11/7/34	0	0	0	0	0	0	0	0	0		
			11/10/34	1	1	0	0	0	0	0	0	0		
			11/15/34	1	1	1	1	0	0	0	0	0		
			11/20/34	1	0	0	0	0	0	0	0	0		
			11/30/34	4	2	1	0	0	0	0	0	0		
			12/13/34	2	1	0	0	0	0	0	0	0		
			12/19/34	3	1	0	0	0	0	0	0	0		
			12/26/34	3	1	1	0	0	0	0	0	0		
			92836	10/25/34	3	10/25/34	NT	2	2	2	1	0		
11/7/34	2	1				1	1	0	0	0	0	0		
11/10/34	2	1				1	1	0	0	0	0	0		
11/15/34	2	2				2	1	0	0	0	0	0		
11/26/34	2	1				1	0	0	0	0	0	0		
11/30/34	2	1				1	0	0	0	0	0	0		
12/13/34	2	2				1	1	0	0	0	0	0		
12/19/34	2	2				1	1	0	0	0	0	0		
12/26/34	3	2				2	1	0	0	0	0	0		
12/31/34	0	0				0	0	0	0	0	0	0		
1/8/35	2	1				1	1	0	0	0	0	0		
1/16/35	2	2				2	1	1	0	0	0	0		
1/23/35	2	1				1	1	0	0	0	0	0		
1/31/35	2	2				2	1	0	0	0	0	0		
2/5/35	3	2	2	2	1	0	0	0	0					
2/15/35	2	2	2	1	0	0	0	0	0					
2/30/35	1	1	1	0	0	0	0	0	0					
92842	10/25/34	0	10/25/34	NT								1*	2/22/35	Negative
			11/7/34	0	0	0	0	0	0	0	0	0		
			11/10/34	0	0	0	0	0	0	0	0	0		
			11/15/34	0	0	0	0	0	0	0	0	0		
			11/20/34	0	0	0	0	0	0	0	0	0		
			11/30/34	0	0	0	0	0	0	0	0	0		
			12/13/34	0	0	0	0	0	0	0	0	0		
			12/19/34	0	0	0	0	0	0	0	0	0		
			12/26/34	0	0	0	0	0	0	0	0	0		
			12/31/34	U	U	U	U	U	U	U	U	U		
			1/8/35	1	0	0	0	0	0	0	0	0		
			1/16/35	0	0	0	0	0	0	0	0	0		
			1/23/35	1	0	0	0	0	0	0	0	0		
			1/31/35	1	0	0	0	0	0	0	0	0		
2/5/35	1	1	1	0	0	0	0	0	0					
2/15/35	0	0	0	0	0	0	0	0	0					
2/20/35	0	0	0	0	0	0	0	0	0					
92898	10/25/34	3	10/25/34	3	3	2	1	0	0	0	0	0	12/27/34	Negative
			11/7/34	2	1	0	0	0	0	0	0	0		
			11/10/34	1	0	0	0	0	0	0	0	0		
			11/15/34	2	1	1	1	0	0	0	0	0		
			11/20/34	1	1	1	0	0	0	0	0	0		
			11/30/34	2	1	1	0	0	0	0	0	0		
			12/13/34	2	1	1	0	0	0	0	0	0		
			12/19/34	2	2	1	0	0	0	0	0	0		
			12/26/34	2	1	1	0	0	0	0	0	0		
			95824	10/25/34	4	10/25/34	NT							
11/7/34	0	0				0	0	0	0	0	0	0		
11/10/34	0	0				0	0	0	0	0	0	0		
11/15/34	0	0				0	0	0	0	0	0	0		
11/20/34	0	0				0	0	0	0	0	0	0		
11/30/34	0	0				0	0	0	0	0	0	0		
12/13/34	0	0				0	0	0	0	0	0	0		
12/19/34	1	0				0	0	0	0	0	0	0		
12/26/34	0	0				0	0	0	0	0	0	0		
12/31/34	0	0				0	0	0	0	0	0	0		
1/8/35	0	0				0	0	0	0	0	0	0		
1/16/35	0	0				0	0	0	0	0	0	0		
1/23/35	0	0				0	0	0	0	0	0	0		
1/31/35	2	1				0	0	0	0	0	0	0		
2/5/35	1	0	0	0	0	0	0	0	0					
2/15/35	1	1	0	0	0	0	0	0	0					
2/20/35	0	0	0	0	0	0	0	0	0					
95825	11/28/34	2	11/28/34	NT								0	12/36/34	Negative
			12/19/34	4	4	2	0	0	0	0	0	0		
			12/26/34	4	3	1	0	0	0	0	0	0		

TABLE 11.--CONTINUED.

Bird No.	Date Detected	Routine Tube Test 1:25	Date of Test	Tube Test								Whole Blood Test	Date of Necropsy	S. pullorum Isolated	
				10	20	40	80	160	320	640	1280				
95829	11/28/34	2	11/28/34	NT									12/31/34	Negative	
			12/19/34	2	1	0	0	0	0	0	0	0			
			12/26/34	1	1	1	0	0	0	0	0	0			
			12/31/34	3	2	1	0	0	0	0	0	1			
95833	10/25/34	U	10/25/34	U	U	U	U	U	U	U	U	U	4	12/26/34	Positive
			11/7/34	4	4	4	4	4	3	1	0	4			
			11/10/34	4	4	4	4	2	1	0	0	4			
			11/15/34	4	4	4	4	2	0	0	0	3			
			11/20/34	4	4	4	4	4	2	0	0	4			
			11/30/34	4	4	4	4	4	2	0	0	4			
			12/13/34	4	4	4	4	4	2	1	0	4			
			12/19/34	4	4	4	4	4	2	1	0	4			
12/26/34	4	4	4	4	4	3	1	0	4						
95842	10/25/34	3	10/25/34	NT	3	2	1	0	0	0	0	0	1*	2/22/35	Positive
			11/7/34	4	4	3	2	0	0	0	0	2			
			11/10/34	4	4	2	1	0	0	0	0	1			
			11/15/34	4	3	1	0	0	0	0	0	0			
			11/20/34	4	4	2	1	0	0	0	0	0			
			11/30/34	3	1	0	0	0	0	0	0	0			
			12/13/34	2	1	1	0	0	0	0	0	0			
			12/19/34	2	1	0	0	0	0	0	0	1			
			12/26/34	2	1	0	0	0	0	0	0	0			
			12/31/34	3	1	0	0	0	0	0	0	0			
			1/8/35	3	2	0	0	0	0	0	0	0			
			1/16/35	2	0	0	0	0	0	0	0	0			
			1/23/35	3	2	0	0	0	0	0	0	0			
			1/31/35	1	0	0	0	0	0	0	0	0			
2/5/35	0	0	0	0	0	0	0	0	0						
2/15/35	2	0	0	0	0	0	0	0	0						
2/20/35	0	0	0	0	0	0	0	0	0						
95928	10/25/34	4	10/25/34	NT	4	3	2	0	0	0	0	0	1*	12/29/34	Positive
			11/7/34	4	4	2	1	0	0	0	0	0			
			11/10/34	4	2	1	0	0	0	0	0	2			
			11/15/34	4	3	2	1	0	0	0	0	1			
			11/20/34	4	4	3	1	0	0	0	0	2			
			11/30/34	4	4	4	4	1	0	0	0	4			
			12/13/34	4	4	4	4	1	0	0	0	4			
			12/19/34	4	4	4	2	1	0	0	0	4			
12/26/34	4	4	4	2	0	0	0	0	3						
95982	10/25/34	4	10/25/34	3	3	2	1	0	0	0	0	0	3	12/29/34	Positive
			11/7/34	4	4	3	1	0	0	0	0	2			
			11/10/34	4	3	2	0	0	0	0	0	4			
			11/15/34	4	3	2	1	0	0	0	0	2			
			11/20/34	4	4	3	2	1	0	0	0	2			
			11/30/34	4	4	4	3	1	0	0	0	4			
			12/13/34	4	4	4	4	2	1	0	0	4			
			12/19/34	4	4	4	4	2	0	0	0	4			
12/26/34	4	4	4	4	3	2	1	0	4						
96027	11/28/34	2	11/28/34	NT									12/31/34	Positive	
			12/19/34	4	2	1	0	0	0	0	0	0			
			12/26/34	1	1	0	0	0	0	0	0	0			
			12/31/34	4	3	1	0	0	0	0	0	0			
96031	10/25/34	3	10/25/34	NT									2/22/35	Negative	
			11/7/34	4	2	1	0	0	0	0	0	0			
			11/10/34	2	1	0	0	0	0	0	0	0			
			11/15/34	4	3	1	0	0	0	0	0	0			
			11/20/34	3	2	2	1	0	0	0	0	0			
			11/30/34	4	4	4	1	0	0	0	0	0			
			12/13/34	4	3	2	1	0	0	0	0	0			
			12/19/34	2	2	1	0	0	0	0	0	0			
			12/26/34	2	1	1	0	0	0	0	0	0			
			12/31/34	4	3	2	0	0	0	0	0	0			
			1/8/35	3	2	1	0	0	0	0	0	0			
			1/16/35	3	2	1	0	0	0	0	0	0			
			1/23/35	4	4	2	1	0	0	0	0	0			
			1/31/35	4	3	2	0	0	0	0	0	0			
2/5/35	4	3	1	0	0	0	0	0	0						
2/15/35	4	3	2	0	0	0	0	0	0						
2/20/35	4	3	1	0	0	0	0	0	0						

*Delayed reaction
 U—unsatisfactory test
 NT—not tested

Discussion

From results obtained in this investigation, the whole blood test, as a diagnostic means of detecting infected pullorum-disease carriers, does not appear to be so efficient as the standard tube method. Antigen and whole blood measurements demonstrated that the quantities of antigen and of whole blood might vary considerably, and that the whole blood-antigen dilution was by no means constant. This variation in the whole blood-antigen dilution had a perceptible influence on the degree of agglutination and was especially significant in birds that possessed a low agglutination titre. Failure of such birds to react might be due to the whole blood-antigen dilution being beyond the agglutination titre. While a definite amount of blood appeared essential to obtain a reaction, yet a greater amount of blood did not seem to increase the intensity and rapidity of the agglutination. Equal amounts of blood and antigen appeared to give the best results, while amounts of blood greater than that of antigen seemed to form a colored mixture in which the clumping was observed with difficulty.

The whole blood and antigen dilution does not appear to be the only factor which influences the accuracy of the whole blood method. While the exact influence which uniform and constant incubation temperature exerted on the agglutination reaction was not definitely determined, it was observed that a controlled heating device was necessary to avoid extremes in temperature which would lead to an unsatisfactory test. The temperature range in which agglutination is not hindered can only be determined when other steps in the test are kept uniform and constant.

An adequate source of light is essential for accurate interpretation of the agglutination reactions. With a suitable light the clumping will appear distinctly and plainly visible, even when the clumps are few in number and small in size. With insufficient light the weak reactions may be completely overlooked.

Interference with the tests due to foreign material, such as dust particles and feathers, can be largely overcome by using a covered test plate. The glass test plate marked off in one-inch squares with a diamond pencil prevents the whole blood-antigen tests from spreading and running together. Rotating the test plate appears to hasten the clumping of the cells as well as to bring about a grouping of the clumps. The latter is especially true in strong reactions.

To what extent humidity, air currents, and other environmental factors influence the agglutination reaction remains a question. The fact that there has been no satisfactory investigation of the influence which any of the apparently important factors exert upon the agglutination reaction, makes it impossible to ascribe to the different factors their full significance in the whole blood agglutination phenomena.

Since it is impossible to produce on the poultry farm conditions which simulate those in the laboratory, it appears reasonable that the quality of the test conducted in the field would not be equal to that conducted in the laboratory. The rate of speed at which the testing is conducted appears to have a marked influence. Some persons are prone to sacrifice accuracy for speed. Such a service may be satisfactory to certain flock owners, but in an eradication program success is based upon accurate and reliable testing results. Sufficient time should be taken to measure the blood and antigen as accurately as possible, to mix them thoroughly and to incubate them for a satisfactory period. The length of the incubation period considered necessary for agglutination to occur varies with different investigators.

Lubbehusen and Beach have observed that agglutination may occur after a period of 10 to 12 minutes. While the majority of positive reactions occur within a very short time, yet some birds give delayed reactions which may be overlooked. These delayed reactions consist of fine clumps, few in number, which are in most cases impossible to distinguish from reactions obtained with birds that are non-specific reactors. As to what factors have a direct or indirect influence on the incubation period, our knowledge appears to be very limited. It is apparent, however, that blood from high-titred birds as a rule produces immediate agglutination. By perfecting the whole blood-antigen dilution and improving the sensitivity of the antigen, we may possibly anticipate encouraging results in the detection of the delayed reactors.

The adoption of the whole blood test as the sole diagnostic means for accrediting flocks free from pullorum disease would seem an inexpedient procedure at this time in certain sections of this country where considerable progress has been made in the establishment of pullorum-clean flocks. In those sections where flock owners have adopted and attained eradication as their goal, the livability of the chicks is not the only benefit derived from a pullorum-disease-clean flock; but the owners are relieved of the frequent retesting that is necessary in a flock from which infection is not completely eliminated. In certain sections where the whole blood test is employed extensively, the benefit claimed from it, and justly so, is the reduced chick mortality. However, that does not mean that pullorum infection is not present in those flocks, because occasionally infected pullets are detected by the tube agglutination test when there has been no previous suspicion of the disease. With the presence of any infection in a breeding flock, little control can be exercised over the amount transmitted to the progeny. The amount of infection passed on to the progeny is influenced by factors such as the number of infected eggs which hatch, effective incubator disinfection, proper care and handling of baby chicks, the virulence of the organism, and the natural resistance of the chicks. One or two infected birds in a breeding flock may be responsible for acute outbreaks of pullorum disease in the progeny.

Since the whole blood test has several desirable features, a concerted effort should be made to improve its diagnostic efficiency, followed by standardization of the different steps in the procedure of the test.

SUMMARY

The whole blood agglutination test was not as efficient as the standard tube agglutination method. Factors such as proper and constant temperature, adequate light, and an effective and constant whole blood-antigen dilution influence the sensitivity and efficiency of the whole blood agglutination test.

Great variation was observed in the whole blood-antigen dilution. The lack of constancy in this dilution exerted a perceptible influence on the degree of agglutination and played an important role in detecting low-titre birds.

Delayed weak reactions occurred in a few instances with blood obtained from birds that harbored *S. pullorum*. The standard tube test detected infected birds which failed to react with the whole blood test.

The adoption of the whole blood test as the sole diagnostic means for establishing and identifying pullorum-disease-free flocks seems inexpedient at the present time.

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