MICROSCOPIC SLIDE FLOCCULATION AND MACROSCOPIC TUBE FLOCCULATION TESTS FOR PULLORUM DISEASE AND A COMPARISON OF THEM WITH THE TUBE AGGLUTINATION TEST

by

Edward Paul Roznowski, Jr.

B. S. in Ed., Illinois State Normal University, 1955

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

TABLE OF CONTENTS

INTRODUCTION

Pullorum disease (bacillary white diarrhea) has been long recognized as a disease of major importance in domestic fowl and a leading cause of loss to the poultry industry in this country. In the young chick it is a highly acute infectious disease, usually affecting the bird a few days after hatching, and in survivors results in chronic infection of the adult bird. The cause is a specific bacterium, Salmonella pullorum. The nature of the disease makes control, by means of curative agents, difficult. For this reason, prevention has been the means of control. The disease is of special economic importance because it is transmitted through the eggs of the adult infected bird, which has the infection localized in the ovary.

It has been found that the agglutination test may be used to detect birds which may act as carriers of the infectious agent. This test is used every year to test thousands of poultry blood samples at various experiment stations and private laboratories throughout the country. Dissatisfaction has been expressed regarding results obtained in different laboratories from testing replicate samples of blood sera. Great variation has been reported in reactions obtained from serum of the same bird tested at different times.

The present investigation was undertaken in the hope that a test could be devised which would speed up the testing program, increase the sensitivity, and standardize the reading of the test from laboratory to laboratory on the same sera.

REVIEW OF LITERATURE

Bunyea (1939) stated that the organized control of pullorum disease began with the introduction of the tube agglutination test in 1913. This

test was, and still is, used to detect infection in fowl. A few years later the so-called wattle test was devised for the detection of the disease but failed to achieve wide acceptance, as its reliability was questionable. In 1927 the rapid serum test was a step in advance of the then pervailing methods. This method was virtually a laboratory test and its usefulness was limited. Realizing the difficulty of conducting a nation-wide campaign against the disease under these conditions, the Bureau of Animal Industry directed its effort toward the development of a test that could be used on the farm as well as in the laboratory. The stained-antigen, rapid, whole-blood agglutination test for pullorum disease resulted from these efforts. This method was effective in the testing of scattered flocks, where a central laboratory was not present. This resulted in a more extensive control program.

The publications of Bushnell (1928), Bushnell and Brandly (1928, 1929), Bushnell and Hudson (1927a, b), Galloway and Holtman (1947), and Reid (1938) reported discrepancies along all of the present techniques being used.

The comparison of the agglutination test with the complement fixation test (Bushnell and Hudson, 1927a) showed that the significance was not marked, but the combination of the two tests did leave smaller numbers of questionable results. The complement fixation test and agglutination test have about the same value for detecting carriers. The difficulty lies in the fact that the agglutination test is difficult to read in borderline cases, and the complement fixation test can not be used with low dilutions of serum such as a dilution of 1:50. During inactivation the anticomplementary effect of the serum is not affected, but the complement fixation substance is destroyed. Galloway and Holtman (1947), following comparison of the rapid and tube antigen in the agglutination test on poultry blood, stated that the oversensitization of the rapid antigen might account for a greater number of positive

tests than were obtained with the tube antigen. A dissociation of strains used in the preparation of the tube antigen might result in fewer positive results with such bacterial suspensions.

Bushnell and Hudson (1927b) believed that the difficulty is in technique, a lack of proper antigen for the performance of the complement fixation test (leading to several different preparations being used) and the difficulty in preparing a stable antigen that retains antigenic properties for a long time and does not become anticomplementary.

Bushnell (1928) believed that low dilutions of serum should be used; and, for this reason, the complement fixation test can not be used to advantage, as the difficulty of technique prevents the use of low dilutions. He further stated that the macroscopic agglutination test is probably the best methodto use to detect reactive fowl. After several years of experience he recorded that the agglutination test proved effective in detecting reactive fowl and thereby led to better eradication of bacillary white diarrhea. He also felt that the agglutination test may be used as a basis of comparison for other methods.

The results of the test carried out by Reid (1938) indicated that the "whole blood" method may under proper conditions be relied upon to detect most, if not all, of the carriers of Salmonella pullorum and may be valuable in a control program. It is further indicated that the reactor birds can be detected before they reach the "production" age.

Bushnell and Brandly (1928), compared the tube and slide agglutination test and stated that the rapid slide test, using concentrated antigen and a short incubation period, may replace the tube agglutination test for the detection of carriers of bacillary white diarrhea. The dilutions should be kept at 1:25, as recommended by the tube test; and a freshly prepared antigen

gives better results than one that has been kept for several days in a refrigerator.

In comparing the pullorin reaction and the agglutination test for bacillary white diarrhea (Bushnell and Brandly, 1929), it was found that the pullorin test as then conducted was not as satisfactory in detecting carriers of bacillary white diarrhea as was the agglutination test. However, the correlation found between the two flocks used indicated the possibility that a pullorin reagent may be developed which will be satisfactory for the purpose. The pullorin test in its present state of development should not be recommended for replacing the agglutination test for the diagnosis of bacillary white diarrhea (Bushnell, 1928). However further research in this field seems desirable in view of the time and expense involved in current methods. Until this is done, the agglutination test should be used (Bushnell and Brandly, 1929). The rapid slide agglutination test was as effective as the tube test and may be used to replace it.

Ferry and Fisher (1925), while testing endotoxin production by organisms, found that the antigenic properties of the filtrates were extremely high in value. The filtrates were equal and in some cases superior in their antigenic value to both original broth and sedimented bacteria. These antigens were loosely bound to the cell or were a component of the ectoplasm. The term "ectoantigen" designates an antigenic substance which is outside the bacterial cell. They are more apt to be found in aqueous solutions in higher concentration by merely washing organisms grown on solid media than attempting to extract them by chemical or physical measures.

The "ectoantigen" has several advantages in complement fixation tests (Bushnell and Hudson, 1927b). In many of the bacterial antigens the antigenic dose is so low that large amounts of heavy suspensions are necessary.

These heavy doses cause a cloud and mask hemolysis and render accurate readings difficult. "Ectoantigens" are water clear and could not be used for agglutination tests but are valuable for use in the complement fixation test. Such antigens are highly antigenic and not anticomplementary. They are very clear and do not mask the test. However, the sediment remains anticomplementary. The bacterial cell is not necessary in the preparation of antigen of S. pullorum, but it is necessary to wash the cells to bring the antigenic substance into solution, increase the antigenic action, and reduce the anticomplementary action of the antigen.

Hunter and Colbert (1954) described a method of preparing an antigen emulsion by coating cholesterol with dialyzed, cell-free, brucella ectoantigen extract. They believed that this antigen emulsion, following the procedure given, will give more consistent results both as to sensitivity and specificity, will be more easily read, and can be reproduced in other laboratories. Kline (1940) reported on the microscopic slide test for syphilis, using an antigen emulsion composed of cholesterol coated with acetone insoluble fraction of beef heart extract; later they used cardiolipin and lecithin. Harris, et al. (1948) developed a macroflocculation test for syphilis using cholesterol coated with cardiolipin and lecithin. These tests for syphilis have been reproducible and have exhibited constant sensitivity and specificity.

Hunter and Colbert (1956) stated that the agglutination test varies from laboratory to laboratory but that the results are in close agreement. The factors that cause these variations are quality and concentration of the bacterial suspension, time and temperature of incubation, diluting fluid, and other factors which cause a variation in the sensitivity and specificity of the test. Even though there are standard tests set up by the Animal Disease and Parasite Research Section of the Agriculture and Research Service of the

U. S. Department of Agriculture, which recommend the use of standard antigen and procedure for both the plate and tube test, variation in results between laboratories still show up.

Hunter and Colbert (1956) stated that the sensitivity and specificity of the antigen emulsion can be adjusted to conform to the standard antigen. The tests are rapidly performed and the results are clear cut, very easily read, and give reproducible results. The heating of serum helps eliminate the reporting of positive sera as negative. The Bureau of Animal Industry, U. S. Department of Agriculture, has a standard antigen and recommended procedure for both the plate and tube agglutination test for pullorum in fowl. However, there is much variation in reading of the test.

In order to overcome the variability of the antigen and technique of performing the agglutination test for pullorum, it was decided to approach this problem by using the procedure of Hunter and Colbert (1954) and substituting the Salmonella pullorum organism in preparing the antigen.

MATERIALS AND METHODS

Source and Description of Organisms

Two cultures of Salmonella pullorum were obtained from the collection of the Department of Bacteriology of Kansas State College; one regular strain and one variant strain. From these cultures were prepared all of the antigens that were used in this study.

Salmonella pullorum, as described in Bergey's Manual of Determinative Bacteriology (Breed, et al., 1948), is a gram negative rod that measures 0.3 to 0.5 by 1.0 to 2.5 microns, occurs singly and is non-motile. The agar colonies are grayish-white, smooth, glistening, and entire to undulate. The

agar slant cultures develop as discrete, translucent colonies. The organism forms acid and gas from glucose, fructose, galactose, mannose, arabinose, xylose, mannitol, and rhamnose, but does not ferment lactose, sucrose, maltose, dextrin, salicin, raffinose, sorbitol, adonitol, dulcitol or inositol. It is a faculative, aerobic organism and has an optimum temperature of 37°C. Its antigenic structure is IX, XII. The complete antigenic formula of S. pullorum is IX, XII_1, XII_2, XII_3. Antigen XII_2 is variable and XII_2^{++} and XII_2^{+} forms occur. The XII_2^{++} forms are synonymous with the X strain of Younie. The organism can be isolated from chickens and other birds, as well as calves, hogs, rabbits, and man. It occasionally produces food poisoning or gastro-enteritis in man. It is the cause of white diarrhea in young chicks and infects the ovaries and eggs of adult birds.

Preparation of Stock Antigen and Reagents

For making the antigen, nutrient agar slants were inoculated with the stock cultures and incubated at 37°C for 48 hours. After incubation the tubes were checked for purity by the gram stain and a plate agglutination test, using a positive serum and adding the organism to it and reading for agglutination. The pure growths were then suspended in 3 ml of sterile saline and used to inoculate Blake bottles, containing 100 ml of thiosulfate-glycerol medium of the following composition:

Water	1000 ml
Sodium chloride	5 g
Beef extract	3 g
Bacto-peptone	20 g
Glycerin	20 ml
Agar-agar	30 g
Sodium thiosulfate	10 ml
Ammonium chloride	10 ml

Mix the water, sodium chloride, beef extract, bacto-peptone

> and agar-agar and heat by means of a steam bath for 30 minutes. The glycerin, sodium thiosulfate and ammonium chloride are added and the pH of the mixture is adjusted to 7.2, using bromthymol blue indicator, with sodium hydroxide. The mixture is heated in a steam bath and filtered through gauze and dispensed into Blake bottles in 100 ml portions. The bottles are then autoclaved under 15 pounds pressure for 30 minutes. After autoclaving, the bottles were layed out to solidify.

The bottles were incubated at 37°C for 72 hours. After testing the apparent purity of each bottle by the gram stain and agglutination test, all the cultures were washed off with 10 ml of phenolized saline (0.25 per cent phenol in normal saline). The bacterial suspensions were screened through gauze to remove large clumps of bacteria and loose media. Phenol was added so the final concentration was 2 per cent. This suspension was allowed to stand in the refrigerator for seven days at approximately 8°C to allow the organisms to be killed. The bacterial suspension was next placed in dialyzing tubing (Aloe Scientific Division of A. S. Aloe Co., Catalog Number 103, Number 22620 cellulose acetate tubing 19 mm) and placed in running tap water for 24 hours or until phenol free. A ferric chloride solution (Simmons and Gentzkow, 1944) was used to test for phenol. The suspension was centrifuged at 5000 rpm for 30 minutes, the supernatant was removed, and the sedimented organisms discarded. The supernatant was made isotonic by the addition of NaCl and merthiolate was added to give a final concentration of 1:10,000. This was bottled and kept in the freezer until needed.

The other reagents necessary are: 1 per cent cholesterol (Pfanstiehl,

ash free, precipitate from alcohol for Kline test) in absolute ethyl alcohol; distilled water (pH approximately 6); 0.85 per cent and 1 per cent solutions of NaCl (Mallinkrodt's, AR); 1 per cent solution of lecithin in absolute alcohol (received from Dr. Sol Rosenberg and diluted so that the amount of lecithin was 10 mg per ml or a 1 per cent solution), and Eagle's buffered saline (Hunter, 1956), pH 7.4.

The other equipment necessary to perform the tests is found in almost any laboratory doing serological tests for syphilis.

Procedure for Making Antigen Emulsion

The antigen emulsion is prepared by: pipetting 0.85 ml of distilled water to the bottom of a 30 ml round glass-stoppered bottle; while rotating add 1 ml of 1 per cent cholesterol solution drop by drop; continue rotating for 20 seconds; add 0.10 to 0.20 ml of lecithin and shake for 15 seconds; add 0.10 to 0.25 ml of dialyzed, cell-free, pullorum ectoantigen and shake vigorously by striking the bottom of the bottle against the palm of the hand for 1 minute; finally add 2.5 ml of Eagle's buffered saline, pH 7.4, and shake less vigorously for 30 seconds. The antigen emulsion can be used immediately. If stored in the refrigerator it may be used any time within 24 hours.

The amount of lecithin and of pullorum ectoantigen to add has to be determined for each lot of antigen and lecithin. Experiments must be performed so that the sensitivity and specificity of the antigen emulsion will be comparable to the standard.

Preparation of Serum

The blood specimen to be examined should be centrifuged and the serum removed by pipetting or decanting. Activate the serum by heating to 56°C for 15 minutes. If the serum is to be re-examined on another day, reheat to 56°C for 5 minutes. Any visible particles should be removed by centrifugation.

Microscopic Slide Flocculation Test

Pipette 0.05 ml of serum into a paraffin or permanent ring on a glass slide. With an 18 gauge needle, the bevel of which has been filed off, held perpendicularly add one drop of antigen emulsion to each portion of serum. The needle should deliver between 80 and 85 drops per ml. The slide should be rotated (Arthur H. Thomas Co., Edition 1950, Number 3623 Electric Rotating Apparatus, A. H. T. Co. Specification) at 180 rpm for four minutes; a longer rotation time will increase the sensitivity and cause rough reactions on negative sera. The reactions should be observed at a magnification of 100 X. A saline (0.85%) control should always be included to determine whether the antigen emulsion alone gives a negative reaction. The reactions are recorded as follows:

Positive:	Large and medium size clumps
Weakly positive:	Small clumps with individual particles
Negative:	No clumps

Plates I through III show the appearance of typical flocculation reaction. In a number of instances, positive reactions will be obtained with the 0.05 ml serum, in which case it is necessary to make additional dilutions of the serum in saline (0.85%). It is recommended that two-fold dilutions be made from 1:10 to 1:640 (see Table 1). The test is then run using 0.05 ml of each

EXPLANATION OF PLATE I

Microscopic Slide Flocculation Test for Pullorum (100 X).

Figs. 1 and 2. Positive: Large and medium size clumps.

PLATE I

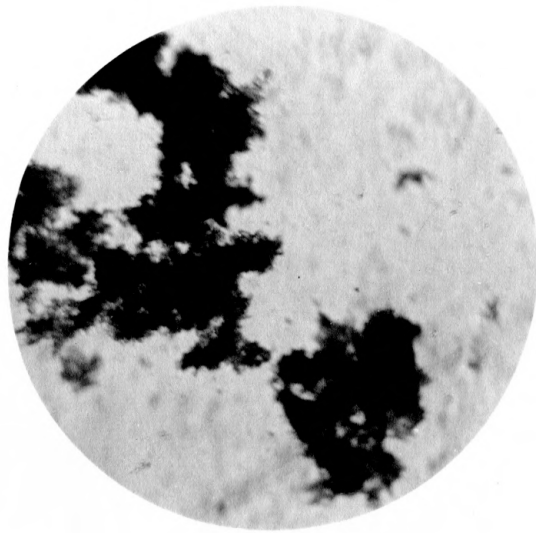

Fig. 1

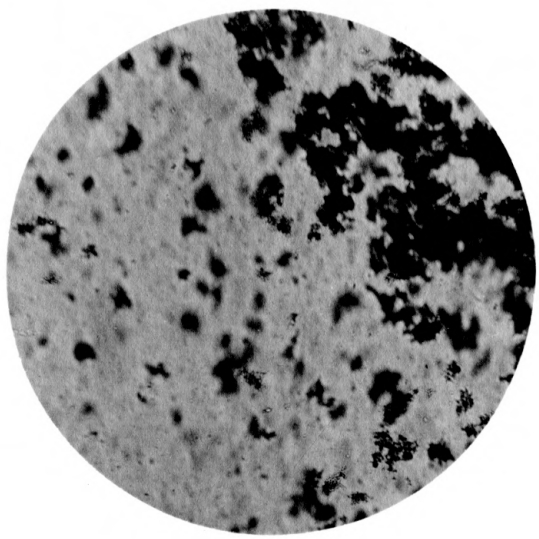

Fig. 2

EXPLANATION OF PLATE II

Microscopic Slide Flocculation Test for Pullorum (100 X).

Figs. 1 and 2. Weakly Positive: Small clumps with individual particles.

PLATE II

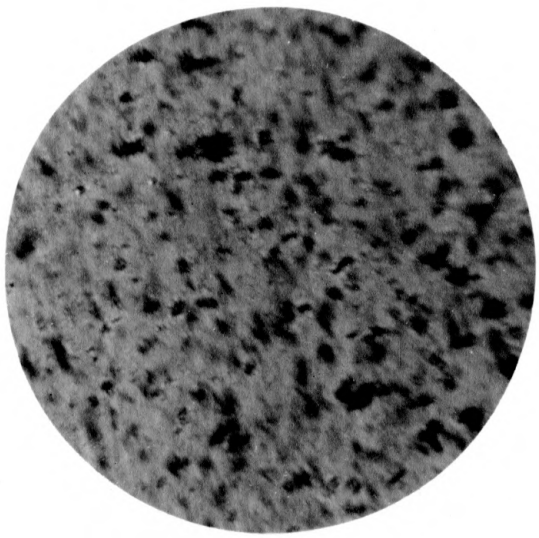

Fig. 1

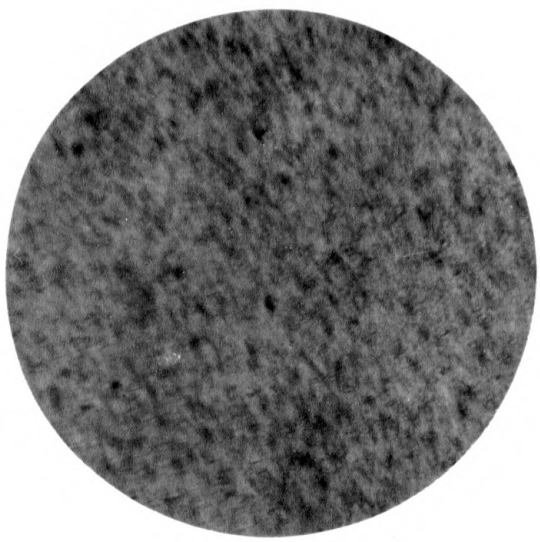

Fig. 2

EXPLANATION OF PLATE III

Microscopic Slide Flocculation Test for Pullorum (100 X).

Negative: No clumps.

PLATE III

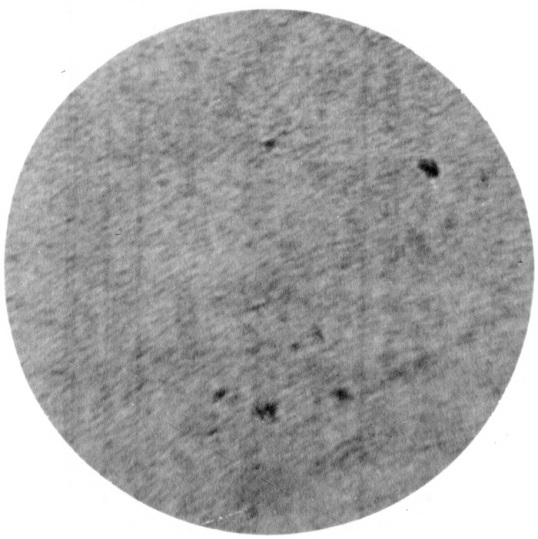

Table 1. Protocol for serum titrations.

	Tube								
	1	2	3	4	5	6	7	8	9
Dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Saline (0.85%)	1.8 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Serum	0.2 ml	. . . . .							

Footnote: Transfer 1 ml of the dilution of tube 1 into tube 2 and mix, transfer 1 ml of the dilution of tube 2 into tube 3 and mix, etc., until the desired dilutions are obtained.

dilution. The titer of the serum run by the microscopic slide test cannot be compared with the titer obtained by the regular agglutination test.

Macroscopic Tube Flocculation Test

The antigen emulsion is prepared as for the microscopic slide test, and is diluted one part with four parts of 1 per cent NaCl solution. This dilution of the antigen emulsion can be used immediately or after standing as long as 24 hours. It is a good practice to let the antigen stand for 5 minutes before using it in the test. The serum is heated at 56°C for 15 minutes, and, if it is re-examined on a later day, reheat it for five minutes at 56°C. Make two-fold dilutions of the serum from 1:10 to 1:640 (see Table 1), using 0.85 per cent saline. In some instances it may be necessary to make further dilutions. To 0.5 ml portions of the diluted serum (see Table 1) add 0.5 ml of the diluted antigen emulsion, so that the final serum dilution will range from 1:20 to 1:5120. The tubes are put on the Kahn shaker and shaken for five minutes. After the shaking, the tubes are centrifuged for 10 minutes at 2000 rpm. After the tubes are removed from the centrifuge, they are again put on the Kahn shaker and shaken for one minute. The following results can be read by holding the tubes in front of a reading lamp with a black background:

Positive:	Large clumps and clear supernatant
Weakly positive:	Clumps but cloudy supernatant
Negative:	No clumps but a cloudy supernatant which gives a swirl similar to that seen in a mixture of blood in saline.

Plate IV shows the appearance of typical tube flocculation reactions.

Methods of Standardizing Antigen

In the preparation of antigen for various flocculation tests for syphilis,

EXPLANATION OF PLATE IV

Tube Flocculation Test for Pullorum.

Reading left to right.

Positive: Large clumps and clear supernatant.

Weakly positive: Clumps but cloudy supernatant.

Negative: No clumps but a cloudy supernatant which gives a swirl similar to that seen in a mixture of blood in saline.

PLATE IV

serologists have found that experiments must be conducted to determine the optimum ratio of reagents used. Antigens must be prepared using varying ratios and concentrations of cardiolipin, lecithin and cholesterol, so that the antigen will give the same sensitivity and specificity as the standard (Hunter and Colbert, 1956). The same is true in standardizing the pullorum extract. The only difference being that pullorum extract is used in place of cardiolipin.

The sera used in the standardization were from a variety of sources, viz. goat, turkey, chicken and human (type "O"). The titers of the sera were determined by the standard Salmonella pullorum tube agglutination test. The tests were set up in varying dilutions of 1:10 to 1:5120 (see Table 2), incubated at 37°C for 20 to 24 hours and read, so that the titer could be established. The tests were read as follows:

4^+	Large clumps with clear supernatant
3^+	Medium clumps with clear supernatant
2^+	Small clumps with cloudy supernatant
1^+	Small clumps with individual particles and cloudy supernatant
Negative	No clumps, but cloudy supernatant.

The results of this work are shown on Tables 3 and 4. Nine sera were selected from the 15 tested to be used in the standardization of the pullorum extract.

The standardization was carried out on heated sera because the final test was to be on heat-activated sera. Thirty-two antigen emulsions were used, varying the ratio of lecithin (1%) and pullorum extract according to Table 5.

The microscopic slide flocculation tests were run on sera numbers one through nine inclusive (see Table 4), with each of the 32 antigen emulsions.

Table 2. Protocol for the determination of the titer of the sera to be used as a standard.

	Tube								
	1	2	3	4	5	6	7	8	9
Dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Antigen	1.8 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Serum	0.2 ml	. . . . .							

Footnote: Transfer 1 ml of the dilution of tube 1 into tube 2 and mix, transfer 1 ml of the dilution of tube 2 into tube 3 and mix, etc. until the desired dilutions are obtained.

Table 3. Results of standardizing unheated serum with the S. pullorum tube test.

Serum Number	Serum Source	Dilutions										
		10	20	40	80	160	320	640	1280	2560	5120	10240
1	Goat Huff & Jackson	4	4	4	4	4	4	4	1	-	-	-
2	Goat Pilson	4	4	4	4	1	1	-	-	-	-	-
3	Chicken pooled positive	2	2	2	2	1	1	-	-	-	-	-
4	S. typhosa	4	4	4	4	1	1	-	-	-	-	-
5	Chicken pooled negative	-	-	-	-	-	-	-	-	-	-	-
6	Human "O" negative	-	-	-	-	-	-	-	-	-	-	-
7	Turkey 1 7-16-56	1	2	3	3	1	1	-	-	-	-	-
8	Turkey 2 7-16-56	2	2	1	-	-	-	-	-	-	-	-
9	Goat Vera & Ward	4	4	4	4	4	4	4	3	2	1	-
10	Goat 90999	4	4	4	4	4	4	4	3	1	1	-
11	Goat X	4	4	4	4	3	3	3	1	-	-	-
12	Goat 7-16-56	4	3	3	3	1	1	1	-	-	-	-
13	Goat Wood & Baldwin	4	4	4	3	2	2	1	1	-	-	-
14	Goat Nelson & Whiteham	4	4	4	4	2	2	2	-	-	-	-
15	Goat Sherrod & Nichols	4	4	3	3	2	1	1	-	-	-	-

Table 4. Results of standardizing heated serum with the *S. pullorum* tube test.

Serum Number	Serum Source	Dilutions										
		10	20	40	80	160	320	640	1280	2560	5120	10240
1	Goat Huff & Jackson	4	4	4	4	4	4	2	1	-	-	-
2	Goat Pilson	4	4	4	4	1	-	-	-	-	-	-
3	Chicken pooled positive	1	1	1	1	-	-	-	-	-	-	-
4	*S. typhosa*	4	4	3	3	2	1	-	-	-	-	-
5	Chicken pooled negative	-	-	-	-	-	-	-	-	-	-	-
6	Human "O" negative	-	-	-	-	-	-	-	-	-	-	-
7	Turkey 1 7-16-56	1	1	2	2	2	-	-	-	-	-	-
8	Turkey 2 7-16-56	2	1	-	-	-	-	-	-	-	-	-
9	Goat Vera & Ward	4	4	4	3	3	3	2	2	1	1	-
10	Goat 90999	4	4	4	4	4	4	2	2	1	-	-
11	Goat X	4	4	3	3	1	1	-	-	-	-	-
12	Goat 7-16-56	4	3	3	3	1	1	-	-	-	-	-
13	Goat Wood & Baldwin	4	4	3	3	3	3	2	1	1	-	-
14	Goat Nelson & Whiteham	4	4	4	4	4	4	1	-	-	-	-
15	Goat Sherrod & Nichols	4	4	4	4	4	4	1	-	-	-	-

Table 5. Varying amounts of lecithin (1%) and pullorum extract used to establish the standard amount needed for the final tests.

		Lecithin 1%					
		None	0.05 ml	0.10 ml	0.15 ml	0.20 ml	0.25 ml	0.30 ml
Pullorum extract	0.05 ml							
	0.10 ml							
	0.15 ml							
	0.20 ml							
	0.25 ml							

The ratio of pullorum extract and lecithin (1%) giving the same sensitivity and specificity as the standard antigen was determined. Once this ratio was determined for a particular lot of pullorum extract and lecithin (1%), it could be used until this lot was exhausted. In other words, it is only necessary to standardize each lot of pullorum extract and lecithin (1%) once. Table 6 gives the results of the standardization of the antigen emulsion. The difference in results among the various concentrations can be readily noted. When an antigen emulsion was prepared without lecithin (1%) all the tests showed definite clumping. This clearly shows the effect of lecithin on the specificity of the test. The exact reason for using the lecithin (1%) is not known, but it is assumed to be a possible wetting agent. Kline used beef heart in his test for syphilis. The extract of the beef heart had lecithin present; however, when cardiolipin was used it did not work without the presence of lecithin. This could demonstrate the necessity for the lecithin in this testing procedure. The lecithin and the cardiolipin made a product similar to the beef heart extract.

The antigen emulsion containing 0.15 ml of lecithin (1%) and 0.15 ml of pullorum extract gave the same sensitivity and specificity as the standard antigen. Salmonella typhosa gave a 3^+ reaction with the pullorum extract as it has the same somatic antigen components IX and XII as is found in S. pullorum, but in addition has a (Vi) component.

RESULTS

The data obtained are shown best by a series of tables. Table 7 presents the results obtained by a group of students using the microscopic slide flocculation test. Ten sera were divided into test tubes, numbered from one

Table 6. Results of titration used for the determination of the amount of lecithin and S. pullorum extract needed to prepare a standard antigen emulsion.

		None										0.5 ml						0.10 ml										0.20 ml										0.25 ml										0.30 ml					
		10	20	40	80	160	320	640	1280	2560	5120	160	320	640	1280	2560	5120	10	20	40	80	160	320	640	1280	2560	5120	10	20	40	80	160	320	640	1280	2560	5120	10	20	40	80	160	320	640	1280	2560	5120	160	320	640	1280	2560	5120
	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	1	-	-	-	-	-	-	-	4	4	4	4	4	3	3	2	2	1	-	-	4	4	4	4	4	3	2	2	1	-	-	-	-
	2	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	-	-	-	-	-	-	-	-	4	4	4	3	2	1	-	-	-	-	-	-	3	3	3	2	2	2	1	-	-	-	-	-	-	-
	3	4	4	4	4	3	3	2	2	1	1	1	4	4	4	4	3	2	1	1	-	-	-	-	-	-	4	4	4	4	3	2	1	-	-	-	-	-	4	4	4	4	3	2	1	-	-	-	-	-	-	-
	4	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	1	-	-	-	-	-	-	-	-	-	3	3	3	2	1	-	-	-	-	-	-	3	3	3	2	1	-	-	-	-	-	-	-	-	-	-	-
0.05 ml	5	3	3	3	3	3	2	2	2	1	1	2	2	2	2	2	2	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	2	2	1	-	-	3	3	3	3	3	2	2	1	-	-	3	3	3	3	2	2	1	-	-	3	3	3	3	2	2	1	-	-	2	2	2	2	1	1	1	-	-	-
	S	1	1	1	1	1	1	1	1	1	1	1						-				-						-				-						-				-						-					
	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	2	2	1	-	-	-	4	4	4	4	4	3	3	1	-	-	4	4	4	4	3	2	-	-	-	4	4	4	4	4	3	2	2	-	-	-	-	-	-
	2	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	3	2	1	-	-	-	4	4	4	3	2	-	-	-	-	-	4	4	4	3	2	1	-	-	-	4	4	4	3	2	1	-	-	-	-	-	-	-
	3	4	4	4	4	3	2	2	2	1	1	4	4	4	4	3	3	2	1	1	-	-	-	4	4	4	4	3	2	1	-	-	4	4	4	4	3	1	-	-	-	4	4	4	4	4	2	1	-	-	-	-	-	-	-
	4	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	-	-	-	-	-	3	3	3	2	-	-	-	-	-	3	3	3	2	1	-	-	-	-	-	3	3	2	1	-	-	-	-	-	-	-	-
0.10 ml	5	3	3	3	3	3	2	2	2	1	1	2	2	2	2	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	1	1	1	1	1	1	1	1	1	1	4	4	4	4	3	3	2	1	-	-	4	4	4	4	4	3	2	1	-	-	4	4	4	4	2	1	1	-	-	3	3	3	3	2	1	1	-	-	-	-	-	-	-
	S	1	1	1	1	1	1	1	1	1	1	1						-				-						-				-						-				-						-					
	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	2	2	-	-	-	4	4	4	4	4	3	2	1	-	-	4	4	4	4	3	2	1	-	-	4	4	4	4	4	4	2	1	-	-	-	-	-	-
	2	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	2	2	1	-	-	-	4	4	4	4	2	-	-	-	-	-	4	4	4	4	3	2	1	-	-	4	4	4	4	3	2	1	-	-	-	-	-	-
	3	4	4	4	4	3	3	2	2	1	1	4	4	4	4	4	3	2	1	1	-	-	-	4	4	4	4	2	1	-	-	-	4	4	4	4	4	3	1	-	-	4	4	4	4	4	3	1	-	-	-	-	-	-
	4	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	1	-	-	-	-	-	-	3	3	2	1	-	-	-	-	-	4	3	3	2	2	-	-	-	-	-	3	3	2	2	-	-	-	-	-	-	-	-
Pullorum 0.15 ml	5	3	3	3	3	3	2	2	1	1	2	2	2	2	2	2	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
extract	6	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	r	-	-	-	-	-	-	-	-	1	r	r	-	-	-	-	-	-	-	-	-	-	-
	8	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	1	1	1	1	1	1	1	1	1	1	4	4	4	4	3	2	2	1	-	-	4	4	4	4	4	3	2	1	-	-	4	4	4	4	2	1	-	-	-	4	4	4	4	4	2	1	-	-	-	-	-	-	-
	S	1	1	1	1	1	1	1	1	1	1	1						-				-						-				-						-				-						-					
	1	1	1	1	1	1	1	1	1	1	1	3	3	3	2	2	2	1	-	-	-	4	4	4	4	3	2	1	-	-	-	4	4	4	4	3	2	1	-	-	4	4	4	4	4	3	2	-	-	-	-	-	-	-
	2	1	1	1	1	1	1	1	1	1	1	3	3	3	3	2	2	-	-	-	-	4	4	4	3	1	-	-	-	-	-	4	4	4	3	2	r	-	-	-	3	3	2	2	1	-	-	-	-	-	-	-	-
	3	4	4	4	4	3	2	2	1	1	1	4	4	4	4	4	3	2	1	-	-	-	4	4	4	4	3	2	1	-	-	-	4	4	4	4	3	1	-	-	-	4	4	4	4	4	2	1	-	-	-	-	-	-	-
	4	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	-	-	-	3	3	2	1	-	-	-	-	-	-	4	3	2	1	-	-	-	-	-	3	3	2	1	-	-	-	-	-	-	-	-
0.20 ml	5	3	3	3	3	3	2	2	1	1	2	2	2	2	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	1	1	1	1	1	1	1	1	1	1	4	4	4	4	3	3	2	1	-	-	4	4	4	3	3	2	r	-	-	-	4	4	4	4	2	1	-	-	-	4	4	4	3	2	1	-	-	-	-	-	-	-
	S	1	1	1	1	1	1	1	1	1	1	1						-				-						-				-						-				-						-					
	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3	2	2	1	-	-	-	4	4	4	4	3	2	-	-	-	-	4																					
	2	1	1	1	1	1	1	1	1	1	1	3	3	3	3	1	-	-	-	-	-	3	3	3	2	1	-	-	-	-	-	4																					
	3	4	4	4	4	3	2	2	2	1	1	4	4	4	4	3	2	1	-	-	-	4	4	4	4	3	2	1	-	-	-	4																					
	4	1	1	1	1	1	1	1	1	1	1	3	2	2	1	-	-	-	-	-	-	3	2	2	1	-	-	-	-	-	-	3																					
0.25 ml	5	4	4	4	4	3	2	2	2	1	1	r	r	r	-	-	-	-	-	-	-	r	r	r	-	-	-	-	-	-	-	r																					
	6	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																					
	7	1	1	1	1	1	1	1	1	1	1	r	r	r	-	-	-	-	-	-	-	r	r	r	-	-	-	-	-	-	-	r																					
	8	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																					
	9	2	2	2	2	1	1	1	1	1	1	4	4	4	4	3	2	1	-	-	-	4	4	4	4	3	2	1	-	-	-	4																					
	S	1	1	1	1	1	1	1	1	1	1	-	-	-	-							-																															

Footnote: Sera 1 through 9 correspond to 1 through 9 of Table 4. (-) indicates no flocculation.

Table 7. Results obtained by students using microscopic slide flocculation test on unheated and heat-activated sera, compared to experimental results as a control standard.

Serum		Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240
Goat 7-16-56												
My results	U	+	+	+	+	+	±	-	-	-	-	-
1		Not used in class										
11		+	+	+	±	±	-	-	-	-	-	-
21		+	+	±	-	-	-	-	-	-	-	-
31		Not used in class										
My results	H	+	+	+	+	±	±	±	-	-	-	-
41		+	+	±	-	-	-	-	-	-	-	-
51		+	±	±	-	-	-	-	-	-	-	-
61		+	+	±	-	-	-	-	-	-	-	-
71		+	+	±	±	±	-	-	-	-	-	-
Goat Wood & Baldwin												
My results	U	±	+	+	+	+	+	+	±	±	-	-
2		Not used in class										
12		+	+	+	+	+	±	±	-	-	-	-
22		+	+	+	+	±	-	-	-	-	-	-
32		+	+	+	+	±	-	-	-	-	-	-
My results	H	+	+	+	±	±	±	±	±	-	-	-
42		+	+	+	+	±	±	-	-	-	-	-
52		Not used in class										
62		+	+	+	±	±	-	-	-	-	-	-
72		+	+	+	+	±	-	-	-	-	-	-
Goat Vera & Ward												
My results	U	+	+	+	+	+	±	±	±	-	-	-
4		Not used in class										
14		+	+	+	+	+	±	-	-	-	-	-
24		+	+	+	+	±	±	-	-	-	-	-
34		+	+	+	+	±	-	-	-	-	-	-
My results	H	+	+	+	+	±	±	±	±	±	±	-
44		Not used in class										
54		+	+	+	+	+	±	±				
64		+	+	+	+	±	-	-	-	-	-	-
74		+	+	+	±	±	±	-	-	-	-	-
Goat Nelson & Whiteham												
My results	U	+	+	+	+	+	±	±	-	-	-	-
3		+	+	+	+	±	±	-	-	-	-	-
13		Not used in class										
23		+	+	+	±	±	-	-	-	-	-	-
33		+	+	+	±	±	-	-	-	-	-	-
My results	H	+	+	+	±	±	±	±	-	-	-	-
43		+	+	+	+	+	±	±	±	±	±	-
53		Not used in class										
63		+	+	+	+	±	±	±	-	-	-	-
73		+	+	+	+	±	-	-	-	-	-	-
Goat 90999												
My results	U	+	+	+	+	±	±	±	-	-	-	-
5		+	+	+	+	±	-	-	-	-	-	-
15		+	+	+	+	±	±	-	-	-	-	-
25		+	+	+	±	±	-	-	-	-	-	-
35		+	+	+	±	±	-	-	-	-	-	-
My results	H	+	+	+	+	±	±	±	±	-	-	-
45		+	+	+	+	±	±	-	-	-	-	-
55		Not used in class										
65		+	+	+	±	±	-	-	-	-	-	-
75		+	+	±	±	-	-	-	-	-	-	-
Goat Huff & Jackson												
My results	U	±	±	±	±	±	±	±	-	-	-	-
6		+	+	+	+	+	±	-	-	-	-	-
16		+	+	+	+	±	-	-	-	-	-	-
26		+	+	+	+	±	-	-	-	-	-	-
36		+	+	+	+	+	±	-	-	-	-	-
My results	H	±	±	±	±	±	-	-	-	-	-	-
46		Not used in class										
56		Not used in class										
66		+	+	+	±	±	-	-	-	-	-	-
76		+	+	+	±	±	±	-	-	-	-	-
Goat X												
My results	U	±	±	±	±	-	-	-	-	-	-	-
7		+	±	±	±	-	-	-	-	-	-	-
17		+	+	±	±	-	-	-	-	-	-	-
27		+	±	-	-	-	-	-	-	-	-	-
37		+	±	±	±	-	-	-	-	-	-	-
My results	H	+	+	+	+	±	-	-	-	-	-	-
47		Not used in class										
57		Not used in class										
67		±	±	-	-	-	-	-	-	-	-	-
77		±	±	±	-	-	-	-	-	-	-	-
Goat Pilson												
My results	U	+	+	+	+	+	±	-	-	-	-	-
8		+	+	+	+	±	±	-	-	-	-	-
18		+	+	+	±	±	-	-	-	-	-	-
28		+	+	+	±	-	-	-	-	-	-	-
38		+	+	+	±	-	-	-	-	-	-	-
My results	H	+	+	+	±	±	-	-	-	-	-	-
48		+	+	+	+	±	±	-	-	-	-	-
58		+	+	+	+	±	±	-	-	-	-	-
68		+	+	±	-	-	-	-	-	-	-	-
78		+	+	±	-	-	-	-	-	-	-	-
S. typhosa												
My results	U	+	+	+	±	±	-	-	-	-	-	-
9		+	+	±	±	-	-	-	-	-	-	-
19		+	+	+	±	±	-	-	-	-	-	-
29		+	±	±	-	-	-	-	-	-	-	-
39		+	+	±	+	-	-	-	-	-	-	-
My results	H	+	+	+	±	±	±	±	-	-	-	-
49		Not used in class										
59		+	+	+	±	±	±	±	±	-	-	-
69		+	+	-	-	-	-	-	-	-	-	-
79		+	±	-	-	-	-	-	-	-	-	-
Human "0" negative												
My results	U	-	-	-	-	-	-	-	-	-	-	-
10		Not used in class										
20		-	-	-	-	-	-	-	-	-	-	-
30		-	-	-	-	-	-	-	-	-	-	-
40		-	-	-	-	-	-	-	-	-	-	-
My results	H	-	-	-	-	-	-	-	-	-	-	-
50		-	-	-	-	-	-	-	-	-	-	-
60		-	-	-	-	-	-	-	-	-	-	-
70		-	-	-	-	-	-	-	-	-	-	-
80		-	-	-	-	-	-	-	-	-	-	-

Footnote: Sera numbers one to 40 are unheated and sera numbers 41 to 80 are heat-activated.
(+) positive; (±) weakly positive; (-) no flocculation.

to 80. Sera numbers one to 40 were not heated but sera numbers 41 to 80 were heat-activated for 15 minutes at 56°C. The students prepared dilutions from 1:10 to 1:5120 and performed the slide flocculation tests.

Table 8 shows a comparison of the agglutination test with the microscopic slide and macroscopic tube flocculation tests for pullorum antibodies on unheated sera. Table 9 shows the results on sera that were heat-activated for 15 minutes at 56°C. The tables represent data from 445 sera (chicken, S. typhosa in goat, Human type "O", turkey, and goat).

In the microscopic slide flocculation test run on the undiluted samples, the tests were considered positive if the floccules were heavy, as seen in Figs. 1 and 2 of Plate I. All other tests were considered negative.

The results of this research showed that the test gave its best results when run on the heat-activated sera, because the positives were distinct and the end points of each dilution were sharp and definite.

The results obtained by the students demonstrated that independent inexperienced workers could duplicate the results on the same sera. The titers obtained by the students differed from my results because a two week period elapsed between the performance of the experiments. The decrease in titer was one of the problems encountered in this research. A comparison of the tests showed that the heat-activated sera gave more distinct results and sharper end points. However, the titers were slightly varied.

Due to the small amount of positive sera available, the indications of the research can only point to the potentiality of this test; and if more positive sera were available, it would have helped prove the full potential of the test.

Table 8. Comparison of agglutination test with microscopic slide and macroscopic tube flocculation test for pullorum with 445 unheated sera.

	Tube Agglutination Test										Tube Flocculation Test										Microscopic Slide Flocculation Test									
	Dilutions										Serum Dilutions*																			
	10	20	40	80	160	320	640	1280	2560	5120	10	20	40	80	160	320	640	1280	2560	5120	U	10	20	40	80	160	320	640	1280	2560	5120
Goat 7-16-56	4	4	4	4	4	2	-	-	-	-	+	+	+	±	-	-	-	-	-	-	+	+	+	±	-	-	-	-	-	-	-
Goat Wood & Baldwin	4	4	4	4	3	3	3	2	1	-	+	+	+	±	+	+	±	-	-	-	+	+	+	±	+	+	-	-	-	-	-
Goat Nelson & Whiteham	4	3	3	3	3	2	1	-	-	-	+	+	+	+	+	±	-	-	-	-	+	+	+	+	+	±	-	-	-	-	-
Goat Vera & Ward	3	3	3	3	3	2	1	1	-	-	+	+	+	+	+	±	±	±	-	-	+	+	+	+	+	±	±	±	-	-	-
Goat 90999	4	4	4	3	2	2	1	-	-	-	+	+	+	+	±	±	-	-	-	-	+	+	+	+	±	-	-	-	-	-	-
Goat Huff & Jackson	2	2	2	2	2	2	2	-	-	-	+	+	+	+	±	±	-	-	-	-	+	+	+	+	±	±	-	-	-	-	-
Goat X	2	2	2	1	-	-	-	-	-	-	+	+	±	-	-	-	-	-	-	-	+	±	±	-	-	-	-	-	-	-	-
Goat Pilson	4	4	4	3	3	2	-	-	-	-	+	+	±	+	-	-	-	-	-	-	+	±	±	±	-	-	-	-	-	-	-
S. typhosa	3	3	3	2	1	-	-	-	-	-	+	+	+	±	-	-	-	-	-	-	+	±	-	-	-	-	-	-	-	-	-
Human "O" negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 10-16-56	3	3	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Turkey 10-23-56																															
30 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 10-24-56																															
30 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 10-27-56																															
60 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-2-56																															
75 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-3-56																															
50 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-8-56																															
50 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-8-56																															
75 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-13-56																															
1 serum	-	-	-	-	-	-	-	-	-	-	+	+	+	±	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-
Chicken 11-8-56																															
16 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-13-56																															
15 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-23-56																															
12 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-29-56																															
20 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Footnote: *Results of microscopic slide flocculation test cannot be compared with the tube test since 0.05 ml of each dilution was used with 0.0125 ml of antigen emulsion.
(+) positive; (±) weakly positive; (-) negative, no flocculation.

Table 9. Comparison of agglutination test with microscopic slide and macroscopic tube flocculation test for pullorum with 445 heated sera.

	Tube Agglutination Test													Tube Flocculation Test								Microscopic Slide Flocculation Test									
	Dilutions																					Serum Dilutions*									
	10	20	40	80	160	320	640	1280	2560	5120	10	20	40	80	160	320	640	1280	2560	5120	U	10	20	40	80	160	320	640	1280	2560	5120
Goat 7-16-56	4	4	4	3	2	1	1	-	-	-	+	+	+	±	-	-	-	-	-	-	+	±	±	-	-	-	-	-	-	-	-
Goat Wood & Baldwin	4	4	3	2	2	2	1	1	-	-	+	+	+	±	+	+	+	±	-	-	+	±	±	+	±	±	-	-	-	-	-
Goat Nelson & Whiteham	3	3	3	2	2	2	1	-	-	-	+	+	+	+	+	+	±	-	-	-	+	+	+	+	±	±	±	-	-	-	-
Goat Vera & Ward	3	3	3	3	2	2	2	2	1	1	+	+	+	+	+	+	±	-	-	-	+	+	+	+	+	±	±	±	-	-	-	-
Goat 90999	4	4	4	3	2	2	1	1	-	-	+	+	+	+	+	+	±	±	-	-	+	+	+	+	+	±	±	-	-	-	-	-
Goat Huff & Jackson	2	2	2	2	2	-	-	-	-	-	+	+	+	+	+	+	+	±	±	-	+	+	+	+	+	±	±	±	±	-	-	-
Goat X	4	4	4	4	1	-	-	-	-	-	+	+	+	+	+	±	-	-	-	-	+	+	±	±	-	-	-	-	-	-	-	-
Goat Pilson	3	3	3	2	1	-	-	-	-	-	+	+	+	+	+	±	-	-	-	-	+	+	±	±	±	±	-	-	-	-	-	-
S. typhosa	3	3	3	2	2	1	1	-	-	-	+	+	+	+	+	±	-	-	-	-	+	±	±	±	±	±	-	-	-	-	-	-
Human "O" negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 10-16-56	3	3	3	2	-	-	-	-	-	-	±	±	±	-	-	-	-	-	-	-	+	±	±	-	-	-	-	-	-	-	-
Turkey 10-23-56																																
30 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 10-24-56																																
30 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 10-27-56																																
60 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-2-56																																
75 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-3-56																																
50 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-8-56																																
50 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-8-56																																
75 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turkey 11-13-56																																
1 serum	2	-	-	-	-	-	-	-	-	-	+	+	±	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-8-56																																
16 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-13-56																																
15 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-23-56																																
12 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chicken 11-29-56																																
20 sera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Footnote: *Results of microscopic slide flocculation test cannot be compared with the tube test since 0.05 ml of each dilution was used with 0.0125 ml of antigen emulsion.
(+) positive; (±) weakly positive; (-) negative, no flocculation.

DISCUSSION

The use of cholesterol coated with cardiolipin and lecithin as an antigen for serological tests for syphilis have shown that the tests can be standardized. They are reproducible and have acceptable sensitivity and specificity (Hunter and Colbert, 1954). No other laboratory procedure has so high a level of reproducibility, from laboratory to laboratory, as this serological test for syphilis. The use of cholesterol coated with a dialyzed, cell-free, ectoantigen extract of S. pullorum should also produce an antigen in which the sensitivity and specificity can be set, thereby producing a standard antigen. The use of this antigen in performing the flocculation tests for pullorum disease will give uniform results that are reproducible in many laboratories. Furthermore, the test can be run in a shorter period of time, than the 24 hours required for the S. pullorum tube agglutination test. The tests are much easier to read than the agglutination tests, and mistakes in interpretation of the titer will be decreased. The pullorum extract used in this work was very satisfactory, but if work is to continue on this problem an effort should be exerted to produce a fraction or fractions which will be more specific. If this test were to be used, especially the microscopic slide flocculation test, it probably would be necessary to re-evaluate the significance of titer with proven cases to establish a "significant standard titer" for diseased fowl.

SUMMARY

1. A method of producing a cholestrol-lecithin-pullorum extract antigen which can be used in performing a microscopic slide flocculation test and a macroscopic tube flocculation test has been described.

2. The sensitivity and specificity of the antigen can be adjusted to conform to the standard S. pullorum tube agglutination test.

3. A method of standardizing the antigen emulsion is described.

4. The tests can be performed rapidly and the results are clear cut and very easily read.

5. The tests can be readily reproduced in any laboratory.

6. Heating the sera to 56°C for 15 minutes gives a better distribution of the floccules and subsequently gives more accuracy in interpretation of the results.

ACKNOWLEDGMENTS

Grateful acknowledgment is hereby made to Professor V. D. Foltz, major professor, who directed this research and for his guidance and constructive criticism; to Dr. C. A. Hunter, Director, and the staff of the Kansas State Public Health Laboratories for their helpful suggestions; to Dr. S. Rosenberg and Dr. L. E. Erwin who furnished material amounts of reagents and sera necessary in this research; to Dr. K. J. McMahon for conducting the class experiment; to Dr. T. H. Lord for his highly constructive criticisms and kind assistance in the preparation of this manuscript; and to my wife who was ever encouraging during the preparation of this thesis.

Appreciation also is expressed to all others who have made helpful criticisms in the preparation of this manuscript and who furnished material so that this work could be carried out.

BIBLIOGRAPHY

Breed, Robert S., E. G. D. Murray and A. Parker Hitchens.
Bergey's manual of determinative bacteriology. 6th ed. Baltimore: Williams and Wilkins Co. p. 521. 1948.

Bunyea, Hubert.
Use of the rapid whole-blood test for pullorum disease. U. S. Dept. of Agr. Misc. Pub. 349 17 p. 1939.

Bushnell, L. D.
Comparison of serologic and ~~pullorum~~ pullorin tests for bacillary white diarrhea. J. Infect. Dis. 43: 60-66. 1928.

Bushnell, L. D. and C. A. Brandly.
Comparison of tube and slide agglutination tests for bacillary white diarrhea. Am. Vet. Med. Assoc. Jour. 73: 844-847. 1928.

Bushnell, L. D. and C. A. Brandly.
A comparison of the pullorin reaction and the agglutination test for bacillary white diarrhea. Am. Vet. Med. Assoc. Jour. 73: 364-371. 1929.

Bushnell, L. D. and C. B. Hudson.
Complement fixation and agglutination tests for Salmonella pullorum infection. J. Infect. Dis. 41: 388-394. 1927 a.

Bushnell, L. D. and C. B. Hudson.
Preparation of Salmonella pullorum antigens for complement fixation tests. J. Infect. Dis. 41: 383-387. 1927 b.

Ferry, N. S. and L. W. Fisher.
Production of immunity with a soluble antigen of the Pneumococcus. J. Lab. and Clin. Med. 10:817-823. 1925.

Galloway, Dale O. and D. Frank Holtman.
A comparison of rapid and tube antigens in agglutination tests on poultry blood. Jour. Bact. 54:36. 1947.

Harris, A., A. A. Rosenberg and E. R. Del Vecchio.
A macroflocculation test for syphilis using cardiolipin-lecithin antigen. J. Ven. Dis. Inform. 29:313-316. 1948.

Hunter, Charles A.
Personal communication. Public Health Laboratory, Kansas State Board of Health. 1956.

Hunter, Charles A. and Bernice Colbert.
Flocculation test for brucellosis. J. Immunol. 77:232-241. 1956.

Hunter, Charles A. and Bernice L. Colbert.
Microscopic slide flocculation and macroscopic tube flocculation tests for brucellosis. The Public Health Laboratory. 12:82-87. 1954.

Kline, B. S.
Microscopic slide precipitation tests for the diagnosis and exclusion of syphilis. Ven. Dis. Inform. Supp. 11:45-55. 1940.

Reid, Roger D.
The agglutination test for detecting Salmonella pullorum infection in chicks. Jour. Bact. 35:62. 1938.

Simmons, James Stevens and Cleon J. Gentzkow.
Laboratory methods of the United States Army. 5th ed. Philadelphia: Lea and Febiger. p. 325. 1944.

MICROSCOPIC SLIDE FLOCCULATION AND MACROSCOPIC TUBE FLOCCULATION TESTS FOR PULLORUM DISEASE AND A COMPARISON OF THEM WITH THE TUBE AGGLUTINATION TEST

by

Edward Paul Roznowski, Jr.

B. S. in Ed., Illinois State Normal University, 1955

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

rotating the bottle one ml of one per cent cholesterol solution was added drop by drop, continuing the rotation for 20 seconds; 0.10 to 0.20 ml of one per cent lecithin was added and the bottle was shaken vigorously for 15 seconds; 0.10 to 0.25 ml of the pullorum ectoantigen was added followed by vigorously striking the bottom of the bottle against the palm of the hand for one minute; and finally 2.5 ml of Eagle's buffered saline (pH 7.4) were added and the bottle was shaken less vigorously for 30 seconds. The antigen emulsion may be used immediately or, if stored in the refrigerator, any time within 24 hours.

The blood serum to be examined was centrifuged and the serum removed by pipetting or decanting. The serum was heat-activated at 56°C for 15 minutes. If the serum were to be re-examined after 24 hours or later, it was reheated to 56°C for five minutes.

Five hundredths ml of the serum was pipetted into a paraffin or permanent ring on a glass slide. With an 18 gauge needle (the bevel of which was filed off) held perpendicularly, one drop of antigen emulsion was added to the serum. The needle delivered 80 to 85 drops per ml. The slides were rotated at 180 rpm for four minutes (a longer rotation time increases the sensitivity and makes the reaction more difficult to read). The reactions were observed at a magnification of 100 X. A saline (0.85%) control was always included in the set up. The reactions were read as follows:

Positive:	Large and medium size clumps
Weakly Positive:	Small clumps with individual particles
Negative:	No clumps

In a number of instances positive reactions were obtained with the 0.05 ml of serum necessitating additional dilutions of the serum in saline (0.85%). (It is recommended that two-fold dilutions be made from 1:10 to 1:640.) The

test was then run using 0.05 ml of each dilution. The titer of the serum run by the microscopic slide test could be compared with the titer obtained by the regular agglutination test.

The antigen emulsion for the tube test was prepared as for the microscopic slide test, and was diluted one part with four parts of one per cent NaCl solution. (This dilution of the antigen emulsion can be used immediately or after standing as long as 24 hours. It was found to be good practice to let the antigen stand for five minutes before using it in the test.) The serum was heated at 56°C for 15 minutes, and, if it were to be re-examined another day, it was reheated for five minutes at 56°C. Two-fold dilutions of the serum from 1:10 to 1:640 were made using 0.85 per cent saline. In some instances it was necessary to prepare higher dilutions. To 0.5 ml portions of the diluted serum 0.5 ml of the diluted antigen emulsion was added, so that the final serum dilution ranged from 1:20 to 1:1280. The tubes were placed on the Kahn shaker and shaken for five minutes. After shaking the tubes were centrifuged for ten minutes at 2000 rpm. After the tubes were removed from the centrifuge, they were again put on the Kahn shaker and shaken for one minute. The following results were read by holding the tubes in front of a reading lamp placed against a black background:

Positive:	Large clumps and clear supernatant
Weakly Positive:	Clumps but cloudy supernatant
Negative:	No clumps but a cloudy supernatant which gives a swirl similar to that seen in a mixture of blood in saline.

In preparing an antigen for a flocculation test, it must be standardized so it will give the same sensitivity and specificity as the standard tube agglutination test. The S. pullorum tube agglutination test was set up in a varying dilutions of 1:10 to 1:5120, and 15 sera were tested to establish their titer. Nine sera were selected so that their titer were varied and

could be used in the standardization of the pullorum extract and lecithin (1%). The standardization was carried out on heated sera, as the final tests were to be run on heated sera. Thirty-two antigen emulsions were used, varying the ratio of lecithin (1%) and pullorum extract. The microscopic slide flocculation test was run on the nine sera with 32 antigen emulsions, and the final ratio used to compare with the standard tube agglutination test was found to be 0.15 ml of lecithin (1%) and 0.15 ml of pullorum extract.

The results of this research indicate: a) that the test will give best reactions when run on heat-activated serum, b) that the positive tests are distinct, and c) that the end points of each dilution is sharp and definite. Due to the small number of positive sera available, the results give only an indication of the possible use of such a method as a test for pullorum disease in fowl.