A Comparison of the Tube, Rapid Serum, and Rapid Blood Drop Agglutination Tests for the Detection of Carriers of Salmonella Pullorum

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Salmonella Pullorum or Bacterium Pullorum was first described by Rettger (1900), though at that time the disease was known as Septicemia instead of White Diarrhea.

Rettger and Harvey in (1908) working with the same organism demonstrated its identity with the disease commonly known as White Diarrhea by the fact that the serum of chicks inoculated with killed cultures would agglutinate the homologous organism.

Jones in (1911) showed that chicks recovering from the original infection may carry the organism in their ovaries. Therefore, in light of available information, Jones suggested the use of the agglutination test for the detection of adult carriers of this disease.

Jones (1911 & '12) described the macroscopic agglutination method of testing fowls to determine the carriers of Bacterium or Salmonella Pullorum. He also described the method of collecting the blood samples and for making the antigen or testing fluid. He reported twenty-one birds as giving a positive reaction to the test and was able to isolate the organism from the ovaries of twenty of these birds.

This agglutination test as developed by Jones has been widely used for testing flocks in order to eliminate the adult birds carrying the infection, and has been generally accepted as the standard test.

Ward and Gallagher (1917) announced the discovery of the intradermal or pullorin test. This test consisted of the injection of a carbolized culture in the wattle. At the expiration of thirty hours readings were made and a swelling was expected in the wattles of infected birds. In their experiment 90 per cent of the artificially infected birds reacted to the test. About 6 percent failed to react from which the organism was recovered and 3 per cent failed to react in which the organism was not recovered, which was as expected.

Field experiments conducted with the intradermal and agglutination tests failed to agree when the reading was made 38 hours after injection. In another experiment the two tests were in agreement in 70 percent of the cases.

The intradermal test has not shown up very favorably when compared with the agglutination tube test as shown by the following references:

Edwards and Hull (1927) compared the agglutination and intradermal tests in which they report very poor correlation results, indicating the agglutination test to be much more reliable for detecting carriers of Bacterium Pullorum than the intradermal test.

Michael and Beach (1929) considered the agglutination test more satisfactory than the intradermal with pullorin.

Graham, Tunnicliff, and McCulloch (1928) found the intradermal test to agree with the agglutination test in 66.9 percent of the cases.

Rettger, McAlpine, and Warren (1930) found the intradermal test to give results which often failed to agree with those obtained by the agglutination methods. The differences were most marked with the negative groups and were so great in the second experiment as to render the method as applied useless.

Stafseth and Thorp (1928) in comparing the agglutination and Pullorin tests, found the pullorin test decidedly inferior.

*Note: The work reported in this bulletin was completed in February 1931, and the manuscript prepared and submitted to the Director on March 1931. Due to changes in personnel and a drastic reduction in Experiment Station funds, publication has been delayed.
Runnells, Coon, Farley, and Thorp (1927) reported the application of the rapid method of agglutination. This method differs from the tube method in that a small quantity of blood serum is placed on slide or pane of glass to which is added a drop of highly concentrated antigen (density about 50 times McFarlane's No. 9). If positive the reaction takes place in one-half to one minute with a distinct clumping throughout the sample.

Gavatkin (1928) reported the tube and rapid agglutination tests equally satisfactory for the detection of B. W. D. carriers.

Beach and Michael (1929) found the tube method and rapid serum method equally reliable for performing the agglutination test.

Stafseth and Thorp (1928) from results obtained in using the rapid agglutination predicted that it may become the most useful and practical test for controlling Bacillary White Diarrhea.

Bunyea, Hall, and Dorset (1929) brought forth still another method of applying the agglutination test, which was described as a rapid field agglutination test. This method is commonly spoken of as the rapid blood drop test and differs from the rapid serum test, in that whole fresh blood is used in the former, blood serum in the latter. The authors called attention to the fact that this method overcomes some of the difficulties encountered with other methods, such as time element, re-handling of fowls, necessity for drawing large blood samples and spoilage of blood sample in transit.

Beach and Michael (1930) reported work based on method devised by Hall, Dorset, and Bunyea in which 3,727 birds were tested. Results reported showed 43.6 percent of which reacted to blood drop test and 39.4 percent reacted to blood film test. Results were secured which varied when different dilutions were used on the same samples.

Sawyer and Hamilton (1930) conducted tube and whole blood tests at four week intervals on thirty birds in conjunction with two other laboratories. They found the results of the two methods to be identical, each showing 15 positive and 15 negative fowls.

The rapid blood drop test has been used for two years on the Mississippi Station flocks. In 1929 and 1930, four positive reactors were found in the 150 birds tested. All of these gave positive reactions to the tube test. In 1930 and 1931 five positive reactors were found in the 375 birds tested. All of these gave positive reaction to the tube test. The 370 birds giving negative reaction were all re-tested by the rapid blood drop method about six weeks later and gave 100 percent negative reactions. To date the chick mortality has been 5.3 percent out of a total of 1,203 chicks. These chicks are now from one to seven weeks old. We have been unable to show White Diarrhea to be responsible for the death of any of the chicks.

PURPOSE OF STUDY

To determine the efficiency of the rapid blood drop agglutination method for detecting carriers of Salmonella pullorum. The tube and rapid serum agglutination methods were used as a check to determine efficiency, and to give a direct comparison of the results of the three methods.

EXPERIMENTAL METHODS

In this study a total of 1,250 birds (hens, mature pullets, and males) were tested by the three agglutination methods. This work was done during the fall and winter seasons of 1929-1930 and 1930-1931.

The antigen used in conducting the tube test was prepared from four pure strains of Bacterium pullorum. The method of preparing the antigen was the same as that described by Gage and Paige (1915) except that NAOH was added. In the first season's work four dilutions, 1-400, 1-200, 1-100, and 1-30 were used. The results of the four checked very closely, but the 1-30 dilution gave slightly better reaction and was used exclusively in the second season's work. The technique of Jones (loc.cit) for carrying out the test was used in the first season's work and that of Beaudette (1923) in second season's work.
Some of the antigen used for the rapid serum and rapid blood drop tests was secured from a commercial laboratory, some from the department of Bio-Chemistry, Washington, D. C. through the courtesy of Dr. Bunyea, and some was prepared in the Mississippi State College laboratory. All of this antigen, regardless of source, was prepared by the method devised by Bunyea, Hall, and Dorset (loc.cit.). It should be noted, however, that the commercial antigen was free of color, while that from the department at Washington and the Mississippi State College laboratory was colored. In conducting both the rapid blood drop and the rapid serum tests, one drop of blood or serum was placed on a pane of glass to which was added one drop of antigen, the two were then stirred together or rotated.

Sixteen flocks embracing 1,250 birds in the territory around the College were used in making these tests. The rapid blood drop test was conducted under field conditions on the farms represented. At the same time a sample of blood was collected from each hen and numbered to correspond with that of the leg band number placed on the hen. The positive or negative reaction of the blood drop test was also recorded on the label of the blood sample by a plus or minus sign. These samples were taken immediately to the laboratory and kept in a refrigerator until sufficient blood serum was available for the tests. A permanent record was made of the results of rapid blood drop test made in the field. This record also contained columns for recording the results of the serum tests. To insure accuracy in conducting the rapid serum test, blocks were used and numbered by rows, six rows deep and twelve rows long, holding seventy-two samples. The pane of glass was lined off in squares with rows corresponding to those of the blocks.

**RESULTS**

**Table I**

<table>
<thead>
<tr>
<th>Flock Number</th>
<th>Number Birds</th>
<th>Positive Reactors</th>
<th>Negative Reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>256</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>114</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>72</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>59</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>88</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>84</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>88</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>60</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>50</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>108</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>96</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1250</td>
<td>265</td>
<td>286</td>
</tr>
</tbody>
</table>

Table One shows the number of flocks tested and the positive and negative reactors to the three methods. It will be noted that the smallest number of positive reactors (265) occurred with the rapid blood drop method, the second smallest (286) with the tube, and the largest (285) with the rapid serum. Expressed in terms of percent positive of total numbers tested, we have for rapid blood drop 21.2 percent, for rapid serum 22.8 percent and for tube 21.44 percent. These figures represent a slight variation in the results of the three tests, leaving very little choice in the methods when the total average results are considered. However, the individual variations occurring between the methods should be a more accurate indication or means of measuring the true efficiency of the different methods than a total average of results secured.

In checking up we find there is a variation of 31 between rapid blood drop and tube, 31 between rapid serum and tube, and 24 between rapid blood drop and rapid serum, or 2.48 percent, and 1.92 percent respectively. Here again we have a rather
close correlation of results, but a considerably greater variation than in the comparison of total average results.

Chart 1 shows graphically the relationship between the positive reactors by the three methods by flocks. It will be noted that there is very little spread between the three curves.

Of the 1,250 birds tested, 77 were males. Two of these, or 2.63 percent, were positive to the rapid blood drop and rapid serum tests, while one was positive and one negative to the tube test. Hence, one male gave positive reaction to all three tests and the other positive to both rapid tests and negative to tube.

Some observations and experiences in applying the rapid blood drop test under field conditions, we feel, are worthy of recording.

Dust particles did not prove to be serious enough to cause the test to be misinterpreted, especially when colored antigen was used. However, it is desirable to do the testing on a table about three feet high, and placed some distance from the poultry house, with any breeze from table toward the house. The helper should remove feathers from under the wing and quiet the bird before approaching the testing table. The observance of these suggestions will reduce the quantity of dust and foreign particles to the extent that it will not interfere with the test in the least.

We also observed that in all cases where the reaction took place immediately in the rapid blood drop test, that the results checked with those of the other two methods in almost all cases. The slow and questionable reactors to the rapid blood drop were also slow and questionable reactors to the tube test. The rapid serum test was more definite in positive reactions on these birds than either of the other two methods as evidenced by the fact that more positive birds (285) were recorded by this method.

It was found that a cold testing surface and low temperature retarded the reaction with the rapid blood drop test, warm weather or artificial heat being necessary to satisfactory tests.
In securing the sample the blood should be taken as it comes from the vein and the antigen applied before coagulation takes place. If coagulation is permitted it is more difficult to read the reaction.

For best results the antigen must be shaken thoroughly at frequent intervals, especially if colored antigen is used.

The clumping in positive reactors was more pronounced with colored antigen and the results checked more closely with the rapid serum and tube tests. A dark background was unnecessary when the colored antigen was used.

**DISCUSSION**

In the historical review given in this paper (loc-Cit) the four known methods for detecting carriers of Bacterium pullorum were considered. The results as reported by numerous workers show the tube method to be extremely valuable in the detection and eradication of White Diarrhea. The close agreement of results secured by the use of the tube method has led to the adoption and acceptance of this method as the standard for conducting the test. It should be pointed out, however, that this method has failed to give identical results when applied by different individuals on blood samples from the same hen. In like manner variations between the results of the test, post-mortem findings, and recovery of the organism from birds having given positive reactions have been reported by numerous workers. It has been shown that this method, if used at frequent intervals in conjunction with sanitary precautions, will rid a flock of the disease. While this method of testing has been adopted as standard and will eradicate the disease from a flock, it is not, by any means, infallible.

The results reported show marked variations between the intradermal and the tube test. The conclusion reached in most cases is that the intradermal test is not satisfactory for the detection of carriers of Bacterium pullorum. Furthermore, the injection of the pullorin has been shown to interfere with subsequent tests conduct-
ed by the tube agglutination method. Then it seems that this method in its present state of development might be eliminated from consideration as a reliable or desirable method for the detection and eradication of white diarrhea from poultry flocks.

The rapid serum method as reported by Runnels, Coon, Farley, and Thorp has been checked by several stations. This method has given uniformly good results in comparison with the tube method. The conclusion having been reached that this method is as reliable as the tube method for detecting carriers of Bacterium pullorum. Several workers have predicted that this method might become the most practical and useful of the two.

Bunyea, Hall, and Dorset reported the rapid blood test in 1929. So far as we know these workers have not reported any comparative data in connection with this and other agglutination methods of testing.

The California Station reported some comparative work on the tube, rapid blood drop and rapid blood film tests in 1930. Their results showed that slightly over half of the birds giving positive reactions to the tube method gave positive reactions to the blood drop and slightly over one-third to the blood film methods.

In contrast to the results reported by the California Station, the Western Washington Station in conjunction with two other laboratories, found complete agreement between the results of the rapid and tube methods. While there were only thirty birds tested by the Washington Station as against 3,727 at the California Station, the fact that three laboratories reported identical results would in a measure compensate for the difference in numbers.

The two citations referred to seem to be the only available data other than that reported in this paper on the comparative efficiency of the tube and rapid blood drop methods for detecting carriers of Bacterium pullorum. Of the three reports the California Station results show a rather wide variation in results secured. The Washington Station report shows complete agreement by three laboratories in results secured, and the results of the work reported in this paper show a very slight variation in results obtained by the rapid blood drop, rapid serum, and tube methods.

In our work there were 31 individual variations between the reactions given by the rapid blood drop and tube tests. Of these there were 17 negative to rapid blood drop and positive to tube, 14 positive to rapid blood drop and negative to tube.

There were 31 individual variations between the reactions given by the rapid serum and tube tests. Of these there were 7 negative to rapid serum and positive to tube, 24 positive to rapid serum and negative to tube.

There were 24 individual variations between the reactions given by the rapid blood drop and rapid serum tests. Of these there were 22 negative to rapid blood drop and positive to rapid serum, 2 positive to rapid blood drop and negative to rapid serum.

There was a very small variation between the three testing methods in the total number of positive and negative reactors as shown in Table I, the rapid serum showing 25 more birds positive than rapid blood drop, and 17 more birds positive than tube. In individual variations there were 31 birds showing a different reaction between rapid blood and tube, the same number (31) between rapid serum and tube, and 24 between rapid blood drop and rapid serum. Each of the rapid tests in comparison with the tube gave a slight increase in individual variations over the combined totals, but in comparison with each other a decrease. In all three methods the individual variations even though slightly larger than the totals suggest less practical difference because the variations were not all positive or negative, being 17 negative, 14 positive between rapid blood drop and tube; 7 negative, 24 positive between rapid serum and tube, and 22 negative, 2 positive, between rapid blood drop and rapid serum. In individual variations the smallest difference occurred between the two rapid methods, 24 against 31, between both rapid methods and tube. This difference may not be especially significant, but is certainly not unfavorable to the rapid blood drop method. The close agreement of results secured by all three methods suggest a high degree of efficiency for all methods in detecting carriers of Salmonella pullorum.

Efficiency is not the only index to the value of a testing method. Economy of
Illustration No. I. The above is a photograph of a positive reaction.

Illustration No. II. The above is a photograph of a negative reaction.
time, labor, and money are also important factors. In addition the possible spoilage of samples and errors in technique must be considered.

The results obtained with the rapid blood drop from the standpoint of efficiency, as measured by the two comparative tests, and the actual results in the field, especially on the College flocks, as measured by a complete negative set of reactions on subsequent test and the livability of the chicks from the community flocks as well as the college flocks, are apparently in agreement. The advantages of the simplified rapid blood drop method have been outlined by those (loc.cit) discovering the method, and no explanation of it is essential here. These advantages along with the efficiency as shown in our comparisons indicate unquestionably that this method is deserving of much consideration of those interested in the practical control and eradication of white diarrhea.

CONCLUSIONS

1. Comparative tests between the rapid blood drop, rapid serum, and tube methods gave very slight variations between the total average positive and negative reactions.

2. There were 31 individual variations between the results of the rapid blood drop and tube, 31 between rapid serum and tube, and 24 between rapid blood drop and rapid serum methods.

3. Colored antigen gave a much more conclusive or positive test than clear antigen.

4. The rapid blood drop method was found to be practical and efficient under field conditions.

5. The rapid blood drop method was more economical in time, labor, and money than the tube or rapid serum tests.

6. The rapid blood drop method when applied to the Mississippi Station flock detected five reactors on the first test, and no reactors on second test six weeks later. The mortality of chicks from these flocks has been 5 percent with no indications of white diarrhea.

7. In view of the fact that the rapid blood drop method was found to be practical, efficient, and economical, it appears that this method should be given recognition by being placed on a parity with the tube and rapid serum methods, and that it might be recommended to the poultry breeders as a reliable, practical method for detecting carriers of Bacterium Pullorum.

ACKNOWLEDGEMENTS

We are indebted to Dr. H. D. Bradshaw for the many valuable suggestions given, and for the use of his laboratory and equipment.

REFERENCES


Beach, J. R. & Michael, S. T.—The Rapid Agglutination Test with Whole Blood for the Detection of Fowls that are Carriers of Bacterium Pullorum—Cal Vet. Sta. Bulletin 286 (1930) P P 18-23 Fig. 1 also North America Vet. 11 (1930) No. 2 P P 43-46 Fig. 2.


