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Comparison of the Tube and Rapid Serum Agglutination Tests for the Detection of Pullorum Disease in Turkeys

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TURKEY producers in recent years have been confronted with the problem of pullorum disease control and eradication. Under the circumstances it is only natural that the agglutination test has become an important part of this program. With the use of the test in its various forms the question has arisen concerning the accuracy and efficiency of the different methods. Hinshaw *et al.* (1940) have shown that the stained-antigen rapid whole-blood test is approximately one half as efficient in detecting turkeys that are carriers of *Salmonella pullorum* as is the tube test.

The object of this report is to present a comparison of the tube and rapid serum agglutination tests conducted on 10,019 blood samples from turkeys during the past two years.

PROCEDURE

The medium used for growing the organisms for antigen production was prepared as follows:

Nutrient agar 1.5 per cent (Difco)	31 grams
Gelatin	20 grams
Peptone	15 grams
Distilled water	1,000 cc

The dry ingredients were dissolved in the water by heating in the autoclave for three 10-minute intervals at 15 pounds pressure. Between each interval the medium was thoroughly agitated by shaking. After the medium was poured into the tubes, they were plugged and sterilized for 20 to 25 minutes at 15 pounds pressure. The tubes were then slanted and the medium allowed to solidify. After solidification the tubes were placed in a bacteriological incubator (37.5° C) in a horizontal position for three or four days. This was to check sterility. It served a dual purpose, however, because after three or four days in this position the surface of the medium was firm and water of condensation was practically eliminated. For producing an abundant growth of *S. pullorum*, this peptone-gelatine fortified medium, freed of water of condensation, has given excellent results.

The antigen was prepared for both tests from four strains of *S. pullorum*, which were obtained originally from the Department of Veterinary Science of Massachusetts State College about 1923. An equal number of 1″ x 8″ tubes of bacteriological medium was seeded with each strain of *S. pullorum*.

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After generous seeding from fresh cultures of *S. pullorum* the tubes were incubated for about 48 hours at 37.5° C. The growth was washed from the medium, using a minimum of an aqueous solution of 12 per cent sodium chloride and 0.25 per cent phenol and dislodging the growth with a cotton swab. The washings were filtered through a pad of gauze, cotton, and glass wool and the density was standardized to a reading of 7 mm by a Gates nephlometer. The tube antigen was prepared in the same manner except that an aqueous solution of 0.85 per cent sodium chloride and 0.5 per cent phenol was used and the density was adjusted to a reading of about 100 mm by a Gates nephlometer. Further, just before using, 1 cc of N/1 sodium hydroxide solution was added to each 1000 cc of tube antigen to produce a pH of about 8.0 to 8.2.

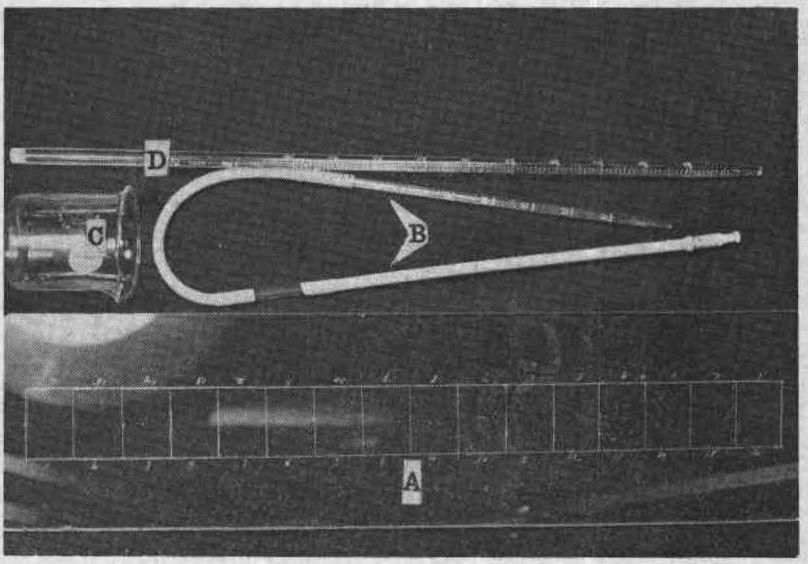

Figure 1. Equipment used in conducting the rapid serum agglutination test. *A:* Plate on which tests are conducted. *B:* Capillary pipette with rubber tube for measuring serum and mixing the test. *C:* Beaker for water to rinse capillary serum pipette. *D:* Pipette (1 cc graduated in hundredths) for measuring antigen.

The rapid serum agglutination test for chickens was conducted on a piece of triple strength window glass marked with a continuous row of rectangles 7/8 x 1¼ inches in size. (See Figure 1.) This space allows for an oval distribution of the test that has certain advantages for stirring and agitation. The dilution was made by placing .05 cc of rapid pullorum antigen in the center of the test space and with a finely calibrated capillary pipette adding .005 cc of serum. The serum pipette was equipped with a rubber tube and mouthpiece so that the serum could be easily controlled and discharged into the antigen. After discharging the serum the end of the pipette was used to mix thoroughly the serum and antigen. After 16 such tests were completed, the plate was rocked to agitate the tests further. Most strong positive reactions were plainly evident within 15 to 20 seconds. The final reading was made within 2 to 3 minutes. (See Figure 2.)

Since Hinshaw *et al.* (1940) found it desirable to use 1:25 instead of 1:50 for the diagnostic dilution for turkey blood samples, all the rapid serum tests on turkey samples were conducted with dilutions of .05 cc of antigen to .01 cc of serum. In other words, a dilution of 1:5 was used for turkeys and a 1:10 dilution was used for chickens.

The standard tube agglutination test used for comparison was conducted with dilutions of 1:50 and 1:100. For these comparisons a complete reaction in 1:50 was considered positive even though the 1:100 dilution showed little or no reaction. This laboratory has always used 1:100 as a tube agglutination diagnostic dilution for chicken samples because the antigen used has been of a

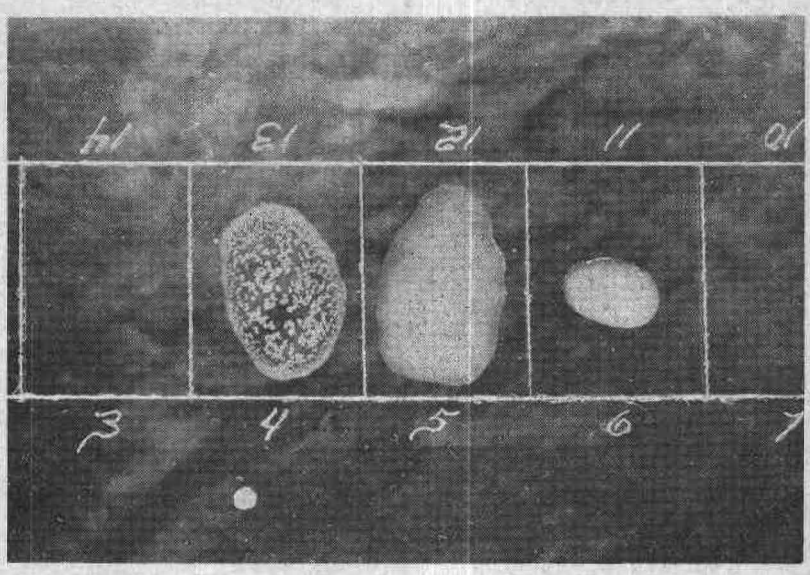

Figure 2. Rapid serum agglutination test (actual size). *4:* Positive reaction. *5:* Negative reaction. *6:* A spot (.05 cc) of antigen before serum has been added and the test stirred and spread on the test space.

lighter density than that used by many laboratories. Further, this dilution has proved highly efficient in detecting carriers of *S. pullorum* as determined by autopsy and bacteriological recovery of the organism from reactor birds.

RESULTS

During the 1941-42 testing season, 162,743 turkey blood samples were tested by the rapid serum method and 9,382 samples were selected for comparison with the tube test. During the 1942-43 testing season, 177,795 turkey blood samples were tested by the rapid serum method and 1,637 samples were selected for comparison. The samples selected for comparison were all those found positive or suspicious on the rapid serum test as well as many totally negative samples. Table 1 gives the comparative results.

Table 1. RESULTS OF COMPARISON OF RAPID SERUM AND TUBE TEST

Season	Total tests	Negative both tests	Positive both tests	Positive rapid negative tube	Positive tube negative rapid	Total positive
1941-42	9,382	8,744	533	49	56	638
1942-43.....	1,637	1,597	38	0	2	40

If the 638 positive reactions recorded during 1941-42 are considered as 100, then 589 positive tube tests gave an accuracy of 92.3 per cent, while 582 positive rapid serum plate tests gave an accuracy of 91.2 per cent. Both tests were positive on 533 samples. The agreement between both tests was 83.5 per cent of the total diagnosed as positive. Since suspicious reactions were regarded as negative, they have not been recorded separately. It was interesting to note, however, that practically all the variations occurred between positive and suspicious reactions rather than between positive and absolutely negative reactions.

For the 1942-43 season, 40 samples were diagnosed as positive. Of these, 38 were positive on the rapid serum test and all 40 were positive on the tube test. Considering the 40 as 100, the rapid serum test was 95.0 per cent as accurate as the tube test.

DISCUSSION

The rapid serum agglutination technique for the detection of chickens that carry *S. pullorum* has been used routinely at the Oregon Agricultural Experiment Station since 1928. The test employed is basically similar to that described by Runnels *et al* (1927).

Before this method was employed as the routine test, more than 35,000 comparison tests (data unpublished) were conducted by the senior author with the rapid serum and the standard tube agglutination tests. Less than 5 per cent disagreement was found between the two tests. Further, bacteriological studies on some of the chickens that reacted to one test and not to the other showed that each test had detected infected birds that the other had missed. Biely *et al.* (1931) in a cooperative testing program, in which the Oregon Agricultural Experiment Station participated, also showed the accuracy of the rapid serum plate test for detecting chickens that were carriers of *S. pullorum.*

It appears from the two years' testing results reported that definite control and eradication is being accomplished. Of the 40 reactors diagnosed in 1942-43, 30 of them were found in one flock. Further, of the hundreds of turkey poults examined during the brooding season of 1943, only 5 cases of pullorum disease were encountered. In all 5 cases the poults had been hatched in hatcheries that also hatch chicks. Three of the cases were from poults custom hatched in the same hatchery. Pullorum disease had been diagnosed in baby chicks from this same hatchery earlier in the season.

Although it is recognized that infected breeding stock may be a serious focus of pullorum infection, at present Oregon turkey growers appear to have a greater danger from pullorum infection by custom hatching turkey poults in hatcheries and incubators that hatch infected baby chicks.

Because of the close agreement of the rapid serum and tube test and the fact that this test has been used successfully in controlling and eradicating pullorum disease in turkeys, it seems desirable that this test be recognized for use in the turkey phase of the National Poultry Improvement Program.

SUMMARY

A comparison of the rapid serum and tube agglutination tests for pullorum disease was conducted on 10,019 turkey blood samples.

Of 678 positive reactions, 92.7 per cent were positive to the tube method and 91.4 per cent were positive to the rapid serum. Of the total diagnosed as positive, both tests were in agreement on 84.2 per cent.

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