LABORATORY

RAPID AGGLUTINATION TECHNIC APPLIED TO *B. PERTUSSIS* AGGLUTINATION

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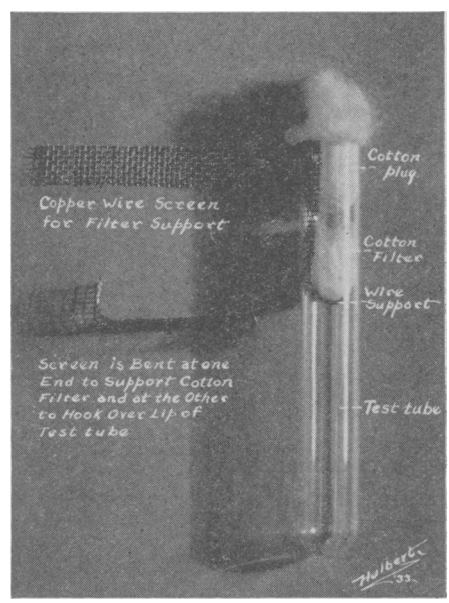
WHEN first using the agglutination reaction in testing cultures of *B. pertussis,* the usual technic was employed. Five tenths c.c. of each serum dilution was mixed with 0.5 c.c. of culture suspension and the mixtures incubated at 55° C. for 4 to 5 hours and then placed at room temperature. Readings were made at the intervals selected by Leslie and Gardner [1]—at 4½ hours—and again after the tests had stood over night. Considerable difficulty was experienced in the interpretation of results because of the tendency of *B. pertussis* suspensions to settle out in a somewhat mucoid clump after standing for a time. Differentiation of this nonspecific factor from true agglutination was not always easy and end-points were indefinite. It seemed possible that the rapid agglutination technic devised by Miss Noble [2] might overcome the difficulty since the readings would be made before the suspensions had an opportunity to settle. Only a few series of tests were needed to demonstrate the superiority of the procedure, and it is now used exclusively in our study of *B. pertussis* agglutination. The details of the procedure follow:

Antigen—The 48-hour growth of *B. pertussis* on a Bordet-Gengou slant is transplanted to a half Petri plate of Bordet-Gengou medium. After 48 hours' incubation, the growth is removed with a stiff, bent needle and emulsified in 1½ c.c. of physiological salt solution. The suspension is filtered and adjusted, if necessary, to a turbidity of approximately 10 billion organisms per c.c. by comparison with a standard.

Filtration is an important step in obtaining satisfactory, smooth suspensions. Test tube filters are prepared for this purpose. A thin layer of moistened cotton is shaped around the finger, placed on a simple copper wire netting support, and inserted in a 5 by ⅝ tube, the free end of the wire strip being bent to hook over the lip of the tube. The cotton is molded against the walls of the tube by means of a wooden applicator. These filter tubes are



plugged with cotton and sterilized. The filter is easily removed after use and the plug reinserted. The accompanying photograph shows the filter support and the completed test tube filter.

For a larger filter, a layer of gauze and cotton is wrapped around the outside of a square of wire netting cut from a bias strip and shaped to fit inside the neck of the bottle or flask of the desired size. The filter is suspended by two wires which cross under it, pass through the netting, and are hooked over the lip of the flask.

Antiserum—For preparation of a diagnostic serum, a rabbit is injected intravenously with a 10 billion per c.c. suspension prepared from a 48-hour growth of a recently isolated *B. pertussis* culture to which merthiolate 1:10,000 has been added at least 24 hours previously. The injection doses are 0.4 c.c., 0.8 c.c., and 1.0 c.c., respectively, at 3- to 4-day intervals. About 1 week after the last injection, the agglutination titer (equivalent, as explained below) has been around 1:20,000 in the six different rabbits treated by this method. The serum dilutions for agglutination tests are chosen according to the particular conditions. For testing cultures isolated by the cough plate method, the dilutions usually employed are 1:10, 1:100, 1:500, 1:750, 1:1,000, 1:1,500, and 1:2,000. For uniformity, the same dilution scheme is always employed and separate pipettes are used for the different dilutions.

The agglutination test—One-tenth c.c. of each serum dilution is mixed with 0.1 c.c. of antigen, the measurements being made with one c.c. pipettes graduated in tenths. For an antigen control, 0.1 c.c. of saline is mixed with 0.1 c.c. of antigen. The mixtures are shaken by hand for 3 minutes. For the shaking process, the racks are rocked at the rate of approximately 60 back-and-forth motions per minute and in such a way that the contents flow up the walls of the tubes for about three-quarters of their length. After the shaking period, physiological salt solution is added for greater ease in reading. Because of its convenience, the Hipple pipetting apparatus, used in the Kahn test and set to deliver 0.5 c.c. of saline, is ordinarily employed.

Reading the tests—The tests are read immediately after the addition of saline and each tube recorded as —, ±, +, + +, + + + or + + + +, according to the degree of agglutination. In the interpretation of the results in comparison with those given by other workers, a question arises because the final dilutions in the rapid test are not strictly comparable with those ordinarily given. This is of small consequence, however, provided the method of expressing the results is clearly stated. In the 0.2 c.c. mixtures of the rapid test, the series of serum dilutions 1:10 to 1:2,000 would give a series of final dilutions from 1:20 to 1:4,000. Any particular final dilution in the usual 1 c.c. test would contain five times the actual quantity of serum and antigen contained in the same dilution of the 0.2 c.c. rapid test. Therefore, if based on the actual quantity of serum present in the mixture, the series of final dilutions 1:20 to 1:4,000 of the rapid test would be equivalent to a series 1:100 to 1:20,000 in the usual 1 c.c. test.

Experience with the method—This rapid agglutination technic has been used for *B. pertussis* agglutinations for about a year. Repeated tests have been made with more than 130 recently isolated cultures and with a variety of stock cultures. The method has been used also for testing various antisera for *B. pertussis* agglutinins. The results have been clear-cut and consistent. The data on the serology of recently isolated cultures given in another communication on the cough plate method by Kendrick and Eldering [3] are based on this rapid method.

SUMMARY

The rapid agglutination technic has proved far superior to the usual 1 c.c. test for *B. pertussis* agglutination. Most important of the advantages, the tests are completed before any difficulty arises from the nonspecific clumping effect observed in so many *B. pertussis* suspensions. The advantage of the rapidity with which the results are obtained is obvious. In addition, the agglutination reaction is more clear-cut and the end-points are more easily determined.

REFERENCES

1. Leslie, P. H., and Gardner, A. D. The Phases of *Haemophilus* pertussis. *J. Hyg.* XXXI:423 (July), 1931.

2. Noble, Arlyle. A rapid method for the macroscopic agglutination test. *J. Bact.* XIV:287 (Nov.), 1927.

3. Kendrick, Pearl, and Eldering, Grace. *Report of a series of cough plate examinations for B. pertussis.* In press.

VITAL STATISTICS

The Importance of Tuberculosis as a Cause of Death in the Various Age Groups in the Population of Michigan, 1932—In spite of the constantly decreasing tuberculosis mortality observable throughout the United States, this disease is still one of the most important causes of death. Its importance varies in the different age groups and according to sex.

In a study of the ten principal causes of death among persons between the ages of 1 and 60 years in the State of Michigan in 1932, some interesting facts come to light. Excluding the deaths among infants under 1 year of age, tuberculosis as a cause of death appears among the ten principal causes in each age group up to 60 years for both males and females.

In the age group from 1 to 4, tuberculosis is the fourth most important cause of death among females and the fifth most important among males. In the age group 5–9, it appears in fifth place for both males and females with the same number of deaths for each sex.

Starting with the age of 10 years and extending to the age of 45, tuberculosis becomes a much more important cause of death than in the ages under 10. For children between the ages of 10 and 14, tuberculosis among females takes first place and among males fourth place; then between 15 and 30 years it remains in first place for females and reaches second place for males. In the age period 15–19 years, this disease was responsible for 119 deaths among girls in Michigan in 1932 and for only 58 among boys. For girls, this number of deaths represents slightly more than 28 per cent of all the deaths in this age group; among the boys of this age, the number of tuberculosis deaths represents not quite 11 per cent of the total deaths.

From ages 30 to 35, it is the most important cause of death for both males and females. In the age group 35–40, tuberculosis becomes relatively less important among females than among males, being superseded among females by heart disease and cancer. Among males, however, it is the most important cause of death, being responsible for more than 15 per cent of the total deaths in this age group, as compared with 10.5 per cent among females of this age. In the age group 40–44, it remains at third place among females, and among males it is superseded only by heart disease. The number of tuberculosis deaths among males is more than twice the number among females, and represents 12 per cent of the total