TECHNICAL METHODS

AGGLUTINATION OF *H. PERTUSSIS* USING CONCAVITY SLIDES

BY

MARION DETLOR

*From the Laboratory of Hygiene, Department of National Health and Welfare,
Ottawa, Canada*

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The agglutination reaction is the easiest to perform and is probably the most widely used of the serological tests for the detection of *H. pertussis* antibodies. Two methods have been used, a slide test and a tube test. A comparison of these techniques has been made by a number of workers (Evans and Maitland, 1939; Mishulow, 1942; Powell and Jamieson, 1942; Powell, 1944; and DeGara and Mayer, 1947). A summation of these results suggests that, for agglutination, the slide test is more useful as a diagnostic or screening test, and tube agglutination is more suitable for quantitative work.

The Laboratory of Hygiene is currently conducting studies on the immunization of children against diphtheria, tetanus, and whooping-cough. The volume of the individual serums is frequently limited, so that tests requiring small amounts of sera are of definite advantage. For this reason the practicability of a slide test for the determination of *H. pertussis* agglutinins was considered. The uncovered slide technique was found unsuitable for quantitative work because the test materials dried rapidly and end-results were difficult to interpret. It was therefore decided to investigate the usefulness of concavity slides, covering the depressions with glass cover slips to prevent evaporation. The results of these tests are described in this paper.

Procedure

Materials.—The following were used:

Concavity Slides.—Slides* measuring 2×3 in., with 10 polished concavities, 15 mm. in diameter and 1.5 mm. deep, were used.

Cover Slips.—Circular microscope cover-slips were used, having the same diameter or a slightly smaller one than that of the depressions. Cover slips with larger diameters were found unsuitable as they were easily displaced during the rotation, resulting in the loss of test materials.

Rotating Machine.—Because of its convenience, a Fisher-Kline rotator timed to run for four minutes at 280 revolutions per minute was used. Other rotators giving a similar rotary motion in a horizontal plane might prove equally satisfactory.

Pipettes.—Pasteur pipettes with tips carefully prepared so as to deliver drops of constant size were used. The number of drops per test varied, depending on the diameter of the tip.

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* Obtained from Clay Adams Co.

Antigen.—A commercial antigen* diluted in normal saline to contain 1×10^{10} organisms per ml. was used. It consists of killed Phase I *H. pertussis*, preserved with ethyl mercuric thiosalicylate at a dilution of 1 : 10,000 and coloured with methylene blue. Other suspensions prepared in this laboratory were tried, but did not prove as satisfactory.

Method.—The method for the two tests is as follows :

Qualitative Test.—The serum to be tested is drawn up in the Pasteur pipette and two drops placed in a cavity. The pipette is then rinsed several times in saline, and the tip is blotted with some absorbent material such as filter paper to ensure more complete drying. The same pipette should be used for a complete test to minimize the error. An equal quantity of vaccine is added and the cover slip placed over the cavity. The cover slip is held in place by the liquid. If the proper amount of material is used, an air space appears under the cover slip, and this aids in mixing the antigen and serum during the rotating. For control purposes, to ensure against auto-agglutination, a mixture of the agglutinable suspension and saline is included on each slide. As a further control, the vaccine is checked with known negative serum. The latter was performed on each day of test but not on each slide.

With a pipette approximately 0.050 to 0.075 in. in diameter, we have found that four drops almost fill the cavity. If too much fluid is added the cover slip will not fit.

Quantitative Test.—Twofold dilutions of the sera to be tested are made directly on the slide. Two drops of saline are placed in each cavity. Two drops of serum are added to the first cavity and the liquid is mixed by raising and expelling it three times with the Pasteur pipette. Two drops of the mixture are then transferred to the next cavity and so on until the required number of dilutions are made. The antigen is added as previously described, and the cover slips are put in place. The slide is then put on the rotating machine.

In both the qualitative and quantitative tests the slides are rotated for four minutes and then held at room temperature for two hours before reading.

Tests conducted over temperature ranges from 5° C. to 54° C. showed that higher titres were obtained at 30° C. and 37° C. Further, it was found that the combination of a four-minute rotation period and incubation at 37° C. yielded the highest titres in these experiments.

The results are most easily read if the slides are held up to the light and read against a dark background. Fig. 1 shows a typical agglutination reaction.

Discussion

The test described has been used successfully for the determination of *H. pertussis* agglutinins in sera from infants and test animals. The technique described was used by Greenberg and Fleming (1950) for the determination of pertussis agglutinins in a series of immunization studies with children. None of the sera was found to have pertussis agglutinins before immunization. Wide variations in titre from 0 to 1:4096 were found in the post-immunization sera.

The test has proved practical and requires only small quantities of material. The reactions were easy to read and yielded reproducible results. The results compared favourably with those in the tube test (Table I), and were obtained in a much shorter time. The procedure used for the tube test was essentially the same as Miller and Silverberg's method (1939).

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* Eli Lilly & Co., Indianapolis.

FIG. 1.—Agglutination on a concavity slide. The agglutination end-point is 1 : 32 dilution. V.C. is the vaccine (antigen) control.

TABLE I
COMPARISON OF RESULTS IN SLIDE AND TUBE TESTS FOR DETERMINATION OF PERTUSSIS AGGLUTININS

Serum Number	Tube Test Titre Reading after 19 Hours' Refrigeration	Concavity Slide Titre Reading after 2 Hours at Room Temperature
25	1 : 16	1 : 16
29	1 : 16	1 : 16
30	1 : 16	1 : 16
63	1 : 128	1 : 128
90	1 : 32	1 : 32
339	1 : 64	1 : 64
343	1 : 128	1 : 256
346	1 : 16	1 : 32
B.T.	1 : 16,384	1 : 16,384

It was also found that rotating accelerated the rate of agglutination, and that by rotating for four minutes the maximum titre was reached 45 minutes earlier than control slides not rotated. The rotation made the reaction stronger and easier to read, but did not change the final agglutination titre.

The described technique has been used only for *H. pertussis* agglutination, but with modifications it could be applied to other agglutination tests performed as a routine in laboratories.

Summary

H. pertussis agglutinations, both qualitative and quantitative, were successfully completed using concavity slides with covered depressions. The results compared favourably with those in the tube test and were obtained in a much shorter time.

The highest agglutination titres were obtained by rotating the slides for four minutes and incubating at 37° C. Final readings could be taken in approximately one hour.

REFERENCES

- DeGara, P., and Mayer, S. A. (1947). *J. Pediat.*, **30**, 171.
Evans, D. G., and Maitland, H. B. (1939). *J. Path. Bact.*, **48**, 468.
Fleming, D. S., and Greenberg, L. (1950). *Canad. med. Ass. J.*, **62**, 146.
Greenberg, L., and Fleming, D. S. (1950). *J. Pediat.*, **36**, 143.
Miller, J. J., and Silverberg, R. J. (1939). *J. Immunol.*, **37**, 207.
Mishulow, L. (1942). *J. Lab. clin. Med.*, **27**, 792.
Powell, H. M. (1944). *J. Indiana med. Ass.*, **37**, 563.
— and Jamieson, W. A. (1942). *J. Immunol.*, **43**, 13.