

## DIAGNOSIS OF WHOOPING-COUGH BY THE COMPLEMENT-DEVIATION TEST \*

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The recognition of such a scourge as whooping-cough, particularly when occurring in institutions in which children are segregated in large numbers, is a matter of great importance.

In his address on this subject before this Society last year, Morse emphasized the difficulty—and the value—of early diagnosis.

By the time the paroxysmal stage is reached, the infection, where children are gathered together, is widespread, and the resulting mortality high, as shown by carefully collated statistics.

The desire to find a definite method of early diagnosis led us to a study of the complement-deviation test. With our modified technic we have been able to make the diagnosis of pertussis in all stages of the disease, catarrhal, paroxysmal and convalescent. Particular emphasis is laid on the point that the diagnosis can be made early in the catarrhal stage, long before any whoop appears, and at a time when prompt isolation of the infected child will prevent the spread of the disease. Thus several children admitted to the open children's ward of the Cincinnati Hospital with a diagnosis of bronchitis, gave a positive early reaction, though there was neither vomiting nor whoop. They were immediately removed to the pertussis ward, and our open ward remained free of pertussis all winter. A preliminary report of our work has already been published<sup>1</sup> from which the following excerpt is taken.

When Bordet and Gengou<sup>2</sup> described the bacillus of whooping-cough in 1906 they used the complement-deviation test to control their bacteriological findings. From this time on, it has been known that the blood of patients in the late stages of pertussis, during convalescence and for some time thereafter, would give a positive test showing definite deviation of complement. More recently it has been shown that in the later stages of atypical cases of pertussis, it is possible to deter-

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\* Read at the meeting of the American Pediatric Society, Stockbridge, Mass., May, 1914.

1. Friedlander, A., and Wagner, E. A.: Jour. Am. Med. Assn., March 28, 1914, p. 1008.

2. Bordet and Gengou: Ann. de l'Inst. Pasteur, 1906, xx, 731.

mine the specific pertussis character of the infection by means of this test.<sup>3</sup>

Several observers have denied the possibility of making a diagnosis of pertussis even during the height of a typical attack or directly after convalescence by means of the complement-deviation test. Working in the Royal Serological Institute of Vienna, Bächer and Menschikoff<sup>4</sup> report that in twenty-seven cases of pertussis, moderate and severe, in the height of the attack, and in convalescence, attempts were made to obtain positive complement-deviation reactions, without success in a single case. Only after vaccines, prepared from pure cultures of the Bordet-Gengou bacillus were given was the test ever positive.

Commenting on these and other similar findings, Bordet<sup>5</sup> himself says: "I repeat that the power (of fixing the complement) is not seen early. In general it does not show itself markedly till toward the period of convalescence or cure." Netter and Weil<sup>6</sup> have reached practically the same conclusion. In fact it would appear to be the consensus of opinion that the test has no diagnostic value in the early stages.

Our own results lead us to the opinion that the complement-deviation test is of the greatest possible value in the diagnosis at all stages. Our results are herewith presented in tabular form.

TABLE 1.—RESULTS OF COMPLEMENT-DEVIATION TESTS FOR WHOOPING-COUGH

	No.	Positive	Negative	Per Cent.
Cases whooping .....	18	18	0	100
Normals .....	16	0	16	100
Early cases catarrhal stage .....	12	11	1	91.6
Not whooping; course otherwise typical .....	1	1	0	100

These cases were taken both from our hospital service and from private practice, and all cases were carefully followed.

It will be noted that the 18 cases tested during the paroxysmal stage gave positive reactions: 3+ in each case. Nine of the cases were in the first week of the whoop, and 3 early in the second week.

3. Bordet and Gengou: *Centralbl. f. Bakteriöl., Abt. I, Orig.*, 1911, lviii, 537; Bordet and Brunard: *Bull. Acad. de méd. Belge*, 1910, xxiv, 320; Delcourt: *Presse méd. Belge*, 1912, lxiv, 19.

4. Bächer and Menschikoff: *Centralbl. f. Bakteriöl., Abt. I, Orig.*, 1912, lxi, 218.

5. Bordet: *Centralbl. f. Bakteriöl., Abt. I, Orig.*, 1912, lxvi, 276.

6. Netter and Weil: *Compt. rend. Soc. de biol.*, 1913, lxxiv, 236.

In no case did a normal give a positive reaction. This series included several cases of bronchitis. Of the 12 early cases, tested in the catarrhal stage, 11 gave positive reactions, and in each instance the definite whoop appeared later, thus confirming the serologic diagnosis clinically. The one case that gave a negative reaction was tested at the very beginning of the cough. The sister had given a positive test. Two weeks later the child with the negative test began to whoop.

TABLE 2.—TABLE OF ELEVEN POSITIVE REACTIONS TESTED IN THE CATARRHAL STAGE

No.	Duration of Cough Without Whoop	Time of Appearance of Whoop After Test
1	3 weeks	1 week
2	3 weeks	3 weeks
3	3 days	2 weeks
4	8 days	About 1 week
5	5 days	2 days
6	1 week	2 days
7	1 week	2 days
8	10 days	2 weeks
9	5 days	2 days
10	10 days	1 week
11	2 weeks	5 days

The one case giving a positive test that never whooped, occurred in a child with paroxysmal cough and vomiting lasting over a period of four weeks, evidently an atypical pertussis.

#### TECHNIC OF TEST

Our technic for the test has been as follows: A small amount of blood—about 15 to 20 drops—was taken from the patient's ear, finger or toe in small test-tubes or the Wright capillary tubes. For young children we have found the great toe very satisfactory.

The blood was kept at room temperature or placed in the incubator until coagulation had taken place. Serum was then separated more completely from the clot in the centrifuge. So far in our test we have used only fresh, active serum. Two drops of the serum were used in each test.

Hemolytic System: The Noguchi system was used because of its extreme delicacy and because of the small amounts of material—especially serum—required. In this system washed human corpuscles, 1 drop to 4 c.c. of salt water, are used.

Amboceptor: The amboceptor employed was prepared according to the Noguchi method, the serum being dried on filter paper. The complement was obtained in the usual way from guinea-pig—dilution 1 to 40. Aside from the delicacy of this hemolytic system, it is of great value in working with children because of the very small quantity of blood required. It is not necessary to take blood from the veins and the small quantity of blood required is easily obtained even from very young children.

Antigen: This is the most important factor in the test. The Bordet-Gengou bacillus was obtained in pure culture from the laboratories of Parke, Davis & Co. Most of our work was carried on with this culture. Cultures were

also obtained from the H. K. Mulford Co., and from Dr. F. B. Mallory of Boston. The latter culture came from a strain grown at Theobald Smith's laboratory.

Subcultures were made on Bordet's medium and an ascitic fluid agar. Later we have used the ascitic fluid agar exclusively and our antigens were made up as follows: Seventy-two-hour growths were taken. The colonies, which were very tenacious, were scraped off the agar with a glass hook into sterile salt water. An emulsion was made and the bacteria again washed in salt water. It is important to do the second washing so as to rid the emulsion of any particles of agar. From this washed emulsion a standard suspension was made, and 0.1 to 0.2 c.c. of this used in the tests. Throughout the test, live bacteria were used. It will be noted that we have always used fresh, active serum and live bacteria, and we consider this innovation of great importance.

Controls: In each test known normal and known positive controls were used. In each series of tests the hemolytic system was tried out in the usual manner, using a water bath at 37 C. for incubation. After primary incubation for half an hour, the amount of amboceptor indicated by the preliminary test was added to our final test-tubes and the tubes again incubated in the water-bath.

In every instance independent readings were taken by each of us, always without previous knowledge of the clinical history of the given case.

The test is not at all difficult to carry out for any one with experience in serologic work. Using the precautions above noted, the readings have always been sharp, clear and unmistakable. It is not difficult to keep the bacillus growing on the ascitic fluid agar, which for the purposes of the test is a much more satisfactory medium than the original Bordet.

The point of particular importance is that by this method and by this test the diagnosis of pertussis can be made with absolute certainty in the catarrhal stage. Finally it is a matter of record that the success of vaccine therapy depends in large measure on the time of its application. The earlier the vaccine is given the better the results. If it be possible to diagnosticate whooping-cough in the catarrhal stage surely and definitely, its rapid cure seems assured. We have just recently begun therapeutic vaccine work. In two cases—father and son—occurring in the private practice of one of us, the test was positive in the catarrhal stage in both instances. Both patients whooped subsequently, confirming the diagnosis clinically. Both of these cases were treated with a vaccine prepared from one of our own subcultures. The father received five injections at four-day intervals, 600,000,000 bacteria being given at a dose. In three weeks his cough had ceased completely. The boy also received five injections of same dosage, and in three and a half weeks his cough was over.

We believe, therefore, that the test as outlined is worthy of full confidence and that it offers definite early diagnosis and thus points the way to a rapid cure of pertussis.

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