LABORATORY

WHOOPING COUGH PLATES IN A PUBLIC HEALTH LABORATORY*

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IN an article published in 1916 Chievitz and Meyer [1] of the Danish Sero-therapeutic Institute described a method of obtaining cultures of *Hemophilus pertussis* from whooping cough patients through "seeding by projection of droplets" crediting the method to the suggestion of their "young colleague M. Emile Mauritzen." This method, commonly called the cough plate method, consists of holding a plate of suitable medium about 5 or 6 inches in front of the mouth of a coughing patient during a paroxysm to catch the material expelled from the deeper parts of the respiratory tract. In case the patient is not having actual paroxysms a series of deep coughs is induced. This method has proved of great value for the diagnosis and study of whooping cough in the hands of Danish workers over a period of several years. It was first used extensively in this country by Lawson and Mueller [2] of the Commission for the Study of Whooping Cough at the Boston Floating Hospital in 1925–1926 with favorable results, and more recently has been highly recommended by several authors, especially Sauer and Hambrecht [3] in this country, Gardner and Leslie [4] in England and Debre [5] and his coworkers in France.

Since studies of the epidemiology of whooping cough in large centers of population are necessarily confused by the many ramifications of the contacts of individuals, a series of studies of whooping cough outbreaks in strictly rural areas was projected a few years ago by members of the staff of the Cattaraugus County Health Department in coöperation with the Division of Research of the Milbank Memorial Fund. Studies of one such outbreak in the spring of 1931 have been reported upon by Burroughs.[6] Reports of further studies during the fall and winter of 1931–1932 are now in preparation. Cough plate examinations were begun toward the end of the first outbreak and continued throughout the studies. The general plan of procedure in these studies was for a field investigator to make contact with all families in the area selected for study, to gather complete data on all respiratory disturbances appearing among the entire population of the district, and to obtain a complete history of all contacts between the various individuals in this population. For this purpose the investigator made semi-weekly visits to each household and school. On these visits cough plates were obtained from all susceptible children in the schools and also from a selected group of other children and adults comprising about one-third of the residents in the area and chosen to represent a cross-section of the population. As soon as a case of whooping cough was recognized, all the immediate contacts of this case were

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added to the list of persons from whom semi-weekly cough plates were collected.

The plates used in this study were prepared in the laboratory using essentially the original Bordet-Gengou formula [7] with human blood. Due to economic conditions we were able to obtain human blood for a reasonable sum, paying $5 for each single bleeding which yielded from 500 to 800 c.c. of blood. Defibrinated rather than citrated blood was used. All plates were inoculated less than 1 week after being prepared. After inoculation in the field they were sealed with a rubber band and brought into the laboratory the same evening. They were then incubated and examined each day for 5 days. If *H. pertussis* was present small mercury droplet colonies appeared in from 2 to 5 days. The appearance of these colonies was quite characteristic and the speed at which they developed after they first could be seen was remarkable. It was nothing unusual to examine a plate with a hand lens on one day, fail to find any evidence of growth, and yet on the next day find the plate studded with colonies from 1 to 2 mm. in diameter. We encountered some trouble from spreaders but little from the ordinary saprophytes of the respiratory tract which usually grew readily in the first day and thereafter enlarged very slowly. It was our practice to cut out, as soon as they were discovered, any colonies which looked as though they might spread, but frequently we would find a plate completely covered with a spreader.

The chief bacteriological difficulty in confirming the presence of *H. pertussis* lies in the fact that colony and individual morphology may easily be confused with that of the closely related Pfeiffer bacillus, *H. influenzae*. For this differentiation we made use primarily of the established difference of the two organisms in their growth characteristics on chocolate agar and blood agar. Transplants of all suspicious colonies were made to tubes of Bordet-Gengou agar and of chocolate agar.[8] The bacillus of influenza grows readily on chocolate agar within 24 to 48 hours while freshly isolated pertussis organisms fail to grow in 4 days, or the growth is so scanty as to be scarcely visible. On Bordet-Gengou agar the influenza bacillus grows as a flat colony with irregular outlines while pertussis has the characteristic mercury droplet appearance. If the suspected colonies seemed to be *H. pertussis* by these differential media, smears were made from the agar slants, stained and examined to determine if the morphology of individual organisms corresponded with that of *H. pertussis*. In our studies all plates that showed colonies that answered these differential tests were called positive.

A total of 1,996 plates were examined of which 14 per cent were unsatisfactory. Of 343 plates taken from clinical cases in all stages of the disease 22, or 6.4 per cent, were positive. Five additional positive plates were obtained from individuals classed as carriers so that our entire series showed 1.4 per cent positives.

The number of colonies of *H. pertussis* on our positive plates varied from 1 to 100. There was little correlation between the stage of the disease and the number of colonies—single as well as large numbers of colonies (over 50) being obtained from all stages of the disease. We must remember, however, that the inoculation of these plates is a variable technic and that probably the number of colonies more nearly represents the success of obtaining material from the deeper parts of the respiratory tract than it does the actual number of organisms expelled.

Table I lists the plates taken from cases of clinical whooping cough in relation to the stage of the disease. We found one instance in which we ob-

TABLE I

COUGH PLATES FROM PERSONS CLASSED AS CLINICAL CASES OF WHOOPING COUGH

	Time of taking plates	Plates examined	Plates positive	Plates negative	Plates unsatisfactory	Percentage positive
	More than one week preceding onset	36	0	27	9	0.0
Days of week preceding onset	Seven	1	0	1	0	
	Six	2	0	2	0	
	Five	0	0	0	0	
	Four	3	0	3	0	12.5
	Three	1	0	1	0	
	Two	1	1	0	0	
	One	0	0	0	0	
Days of week following onset	One (date of onset)	3	1	1	1	
	Two	3	2	1	0	
	Three	2	1	0	1	
	Four	4	2	2	0	43.7
	Five	3	1	2	0	
	Six	0	0	0	0	
	Seven	1	0	1	0	
Weeks after onset	Second	23	9	10	4	39.1
	Third	23	4	17	2	17.4
	Fourth	28	1	26	1	3.5
	Fifth	27	0	26	1	0.0
	Sixth	24	0	22	2	0.0
	Seventh	24	0	19	5	0.0
	Eighth	22	0	19	3	0.0
	Ninth	19	0	17	2	0.0
	Thereafter	92	0	79	13	0.0
	Total	343	22	276	45	6.4

tained a positive plate in the week preceding the first catarrhal symptoms of the disease. The highest percentage of positive plates was found in the week beginning with the day of onset. A gradually decreasing percentage occurred during the next 3 weeks. No positive plates were obtained after the 4th week of the disease.

Table II shows the positive plates in relation to the number of individuals furnishing them. We found that semi-weekly plates taken through the first 3 weeks of the disease were sufficient to give positive results in 93 per cent of our cases. It is obvious that if plates were taken 4 times a week or daily our chances of raising this figure to 100 per cent would be increased.

These findings are in general agreement with those of other investigators. Chievitz and Meyer [1] in a series of 32 cases obtained 84 per cent positives in the week of onset and 71 per cent in the next 2 weeks, with no positives after that period. Sauer and Hambrecht [3] in a series of 200 cases obtained 98 per cent positives in the week of onset, 65 per cent from the 2nd to the 5th week and no positives thereafter. McGee [9] in a series of 35 cases obtained 56 per cent positives in the week of onset, 63 per cent in the 2nd week, 71 per cent in the 3rd week and 17 per cent in the 5th week. Marie [10] in a series of 48 cases obtained 88 per cent positives in the week of onset, 55 per cent in the 2nd week, 50 per cent in the 3rd week and none thereafter. Gardner and Leslie [4] in a series of 47 cases obtained 75 per cent positives in the week of onset, 67 per cent in the 2nd week, 75 per cent in

TABLE II

CLINICAL CASES FURNISHING PLATES

	Total cases	*Cases with positive plates*	*Percentage positive*
More than one week preceding onset	5	0	
A. Week preceding onset	5	1	20.0
B. Week of onset	10	6	60.0
C. Second week after onset	12	7	58.0
D. Third week after onset	13	4	30.7
E. Fourth week after onset	14	1	7.2
A and B	10	7	70.0
A, B and C	14	13	93.0
A, B, C and D	14	13	93.0
A, B, C, D and E	15	13	87.0

No positive plates were obtained after the 4th week following onset.

the 3rd week and 25 per cent in the 4th week.

All of the cases from which we obtained positive plates would have been diagnosed as whooping cough by clinical manifestations alone. However, in certain cases positive diagnoses could be made by cough plates before clinical symptoms were decisive. Although it requires a minimum period of 7 to 10 days to render a laboratory report on a cough plate, we find that the time elapsing between the first prodromal symptoms and the date on which a positive diagnosis could be made by clinical symptoms alone averaged about 14 days for our series of 16 cases. Actually there were 5 cases in this series on which the laboratory reported a positive plate before the final diagnosis could be made.

We also found positive cough plates from 4 persons who did not develop any symptoms characteristic of whooping cough and who were classed as carriers. These findings have recently been reported in detail by Burroughs and Kline to the American Public Health Association in Washington. The epidemiological evidence of our studies convinced us that these carriers were not responsible for any additional infection and that they are probably of little consequence in the spread of the disease. Post-convalescent carriers were not found.

The possibility of detecting missed or atypical cases by means of cough plates has frequently been asserted in the literature. There were only 2 additional cases in the area studied which could be classified as probable sub-clinical whooping cough, and the laboratory did not obtain plates from either of these individuals before the 2nd week following onset. All plates from them were negative. As in the case of carriers there was no epidemiological evidence that these subclinical cases were in any way responsible for transmitting infection.

On the other hand, there were many persons in the area studied who during the course of our investigations developed some respiratory symptoms, usually suggestive of common colds. We have epidemiological histories of 130 residents of the community not included in the above groups of whooping cough cases or carriers. Although 71 per cent of them showed some respiratory disturbances no positive cough plates were obtained from any of these persons. Many negative cough plates were obtained from them, including 434 plates from 46 persons dur-

ing the course of some respiratory disturbance.

The cough plate method should be of great value in the differential diagnosis between sub-clinical whooping cough and common colds since the early manifestations of whooping cough are clinically catarrhs and coryzas which cannot be differentiated from the symptoms of common colds. If sub-clinical cases were frequent in whooping cough outbreaks, as is commonly stated, we should expect that our series of cough plates from persons residing in whooping cough areas and suffering from respiratory disturbances would serve to identify such cases. The time relationship between the appearance and type of respiratory disturbance and the negative cough plates is shown in Table III. Here we find a large series of plates taken during all stages of respiratory disturbances without a single positive finding. If this is contrasted with the results shown in Table II where we found positive plates from 93 per cent of the cases finally diagnosed as whooping cough, it is apparent that not only does this group of negative findings offer an excellent control of the accuracy of our methods but it also tends to minimize the importance of the rôle played by sub-clinical cases in whooping cough outbreaks.

TABLE III

GENERAL POPULATION COUGH PLATES

No positive cough plates were obtained—All figures represent negative plates

Persons with Respiratory Symptoms	*Week preceding cold*	*During cold*	*Week after cold*	*Totals*
Colds of less than one week duration without cough	10	8	10	28
with cough	2	4	3	9
Colds of one to four weeks' duration without cough	16	47	15	78
with cough	25	79	20	124
Colds of more than four weeks' duration without cough	4	28	1	33
with cough	14	138	10	162
Total number of plates taken during or near colds	71	304	59	434
Number of plates on above persons at other times				311
Persons Who Showed No Respiratory Symptoms				
Number of plates from persons showing no symptoms				75
Total number of plates from the general population				820

DISCUSSION

In considering our experience in connection with the question as to what place the cough plate procedure should be given in the program of a public health laboratory we are at once struck with the uncertainty of the method. Repeated negative cultures have been obtained from patients in the early stages of the disease. On the other hand, the identification of 93 per cent of our cases by the cough plate method has been quite satisfactory. It would seem that the first requirement for diagnosis should be that repeated plates be submitted at frequent intervals early in the disease. We would recommend daily plates. In the next place it is also apparent that errors in the technic of inoculating plates will greatly modify the result. Therefore, it might be well to make the additional requirement that all plates be inoculated in the presence of some person with special knowledge of this procedure. With these two safeguards, the cough plate method may well be included in the program of a

public health laboratory as a means of the diagnosis of whooping cough.

We have shown that the method may serve to detect carriers and it seems quite likely that sub-clinical cases would also have been detected had sufficient plates been submitted at the proper stages of the infection. However, the practical value of detecting either carriers or sub-clinical cases is not established since epidemiological evidence indicates that neither of them are of significance in the spread of the disease.

We know of no application of laboratory findings that would aid in controlling the course of the disease in individuals.

As a means of release from quarantine the findings by cough plate methods seem to be of very little value. Comparison of results with the findings of various investigators using the sputum method of isolating *H. pertussis* show that the latter method is much superior toward the end of the disease, although not so satisfactory in the early stages. Neither method approaches the degree of accuracy necessary in quarantine release technic where dependence must be placed on negative findings.

The most important value of the more general use of cough plates is the research value. There are many disputed questions concerning whooping cough and a large series of cough plates from many laboratories would do much to answer them. For instance, we might be able to rationalize our present quarantine requirements. There are also many important bacteriological questions to be settled. Weill [11] and Debre [12] indicate that the organisms lose their virulence during the course of the disease. The serology of the group is not completely worked out and but little study has been made of dissociation variants.[13] The accessory food factor requirements of other species of Hemophilus have been the subject of some recent investigations, which should be broadened to include the entire group.

Considering all of the above factors it would seem to us that at the present time cough plates are of greater importance to the research than to the routine public health laboratory.

CONCLUSIONS

In times of epidemics of whooping cough the routine public health laboratory can be of considerable service to the physician and to the community in occasionally making a bacteriological diagnosis of whooping cough before a clinical diagnosis can be made. As a means of controlling the spread of the disease, however, this will be of relatively little value since even the earliest cough plate diagnosis will be made after the individual will have passed his period of greatest infectivity. There is at present no evidence that cough plates have any special public health significance in other directions than early diagnosis.

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STANDARDIZATION OF THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

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PERSONAL preference for particular details in the complement-fixation test for syphilis renders improbable the general adoption, at this time, of any one technic *in toto*. If agreement on basic principles can be secured, however, a nucleus for a standard method will be provided. Coöperative studies made prior to 1930 indicated that, even with manifold variations in details, the complement-fixation tests for syphilis extensively used in Canada and the United States had much in common; also, that the least satisfactory of them, although generally lacking in sensitivity, seldom gave definite reactions with specimens from persons believed to be free from syphilis. Consequently, the conclusion seems warranted that, if precautions are observed to prevent technical and clerical errors, the chief concern in standardization will be the incorporation of factors found essential for obtaining significant results with weakly reacting sera.

Experience has shown that the outline for a standard procedure proposed in 1924, when the problem of standardization was first undertaken, meets such a requirement in most particulars. In 1930, this outline was modified to incorporate features of technic believed to be desirable for insuring further the delicacy of the test, and was then submitted to representative serologists, the majority of whom signified hearty accord with the general plan for a standard procedure. The Committee on Standard Methods of the Laboratory Section of the American Public Health Association, at the meeting held in Washington, October 24, 1932, approved the technic recommended as a standard method, and referred the report to the section for final action next year.

Provided the test is performed by a capable personnel and is suitably controlled, and precautions to avoid technical inaccuracies are observed, the outstanding features of the procedure offered as a standard may be summarized as follows: the distribution of standard reagents by central laboratories; the employment of a cholesterolized antigen having a high degree of selectivity with serum from cases of syphilis; the performance of an independent complement-fixation test with a second antigen or of another generally accepted serological test for syphilis (precipitation or flocculation), for

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purposes of control; the use of amboceptor that is markedly hemolytic and practically free from agglutinative properties in the dilutions used; complement that has been proved by preliminary tests to be actively hemolytic and to be readily fixed in the presence of antigen and syphilitic serum and which fails to react appreciably with antigen alone; a comparatively large amount of patient's serum as well as a smaller quantity to detect so-called prezone reactions; and an extended preliminary period for fixation at a low temperature with an additional exposure for a few minutes at 37° C., or provision for a second test with fixation at 37° C. for from ½ to 1 hour, in case the history indicates the possibility of recent infection.

VITAL STATISTICS

The Census of 1930 and the Growth of Population in Finland During the Last Decade—Some preliminary data concerning the results of the general census of 1930 in Finland are now available. The total registered population of Finland amounted to 3,667,067 persons on December 31, 1930. During the last half century, the population has increased from 2,060,782 in 1880, to the 1930 figure of 3,667,067. The decennial increases during this period have varied considerably. Between 1880 and 1890, the total increase was 319,358, or 15.5 per cent; between 1890 and 1900, 332,422 or 14.0; from 1900 to 1910, the increase amounted to 402,635 persons, or 14.8 per cent; between 1911 and 1920, to 249,610, or 8.0 per cent; and between 1920 and 1930, the increase was 302,260, or 8.2 per cent.

During the decade 1921–1930 the increase in population was slightly higher than during the preceding period of 10 years. In comparison with 1901–1910, however, the increase in population has fallen off very much. When it is considered that the civil war and severe epidemics occurred during 1911–1920, which greatly retarded the growth of the population, the increase in population of the latest period appears very small, for during the years 1921–1930 the conditions were in every respect normal. The mortality was very favorable. The poor growth of population was consequently due entirely to the great falling off in births.

Although the growth of population in Finland during the last 10 years proceeded more slowly than during earlier normal periods it is nevertheless quite large in comparison with conditions in the other countries of Western and Northern Europe. During 1921–1930, the growth of the population for Hungary amounted to 9.0 per cent; for Denmark 8.7 per cent; Belgium 8.2 per cent; France 6.9 per cent; Norway 6.2 per cent; England and Wales 5.3 per cent; Switzerland 5.0 per cent; and Sweden 4.0 per cent.

These figures of population growth are the results of greatly varied conditions of population. Together with Hungary, Finland has the highest rate of natality of these countries, but the surplus of population, for instance in Denmark, is approximately as large in spite of the lower birth rate in this country, because the conditions of mortality in Denmark are more favorable than in Hungary and Finland. In France, immigration from other countries has also contributed toward the increase in population.

Of the total population in Finland in