THE NEUFELD METHOD OF PNEUMOCOCCUS TYPE DETERMINATION AS CARRIED OUT IN A PUBLIC HEALTH LABORATORY: A STUDY OF 760 TYPINGS[1]

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As early as 1902 Neufeld (1), in Germany, noticed that when pneumococci are mixed with homologous immune serum, there occurs a pronounced swelling (quellung) of the pneumococcus capsule. In 1931 and 1932, Armstrong (2) (3), in England, reported his results in typing pneumococci in sputum and mouse peritoneal exudate using a method based on this phenomenon: these results were confirmed by Logan and Smeall (4). Last year, Sabin (5) of the Bellevue Hospital, New York, reported his findings using the Neufeld method for the typing of pneumococci in sputa. During the past 14 months this method has been used as a routine procedure at the Bacteriological Laboratory of the Massachusetts Department of Public Health—and has been found to be simple and accurate.

The technic used during this period was demonstrated to us by Dr. Kenneth Goodner of the Hospital of the Rockefeller Institute. His technic has been modified to the extent that plane slide preparations have been used in preference to hanging drops. Furthermore, we have extended the Neufeld method, previously used only for the determination of Types I, II and III, to the determination of the other 29 specific types of pneumococci.

Our technic is as follows: upon receipt of the sputum at the laboratory, stained liquid mounts of the specimen are mixed with rabbit antisera (Types I to XXXII) used undiluted. Combinations of monovalent antisera (rabbit) are used instead of making thirty-two preparations of the sputum with the thirty-two monovalent sera. The combinations of sera that we use are the following:

Type I
A, Types II, IV, V and VII
B, " III and VIII
C, " IX, XI, XIII and XV
D, " VIa, VIb, XVII and XVIII

[1] With the financial assistance of the Commonwealth Fund, New York.

E, Types XII, XIV, XVI and XXVIII
F, " X, XIX, XX and XXI
G, " XXII, XXIII, XXIV and XXV
H, " XXVII, XXIX, XXX, XXXI and XXXII

Nine loopfuls of sputum are placed approximately one inch apart on a 9 x 2 inch plane glass slide; to each drop is added two loopfuls of the antiserum, i.e. the first drop is mixed with Type I antiserum, the second drop is mixed with combined serum A (II, IV, V and VII), the third drop with B, etc. These preparations are stained with Loeffler's alkaline methylene blue (two drops to each mixture) and are covered at once with cover slips to prevent drying. Examination is made with the oil immersion lens, with the light dimmed. When a positive reaction occurs, which is usually within a few minutes, there is a decided swelling of the capsule of the pneumococcus present. The swollen capsule is of a light greenish-grey color, is much less translucent than one that is not swollen, and has a very

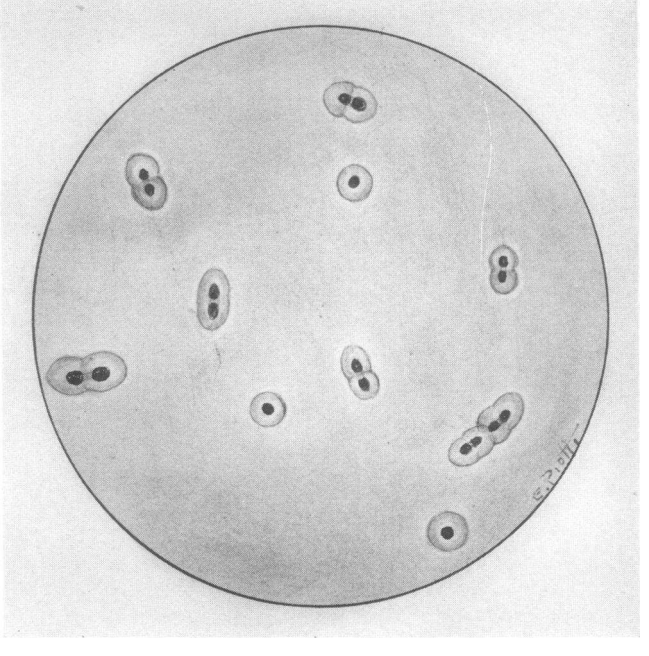

PLATE A. Positive reaction showing swollen capsules.

definite outline which is one of the most characteristic features of a positive reaction (Plate A). In the preparations in which no reaction is evident, the capsule of the pneumococcus appears as a halo of refracted light (Plate B). In all preparations the body of the pneumococcus stains a definite blue. If the reaction is observed in drop 1, then the pneumococcus present is Type I and can be reported immediately; if the reaction is ob-

served in drop 3, for example, the test is repeated using two drops of the sputum mixed with Type III and Type VIII undiluted monovalent rabbit sera, respectively; if the reaction is observed in drop 9, five loopfuls of the sputum are mixed with Types XXVII, XXIX, XXX, XXXI and XXXII undiluted monovalent sera, respectively. Should no reaction be seen on the first examination, the preparations are reexamined at the end of thirty minutes.

When dealing with sputa containing many Type III organisms, it has been necessary, occasionally, to dilute the sputum with saline before any

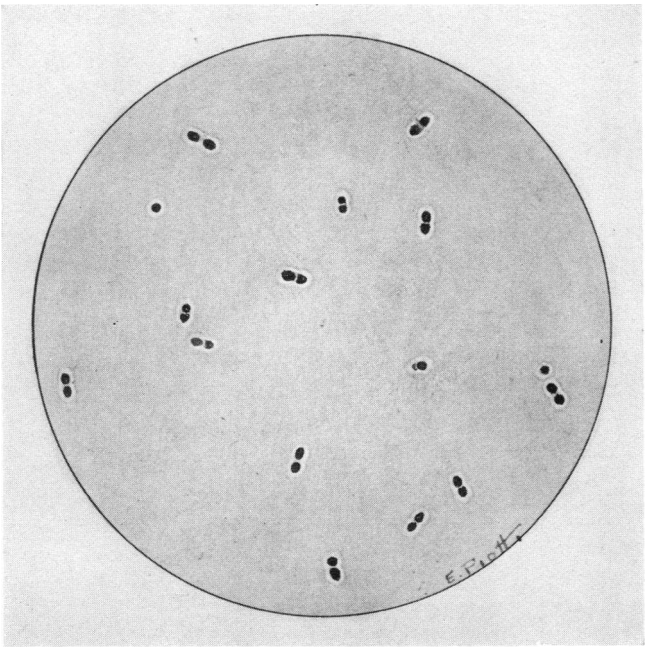

PLATE B. Negative reaction (capsules not swollen).

"quellung" of the pneumococcus capsule became evident. In such instances, when we have used the regular Neufeld technic, the organisms formed large masses which were surrounded by much precipitated material; and no definite swelling of the capsules could be seen. However, upon dilution of the sputum with saline and the test repeated, the individual diplococci have shown a typical positive reaction.

The specimens of sputum that we receive are often at least 24 hours old, since they are sent by mail from various parts of the State. In our hands, the age of the specimen has made little difference in relation to the success with which the Neufeld method has been applied. We have demonstrated a typical positive reaction on sputum over 48 hours after the time of its collection from the patient.

During the past 16 months, 760 specimens of sputum have been examined by this method; of these, 246 were diagnosed immediately as Types I, II and III, and 110 as pneumococci of the higher types.[2] Nine others were questioned, two as Type I's, three as Type III's and four as Group IV's. These later proved to be the types suspected.

TABLE I

Results of Neufeld method on 760 specimens

Number of sputa found to have specific types (I–XXXII)	356
" " " in which specific type was doubtful	9
" " " set up for Types I, II and III only, later classified as higher types	148
" " " showing typical pneumococci, negative on Neufeld	25
" " " in which typical pneumococci were not seen, pneumococci cultivated later	102*
" " " not containing pneumococci	120
Total	760

* Of these, 9 showed Type I pneumococci and 4 Type II, and the remainder were those of higher types.

The results of the 356 positive Neufeld typings have been confirmed by the various methods shown in Table II.
Whenever the amount of sputum was sufficient, as many of these methods were used on the individual specimen as was possible—thus making as

TABLE II

Confirmation of Neufeld results by other methods

Type	Mouse methods only	Krumwiede only	Methods on Avery broth only	Mouse methods and Krumwiede	Mouse methods and Avery broth	Krumwiede and Avery broth	Krumwiede, mouse methods and Avery broth	Other methods*	Not confirmed by any method	Total
I	49	16	4	32	13	4	8	1	8	135
II	9	2	0	9	6	2	1	0	0	29
III	41	6	3	12	12	1	4	1	2	82
IV–XXXII	67	1	3	1	27	1	0	2	8	110
Total	166	25	10	54	58	8	13	4	18	356

* 1 Rosenthal and Sternberg's method on sputum (9).
3 direct microscopical agglutination on sputum.

thorough and complete a study of the accuracy and efficiency of the Neufeld method as the specimens allowed.

[2] The Group IV examination by the Neufeld method has been a routine procedure for only two months.

Of the 135 sputa diagnosed Type I by the Neufeld, the standard mouse methods [3] failed to give a Type I diagnosis in 25 instances: in 12 of these, pneumococci of other types (from Types IV to XXXII) were recovered, and in the remaining 13, the mice failed to develop a pneumococcus infection. However, an agreement with the Neufeld was obtained by other methods in 17 of these cases: 7 were diagnosed Type I by the Krumwiede method (10); 4 by various methods used on the Avery (6) broth culture of the sputum; and 6 by using the Neufeld method on the peritoneal exudate of the inoculated mice. In 4 of these 6 cases, the mice died of a pneumococcus infection other than Type I, whereas the peritoneal and heart's blood cultures from the other two mice were so mixed it was impossible to isolate any pneumococci from them. Nevertheless, occasional Type I pneumococci were seen in the peritoneal exudate using the Neufeld technic on these 6 mice.

Of the 8 specimens reported Type I by the Neufeld in which we were unable to obtain a Type I diagnosis by other methods, 4 showed other types of pneumococci in the mice, and in the remaining 4, no pneumococci of any type were isolated (Table III).

In only 1 of the 29 sputa diagnosed Type II by the Neufeld method did the inoculated mouse die of infection from pneumococci other than Type II. In this instance, both the Neufeld and Krumwiede methods gave a Type II reaction on the sputum, whereas the mouse developed a Type I

TABLE III

Results of typing 246 Type I, II and III specimens by the Neufeld method

Type	Total typed by Neufeld	Checked	Not checked	Per cent checked
I.....	135	127	8*	94
II.....	29	29		100
III.....	82	80	2†	98
Total.....	246	236	10	96

* 1—Type III.
1— " IV.
1— " VIII.
1— " XXI.
2—No pneumococci.
2—Mouse survived.
† 2—Type VIII.

[3] The standard mouse methods used were
(1) The Sabin stained slide microscopic agglutination test (7),
(2) The tube agglutination and precipitin methods (8),
(3) Microscopic or macroscopic agglutination of the heart's blood culture.

septicemia. Particular mention is made of the Type I and Type II cases because of the necessity of their early and correct diagnosis in view of the serum treatment of these patients. In 2 of the sputa diagnosed Type III by the Neufeld, Type VIII pneumococci were recovered from the mice (Table III).

Because of their scarcity, pneumococci may not be seen in some sputa on microscopic examination. Satisfactory results can frequently be obtained in such instances by inoculating an Avery broth culture with 0.5 cc. of the sputum. This is incubated for from 4 to 6 hours at 37° C. Pneumococci obtained from such cultures may then be typed by the Neufeld method.

On some occasions, where 2 types of pneumococci were present in the sputum in unequal numbers, only one type was found by the Neufeld technic. At such times pneumococci of the predominant type were readily found while those of the less common type were overlooked and only found later. In these, the second type was found by the Neufeld technic applied to pneumococci obtained from an Avery broth culture, mouse peritoneal exudate, or culture of the heart's blood of the mouse inoculated with the specimen in question. Such instances were uncommon and occurred only a few times among the 760 specimens examined. Conversely, however, as noted above, pneumococci of Types I or II were in some instances found by the Neufeld method that would have been missed by other technics, owing to the heavy contamination of the specimen with other organisms.

By use of the Neufeld typing method we have been able to give physicians an immediate report on 94.6 per cent of our Type I, II and III specimens in which pneumococci were seen in the sputum, and in 85.2 per cent of all Type I, II and III specimens on which this method was tried. Particular interest is attached to the rapid determination of Types I and II because of the availability of therapeutic serum; in 89 per cent of all specimens containing these types, immediate type diagnosis was made possible by this method.

SUMMARY

The Neufeld method of pneumococcus type determination has been used for the diagnosis of Type I, II and III pneumococci for 16 months. The results obtained have been confirmed by other methods in 96 per cent of the instances.

The use of this method of typing has enabled us to give to physicians an immediate type diagnosis on 94.6 per cent of specimens containing pneumococci of Types I, II or III, when typical pneumococci were seen in the sputum.

During the past 2 months the use of the method has been extended to include the determination of the higher types (Types IV to XXXII).

This method of typing may be carried out on a very small amount of sputum, is simple, rapid and accurate, and does not require the use of mice.

CONCLUSIONS

We believe the Neufeld method of typing to be very valuable. It has enabled us to give immediate reports to physicians in 89 per cent of Type I and II cases, thus leading to earlier and consequently more effective serum therapy.

The use of the Neufeld technic for typing pneumococci contained in sputum and Avery broth cultures of it has proved an efficient routine procedure for pneumococcus type determination.

This method of typing has proved as accurate as other more generally used methods. Its outstanding advantage is that a type diagnosis can be made almost immediately (5 to 30 minutes).

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