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Laboratory Diagnosis of *Pneumococcus* Infections *

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CONTENTS

INTRODUCTION

The pneumococci belong to the coccus family, where they may be regarded as a subdivision of the streptococcal tribe. Pneumococci are Gram-positive capsulated diplococci (often growing in short, straight chains), soluble in bile, sensitive to optochin, catalase-negative, growing as smooth colonies on blood-agar showing α-haemolysis, homogeneous in serum broth, virulent for mice, killed by heat at 60°C for 30 minutes and easily giving lysis. Pneumococci are facultative aerobic organisms, but in very rare cases a strain is found to be anaerobic.

COLLECTION OF SPECIMENS

Pneumococci are especially found in infections of the respiratory tract—ear, nose, throat, pleura and lungs—but may be seen in meningitis, peritonitis, endocarditis and arthritis. Pneumococci are found, too, in the throat of about 50% of healthy people.

In the case of sputum, pleural and spinal fluids, a few millilitres are taken in sterile tubes. From infections in the ear or nose a specimen of pus is collected; if there are only small amounts of pus a swab is taken with a dry, sterile swab, placed in a dry, sterile tube. Blood samples may be sent as coagulated or citrated blood. It is preferable to take the samples before antibiotic treatment is started.

* This is one of a series of studies on the laboratory diagnosis of various diseases which, it is hoped, will eventually be revised and published in monograph form. An effort is made to ensure that the diagnostic methods recommended in these studies are as internationally representative and acceptable as possible by securing the co-operation of a number of experts from different countries. A list of the reviewers of the study presented here is given in the Annex on page 13. To all of these, and to the author herself, the World Health Organization is greatly indebted.—ED.

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CULTURE MEDIA AND REAGENTS

Blood-agar (5% or 10%)

5% or 10% defibrinated or citrated horse (or rabbit) blood in beef agar, i.e., 1.8% agar in beef broth containing 1% peptone, 0.3% NaCl and 0.2% $Na_2HPO_4,12H_2O$; pH 7.3-7.4.

Serum broth

Broth containing 5% ox or horse serum, 1% peptone, 0.3% NaCl, 0.2% $Na_2HPO_4,12H_2O$, 0.1% glucose and 0.05% haemoglobin; pH 7.3-7.4.

Ascitic broth

Broth containing 5-10% ascites-fluid.

Sodium taurocholate (10%)

Beef broth containing 10% sodium taurocholate.

Optochin-blood-agar

10% horse-blood-agar containing optochin (ethyl hydrocuprein hydrochloride) 1 : 50 000.

Hiss's medium

80 ml of ox serum, 160 ml of distilled water and 3 ml of phenol red; pH 8.0.

Truche medium

Peptone (Chapoteaut), 4%; sodium chloride, 0 5%; and glucose, 0.2%; dissolved in tap water.

Gelatin

Truche medium with addition of 10% gelatin.

EXAMINATION OF SPECIMENS

DIAGNOSIS OF PNEUMOCOCCI

Fluid specimens and swabs are inoculated on 5% or 10% horse-blood-agar and in 5% ox (or horse) serum broth, the swabs being left in the serum broth. The cultures are incubated at 37°C for 6-8 hours or overnight. On blood-agar typical colonies of pneumococci may be observed: round, flat, smooth, translucent, often with a central pitting and with a greenish discoloration of the medium around the colony. If it is a pure culture the diagnosis may be confirmed by tests with optochin and bile. If it is a mixed culture a single pneumococcal colony has to be isolated on blood-agar and in serum broth. If a strain is growing with typical colonies on blood-agar, diffuse in serum broth, and is sensitive to optochin, it will practically always be a *Pneumococcus*. The above description covers the capsulated form, the "smooth" (S) form. If precautions are not taken by subcultivation, the pneumococcal capsules will easily degenerate into forms more or less atypical, ending in a decapsulated "rough" (R) form.

Pneumococci are related to non-haemolytic streptococci very closely, and in several cases common antigens are found (Lund, 1950). For the differentiation the following characteristics may be used:

(1) sensitivity to optochin;

(2) solubility in bile;

(3) virulence for mice.

(1) *Optochin test* (Lund, 1959). The sensitivity to optochin may be examined by inoculation of a young culture on blood-agar containing optochin 1: 50 000. As a control the same culture is inoculated on blood-agar without optochin. The pneumococci are not able to grow on this optochin-blood-agar, while all streptococci grow well. This test may be performed by means of tablets [1] or paper discs [2] containing optochin in a certain amount. On blood-agar cultures pneumococci show zones of inhibition around such a tablet or disc, while streptococci give no such zones, the culture growing right up to the tablet or disc.

(2) *Bile test.* 1.0 ml of a young fluid culture is mixed with 1.0 ml of 10% sodium taurocholate or animal bile. After 10 minutes in an incubator at 37°C, the fluid is examined macroscopically for clearness, after which it is inoculated on blood-agar. If the fluid is quite clear and gives no growth on blood-agar, the diagnosis is pneumococci. A control with broth without taurocholate is desirable.

(3) *Virulence test.* All types of pneumococci are able to kill white mice, when 0.5 ml of an undiluted, young fluid culture is inoculated intraperitoneally (Mørch, 1943b). In most cases a pneumococcal culture diluted 10^{-5}-10^{-6} will be able to kill a mouse,

[1] Produced by the Roskilde Medical Company, Roskilde, Denmark.

[2] Produced by Baltimore Biological Laboratory, Baltimore 18, Md., USA.

the virulence of pneumococci being much greater than that of streptococci.

Neufeld test. If diagnostic pneumococcal sera are available (Mørch, 1942), the diagnosis may be made by the Neufeld test (Neufeld, 1902). Sputa, spinal fluids, pleural exudates and similar fluid material may be examined directly. Swabs have to be grown in serum or ascitic broth before the Neufeld test can be performed.

The Neufeld test is made first with nine pooled sera and with the serum for type 3; the sera are composed in such a way that each of the 80 *Pneumococcus* types known (Lund, 1957) will give a capsular reaction in one of these sera. An examination is made with the serum of type 3 especially, as it is difficult to produce a strong serum for this type. When a positive reaction is obtained in one of the pooled sera, the specimen is examined with the sera corresponding to the individual types (or groups) that make up the mixture.

The Neufeld test is carried out as follows: A tiny amount of the specimen is placed on a slide by means of a platinum loop. With another loop a little serum is added to the droplet on the slide, and the two elements are mixed thoroughly. It is preferable to have relatively few organisms per microscope field, so it is recommended that a loop of 1-mm diameter be taken for the specimen and a loop of 4-mm diameter for the serum. A cover-slip is placed on the mixture, and the preparation is examined under the microscope with an oil-immersion lens. To the serum is added 0.2 ‰ methylene blue, which colours the corpus, but not the capsular substance. The Neufeld reaction is not a swelling of the capsule, but a reaction between the type-specific serum and the capsular substance, making the capsule visible as a dark outline. In an India-ink preparation the capsule will be of the same size as it is in a positive Neufeld reaction.

The type diagnosis may be made by agglutination (macroscopical or microscopical). An R form will be able to give non-specific agglutination in the sera of several types.

Mouse test. If no pneumococci are demonstrated by a direct Neufeld test, white mice (18-20 g) may be inoculated intraperitoneally with 0.5 ml of the material; a blood-agar plate is inoculated at the same time. If the mice become ill or die, an autopsy is performed under sterile conditions. A blood-agar plate is inoculated with heart blood and with peritoneal exudate; in addition, a culture is made from the heart blood in 5% ox-serum broth. The peritoneal cavity is washed with sterile saline, and this lavage is examined by a Neufeld test. The results obtained in this way may be confirmed on the following day by examination of the serum-broth culture and the blood-agar plate. Mice injected with some strains of pneumococci develop illness only after several days or even remain well. Exudate taken from the peritoneal cavity of these mice after 18-24 hours by means of a capillary tube drawn to a point frequently shows organisms which could be typed. The mice remain alive for further examination.

FURTHER METHODS FOR EXAMINING PNEUMOCOCCI

Haemolysis

All pneumococci give some degree of α-haemolysis on blood-agar. Pneumococci grow best on 10% blood-agar, but haemolysis is best observed on blood-agar containing only 5% blood. Most of the strains produce a haemolysin—pneumolysin (Mørch, 1946b)—which is best examined after cultivation in ox-heart broth. Serum broth cannot be used, because normal serum inhibits the production of pneumolysin.

Fermentation

All pneumococci ferment glucose, galactose, lactose, sucrose and maltose. None of the strains are able to ferment arabinose, xylose, dulcitol, inositol or sorbitol. Only a proportion of the strains ferment inulin (60%), mannitol (20%), salicin (71%) and aesculin (23%) (Mørch-Lund, 1949).

The substances to be fermented are added in a proportion of 0.5% (aesculin, 0.2%) to Hiss's medium (Kauffmann et al., 1940) with phenol red as indicator. The medium is steamed for about one minute before the addition of the sugars, and is then tubed 2 cm high into narrow test-tubes and steamed for a further 10 minutes.

Two drops of a young culture in serum broth are added to each tube and to a control tube containing pure Hiss's medium. The tubes are shaken, incubated at 37°C for five days and inspected daily. Only a distinct change in colour (red to yellow) together with coagulation is regarded as positive. In most cases the fermentation is completed within 24 hours.

Capsule-staining of pneumococci

The different methods described for staining the pneumococcal capsule do not give better results than a well-made India-ink preparation, counter-stained with some red colour (methyl red). It is important to use young cultures with good capsules for these preparations.

In a Gram-stain preparation the capsular (S) pneumococci are Gram-positive, the non-capsular (R) forms Gram-negative, and the intermediate (S-R) forms are Gram-positive.

Sensitivity to antibiotics

All types of pneumococci are sensitive to penicillin, the tetracyclines, chloramphenicol, bacitracin, and erythromycin and show moderate sensitivity to streptomycin and neomycin; to polymyxin B they are rather resistant. Pneumococci are practically always sensitive to sulfonamides. It is possible *in vitro* to make pneumococci resistant to penicillin, but *in vivo* this has never been observed.

The sensitivity test may be performed by the tablet method (Lund, 1955), by the disc method or by the dilution method.

PNEUMOCOCCAL ANTIBODIES IN HUMAN SERA

During an infection with pneumococci type-specific antibodies are produced and may be found in the serum of the patient. For this test nine pooled vaccines may be used which react with the antisera for all known pneumococcal types. The serum is examined by a Neufeld test with these vaccines. If one of the mixtures gives a positive reaction, the serum is tested with vaccines of the types therein. When the reacting type or group has been found, the capsular and agglutination titres may be determined. If a group is reacting the titration is performed with all the types within the group. The method of titration is described under " Production of diagnostic pneumococcal sera ".

In human sera containing pneumococcal antibodies the capsular reaction in most cases is well defined, whereas the corresponding agglutination is often weak and difficult to observe. After pneumonia of pneumococcus origin the capsular and agglutination titres—as a rule of the same size—are in most cases from 1:4 to 1:32

As to non-specific reactions, Löfström (1939) states that in human sera non-specific capsular reactions are often seen with pneumococci of types 16, 27 and 28. These reactions are found in patients with various diseases in the first days of illness, but disappear as soon as the specific antibodies are formed. No agglutination reactions are found corresponding to these weak non-specific capsular reactions.

TYPE-SPECIFIC POLYSACCHARIDES IN URINE

The greater part of the pneumococcal capsule consists of polysaccharides, differing in composition with each individual type and giving the organism its specific type character. During the growth of the pneumococci these polysaccharides diffuse into the culture medium—in patients into the body fluids and urine, where their presence may be demonstrated by precipitation with the homologous sera (Mørch, 1943a). These substances are water-soluble and designated " soluble specific substances " (SSS).

This precipitation of SSS in urine from patients with a pneumococcal infection has no practical diagnostic value, as it is very seldom positive, even in severe infections.

Preparation of SSS (Roesgaard, 1945)

The type-specific polysaccharides, SSS, may be obtained from strains of pneumococci by the following procedure: The strain is added to 100 ml of 5% serum broth and incubated at 37°C for 18 hours; 5-10 ml of ox-bile is then added. After two hours at 37°C the culture will be autolysed, 1 ml of 50% acetic acid is added and the mixture heated to boiling. After cooling, sodium hydroxide is added for neutralization, and the fluid is centrifuged for 20 minutes at 4500 r.p.m. The clear supernatant fluid containing the type-specific polysaccharide is mixed with two volumes of absolute alcohol. The mixture is shaken and left in the refrigerator (5°C) overnight. Next day the precipitated polysaccharide is separated by centrifuging for 10 minutes at 3000 r.p.m. The sediment is mixed with 10-20 ml of absolute alcohol, centrifuged, and dried in a desiccator for 10-12 hours. The drying may be completed by shaking with ether and leaving in the incubator. The dried sediment is ground.

The pneumococcal polysaccharides in urines may be isolated by precipitation with 5 volumes of 96% alcohol (80% alcohol in the mixture). The precipitation is complete after 30 minutes at about 5°C. The precipitate is separated by centrifuging and dried with ether, as above.

STORAGE OF PNEUMOCOCCAL STRAINS

Pneumococcal strains are best stored for long periods as freeze-dried cultures (Mørch, 1946a). For a shorter period of storage it is practical to use gelatin. For this purpose the strain may be cultured in serum broth or better in Truche medium (Truche & Cotoni, 1912). When optimal growth is obtained at 37°C the culture is mixed with an equal amount of 10% gelatin in Truche medium. The tubes are stored in an ice-box, and the cultures are transplanted at intervals of 3-4 months. Before a transplantation

the culture is made fluid at 37°C and a few drops of the culture are transferred to serum broth, before the strain is again inoculated into Truche medium.

The quality of the peptone in the medium is decisive for the growth of the pneumococci (Lund, 1954). If a good peptone cannot be obtained, it is better not to use Truche medium, but to use serum broth instead.

Pneumococcal strains which preserve their capsule retain their virulence for mice (Mørch, 1945).

PRODUCTION OF DIAGNOSTIC PNEUMOCOCCAL SERA

At present there is no place where reliable diagnostic pneumococcal sera may be obtained. The method for the production of these sera (Mørch, 1942) will therefore be described here.

Vaccine

The strain employed for the preparation of vaccine must have well-developed capsules which give a clear capsular reaction with the homologous serum. Culture is made in 5% ox-serum broth in a quantity sufficient for the inoculation. As a rule, 15-18 litres of 5% serum broth are inoculated for each strain (5-6 flasks of 3 litres). On the day before the inoculation these flasks are left in the incubator at 37°C for 24 hours; this gives a control of their sterility. In the morning each 3-litre flask is inoculated with 20 ml of the serum-broth culture. The flasks are shaken and incubated again at 37°C, and the growth is sampled by culture from time to time (5-10 ml in a test-tube). The density of the growth in the sample is estimated, and the capsule formation is tested by a Neufeld reaction with the homologous serum. Particular attention must be paid to the cultures of types 3 and 37, as the capsules of these types degenerate very rapidly. On this account it may be necessary to interrupt the growth before it becomes maximal. The capsules are in most cases found to be well preserved when their optimal density is reached after 4-7 hours' growth.

The growth is stopped by the addition of 2% formol, after subcultures on blood-agar plates have been made from each flask for control of the purity. The flasks remain in the incubator at 37°C until the following morning, when the cultures are centrifuged in a high-speed milk separator. The resulting sediment is ground in a mortar and suspended in Sørensen buffer solution (20 ml of M/15 KH_2PO_4+ 80 ml of M/15 $Na_2HPO_4,2H_2O$+300 ml of 0.9% NaCl solution), to which is added 0.5% formol. The density of the finished vaccine is estimated by means of a standard containing 1000-2000 million pneumococci per ml; this density is designated as standard 1. The vaccines are stored in suspensions that are 15-25 times denser than standard 1.

The vaccine is controlled for capsular reaction with the homologous serum, and a smear is stained by Gram's method. In a successful vaccine all the bacteria are Gram-positive. If a greater part of the pneumococci are Gram-negative, the vaccine has undergone too much autolysis and is unserviceable. For immunization the vaccine is most often used with a density of standard 1, containing 0.2% formol. When stored in dense suspensions in the ice-box, these vaccines have for the greater part of the types been found to preserve their capacity for capsular reaction and agglutination through several years. Exceptions to this rule, however, are the vaccines of types 3 and 37 and of group 19, which have to be renewed after 2-3 months.

Immunization

White rabbits (or other breeds), weighing 2-3 kg, are used for the immunization. The vaccine (with a density of standard 1) is heated to 37°C prior to the injection, which is given slowly intravenously in the ear. The usual procedure is to give the rabbits three injections a week (i.e., every other day). The dosage may be arranged as follows:

1st week:	0.25	0.5	0.5 ml,	standard 1		
2nd week:	1.0	1.0	1.0 „	„	„	
3rd week:	1.5	1.5	1.5 „	„	„	
4th week:	2.0	2.0	2.0 „	„	„	
5th week:	2.0	2.0	2.0 „	„	„	and so on.

As a rule the animals do not stand higher doses than 2 ml per injection. It is not practicable to establish a general dosage scheme, as allowance has to be made for the varying sensitivity of the individual animals, in which also the vaccine may have a more or less toxic effect.

After immunization for 5-6 weeks a sample of blood is taken for estimation of the potency of the serum. As the various animals do not form the same amounts of antibody, the sera of three animals are pooled. If this pooled serum is found to possess a

sufficiently high titre, the animals are bled by heart puncture 5-6 days after the last injection of vaccine. About 50 ml of blood is taken from each rabbit. Two days after the heart puncture the immunization is resumed, and 3-4 weeks later the heart puncture is repeated. When the immunization period is more protracted, the number of injections of vaccine may be reduced to two a week. At the conclusion of the immunization the animals are bled out.

It is possible by immunizing rabbits with a vaccine mixture to produce sera that react with several types. A serum that is to be used, for instance, for diagnosis of group 7 (types 7, 7A, 7B and 7C) may be obtained by immunization with a vaccine containing equal parts of vaccine of types 7, 7A, 7B and 7C. The dosage for such immunization may be the same as that given for a vaccine of one type. If necessary, higher doses may be given, possibly with a denser vaccine (standard 2 = twice the density of standard 1); up to 2 ml of standard 2 has been tolerated.

Production of diagnostic serum

The blood from the rabbits is left standing in the ice-box for about 24 hours. The serum is poured off and centrifuged, and 1% thiomersal is then added to the supernate. The antibody content of such a serum remains unchanged for years or is not lowered significantly. Serum for therapeutic use is no longer produced.

The serum meant for diagnostic use is examined for homologous capsular and agglutination titres. The titration is carried out in dwarf tubes, 0.2 ml in each tube: 1 : 1, 1 : 2, 1 : 4, etc. A control test for auto-agglutination of the vaccine is made in a tube with 0.2 ml of physiological saline. To each tube is added 0.2 ml of pneumococcal vaccine of a standardized density (about 100 million per ml). The tubes are shaken and a loopful of the contents of each is examined under the microscope. The highest dilution of serum giving a distinct capsular reaction indicates the " capsular titre ". The tubes are placed in a water-bath at 50°C for 20 hours, after which the agglutination is read (macroscopic flocculation).

A diagnostic serum must be specific, i.e., must react only with the type or group it is adjusted to. Each portion of serum has to be examined for cross-reactions. This is done by means of Neufeld tests with all known pneumococcus types. This determination is carried out with the same diluted vaccines as used for determining capsular and agglutination titres. The degree of the cross-reactions found is assessed by titration, and the capacity for these reactions is then eliminated by absorption.

Absorption

The absorption is carried out with the dense formolized vaccines. When the degree of the cross-reactions is known, it is possible with some experience to estimate how much vaccine is required to remove a reaction of a certain degree. The vaccine is put into centrifuge tubes that are centrifuged for 30-60 minutes at a rate of 500 r.p.m. The clear supernatant fluid is then poured off. The tube is filled with the serum that is to be absorbed. The serum and the vaccine are mixed thoroughly by means of a Pasteur pipette. The absorption is completed within 5-10 minutes, irrespective of the temperature and serum concentration.

The mixtures of serum and vaccine are centrifuged for 30-60 minutes at the same rate as before. The supernatant clear serum is pipetted off, and a control test is made to ensure that absorption is complete by testing the serum for capsular swelling with the absorbing type. A cross-reaction with a capsular titre of 32-64 can usually be removed by one absorption. With higher titres the absorption has to be repeated. A control test is again made on the serum in order to ascertain the completeness of the absorption. If several reactions with low titres (2-8) have to be removed from the same serum, 2-3 different vaccines may be mixed in the centrifuge tubes, and thus the reactions with 2-3 types can be removed from the serum by one absorption.

After filtration of the sera, methylene blue is added to the absorbed sera (1 ml of a 2% methylene blue solution per 100 ml of serum) and the capsular titres are finally determined. It is the general experience that absorbed sera decrease in potency more rapidly than non-absorbed sera, and hence it is necessary at intervals of a few months to make control tests for capsular titre on the diagnostic sera, nearly all of which are absorbed.

Four kinds of pneumococcal serum are employed for diagnostic purposes: (*a*) type sera, reacting with a single type; (*b*) group sera, reacting with all the types within one group; (*c*) pooled sera, reacting with several different types; and (*d*) the so-called " factor " sera, absorbed sera employed for differential diagnosis within a group.

Polyvalent sera, sera reacting with several types, may be produced by (1) pooling monovalent sera, (2) immunization with several types, or (3) pooling sera obtained by immunization with several types.

Polyvalent group sera (*b*) may be obtained by pooling the monovalent sera corresponding to the individual types making up the group. In order to avoid the dilution brought about by the mixture it is possible to produce these sera by immunization with several types injected simultaneously (up to five different types). The immune serum is freed from heterologous reactions by absorption, and the capsular titre is determined for each type of the group.

For diagnosis of group 35 it is preferable to use a serum produced with type 35 alone; such a serum gives good reactions with all the types of the group (35, 35A, 35B, 35C). A group-35 serum produced by immunization with all the types in the group will give such strong heterologous reactions that the removal of these reactions will leave a rather weak diagnostic serum.

The 9 pooled sera A-I (*c*) used for the diagnosis of pneumococci are composed in such a way that each of the 80 types known gives a capsular reaction in one of these sera (type-3 serum is used monovalent). The distribution is planned with a view to the cross-reactions, so that the types which have strong reactions with each other are gathered in the same pool, and thus it is not necessary to remove these reactions.

These pooled sera are produced by immunization with up to five different types simultaneously. In the case of immunization with more than five strains there will be a reduction in the amount of antibody formed per type. The pooled sera A-I consist of the following types or groups:

Pool	*Types or groups*
A . . .	1, 2, 4, 5, 18
B . . .	6, 8, 19
C . . .	7, 20, 24, 31, 40
D . . .	9, 11, 16, 36, 37
E . . .	10, 21, 33, 39
F . . .	12, 17, 22, 27, 32, 41
G . . .	13, 25, 29, 34, 35, 38, 42, 47
H . . .	14, 15, 23, 28
I . . .	43, 44, 45, 46

Factor sera (*d*) are especially absorbed sera used for the differential diagnosis within a pneumococcal group. If we have, for instance, to distinguish between the two types in group 6, 6A and 6B, two sera are required, one of which reacts only with type 6A, the other only with type 6B. On absorption of serum 6A with type 6B, the serum components that react with 6B are removed, and the absorbed serum will then give a reaction only with type 6A. Correspondingly, the serum specific for type B is obtained by absorption of serum 6B with type 6A. The two types are set up with the following formulae for capsular antigens:

$$6A = 6a, 6b$$

$$6B = 6a, 6c$$

6a signifies the antigen (or antigens) common to the two types, while 6b and 6c signify the special antigen content of each type. By absorption of serum 6A with type 6B, the mutual component 6a is removed, leaving a serum 6b that is specific for type 6A. Absorption of serum 6B with type 6A removes the factor 6a too, leaving serum 6c that reacts only with type 6B. By means of these two sera, 6b and 6c, it is possible to make the differential diagnosis within group 6.

In the case of a group consisting of more than two types, the procedure is fundamentally the same. From the antigenic formulae it is possible to calculate the absorption processes required.

These strongly absorbed sera weaken rather rapidly; hence it is necessary at intervals of a few months to check these factor sera for capsular titre.

CAPSULAR ANTIGENS OF THE PNEUMOCOCCI

Table 1 gives the principal capsular antigens of the 80 pneumococcus types known. This table is not to be regarded as being complete; the discovery of new types involves the addition of new antigens. Some of the types have no or very few cross-reactions (= common antigens), e.g., type 1 = 1a, type 2 = 2a, while other types have antigens in common with several types—for example, types 7B, 7C, 19B, 19C, 24, 24B and 40 have the antigen 7h in common.

Some of the types are serologically so closely related that it is impossible to produce monovalent diagnostic sera corresponding to each type. Some types, contain, as far as we know at present, no antigen particular to the type, but consist exclusively of antigens that are present also in other pneumococcus types (e.g., 7, 32, 33, 35A). For practical reasons such types have therefore been placed in groups together with related types. Group 7, for

TABLE 1

CAPSULAR ANTIGENS OF THE PNEUMOCOCCI

Type	Antigenic formula	Type	Antigenic formula
1	1a	20	20a, 20b, 7g
2	2a	21	21a
3	3a	22	22a, 22b
4	4a	22A	22a, 22c
5	5a	23	23a, 23b, 18b
6A	6a, 6b	23A	23a, 23c, 15a
6B	6a, 6c	23B	23a, 23b, 23d
7	7a, 7b	24	24a, 24b, 24d, 7h
7A	7a, 7b, 7c	24A	24a, 24c, 24d
7B	7a, 7d, 7e, 7h	24B	24a, 24b, 24e, 7h
7C	7a, 7d, 7f, 7g, 7h	25	25a, 25b
8	8a	27	27a, 27b
9A	9a, 9c, 9d	28	28a, 28b, 16b, 23d
9L	9a, 9b, 9c, 9f	28A	28a, 28c, 23d
9N	9a, 9b, 9e	29	29a, 29b, 13b
9V	9a, 9c, 9d, 9g	31	31a, 20b
10	10a, 10b	32	32a, 27b
10A	10a, 10c, 10d	32A	32a, 32b, 27b
11	11a, 11b, 11e, 11g	33	33a, 33b, 33d
11A	11a, 11c, 11d, 11e	33A	33a, 33b, 33d, 20b
11B	11a, 11b, 11f, 11g	33B	33a, 33c, 33d, 33f
11C	11a, 11b, 11c, 11d, 11f	33C	33a, 33c, 33e
12	12a, 12b	34	34a, 34b
13	13a, 13b	35	35a, 35b, 34b
14	14a	35A	35a, 35c, 20b
15	15a, 15b, 15c, 15f	35B	35a, 35c, 29b
15A	15a, 15c, 15d, 15g	35C	35a, 35c, 20b, 42a
15B	15a, 15b, 15d, 15e, 15h	36	36a, 9e
15C	15a, 15d, 15e	37	37a
16	16a, 16b, 11d	38	38a, 25b
17	17a, 17b	39	39a, 10d
17A	17a, 17c	40	40a, 7g, 7h
18	18a, 18b, 18c, 18f	41	41a, 41b
18A	18a, 18b, 18d	41A	41a
18B	18a, 18b, 18e, 18g	42	42a, 20b, 35c
18C	18a, 18b, 18c, 18e	43	43a
19	19a, 19b, 19d	44	44a, 44b, 12b
19A	19a, 19c, 19d	45	45a
19B	19a, 19c, 19e, 7h	46	46a, 44b
19C	19a, 19c, 19f, 7h	47	47a, 35a, 35b

instance, consists of four serologically near-related types, 7, 7A, 7B, and 7C, which have more antigens in common. The diagnostic group sera react with all the types in the group.

In 1944 Eddy described some new pneumococcus types found in the USA, using her own nomenclature with consecutive numbers from 1 to 75. The new types put up by Eddy were later examined by Lund (1957) and are included in the Danish nomenclature.

TABLE 2

CORRELATION BETWEEN THE AMERICAN AND THE DANISH NOMENCLATURE OF THE GENUS *PNEUMOCOCCUS*

USA	Denmark	USA	Denmark
1	1	41	34
2	2	42	33B
3	3	43	11A
4	4	44	18A
5	5	45	40
6	6A	46	23A
7	7A	47	35A
8	8	48	7B
9	9N	49	9L
10	10	50	7C
11	11	51	7
12	12	52	47
13	13	53	11C
14	14	54	15B
15	15	55	18B
16	16	56	18C
17	17	57	19A
18	18	58	19B
19	19	59	19C
20	20	60	24B?
21	21	61	35C
22	22	62	35A
23	23	63	22A
24	24	64	23B
25	25	65	24A
26	6B	66	35B
27	27	67	32A
28	28	68	9V
29	29	69	39
30	15A	70	33
31	31	71	38
32	32	72	45
33	9A	73	46
34	10A	74	41A
35	35	75	43
36	36	76	11B
37	37	77	15C
38	41	78	17A
39	33C	79	28A
40	33A	80	42
		81	44

A comparison of the Danish and American designations (see Table 2) has been made by Kauffmann, Lund & Eddy (1960).

Reliable diagnostic pneumococcal sera are not at present on sale, but will in the near future be produced at the Statens Seruminstitut, Copenhagen, Denmark.

SUMMARY

All types of pneumococci have been found to be sensitive to the commonly used antibiotics and sulfonamides (they are moderately sensitive to streptomycin). The typing of pneumococci (by the Neufeld reaction) has therefore only little clinical interest. The differentiation of pneumococci from other sorts of streptococci may be made by observing (1) typical colonies on blood-agar, (2) diffuse growth in serum broth, (3) sensitivity to optochin, and (4) solubility in bile. Examination for haemolysis, fermentation and sensitivity to antibiotics has but little practical value. The pneumococcal antibodies in human serum are not found until the 4th to 5th day of illness, and the precipitation of type-specific polysaccharides in urine is very seldom positive, so these tests are not important for the diagnosis. Pneumococcal strains are best stored as freeze-dried cultures, but for shorter periods gelatin may be used.

Annex

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