Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1961, **24**, 771-784

Phage Typing of Staphylococci *

J. E. BLAIR, Ph.D.[1] & R. E. O. WILLIAMS, M.D., M.R.C.P.[2]

Standardized methods are essential if phage typing of staphylococci is to be reliable and if the results obtained in different laboratories are to be compared. This paper, prepared on behalf of the Subcommittee on Phage Typing of Staphylococcus of the Nomenclature Committee of the International Association of Microbiological Societies, gives a detailed account of methods that have been found satisfactory for propagating the phages and defines a standard testing routine by which the stability of the phage preparations can be verified. Technical details are given of methods recommended for determining the phage type of staphylococci and the interpretation of differences between the types is discussed. The need for close collaboration between the epidemiologist and the laboratory worker in the use of phage typing is emphasized.

INTRODUCTION

The wide adoption of phage typing for the characterization of coagulase-positive staphylococci in public health and hospital laboratories makes it highly desirable to have a common basis upon which an intelligent comparison of the results reported by those laboratories can be made. This goal can be approached only if all laboratories employ those reagents and procedures which have been demonstrated to be the most acceptable. Of fundamental importance are the test methods by which the suitability of the phages for typing is determined. It is the purpose here to describe in some detail the procedures for propagating and testing the phages and for typing, to indicate those which are regarded as essential, and to point out the limits of any permissible deviations.

The propagation methods to be described are designed primarily for the large-scale production of phage and for the testing of phages so prepared. The production of phage in bulk (e.g., 500 ml) has a distinct advantage over its preparation on a small scale: once a suitable preparation has been obtained, the stability of the phage ensures a uniform source of supply for many months, and the tedious task of determining the lytic spectrum (essential for each new batch) is reduced to a minimum. It is now the practice in certain countries for central laboratories to produce the phages in bulk and to distribute sets of phages to co-operating laboratories for routine use. There are also indications that the typing phages may be made available eventually by some commercial manufacturers. It is clearly most important that the full complement of control tests be carried out for every batch of phage prepared for such purposes. The individual laboratory which purchases commercially prepared phages should require evidence from the manufacturer that the testing requirements for bulk preparations have been satisfied in every detail. When laboratories have to prepare the phages for their own use and propose to use them within their own institution, some tests may be modified in certain details, as will be mentioned below.

Regardless of whether phages are obtained from a central distributing laboratory, from a commercial manufacturer, or are prepared for local use on a small scale, the individual laboratory has the responsibility of performing certain tests on the

* This report was prepared on behalf of the Subcommittee on Phage Typing of Staphylococcus of the Nomenclature Committee of the International Association of Microbiological Societies by the above authors in collaboration with Elizabeth H. Asheshov, M. Patricia Jevons and Elaine L. Updyke, and has been seen and approved by the members of the Subcommittee, a list of whom is given in the Annex on page 783.

[1] Department of Bacteriology, Hospital for Joint Diseases, New York, N.Y., USA; Chairman of the Subcommittee on Phage Typing of Staphylococcus.

[2] Professor of Bacteriology, Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, University of London, London, England (formerly, Director, Streptococcus and Staphylococcus Reference Laboratory, Public Health Laboratory Service, Colindale, London, England); Secretary of the Subcommittee on Phage Typing of Staphylococcus.

phages before they are used for typing. The laboratory must determine the routine test dilution (RTD) [1] of each phage by titration against the specific strain used for its propagation; this requires that a distributing laboratory or a commercial manufacturer shall provide proper test strains together with the phages. The laboratory must also check the potency and stability of the routine test dilutions by frequent tests.

All experience shows that phage typing of staphylococci can only be used satisfactorily when the laboratory is kept fully informed of the epidemiological inquiries for which it is needed. The interpretation of possible relations between different strains can only be made by the laboratory working in collaboration with the epidemiologist.

Phages used

There would be a distinct advantage in using a set of phages each of which was type-specific in the sense that it would lyse only a single type of *Staphylococcus*. Naturally occurring, type-specific phages are not common, nor has it been possible to produce from a single parent staphylococcal phage a series of specifically adapted phages such as were derived from the typhoid Vi phage II. Most strains of staphylococci, therefore, must be characterized by " pattern reactions ", which represent the susceptibility of the strains to various combinations of the typing phages. The number of patterns that are observed in the course of routine typing of staphylococci is very large and it would be impracticable to attempt to employ an equally large series of phages for routine typing.

The typing phages are liable to undergo variations and, while this should not ordinarily occur when they are handled according to accepted methods, the chance that it might occur is always present. For this reason detailed tests of the host range (lytic spectrum) of each new batch of phage are necessary to detect either a variation or a contamination. It is most inadvisable to substitute a new strain of *Staphylococcus* for any one of the established propagating strains. To do so is to risk a modification of the typing phage.

A relatively small, conveniently handled, set of phages has been found to be adequate for the identification of the majority of strains of staphylococci. For this purpose a set of 19 " basic " phages was selected by the Subcommittee on Phage Typing of Staphylococcus of the Nomenclature Committee of the International Association of Microbiological Societies at its meeting in Rome in 1953, and this set was extended to include 21 basic phages when the Subcommittee met in Stockholm in 1958. The set of basic phages has been selected so that, as far as possible, all strains of staphylococci are lysed by at least one phage, and yet the phages are sufficiently specific for relatively few strains to be lysed by many of them.

The Subcommittee now recommends that a set of 21 basic phages should be the minimum number used for routine typing. These are:

29, 52, 52A, 79, 80	(Group I)
3A, 3B, 3C, 55, 71	(Group II)
6, 7, 42E, 47, 53, 54, 75, 77	(Group III)
42D	(Group IV)
81, 187	(Miscellaneous)

There is no objection to the use by the individual laboratory of such additional phages as may be found to be useful locally—for example, some of the additional phages listed below. However, it is recommended that all publications which report the results of phage typing should include a list of all phages employed.

Under certain circumstances strains which do not react with the routine series of typing phages, even when used at 1000 times the normal working strength, may be tested with a group of additional phages. The phages available are: 42B, 47C, 52B, 69, 73, and 78. If desired, they may be combined into pools, each phage in a pool being present at its working dilution. Since only a very small proportion of cultures are typable with these extra phages when not typable with the basic set, the results do not appear to justify the added burden of attempting to maintain them in the average laboratory. It is recommended that the extra phages be restricted to central reference laboratories, where they may be used for special studies.

It may be noted here that the propagating strains are identified by the same numbers as are used to designate the phages. Thus, " PS 29 " is used for the propagation of phage 29, " PS 52 " for the propagation of phage 52, and so on. Phages 52A and 79 are both propagated on " PS 52A/79 ".

" Screening " of cultures by typing with a selected few of the phages rather than with the entire series cannot be recommended. The establishment of a strain as, for example, type 52A/79 demands not only the demonstration of lysis by phages 52A and 79 but also the absence of lysis by other phages.

[1] For definition of the routine test dilution, see page 775.

CULTURE MEDIA

In general, any good nutrient media can be employed for phage propagation and typing. Dehydrated media such as BBL (Baltimore Biological Laboratories) trypticase soy broth and agar, Difco "Bacto" nutrient broth, and Oxoid nutrient broth, and solid media prepared from them, have all been widely used with success. Alternatively, good nutrient broth prepared from fresh meat is satisfactory.

The phages require calcium ions for adsorption to or growth in the cocci; broth media used for propagation are best enriched with 400 μg per ml of calcium chloride. Media made with shredded agar do not usually require additional calcium. Recent observations at the Staphylococcus Reference Laboratory, Colindale, London, suggest that even a slight excess heating of the medium in preparation may "bind" the calcium in some way so that there is insufficient available for the more calcium-dependent phages. This defect can be rectified by the addition of calcium (400 μg per ml), but since the resulting agar may be slightly cloudy it seems better not to add the calcium unless it is found to be necessary.

Solid media for propagation and typing should be kept rather soft to facilitate the recognition of small phage plaques; if shredded agar is used, 1-1.2% is usually quite sufficient. Care should be taken not to dry the plates more than enough to remove surface moisture.

Each batch of medium should be tested for suitability for typing before use; this can be readily accomplished by setting up a plate of the new medium in comparison with the old by the method used for routine testing of the working phages, as described below (page 779).

PROPAGATION OF PHAGES

There are basically two different methods for propagating the phages: (*a*) in a liquid medium (broth) and (b) on the surface of a solid medium. Broth propagation is undoubtedly the simpler method and can be used with most of the basic set of typing phages, yielding phage concentrations between 5×10^8 and 10^{10} particles per ml, corresponding to RTD (as defined on page 775) levels between 2×10^{-4} (1 : 5000) and 10^{-5} (1 : 100 000). However, where higher titres are required, and also in particular with some of the typing phages (see Table 1), propagation on solid medium is preferable; it can be used as an alternative to broth propagation for all phages.

Several methods for propagation on solid medium have been described—the soft-agar layer method (Swanstrom & Adams, 1951), the freeze-and-thaw method (Williams & Rippon, 1952), the Cellophane method (Liu, 1958) and a surface method described recently by Zierdt (1959). These different methods have been compared at the Staphylococcus Reference Laboratory, Colindale, and the details will be published elsewhere by Asheshov; in these experiments the soft-agar method was found the easiest to perform and yielded the highest titres. In other circumstances the freeze-and-thaw or broth methods may be found preferable.

Culture and phage

Stock cultures and phages should be used as the starting material for the propagation of each new batch. It is important that neither the propagating strains nor the phages should undergo changes and freeze-drying is the most suitable method for storage. It is advisable, therefore, for laboratories undertaking phage propagation to dry the propagating strains and some of their first batch of each of the typing phages and to use this as starting material for all subsequent propagations. Laboratories with no facilities for freeze-drying should preserve a number of small samples of their first batch of each phage in the liquid state at 4°C. The staphylococci may be preserved in agar stab cultures. The practice of propagating phage serially from batch to batch should be avoided, since it entails dangers of propagating mutations and modifications of the phages.

The propagating strain is first subcultured from the freeze-dried ampoule or agar stab to a blood agar plate. A single colony is picked for use and the phage pattern is checked, using the phages of the basic set at the RTD (see definition below) and also at a concentration 1000 times stronger than the RTD. The phage pattern should conform to the standard pattern given in Table 2. The appearance of a strong (++) reaction at 1000 RTD where one is not recorded in the standard pattern, or the loss of a strong reaction that should be present, or any more marked deviation, suggests that the strain has undergone modification. Since the typing phages are very liable to undergo host-induced modification, a change in the propagating strain may be reflected in a change in the phage propagated on that strain; such propagating strains should therefore not be used.

TABLE 1
CHARACTERISTICS OF THE TYPING PHAGES

Phage		Propagating strain		Serological group of phage	Calcium requirement	Propagation method			
Phage No.	NCTC No.	PS No.	NCTC No.			Method of choice [a]	Optimal phage conc. per ml	Temperature (°C)	Incubation period (hours)
29	8413	29	8331	B	absolute	soft agar	1×10^6	30	18
52	8401	52	8507	B	"	"	1×10^4	30	18
52A	8420	52A/79	8363	B	"	"	1×10^4	37	18
79	8290	52A/79	8363	B	"	"	1×10^4	37	18
80	9788	80	9789	B	"	"	5×10^4	37	18
3A	8408	3A	8319	A	partial	soft agar or broth	1×10^5	37	18
3B	8410	3B	8321	A	"	soft agar or broth	1×10^5	37	18 6
3C	8411	3C	8327	A	"	soft agar or broth	1×10^5	37	18 6
55	8429	55	8358	B	absolute	broth	1×10^5	37	6
71	9316	71	9315	B	"	"	1×10^5	37	6
6	8403	6	8509	A	partial	soft agar or broth	1×10^5	37	18 6
7	8404	7	8510	A	"	broth	1×10^5	37	6
42E	8418	42E	8357	A	"	"	1×10^5	37	6
47	8409	47	8325	A	"	soft agar	1×10^5	37	18
53	8406	53	8511	B	absolute	broth	1×10^5	37	6
54	8412	54	8329	A	partial	"	1×10^5	37	6
75	8427	75/76	8354	A	"	"	1×10^5	37	6
77	8428	77	8356	F	"	"	1×10^5	37	6
42D	10032	42D	10033	F	"	soft agar	1×10^4	37	18
81	9716	81	9717	A	"	broth or soft agar	1×10^5	37	6 18
187	9753	187	9754	L	absolute	soft agar	2×10^5	30	18
42B	8419	42B/47C	8355	A	partial	soft agar	1×10^5	37	18
47C	8421	42B/47C	8355	A	absolute	"	1×10^5	37	18
52B	9304	52B	9303	B	"	"	1×10^5	37	18
69	8398	69	8397	B	"	"	1×10^5	37	18
73	8430	73	8360	A	partial	broth	5×10^5	37	18 [b]
78	9314	78	9313	A	"	broth or soft agar	1×10^5	37	6 18

[a] Based on experience at the Staphylococcus Reference Laboratory, Colindale.
[b] Phage 73 withstands heating to 50°C for 60 minutes, and this method may be used for the sterilization of its lysates.

Titration of phage

Before propagation is commenced, the titre of the phage has to be measured to indicate the dose of phage to be added to the propagation medium. The dried phage is suspended in 1.0 ml of broth, and this or a fluid stock is diluted in tenfold steps to 1 : 1 000 000 (10^{-6}); separate pipettes must be used for each dilution step. One drop (i.e., 0.02 ml) of each dilution is then applied to the surface of an agar plate previously spread with a 4- to 5-hour

TABLE 2
PHAGE PATTERNS OF THE PROPAGATING AND TEST STRAINS OF STAPHYLOCOCCI

Basic set PS No.	NCTC No.	RTD	1000 RTD
29	8331	29++	29++ 52° 52A° 79° 80°
52	8507	52++ 52A± 80±	52++ 52A++ 79° 80++
52A/79	8363	52A++ 79++	52++ 52A++ 79++ 80++
80	9789	80++ 81++	29° 52± 52A± 80++ 81++
3A	8319	3A++ 55± 71±	3A++ 3B++ 3C++ 55++ 71++
3B	8321	3B++ 3C++ 55++ 71++	3A++ 3B++ 3C++ 55++ 71++
3C	8327	3B++ 3C++ 55++ 71++	3A++ 3B++ 3C++ 55++ 71++
55	8358	3B++ 3C++ 55++ 71++	3A++ 3B++ 3C++ 55++ 71++
71	9315	3C++ 55++ 71++	3C++ 55++ 71++
6	8509	6++ 7++ 42E± 47++ 53++ 54++ 75++ 77++ 81±	6++ 7++ 42E++ 47++ 53++ 54++ 75++ 77++ 81++
7	8510	6++ 7++ 42E± 47++ 53++ 54++ 75++ 77++ 81±	6++ 7++ 42E++ 47++ 53++ 54++ 75++ 77++ 81++
42E	8357	42E++ 81±	42E++ 53++ 81++
47	8325	47++ 53++ 75++ 77++	29++ 52+ 52A+ 79++ 80++ 7++ 47++ 53++ 54++ 75++ 77++
53	8511	53++ 54+ 75++ 77++	53++ 54++ 75++ 77++
54	8329	7++ 47++ 53++ 54++ 75++ 77++ 81±	79+ 3B+ 7++ 42E++ 47++ 53++ 54++ 75++ 77++ 81++
75/76	8354	53++ 75++ 77++	79+ 7° 47° 53++ 54° 75++ 77++
77	8356	77++	80+ 47++ 53++ 54° 77++
42D	10033	42D++	42D++
81	9717	80++ 81++	52± 80++ 42E± 81++
187	9754	187++	187++
42B/47C	8355	81++	52++ 79± 80+ 7± 42E± 47± 53± 75± 77± 81++
52B	9303	47± 53+ 77++ 81±	52+ 6° 7° 42E++ 47++ 53++ 54° 75++ 77++ 81++
69	8397	Not typable	52°
73	8360	3C++ 6++ 7++ 42E++ 47++ 54++ 75++ 77++ 81++	29° 52° 52A° 79++ 80° 3B++ 3C++ 55++ 71° 6++ 7++ 42E++ 47++ 53° 54++ 75++ 77++ 81++
78	9313	Not typable	54++
42C	8353	3C± 71±	29± 3A++ 3B++ 3C++ 71++ 42E+ 47± 53++ 54++ 75+ 81±
2009	10019	52++	29++ 52++
8719	10017	71++	3B° 71++

± = less than 20 plaques.
+ = 20-50 plaques.
++ = more than 50 plaques.
° = inhibition (used at 1000 RTD only).

broth culture of the propagating strain and the plate is incubated at 30°C overnight. The plate is examined the following day and the phage titre and the RTD are determined. The RTD is the highest dilution that just fails to give confluent (complete) lysis, ideally as in Fig. 1A. Near-confluent lysis is preferred to confluent lysis for defining the RTD because it is more exactly determined; confluent lysis can be produced by all phage dilutions up to a certain point, while near-confluent lysis is produced

only in one narrow range of dilutions. Where none of the tenfold dilutions tested gives the required phage concentration for RTD, simple interpolation may be used (see Fig. 1B). In reading titrations, secondary growth of staphylococci developing in the titration drop area is ignored. The concentration of phage sufficient to give near-confluent lysis varies somewhat, depending on the plaque size of the particular phage, but lies between 5×10^4 and 2×10^5 particles per ml. The phage concentration required for confluent lysis is between 2 and 10 times greater than that required to produce the RTD as defined above.

Propagation in liquid medium

The optimal phage : culture ratio varies slightly with different phages, but a standard procedure, which gives satisfactory results with most of the phages, consists in adding a broth culture of the propagating strain grown overnight at 37°C to the medium to give a final dilution of 1 : 100; phage is then added to give a final dilution equivalent to the RTD. The mixtures are incubated at 37°C, with shaking, for 6 hours.

Immediately after incubation the lysate is centrifuged and the supernatant fluid is taken off and titrated by spotting serial tenfold dilutions on a plate spread with the propagating strain. The lysate is then stored overnight at 4°C and, if the titre is sufficiently high, is filtered and retitrated. The filtrate is then distributed into sterile screw-cap bottles which are kept at 4° C without preservative.

Titres of 10^9-10^{10} phage particles per ml, corresponding to an RTD of 10^{-4}-10^{-5}, should be obtained by this method; ordinarily, filtrates with an RTD lower than 10^{-3} are discarded.

Propagation in soft-agar layer

Six-inch (15-cm) Petri dishes containing nutrient agar to a depth of about 5 mm are used. Nutrient broth containing 0.5% shredded or granular agar and 400 μg/ml of calcium chloride is prepared and cooled to 45°C. Cells from a 4- to 6-hour broth culture, or the growth from an overnight agar slope culture, of the propagating strain are added to the melted agar to give a final concentration of about $2\text{-}5 \times 10^7$ cells per ml, followed by phage in a concentration sufficient to produce near-confluent lysis after overnight incubation. This phage concentration varies somewhat; the concentration found to be satisfactory for the different phages is given in Table 1. After mixing, 7.5-10 ml of the cell-phage-agar mixture is transferred to the surface of the agar plate. The plates are incubated for 18 hours; the optimal incubation temperature varies with the phage, some phages yielding maximum titres when incubated at 37°C, others giving the best results at 30°C (see Table 1). After incubation, 20 ml of broth are added to each plate. The soft-agar layer is stirred up with a sterile bent glass rod and both agar and broth are taken off. The agar lumps are broken up by rapid pipetting or shaking and the mixture is then centrifuged and the supernatant removed. Alternatively, the whole plate may be frozen at −60°C, thawed at room temperature, and the expressed fluid removed and centrifuged. The supernatant fluid prepared by either of these methods is titrated and stored overnight at 4°C. If the RTD is 10^{-3} or more the lysate is filtered the following day and retitrated after filtration.

It is undesirable to carry out more than two serial propagations from the stock material. Provided that the medium and inoculum are satisfactory, it is found that high-titre phage lysates can be prepared directly from the freeze-dried stock material.

Sterilization of lysates

It is not sufficient to rely on lysis by the phage to produce a lysate free from staphylococci, nor is it possible to remove all the staphylococci by centrifugation. Cocci remaining in a lysate can liberate their carried phage, or may adsorb some of the specific phage and lower the titre. Sterilization is best achieved by filtration and any bacteriological filter capable of removing bacteria without adsorbing too much phage can be used. Sintered-glass ("5/3") and porcelain filters appear to be the best. Berkefeld candles and membrane filters are satisfactory in that they do not adsorb phage, but difficulty may be experienced in obtaining sterile filtrates. Seitz filters are good for sterilization but tend to adsorb too much phage.

Sterilization of phage lysates by chemical means has been widely used, but generally has the disadvantage that there is some residual bactericidal effect in the undiluted lysate.

TESTING OF PHAGE FILTRATES

The proper testing of new batches of phage filtrates is probably the most important step in the production of typing materials. *Staphylococcus* typing phages are very liable to undergo host-induced

modifications, so that alteration in the propagating strain used may have a profound effect on the phage produced. And almost all the propagating strains of staphylococci are themselves lysogenic, so that there is always a risk that the phages from the propagating strain may be present in significant concentration in the final lysate. The fact that the types of staphylococci are determined by pattern reactions makes it important that the typing phages should conform to the standard in their minor as well as their major reactions.

The standard testing routine comprises three phases: (1) the preliminary titration to determine the RTD, carried out by the method given on page 774; (2) the comparison of the " lytic spectrum " of the phage—i.e., its lytic activity on a defined range of staphylococci—with that regarded as standard for the particular phage; and (3) the routine testing of the diluted phage in use for typing to verify that its titre remains adequate.

Determination of lytic spectrum

The lytic spectrum is determined by testing the phage against its propagating strain and a set of standard test strains (see Table 4 overleaf) and is carried out to detect any possible mutations or modifications which may have occurred during propagation. The 16 test strains are chosen so that phages with similar lytic spectra can be differentiated.

Stock filtrates with an RTD of 10^{-4} or less are tested undiluted. Stronger filtrates are first diluted: tenfold if the RTD is 10^{-5}, 100-fold if it is 10^{-6}, etc. This preliminary use of a dilution not more than 10 000 times more concentrated than the RTD eliminates many " inhibitory effects " and reduces the number of titrations needed subsequently. Testing is carried out on the same agar medium as is used for routine typing and plates are incubated for the same time and at the same temperature as routine typing plates.

The phage in the concentration just mentioned is first tested against all the test strains ("LS1") and is subsequently titrated ("LS2") on those strains that give any degree of lysis or inhibition (see Fig. 2). The dilutions giving minimal ++ reactions on the various test strains are compared with the dilution giving approximately the same reaction on the homologous propagating strain and recorded as below:

5 = a ++ reaction in the same dilution as on the propagating strain.

4 = a ++ reaction in a dilution 10-10^2 times more concentrated than that giving ++ on the propagating strain.

3 = a ++ reaction in a dilution 10^3-10^4 times more concentrated than that giving ++ on the propagating strain.

2 = a ++ reaction in a dilution 10^5-10^6 times more concentrated than that giving ++ on the propagating strain.

1 = very weak lysis.

High-titre phages may " inhibit " the growth of many of the strains when used undiluted, but produce no discrete plaques when diluted. In some cases this inhibition may simulate confluent lysis, but generally it appears as a thinning of the growth in the drop area. Such reactions are recorded as " 0 " in Tables 3 and 4.

In general, the appearance of a grade 3, 4 or 5 reaction where none should exist, or the complete absence of such a reaction where one should exist, is an indication for rejection of a batch of phage. Variations of grade 4 to 5, 3 to 4, etc., and loss or gain of a grade 1 reaction are permissible. Before rejecting a batch of phage it is, of course, necessary to be certain that it is not one of the indicator strains of staphylococci that is at fault.

An example of the lytic spectrum of phage 3B is given in Table 3 and the standard spectra for the typing phages appear in Table 4.

TABLE 3

LYTIC SPECTRUM OF PHAGE 3B

LS1. Phage 3B, at a concentration of 10 000 × RTD, gave the following reactions on the test strains:

3A,++; 3B, ++; 8719, 0; 42C, 0; [a] 54, ++; 75, +

LS2. When phage 3B was titrated against these strains the results were as follows:

Strain	Phage dilutions							Reaction coded as
	Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
3B	++	++	++	++	++	++	+	5
3A	++	++	++	+	±	.	.	3
8719	0	.	.	.	.	.	.	0
42C	++	++	+	±	.	.	.	3
54	++	+	±	.	.	.	.	2
75	+	±	.	.	.	.	.	2

[a] The reaction on strains 8719 and 42C in the LS1 tests both appeared to be due to inhibition (0), but titration in the LS2 tests showed that the reaction on 42C was due to true phage lysis while that on 8719 was due to inhibition.

TABLE 4

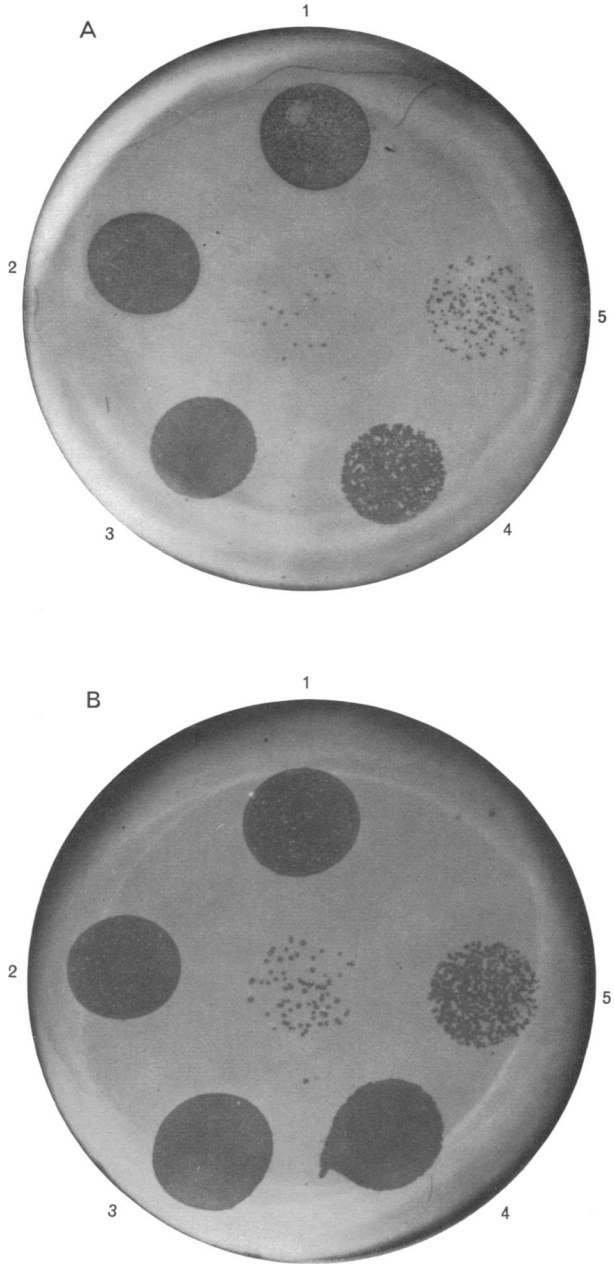
LYTIC SPECTRA OF THE PHAGES

Test strain	Phages																										
	29	52	52A	79	80	3A	3B	3C	55	71	6	7	42E	47	53	54	75	77	42D	81	187	42B	47C	52B	69	73	78
29	5	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.
52	0	5	4	0	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	3	.	.	.
52A/79	.	3	5	5	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
80	.	1	1	.	5	.	.	.	.	.	.	1	2	.	.	.	.	.	.	5	.	0	0	.	.	3	.
2009	3	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	4	3	.	.
3A	1	1	1	1	1	5	3	4	4	4	1	1	1	1	1	1	.	1	1	1	.	1	1	2	1	1	1
3B	.	.	.	.	.	3	5	5	5	5	.	.	.	.	.	.	1	.	.	.	.	.	1	.	.	.	.
71	1	.	.	.	.	.	.	5	5	5	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
8719	.	.	.	.	.	.	0	.	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
42C	2	0	0	0	0	3	3	4	0/2	4	.	0	3	2	3	3	2	0	.	3	.	2	.	.	.	.	.
42E	0	0	0	0	0	.	.	.	.	.	.	.	5	.	3	2	.	.	0/2	3	.	2	1	.	.	.	3
47	3	3	3	3	3	.	.	.	.	.	2	2	.	5	5	3	5	5	.	0	.	2	4	5	1	2	.
53	.	.	.	.	.	.	.	.	.	.	0	0	.	0	5	4	5	5	.	1	.	.	.	.	.	0	.
54	1	.	.	2	.	2	2	2	.	.	.	5	3	5	5	5	5	5	.	3	.	4	4	2	1	4	3
75	.	.	.	2	1	.	2	2	.	.	.	0	1	0	4	0	5	5	.	.	.	.	.	.	1	0	.
77	.	.	.	.	2	.	.	.	.	.	.	0	.	2	4	0	.	5	.	.	.	.	.	4	1	.	0

NOTES:

1. Phages propagated on strains not included in the test set are also tested on their propagating strains, on which the reaction is by definition " 5 ".
2. The few minor differences between this table and the corresponding tables previously published derive partly from further experience with the phages and partly from the results of a comparative test of the phages currently in use in five national laboratories.
3. The notation 0/2 is used for reactions which are variable and which may appear as inhibition reactions on one occasion and as true lytic reactions on another.

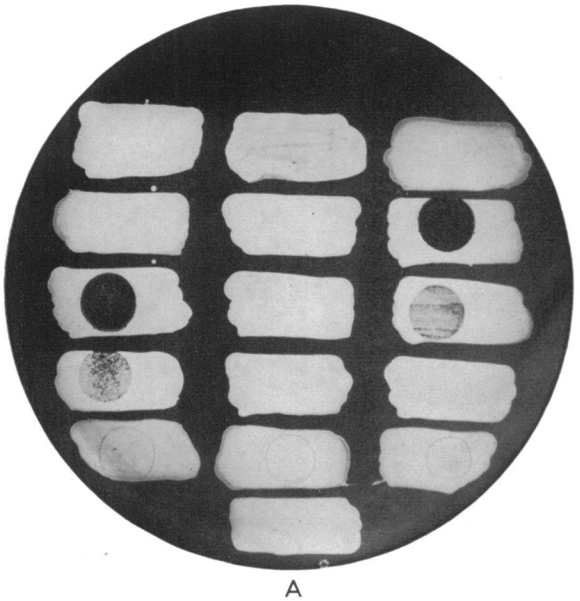
FIG. 1
TITRATION OF PHAGE FILTRATES [a]



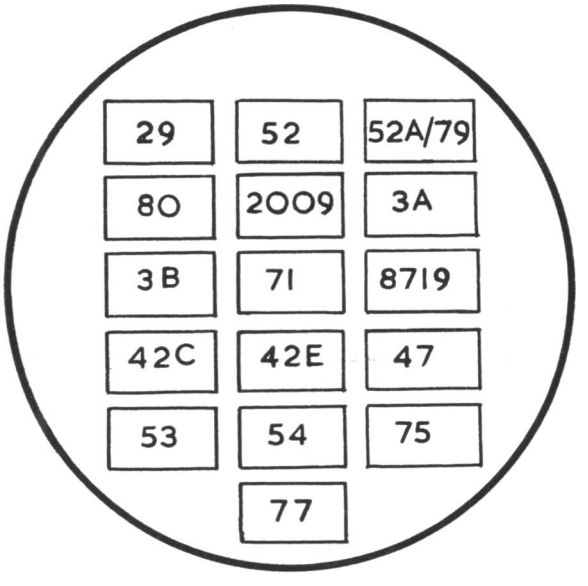
[a] In A the RTD is the 4th dilution; in B it would be interpolated as between the 4th and the 5th dilution.

FIG. 2

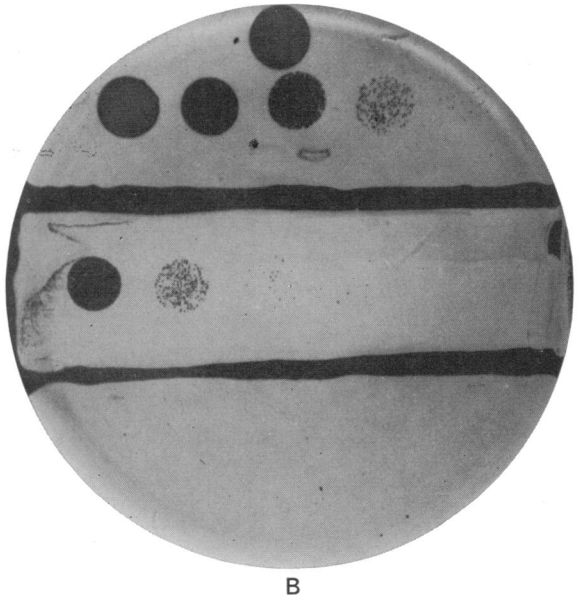
DETERMINATION OF THE LYTIC SPECTRUM OF PHAGE 3B



A



KEY TO STRAINS



B

A. Determination of LS1, with key to the arrangement of the test strains.

B. Titration for LS2 10^{-1} to 10^{-6} dilutions on strains PS 3B, PS 3A and 8719. The reaction on 8719 is entirely due to inhibition.

FIG. 3

ROUTINE TESTING OF PHAGES AT RTD

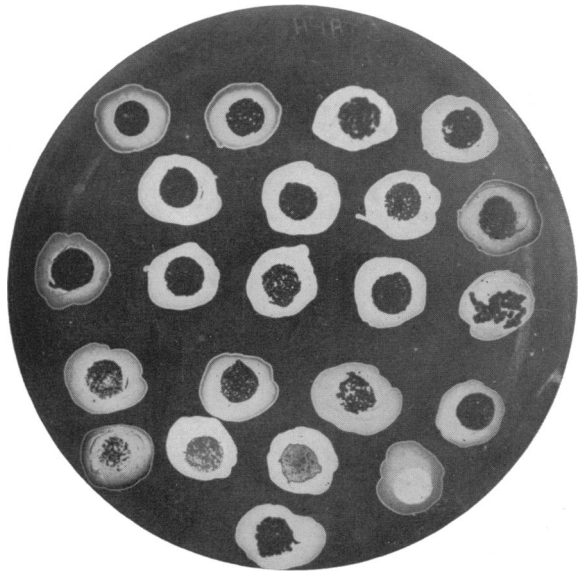
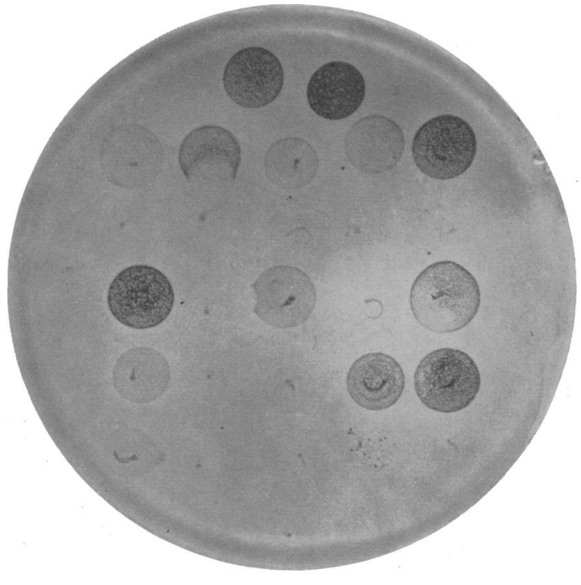


FIG. 4

TYPING PLATE OF A STRAIN OF *STAPHYLOCOCCUS* THAT IS PARTICULARLY SENSITIVE TO INHIBITION BY 1000 RTD FILTRATES[a]



[a] All the apparently ++ reactions on this plate, except that at the top right and the fourth drop in the bottom row, are due to inhibition. Several other phages have produced lesser degrees of inhibition.

Experiments are at present in progress to discover whether it would be practicable to prepare samples of a "standard" phage which could be tested in parallel with any new batch of phage. The lytic spectrum of the new batch would then have to conform to that of the standard tested at the same time and on the same cultures and there would be less need for the sometimes difficult comparison with the recorded results of previous tests.

The testing routine described is necessarily somewhat complex if possible variations in the typing phage, with consequent differences in the typing pattern reported, are to be recognized. It may be emphasized, however, that by the use of either of the propagation methods described it is possible to prepare large batches of phage and that almost all of the typing phages are sufficiently stable to be stored in the fluid state for at least 2-3 years before use. With batches of this size, even an elaborate testing scheme should not prove too onerous.

Where phage is prepared centrally for distribution to several laboratories it is most important that no step in the testing scheme be omitted. A hospital preparing phage simply for its own use could consider the use of a single dilution (e.g., RTD) for estimation of the lytic spectrum. Even for laboratories that do not have to distribute phage, however, it seems preferable to make relatively large batches and carry out the full testing.

Routine testing

The routine test dilutions are stored at 4°C and are tested for potency at least once a week. An agar plate is inoculated in small areas with 4- to 5-hour broth cultures of the propagating strains; a drop of the test dilution of each phage is spotted on to its own propagating strain (Fig. 3).

A test dilution is satisfactory for typing as long as the spot test shows near-confluent lysis. When near-confluent lysis is no longer produced, the dilution is discarded and a fresh dilution is made from the stock phage, its potency being confirmed by a spot test before it is placed in the set of dilutions used for typing. A test dilution should not be used for typing unless it has been spot-checked within at least 7 days just prior to typing. The test dilutions of the majority of phages usually remain satisfactory for typing for from 4 to 6 weeks and sometimes longer. However, the stability of the test dilution of any given phage is not predictable, and frequent periodic checks of the test dilutions of all phages are essential.

TYPING TECHNIQUE

Typing

The typing phages under discussion are designed for the examination of staphylococci from human sources or closely related environmental sources. While many bovine strains are susceptible to these phages, most strains from other animal species are not; all must be examined with phages derived specifically for use with the appropriate animal strains of staphylococci.

Only pure cultures of coagulase-positive staphylococci should be submitted to typing; coagulase-negative strains are not susceptible to these phages. The cultures should be obtained by fishing from one or more well-isolated colonies according to the established practice of the individual laboratory. Impure or mixed cultures should not be submitted to a central laboratory for typing, for the usual work load of the central laboratory precludes the added burden of purifying cultures.

Cultures are always typed first with the phages at RTD. They are submitted to further testing only when no significant lysis is produced by any phage of the typing series at RTD.

One standard 9-cm agar plate is used for each culture to be typed. The agar should be free from surface moisture.

When the phages are applied individually by hand, rulings in the form of a grid of 25 or more squares on the bottom of the plate greatly facilitate both the application of the phage dilutions to the plate and the orientation of the phages when the lytic reactions are read subsequently. The grid may be etched permanently on the plate, ruled with a glass-marking pencil, or applied with a rubber stamp. Alternatively, a template containing the grid may be placed beneath the plate as a guide when the phages are applied.

Cultures to be typed are inoculated into broth and incubated at 37°C for 4-5 hours, or sufficiently long to produce distinct turbidity. When cultures of this age are used, the broth is usually inoculated in the forenoon and the typings are set up in the afternoon. If the work load is so large that typing must be started in the forenoon, the broth cultures may be prepared on the previous day. Broth cultures incubated at 37°C for 16-18 hours have been found to be satisfactory for seeding the plates. Alternatively, the culture may be incubated at 37°C for 4-5 hours, held in the refrigerator overnight, and reincubated for about 30 minutes just prior to

inoculation of the plates. The aim is to produce on the surface of the agar a uniform lawn of staphylococcal growth which supplies an adequate substrate for phage action but is not so heavy as to obscure the plaques.

The plates are inoculated by either (*a*) flooding the surface of the agar with 1-2 ml of the broth culture and drawing off the excess with a pipette, or (*b*) spreading the inoculum evenly over the entire surface of the agar with a swab that has been moistened thoroughly with the broth culture. The plates are then allowed to dry at room temperature; on plates that have been previously freed adequately from surface moisture, the inoculum should dry within 10-30 minutes.

The phages are then applied to the seeded plate, a small drop of the RTD of each phage being placed over the centre of a ruled square. The phages are always placed on the plates in an established sequence so that each square corresponds to a particular phage. Pasteur pipettes drawn out to a fine tip, or 1-ml syringes equipped with 27-gauge (0.40 mm) needles, are used to apply the phages, which are best dropped off on to the agar. Care should be taken not to touch the agar with the tip of the pipette or needle. Touching the agar may transfer staphylococci from one plate to the next and result in the appearance of plaques of non-specific lysis produced by phage that is harboured by the cocci so transferred; usually, plaques of non-specific lysis are few in number and are distributed irregularly over the area of the drop.

When the drops of phage have dried, the plates are incubated at 30°C for 18 hours. Alternatively, they may be incubated at 37°C for 4-6 hours and then held at room temperature overnight. The plates should not be incubated continuously at 37°C overnight since heavy growth of the staphylococci tends to obscure the phage plaques.

Several mechanical devices have been proposed for the simultaneous application of all the phages to the plate. These devices offer considerable advantages over the application of the phages individually by hand, including the saving of time spent in monotonous work (especially important when many cultures are typed daily) and of the need for specially ruled plates.

One such device is a " multiple-loop " applicator, as devised by Tarr (1958) and Lidwell (1959), which simultaneously deposits 3-mm loopfuls of each phage preparation on the previously seeded plate by means of wire loops carried on a horizontal arm. Another type of applicator consists of steel or aluminium pins set in a template. The phages are transferred on the pins from an appropriate receptacle to the surface of the uninoculated plate; after the phage drops have dried the plate is seeded by flooding the surface with a broth culture and drawing off the excess with a pipette. Other devices for application of the phage are doubtless practicable. With any device it is important that tests of reproducibility should be carried out and it may be preferable to titrate the phages specifically for use with the particular equipment.

If a culture cannot be characterized by a pattern of significant reactions when typed at RTD it may be retested with the same phages in stronger concentrations or submitted to a wider range of phages. The former alternative is the more successful. Cultures which show no significant lysis or which are not lysed in any degree by any phage of the typing series when typed at RTD are retyped with more concentrated phage preparations. The dilution recommended for this purpose is 1000 times stronger than the RTD; this implies that, with phages of relatively low titre, it may be necessary to retype at a 1 : 10 dilution or even undiluted.

As mentioned above, retyping with the " extra " phages should be restricted to central reference laboratories, where these phages should be used only for special studies. Lysis by any of these phages is to be reported only when a lytic pattern is not obtained with any phage of the regular typing series, either at RTD or at 1000 RTD.

Reading and reporting of results

The plates are examined by indirectly transmitted light against a dark background with the aid of a hand lens of moderate magnification. Alternatively, they may be read on a Quebec colony counter, or some similar device which can be fitted with a glass plate suitably etched with a grid and the phage numbers.

When strains are typed at RTD, susceptibility to the phages is indicated by varying degrees of lysis, from a few discrete plaques to completely confluent lysis, which coincides with the area of the phage drop. Secondary phage-resistant growth may occur in the area of confluent lysis.

Similar reactions ranging from discrete plaques to confluent lysis are also produced when strains are typed at 1000 RTD. In addition, the more concentrated phages may produce a reaction of " inhibition " which is sometimes difficult to distinguish

from secondary growth due to phage-resistant cocci. Its appearance is frequently that of a somewhat translucent film or " veil ", covering the area of the drop, or a distinct thinning of the growth in the area (Fig. 4). Plaques are sometimes superimposed upon the area of inhibition.

When certain phages of high titre are spotted at 1000 RTD on some strains of staphylococci they may produce an inhibitory effect which is represented by a clearing in the drop area entirely like the confluent lysis which results from true phage action. This effect, and inhibition that simulates confluent lysis with secondary growth, can be distinguished from true lysis by titration of the phage against the inhibited strain; no plaques will be produced in any dilution if the reaction is one of inhibition, but plaques will occur in some dilution if the reaction is one of lysis. Such detailed investigation is only needed in special cases.

The degrees of lysis observed both at RTD and at 1000 RTD are entered in the laboratory records by the following symbols:

++ = more than 50 plaques; semi-confluent lysis; confluent lysis (with or without secondary growth).

\+ = 20-50 plaques.

± = less than 20 plaques.

Reactions of inhibition which are encountered with the phages at 1000 RTD are recorded as " 0 ".

All lytic reactions from over 50 plaques to confluent lysis are regarded as " strong " reactions. Lesser degrees of lysis are considered as " weak " reactions.

The results of phage typing of a strain are reported in terms of those phages that produce strong lysis of the strain—i.e., the phages that produce reactions of any degree from over 50 plaques to confluent lysis. This is the " phage pattern " of the strain, sometimes referred to as the " type ". The pattern is usually reported in a form such as: 52/52A/80, or 6/47/53/54/77, or 71. In some laboratories it is the practice when making a report to indicate the presence of weak reactions by placing a + sign after the pattern of significant lysis, e.g., 6/47/53/77/+; in this case it may be well to append to the report a list of those phages which produced the weak lysis.

Individual cultures of the same strain, especially those of a set from presumably related sources, may exhibit some slight differences of pattern. When the results of typing of strains isolated in an epidemiological study are reported it is often helpful to indicate those cultures that are considered to be the same. The criteria by which the probable identity of strains is determined are described below.

When strains are retyped at 1000 RTD, the pattern is also reported in terms of those phages which produce from over 50 plaques to confluent lysis. Lesser degrees of lysis and reactions of inhibition are disregarded. The report may well include an indication that the reactions were obtained with concentrated phages but not with the routine test dilution. In exceptional cases when only a pattern of inhibitory reactions occurs, the pattern may be reported in terms of inhibition by the phages.

Interpretation

It cannot be too strongly emphasized that the laboratory can give a useful interpretation of the phage-typing results only if it is provided with full details of the source of the strains and the precise question that the typing is required to answer. Moreover, although, for convenience and clarity, the reported " type " of a *Staphylococcus* is given in terms of the pattern of strong lysis that it shows, the laboratory is sometimes able to elucidate the epidemiological relations further by consideration of additional weak reactions.

For the most part, the activity of a phage belonging to one of the broad phage groups is restricted to its appearance in patterns with some of the other phages of the same group, and most of the coagulase-positive staphylococci can be separated into four principal groups which correspond to the broad subdivisions of the phages. A certain proportion, from 1% to 10%, show patterns that involve phages of groups I and III, even at RTD. Exceptional strains may exhibit patterns composed of phages of several groups.

The assignment of staphylococci to broad groups is not sufficient to demonstrate differences between individual strains, especially for purposes of epidemiological study. The finer distinction that is necessary for their differentiation is obtained by a comparison of the individual phage patterns. The patterns that are encountered in routine typing are numerous, and there is considerable overlap between them. It is therefore the usual practice to characterize each individual culture in terms of its particular phage pattern; this is customarily done by listing those phages which produce strong lysis of the culture. The term " type " is often used to refer to a phage pattern—e.g., " type 75/77 " or " type 3B/3C/55 ".

TABLE 5
REPORTING AND INTERPRETATION OF PHAGE PATTERNS

Strain No.	Reactions with phages: 6	7	42E	47	53	54	75	77	Reported pattern	Interpretation
1	++	++	±	++	++	++	++		6/7/47/53/54/75/+	One type
2	++	+		++	++	++	++		6/47/53/54/75/+	
3	++	±		++	++	++	±	±	6/47/53/54/+	
4	++	±		++	++	±	±		6/47/53/+	
5	++				++			++	6/53/77	Different from strains 1-4 and from one another
6				++	++		++	++	47/53/75/77	
7							++	++	75/77	

The validity of phage typing as a means of identification depends closely upon the stability of the phage pattern. Detailed studies have shown that the phage pattern of a given strain is, in fact, essentially stable. However, some variation is likely to occur in the phage patterns of a set of presumably related cultures, the degree of variation between the several patterns tending to increase with the remoteness of the individual cultures from their probable common source. Thus the basic phage pattern of a strain is reproducible in replicate cultures with, at the most, only minor differences from the pattern of the parent strain. This is usually true also of cultures of the same strain isolated at the same time from different sites in one individual. In an acute epidemic of relatively brief duration, such as might occur in an obstetric unit or a surgical ward, or in an outbreak of food-poisoning, the several cultures of the responsible strain isolated from the patients usually show only slight variations in their phage patterns. However, when an epidemic is prolonged and when the responsible strain is transmitted successively through a series of infected patients or healthy carriers, the several isolates of the strain may tend to show greater variations from the pattern of the original parent strain. To reduce the chance of additional variation due to different batches of culture media or other environmental conditions, it is advisable, whenever possible, to type all cultures of a related set on the same day.

Many sets of strains thus present no special problems of interpretation, for identical cultures can be recognized by their identical or closely similar phage patterns, while distinct differences between the patterns serve to distinguish the unrelated cultures. The chief problems of interpretation arise among cultures, usually those of a set that are presumed to be related, which exhibit patterns that are rather similar but which nevertheless do not show complete correspondence. Often these differences are represented by one or two strong reactions which are present in certain patterns of the set but are absent from the others. When such differences occur in the patterns of presumably related cultures, it becomes necessary to decide whether the divergence is sufficient to indicate that the cultures are different or whether the resemblance is close enough to suggest that they are probably related.

As a working guide to the interpretation of such patterns, when typing is done at RTD two cultures are considered to be different when one is lysed strongly by at least two phages which produce no lysis of the other to any degree. While a difference of only one strong reaction may be shown by related cultures, it should always be interpreted with caution (Table 5). These criteria are based upon the variations that are known to occur. While they have proved to be useful in many laboratories, they must be regarded only as a rough guide and subject to modification under certain circumstances. For example, although only the strong reactions are usually considered significant in the phage pattern, two presumably related strains may be regarded as probably identical when the pattern of one exhibits some weak reactions by phages that produce strong lysis of the other. A difference of two strong reactions can occasionally be interpreted rather liberally, but *only* when knowledge of the history and source of the cultures strongly suggests that they are probably related. In any case, the whole set of cultures needs to be considered; if many strains of identical phage

pattern have been received from one source, a strain showing a difference of only one strong reaction could probably be safely assumed to be a different strain. If the set of cultures included many strains showing closely similar but not identical patterns, then a new culture would have to show a wider variation before it could be accepted as a different type.

When cultures are retested with the more concentrated phages, e.g., at 1000 RTD, the criteria mentioned above may be applied if the reactions are those of strong lysis. Often the concentrated preparations produce a number of weak reactions of less than 50 plaques, or reactions of inhibition, which are not reproducible enough to be useful. It is therefore necessary to interpret conservatively the results obtained with the more concentrated phages; it is advisable to give weight only to the strong reactions of more than 50 plaques and to disregard all lesser degrees of reaction. Occasionally, a reaction of inhibition may provide the only indication of susceptibility to the phages. In such a case, identical patterns of inhibition may serve to identify related strains; the report should indicate that the probable relationship is assumed on this basis.

Provided that it has adequate epidemiological data, the laboratory can indicate on the report those strains that could reasonably be considered to have a common source. For this reason, it is most important that there should be close co-operation between the laboratory and the epidemiologist, for it is only by mutual exchange of information that a proper evaluation and interpretation of phage typing can be reached.

SUPPLY OF PHAGES

At the meeting of the Subcommittee on Phage Typing of Staphylococcus held at Stockholm in 1958, it was agreed that the Staphylococcus Reference Laboratory, Colindale, should serve as the International Staphylococcus Reference Laboratory and should be responsible for the supply of typing phages, propagating strains and test strains to national laboratories throughout the world (Subcommittee on Phage Typing of Staphylococcus, 1959). It was envisaged that in each country a laboratory carrying out phage typing for public health workers would be recognized as the National Laboratory and would be prepared to distribute material to other laboratories in that country. The supply of materials to workers in other countries is a function of the Staphylococcus Reference Laboratory at Colindale. The Subcommittee on Phage Typing of Staphylococcus is composed of representatives from the various national laboratories; the members of the Subcommittee and their respective laboratories are listed in the Annex to this report.

In several parts of this report we have emphasized the advantages to be gained by bulk production of phages and their distribution to laboratories undertaking phage typing as compared with the propagation of the phages in each separate laboratory. In the United Kingdom this function is undertaken by the Public Health Laboratory Service; and in the USA arrangements are made for the bulk production of phage by the Communicable Disease Center for use in a number of State health laboratories which serve as regional typing laboratories.

Annex

MEMBERS OF THE SUBCOMMITTEE ON PHAGE TYPING OF STAPHYLOCOCCUS AND RESPECTIVE NATIONAL LABORATORIES *

Dr V. D. Allison, The Laboratories, Belfast City Hospital, Belfast, Northern Ireland.

Dr J. E. Blair, Department of Bacteriology, Hospital for Joint Diseases, New York, N.Y., USA.[1]

Dr E. T. Bynoe, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada.

Dr M. Grigorova, Research Institute of Epidemiology and Microbiology, Sofia, Bulgaria.

Professor K. Ishihara, University of Gunma College of Medicine, Maebashi, Japan.

Dr J. D. Manning, National Health Institute, Wellington, New Zealand.

Dr H. Milch, Staatl. f. Hygiene, Budapest, Hungary.

Dr R. Pakula, Państwowy Zakład Higieny Centrala, Warsaw, Poland.

Dr H. P. Pöhn, Hygienisches Institut der Universität, Göttingen, Germany.

Dr M. Popovici, Institut " Dr I. Cantacuzino ", Bucharest, Romania.

* As at October 1960.

[1] Responsible for supply of phages to hospitals, research institutes and universities.

Dr K. Raška, Státní Zdravotnicky Ústav, Prague, Czechoslovakia.

Dr H. Rische, Zentral Laboratorium für Lysotypie beim Bezirks-Hygiene-Institut Wernigerode, Wernigerode, Germany.

Dr K. Rosendal, Statens Seruminstitut, Copenhagen, Denmark.

Dr Phyllis Rountree, Fairfax Institute of Pathology, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia.

Dr R. Th. Scholtens, Rijks Instituut voor de Volksgezondheid, Utrecht, Netherlands.

Dr R. Skalova, School of Public Health, " Andrija Stampar " University of Zagreb, Zagreb, Yugoslavia.

Dr Elaine L. Updyke, Streptococcus Laboratory, Communicable Disease Center, Chamblee, Ga., USA.[1]

Professor Th. M. Vogelsang, Gade's Institute, Bergen, Norway.

Dr R. Wahl, Institut Pasteur, Paris, France.

Dr G. Wallmark, State Bacteriological Laboratory, Stockholm, Sweden.

Dr R. E. O. Williams, Streptococcus and Staphylococcus Reference Laboratory, Public Health Laboratory Service, Colindale, London, England.[2]

[1] Responsible for supply of phages to State, County, City and Federal agencies.

[2] Dr Williams is now Professor of Bacteriology at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, University of London, but the Staphylococcus Reference Laboratory, Colindale, continues to serve as the national laboratory.

RÉSUMÉ

Pour être comparable d'un laboratoire à l'autre et utile en épidémiologie, la détermination par les phages des staphylocoques coagulase-positifs exige des méthodes reconnues et éprouvées.

Les auteurs, au nom du Sous-comité de lysotypie des staphylocoques, du Comité de Nomenclature de l'Association internationale des Sociétés de Microbiologie, exposent dans cet article les méthodes qui ont donné satisfaction pour la propagation des phages et la vérification de la stabilité de leurs préparations.

Une série de 21 phages est recommandée pour être employée dans tous les cas. Quelques phages supplémentaires peuvent être utiles dans certaines régions, mais il ne paraît pas justifié d'en recommander beaucoup plus, étant donné le travail qu'implique leur maintien et leur propagation. La plupart de ces phages peuvent être entretenus et multipliés sur des milieux de routine, tel le bouillon additionné de 0,4 mg/ml de chlorure de calcium; la gélose molle donne le rendement le plus élevé.

La stabilité de tous les nouveaux lots de phages doit être vérifiée. Ces tests impliquent: une titration, afin d'établir la dilution de routine pour le typage; la détermination du spectre de lyse, c'est-à-dire l'étendue de l'action lytique éprouvée sur une série de souches de staphylocoques; la vérification répétée de l'activité des phages, au cours de la période où on les utilise. Si les phages proviennent d'entreprises commerciales ou d'un autre laboratoire, il y a lieu de s'assurer qu'ils ont subi les épreuves nécessaires.

Toutes les cultures de staphylocoques soumises à la lysotypie doivent être éprouvées d'abord par les phages à la dilution de routine, puis si la réaction escomptée ne se produit pas, être mises en présence de préparations 1000 fois plus concentrées. Certaines méthodes mécaniques sont proposées, permettant l'application simultanée de tous les phages sur une plaque, qui ont de grands avantages sur les procédés manuels.

Cinquante plages de lyse sur une plaque sont considérées comme une réaction significative du staphylocoque au phage en question. Une liste des phages donnant une telle réaction caractérise le lysotype d'un staphylocoque. Les filtrats de phages très concentrés peuvent donner lieu à des phénomènes d'inhibition, sans lyse vraie, qui peuvent, dans certains cas, être utiles pour rapprocher certaines souches les unes des autres, mais qui ne sont pas des critères de lyse.

S'il dispose de renseignements d'ordre épidémiologique sur les souches à typer, un laboratoire de typage peut indiquer les souches qui paraissent avoir une provenance commune. Il est donc essentiel d'établir une collaboration étroite entre les épidémiologistes et le laboratoire, afin de donner à la lysotypie toute sa signification pratique.

REFERENCES

Lidwell, O. M. (1959) *Monthly Bull. Minist. Hlth Lab. Serv.*, **18**, 49

Liu, P. V. (1958) *Amer. J. clin. Path.*, **29**, 176

Subcommittee on Phage Typing of Staphylococcus (1959) *Int. Bull. bact. Nomencl.*, **9**, 115

Swanstrom, M. & Adams, M. H. (1951) *Proc. Soc. exp. Biol. (N.Y.)*, **78**, 372

Tarr, H. A. (1958) *Monthly Bull. Minist. Hlth Lab. Serv.*, **17**, 64

Williams, R. E. O. & Rippon, J. E. (1952) *J. Hyg. (Lond.)*, **50**, 32

Zierdt, C. H. (1959) *Amer. J. clin. Path.*, **31**, 326