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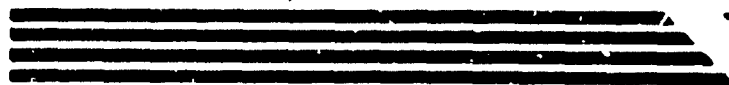
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REPORT NO. 450

RAPID METHODS FOR THE BACTERIOPHAGE TYPING OF STAPHYLOCOCCI

Elmo S. Dooley, Ph. D.
Sp 4 Edward H. Pine
Sp 4 Walter L. Betts

Medical Problems of Arctic Field Operations
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ABSTRACT

RAPID METHODS FOR THE BACTERIOPHAGE TYPING OF STAPHYLOCOCCI

OBJECT

To review the progress made in the development of rapid methods for the bacteriophage typing of staphylococci. New techniques developed in this laboratory, as well as modifications of conventional procedures, which reduce the total time to type staphylococci by the bacteriophage method are presented.

RESULTS

The time required to type staphylococci by the bacteriophage method can be substantially reduced through the application of rapid methods for propagating the typing phages, rapid visualization of test results, and through the use of self-contained typing systems.

CONCLUSIONS

It is apparent that conventional methods for bacteriophage typing are subject to improvement and that such improvement will bring this important epidemiological tool within the capability of the average hospital. The development of a dependable and inexpensive self-contained typing systems represents a step toward the achievement of this goal.

RECOMMENDATIONS

None.

APPROVED:

Thomas R. A. Davis

THOMAS R. A. DAVIS, M. D.
Director, Environmental
Medicine Division

APPROVED:

Floyd A. Odell

FLOYD A. ODELL, Ph. D.
Technical Director of Research

APPROVED:

Harold W. Glascock, Jr.

HAROLD W. GLASCOCK, JR.
Colonel, Medical Corps
Commanding

RAPID METHODS FOR THE BACTERIOPHAGE TYPING OF STAPHYLOCOCCI

I. INTRODUCTION

The importance of bacteriophage typing in the prevention and control of staphylococcal disease is best emphasized by the fact that it is the only reliable technique presently available for tracing the sources of infections caused by strains of *Staphylococcus aureus*²⁶. Since prompt recognition of the existence of a staphylococcal problem and the ultimate elimination of the causative strains from the hospital depend upon rapid identification of the strain, it is essential that this important epidemiological tool be readily available to all hospitals. The speed with which an epidemic of staphylococcal infections may sweep through a nursery, ward, or entire hospital is well known and serves to emphasize the need for rapid identification procedures at the local level. At the present time, however, the expensive and time-consuming phage typing service is confined to typing centers located in metropolitan hospitals and medical centers, state public health laboratories, and research institutions. Although most hospitals may submit cultures to these typing centers for identification, the long delays associated with collection, transportation and processing of such cultures makes such information of little immediate value in the control or prevention of epidemics. Final information concerning cultures submitted to a distant center may be delayed for periods ranging from 10 days to 6 weeks.

Before phage typing can be utilized to the fullest extent in the prevention and control of staphylococcal disease the typing procedure must be simplified and brought within the capability of the average hospital laboratory. Very little progress has been made toward simplification since the classical studies of Williams and Timmins³⁶, Fisk¹⁴, and Williams and his associates^{1, 34, 35}. The unpublished recommendations of the Subcommittee on Phage Typing, International Committee on Bacterial Nomenclature, adopted in 1953, were general in nature and were designed to give uniformity to the interpretation of results. No rigid procedural rules were established although the recommendations were essentially based on methods developed in the Central Public Health Laboratory in London³⁴.

Operating within the freedom permitted by the recommendations of the Subcommittee on Phage Typing a program was undertaken in this laboratory in 1958 to investigate possible ways of simplifying the staphylococcal typing procedure and of reducing the time required to

obtain the results of typing tests. Although the main objective was the design of a typing system for use in small military hospitals and clinics, it was recognized early in the investigation that most of the problems and needs in connection with phage typing were common to all hospitals, civilian or military. Many of our findings appeared to be applicable to the general problem of phage typing. This paper describes some of the new techniques evolved in our laboratory, as well as modifications of conventional procedures, which have reduced the time required to obtain phage patterns of staphylococci. For details concerning the development of the conventional typing procedures the reader is referred to the many excellent papers in the literature 3, 4, 14, 15, 32, 34, 35.

II. PROPAGATION OF TYPING PHAGES

The most time-consuming operation in the phage typing procedure is the propagation of the phages. To develop a complete set of typing phages at the critical test dilution may require the services of a skilled technician for a period ranging between 1 and 6 months. In addition to requiring a great deal of time, the propagation step also involves the use of expensive equipment and large amounts of laboratory space. These and other technical and mechanical problems require solution before the typing procedure can be utilized to its most practical potential.

Propagation of the typing phages is most often performed by the addition of a suspension of the stock phage to its specific propagating strain of the surface of agar plates³⁴ or within a semisolid agar overlay³. Following incubation the phage is harvested by washing the surface of the agar plate with sterile broth or by extraction of the phage from the agar overlay by a variety of complicated techniques⁴. After filtration and titration the phage suspensions are adjusted to the critical test dilution for use in the test procedure.

Most of the typing phages can be propagated in broth when proper precautions are observed^{14, 20, 26, 32-34}. In some instances, however, it is difficult to obtain high titers in liquid media. Recently, it was shown that aeration and agitation of liquid cultures during incubation substantially reduced the time required to obtain acceptable titers of phage⁷.

Liu¹⁸ applied the cellophane plate technique, originally developed by Birch-Hirshfeld² during an investigation of staphylococcal hemolysin, to the production of typing phage 3A. Although the technique gives satisfactory results when applied to other typing phages (Table 2), we have found that harvesting from the cellophane plates presents many problems⁹.

Soft agar propagations. Studies conducted in the laboratory have recently shown that propagations may be carried out in thin layers of soft agar without the use of the firm agar base. Very soft agar (0.15 per cent) is used. The media is dispensed in appropriate amounts in test tubes following preparation and cooled to 47°C. The tubes are then inoculated with suitable amounts of the propagating strain and stock phage suspension. After mixing thoroughly, the contents of the tubes are poured into sterile petri dishes to a depth of approximately 8 mm. Since this system remains very soft on cooling, the plates are incubated in the upright position at 37°C. for 4 hours. The plates are then permitted to stand at room temperature overnight prior to harvesting. On the following morning the contents of the plates are returned to sterile test tubes without the addition of extra broth and the tubes are chilled in a refrigerator. Following centrifugation for 40 minutes, during which time the agar, host cells, and cell debris becomes packed, the relatively clear, concentrated supernatant can be harvested. After filtration and titration these concentrates suspensions may be used to prepare critical test dilutions.

The results of a typical propagation experiment involving phages of groups I, II, III, and Miscellaneous are presented in Table I, page 16. Photographs of titration plates are presented in Figures 1, 2, and 3, pages 18, 19, and 20. It may be seen that propagations may be carried out effectively in very soft agar and that a single passage produces acceptable titers. The results also show that the thickness of the agar layer influences the titer of the yield (Figs. 2 and 3). The addition of calcium chloride to the agar (400 mcg/ml) did not appear to influence the results obtained in thick agar layers under the conditions of this experiment.

Advantages of propagating in soft agar include saving of time and money. Since a single passage in soft agar usually produces acceptable concentrations for typing, larger numbers of phages may be propagated simultaneously with a resulting economy of materials and time. Harvesting is simplified in that the procedure is repeated a minimum number of times to produce a complete set of phages at the critical test dilution. Because it is not necessary to use additional broth during the harvesting procedure, the resulting suspensions are more concentrated than those usually obtained when the conventional procedure is used.

Dialysis bag propagations. A technique for the production of staphylococcal typing phages in bags made from seamless dialysis tubing has recently been described⁹. This newly developed technique

combines the advantages of propagations in liquid media with the reduced incubation times which characterize aerated cultures and the cellophane plate procedure. The bags are prepared from seamless dialysis tubing measuring approximately 3/4 inch in diameter and cut into lengths sufficient to accommodate approximately 10 ml. of broth. The bags are sealed by tying at one end with a simple overhand knot and are wrapped in damp towels for sterilization in a pipette canister under steam pressure.

The propagation system consists of 0.2 ml of a stock suspension of phage (CTD ranging between 10^{-1} and 10^{-2} and .3 ml. of a 4-hour broth culture of the appropriate propagating strains adjusted to contain between 10^3 and 10^5 cells per ml. The volume of the contents is then made to 5 ml. by the addition of sterile trypticase soy broth containing added calcium (400 mcg/ml). The bags are then sealed following the removal of all air and bubbles by tying (Fig. 4, page 21). After being properly marked for identification the bags are submerged in a flask containing a liter of trypticase broth with added calcium. To get maximum aeration and agitation, the broth in the flask is magnetically stirred during incubation (Fig. 5, page 24). The incubation period lasts 4 hours and is carried out at 35°C. Contents of the bags are harvested following incubation by removing the bags from the flask, rinsing with distilled water, and clipping off one end of the tubing. The contents are then poured directly into a Seitz filter. An alternate method of harvesting consists of the use of a Swinny filter (modified to accommodate 0.45 μ pore size molecular membrane filters) (Fig. 6, page 23). The latter method of harvesting is especially useful when the volume of the propagating system does not exceed 10 ml. The resulting suspensions are obtained with a minimum loss of volume and potency.

The results of an experiment involving the propagation of Group III phages in dialysis bags are presented in Table II, page 16. For comparative purposes the results obtained when the same phages were propagated on cellophane plates and in semisolid agar are presented. The same inoculum was used in all experiments. Since the figures in the table are reciprocals of the highest dilutions producing confluent lysis of the appropriate propagating strains, they may be used for comparative purposes. It may be seen that a single passage of the phages in dialysis bags produced acceptable titers approximately equal to those obtained by three passages using the other methods.

Among the advantages of propagation in dialysis bags is the complete elimination of solid media from the procedure. The simplified harvesting procedure saves time and money. Perhaps the greatest

advantage, however, is derived from the fact that all of the typing phages may be propagated simultaneously under uniform conditions. Suspensions harvested from the dialysis bags are easily prepared for use in the typing procedure since complicated extraction procedures are eliminated.

III. RAPID PHAGE TYPING

The second most-time consuming step in the phage typing procedure is the incubation of the completed test system. A review of the recommendations concerning incubation in the literature reveals many variations in times and temperatures. Normally, the incubation period ranges between 18 and 30 hours and is carried out at temperatures between 30 and 37° C. In some cases incubations for 4 hours at 35° C. is followed by a period during which the test plates are permitted to stand at room temperatures. In every instance, however, the extended periods of incubation have made it impossible to complete a typing test and read the results within the hours of a normal workday. In an effort to reduce the time required to obtain the final results of typing tests, a technique has been developed in this laboratory which enables the technician to read the results of typing tests, with acceptable accuracy, within 4 to 6 hours following inoculation. The technique may be used in connection with the conventional microdroplet typing procedure by the addition of a simple step.

Rapid visualization technique. The use of synthetic and natural oxidation-reduction indicators in connection with drug sensitivity tests has been reported^{6, 17, 19, 22-24, 27}. However, there have been few, if any, attempts to use similar techniques to reduce the time required to obtain the results of phage typing tests. Menolasino²¹ recently reported that the addition of minute amounts of tetrazolium chloride to the test agar resulted in a contrast which greatly facilitated the reading and evaluation of the results of staphylococcal typing tests. The incubation period, however, was not shortened by the use of tetrazolium-tryptone soy agar. Sorenson²⁸ found that the incubation time for antibiotic sensitivity test could be reduced through the use of an oxidation-reduction indicator incorporated into a soft agar overlay. Appropriate drugs could be selected with accuracy in 2 to 6 hours following inoculation, depending upon the species of bacteria involved.

In both antibiotic sensitivity and bacteriophage typing tests and determination of the final results depends upon the detection, within a lawn of cells growing normally, of zones of inhibited bacterial growth. In both tests the fundamental reactions leading to the ultimate formation

of zones of inhibited growth occur within a few hours after inoculation. The remainder of the incubation is therefore devoted to promotion of sufficient cellular growth on the surface of the test plate to enable the technician to distinguish, visually, the zones of inhibited growth produced by drugs or bacteriophages. Our efforts to reduce the incubation period have been directed toward the visualization of the test lawn as rapidly as possible after the occurrence of the fundamental reactions leading to the formation of zones of bacterial lysis.

The preliminary results of our investigation of rapid visualization methods applicable to staphylococcal phage typing tests have been reported¹². Essentially, the technique involves the use of synthetic oxidation-reduction indicators incorporated into semisolid agar overlayers. Two indicators, resazurin and tetrazolium chloride have been used successfully in routine typing tests.

A stock solution of resazurin to be used in the preparation of the indicator agar was prepared in advance by dissolving 200 mg. of resazurin reagent (Allied Chemical and Dye Corp.) in 10 ml. of ethanol. This solution was then made up to a volume of 200 ml. by the addition of boiling water. The solution was then cooled, passed through a Seitz filter, and transferred to a brown glass bottle for storage under refrigeration.

The stock solution of tetrazolium indicator was prepared in advance of use by dissolving 1.0 Gm. of 2, 3, 5-triphenyl-2H-tetrazolium chloride (Allied Chemical and Dye Corp.) in 100 ml. of distilled water. The solution was then passed through a Seitz filter and transferred to a brown glass bottle for storage under refrigeration.

The overlayer is composed of 1.5 per cent agar. Base, nutrient, trypticase soy, or heart infusion agar may be used. The medium is prepared in advance of use and for convenience is dispensed in 15 ml. amounts in test tubes prior to sterilization.

Rapid visualization is used in connection with the conventional microdroplet typing procedure and the test plates are incubated for 2 hours at 35° C. prior to application of the indicator agar overlayer. The preliminary incubation period may be more accurately determined by applying a small drop of the resazurin on the surface of the test plate and incubating until the blue area turns pink. In most cases, however, a standardized 2 hour pre-incubation period is adequate for staphylococcal typing.

During the pre-incubation period the agar for the overlay is prepared by adding 1.0 ml. of the stock solution of resazurin or of the stock solution of tetrazolium chloride to each of the tubes of semi-solid agar previously melted and cooled to 47° C. Tubes of temperature indicator agar may be conveniently prepared in advance of use and held in a water bath until the completion of the preliminary incubation period.

Upon completion of the pre-incubation period the test plates are removed from the incubator and a sufficient amount of indicator agar is carefully poured over the surface of each plate to give a depth of approximately 1 mm. (Fig. 7, page 24). After the overlayer has solidified the plates are returned to the incubator and observed at 15 minute intervals. Zones of lysis appear as blue circular areas of a background of pink agar (Fig. 8, page 25) when resazurin is used and maximum color contrast usually occurs within 90 minutes following the addition of the indicator overlayer. When tetrazolium is selected as the indicator the zones of lysis appear as clear circular areas on a background of red agar (Fig. 9, page 26) and maximum color development usually requires 1 to 4 hours of additional incubation.

The possibility of growth inhibition due to the presence of the oxidation-reduction indicators in the overlayer is minimized by the preliminary incubation period. This period permits the establishment of a substantial lawn growth and the occurrence of the essential reactions leading to the lysis of areas of the lawn by phage prior to the application of the indicator agar.

Certain precautions should be observed when the rapid visualization procedure is to be applied to bacteriophage typing tests. These include: 1) the use of agar test plates with very dry surfaces, 2) application of the overlayer with the plates resting on a level surface to assure uniform layer thickness, 3) the use of extreme care during the application of the overlayer to prevent disturbance of the integrity of the test lawn and plaque pattern (Fig. 7, 24), 4) the indicator agar should be cooled to 45° C. before pouring, and 5) the overlayer must solidify completely before the test plates are moved to the incubator. The observance of these precautions will prevent the slippage of the indicator overlayer during the test period and will minimize the distortion of the plaque pattern.

The results obtained when 10 coagulase-positive strains of staphylococci were typed by the conventional and rapid visualization procedures are presented in Table III, page 17. It may be seen that major patterns

were easily determined within 4 to 6 hours after inoculation. Interpretation of the test results presented no serious problems because the distinction between strong and weak lytic reactions is easily made. With experience intermediate reactions may also be observed and recorded with accuracy. We have found that the interpretation of results may be further simplified through the use of the typing phages adjusted to 100 times the critical test dilution (100 x CTD) as recommended by Wahl and Fouace^{30, 31}.

The principal advantage of rapid visualization lies in the fact that it reduces the time required to obtain the final results of typing tests by reducing the incubation period from 18 to 30 hours to 4 to 6 hours. Using this new technique the results of staphylococcal typing test may be easily obtained within the hours of a normal workday.

IV. SELF-CONTAINED TYPING SYSTEMS

The feasibility of typing through the use of self-contained typing systems has been under investigation in the laboratory since 1958 and the preliminary results have been reported¹⁰. Since such systems would relieve clinical laboratories of the tedious, expensive, and time-consuming steps involved in the conventional typing procedure, they would also place staphylococcal typing within the capability of the average hospital laboratory. Important contributions to the development of simplified typing systems have been made by Cavanaugh and Quan⁸. These investigators demonstrated the feasibility of using lyophilized bacteriophage-impregnated paper strips for typing strains of Pasteurella pestis.

Mora and Eisenstark²³ extended the paper disc system to staphylococcal typing and obtained results equal to those obtained by the conventional microdroplet procedure. They also demonstrated that phage-impregnated discs could be prepared in advance of use and stored for periods of 90 days without loss of usable titer.

More recently a self-contained paper disc system for determining the sensitivity patterns of bacteria to various antibiotics has been developed by Brown and his associates⁶. Through the use of an oxidation-reduction indicator incorporated into antibiotic-impregnated discs, these investigators have shown that accurate results could be obtained within 2 to 6 hours following the inoculation of the discs with a strain of bacteria.

The incorporation of an indicator system into bacteriophage-impregnated discs was recently suggested¹³ and the preliminary results

of experiments conducted with single component discs have been reported¹⁰. Although acceptable results were obtained with the original discs, instability during storage presented many problems and limited the usefulness of the typing system. Subsequent investigations have shown that failure to achieve a very high degree of dehydration during preparation and storage led to interaction between the indicator and the phages with a resultant reduction in the shelf life of the discs. Such interaction was possible because of the direct contact between the indicator and the phages in the single component discs.

We have developed a multi-component disc system in our laboratory which alleviates many of the difficulties encountered in the original discs. The new system provides for the mechanical separation of the phages and the indicator until the time of inoculation (Fig. 10, page 27) and thereby extends the shelf life of the discs. Shortly after inoculation the separators lose their ability to act as mechanical barriers and the constituents of the system diffuse freely in the matrix of moistened filter paper.

Preparation of laminated phage typing discs. Sterile filter paper discs (S & S 740-E) measuring 12.7 mm. in diameter are used as major components for impregnation. Separators are cut from thin facial type tissue. The indicator discs are impregnated with 0.5 mg. of 2, 3, 5-triphenyl-2H-tetrazolium chloride in an aqueous solution. Following the impregnation and drying these discs are stored in brown glass bottles over a drying agent. Large numbers of these discs may be prepared in advance of use and stored.

The phage component of the test system is prepared by impregnating sterile filter paper discs with approximately 10^7 phage particles. Suspensions of the typing phages are adjusted to titers ranging between 10^7 and 10^8 particles in trypticase soy broth containing added calcium (400 mcg. per ml.). Purified ovalbumin is then added to the adjusted phage suspensions in amounts necessary to give a final concentration of 7 per cent albumin. After thorough mixing, impregnation is accomplished by adding 0.2 ml. of the suspensions to sterile discs. The discs are then shell frozen and lyophilized⁸. Success in the preparation of phage-impregnated typing discs depends, to a large extent, upon achieving and maintaining a high degree of dehydration following impregnation and during storage. The finished phage-impregnated discs are stored in wax sealed glass vials over a drying agent at temperatures ranging between -10 and -15° C.

The laminated test discs are assembled shortly before use by placing one of the indicator discs in each well of a plastic depression dish (Fig. 11, page 28). Separator discs are then placed on top of the indicator components in each well. Finally, the phage impregnated discs representing the typing phages are placed in the appropriate wells. Control discs consisting of only the indicator component are included with each series. Test systems may be prepared in advance of use and stored in vacuo over a drying agent and under refrigeration.

Typing is accomplished by inoculating each of the laminated discs with 0.5 ml. of a slightly turbid culture of the test strain. To prevent overpowering of the disc system we have found it convenient to adjust the turbidity of the culture to an optical density between 0.02 and 0.04 on the Coleman Junior spectrophotometer and 485 m μ . This represents approximately 10^4 organisms per ml.

Following inoculation the test system is incubated at 35°C. in a high humidity incubator. The system is checked at 15 minute intervals until the control discs show maximum color development. Results are then read by recording the numbers of the discs that did not undergo a change of color from white to pink. These numbers represent the phage pattern of the test strain.

Interpretation of the results obtained from typing tests carried out by the disc technique present no serious problems. In staphylococcal typing the distinguishing characteristic is the visual appearance of zones of lysis. Some strains are lysed so that the zone is confluent while other strains are lysed with a semiconfluent zone. Both of these types of reactions are readily detectable when laminated discs are used and reference is made to the color intensity of the control discs. With experience, intermediate types of reactions may also be determined. The results obtained when a series of cultures were typed by the disc and conventional techniques are presented in Table IV, page 17. It may be seen that acceptable results may be obtained by the disc technique within 4 hours after inoculation.

Self-contained typing systems provide many potential technical advantages including: 1) Large numbers of discs can be prepared in advance of use. 2) Variations arising in culturing and subculturing of the host cells and phages in the laboratory during propagations can be eliminated. 3) Closely controlled batches of discs can be prepared and predictable results can be expected. 4) Problems of contamination can be minimized during storage and use of the discs, and 5). These

systems would make phage typing available to laboratories of most hospitals in a simple, accurate and inexpensive form.

The main mechanical advantage to be gained through the use of self-contained typing systems is the saving of time. The time now required to prepare and standardize a complete set of typing phages (1 to 6 months) would be eliminated entirely at the local level. The requirement for space and equipment would also be reduced or eliminated. The costs of typing would, of course, be reduced as a result of the above mentioned advantages. It is hoped that the manufacture of self-contained typing systems can be arranged in the near future.

V. SUMMARY

Two new techniques for the propagation of staphylococcal typing phages developed at the U. S. Army Medical Research Laboratory have been described. Application of these techniques reduces the time required to obtain a complete set of phages at the critical test dilution by simplifying the propagation and harvesting procedures.

A new technique for the rapid visualization of the results of staphylococcal phage typing test has been presented. Using this technique the results of typing test may be easily obtained within the hours of a normal workday.

The results obtained in studies of the development of self-contained phage typing systems have been presented and discussed. The development and production of such systems could provide many technical and mechanical advantages over the conventional microdroplet typing procedure and would make phage typing available to the staffs of all hospitals in the form of an economically feasible routine laboratory procedure.

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TABLE I
TITERS OF PHAGES PROPAGATED IN SOFT AGAR*

Phage	Soft Agar (Tube)	Soft Agar + Ca (Tube)	Soft Agar (Petri dish)
29	$10^{2.5}$	10^2	10^5
52	10^2	10^2	10^4
52A	10^2	10^2	10^4
79	10^2	10^2	10^5
80	10^2	10^2	10^5
81	10^3	10^2	10^4
71	10^2	10^2	10^3
47	10^3	10^3	10^5
53	10^2	10^2	10^5
54	10^2	10^3	10^4
			10^5

*0.15 per cent tryptic soy agar (Difco)

**Figures are reciprocals of the dilutions producing confluent lysis of the propagating strains.

TABLE II
TITERS OF PHAGES PROPAGATED IN DIALYSIS BAGS, CELLOPHANE PLATES AND SEMISOLID AGAR

Phage	Semisolid Agar (3 passages)	Range* Cellophane Plates (3 passages)	Dialysis Bags (1 passage)
6	$10^2 - 10^5$	$10^2 - 10^4$	$10^5 - 10^6$
7	$10^3 - 10^3$	$10^3 - 10^4$	$10^3 - 10^4$
42E	$10^3 - 10^4$	$10^3 - 10^5$	$10^4 - 10^4$
47	$10^3 - 10^5$	$10^2 - 10^5$	$10^4 - 10^7$
57	$10^2 - 10^4$	$10^3 - 10^5$	$10^5 - 10^7$
54	$10^2 - 10^5$	$10^2 - 10^6$	$10^4 - 10^6$
73	$10^2 - 10^4$	$10^2 - 10^4$	$10^4 - 10^5$
75	$10^3 - 10^5$	$10^3 - 10^6$	$10^3 - 10^7$
77	$10^2 - 10^5$	$10^3 - 10^5$	$10^4 - 10^7$
83	$10^2 - 10^5$	$10^3 - 10^5$	$10^4 - 10^8$

*Figures are reciprocals of the dilutions producing confluent lysis of the propagating strains.

Inoculum for the entire series consisted of 0.2 ml. of a stock phage suspension (CFU between 10^{-1} and 10^{-2}) and 0.3 ml. of broth culture containing between 10^3 and 10^5 cells per ml.

TABLE III
PATTERNS OF COAGULASE-POSITIVE STAPHYLOCOCCI AS DETERMINED BY
CONVENTIONAL AND RAPID VISUALIZATION TECHNIQUES

Strain No.	Phage Pattern	
	Conventional*	Rapid Visualization**
1	29 52 52A 80 81	29 52 52A 80 81
2	29 52 52A 79 80	29 52 52A 79 80
3	6 47	6 47
4	3A 3B 3C 71	3B 3C 71 (weak 3A)
5	80 81	80 81
6	6 47 54 75 83	6 47/54/75 83
7	Untypable	Untypable
8	29 52 52A 80	29 52 52A/80 (weak 81)
9	47 53 54 77 83	47 53/77 83 (weak 54)
10	3B 3C 55 71	3B/3C 55/71

*Incubation-30 hours, 35°C., Typing phages - 100 x CTD.

**Incubation - 4 to 6 hours, 35°C., Typing phages - 100 x CTD.

TABLE IV
PATTERNS OF COAGULASE-POSITIVE STAPHYLOCOCCI AS DETERMINED BY
CONVENTIONAL AND LAMINATED DISC TECHNIQUES

Strain No.	Phage Patterns	
	Conventional*	Laminated Disc**
1	29/52/52A/80/81	29/52/52A/80/81
2	29/52/52A/79/80	29/52/52A/80 (weak 79)
3	6/47	6/47
4	3A 3B/3C/71	3B/71 (weak 3A/3C)
5	80 81	80/81
6	6 47/54/75/83	6/47/54/83 (weak 75)
7	Untypable [†]	Untypable (very weak 42E)
8	29/52/52A/80	29/52/52A/80 (weak 81)
9	47/53/54/77 83	47/53/77/83 (weak 54)
10	3B 3C 51/71	3B/51/71 (weak 3C)

*Significant reactions at the CTD. Incubation: 30 hours at 35°C.

**Significant reactions after 4 hours incubation at 35°C.

[†]Untypable at CTD; 42E at 1000 x CTD.

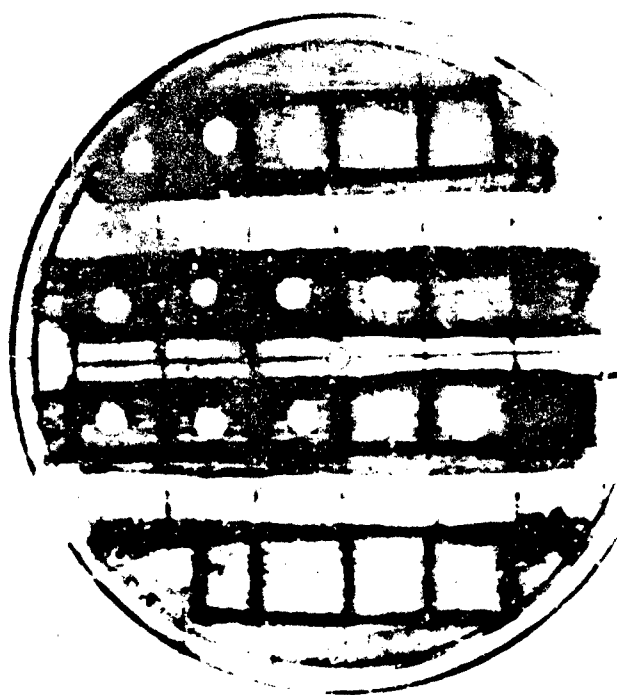


Fig. 1. Titration of phages 71, 47, 53, and 54 propagated in 0.15 per cent agar. Decimal dilutions range from 10^{-1} to 10^{-6} .

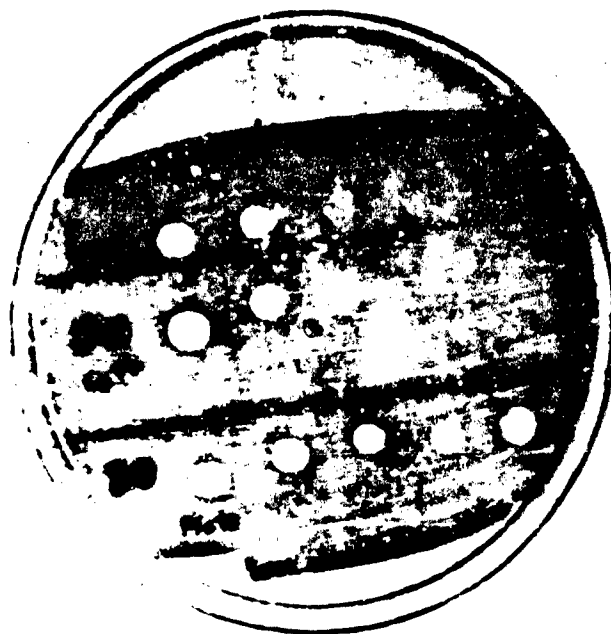


Fig. 2. Titration of phage 80 in soft (0.15 per cent) agar showing the effect of layer thickness on yields. Propagations were carried out in thick layers (6 - 7 cm.) in test tubes without added calcium (top row) and with added calcium (middle row). Bottom row shows the yield obtained from a single passage in thin layers (8 mm.) in petri dishes. Decimal dilutions range from 10^{-1} (left) to 10^{-6} .



Fig. 3. Titration of phage 29 in soft (0.15) per cent agar showing the effect of layer thickness on yields. Propagations were carried out in thick layers (6 - 7 cm.) in test tubes without added calcium (top row) and with added calcium (middle row). Bottom row shows the yield obtained from a single passage in thin layers (8 mm.) in petri dishes. Decimal dilutions range from 10^{-1} (left) to 10^{-6} .



Fig. 4. Dialysis bags for the propagation of staphylococcal typing phages in liquid medium. Bags are sealed by tying.

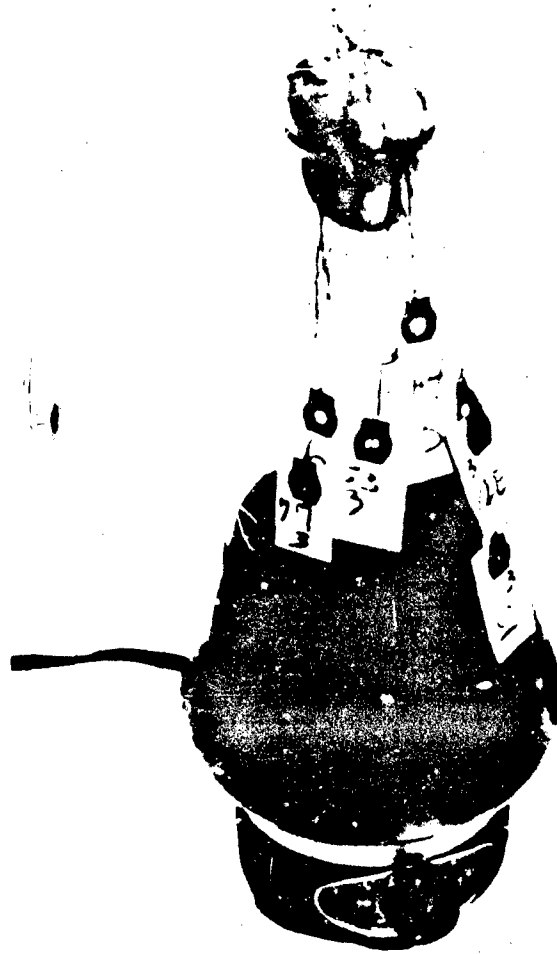


Fig. 5. Propagation of staphylococcal typing phages in submerged liquid cultures. Contents of the flask are stirred during incubation to promote aeration.



Fig. 6. Harvesting staphylococcal typing phages propagated in dialysis bags. Swinny adapters fitted on syringes are used to obtain suspensions of high potency with a minimum loss in volume.

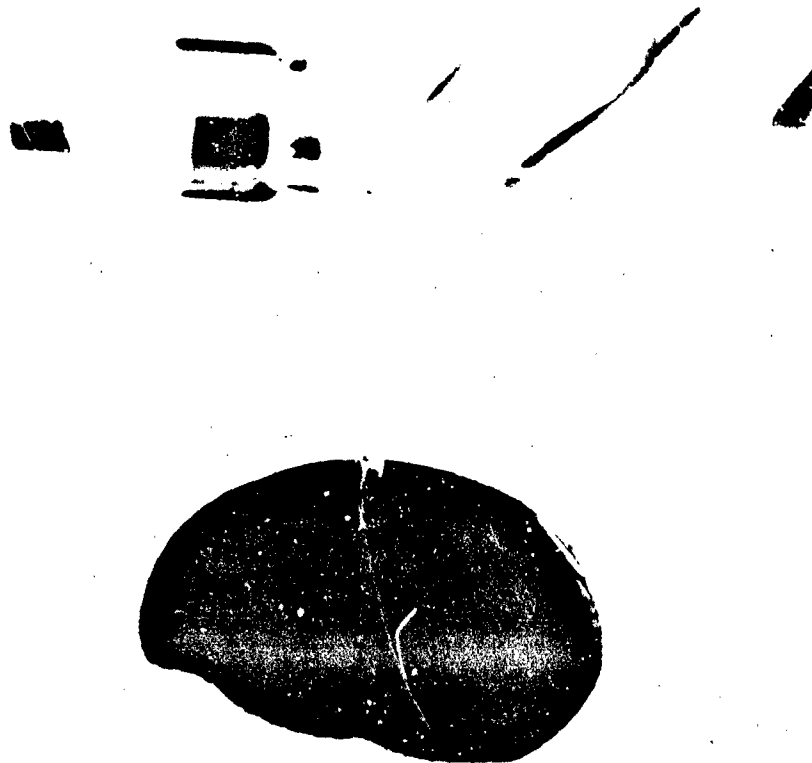


Fig. 7. Technique for applying the indicator overlayer to phage test plates. The use of a sterile glass rod to direct the stream of melted agar to the center of the surface minimizes the disturbance of the lawn and distortion of the plaque pattern.

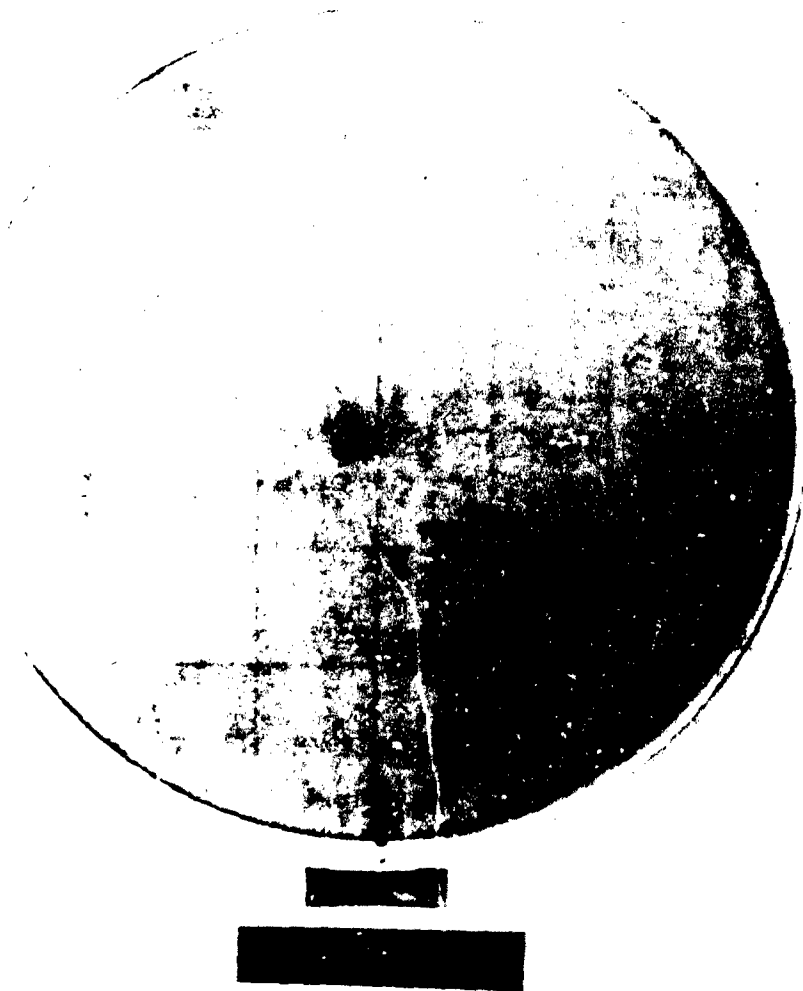


Fig. 8. Rapid visualization of the results of a staphylococcal phage typing test through the use of resazurin indicator agar. The plate was incubated for 90 minutes following application of the overlayer. (Pattern: 29/52/52A/80). The irregular shaped spot in the center of the plate was caused by a pile up of agar at the pour point.

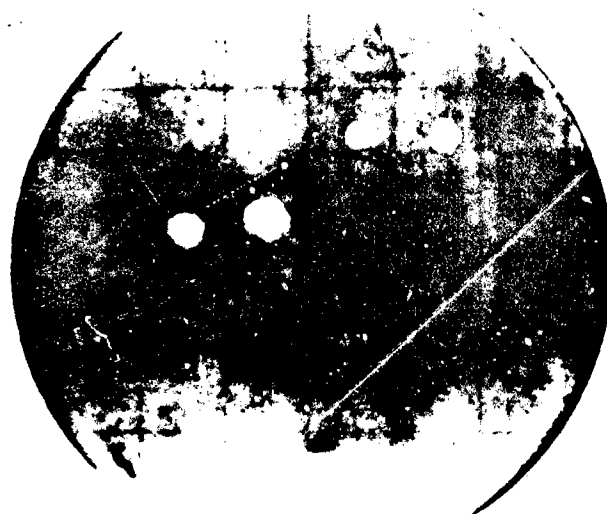
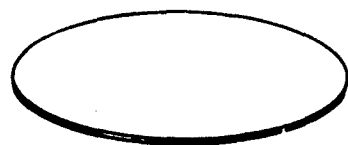


Fig. 9. Rapid visualization of the results of a staphylococcal phage typing test through the use of tetrazolium indicator agar. The plate was incubated for 180 minutes following the application of the over-layer. (Patt. -n: 3B/3C/55/71).



FILTER PAPER DISC
PHAGE COMPONENT



TISSUE SEPARATOR



FILTER PAPER DISC
INDICATOR COMPONENT

Fig. 10. Exploded view of laminated typing discs.

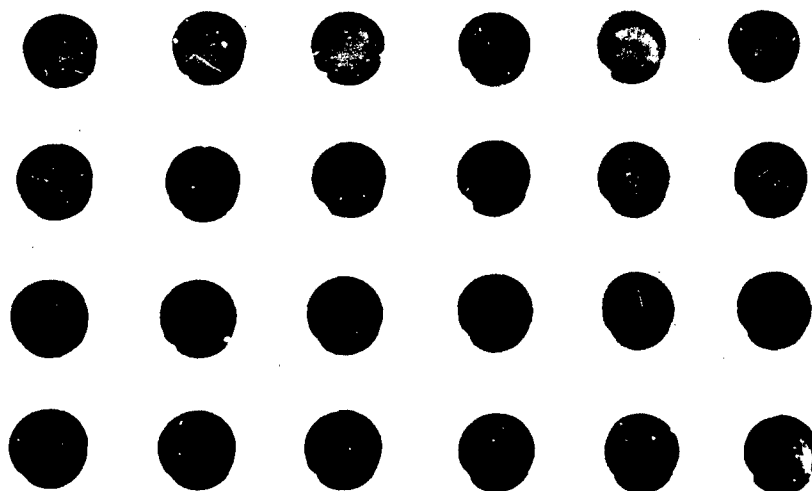


Fig. 11. Photograph of the laminated disc typing system showing the degree of contrast between positive and negative reactions after 4 hours of incubation at 35°C. Phage pattern: 29/52/52A/30/81.