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PROCESS FOR PREPARING BACTERIOPHAGE-IMPREGNATED PAPER DISCS FOR RAPID IDENTIFICATION OF BACTERIAL STRAINS

Elmo S. Dooley, 105 E. Adams St., Crossville, Tenn.

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The invention described herein may be manufactured and used by or for the Government for governmental purposes without the payment of any royalty thereon.

The importance of phage typing as an epidemiological tool in the control and prevention of drug-resistant staphylococcal infections in hospitals and the usefulness of bacteriophage specificity for identifying bacteria is well documented in the scientific and medical literature. Bacteriophage typing is of prime importance in tracing the source of maternity and nursery ward epidemics as well as post-operative staphylococcal infections, and exceptionally virulent strains can be recognized and identified and typing of staphylococci isolated from the anterior nares has been of particular value in the recognition of carriers.

The present invention provides a process for preparing monovalent and polyvalent bacteriophage-impregnated paper discs for effecting a rapid identification of epidemic or hospital strains of *Staphylococcus aureus* and other bacteria, the instant process obviating prior slower and more expensive techniques which have been recognized heretofore as standard procedures.

Before proceeding with a detailed description and explanation of the instant procedure, for purposes of comparison it is thought to be desirable to outline what has been regarded as the conventional or standard procedure for arriving at the same ultimate results as are reached by the much more rapid, simpler, and less expensive procedure of the present invention.

In practice, the conventional or standard procedure for accomplishing the same results as are obtained by the instant invention usually is referred to as a "microdrop" method; and in practice this conventional microdrop method for bacteriophage typing of bacteria substantially follows the hereinafter described procedure. This conventional "microdrop" method for bacteriophage typing of bacteria is substantially as follows:

Twenty-two bacteriophages usually are used for routine typing, although this number may vary with individual typing laboratories. This number includes the so-called "basic" phages that are recommended by the International Committee on Bacteriological Nomenclature.

Following propagation and titration of the individual bacteriophages, typing usually is performed only on coagulase-positive strains of *Staphylococcus aureus*, the general procedure being as follows:

(1) Subcultures of the isolates to be typed are grown overnight in trypticase soy broth or other suitable liquid media.

(2) Modified trypticase soy agar plates prepared on the previous day are surface-dried at 37° C. for two hours in the inverted position. The bottoms of the prepared agar plates are marked in a cross-hatched manner and each of the resulting squares is then marked with a number corresponding to the numbers of the typing phages.

(3) The test plate is streaked with culture, for which purpose the liquid culture of *Staphylococcus* to be typed is agitated vigorously, and a sterile cotton swab then is saturated with the culture and applied gently to the entire surface of the test plate or plates. An alternate method of inoculating the test plate involves flooding the surface with approximately one ml. of the liquid culture and

draining off the excess liquid. In either case the inoculum absorbs into the agar within a few minutes.

(4) In the application of the bacteria phage, approximately 0.25 ml. of the phage suspensions, previously checked for activity and titrated, are used for typing. The material is drawn up into a small syringe with a 27-gauge x 1-inch needle. Holding the syringe vertically, a single drop of the phage suspension is placed in the center of the appropriately-numbered square of the agar test plate, as referred to in (2) above, care being taken not to stab the agar with the needle and to prevent the phage suspension from "bouncing" as it is dropped from the needle. A uniform full drop must be applied to each square without contamination. When the phage is absorbed, the plates are incubated overnight at 30° C. An alternate period of incubation, i.e., 37° C. for 18 hours, may be used.

(5) A strain of Staphylococcus which is highly susceptible to the various typing phages is used as a control.

(6) Following incubation, the plates are removed from the incubator. Lysis is read by transmitted illumination. When discrete plaques are visible, they are counted. Typing is established when 50 or more plaques, through confluent lysis, are formed within the area of the phage drop. If less than 50 plaques are counted, the strain is reported as being untypable at the phage concentration employed. Typing may be repeated with more concentrated phage preparations, usually 1000 times the routine test dilution (RTD×1000).

In the "Proceedings of the National Conference on Hospital-Acquired Staphylococcal Disease," September 1958, page XIX, the following statement concerning bacteriophage typing by conventional means is made:

"This is the only reliable epidemiological tool practicable for tracing the sources of staphylococcal infections. However, materials for phage typing are not readily available to hospital laboratories, the procedure is time-consuming, and the interpretation of the tests are difficult and sometimes subjective. Moreover the results are of no diagnostic or therapeutic significance and therefore the procedure is not included in this manual. Its use should be limited to epidemiological or research studies. Phage typing should not be attempted by the average hospital laboratory but large hospitals with adequate personnel and facilities may wish to establish their own phage typing laboratory for research purposes.

"There is no direct relationship between antibiotic resistance per se and either virulence or the phage type of the Staphylococci."

The objects and advantages of the present invention comprising phage-impregnated paper discs containing a metabolic indicator, as compared with the foregoing "standard" procedure, may be listed as comprising the following, among others:

(1) The use of phage-impregnated discs containing a metabolic indicator places bacteriophage typing, an important epidemiological tool, within the capability of small hospital and clinical laboratories, standardized phage-impregnated discs being easily prepared in a central laboratory and distributed to small hospitals and clinics.

(2) The use of phage-impregnated discs with a metabolic indicator places phage typing in the hands of the epidemiologist or infection control officer at a time when such information is most meaningful in the prevention and spread of resistant-type infections. At the present time, small hospitals must await the results of typing carried out in the large typing centers. This waiting period may range from one to two weeks, and an epidemic can be well advanced before it is actually associated with resistant-type organisms.

(3) It is time-saving. The amount of time required

to obtain the complete phage pattern of a strain of coagulase-positive Staphylococci starting with a broth culture of the strain, is reduced from 72 hours to approximately 4 hours when phage-impregnated discs are used.

(4) It is labor-saving. Page discs with a metabolic indicator represent a self-contained test system which may be prepared in large numbers in advance of use. Pre-test titrations and spot checks for activity of phage suspensions are eliminated when phage-impregnated discs containing a metabolic indicator are used. When the test discs are prepared and distributed from a central laboratory, small hospitals and clinics are relieved of the tedious, expensive, and time-consuming process of propagating and maintaining stocks of the typing bacteriophages. Using the phage disc method, the complete typing procedure may be accomplished within a fraction of the time required when the above-referred to microdrop technique is used. The complete set of phage discs may be inoculated in a fraction of the time required to apply typing suspensions by syringe as practiced in the microdrop procedure. Since the use of solid media is not a part of the phage disc technique, there is an additional saving of time and labor in this respect.

(5) In is money-saving. Since the use of solid media is not involved in the phage disc procedure, there is a direct saving in the cost of materials. Since the phage disc technique has been shown to be time and labor saving, it also is money saving. The typing of bacteria by the above-outlined microdrop technique generally requires the full time service of one skilled person to propagate, titrate, and maintain phage stocks and to carry out the typing. Through the use of phage-impregnated paper discs with a metabolic indicator, typing can be carried out by laboratory technicians without special training.

(6) So far as is known, bacteriophage-impregnated paper discs containing a metabolic indicator represent the first use of these indicators (electron acceptors) as a marker or key to the interaction of two complex living systems, namely, bacteria and bacteriophage. The use of color indicators is common in test systems involving one organism or one enzyme, but, as is the practice employed in the present invention, the use of tetrazolium indicators (electron acceptors) employed in the instant invention to determine the reactivity of two living systems to each other, is thought to be entirely new and novel, and it is thought to embody more than a slight degree of novelty.

(7) Phage-impregnated discs containing a metabolic indicator represent a self-contained system for detecting the sensitivity of microorganisms to specific bacteriophage. As practiced in accordance with the instant invention, these discs contain a specific bacteriophage, a supporting growth medium, and a metabolic indicator (tetrazolium compound) for the rapid determination of the effect of phage on the test organism. The use of these discs requires no additional media or equipment, the results are read by observing a color change from white to pink, red, or blue, depending upon the specific tetrazolium indicator used, and the test procedure is simple as compared to the conventional microdrop technique.

Other objects and advantages of the instant invention will become apparent as the description proceeds, and the features of novelty will be pointed out in particularity in the appended claims.

The procedure employed in carrying out the instant invention is as follows:

METHODS

The phages employed in the experimental work leading to the instant invention were the twenty-two "basic" phages recommended by the International Committee plus two additional phages currently being used in the United States. The phages were obtained originally from Dr. J. E. Blair, Laboratory Division, Hospital for Joint Diseases, New York, N.Y.

DISC MATERIALS

For production, the laboratory discs were cut from Watman No. 3 mm. filter paper. In early trials, squares measuring substantially ½ inch x ½ inch were cut from strips of filter paper. The discs or squares were wrapped and sterilized with dry heat. For large scale production, filter paper discs (Carl Schleicher & Schuell, 12.7 mm. penicillin assay) were used. In commercial production, the discs are cut or stamped from sheets of filter paper following impregnation.

PREPARATION OF THE DISCS

Trypticase soy broth filtrates of each of the phages composing the broad group were titrated and adjusted to the decimal dilution representing 1,000 times the routine typing dilution as determined by the microdrop technique (RTD×1000). Equal amounts of the filtrates representing the phages within a broad group are mixed together under aseptic conditions. Purified egg albumin is added to the phage mixture in the amount to give a final concentration of five percent. The sterile filter paper discs are dipped in the phage-albumin broth suspension and are dried in vacuo. In some of the early trials, the discs were dried in an incubator at 37° C. Dried discs are stored in sterile petri dishes. Polyvalent discs are stable for at least 90 days when stored at room temperature, but storage at temperatures ranging from 0° C. to 10° C. increases the effective life of the discs.

The process as outlined above is repeated to prepare discs representing each of the broad groups of phages and thus, with five discs, there may be identified to the group level any strain of *Staphylococcus aureus*.

PROCEDURE FOR THE USE OF IMPREGNATED DISCS

Pour plates are prepared from trypticase soy agar using the two-layer technique. The second layer of agar is seeded with the strain of bacteria to be identified. Surface-streaked plates may be used instead of the two-layer plates, but care must be taken to obtain complete coverage of the surface. Discs representing each of the five major groups then are placed on the surface of the test plates using sterile forceps. The surface of the plates must be dry before the test discs are transferred to the test plates.

Test plates are incubated at 37° C. and the results are read after 20 hours.

RECORDING AND INTERPRETATION OF RESULTS

The presence of a clear zone around the test discs indicates that the test organism has been lysed by the phage or phages composing the broad group represented by the disc. Thus, by inspection, the test strain may be classified into the phage group or groups. Since most of the resistant or "epidemic" strains of *Staphylococcus aureus* belong in the broad phage group III, the use of polyvalent phage discs may provide the infection control officer in small hospitals with sufficient information on the nature of an outbreak of infections.

The use of polyvalent discs can be employed to eliminate unnecessary use of test discs for the determination of the complete phage pattern of the test organism. The use of polyvalent discs at the local level also simplifies the work of central typing laboratories by confining test procedures to the phages within the broad groups as determined through the use of polyvalent discs.

At this point, it may be noted that methods involving the use of paper discs for the determination of the antibiotic susceptibility patterns of bacteria have been shown to be reliable and simple. Recently, in accordance with the procedure of the instant invention, the flexibility and usefulness of the paper disc method for antibiotic sensitivity testing in the clinical laboratory was extended by the addition of a color indicator to the test system. In

accordance with the present invention, by incorporating tetrazolium chloride and a growth medium into the paper discs containing antibiotics, there is eliminated the need for solid media from the test procedure, and thereby there is effected a reduction of the time required to determine the antibiograms of bacteria.

E. C. Mora and A. Eisenstark, "Use of Phage-Impregnated Paper Discs for Bacterial Specificity Tests," J. Lab. and Clin. Med., vol. 51, page 802 (1958), successfully adapted the paper disc method to the differentiation of bacterial strains with bacteriophage. The phage-susceptibility patterns were determined by observing the appearance of a clear zone around paper discs placed on the surface of seeded agar plates. The results obtained by this method were the same as those obtained by other investigators using the same bacteriophages.

As has been indicated above herein, the instant invention includes the addition of a metabolic indicator and a growth medium to phage-impregnated paper discs to create a self-contained system for the identification and typing of bacteria. This development of a self-contained test system for phage typing eliminates the need for laboratories to propagate phage stocks, reduces the time required to obtain complete phage susceptibility patterns to approximately four hours, and has placed phage typing within the capability of most hospital laboratories. Phage-sensitivity patterns may be determined directly from discs inoculated with a broth culture of the test organism by observing the color reaction of the discs following incubation.

The phages used in the experimental work leading to the present invention, as has been referred to above, were the twenty-two "basic" phages recommended by the International Committee, and two additional phages being used in the United States.

The test strains of *Staphylococcus aureus* used in such experimental work included 25 strains isolated during outbreaks of hospital-acquired infections and 15 additional strains. All strains were coagulase positive. They were maintained on trypticase soy agar slants and were transferred in broth three times prior to typing.

Phage-impregnated discs were prepared in accordance with the following specific procedure:

Trypticase soy broth filtrates of each of the twenty-four phages were prepared and adjusted to the decimal dilution representing 1000 times the routine test dilution as determined by the routine microdrop technique. To each of the tubes of phage filtrates amounts of 2,3,5-triphenyl-tetrazolium chloride or 2,3-diphenyl-5-methyl tetrazolium chloride are added to give a final concentration of 0.5 percent of the indicator. The contents of the tubes are mixed thoroughly and the sterile filter paper discs are dipped into the phage-indicator broth mixture. The impregnated discs are dried in vacuo. In early trials the discs were dried in an incubator at 37° C., but it has been found that in vacuo drying gives discs of more uniform quality. The test discs, each representing a single phage, may be stored in sterile petri dishes. Stability is comparable to that of the polyvalent test discs.

RESULTS

Table I below shows the results obtained when 15 strains of *Staphylococcus aureus*, previously typed elsewhere, were typed with phage-impregnated discs containing tetrazolium chloride. As will be seen from Table I below, the patterns obtained by the two methods were in excellent agreement.

Table I

PHAGE SENSITIVITY PATTERNS OF STAPHYLOCOCCAL STRAINS AS DETERMINED BY PHAGE DISC AND MICRODROP PROCEDURES

Strain	Source	Phage Pattern		
		Disc(RTD)	Disc (RTD×1,000)	Microdrop
1	UC wound	77/VA4/80	77/VA4/80	77/VA4/80.
2	UC OR air	77/VA4/80/81	77/VA4/80/81	77/VA4/80/81.
3	UC nursery	77/VA4/80	77/VA4/80	77/VA4/80.
4	UC wound	53/54/77	53/54/77	53/54/77.
5	CH wound	47/54/75	47/54/75	47/54/75.
6	CH wound	47/54/75	47/54/75	47/54/75.
7	CH nose	47/54/75	47/54/75	47/54/75.
8	MH nursery	6/47	6/47	6/47.
9	MH nose	6/47	6/47	6/47.
10	MH OR air	6/47	6/47	6/47.
11	MH carafe	6/47	6/47	6/47.
12	MH B, abscess	6/47	6/47	6/47.
13	PH OR air	3A/29/52/80	3A/29/52/80/81	3A/29/52/80/81.
14	PH wound	3A/29/52/80	3A/29/52/80	3A/29/52/80.
15	PH nose	3A/29/52/80	3A/29/52/80/81	3A/29/52/80/81.
16	PH nose	3A/29/52/80	3A/29/52/80	3A/29/52/80.
17	SH nursery	52/47/80/81	52/47/80/81	52/47/80/81.
18	SH wound	52/47/80	52/47/80	52/47/80.
19	SH nose	52/47/80/81	52/47/80/81	52/47/80/81.
20	SH OR air	52/47/80/81	52/47/80/81	52/47/80/81.
21	RH OR air	29/47/53/80	29/47/53/80	29/47/53/80.
22	RH wound	29/47/53/80	29/47/53/80	29/47/53/80.
23	RH nose	29/47/53/80	29/47/53/80	29/47/53/80.
24	RH throat	29/47/53/80	29/47/53/80	29/47/53/80.
25	RH carafe	29/47/53/80	29/47/53/80	29/47/53/80.

Table II below shows the phage patterns of 25 strains of coagulase-positive *Staphylococcus aureus* isolated during outbreaks of hospital acquired infections in six rural hospitals. For purposes of comparison, these strains were also typed by the microdrop procedure. The agreement of the results obtained by these two different methods was excellent.

Table II

PHAGE SENSITIVITY PATTERNS OF TYPED STAPHYLOCOCCAL STRAINS AS DETERMINED BY THE PHAGE DISC PROCEDURE

Strain	Reported pattern	Phage disc patterns	
		RTD	RTD×1,000
31	52/79/81	52/79/81	52/79/81/80.
32	52A/79/VA4	52A/79/VA4	52A/79/VA4.
33	80/81	80/81	80/81.
34	80/81	80/81	80/81.
35	3A/3C/53	3A/3C/53	3A/3C/53.
36	52A/79	52A/79	52A/79.
37	52A/79/80	52A/79/80	52A/79/80.
38	6/7/47	6/7/47	6/7/47.
39	29/52/80/81	29/52/80	29/52/80/81.
40	52/47/53/80/81	52/47/53/80	52/47/53/80/81.
41	53/77/VA4	53/77/VA4	53/77/VA.
42	53/54/75/VA4	53/54/75/VA4	53/54/75/VA4.
43	52/47/80/81	52/47/80	52/47/80/81.
44	47/53/54/75	47/53/75	47/53/54/75.
45	7	7	7.

DISCUSSION

From the foregoing considerations, bacteriophage typing may be considered to be the only reliable epidemiologic tool practicable for tracing the sources of staphylococcal infections. However, conventional methods for phage typing are not within the capability of most hospital laboratories, the materials are not readily available, the procedure is expensive and time-consuming, and the reading and interpretation of the tests is difficult and often subjective. For these reasons, the use of phage typing has been largely limited to epidemiologic or research studies. Thus, the epidemiologist or hospital infection control officer at the local level in small hospitals is deprived of the use of this valuable tool or must await the receipt of the information from the typing center. In many cases epidemics are well advanced before positive identification can be made.

In accordance with the present invention, the development of phage-impregnated discs containing a metabolic indicator and a supporting growth medium represents a simplification of the typing procedure and places it within the capability of most hospital laboratories. These discs have demonstrated excellent storage characteristics and their production and distribution can be carried out readily in a central laboratory. The distribution of standardized phage test discs to all hospital laboratories reduces the expense of phage typing and places all epidemiological studies on a comparable basis.

Conventional methods of phage typing are characterized by their dependence upon the appearance of a zone of clearing or lysis or agar plates seeded with the test strain. Some strains are lysed so that a confluent clear zone appears on the test plate, while with other strains there may be observed only semi-confluent zones. Both of these reactions may also be observed when phage-impregnated discs are used by noting the degree of color development in the test discs as compared to known positive and negative controls.

A comparison of the results obtained when 40 strains of coagulase-positive *Staphylococcus aureus* were typed by the phage disc and microdrop techniques (Tables I and II above) indicates that the former method gives satisfactory results. Discs prepared from suspensions 1000 times more concentrated than the routine typing dilutions enable the complete sensitivity patterns to be easily obtained. Staphylococci specifically susceptible to phage 80 were isolated by Roundtree and Freeman in 1955 (P. M. Roundtree and B. M. Freeman, "Infections Caused by Particular Phage Type of *Staphylococcus aureus*," M. J. Aust. 42: 157, 1955), and strains susceptible to phage 81 were reported by Bynoe and associates in 1956 (E. T. Bynoe, R. H. Elder, and R. D. Comtois, "Phage-Typing and Antibiotic-Resistance of Staphylococci Isolated in a General Hospital," Canadian J. Microbiology 2: 346, 1956). These phages appear to be very similar, and for all practical purposes strains lysed by either of these phages may be considered to be identical, (J. E. Blair and M. Carr, "Staphylococci in Hospital-Acquired Infections. Types Encountered in the United States," J.A.M.A. 166: 1192, 1958).

In the case of strain 44 (Table II), phage 54 did not give a significant reaction when discs prepared from the routine typing dilution were used. However, as in the case of phage 81, a significant reaction was observed when discs prepared from suspensions 1000 times stronger than the RTD were employed.

Phage-impregnated discs containing tetrazolium chloride as a metabolic indicator have been shown to be stable after storage for 60 days at room temperatures. The suspensions used in the microdrop procedure usually remain usable for a period ranging from 2 to 6 weeks. However, suspensions older than 7 days must be spot-checked for activity before they can be used for typing purposes, and this requirement serves to extend the time required to obtain the complete phage sensitivity pattern of an organism.

Phage-discs of the instant invention represent a self-contained test system which may be prepared in advance in large numbers, can be used when needed with a minimum of effort, and from which the results may be observed directly following 4 to 6 hours of incubation. Since the time required to obtain a complete pattern by the conventional microdrop technique is approximately three days, the time-saving factor of the phage discs is enormous. Also, the results of any typing may be placed in the hands of the epidemiologist at the local level before an outbreak reaches epidemic proportions and at a time when corrective measures will be most effective.

SUMMARY

From the foregoing, it will be seen in accordance with the instant invention, that bacteriophage-impregnated paper discs containing tetrazolium chloride have been employed successfully in typing 40 strains of coagulase-positive *Staphylococcus aureus*, the results obtained through the use of these phage discs being essentially the same as obtained by the conventional microdrop technique.

The phage disc method of the present invention has advantages over the conventionally used microdrop procedure in that large numbers of standardized discs can be prepared in advance, they may be used from storage as needed, and they reduce the work required to obtain satisfactory results.

The use of phage discs places Staphylococci typing within the capability of most hospital laboratories and makes the results of typing readily available to the epidemiologist.

While the foregoing description is directed to the preferred operational procedure of the present invention, it will be apparent that certain procedural details as herein described may be modified by one skilled in the art without departing from the substance of the actual invention; and it will be understood, therefore, that it is intended and desired to embrace within the scope of the invention such modifications and changes as may be desirable or necessary to adapt the invention to varying conditions and uses as defined by the scope of the appended claims.

I claim:

1. The process of typing strains of micro-organisms, which comprises impregnating flat shapes of porous material with aqueous liquid materials, successive portions of which contain a bacteriophage material adapted to lyse strains of organisms to be typed, a growth-supporting medium for the bacteriophage material, and a redox indicator, and applying the resulting impregnated flat shapes to cultures of the organisms to be typed.

2. The process of typing strains of micro-organisms, which comprises impregnating flat bodies of porous material with aqueous liquid materials, successive portions of such impregnating liquid materials containing a bacteriophage material adapted to lyse strains of the organism to be typed, a growth-supporting medium for the bacteriophage material, and a redox indicator, incubating and drying the resulting impregnated flat bodies for developing the bacteriophage material, and applying the resulting incubated and dried bodies to cultures of organisms to be typed.

3. The process of typing strains of *Staphylococcus aureus*, which comprises impregnating bodies of porous paper wtih successive liquid portions of an aqueous material, each portion containing respectively, a bacteriophage material adapted to lyse known strains of *Staphylococcus aureus*, a growth-supporting medium for the bacteriophage material, and a redox indicator comprising a tetrazolium chloride, incubating the resulting impregnated paper bodies to develop the bacteriophage therein, applying resulting impregnated and incubated paper bodies to cultures of *Staphylococcus aureus* to be typed, and incu-

bating the paper bodies in the presence of the cultures.

4. The process of claim 3, wherein the strains of *Staphylococcus aureus* being typed are coagulase-positive strains.

5. The process of claim 3 wherein the redox indicator is selected from the group consisting of 2,3,5-triphenyltetrazolium chloride and 2,3-diphenyl-5-methyltetrazolium chloride.

6. A testing device for use in typing micro-organisms, which comprises an essentially flat, integral piece of absorbent material containing a bacteriophage material and a redox indicator.

7. A testing device for use in typing coagulase-positive strains of *Staphylococcus aureus,* which comprises an essentially flat, integral piece of absorbent material containing a bacteriophage material active with respect to the strain of *Staphylococcus aureus* to be typed and a redox indicator selected from the group consisting of 2,3,5-triphenyltetrazolium chloride and 2,3-diphenyl-5-methyltetrazolium chloride.

8. A testing device for use in typing micro-organisms, which comprises an essentially flat piece of absorbent material containing a bacteriophage material, a growth-promoting substance therefor, and a redox indicator.

9. A process for making testing devices for use in typing microorganisms, which comprises impregnating essentially flat porous pieces of adsorbent material with a bacteriophage material reactive with respect to organisms to be typed, a growth-promoting substance therefor, and a redox indicator, incubating the resulting impregnated material until the bacteriophage material therein has become developed, and sterilizing the resulting impregnated devices for storage and use.

10. A process for making testing devices for typing strains of *Staphylococcus aureus,* which comprises impregnating successively flat pieces of absorbent material with bacteriophage material reactive with respect to *Staphylococcus aureus,* a growth-promoter therefor, and a redox indicator selected from the group consisting of 2,3,5-triphenyltetrazolium chloride and 2,3-diphenyl-5-methyltetrazolium chloride, incubating the resulting impregnated pieces of absorbent material until development of the bacteriophage material is effected, and sterilizing the resulting substantially flat impregnated devices for storage and use.

11. Bacteriophage-impregnated discs of porous paper for typing strains of *Staphylococcus aureus,* the sand discs containing also a redox indicator adapted to assume a definite color change in absence of lysis of the particular strain of *Staphylococcus aureus* being typed by the impregnated discs.

12. Bacteriophage-impregnated porous paper discs for lysing selected strains of *Staphylococcus aureus,* the paper discs including also a growth-promoting material for the bacteriophage and a tetrazolium chloride as a redox indicator adapted to assume a definite color change in absence of lysis of the particular strain of *Staphylococcus aureus* being typed by means of the impregnated discs.

13. Bacteriophage-impregnated porous paper discs as claimed in claim 11, wherein the redox indicator is selected from the group consisting of 2,3,5-triphenyltetrazolium chloride and 2,3-diphenyl-5-methyltetrazolium chloride.

14. Bacteriophage-impregnated absorbent paper discs containing also a redox indicator adapted to assume a definite color change in absence of lysis of a selected microorganism by the bacteriophage in the paper discs.

15. Bacteriophage-impregnated absorbent paper discs adapted to contain a phage for lysing a selected strain of *Staphylococcus aureus,* the discs containing a redox indicator adapted to assume a definite color change in absence of lysis of cultured strain of *Staphylococcus aureus* in contact with the discs.

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