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3,094,466

PREPARATION FOR TYPING OF STAPHYLOCOCCI AND PROCESS THEREFOR

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No Drawing. Filed July 15, 1959, Ser. No. 827,140
4 Claims. (Cl. 195—103.5)

This invention relates to a new and novel preparation useful in the routine typing and identification of Staphylococci organisms and to a method of producing said preparation.

In recent years, a serious problem has developed in hospitals as a result of infections caused by antibiotic-resistant strains of staphylococcus, and particularly strains of *Staphylococci aureus*. These strains are harbored not only among professional personnel and patients but may also be found in the physical environment of the hospital, for example on bedding and furniture, in dust or in the air. The presence of these antibiotic-resistant strains represents a reservoir which acts as a constant potential source of infection, which is manifested by the frequent outbreaks of Staphylococci infections among hospitalized maternity patients and infants. It is frequently found that a single strain is responsible for the major problem in any one hospital and prompt and accurate identification of this particular strain and its source would greatly aid in controlling this problem.

Bacteriophages, usually known as phages, are viral agents which produce transmissible dissolution of specific bacterial cells. Phage typing of Staphylococci is a well-known bacteriological technique used to identify an unknown strain of this organism. Most strains of an organism, for example the strains of Staphylococci, are sensitive to a particular phage or group of phages with the result that a culture of an unknown strain may be typed by observing the action upon the strain of a series of known bacteriophages. Where lysis of the unknown strain occurs in the presence of a known phage or group of phages, it is established that the strain is sensitive to the particular phage or group of phages and enables one to thereby identify the strain.

Fisk, Roy T., J. Infect. Dis. 71, 161-165 (1942), Williams et al., J. of Hygiene 50, 320-353 (1952), and Blair et al., J. Infect. Dis. 93, 1-13 (1953) discuss the general principles involved in phage typing of strains of Staphylococci. The procedures described, while adapted to use by specialized typing laboratories, are too cumbersome and time consuming for routine use in a hospital.

Recently there have been made commercially available lyophilized stable phages of 29 strains of Staphylococci, including the strains most commonly encountered in recent hospital infections. These phages are in the form of dry powders and thus represent a source of stock phages which avoid the need for extensive propagation by the laboratory. However, even these preparations present problems in use. The lyophilized strain must be reconstituted in broth, cultured in the presence of its propagating strain, harvested and titrated to standardize the phage content of the filtrate. These procedures must all be carried out before typing can begin. Test dilutions of the stock filtrate are not stable for more than a few days and frequent restandardization is required. Thus, even these preparations do not solve the problem of providing a tool for rapid typing which may be carried out within a hospital laboratory without highly specialized training.

It is apparent that effective control of an outbreak of staphylococcal infection depends upon prompt identification of the strain responsible and also the location

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of carriers or other sources of the organism within the hospital community.

It is, therefore, a particular object of this invention to provide a preparation adapted to the rapid typing of Staphylococci and which may be used on a routine basis without highly specialized training.

Other objects and the advantages of this invention will become apparent from the following detailed description.

In accordance with this invention semi-porous solid beads are impregnated with high titer filtrate of a specific staphylococcal phage and the impregnated beads are then dried to provide beads each containing a specific quantity of viable phage organisms. In use, one or more of these impregnated beads are shaken with an appropriate liquid medium, thereby providing a standardized solution containing the specific phage. This phage solution may then be used directly in typing by applying a drop to a culture of the unknown organism and observing whether lysis of the organism occurs.

The initial step in the preparation of the impregnated beads of this invention is the preparation of a high titer broth culture filtrate (titer of at least 10^6) containing a specific staphylococcal phage. This step is conventional and any of the techniques described in the prior art may be used, for example those described by Williams et al., J. of Hygiene 50, 320-353 (1952), Blair et al., J. Infect. Dis. 93, 1-13 (1953), and Swanstrom et al., Proc. Soc. Exp. Biol. & Med. 78, 372-375 (1951). The phage is recultured repeatedly on its propagating strain until serial titrations indicate that a satisfactory potency has been obtained. The phage suspensions are ordinarily centrifuged at high speed and the supernatant filtered under sterile conditions. Such fresh high titer filtrates are used to coat the beads.

The typing preparation of this invention is prepared by impregnating beads with the high titer filtrate containing a specific staphylococcal phage. The beads used are formed of a semi-porous inert solid material. The term "semi-porous" as used herein refers to the surface characteristics of the solid material used to form the beads and means that the solid material should have the ability to absorb and hold phage organisms during the impregnation step. Solids having microscopically rough surfaces, thus presenting myriad crevices which can hold the organisms, are desirable. Beads which present a smooth glazed surface are generally undesirable since an insufficient number of phage organisms can be deposited during the impregnation step to provide a useful typing preparation when reconstituted. The term "inert" as used herein means that the solid material must contain no substance which is capable of destroying or impairing the potency of the phage.

A number of solid materials which are inert and which may be formed into semi-porous beads are available and include synthetic resins, such as phenolic resins, melamine resins and the like, hard rubber, cementitious or ceramic materials such as unglazed porcelain, and the like. Unglazed porcelain is generally preferred.

The beads may be of any convenient geometric shape, such as spherical, cylindrical and the like. It is desirable that the beads have a maximum exposed surface area for their size. Thus, beads in the shape of doughnuts, cups, hollow cylinders, saddles and the like are preferred. With this type of construction, the filtrate containing the phage can contact both external and internal surfaces during impregnation. This insures that a maximum number of phage organisms are deposited on the beads.

In typing, small volumes of solutions are normally used. Since the viable phage organisms must be removed from the beads to form a useful typing solution, it is desirable that the beads be small in size so that all

surfaces thereof may be contacted with the small volume of liquid used in reconstitution. Normally, the maximum dimension of the beads should not exceed 15 millimeters.

The titer of the phage filtrate must be carefully controlled to insure a constant, standard concentration of phage organisms in the impregnating medium. In addition, it is apparent that the physical characteristics of the beads, such as shape, dimensions, roughness and absorbency should be constant to insure that the surface of each bead is impregnated with a uniform number of phage organisms.

The impregnation may be effected conveniently by contacting sterile beads with the filtrate containing the desired phage. The beads should preferably remain in contact with the filtrate for a sufficient time to insure uniform impregnation of the beads. The impregnated beads are then removed from the filtrate.

The final step involves drying the impregnated beads and packaging in suitable containers. Drying may be conveniently effected at room temperature under high vacuum or by conventional lyophilization (freeze-drying) techniques. In lyophilization, the wet impregnated beads are quick frozen and then subjected to an absolute pressure below the vapor pressure of water at the temperature of the frozen beads. To prevent undue loss in potency during storage, it is essential that the impregnated beads be completely dry.

The dried impregnated beads may be packaged in several ways, each adapted to insure complete absence of moisture during storage. A quantity of beads may be packaged in a sterile vial or tube, with the beads separated by a porous plug from a mass of a desiccant such as silica gel at the bottom of the containers. Alternately, the sterile vial or tube containing the beads may be packaged within a larger sealed container containing the desiccant. Another convenient packaging technique is to seal one or more beads within a length of sterile glass tubing to form a sealed ampule-like container. In this type of package, no desiccant is needed since the sealed glass prevents the entry of any moisture. In this method of packaging, each ampule-like container contains the number of beads (one or more) required for a single reconstitution.

In use, one or more beads are removed aseptically from the container and thoroughly contacted with a liquid. Useful liquids which may be used in reconstitution include water or any general purpose medium of the type used in culturing staphylococcal phage organisms. A typical general purpose medium useful in reconstitution is a broth containing dextrose, salt, buffers and one or more peptones, that is enzymatic hydrolysates of proteinaceous materials such as soybean meal, casein and the like. During reconstitution, the viable phage organisms with which the surface of the beads are impregnated become suspended in the liquid, and the resulting solution may be utilized in typing without the necessity of culturing and standardizing the phage.

The method of typing after reconstitution of the phage is conventional, the procedure described by Blair et al., J. Infect. Dis. 93, 1-13 (1953) being typical. One agar plate is used for each strain to be typed. With a swab or loop, the surface of the plate is seeded uniformly from a 4-6 hour broth culture of the unknown strain and the inoculation is allowed to dry. With a 4 mm. loop, or a capillary pipette, one loopful or one drop of reconstituted phage is placed on the seeded area at spaced intervals about 1 cm. apart. After the phages have dried, the plate is inverted and incubated for 18-24 hours at 30° C. From 30-36 phages can be spotted on a single plate. The reactions are recorded according to the degree of lysis. Strains are differentiated on the basis of their sensitivity to one or more members of several distinct groups of phages.

The specific advantage of the impregnated beads of the

present invention is that the laboratory wishing to type an unknown strain of Staphylococci need not carry out the cumbersome and laborious procedures of culturing and standardizing numerous phages, since the impregnated beads may be easily reconstituted and the resulting solution used directly in typing.

In some circumstances where it is desired to determine whether an unknown organism is one of a group of strains of Staphylococci, a pooled reconstituted phage solution may be used. In this technique, a series of beads, each impregnated with a specific phage, may be reconstituted together to form a single pooled solution which may then be used in typing. Sufficient phages are represented to insure positive identification of the unknown organism as one of the group of strains.

The following examples are included in order further to illustrate this invention:

Example I

Small cup-shaped unglazed porcelain beads ("Fish Spine" beads No. 2 from Taylor, Tunnicliffe and Co., London, England) having a maximum dimension of 4 mm. are immersed in a high titer filtrate of staphylococcal phage strain No. 80. The beads are separated aseptically from the filtrate, dried under high vacuum at room temperature, and finally inserted into sterile tubes containing a mass of silica gel covered by a sterile glass wool plug. The tubes are then closed with sterile stoppers.

Example II

Unglazed porcelain beads are impregnated as described in Example I and then subject to lyophilization in a Dry Ice bath. The lyophilized beads are then packaged as described in Example I.

Example III

Lyophilized beads prepared as described in Example II are placed within a length of sterile glass tubing having an inside diameter slightly in excess of the size of the beads. The beads are arranged within the tubing in a series of spaced pairs of beads. The glass in the area of the spaces is sealed to provide a chain of connected sealed ampule-like containers, each containing two of the lyophilized beads. In use in typing, an ampule may be broken from the chain and the two beads reconstituted with broth to provide a standardized solution of staphylococcal phage, strain No. 80.

It is understood that the foregoing detailed description is given merely by way of illustration and that many variations may be made therein without departing from the spirit of my invention.

Having described my invention, what I desire to secure by Letters Patent is:

1. A preparation useful in the rapid routine typing of Staphylococci consisting of an unglazed dried porcelain bead the surface of which is uniformly coated and impregnated with a controlled and calibrated quantity of viable and removable bacteriophage organisms effective against a strain of Staphylococci.

2. A method of producing a bacteriophage preparation useful in the rapid routine typing of Staphylococci which comprises immersing a plurality of beads of a semi-porous inert solid material into a liquid medium comprising a filtrate containing viable bacteriophage organisms effective against a strain of Staphylococci, separating wetted beads from the liquid medium and drying the beads.

3. A method of preparing a solution useful in the rapid routine typing of Staphylococci which comprises contacting with a liquid at least one dried bead of a semi-porous inert solid material the surface of which is impregnated with viable bacteriophage organisms effective against a strain of Staphylococci, whereby said viable bacteriophage organisms are released from said bead and distributed throughout said liquid to form said solution.

4. A method of preparing a pooled bacteriophage solu-

tion useful in the identification of an unknown organism as a member of a group of strains of Staphylococci which comprises contacting with a liquid a plurality of dried beads of a semi-porous inert solid, the surfaces of said beads being impregnated with viable bacteriophage organisms effective against said strains of Staphylococci, no single bead being impregnated with more than a single bacteriophage strain, there being at least one impregnated bead for each strain of bacteriophage and sufficient bacteriophage strains being represented to insure positive typing of said unknown organism as a member of said group, whereby said viable bacteriophage organisms are released from said beads and distributed throughout said liquid to form said pooled bacteriophage solution.

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