Bacteriophage Typing of *Salmonella typhimurium* by Use of a Mechanized Technique

P. A. M. GUINÉE, W. H. JANSEN, A. VAN SCHUYLENBURG, AND W. J. VAN LEEUWEN

*Rijks Instituut voor de Volksgezondheid, Bilthoven, The Netherlands*

Received for publication 20 July 1973

A new mechanized technique for the application of drops of phages on agar plates is described. Drops of equal size are delivered by needles with the aid of filtered pressurized air. The part of the device which is in contact with phage is interchangeable as a whole.

For the phage typing of *Salmonella typhimurium* by the scheme of Scholtens (2), two sets of 27 phages each must be applied on two agar plates. The phage applicator of Lidwell (1) has been used for this purpose. Particularly when large numbers of strains are phagetypered, the working speed of the Lidwell apparatus is felt to be limited by the cooling time of the applying loops. A more rapid technique was therefore developed.

**MATERIALS AND METHODS**

**Description of the phage applicator.** The basis of the new applying device is the multiple reagent dispenser (Canalco, Rockville, Md.). The operating principle of the multiple reagent dispenser (MRD) is outlined in Fig. 1. A bottle containing the reagent to be dispensed is pressurized by means of compressed air. The tube leading from this bottle to the receptacle is channeled through the MRD and pinched off by means of a bar against a plate. The bar is pressed against the plate by means of a wheel with a detent driven by a foot switch-operated electric motor. A firm pressing on the foot switch triggers half a rotation of the wheel. When the detent in the wheel passes the bar 0.8 s after the motor has been started, the bar is pulled back by return springs and the “bridge” in the channel is opened. A light touch on the foot switch triggers a continuous cycle. The opening time of the “bridge” is determined by the size of the detent in the wheel. The amount of reagent to be dispensed by the MRD depends on the pressure in the bottle containing the reagent, the internal diameter of the tubing, and the opening time of the “bridge.” In the original MRD, nine different reagents could be dispensed from nine bottles which were pressurized by means of separate tubes. The minimal amount dispensed by the MRD in its original form is claimed to be 0.025 ml. It was established in preliminary experiments that the amount of phage suspension required to give a spot about 9 mm in diameter on the surface of an agar plate was 0.0025 ml.

The MRD was modified to deliver drops of about 0.0025 ml from 27 different reagents as follows: (i) The internal diameter of the tubing was reduced from 1.8 mm to 0.8 mm, and the number of channels was increased from 9 to 27 mm. (ii) The wheel was replaced by another with a smaller detent, which resulted in a shorter opening time of the “bridge” (Fig. 1). When the pressure was set at 3 psi, the MRD dispensed drops of about 0.0025 ml. (iii) The 27 glass tubes with phage suspensions were not separately pressurized but were placed in a closed box pressurized by one tube only. For this purpose, a round Perspex block was drilled to form 27 reservoirs for the glass tubes containing the phage suspensions. Instead of bringing the phage suspensions directly into the reservoirs drilled in the Perspex block, the use of glass tubes was preferred because they can be sterilized and handled individually. The glass tubes are 9 cm in length, 0.8 cm in internal width, and have an “efficient” content of about 4 ml. The Perspex block can be closed air tight by means of a Perspex cover, a rubber O ring, and an aluminum screw ring. Twenty-seven needles have been cemented in the Perspex cover in an asymmetrical pattern similar to that of the Lidwell apparatus (1). Two holes in the cover match two pins on the Perspex block so that the cover with the needles can be placed on the Perspex block only in such a way that the needles fit into the glass tubes and nearly touch the bottom. The tubes connected to the needles lead to an aluminum frame which can be connected to the MRD so that the 27 tubes are channeled through the “bridge” of the MRD. The tubes are finally connected to 27 delivering needles set asymmetrically in a Perspex frame and spaced so that they match a 9-cm outer diameter (OD) petri dish.

The “phage set,” consisting of a Perspex block with glass tubes containing the typing phages and the attached tubes and frames, can be stored at 4°C as a whole. During storage, the tubes in the aluminum frame are closed by means of a crossbar and a knob on the frame to prevent siphoning of phage suspension. Figure 2 shows the details of a “phage set.” An empty “phage set” can be disinfected with a proper disinfectant.

With this modified MRD, 27 different phage suspensions can be dispensed at the same time. Figure 3 shows the phage-applicating device. To avoid spatter-
Fig. 1. Operating principle of the multiple reagent dispenser (modified). Key: 1, filtered compressed air; 2, reagent to be dispensed; 3, plate; 4, return springs; 5, bar; 6, wheel (modified; measures given in millimeters); 7, receptacle; 8, original wheel.

Fig. 2. Use of the multiple reagent dispenser and "petri dish lifter" for the application of typing phages. Key: 1, filtered compressed air; 2, Perspex block containing 27 glass tubes with the typing phages; 3, multiple reagent dispenser (modified); 4, electric bridge belonging to the multiple reagent dispenser; 5, aluminum frame (Fig. 3, part 10) with crossbar (Fig. 3, part 11); 6, wing screws, connecting the aluminum frame to the multiple reagent dispenser; 7, Perspex frame with 27 dispensing needles; 8, "petri dish lifter," 9, pneumatically lifted table (foot switch operated); 10, air values for speed regulator of the table (9); 11, connections to compressed air.

The combined foot switch triggers a continuous cycle of the MRD. When the foot switch is pressed down, the table is elevated, followed after 0.8 s by one opening of the "bridge." When the foot switch is released, the table returns to its original position.

Working procedure. A "phage set" is connected to the MRD and the "petri dish lifter" as follows. The plate is unscrewed from the "bridge" of the MRD. The aluminum frame with 27 tubes is fixed to the MRD by means of four wing screws. The plate is replaced. The Perspex frame with 27 delivering needles is placed on the "petri dish lifter." The compressed air tubing is connected to the Perspex block, and the pressure is set to 3 psi. The crossbar (Fig. 3, part 11) which shuts off the tubes during storage is loosened. By touching the foot switch lightly, a continuous cycle starts and the tubes are filled with phage suspension until each needle delivers. By pressing the foot switch, the continuous cycle is stopped. The device is then ready for use. An agar plate inoculated with the culture to be phage typed is placed on the table. Pressing the foot switch results in lifting of the table, after 0.8 s, followed by dispensing the 27 drops (0.0025 ml each) of phage suspensions. The plate is removed and the procedure is repeated. After completing the application of the first set of phage suspensions, the "phage set" is removed, after shutting off the tubes with the knob and the crossbar (Fig. 3, part 11). The second "phage set" with the remaining 27 typing phages is connected as described above, and the whole procedure is repeated.

RESULTS

The new phage applicator has been used for over a year, and about 30,000 strains of S.
typhimurium have been phage typed. About 2,000 of these strains were also phage typed by using the Lidwell applicator. There were no significant differences in the phage patterns obtained with the two techniques on the same strain.

The greatest advantage of the new apparatus over the Lidwell applicator was its speed. In comparative tests by experienced operators, the time required for the application of the phage suspensions appeared to be reduced to 25%.

The volumes of phage delivered were more constant and equal than those applied by means of the Lidwell applicator. Negative and weakly positive reactions by phages applied with the new applicator showed a bald spot of about 1 mm in diameter in the center of the drop because the application of the “pressurized” phage suspensions appears to remove locally the inoculated bacteria (Fig. 5). The marks made by the loops touching the agar when using the Lidwell applicator were more irregular in shape than those produced by the new applying device (Fig. 5).

With 4 ml of phage suspension per glass tube, about 1,500 applications could be done. It had been established earlier that the 1.75-ml reservoirs of the Lidwell applicator were sufficient for about 150 applications, on an average. Although the same phage suspension was used much longer when the new applicator was employed, there was no detectable contamination of the typing phages.

DISCUSSION

Although, as expected, time was the major advantage of the mechanized procedure described here, the system is felt to have some other minor advantages. The phage reservoirs in the form of 4-ml glass tubes are larger than those of the Lidwell applicator. Moreover, they can be replenished under sterile conditions. Many reserve tubes with phage suspension can be held in stock.

It is a closed system, preventing contamination of the phage suspensions with airborne bacteria, as well as cross-contaminations of the
phage suspensions. The new device was compared only with the Lidwell applicator and not with the many other devices which have been described (3), since the Lidwell applicator was the only apparatus available.

LITERATURE CITED
1. Lidwell, O. M. 1959. Apparatus for phagetyping of Staphylococcus aureus. Mon. Bull. Min. Health 18:49-52.
2. Scholtens, R. T., and J. A. Rost. 1972. Subdivision of Salmonella typhimurium into phage types and bio types. Characterization of transferable factors by means of phage typing. The increase of drug-resistance in Salmonella. Ann. Sclavo 14:309-344.
3. Smith, P. B. 1972. Bacteriophage typing of Staphylococcus aureus, p. 431-441. In J. Cohen and O. Wiley (ed.), The staphylococci. Interscience Publishers, Inc., New York.