New Technique for Biotyping

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

Rijks Instituut voor de Volksgezondheid, Bilthoven, Netherlands

Received for publication 6 March 1972

An efficient technique for biotyping, using disposable trays instead of glass tubes and agar instead of liquid media, is described.

In the conventional method of biotyping, liquid media in glass tubes are usually employed. A more efficient technique is described here.

We use clear disposable plastic trays (type 96 sc, Gateway International Inc., Los Angeles, Calif.) with 96 cups instead of glass tubes, and agar (1%) instead of liquid media to allow easier handling and transport of the filled trays. The media are dispensed in 0.5-ml samples into the cups. This can be easily done by means of a multiple reagent dispenser (Canalco, Rockville, Md.) (Fig. 1 and 2).

The filled trays are covered with adhesive tape (58 ps, Gateway International Inc., Los Angeles, Calif.) of the same size as the trays and stored at 4°C. The media in the trays are inoculated by means of a flame-sterilized injection needle (2 mm external diameter), without conus placed in a common loopholder, through the adhesive tape which has to be cleaned before and after inoculation with a proper disinfectant. The inoculum is preferably taken from a broth culture or suspension, containing about $10^8$ organisms/ml.

This technique was primarily developed for the biotyping of Salmonella typhimurium. Only 6 reactions out of 20 originally described by Harhoff (1) and Kallings and Laurell (2) are required to distinguish seven biotypes (Table 1). Therefore 16 strains can be biotyped in one tray.

---

**Fig. 1. Use of “multiple reagent dispenser” for dispensing culture media into tray cups.**

**Fig. 2. Operating principle of the “multiple reagent dispenser.”**
The liquid D-tartrate medium could not be replaced by a solid medium because the reaction is based on estimation of the quantity of precipitate which is formed after addition of saturated lead-acetate solution (Brocades ACF). Trehalose agar according to Scholtens (3) was used. The other reaction media, composed of basal nutrient agar medium, carbohydrate, and indicator, have been adapted to this technique and are listed in Tables 2 and 3.

For preparation of the reaction media, basal agar media are melted by heating at 100 C for 30 min, cooled to 60 C, and carbohydrate and indicator solutions are added and dispensed in the trays. If the tubings of the dispenser are prewarmed to 60 C, no special precautions have to be taken by an experienced worker to prevent the coagulation of the media. After sealing, the trays can be stored at 4 C for at least 14 days. For cleaning before and after inoculation, we used an aqueous solution (1%) of Sanosept.

After 16 to 20 hr of incubation at 37 C, 0.05 ml of saturated lead-acetate solution is injected through the adhesive tape into the cups containing D-tartrate. The reactions are then read.

A comparative study revealed no differences in results between conventional biotyping in tubes with liquid media and the newly developed procedure.

The new technique has several advantages. Smaller amounts of the media are required, dispensing of the media takes less time, less space is required in the laboratory as well as in

| Biotype according to Harhoff | Biotype as indicated by us | Reaction |
|-----------------------------|----------------------------|----------|
|                             | Rhamnose | Xylose | Inositol | Trehalose | D-Tartrate (liquid) | Xylose |
| 1a                          | I        | +      | +        | +        | +                   | +       |
| 1b                          | II       | +      | +        | +        | -                   | +       |
| 6                           | VI       | +      | +        | +        | +                   | -       |
| 8                           | VIII     | +      | +        | +        | -                   | -       |
| 9                           | IX       | +      | +        | -        | +                   | -       |
| 10 (11, 12)                 | X        | +      | +        | -        | +                   | -       |
| 17                          | XX       | -      | +        | +        | -                   | -       |

* According to Bitter.

**TABLE 2. Basal agar media**

| Basal medium | Composition            | Percent |
|--------------|-----------------------|---------|
| I            | Tryptone (Difco)      | 0.06    |
|              | Agar (Oxoid nr. 1)    | 1       |
|              | NaCl (B.D.H.)*        | 0.6     |
| II           | Peptone (Oxoid)       | 1.25    |
|              | Agar                  | 1       |
|              | NaCl                  | 0.6     |
| III          | Peptone               | 0.01    |
|              | Agar                  | 1       |
|              | NH4Cl (Merck)         | 0.25    |
|              | Na2HPO4·2H2O (Merck)  | 0.125   |
|              | Na2C4H4O6·2H2O (B.D.H.) | 0.5 |

* Basal media can be stored at 4 C for several months. All were pH 7.4.
* British Drug Houses.

**TABLE 3. Reaction media used in the new technique of biotyping of Salmonella typhimurium**

| Reaction            | Composition* |
|---------------------|--------------|
| Rhamnose            | 80 ml of basal medium I + 20 ml of 15% carbohydrate solution (w/v) + 1.2 ml of indicator | |
| Xylose              | ml of 5% carbohydrate solution (w/v) + 1.2 ml of indicator | |
| Inositol            | 80 ml of basal medium II + 20 ml of 6% carbohydrate solution (w/v) + 1.2 ml of indicator | |
| Trehalose           | 10 g of peptone + 10 g of D-tartrate (Merck) + 12 ml of indicator in 988 ml of distilled water | |
| Xylose according to Bitter | 80 ml of basal medium III + 20 ml of 15% carbohydrate solution (w/v) + 1.2 ml of indicator | |
| D-Tartrate          | | |

* Final pH = 7.4.
* All carbohydrates were purchased from British Drug Houses (B.D.H.).
* Aqueous bromine thymol blue (B.D.H.) solution 0.2% (w/v).
the incubator for the trays compared with the conventional method, and inoculation and reading take less time. The procedure is probably also applicable for other biochemical tests, but, if prolonged incubation is required, another inoculation technique which avoids puncturing of the adhesive tape should be employed.

LITERATURE CITED

1. Harhoff, N. 1948. Gastroenteritisbaciller af Salmonellagruppen i Danmark. Nyt Nordisk Forlag, Copenhagen.
2. Kallings, L. O., and A. Laurell. 1957. Relation between phage types and fermentation types of Salmonella typhimurium. Acta Pathol. Microbiol. Scand. 40:328-341.
3. Scholtens, R. T. 1969. Une subdivision de S. typhimurium en lysotypes et en biotypes. Arch. Roum. Pathol. Exp. Microbiol. 28:984-989.