Technical methods

A mechanised microtechnique for salmonella serotyping

CHRISTINE R SHIPP AND B ROWE *Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT*

In the reference laboratory complete serological identification of salmonella requires the identification of 59 'O' (somatic) and 87 'H' (flagellar) antigens. When large numbers of strains are studied mechanisation of the serological techniques would be advantageous.

Normally, slide and tube agglutination methods are used,[1 2] and the latter is an essential confirmatory test. The antisera used are costly to produce, and although a mechanised system based on tube agglutination could be developed, an alternative system using smaller volumes of antisera would be preferable. A technique using microagglutination trays combined with a mechanised system to dispense antisera and suspensions is described.

Material and method

Both O and H suspensions are prepared using Brain Heart Infusion Broth (Oxoid Ltd). After incubation at 37°C for 4-5 hours the broths used for O suspensions are steamed for 30 minutes and diluted with an equal volume of saline; those for H suspensions are killed by the addition of an equal volume of 1% formol saline and are left overnight before use. Antisera are dispensed into 96-well round-bottomed microtitre plates using a Dynadrop Multi-reagent Dispenser (Dynatech Laboratories Ltd) (Fig. 1). Suspensions are manually pipetted into 8-channel reservoirs. A 96-channel Automatic Pipetter (Dynatech Laboratories Ltd) (Fig. 2) is used to transfer suspension from the reservoirs into the microtitre plates; 0·025 ml volumes are used for both antisera and suspensions. O agglutination plates are sealed with plate-sealing tape (Flow Laboratories Ltd) and placed in an incubator for 2 hours at 50°C followed by 18 hours (overnight) at 4°C. H agglutinations are incubated for 2 hours at 50°C in a waterbath. The plates can either be floated on the water or, preferably, put on a special shelf without holes which is installed at the same level as the water, thus preventing the plates becoming wet. Agglutinations are examined using a test reading mirror (Dynatech Laboratories Ltd) which allows easy inspection from below of the agglutination pattern (Figs 3 and 4) in the bottom of the well. The use of a strip light in a darkened area makes reading easier.

Approximately 2000 salmonella serotypes have been described, but about 10 serotypes account for between 60% and 70% of salmonella isolates from humans in the United Kingdom. Furthermore, all the common serotypes belong to the first five groups out of the 39 O groups of the Kauffmann-White Scheme. The system described above identifies the antigens of these common O groups and all the commonly occurring H antigens. The rarer antigens not included in the mechanised scheme can be identified using microtitre techniques or traditional slide and tube agglutination methods, but the use of the latter is kept to a minimum.

Before use all antisera are tested in microtitre plates, the homologous titres are determined, and suitable dilutions prepared using a 1 in 10^4 dilution of merthiolate in saline. Twenty-three O antisera are used and they are arranged in two sets with a saline control in the second set. The first set contains polyvalent salmonella O antiserum for groups A to H of the Kauffmann-White Scheme, seven antisera for groups B (4, 5, 12), C_1 (6, 7), C_2 (6, 8), D_1 (9, 12), E_1 (3, 10), G_1 (13, 22), and H_1 (6, 14, 24), and four absorbed antisera 1, 20, 27, and 46. Set 2 contains absorbed antisera 4, 5, 7, 8, 9, 10, 14, 15, 19, 22, and 23. All strains are tested against these two sets of antisera.

To identify the H antigens each strain is initially tested against 11 antisera and a saline control. The antisera are polyvalent salmonella H (PSH) containing H antibodies for H = a through to H = z_{29} to cover phase 1 and antibodies for the phase 2 complex H = 1, 2, 5, 6, 7, four antisera for the flagellar complexes H = E; G; L; 1, 2, 5, 6, 7, and six absorbed antisera for H = b, d, i, r, z, z_{10}. The identification of phase 1 is complete in those strains which react with PSH and one of the six absorbed antisera, and no further testing is needed at this stage. Those strains reacting with PSH and one of the four complexes H = E; G; L; 1, 2, 5, 6, 7 need further testing to identify the single factor antigens, eg, H = z_{15}, s, v, and 2. Strains reacting with PSH only are tested against H = a, c, k, y, z_4, z_6, and z_{29} antisera. Phase

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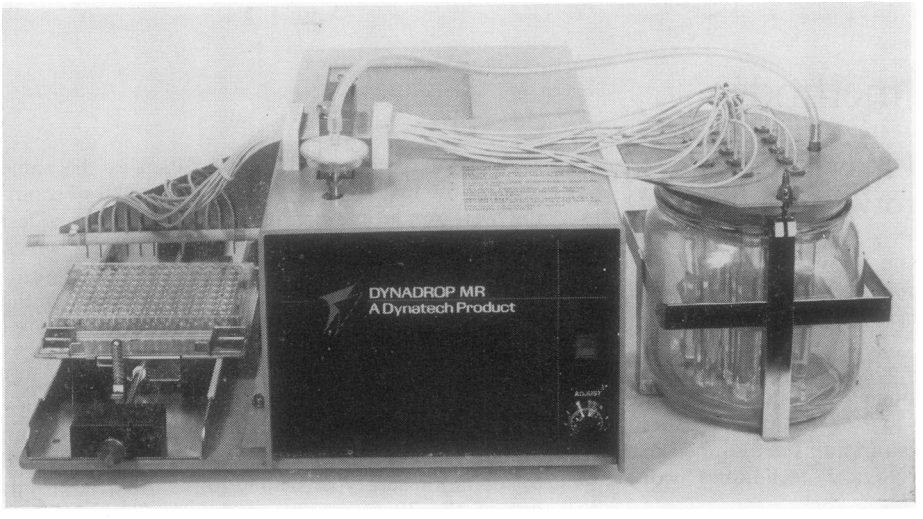

Fig. 1
Dynadrop Multi-reagent Dispenser.

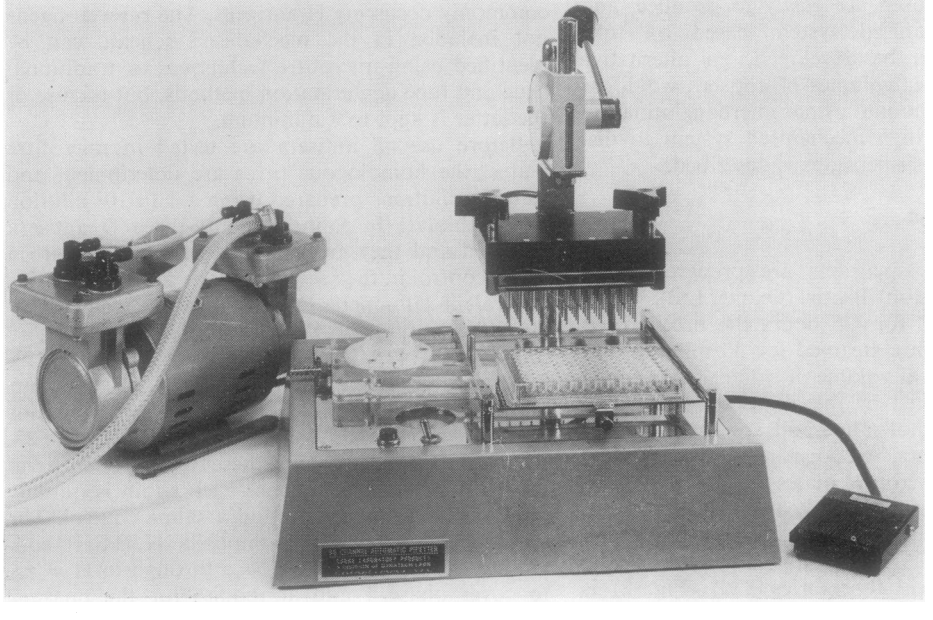

Fig. 2
96-channel automatic pipetter.

reversal is accomplished using an antiserum semi-solid agar technique.[3,4] After phase reversal the H antigen testing procedure is repeated to determine the antigens of the alternative phase.

Conclusion

The mechanised microtitre method for salmonella serotyping has been used to study over 15 000 strains isolated in the British Isles, and 98% of them have been successfully identified without recourse to slide agglutination. Slide agglutination required the use of living bacterial suspensions, a potentially hazardous procedure, while in the mechanised method killed suspensions are used. Furthermore, slide agglutination is tedious and labour-intensive,

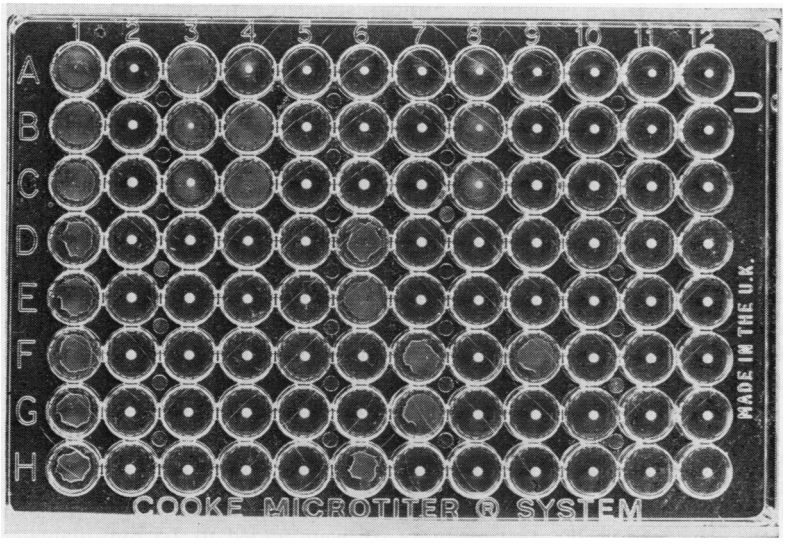

Fig. 3 *Salmonella O agglutination.*

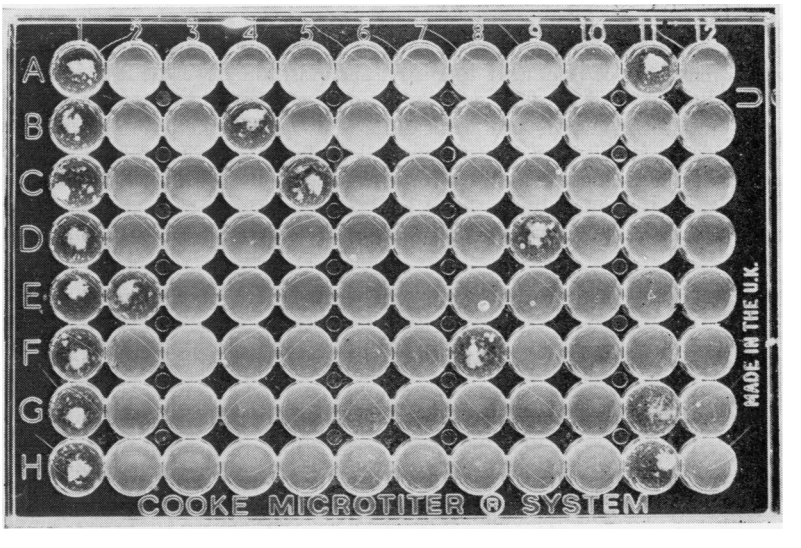

Fig. 4 *Salmonella H agglutination.*

and requires highly trained staff for accurate work. The mechanised system does not have these characteristics. Additionally, when compared with tube agglutination methods, the mechanised system uses smaller quantities of costly antisera and therefore is more economical. The inbuilt flexibility of the system allows for constituent antisera of the sets to be altered to allow for changes in the frequency of serotypes.

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Requests for reprints to: Christine R Shipp, Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT.