Evaluation of Plastic Multi-Well Plates for Serological Screening of Salmonella Cultures with Spicer-Edwards Pooled Antisera

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We compared the relative advantages of using glass test tubes and plastic multi-well plates in the serological identification of Salmonella cultures by the Spicer-Edwards method, and we conclude that the advantages of multi-well plates outweigh those of test tubes.

Serological screening of Salmonella cultures by the method of Spicer (3) as modified by Edwards (1) has proved to be a useful step in serological identification of salmonellae in this laboratory. The method is designed to screen for flagellar antigens on the basis of an agglutination scheme by using a maximum of seven tubes. The purpose of this study was to compare results with glass test tubes and with plastic multi-well plates in the procedure.

Salmonella cultures isolated from processed fish meal, fish matter being processed, fish meal plant environment, and river water were selected to be analyzed in this study because they offer the maximal challenge normally encountered in H serological screening. This difficulty in serotyping is due to the wide variety of serotypes present, the frequent occurrence of rare serotypes, and the high frequency of organisms that agglutinate weakly in H antisera prior to growth in the semisolid medium. The cultures were inoculated from a triple sugar iron agar slant to Trypticase soy tryptose broth (prepared by adding 15 g of dehydrated Trypticase soy broth [BBL] and 13 g of Tryptose [Difco] to 1 liter of distilled water), incubated at 37 C for 18 to 24 hr, and killed by adding an equal volume of 0.6% Formalin in saline.

Antiserum was prepared by pooling individual antiserum samples obtained from the Biological Reagents Section, Center for Disease Control. Working dilutions (1:20) of the pooled antiserum were prepared so that all sera, when added in amounts of 0.02 ml to 1 ml of Formalinized broth culture, represented a 1:1,000 dilution. The tests were conducted with 13 by 100 mm test tubes and plastic plates, each containing 12 rows of eight 2-ml wells (Linbro Chemical Co., Inc., New Haven, Conn.). The tubed antigen-antibody suspensions were incubated in a water bath at 50 C, whereas the suspensions in multi-well plates were incubated in an air incubator at 50 C. The shallow wells on the multi-well plates and the fogging of plates in the water bath made the air incubator more practical than the water bath for incubation of the multi-well plates. All reactions were read at 15, 30, 60, and 120 min. The tubes were read with the aid of a fluorescent light source and a black background. The multi-well plates were read over a glass-topped box providing indirect light (Fig. 1). The cultures were identified by the O and H serological grouping, as described by Edwards (1) (Table 1), followed by definitive serological typing (2).

Examination of 79 isolates resulted in the identification of 22 serotypes (Table 2). All 79 cultures reacted with at least one H-phase antiserum. The number of positive reactions increased progressively as incubation time increased. Compared with the tube method, slightly fewer reactions occurred with the multi-well plate method, and the reaction time was longer (Table 3). The multi-well plates were made of plastic, which is a poorer conductor of heat than glass, and were incubated in an air incubator rather than in a water bath. Both of these factors probably caused more time to be required for the antigen-antibody suspensions to equilibrate to 50 C, thus resulting in a longer reaction time.

All 79 cultures examined in this study reacted with the Spicer-Edwards pooled antiserum by both the tube and the multi-well plate method. One culture (S. sera) failed to react by the multi-well plate method within 1 hr. Subse-
Fig. 1. Serological screening of Salmonella cultures by using the multi-well plate method. All cultures were positive (from right to left) as follows: first row (horizontal), tubes 1, 3, and 4 (d); second row, tubes 2 and 3 (z); third row, tube 5 (L complex); fourth row, tube 4 (z); fifth row, tube 6 (1 complex); sixth row, tube 2 (y); seventh row, tubes 1 and 6 (i: 1 complex); eighth row, tubes 1 and 4 (G complex); ninth row, tube 1 (i); tenth row, tube 3 (z complex); eleventh row, tubes 1, 2, and 4 (b); and twelfth row, tubes 1, 3, and 7 (e, h: e, n complex).
TABLE 1. Reactions of H antigens in Spicer-Edwards polyvalent antiseras

| Antigen | Antiseras pool no.* | 1   | 2   | 3   | 4   |
|---------|---------------------|-----|-----|-----|-----|
| a       | +                   | +   | +   | +   | +   |
| b       | +                   | +   | +   | +   | +   |
| c       | +                   | +   | +   | +   | +   |
| d       | -                   | -   | -   | -   | -   |
| e, h    | +                   | +   | +   | +   | +   |
| G complex |               | +   | -   | -   | +   |
| i       | +                   | +   | -   | -   | +   |
| k       | -                   | +   | +   | +   | +   |
| r       | -                   | -   | -   | -   | -   |
| y       | -                   | +   | +   | +   | +   |
| z       | -                   | -   | +   | +   | +   |
| z⁺, complex |             | -   | -   | +   | +   |
| z⁻, 22  | -                   | +   | +   | +   | +   |

* Individual sera were added to three tubes as follows: L complex, tube 5; 1 complex, tube 6; e,n complex, tube 7.

TABLE 2. Salmonella serotypes identified

| Salmonella serotype | Antigen | No. of cultures |
|---------------------|---------|-----------------|
| bareilly            | 6,7:y:1,5 | 4               |
| cerro               | 18:z₂,2₂   | 7               |
| cubana              | 1,13:23:zz | 3               |
| derby               | 1,4,5,12:fg | 4               |
| eimsbuettel         | 6,7,14:d:1,w | 7             |
| gaminara            | 16:d:1,7 | 3               |
| gera                | 42:z₂,2₂ | 2               |
| give                | 3,10:1;v:1,7 | 3             |
| havana              | 1,13,23:fg | 2               |
| inverness           | 38:k:1,6 | 2               |
| johannesburg        | 1,40:bc:en,x | 5           |
| lexington           | 3,10:x:2,1,5 | 2          |
| manhattan           | 6,18:1,5 | 3               |
| minnesota           | 21:bc:en,x | 2               |
| molade              | 8,20:z₁₀:z₁ | 1               |
| montevideo          | 6,7:gm,s | 1               |
| muenchen            | 6,8:d:1,2 | 3               |
| new brunswick       | 3,15:1,v:1,7 | 5          |
| newport             | 6,8:e,h:1,2 | 2               |
| oranienburg         | 6,7:m,t | 16              |
| ruiru               | 21:y:e,n,x | 1               |
| tennessee           | 6,7:z₂₂ | 1               |

TABLE 3. Number of cultures yielding positive reactions

| Phase   | Time (min) | Tube | Multi-well plate |
|---------|------------|------|------------------|
|         | No. (%)    |      | No. (%)          |
| Strong H | 15 | 77⁺ (97.5) | 67 (84.8) |
|         | 30 | 78 (98.7) | 75 (94.9) |
|         | 60 | 79 (100.0) | 78 (98.7) |
|         | 120 | 79 (100.0) | 79 (100.0) |
| Weak H  | 15 | 2⁻ (14.2) | 2 (14.2) |
|         | 30 | 6 (42.9) | 3 (21.4) |
|         | 60 | 8 (57.1) | 7 (50.0) |
|         | 120 | 11 (78.6) | 10 (71.4) |

*A total of 79 cultures was examined.

14. Fourteen cultures reacted weakly in a second H phase.

Querent examination of this culture indicated that it was weakly motile, and after passage through motility agar it reacted strongly by both methods. Of the 79 cultures, 43 were diphase; 14 of these reacted weakly with a second H-phase antiserum in this initial screening in addition to reacting strongly in one phase (as did all cultures). These weak reactions were also recorded and summarized (Table 3). No cross-reactions because of O antigens were noted in these studies, even after 2 hr of incubation.

Microtitration plates were also evaluated with a few cultures. These plates have two advantages: they can be sealed with cellophane tape and incubated in a water bath, rather than in an air incubator, and they require less antiserum; but it is harder to read the reactions in the small trays than in the large multi-well plates used in this study.

Multi-well plates offer several advantages over the customary 13 by 100 mm glass tubes. They are more economical (multi-well plates cost one-sixth as much as tubes), eliminate the time required to assemble tubes into test tube racks, eliminate the necessity for removing the tubes from a test tube rack and holding the tube up to a light source for reading (holding seven tubes up to a light source is cumbersome, and occasionally a tube may be dropped), and reduce the chance of mix-up of tubes because wells in the plates are stationary. The disadvantages are that plastic is a poorer conductor of heat than glass and that the trays are incubated in an air incubator rather than the water bath used for tubes, which increases the time required for agglutination to occur. After incubation for 1 hr, only one false-negative reaction was noted with the multi-well plates. The advantages of using multi-well plates appeared to outweigh the disadvantages.

LITERATURE CITED

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