Typing of O Antigens of Vibrio parahaemolyticus by a Slide Agglutination Test

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O antigens of Vibrio parahaemolyticus can be detected by a slide agglutination test.

Serological typing of isolates of Vibrio parahaemolyticus has provided data related to the epidemiology and ecology of this enteropathogenic, facultatively halophilic bacterium. The annual distribution and frequency of occurrence of serotypes among patients with gastroenteritis due to V. parahaemolyticus have been, in general, based on typing for K antigens, since this can be easily done by a slide test. Antigenic studies with O groups, however, have been undertaken with the tube agglutination test (3).

Japanese microbiologists have developed a slide agglutination test, similar to that employed for K typing, for the O grouping of V. parahaemolyticus. The present report pertains to results obtained with this test on 82 isolates of V. parahaemolyticus.

Eighty-two isolates of V. parahaemolyticus with typical bacteriological and biochemical properties (1), and representing all known O groups (3), were selected from our stock culture collection. All isolates had been previously tested for O antigens by the tube agglutination test and for K antigens by the slide agglutination test (Table 1).

Absorbed O antisera were prepared as described by Miwatani et al. (2) with the following modifications. Antigens for the immunization of rabbits were prepared by suspending the organisms in 3% NaCl solution with 5% glycerol (pH 9.4) at a concentration of 10 mg/ml (wet weight). The cell suspensions were heated at 121 C for 15 min and centrifuged; the sedimented cells were washed twice with 3% NaCl (pH 7.0) and resuspended in 3% NaCl (10 mg/ml).

Slide tests were performed and interpreted as follows. Cultures were grown on Brain Heart Infusion Agar (Difco) which contained 3% NaCl (for 18 hr at 37 C). Dense suspensions of growth from the slants were prepared by adding 3% NaCl solution which contained 5% glycerol (pH 9.4). Suspensions were heated in an autoclave at 121 C for 60 min, allowed to cool, and centrifuged at 2,500 × g for 15 min. The sediment was employed as antigen. One loopful (0.001 ml) of antigen was placed on a glass slide, and one drop of antiserum was added. In the event a culture did not react with any of the O antisera, it was reheated at 121 C for 60 min and retested. Agglutination appearing within 1 min was considered positive; delayed or partial agglutination was considered negative.

Organisms having O10 antigen reacted with O10 and O12 antisera; therefore, organisms displaying such a reaction were classified as O10.

Table 1. O and K antigens of 82 isolates of Vibrio parahaemolyticus

| O group: K type | No. of isolates | O group: K type | No. of isolates |
|----------------|----------------|----------------|----------------|
| 01:K1          | 4              | 04:K42         | 1              |
| 01:K32         | 6              | 04:K49         | 1              |
| 02:K3          | 8              | 05:K15         | 6              |
| 02:K28         | 2              | 05:K17         | 4              |
| 03:K6          | 2              | 06:K18         | 2              |
| 03:K7          | 3              | 06:K46         | 3              |
| 03:K29         | 2              | 07:K19         | 1              |
| 03:K33         | 1              | 08:K20         | 1              |
| 03:K48         | 1              | 08:K21         | 1              |
| 04:K4          | 1              | 08:K22         | 6              |
| 04:K8          | 1              | 08:K39         | 1              |
| 04:K9          | 1              | 09:K44         | 10             |
| 04:K10         | 1              | 010:K24        | 2              |
| 04:K11         | 1              | 011:K50        | 1              |
| 04:K12         | 2              | 011:K51        | 1              |
| 04:K13         | 1              | 012:K52        | 3              |
| 04:K34         | 1              |                |                |

a K4 may be either 03 or 04.
The percentage of agreement between the tube agglutination and slide agglutination tests, taking into consideration the above correction, was 100%. When 20 randomly taken isolates were retested, the results were in complete agreement, indicating that the slide test has an acceptable level of reproducibility. In no instances was it necessary to reheat and retest.

Tube agglutination tests for O antigens of *V. parahaemolyticus* require 18 hr of incubation at 50°C before they can be observed for evidence of agglutination. In contrast, slide agglutination tests can be performed in approximately 1.5 hr. Since the results obtained on the 82 cultures examined had 100% agreement by both tests, it would appear that the slide test can be routinely used as a replacement for the more cumbersome and lengthy tube test.

**LITERATURE CITED**

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