

# A thermoprecipitin test for rapid diagnosis of cholera

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**THERMOPRECIPITIN TESTS**, in which antigen extracted by boiling a sample of infected tissue is layered on antibacterial serum to obtain a ring of precipitate, are well established in veterinary diagnosis, e.g., in anthrax and plague. They are simple, rapid, and usable even when the pathogen has become nonviable or overgrown with contaminants. Such tests have not been used in man, except that ring tests can be made with unheated cerebrospinal fluid for instant diagnosis of meningococcal and haemophilus infections.

Since cholera stool is almost watery and contains a high concentration of dissolved antigen (Finkelstein et al., 1963) the ring test appeared suitable for rapid diagnosis. In the absence of cholera cases animal experiments were made, the stool of infected baby rabbits being virtually identical with that of cholera patients (Burrows, 1968).

## Materials and Methods

A strain of *Vibrio cholerae* Inaba and another El Tor vibrio of Ogawa serotype, both isolated from

patients, were used to infect baby rabbits by inoculation into the duodenum (Ghosh, 1969a). As soon as diarrhoea developed, the watery fluid in the colon containing flakes of mucin and other debris was collected. About 2 ml fluid was boiled over a spirit lamp for about a minute, and then centrifuged for 2-3 minutes at c. 1000 x g. The coagulated debris settled or floated, leaving a faintly opalescent middle layer. Antigen controls were broth cultures of 2 strains each of *Escherichia coli*, *Alcaligenes faecalis*, *Shigella flexneri*, *Salmonella enteritidis* and NAV of Heiberg's Group I incubated for 7 days at 37° C.

The antiserum was made by injecting intravenously 2 adult rabbits with agar culture of the cholera vibrio strain suspended in saline to contain c. 10<sup>9</sup> cells per ml, and steamed for 2 hours. An initial dose of 0.5 ml was followed by 3 weekly 1 ml doses. The serum, collected 7 days later and pooled, had an agglutinin titre of 1:3200. This was diluted with 3 volumes of normal serum.

The test was performed at c. 26° C by layering 2-3 drops of heated stool or broth culture on a drop of

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antiserum or normal serum in a narrow tube made by cutting a pasteur pipette at the neck and sticking the shaft in plasticene.

### Results

In view of the consistent results and the exploratory nature of the experiments, each vibrio strain was used to infect only 6 baby rabbits: 2 at a time. All stools gave a distinct precipitation within 5 minutes, usually even when diluted with 2 volumes of saline (figure). With the noncholera broth cultures or with normal serum, nonspecific precipitation sometimes appeared after c. 15 minutes.

### Discussion

With increasing incidence of cholera and the popularity of fast modes of travel, there is considerable interest in rapid laboratory confirmation of suspected cholera. The quickest method is based on immobilisation of vibrios by antiserum (Pfeiffer and Vagedes, 1896). Using dark-ground microscopy to observe motility, about 75% of culture-positive cases can be diagnosed rapidly (Benenson et al., 1964). Some culture-negative cases also give positive results. This test has not been popular presumably owing to operational problems. The same appears true of fluorescent-antibody staining of stool smears (Finkelstein and Gomes, 1963).

The ring test described here can be performed with portable apparatus. The ratio of antigen to antibody is not critical in ring tests, and they would detect traces of antigen. It is likely that boiling the stool for clarification also increases the concentration of dissolved antigens (Ghosh, 1969b).

It is hoped that the test will be evaluated clinically by workers in endemic areas, using stool from severe non-cholera diarrhoea as controls. It would be an advantage to use a serum with high precipitin titre instead of depending on the agglutination test.

### Summary

On gentle boiling and centrifuging the stool of



From left to right: (i) Saline on antiserum, (ii) Cholera vibrio stool on antiserum, (iii) El Tor stool on antiserum, (iv) El Tor stool diluted threefold, and (v) Cholera stool on normal serum.

baby rabbits infected with cholera vibrio, one obtains a clear fluid that gives a specific ring of precipitate when layered on high-titre serum. A plea is made for evaluation of this simple and rapid diagnostic procedure in natural cholera.

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