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# Evaluation of the Cholera Spot Test: a chromatographic immunoassay for the rapid detection of Cholera antigen

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***Abstract***

A chromatographic immunoassay cholera antigen detection kit, the Cholera Spot test, was evaluated. The test was found to be specific with a sensitivity of $10^6$ cfu/ml for the direct detection of V. ***cholerae*** in simulated stool specimens and 10 cfu/ml in simulated cotton-tipped swab specimens after overnight incubation in alkaline peptone water. This enables early recognition of cholera cases and their contacts so that prevention and control measures can be promptly instituted.

***Key words:*** Immunoassay, cholera antigen, rapid test

## INTRODUCTION

Cholera is maintained by continuous transmission of inapparent or mild infection until climatic conditions become favourable for a new outbreak to occur. Individuals with mild illness and in convalescence are primary sources of cholera during the epidemic spread of cholera. Another important group although rare are chronic carriers who shed the organisms intermittently from the gall bladder to the intestine and finally to the environment and may be an important secondary source of cholera.

The laboratory diagnosis of cholera is primarily based on the isolation of V. ***cholerae*** from the stool or vomitus of the patient. The technique is laborious and time consuming. In general, the laboratory diagnosis of cholera or detection of cholera carrier takes between 48 to 72 hours. Thus a rapid, specific, simple and economical diagnostic method is required for the control and prevention of cholera outbreak and for disease surveillance. Detection of cholera antigen using monoclonal antibody has been found to have an excellent degree of agreement with culture method.'

We evaluated the cholera spot test to determine its sensitivity, specificity and ability to detect V. ***cholerae*** antigen from a swab inoculum after overnight incubation in alkaline peptone water.

## MATERIALS AND METHODS

### *Cholera spot test*

The cholera spot test is a chromatographic immunoassay test for the qualitative detection of V. ***cholerae*** specific antigen obtained from Malaysian Bio-Diagnostics Research (MBDr). The test was developed by the Communicable Diseases Centres (CDC) of the United States. It consists of a long narrow plastic strip of 80mm long x 5mm wide (Fig. 1). On the plastic strip is a fluffy white area for specimen sampling at the lower portion which is coated with V. ***cholerae*** monoclonal antibody (McAb) tagged with gold, which is the detection system. The middle portion is designated the reaction zone for antigen detection and test control, while the upper orange portion is for holding and handling. The reaction zone is coated with second V. ***cholerae*** antibody and anti-mouse antibody located at 2 different bands. The second VC antibody will bind to the antigen site of the V. ***cholerae*** antigen-McAb complex while the anti-mouse antibody to the McAb site and produce two pink bands. In the test procedure, the fluffy area of the smp is immersed into the test specimen for thirty seconds. It is then removed and placed on a tissue or blotting paper. A time of ten minutes is given for the antigen-antibody reaction to take place after which the test is read. The appearance of only

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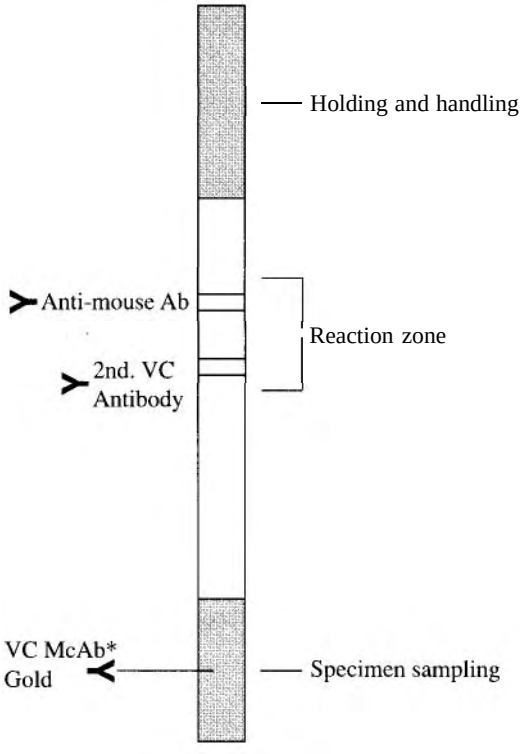


McAb = Monoclonal Antibody

FIG. 1: Cholera spot test strip – basic components

one pink band at the anti-mouse antibody band denotes that the test control is working but the test is negative. The appearance of two pink bands indicates that cholera specific antigen is present in the test specimen.

*Sensitivity of the test using Vcholerae culture*

A few colonies of *V. cholerae* were used to make a suspension in sterile normal saline. The turbidity of the suspension was adjusted to 0.5 MacFarland standard which is equivalent to $10^8$ cfu/ml. A serial dilution was made until the concentration of 10' cfu/ml was obtained. Each dilution was tested with a test strip.

*Sensitivity of the test in the presence of fresh normal stool*

This was carried out by dispensing 1.8 ml sterile normal saline into 8 sterile bijou bottles which were labeled as 'Neat', $10^7$ through 10'. Approximately 0.3 gm of fresh normal stool were added into each bottle. 0.2 ml of the 10 fold dilutions of V *cholerae* suspensions prepared earlier were added into their respective labeled bijou bottle, except to the one labeled 'Neat'. The bottles were shaked for about 10 seconds. A cholera spot test strip was added into each bottle, once the stool debris had settled and the result was recorded.

*Sensitivity of the test in the presence of fresh stool after overnight incubation in alkaline peptone water (APW)*

A cotton swab was dipped into each of the dilutions of V *cholerae* suspensions. They were then placed in 5.0 ml of APW and incubated at 37°C overnight. The volume of bacterial suspension sampled by each swab from the bottles was estimated to be about 100 microlitres (0.1 ml). After incubation, the cultures were tested for the presence of *V cholerae* antigens.

*Specificity of the test in the presence of various known enteric pathogens*

Pure nutrient broth cultures of 22 different strains of known enteric bacterial pathogens including *Salmonella typhi, Salmonella paratyphi A, Salmonella paratyplzi B, Salmonella paratyphi C, Salmonella typhimurium, Salmonella honten, Salmonella aberdeen, Salmonella chingola, Shigella sonnei, Shigella boydii, Shigella dysenteriae, Vibrio paraheamolyticus, Vibrio vulnificus, Vibrio alginolyticus, Vibrio fluvialis, Aeromonas hydrophila, Campylobacter jejuni, Campylobacter coli*, two different strains of enteropathogenic *E.coli* (EPEC) and two different strains of enterotoxigenic *E.coli* (ETEC) were used. After overnight incubation, a 1:10 dilution of each broth culture was made in sterile normal saline and tested for cholera antigen.

## RESULTS

The cholera spot test were positive for *V cholerae* suspensions at the concentrations of $10^6$ cfu/ml and above. Negative results were obtained from cell concentrations of $10^5$cfu/ml or less. Fresh normal stools without *V cholerae* gave negative results. Positive results were seen in the bottles containing more or equal to $10^6$ cfu/ml *V. cholerae.*

Table 1 shows the results of the test after overnight incubation in APW which indicates that even if a swab specimen is to contain one or two cells, incubation overnight in APW will increase the cell concentration high enough for the cholera spot test to be positive. All the overnight nutrient broth cultures of the enteric bacterial pathogens tested negative with the cholera spot test.

**TABLE 1: Results of swab samples from various concentrations of *V. cholerae* in the presence of fresh stool**

| *V. cholerae* concentration (cfu/ml) | Estimated no. of cfu in swab sample | Cholera antigen detection test* |
|---|---|---|
| $10^7$ | $10^7 \times 0.1 = 1{,}000{,}000$ | Positive |
| $10^6$ | $10^6 \times 0.1 = 100{,}000$ | Positive |
| $10^5$ | $10^5 \times 0.1 = 10{,}000$ | Positive |
| $10^4$ | $10^4 \times 0.1 = 1{,}000$ | Positive |
| $10^3$ | $10^3 \times 0.1 = 100$ | Positive |
| $10^2$ | $10^2 \times 0.1 = 10$ | Positive |
| $10^1$ | $10^1 \times 0.1 = 1$ | Negative |

Note: * = After overnight incubation of the swab sample in alkaline peptone water (APW)

## DISCUSSION

The cholera spot test is easy to perform and does not require additional reagent or laboratory equipment, except when the specimens needed to be incubated at 37°C. The results are easy to interpret and can be obtained in less than 20 minutes. Although a direct fluorescent antibody test using monoclonal antibody developed by Hasan *et al.*,[2] is as rapid as this test, it requires additional reagent and a fluorescent microscope.

The test is very specific for *V. cholerae* and there was no false positive result with any of the twenty-two nutrient broth cultures of enteric bacterial pathogens tested. This is relevant as the stool specimen may contain high concentrations of other bacterial pathogens, especially in cases of diarrhoea. From this evaluation it has been shown that the presence of fresh normal stool did not give false positive results. However, the test needs to be evaluated further in a proper trial in a clinical setting with actual patients.

The test was able to detect as low as $10^6$ cfu/ml, both in the presence or absence of fresh stool. This is in contrast to the manufacturer's claim i.e. $10^5$ cfu/ml. It is, however, within acceptable limits because the difference is only in one dilution which could be due to technical factors. Polymerase chain reaction is able to detect as few as $10^2$ cfu/ml[3] but this technique is not as simple and needs expensive equipment and reagents.

This evaluation was designed to create a set up which simulates, as closely as possible, the actual field situations, where screening for cholera carriers by health authorities is done by taking rectal swabs and inoculating the swabs in APW. The test is able to detect cholera antigen even if only a few *V. cholerae* cells are present, provided the swab is incubated overnight in APW which will allow the bacteria to grow and multiply into numbers detectable by the test.

The test is easy to perform thus it can be carried out by field personnel with very minimum training. It allows field screening of a large population to be done such as in the investigation of a cholera outbreak. This approach has an additional advantage in that it able to detect large number of cholera carriers as the larger the sample or the more frequent the sampling, the greater the chances of detecting cholera carriers. The test is of value for the detection of cholera carriers if the rectal swabs are incubated overnight in APW. This will allow early detection of cholera cases and their contacts so that other action, i.e. prevention and control of outbreaks and surveillance can be promptly carried out.

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