Rapid Detection of Vibrio Cholerae O1 And O139 In Stool Samples By One-step Immunochromatographic Dip-stick Test.

DR VIJAYA S, DR DHANALAKSHMI TA
Dept. of Microbiology, A.I.M.S., B.G.Nagara-571448

Background and objectives: Cholera can occur in any form; sporadic, endemic or epidemic in India. The present study compares the performance of rapid dipstick assay and conventional culture methods for the detection of Vibrio cholerae in stool samples. Materials and methods: 20 stool samples were collected from patients having acute watery, non-bloody diarrhea and subjected to Crystal VC dipstick test and conventional culture method. Results: Out of 20 stool samples, 15 were positive for Vibrio cholerae O1 strain in rapid dipstick test and 10 were culture positive. The sensitivity and specificity of this dipstick for detection of O1 strain was 100% and 66.66% respectively. None of samples were O139 positive by both dipstick and culture methods. Conclusion: This one-step dipstick test performed well in the diagnosis of Vibrio cholerae O1 in a setting with seasonal outbreaks where rapid tests are most urgently needed.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology for a period of 6 months. Ethical clearance was obtained from the Institution for the study.

A total of 20 stool samples (18 bulk stool and 2 rectal swabs) were collected from patients having acute watery, non-bloody diarrhea attending a tertiary care hospital. All the specimens were transported to the laboratory without any delay. The stool samples were subjected to hanging drop, rapid dipstick test and conventional culture methods.

Rapid dipstick test:

This test was carried out as per the manufacturer’s instructions. Briefly, around 200µl of faeces was added to a test tube containing buffer. The rectal swabs were incubated for 6 hours in alkaline peptone water (APW) at 37°C, after which 200 µl of the enrichment medium was added to a test tube containing buffer. A Crystal VC dipstick was placed vertically into the test tube, such that the last centimeter of the strip was immersed in the faeces. The results were read at the end of 15-20 minutes, after removing the strip from the test tube. The test was defined as positive, when both a test line and control line appeared on the test strip.

Bacteriological culture:

Bulk stool and rectal swabs after enrichment in APW for 6 hours at 37°C were plated on Mac-Conkey agar, blood agar and...
thiosulfate citrate bile salt sucrose (TCBS) agar. After overnight incubation, suspected colonies were confirmed by biochemical tests and agglutination with polyvalent O1 and O139 antisera.  

RESULTS  

Out of 20 samples included in this study, only 6 samples showed darting motility in hanging drop examination.

The results of dipstick test and culture methods are shown in table 1. None of the samples were positive for O139 strain in the dipstick test and culture method. The sensitivity and specificity of dipstick with culture as gold standard was 100% and 66.66% respectively. The positive predictive value (PPV) and negative predictive value (NPV) of the test was 67% and 100% respectively. The false positivity rate was 33.3%.

Table 1: Detection of Vibrio cholerae O1 in 20 stool samples by rapid dipstick test versus conventional culture

<table>
<thead>
<tr>
<th>TEST BACTERIOLOGICAL CULTURE</th>
<th>CRYSTAL VC DIPSTICK POSITIVE</th>
<th>CRYSTAL VC DIPSTICK NEGATIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACTERIOLOGICAL CULTURE POSITIVE</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>BACTERIOLOGICAL CULTURE NEGATIVE</td>
<td>05</td>
<td>05</td>
<td>10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>15</td>
<td>05</td>
<td>20</td>
</tr>
</tbody>
</table>

DISCUSSION:  

The detection of infectious diseases like cholera is revolutionized by simple, quick and economical near point-of-care tests and rapid diagnostic tests. The present study showed a high sensitivity of 100% and a moderate specificity of 66.66% as compared to the conventional culture methods. The manufacturers’ (Span Diagnostics Ltd, India) claim a sensitivity of 88-100% and specificity of 61-87% respectively, which co-related well with our results. Previous evaluations of the Crystal VC dipstick have also shown a sensitivity ranging from 92-100% and a specificity of 50-100%, respectively. These studies varied in the sample size, bacterial load in the sample, RDT kit version, methodology and qualifications/skill levels of the personnel performing RDTs.

Because of the severity of cholera, the sensitivity of a rapid diagnostic test is important in correctly identifying the true cases. A predominance of positive test results indicates Vibrio cholerae as the etiologic agent of the outbreak. But at least some cases must be culture confirmed to validate the results. A highly specific assay is important to rule out cholera in individual patients, where the disease is rare, but not in endemic countries.

Our study showed a PPV and NPV of 67% and 100% respectively. Earlier studies had a PPV and NPV of 47-93% and 91-95% respectively. PPV and NPV of any test varies with the prevalence of the disease in the population.

This study had 5 dip-stick positive but culture negative samples, which could be due to:

- Dead /low number of organisms in the sample.
- Prior treatment with antibiotics.
- Changes in pH or osmolarity in the lower gut can induce transformation of Vibrio bacilli into coccoid non-culturable form.
- Phage elimination of Vibrio from the guts of infected people.

Even though, stool culture remains the gold standard for laboratory diagnosis of cholera, it lacks sensitivity. So any evaluation against stool culture leads to underestimation of the specificity. To overcome this, polymerase chain reaction (PCR) can be done, which detects low number of organisms or dead cells, improving the sensitivity of the reference standard. Alternatively statistical approaches using latent class model/ Bayesian inferences have shown an overall better performance of the dipstick test.

The limitations of this study were:

- PCR, which could have been more sensitive than the culture was not done, due to financial constrains.
- The sample size was smaller for much wider analysis.
- We were unable to evaluate the performance of dipstick for the detection of O139, as none of the samples were O139 positive.
- The trial was not done in field conditions.
- The test validity was not related to the skill level of the technicians.

CONCLUSION:  

The importance of an efficient cholera surveillance system continues to be stressed by WHO with regard to quick diagnosis of cholera in outbreak situations. Rapid diagnosis is essential for mobilization of resources for treatment and containment of the outbreak. So the dipstick test is a sensitive and moderately specific test which is easy to perform without any laboratory infrastructure. Further studies are indicated to evaluate this in crisis situations.

REFERENCES:


