
One-Step Immunochromatographic Dipstick Test for Rapid Detection of *Vibrio cholerae* O1 in Stool Samples

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Abstract:

Background: Early detection of cholera outbreaks is crucial for the implementation of the most appropriate control strategies.

Objectives: The aim of this study was to evaluate the sensitivity and specificity of a Lateral Flow Immunochromatographic test for the qualitative determination of Lipopolysaccharide (LPS) antigen of *Vibrio cholerae* O1, from the stool specimen, using specific monoclonal antibodies in comparison to the conventional culture methods.

Subjects & Methods: The performance of an immunochromatographic dipstick test (Span, India) specific for *Vibrio cholerae* was evaluated in Iraq during the 2007 to 25th January/2009 in cholera outbreak seasons. Fecal specimens were collected from (100) patients with acute watery non bloody diarrhea and tested by dipstick and conventional culture.

Results: The results of this study showed that the males prone to be infected with *V. cholerae* O1 more than females, (61% vs. 39 %) ($P > 0.05$). The mean $\pm$ SD age of patients with *V. cholerae* O1 was (13.5 $\pm$ 5.5 years) with no significant difference ($P > 0.05$). Patients under 20 years age group constituted the highest number (57%) with highly significant difference between the age groups ($P < 0.01$). Patients infection with *V. cholerae* O1 was clearly spread in rural areas with highest percentage (78 %) compared to only (22 %) of patients infected with *V. cholerae* O1 spread in urban areas with statistical analysis of high significant difference ($P < 0.01$). The sensitivity of the O1 dipstick on stool samples compared to culture was (94%), with a specificity of (84%).

Conclusion: This one-step dipstick test performed well in the diagnosis of *V. cholerae* in a setting with seasonal outbreaks where rapid tests are most urgently needed.

Keywords: *Vibrio cholerae*, Dipstick, Sensitivity, Specificity.

Introduction

Cholera is an acute intestinal infection caused by the bacterium *Vibrio cholerae*. The bacteria produce a toxin that causes severe dehydration through vomiting and profuse watery diarrhea^[1].

Two strains of Cholera are now associated with infection; *V. cholerae* serogroup O1 and *V. cholerae* serogroup O139^[2]. The O1 serogroup is subdivided into two serotypes, Ogawa and Inaba^[3]. Serogroup O139, which appeared in India in 1992, has spread rapidly throughout Asian countries and is considered to be the potential eighth pandemic strain of cholera^[4]. Cholera is acquired directly through contaminated water or food, or indirectly from exposure to feces or vomit of an infected person. Cholera is found in many tropical countries around the world where outbreaks are common.

The incubation period for Cholera ranges from less than 1 day to 5 days^[5]. Cholera can spread as an epidemic, endemic or pandemic disease^[6]. Despite all the major advances in medical sciences Cholera still remains a challenge to the modern medical world; Successful intervention and containment depends largely on early detection of Cholera outbreaks. Prompt diagnosis of cholera is of key importance to initiate effective therapy and to institute proper epidemiological measures^[7]. The diagnosis is suggested by strikingly severe, watery diarrhea. For rapid diagnosis, a wet mount of liquid stool is examined microscopically.

The characteristic motility of vibrios is stopped by specific antisomatic antibody^[8]. Other

methods are culture of stool or rectal swab samples on thiosulfate citrate bile salt sucrose TCBS agar and other selective and nonselective media; the slide agglutination test of colonies with specific antiserum; fermentation tests (oxidase positive); and enrichment in peptone broth followed by fluorescent antibody tests, or retrospective serologic diagnosis^[9]. More recently the polymerase chain reaction (PCR) and additional genetically-based rapid techniques have been recommended for use in specialized laboratories^[10]. Several rapid diagnostic tests for cholera have been described; Some detect the cholera toxin. The others detect the lipopolysaccharide (LPS) antigen of *V. cholerae* O1 or O139. Recently, a multi step colloidal-gold-based colorimetric immunoassay was also developed for direct detection of *V. cholerae* O1 or *V. cholerae* O139 in stool specimens^[11].

Materials & Methods

The present study was conducted for the period from 1 September / 2007 to 20 January / 2009 in Central public Health Laboratory / Baghdad and Teaching Laboratories of Medical City / Baghdad.

Bacteriological culture

Conventional bacteriological culture was applied as gold standard against which we evaluated the accuracy of the dipstick test.

Stool samples were plated directly onto thiosulfate citrate bile salt sucrose (TCBS) agar after enrichment in alkaline peptone water for 6

hours (pH 8.6, 37°C) after overnight incubation, suspected colonies on the agar plates were selected for biochemical test specially for oxidase test (filter strip method/MAST/USA) and string test other biochemical test have been doing by Iapi® E 20 (biomerieux, France).

Serological identification of *V. cholerae* antigens by antisera (MAST/USA) agglutination with polyvalent, Ogawa, and Inaba antisera.

Dipstick test

Dipstick test is based on the principle of immunochromatography, in which nitrocellulose membrane is coated with monoclonal antibodies to *V. cholerae* O1 and O139 LPS as two separate bands. When the test sample (200 µl of stool after dilution with normal saline was pipetted into a tube

which the test strip was inserted) flows through the nitrocellulose membrane, the Colloidal Gold, coupled with anti-*V. cholerae* O1 / *V. cholerae* O139 LPS monoclonal antibodies, binds to the respective antigens from the test sample. This antigen-antibody complex moves through the nitrocellulose membrane and binds to the corresponding immobilised antibodies against *V. cholerae* O1 / O139 and forms a magenta red color band, which confirms reactive results. A Control band always appears, irrespective of reactive or non-reactive sample, so as to validate the test procedure this presented in **Figure (2)**.

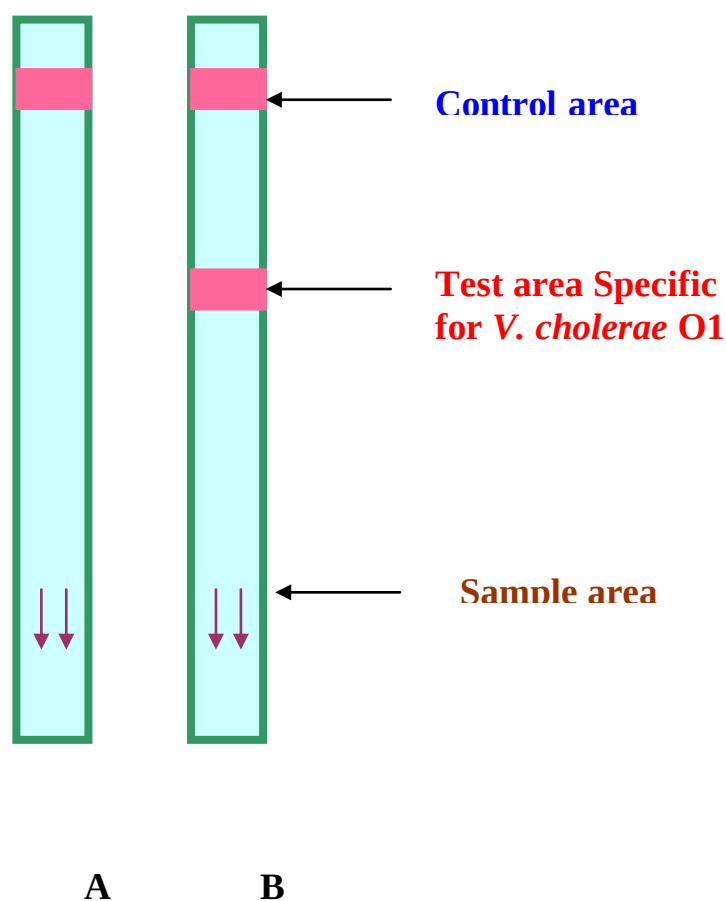


Figure (2): Immunochromatographic dipstick tests for *Vibrio cholerae* O1.

(A) Negative test display one pink band in control area.

(B) Positive test display two pink bands in test and control area.

Antimicrobial Resistance

All *V. cholerae* isolates were tested for resistance to antimicrobial drugs by using the method of Bauer et al.^[12] with standard antibiotic disks at the following antibiotic concentrations (mg/disc): ampicillin (10); streptomycin (10); tetracycline (30); sulfamethoxazole (23.5); kanamycin (30); gentamicin (10); ciprofloxacin (5); chloramphenicol (30); trimethoprim (1.25) and nalidixic acid (30).

Data management and analysis

The suitable statistical methods were used in order to analyze and assess the results; they include descriptive statistics, inferential statistics. All the statistical analysis was done by using Pentium-4 computer through the SPSS program (version-10) and Excel application. Note: The comparison of significant (P-value) in any test were:

Significant difference ($P < 0.05$), highly significant difference ($P < 0.01$) and non significant difference ($P > 0.05$).

Results

During the period of study, stool specimens from 100 patients were tested by conventional culture and by the dipstick test. This dipstick developed for direct detection of the

lipopolysaccharide (LPS) antigen of *V. cholerae* O1 in stool specimens. This study showed all cases of cholera patients attributed to *V. cholerae* serogroup O1 serotypes Inaba. On the other hand, the study showed a marked increase in cholera cases associated with *V. cholerae* O1 from early September to the end of January next year during the cholera outbreak for years (2007, 2008 and 2009).

During late 2007, *V. cholerae* O1 spread in the capital city Baghdad and adjoining areas specially (Giser-Diyala) scored most cases of cholera infection. The epidemiology of cholera in Iraq changed again recently, and a large outbreak of cholera caused predominantly by *V. cholerae* O1 occurred in the middle or south of Baghdad especially in Babylon and adjoining areas as presented in table (1).

The results of this study showed that the males prone to be infected with *V. cholerae* O1 more than females, since the males represented (61%) of the total positive results while the females represented (39%) only. On the other hand, mean age of patients with *V. cholerae* O1 was (13.5 ± 5.5 Years) with no significant difference ($P > 0.05$) as referred in table (2).

Table (1): Distribution of patients infected with *V. cholerae* O1 according to Governorates and date (Years)

Governorates	Years				Total	
	2007 N	%	2008 N	%	2009 25 th January N	%
Baghdad	30	100	11	17.5		
Babylon			22	34.9	7	100
Basra			10	15.9		
Karbala			11	17.9		
Najaf			4	6.3		
AL-Qadeseya			2	3.2		
Wasit			2	3.2		
Thi kar			1	1.6		
Total	30	100	63	100	7	100

Table (2): Distribution of patients infected with *V. cholerae* O1 according to gender and mean of age (Years).

Gender	No. of patients	%	AGE Mean $\pm$ SD	P-Value	Comparison of Significant for Gender
Male	61	61	13.5 $\pm$ 5.5	.966	Non-Sign. ($P > 0.05$)
Female	39	39			
Total	100	100			

Study population was divided into four different age groups; under 20 year, 20-39 years, 40-59 years, and above 60 years. Among the patients, under 20 years age group constituted the highest number (57%) followed by 20-39 years (24%). There were only (5 %) patients over 60 years of age in this group; in addition statistical analysis showed highly significant difference between the

age groups ($P < 0.01$) as presented in **figure (1)**. Patients infection with *V. cholerae* O1 was clearly spread in rural areas with highest percentage (78 %) compared to only (22 %) of patients infected with *V. cholerae* O1 spread in urban areas with statistical analysis of high significant difference ($P < 0.01$) as presented in **table (3)**.

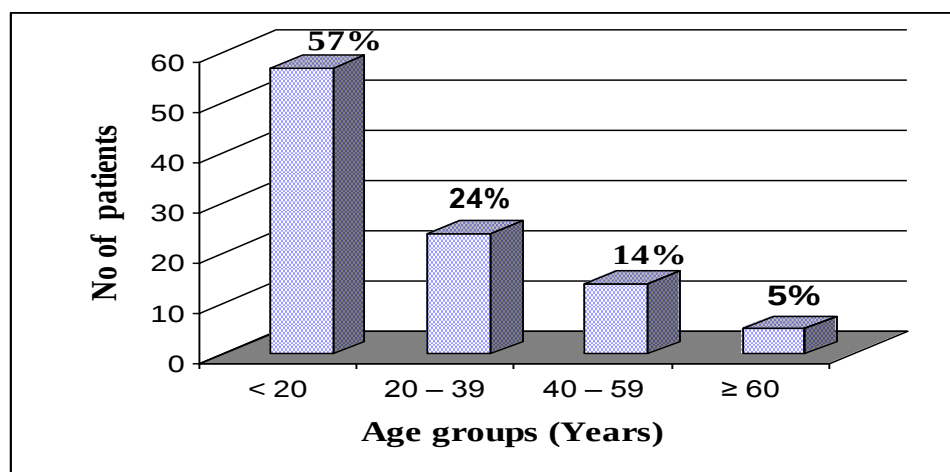


Figure (1): Histogram of patients infected with *V. cholerae* O1 according to age groups (Years).
 $\chi^2=61.840$, $P\text{-value}=0.004$

Table (3): Distribution of Patients infection with *V. cholerae* O1 according to the area of residency

life style	No. of Patients	%	Chi - Square	Comparison of Significant	
				P-value	Sig.
Urban	22	22	49.560	0.00	Highly Sig. ($P < 0.01$)
Rural	78	78			
Total	100	100			

For the dipstick evaluation, of the (100) stool samples, (83) were both dipstick and culture positive, (11) were dipstick positive but culture negative, (6) were dipstick negative but culture positive as presented in **table (4)**. The overall the sensitivity and specificity of the dipstick tests for detection of *V. cholerae* O1 from stool samples were excellent, since the sensitivity of the O1

dipstick on stool samples compared to culture was (94%), with a specificity of (84%).

All strains isolated from the recent epidemic were resistant to nalidixic acid and were susceptible to ampicillin, gentamicin, tetracycline, ciprofloxacin, streptomycin, sulfamethoxazole, trimethoprim, and chloramphenicol.

Table (4): Frequencies of *V. cholerae* O1, test results by dipstick test versus conventional culture.

Bacteriological culture	No. of specimens with <i>V. cholerae</i> O1 dipstick test result		Total No. of specimens tested
	Positive	Negative	
Positive	83	6	89
Negative	11	0	11
Total	94	6	100

Discussion

The conventional culture method to detect cholera takes at least 2 days, by which time the disease might have spread widely. The rapid detection of cholera cases or asymptomatic carriers is needed for epidemic control and disease surveillance^[13]. The increase in total number of cases seen during early September and end of January was not unusual, since in this months represented the proper ecology for be proliferated and multiplied easily of *Vibrio cholerae*.

According to the results presented in this study, Patients infection with *V. cholerae* O1 was predominated in the age group under 20 year and less existence in age group above 60 years, explained by a lack of immunity of patients under 20 year comparing with likely partially immune in adults^[14].

Area of residency factor plays an important role in the infection with *V. cholerae* O1, since most studies showed that Patients infection with *V. cholerae* O1 was more common in the rural area^[14, 15].

This study go along with this report, since (78%) of patients infected with *V. cholerae* O1 occupation in rural area, while (22%) of patients occupation in urban area with statistical analysis of high significant difference ($P < 0.01$).

In outbreak situations, a quick diagnosis of cholera is essential for mobilization of resources for treatment and containment of the outbreak. Therefore, the need for sensitive and specific diagnostic tests that can be utilized by minimally skilled personnel and that require negligible laboratory infrastructure is very real^[16].

We embarked on this study to fulfill this need, with our priority being the development of a bedside detection test that can be performed by any health care worker and that comes in a format ideally suited for a resource-poor setting.

In the earlier studies^[17, 18, 19], the sensitivity and specificity of the dipstick test were 94%–100% and 84%–100%, respectively, very similar to the characteristics we found. The high sensitivity of step dipstick test because specific antibodies to the serogroup O1 do not react with the *V. cholerae* O139 or their **LPS**.

This simple, one-step dipstick test performed well in the diagnosis of cholera in a setting with minimal facilities, where rapid tests are most urgently needed. Diagnostic testing may not be necessary for the clinical management of each diarrhea patient during an outbreak. The dipstick test may be helpful to confirm clinically suspected cholera cases, especially during the start of an outbreak^[20].

In our study, the results of dipstick assay could be interpreted objectively with stool culture as the reference standard. Thus, other common confounders or biases of diagnostic studies, namely

influence of clinical factors on test interpretation and reference standard error were unlikely to affect the validity of our evaluation.

In the evaluation of dipstick test were few samples which were positive by the dipstick test but negative by culture for the O1 strains. We did not have a method such as polymerase chain reaction (**PCR**) to prove whether these results were true or false positives. However, it is possible that some of these stool samples lacked live organisms but contained enough LPS to react with the dipsticks, due to prior treatment with antibiotics or long delay and bad field conditions during the conveying of the samples. Finally, a suspected outbreak of cholera, whether detected by clinical diagnosis or by rapid diagnostic assays, should be confirmed with culture of stools from a sample of typical patients. Culture confirmation will allow antimicrobial susceptibility testing to be performed on *V. cholerae* O1 isolates in order to guide treatment of severely ill patients.

In conclusion, the dipstick test for detection of *V. cholerae* is a sensitive, specific, rapid, reliable, accurate, easy-to-perform assay, successfully used for diagnose cholera and that does not need special equipment comparison to the conventional culture methods. The simplicity and efficacy of these dipsticks should improve patient care and epidemiological surveillance, even in the remotest regions, though, molecular technique such as multiplex PCR for diagnosis of *V. cholerae* O1 and *V. cholerae* O139 should not be overlooked.

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