

TECHNICAL REPORT

Rapid identification of *Vibrio cholerae* serotype O1 from primary isolation plates by a coagglutination test

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Summary. A coagglutination test was developed for identifying suspected colonies of *Vibrio cholerae* serotype O1 directly from primary isolation plates. Visible agglutination occurs when *V. cholerae* O1 antibody attached to cell-wall protein A of *Staphylococcus aureus* reacts with its homologous antigen. From 314 faecal samples from clinically suspected cases of cholera, 210 colonies from thiosulphate citrate bile salts sucrose (TCBS) agar and 222 colonies from taurocholate tellurite gelatin (TTG) agar were tested as suspect *V. cholerae*. In each case 204 isolates were identified as *V. cholerae* O1 by conventional methods and also gave positive results for *V. cholerae* O1 in the coagglutination test; with one partial exception, no other colonies tested gave positive results. The coagglutination test is simple and inexpensive and provides a result 24 h earlier than conventional methods.

Introduction

Cholera is still a major public health problem in many developing countries (Samadi *et al.*, 1983). Early diagnosis is essential so that effective therapy and epidemiological measures can be initiated. *Vibrio cholerae* serotype O1 is the causative agent (Farmer *et al.*, 1985) and conventional laboratory diagnosis depends upon isolation by culture and identification by biochemical and serological tests (Farmer *et al.*, 1985). These procedures are expensive and normally take about 2 days to obtain a result. Moreover, the limited facilities available for expensive biochemical tests in small laboratories in many developing countries make the identification of this organism difficult in such conditions. Morris *et al.* (1979) described a slide agglutination test for the identification of *V. cholerae* O1, by testing colonies taken directly from thiosulphate citrate bile-salts sucrose (TCBS) agar and taurocholate tellurite gelatin (TTG) agar with specific antiserum, in order to reduce identification time and costs. However, although the less frequently used TTG agar was suitable for this purpose, *V. cholerae* O1 colonies from the more widely used TCBS agar gave negative or doubtful results in the majority of

cases (Monsur, 1961; Kobayashi *et al.*, 1963; Morris *et al.*, 1979).

Coagglutination, another rapid serological test, is used to detect antigens of the causative bacteria of pneumonia, meningitis and typhoid in clinical specimens (Kronvall, 1973; Thirumoorthi and Dajani, 1979; Sivadasan *et al.*, 1984; Fung and Tilton, 1985). Formaldehyde-stabilised, heat-treated cells of *Staphylococcus aureus* Cowan 1 (ATCC 12598), which contains protein A in its cell wall, are treated with high-titre specific antiserum. Protein A binds IgG by the Fc fragment and, in the presence of homologous antigen, the antibody-coated staphylococcal-cell reagent produces visible agglutination. We have previously described the use of a coagglutination test with 4-h faecal enrichment cultures for the rapid diagnosis of cholera (Rahman *et al.*, 1987). In this paper we describe its application to the identification of colonies taken from primary isolation plates to facilitate rapid screening of faecal samples for *V. cholerae* O1.

Materials and methods

Detection of V. cholerae in faeces

Faecal samples from 314 suspected cases of cholera were cultured on TCBS (Eiken Chemical Co. Ltd, Tokyo,

Japan) and TTG agar (Monsur, 1961). *V. cholerae* O1 was identified by conventional biochemical and serological tests (Farmer *et al.*, 1985).

Preparation of coagglutination reagents

A modification of the method of Kronvall (1973) was used to prepare coagglutination reagents (Rahman *et al.*, 1987). High-titre *V. cholerae* O1 antiserum was prepared by multiple inoculation of rabbits with live *V. cholerae* O1. *S. aureus* Cowan 1, grown overnight in trypticase soya broth with shaking at 36°C, was washed with phosphate-buffered saline (PBS; 0.03 M phosphate, 0.12 M NaCl, pH 7.3) and stabilised with formaldehyde 0.5% in PBS. The cells were heated for 1 h at 80°C, washed and 1 ml of a 10% v/v suspension of cells in PBS was mixed with 0.2 ml of *V. cholerae* O1 antiserum and incubated for 3 h at room temperature (25°C) with shaking. The antibody-coated staphylococci were washed with PBS and resuspended at 2% v/v in PBS containing sodium azide 0.1% to form the coagglutination reagent. A formaldehyde-stabilised, heat-treated 2% v/v suspension of staphylococci in PBS containing sodium azide 0.1% was used as a negative control reagent. Reagents were stored at 4°C until used. *V. cholerae* O1 lipopolysaccharide (List Biological Laboratory, USA) was used as a positive antigen control; 0.5 mg/ml in PBS gave easily visible agglutination.

Slide coagglutination test

The reagents were brought to room temperature and shaken to obtain a uniform bacterial suspension. Four rectangles were made with a wax pencil on a glass slide. In the first two rectangles, a suspension of bacterial cells in normal saline was prepared from suspected *V. cholerae* colonies on the TCBS agar plate, i.e., sucrose-positive, yellow colonies. In the other two rectangles, a suspension was made with suspected colonies from the TTG agar plate, i.e., gelatinase-positive colonies (halo-positive). One drop of coagglutination reagent was added to the first and third rectangles and one drop of the negative control reagent to the second and fourth rectangles and the drops were mixed well with sticks. After rotation by hand for 2 min, the slides were examined against a dark background with a viewing lamp. The coagglutination test was considered positive if macroscopic agglutination was seen with coagglutination reagent but not with the control reagent.

Results

Suspected colonies of *V. cholerae* from the primary isolation plates were screened by conventional methods and by the coagglutination test. The results are shown in the table. *V. cholerae* O1 was detected in 204 (65%) of 314 faecal samples by conventional methods. The coagglutination test identified all 204 of these isolates as *V. cholerae* O1,

with colonies from both TCBS and TTG agar plates; there was no difference in the results obtained from either plate. The suspected colonies of *V. cholerae* from the six TCBS agar plates that gave negative results in the coagglutination test were all identified as other than *V. cholerae* O1 by conventional methods (table). With TTG medium, there were suspect colonies from 18 plates that proved to be other than *V. cholerae* O1 by conventional methods and 17 of these gave negative coagglutination reactions, the other giving a weakly positive result although it was identified as *V. mimicus* by conventional methods (table). None of the strains identified as *V. cholerae* O1 gave negative or even weakly positive coagglutination reactions; all gave moderate or strong (some clumping) reactions with colonies from either isolation medium; four TCBS isolates and 30 TTG isolates gave very strong reactions (coarse clumping).

Discussion

The development of more rapid, specific and economic means of detection of diarrhoeal pathogens is desirable, in order to provide the early aetiological diagnosis needed for initiation of proper treatment and public health measures. The

Table. Comparison of results obtained with suspected colonies* of *V. cholerae* in conventional tests and in the coagglutination test

Number of specimens with suspected colonies on the indicated medium	Identity and (number) of isolates determined by	
	Conventional methods	Coagglutination-test
<i>TCBS agar</i>		
210	<i>V. cholerae</i> O1 (204)	+ (204)
	<i>V. fluvialis</i> (4)	– (4)
	<i>V. cholerae</i> non O1 (2)	– (2)
<i>TTG agar</i>		
222	<i>V. cholerae</i> O1 (204)	+ (204)
	<i>V. fluvialis</i> (4)	– (4)
	<i>V. cholerae</i> non O1 (2)	– (2)
	<i>V. parahaemolyticus</i> (3)	– (3)
	<i>V. mimicus</i> (3)	– (2)†
	<i>Aeromonas hydrophila</i> (3)	– (3)
	<i>A. sobria</i> (3)	– (3)

+ = Positive result; – = negative result.

* Sucrose-positive yellow colonies from TCBS and gelatinase positive colonies (with halo) from TTG agar.

† One strain from TTG agar gave a weak reaction in the coagglutination test.

isolation and identification of *V. cholerae* O1 from faeces by conventional culture methods is time-consuming, laborious and expensive. The coagglutination test described here is performed directly from the primary isolation plates and can, therefore, be used to diagnose cholera one day earlier than hitherto. The sensitivity and specificity of the test were both equal to those obtained with conventional methods. Furthermore, no cross-reactions were observed in preliminary slide coagglutination tests with colonies of commonly isolated species of Vibrionaceae held in our stock culture. Unfortunately, the number of *V. cholerae* non O1 and other vibrios isolated in the study was small.

V. cholerae O1 was identified by the coagglutination test in 18–24 h with colonies from either TCBS or TTG agar. The preparation of coagglutination reagents is simple and whole *V. cholerae* O1 hyperimmune serum can be used to coat the staphylococci which form the basis of the test. Because only 0.2 ml of antiserum is required for 5 ml of coagglutination reagent, the test conserves antiserum, allowing about 25 times as many tests for a given volume of antiserum as does the conventional slide agglutination method. It is easy to perform, requires few materials and does not require highly trained or skilled laboratory workers. The results of the coagglutination test were easily readable, producing clear aggregation on the test slide in all positive samples that we examined.

The test has several potentially useful advantages

and applications. Firstly, as already indicated, it can be used to confirm the diagnosis of cholera earlier than by conventional methods. Secondly, as a reliable test, it could eliminate the need for expensive and tedious routine biochemical tests. Thirdly, either of the two isolation media (TCBS and TTG agar) recommended by the World Health Organisation (1974) can be used for the test, although TCBS has special advantages. As few *Vibrio* spp. produce yellow colonies on TCBS, the possibility of a false identification is very low (table), giving this medium an advantage over others. Moreover, TCBS agar does not require sterilisation during preparation; hence the coagglutination test with TCBS agar is practicable for field use, as well as for use in small laboratories with minimal facilities. Finally, identification of *V. cholerae* O1 by this method would allow antibiotic susceptibility tests to be performed earlier, with evident potential clinical benefits. Considering these advantages, together with its high sensitivity and specificity, we recommend the routine use of the rapid coagglutination test described here for the laboratory diagnosis of cholera.

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