# CHAPTER VI
# DISCUSSION

Rapid detection of cholera is necessary for controlling an explosive outbreak which usually occurs within a day or two after a single unrecognized case. Cholera vibrios are commomly and rapidly disseminated by either a patient, convalescent carrier or individual with inapparent or mild infection whose fecal matter contaminates water supplies, food, or household contacts who are under poor personal hyginene. Conventional culture method to detect *Vibrio cholerae* takes at least two 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.

The PCR technique has been recommended for rapid detection of *V. cholerae* O1 in stool specimens (Shirai *et al.*, 1991). PCR with its powerful amplification of small amount of DNA yields improved diagnosis systems (Bell, 1998). However, the high level of amplification can also create a significant problem with contaminating sequences and unexpected results will be occurred. This type of detection sytem is unlikely to be adapted to a field kit as yet because of the necessary for heating and cooling cycles and the difficulty of extracting the bacterial DNA in the field condition (Al-Riyami *et al.*, 1991). The PCR technique is also useful in detecting the cholera toxin gene when cells of *V. cholerae* are in viable, but non-culturable state. Unfortunately, in developing countries where cholera is endemic, DNA probe and PCR technique is not readily available and will not be for the immediate future, at least until the procedure is simplified with reduced cost (Cowell *et al.*, 1992). Usually, samples for PCR contain inhibitors. The hemi-nested PCR was developed by Verela *et al.* (1994) to directly detect toxigenic *V. cholerae* by PCR in stool specimens from patients with no pervious cultures. The inhibition of PCR by substances presented in the samples was prevented by diluting the samples and applying the sample to the hemi-nested PCR. The external primers were designed spanning *ctxA* and *ctxB* (**Table 3**). The internal reverse primer was designed to perform a second PCR amplification together with the external forward primer (**Table 3**). The products after a single and hemi-nested PCR amplification were 519 bp and 452 bp. The sensitivity of hemi-

nested PCR was as low as a single organism. Twenty seven stool samples which were positive for CT by GM1-ELISA and bacteriological assays were tested. Only 40% of undiluted stool samples were positive by one step PCR and negative samples could not further amplified in the second hemi-nested PCR. On the other hand, when stool samples were diluted 1/ 100, only 10% was positive after the first PCR, but 100% was positive after the second hemi-nested reaction. In this study the PCR result that shown no expected bands for *ctxA, ctxB. tcpA*, or even *toxR* in positive culture of *V. cholerae* O1 of sample no.5 may be due to the inhibition of PCR by sunstances presented in the samples. Further study in hemi-nested PCR should be performed.

Patient no. 5 whose sample was positive for *V. cholerae* O1 by culture and dot-ELISA was found to be infected with *Strongyloides* parasite and the parasite larvae in his stool. He was admitted to the institute for treated of acute watery diarrhea (more than 10 stools/ day). He was treated with norfloxacin and albendazone after the lab result revealed positive for *V. cholerae* O1 and *Strongyloides* lavae. For sample no. 376, the patient admitted due to acute diarrhea but enteric bacterial pathogens and parasite were not found. Patient has been infected with HIV for more than 10 years and got abdominal tuberculosis infection. For sample no.999, patient has mixed infection in stool sample with *Isospora belli* mature oocytes, *Nocardia*, *V. cholerae* O139, and also acid fast bacilli were found.

Rapid diagnosis of *V. cholerae* O1 has been achieved by coagglutination tests utilizing antibodies agaist the O1 CT B-subunit antigens (Jesudason *et al.*, 1984; Rahman *et al.*, 1989). A bead ELISA for diagnosis of CT has been shown to detect the toxin directly from stool specimens, yielding positive result in 85% of culture-positive specimens (Ramamurthy *et al.*, 1992). There have been several reports on the use of polyclonal antibodies in the detection of *V. cholerae* O1 antigens and the differentiation of strains between the Ogawa and Inaba serotypes. These immunoassays include indirect immunofluorescence (Finkelstain *et al.*, 1959), slide agglutination (Mukerjee, 1978) and coagglutination tests (Jesudason *et al.*, 1984). The preparation of the specific polyclonal antiserum depends on prolonged immunization and the sequential absorptions of antiserum with related (cross-reactive) antigens; the titre of specific antibodies was decreased. Therefore monoclonal antibodies directed against a unique determinant of *V. cholerae* O1 should represent more reliable source

of highly specific reagent for the immunological assay (Gustafsson and Holme, 1983). However, cross-reaction may also occur with monoclonal antibodies if they recognize related antigen determinant on different antigens and such cross-reaction can not be eliminated by absorption (since antibodies derived from one clone are identical and are mono-epitope specific; removal of the cross-reactivity would remove all of the antibody activity). Thus, the most specific monoclonal antibody of *V. cholerae* should be the one that react only a unique antigen of *V. cholerae* O1 such as antigen A.

Non-specific results can occur in MAb-based dot-ELISA due to long term keeping of boiled samples at 4 °C. The boiled samples should be used as quick as possible. Supernatant of boiled sample is recommended for dotting on NCM instead of vortexing.

Application of monoclonal antibodies specific to *V. cholerae* O1 has been partly outlined by Home and Gustafsson in 1985. A coagglutination test using monoclonal antibodies against the O1 antigen has recently become available commercially (Colwell *et al.*, 1992, Abbott and Janda, 1993, Nataro *et al.*, 1992, Hasen *et al*, 1994). All of rapid detection methods are of great epidemiological importance as actions for prevention of cholera explosive and control of epidemics and pandemics can be promptly arranged. However the cost per test is extremely high which is not affordable by the cholera endemic countries.

After the eight pandemic of cholera caused by *V. cholerae* O139 in 1992, Thailand has no cases of infection until January 2005 to March 2006, 2 cases of *V. cholerae* O139 infection were reported by The Division of Epidemiology, MOPH, 2005-2006. From January to December, 2005, *V. cholerae* O1 infection were 272 cases. Among 272 cases found 245 cases infected by *V. cholerae* O1 El Tor, Ogawa serotype, and 26 cases infected by *V. cholerae* O1 El Tor, Inaba serotype (Division of Epidemiology, MOPH, 2005-2006).

As reported in **Table 7**, the diagnostic sensitivity, diagnostic specificity, and efficacy of the MAb-based dot-ELISA for detecting of *V. cholerae* O1 compared to the cultured method were all 100.00%. **Table 10**, the diagnostic sensitivity, diagnostic specificity, and efficacy of the MAb-based dot-ELISA for detecting of *V. cholerae*O139 compared to the cultured method were 100.00%, 99.9% and 99.9%, respectively, which indicates perfect agreement between the two tests. The ELISA is

easy to performed, relatively inexpensive, sensitive and specific. It permits multisample analysis in a single time, required no special equipment and does not pose any disposal problem (compared with culture method). It reduces the test time from at least two days of the conventional culture method to three hours. Thus, this method is recommended as a rapid screening test of cholera cases. The test can be carried out in the field, hospital, clinic or minimally equipped laboratory, *e.g.* laboratories at the district level or health centres for epidemiological investigation.