One-Step Immunochromatographic Dipstick Tests for Rapid Detection of Vibrio cholerae O1 and O139 in Stool Samples

F. Nato,1,* A. Boutonnier,1 M. Rajerison,2 P. Grosjean,2 S. Dartevelle,1 A. Guénoé,1 N. A. Bhuiyan,3 D. A. Sack,3 G. B. Nair,3 J. M. Fournier,1 and S. Chanteau2†

Institut Pasteur, Paris, France; Institut Pasteur, Antananarivo, Madagascar; and Laboratory Sciences Division, International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

Received 24 October 2002/Returned for modification 10 December 2002/Accepted 28 January 2003

We describe the development and evaluation of a rapid diagnostic test for Vibrio cholerae O1 and O139 based on lipopolysaccharide detection using gold particles. The specificity ranged between 84 and 100%. The sensitivity of the dipsticks ranged from 94.2 to 100% when evaluated with stool samples obtained in Madagascar and Bangladesh. The dipstick can provide a simple tool for epidemiological surveys.

_Vibrio cholerae_ strains belonging to the O1 and O139 serogroups are capable of causing epidemic and pandemic cholera. The O1 serogroup is subdivided into two serotypes, Ogawa and Inaba. 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. Prompt diagnosis of cholera is of key importance to initiate effective control measures. There are definitive indications that the incidence of this serogroup is of key importance to initiate effective control measures.

Several rapid diagnostic tests for cholera have been described. Some detect the cholera toxin (2, 19). Others detect _Vibrio cholerae_ (3, 6, 12, 16) or _O139_ (1, 10, 14). Recently, a multistep colloidal-gold-based colorimetric immunoassay known as SMART was also developed for direct detection of _V. cholerae_ O1 (9, 11) or _V. cholerae_ O139 (15) in stool specimens and has demonstrated 95% sensitivity and 100% specificity for O1 strains (11) and 97% sensitivity and 98% specificity for O139 strains (15).

In our effort to develop a conjugate vaccine that targets _V. cholerae_ O1 and O139, we have developed monoclonal antibodies specific to _V. cholerae_ O1 or O139 LPS (4, 5). Here we have exploited the specificity of the monoclonal antibodies to develop rapid diagnostic tests for cholera O1 or O139 using colloidal gold particles and based on a recently optimized one-step, vertical-flow immunochromatography principle (13). The detection threshold with purified LPS was 10 ng/ml for _V. cholerae_ O1 and 50 ng/ml for _V. cholerae_ O139. The dipsticks were stable after storage for 21 days at 60, 4, –20, and –80°C.

We have evaluated the sensitivity and specificity of the rapid dipstick tests in the laboratory setting and in two areas of cholera endemicity, namely, in Madagascar and in Bangladesh. The specificity was assessed using 14 pure cultures of _V. cholerae_ non-O1/non-O139 and 16 strains belonging to six other species of the genus _Vibrio_ ( _V. alginolyticus, V. fluvialis, V. para-haemolyticus, V. furnissii, V. hollisae, and V. mimicus_), seven strains of _Aeromonas_ species ( _A. caviae, A. enteropelogenes, A. hydrophila, A. sobria_, and _A. trota_), two strains of _Plesiomonas shigeloides_, and two strains of _Campylobacter jejuni_. Additionally, eight strains of _Yersinia pseudotuberculosis_, 10 strains of _Yersinia enterocolitica_, 35 other strains of _Yersinia_ belonging to seven species, and another 47 strains belonging to 11 other genera of _Enterobacteriaceae_ were included. The specificity of both dipsticks was 100% for all bacterial cultures. When tested for sensitivity, O1 dipsticks were positive with all 12 strains of _V. cholerae_ O1 (100%) and O139 dipsticks were positive with 17 of the 19 strains of _V. cholerae_ O139 (89.5%). A minimum of 107 CFU of _V. cholerae_ O1/ml or 106 CFU of _V. cholerae_ O139/ml is required to give an unequivocal positive reaction.

In Madagascar, the cholera O1 dipsticks were tested on 140 frozen stools samples or on rapid cultures (6 h, 37°C) of stool collected in the field on filter paper. Sixty-five samples were dipsticks and culture positive, 1 sample was dipsticks negative and culture positive, 3 samples were dipsticks positive and culture negative, and 71 samples were negative by both tests (Table 1). From the three dipsticks-positive and culture-negative samples, one was positive for _Shigella_ spp. The specificity in this field trial was 96%, and the sensitivity was 83.5%. The specificity of O139 dipsticks was also tested with the same 74 noncholera samples. Seventy-one out of the 74 samples tested positive (96%). The three samples which were positive by the dipstick test and negative in conventional culture were positive for _Shigella_.

In Bangladesh, fresh stool samples from suspected cholera patients were cultured for vibrios and other enteric pathogens as described previously (14, 15). Frozen stool samples in which the etiology was known were made available for this study from the specimen bank of the International Centre for Diarrhoeal Diseases Research, Bangladesh. The dipstick test was performed simultaneously by introducing either the O1 or the O139 dipsticks into 200 μl of stool.

For the O1 dipstick evaluation, 102 stool samples were used. Forty-nine were dipstick-positive and culture positive, 3 were dipstick negative and culture positive, 42 were negative by both tests (Table 2). The sensitivity of the O1 dipstick compared to culture was 96.1%, with a specificity of 84%. We further analyzed the eight
stool samples which were O1 dipstick positive but O1 culture negative; six were negative for V. cholerae, one was positive for V. cholerae O139 and Pseudomonas spp., and another was positive for Pseudomonas spp. All three stool samples which were negative by the dipstick test were positive for V. cholerae O1. One of the reasons for such a result might be that the amount of LPS in these stool samples is lower than threshold levels but such stool samples were not many in numbers.

For the O139 dipstick evaluation, of the 158 stool samples, 91 were dipstick and culture positive, 5 were dipstick positive and culture negative, and 60 were negative by both dipstick and culture (Table 3). The sensitivity of the O139 dipstick compared to culture was 100%, with a specificity of 92.5%. We further analyzed the five stool samples which were O139 dipstick positive but O139 culture negative and found three samples were positive for V. cholerae O1 Inaba by culture while the other two were negative for vibrios.

In the evaluation of Madagascar as well as that in Bangladesh there were few samples which were positive by the dipstick test but negative by culture for both the O1 and O139 strains. We did not have a method to prove whether these results were true or false positives as we did not perform a PCR on these samples. 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 in Madagascar. Plans are under way to conduct a more rigid evaluation to understand whether these were false-positive results.

### TABLE 2. Detection of V. cholerae O1 in fresh and frozen stool samples by O1 dipstick test versus conventional culture (Bangladesh)

| Bacteriological culture | No. of specimens with O1 dipstick test result | Total no. of specimens |
|-------------------------|---------------------------------------------|-------------------------|
|                         | Positive  | Negative | Positive  | Negative |
| Positive                | 49        | 3        | 52        |          |
| Negative                | 8         | 42       | 50        |          |
| Total                   | 57        | 45       | 102       |          |

*a Sensitivity was 94.2%; specificity was 84%.

Of the 102 samples, 77 were fresh and the remaining 25 were frozen for various periods.

### TABLE 3. Detection of V. cholerae O139 in fresh and frozen stool samples by O139 dipstick test versus conventional culture (Bangladesh)

| Bacteriological culture | No. of specimens with O139 dipstick test result | Total no. of specimens tested |
|-------------------------|-----------------------------------------------|-------------------------------|
|                         | Positive | Negative | Positive | Negative |
| Positive                | 91       | 0        | 91       |          |
| Negative                | 5        | 62       | 67       |          |
| Total                   | 96       | 62       | 158      |          |

*a Sensitivity was 100%; specificity was 92.5%.

Of the 158 samples, 100 were fresh and the remaining 58 were frozen for various periods.

In summary the importance of an efficient cholera surveillance system continues to be stressed by World Health Organization with regard to improving risk assessment of potential cholera outbreaks (18). Further, 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. 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.

This work was supported by the Institut Pasteur, Paris (grant PTR 2000-11). Part of the work was funded by a grant from the Government of Japan to the Laboratory Science Division of the ICDDR,B: Centre for Health and Population Research, and by other core donors of the Centre, who share its concern for the health problems of developing countries.

We are grateful to F. Qadri, ICDDR,B, for providing us the frozen stool samples. We are grateful to Elisabeth Carniel (Institut Pasteur, WHO Collaborating Centre for Yersinia) for provision of strains used in our specificity studies.

### REFERENCES

1. Agarwal, V., M. Biswas, A. A. Pathak, and A. M. Sanji. 1995. Rapid detection of Vibrio cholerae O139 in faecal specimens by coagglutination. Indian J. Med. Res. 101:55-56.

2. Almeida, R. J., F. W. Hickmanbrenner, E. G. Sowers, N. D. Puhr, J. J. Turner, and I. K. Wachsmuth. 1990. Comparison of a latex agglutination assay and an enzyme-linked immunosorbent assay for detecting cholera toxin. J. Clin. Microbiol. 28:128-130.

3. Andrade, J. R. C., J. L. B. Dasilva, M. F. L. Barbosa, and C. A. R. Caldas. 1992. Preliminary report on the application of the coagglutination test for rapid diagnosis of cholera in the Upper Solimoes River area in the Brazilian Amazon region. Braz. J. Med. Biol. Res. 25:375-378.

4. Bougourdgo, F., F. Vely, F. Nato, A. Boutonnier, P. Gounon, J. C. Mazie, and J. M. Fournier. 1995. Protective activities of serum immunoglobulin G on the mucosal surface to Vibrio cholerae O1. Bull. Inst. Pasteur 93:273-283.

5. Boutonnier, A., S. Villeneuve, F. Nato, B. Dassy, and J. M. Fournier. 2001. Preparation, immunogenicity, and protective efficacy, in a murine model, of a conjugate vaccine composed of the polysaccharide moiety of the lipopolysaccharide of Vibrio cholerae O139 bound to tetanus toxoid. Infect. Immun. 69:3488-3493.

6. Carillo, L., R. H. Gilman, R. E. Mantle, N. Nunez, J. Watanabe, J. Moron, V. Quispe, A. Ramirez-Ramos, et al. 1994. Rapid detection of Vibrio cholerae O1 in stools of Peruvian cholera patients by using monoclonal immunodiagnostic kits. J. Clin. Microbiol. 32:856-857.

7. Chanteau, S., L. Rahalison, L. Ralafiarisoa, J. Foulon, M. Ratsitorahina, L. Ratsifsasamanana, E. Carniel, and F. Nato. 2003. A rapid and reliable diagnosis of pneumonic and bubonic plague, easily achievable by health workers in the natural endemic foci of Madagascar. Lancet 361:211-216.

8. Colwell, R. R., J. A. K. Hasan, A. Hsing, L. Loomis, R. J. Siebeling, M. Torres, S. Galvez, S. Islam, M. T. Tamplin, and D. Bernstein. 1992. Development.
and evaluation of a rapid, simple, sensitive, monoclonal antibody-based co-agglutination test for direct detection of *Vibrio cholerae* O1. FEMS Microbiol. Lett. 97:215–220.

9. Hasan, J. A., D. Bernstein, A. Huq, L. Loomis, M. L. Tamplin, and R. R. Colwell. 1994. Cholera DFA: an improved direct fluorescent monoclonal antibody staining kit for rapid detection and enumeration of *Vibrio cholerae* O1. FEMS Microbiol. Lett. 120:143–148.

10. Hasan, J. A. K., A. Huq, G. B. Nair, S. Garg, A. K. Mukhopadhyay, L. Loomis, D. Bernstein, and R. R. Colwell. 1995. Development and testing of monoclonal antibody-based rapid immunodiagnostic test kits for direct detection of *Vibrio cholerae* O139 synonym Bengal. J. Clin. Microbiol. 33:2935–2939.

11. Hasan, J. A. K., A. Huq, M. L. Tamplin, R. J. Siebeling, and R. R. Colwell. 1994. A Novel Kit for Rapid Detection of *Vibrio cholerae* O1. J. Clin. Microbiol. 32:249–252.

12. Jesudason, M. V., C. P. Thangavelu, and M. K. Lalitha. 1984. Rapid screening of fecal samples for *Vibrio cholerae* by a coagglutination technique. J. Clin. Microbiol. 19:712–713.

13. Park, S. H., S. H. Lee, J. H. Cho, and Y. S. Kim. 2000. Development of rapid one-step immuno-chromatographic assay. Methods 22:53–60.

14. Qadri, F., A. Choudhury, J. Hossain, K. Chowdhury, T. Azim, T. Shimada, K. M. N. Islam, R. B. Sack, and M. J. Albert. 1994. Development and evaluation of rapid monoclonal antibody-based coagglutination test for direct detection of *Vibrio cholerae* O139 synonym Bengal in stool samples. J. Clin. Microbiol. 32:1589–1590.

15. Qadri, F., J. A. K. Hasan, J. Hossain, A. Chowdhury, Y. A. Begum, T. Azim, L. Loomis, R. B. Sack, and M. J. Albert. 1995. Evaluation of the monoclonal antibody-based kit Bengal SMART for rapid detection of *Vibrio cholerae* O139 synonym Bengal in stool samples. J. Clin. Microbiol. 33:732–734.

16. Rahman, M., D. A. Sack, A. Wadood, M. Yasmin, and A. Latif. 1989. Rapid identification of *Vibrio cholerae* serotype O1 from primary isolation plates by a coagglutination test. J. Med. Microbiol. 28:39–41.

17. Sinha, S., R. Chakraborty, K. Y. De, A. Khan, S. Datta, T. Ramamurthy, S. K. Bhattacharya, Y. Takeda, and G. B. Nair. 2002. Escalating association of *Vibrio cholerae* O139 with cholera outbreaks in India. J. Clin. Microbiol. 40:2635–2637.

18. World Health Organization. 2001. Cholera 2000. Wkly. Epidemiol. Rec. 76:233–240.

19. Yam, W. C., M. L. Lung, and M. H. Ng. 1992. Evaluation and optimization of a latex agglutination assay for detection of cholera toxin and *Escherichia coli* heat-labile toxin. J. Clin. Microbiol. 30:2518–2520.