Attachment 1.

Date July 7, 1991
Sept. 17, 1991

ETHICAL REVIEW COMMITTEE, ICDDR,B

Principal Investigator DR. ANWARUL HUQ Trainee Investigator (if any) _____

Application No. 91-012 (Revised) Supporting Agency (if Non-ICDDR,B) UNIV. MD

Title of Study: A COMPARATIVE STUDY OF THREE DIFFERENT METHODS FOR THE IDENTIFICATION OF V. CHOLERAE 01 IN STOOL AND WATER SAMPLES

Project status:
(X) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects — (Yes) No
   (b) Non-ill subjects — Yes No
   (c) Minors or persons under guardianship — Yes No
2. Does the study involve:
   (a) Physical risks to the subjects — Yes (No)
   (b) Social Risks — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — Yes (No)
   (e) Invasion of privacy — Yes (No)
   (f) Disclosure of information damaging to subject or others — Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — Yes (No)
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — Yes (No)
4. Are subjects clearly informed about: (NA)
   (a) Nature and purposes of study — Yes No
   (b) Procedures to be followed including alternatives used — Yes No
   (c) Physical risks — Yes No
   (d) Sensitive questions — Yes No
   (e) Benefits to be derived — Yes No
   (f) Right to refuse to participate or to withdraw from study — Yes No
   (g) Confidential handling of data — Yes No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes No
5. Will signed consent form be required: (NA)
   (a) From subjects — Yes No
   (b) From parent or guardian (if subjects are minors) — Yes No
6. Will precautions be taken to protect anonymity of subjects (NA) — Yes No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   X Protocol (Required)
   X Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required).
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Ctte. for review.

(PTO)

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Anwarul

Principal Investigator

Trainee

91-012 (Rev)
17.9.91

SECTION I - RESEARCH PROTOCOL

1. Title: A comparative study of four different methods for the identification of V. cholerae 01 in stool and water samples.

2. Principal Investigator: Dr. Anwarul Huq

   Co-Investigators: Dr. Rita Colwell
   Dr. Larry Loomis
   Dr. Brad Sack (ICDDR,B)
   Dr. Sirajul Islam

3. Starting Date: August 18, 1991

4. Completion Date: ~~September~~ December 30, 1991

5. Total Direct Cost:

6. Scientific Program: Laboratory Sciences Division

7. Abstract Summary:

A comparative study of ~~three~~ four different laboratory techniques for the qualitative identification of Vibrio cholerae 01 in stool and environmental water samples will be done. The techniques, conventional culture method, fluorescent antibody method, quick coagglutination method and "dipstick", a newly developed rapid method, will be evaluated. Approximately 100 cholera suspected stool and 100 water samples from cholera prone areas will be tested.

8. i. Ethical Review Committee
   ii. Research Review committee
   iii. Director

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives

To evaluate the efficacy of a "dipstick" type of test and a coagglutination test for the rapid identification of V. cholerae 01 in water and stool samples.

2. Background

There are various methods or tests known to provide rapid identification of V. cholerae 01 from clinical or environmental samples (Rahman et al. 1989; Brayton et al. 1987; and Xu et al. 1984). A conventional culture method requires at least 24 - 48 hours for results to be obtained and it may not provide any useful information in the event that the organisms are in non-culturable stage. A serological method developed by Rahman et al. is rapid, compared to a conventional culture method, however, it requires 24 hours for the result. Recently, Simmonson and Siebling (1991) reported the development of a dipstick - Elisa method for the detection of V. cholerae in oyster meat homogenate that requires one full working day. More over, an enrichment step that essentially detects only culturable organisms by this method. A quick identification of V. cholerae and diagnosis of cholera could be very important for control of epidemic, particularly by routine monitoring of water system in the epidemic and pandemic areas for cholera using a rapid but inexpensive kit.

A "dipstick" method now being developed through collaboration between the University of Maryland and New Horizon Diagnostic Company, can provide results within 30 minutes. This immunological method is prepared using polyclonal and monoclonal antibody against V. cholerae 01 tagged with gold that would give a red coloration upon positive reaction. This is a simple, inexpensive method, and does not require addition of chemicals or full laboratory facilities to perform the test. Also a simple co-agglutination test that we have is based on polyclonal and monoclonal antibody tagged on Staph cells may require atmost 4 hours to detect V. cholerae in stool or environmental samples. Promising results in the field will be helpful for further development of this test and, ultimately, to make it available for general use.

3. Rationale:

Most countries dealing with the problem of cholera outbreaks do not have laboratory facilities, notably in rural areas, nor the money to perform high technology methods to detect and identify V. cholerae 01 in the stool. The dipstick test kit we have developed does not require any refrigeration or any equipment to use. More over it has a long shelf life. Also, in an epidemic situation, large numbers of stool samples may have to be tested at the same time, requiring the water supply also to be tested for the presence of causative agents of the disease.

B. SPECIFIC AIMS:

Hypothesis to be tested:

(a) The "dipstick" method and coagglutination kit, developed by the University of Maryland and New Horizon Diagnostic Company, requires full evaluation before general use can be made.

(b) Promising results obtained in the laboratory must be evaluated under practical conditions, i.e., in the field.

C. METHODS AND PROCEDURE:

Field Sites: Dhaka treatment Center and Matlab Field hospital of ICDDR,B.

Samples and Specimen: 100 cholera suspected stool specimens arriving at the diagnostic laboratories from suspected cholera patients will be tested. Also 100 water samples will be collected from river, pond, and lakes in and around Dhaka and Matlab Treatment Center for testing.

Bacteriological Method: Each specimen will be tested simultaneously using four different methods. The conventional culture method, involving culture plates will be used, following the method described by Farmer et al. (1985) and the fluorescent antibody (FA) technique using the monoclonal antibody which was developed in our laboratory (Brayton et al., 1987). The FA slides will be prepared at the ICDDR,B and will be stained in Maryland for observation.

Dipstick method: This is an approximately 2" x 2" plastic chamber with two folding compartments. Stool sample or water sample (preferably concentrated by using filter membrane) will be soaked with a cotton swab. This swab will be placed in one of the chambers and within 10 minutes after closing it, a color reaction will be produced in the other chamber in case of positive result. Stool and water samples

will also be enriched for 4 - 6 hours in alkaline peptone water and then swabs will be prepared and processed in the same chamber for comparative study.

Rapid coagglutination method: The coagglutination reagents developed in Maryland will be tested on 4 hour enrichments as well as unenriched stool and environmental specimen for glass slide agglutination. A drop of sample such as, undiluted stool, 1:10 dilution with PBS, 4 ours enrichment in alkaline peptone water will be placed on a glass slide. Similarly one drop of enrichment from 100 ml filtered water membrane will be placed on glass slide. Then one drop of M-I, MIII and LI coagglutination reagents will be added on to the glass slide and will be agitated for the development of agglutination. Reading will be taken at 5 and 10 minutes. In case of positive agglutination reaction, same sample will be tested with staph reagent to rule out non-specific reaction. Cells of Vibrio cholerae 569-B as positive control and E. coli or Aeromonas species as negative control will be used in the study.

Isolates from cultures giving positive reaction in any of the test will be stocked for future study.

F. COLLABORATION ARRANGEMENT:

The principal investigator will come to Dhaka for about two weeks to perform the field trial himself and Dr. Sirajul Islam of ICDDR,B will be involved in the study. Technical help may be required and the cost will be reimbursed. The findings of this study will be published with co-authorship from ICDDR,B. This project will reimburse ICDDR,B all the expenses in the laboratory including technician's time.

LITERATURE CITED

Brayton P. and R.R. Colwell. 1987. Fluorescent antibody staining method for enumeration of viable environmental Vibrio cholerae 01. J. Microbiol. Methods 6: 309-314.

Farmer, III, J.J., F.W. Hickman-Brenner and M.T. Kelly. 1985. Vibrio p. 282-301. In Lennette, E.H., Balows, A., Housler, W.J., Jr., and Shadomy, H.J. (eds), Manual of clinical Microbiology, 4th ed. ASM, Washington D.C.

Rahman, M., D.A. Sack, A. Wadood, M. Yasmin and A. Latif. 1989. Raid identification of Vibrio cholerae serotype 01from primary isolation plates by a coagglutination test. J. Med. Microbiol. 28: 39-41.

Simmonson, J.G. and r.J. Siebling. 1991. Detection of V. cholerae 01 serovars in oyster meat homogenate by a dipstick-ELISA method. MTS Journal 25: 52-59.

Xu, H.S., N.C. Roberts, L.B. Adams, P.A. West, R.J. Siebling, A. Huq, M.I. Huq, R. Rahman and R.R. Colwell. 1984. An indirect fluorescent antibody staining procedure for direct detection of V.cholerae serovar 01 cells in aquatic environmental samples. J. Microbiol. Methods. 2: 221-231.

BUDGET

All the expenses in the ICDDR,B will be reimbursed on cost basis by the University of Maryland.

Salary:

Technician, ICDDR,B 100% (for two weeks).	$300

Supplies:

TCBS plates, Alkaline peptone water etc.	$400

Travel:

Dhaka-Matlab-Dhaka two trips	$100

Miscellaneous

Transport, Telephone, FAX etc.	$200
Total:	$1000

بسم الله الرحمن الرحيم

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Date: 15 Sep'91

Dr. Demissie Habte, MD
Chairman, Research Review Committee

Dear Dr. Habte,

1. Many thanks for forwarding the protocol entitled 'A comparative study of four different methods for the identification of V.cholerae 01 in stool and water samples' for my comments.

2. The four different techniques which the investigators wish to employ are : conventional culture method, fluorescent antibody method, quick coaggulation method and the newly developed rapid dip-stick method. They will test 100 stool samples from suspected cholera patients and 100 water samples from rivers, ponds and lakes from cholera prone areas (Dhaka and Matlab). They will use V.cholerae 569-B as positive control and E.co or Aeromonas spp.es as negative control.

3. The following are my comments:

3.1. Composition of the Dip-stick and the exact mechanism of action has not been indicated. It has been simply mentioned that it is an immunological method.

3.2. Will the shelf-life of sticks be affected by the hot and humid climate of Bangladesh ?

3.3. What will be the approximate cost per test ?

3.4. Being an immunological test will it be able to indicate beyond '01' i.e. serotypes of the organism such as Inaba, Ogawa etc ?

3.5. What about its capability in detecting the ElTor bio-types.

3.6. This protocol lacks the design for evaluating the method (Rapid Dipstick Method) for analytic specificity, sensitivity, accura and precision.

4. I am however sure that the investigators will take the above points into consideration while carrying out the study.

With warm personal regards.

Sincerely yours

(M R CHOUDHURY)

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

Memorandum

TO : Chairman, RRC

DATE: September 15,1991

FROM : M. John Albert

SUBJECT: Review of protocol entitled "A comparative study of four different methods for the identification of V.cholerae O1 in stool and water samples" by Anwarul Hug et al.

My comments on the protocol are as follows :-

Page-1: Title says four different methods will be compared, but abstract summary says only three. Abstract summary says 100 stool samples will be tested, but it is not clear what is the type of stool that will be tested.

Page-2: In the background section it is stated that the rapid method developed by Rahman et al require 24 hours for the result. This statement is wrong and the results are available in few hours.

In the background section, it is not clear what is being conveyed through the fifth sentence.

Page-4: First paragraph: What are the natures of the reagents MI, MIII and LI ?

I would rather find it uneasy to look for agglutination after 10 minutes.

In conclusion, the protocol is written in a vague manner making the reader guess about the details.

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

Memorandum

TO : Dr. Demissie Habte
Chairman, RRC

DATE: 16 Sept 199

FROM : Dr. P.K. Bardhan

SUBJECT : Protocol review for RRC

Thank you very much for giving me the opportunity to review the protocol entitled "A comparative study of four different methods for the identification of V. cholerae 01 in stool and water samples" by Anwarul Huq et al.

The protocol is brief and is more like a project outline. However, considering the time constraints and noting the exploratory nature, it can be overlooked. The stated aim of rapid identification of V. cholerae in stool and water is important. Still, I think the principles and procedure of the two methods ("Dipstick" and coagglutination methods) should be provided with more details along with the respective references.

I support the protocol.

INTERNATIONAL CENTRE FOR
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September 17, 1991

Dr. D. Habte
Chairman RRC,
ICDDR,B

Dear Dr. Habte:

Thanks for giving me an opportunity to improve our protocol on "A comparative study of 4 different methods for identification of *V. cholerae* 01 in stool and water samples".

1. Reviewer 1

3.1 Exact composition of the dipstick can not be spelled out at this point for technical reasons, but the mechanism of action has been provided.

3.2 It is expected that humidity and hot temperature should not interfere with a long shelf life, (more than a year). Since it is in developmental stage, research is in progress.

3.3 Cost has not yet been evaluated. But it is expected that the test will be much cheaper than the cost of conventional culture method.

3.4 At present we are not thinking beyond 01 detection.

3.5 Both Eltor and Classical biotypes will be detected by this method.

3.6 Simple analysis of data will be made on the basis of positive and negative results only at this point.

Reviewer II

Page 1 : Correction has been made on the number of stool samples to be listed. Stool from suspected cholera patients will be listed.

Page 11 : " *V.cholerae* 01 was identified by coagglutination test in 18-24 hours will colonies from within TCBS or TTG agar" - quoted from Rahman *et al* J. Med. Microbiol 28: 1989, page 41, first sentence of the second paragraph, fifth sentence has been modified.

Page 4 : These reagents are based on monoclonal and polyclonal antibody against *V. cholerae* 01 tagged with Staph cells for co-agglutination test.

Although we are aiming to see agglutination in 2 minutes howwever, we believe it would be quite interesting to see the result beyond that time.

Reviewer 3

The reviewer is very right that the protocol is of the exploratory nature. Hence, detail of the composition could not be provided at present time. Principles and procedures of the two methods has been provided.

I hope the answer will satisfy the reviewers. Revised protocol is endosed

Thanking you.

Sincerely yours,

Dr. Anwarul Huq

Anwar:D.Habte