Attachment 1.

Date 13.12.79

REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

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Principal Investigator MR. STEVE MOSLEY MR. IMDADUL HUQ Trainee Investigator (if any) x

Application No. 80-001 Supporting Agency (if Non-ICDDR,B) x

Title of Study Detection of ETEC from Stool Culture and environmental samples by hybridization with specific 32p labelled DNA probe

Project status:
(✓) 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:
   (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:
   (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 — (Yes) No
7. Check documents being submitted herewith to Board:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ 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 Board for review.

I agree to obtain approval of the Review Board on the Use of Human Subjects for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator — Trainee

80-001
Recd 20/12/79

ABSTRACT SUMMARY

The purpose of this study is to find out wide ranging more specific method of detecting enterotoxigenic *Escherichia coli* from the isolation plate rather than testing individual colonies for toxin production by using conventional expensive bioassay methods. This will also give us an opportunity to study the presence of toxigenic strains from diseased to convalescent stage. The different steps involved in this process is to inoculate the stool directly onto nitrocellulose filters placed on medium and fixing the bacterial colonies on filters by treating with different grades of NaOH. The dried filter is then hybridized with P32 labelled DNA which is specific to LT or ST of *Escherichia coli*. The hybridized filters are radiographed and positive colony appears as black spots. A total of 50 patients will be followed in Dacca hospital and stool will be cultured everyday for 5 days. 15-20 patients will be followed-up and family members cultured on 1, 3, 7 and 14th day. In Matlab, 10-15 Index cases positive for *E.coli* will be taken and families studied for follow-up up to 2 weeks. There is no potential risk to the patients as only one stool sample will be cultured perday. There will be no formal interview with the patients only they will be volunteered to stay in the ward for 5 days. No part hospital records etc., will be needed. The study will open a new simplified method for the quick and cheaper method for the detection of Enterotoxigenic and *E.coli*.

SECTION I - RESEARCH PROTOCOL

1. Title: DETECTION OF ENTEROTOXIGENIC *E.COLI* FROM STOOL CULTURE AND ENVIRONMENTAL SAMPLES BY HYBRIDIZATION WITH SPECIFIC 32_p LABELLED DNA PROBE

2. Principal Investigators: Mr. Steeve Mosley
   Mr. Imdadul Huq

3. Starting Date: February 1, 1980

4. Completion Date: April 30, 1980

5. Total Direct Cost:

6. Availability of Funds: (a) Scientific Director's Remarks:-

   (b) Controller's Remarks:-

7. Abstract Summary:

It has been observed by Mosley *et al.*, (personal communication and ASM abstract) that the heat labile enterotoxin (LT) genes labelled with 32_p deoxyneucleotides can be hybridized with plasmid DNA from unknown LT producing strains of *E.coli*. The *E.coli* strains which are LT producing hybridizes on a nitrocellulose filter and can be detected on radiography of the filter blot as black spots. Mosley *et al.*, suggested that this technique can be used for the examination of a large number of strains of *E.coli* for the presence of LT genes and should be of value in studies of molecular epidemiology of LT. It has also been found recently that using the same technique with ST probe, the Stable toxin producing cultures of *E.coli* can also be detected.

In the standard methods used to detect LT and ST the *E.coli* cultures are picked and toxin is produced in TSBC Yeast Extract broth and tested using standard methods. In this method a few colonies are tested from the culture plate. Using this new technique we will be able to locate any LT or ST producing colonies directly on the nitrocellulose filter on which the stool from the patient or contacts will be streaked thus allowing even a small percentage of toxin producing strain, if present, to be detected.

8. Reviews:

   (a) Research Involving Human Subjects: _____
   (b) Research Review Committee: _____
   (c) Director: _____
   (d) BMRC: _____
   (e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. Introduction:

1. Objective:

The overall objective of this study is to (1) find our more specific and wide ranging method of detecting enterotoxin producing *Escherichia coli* colonies from original isolation plates rather than testing picked colonies. This will give a better chance of isolation of the ETEC from household contacts of the cases where there are fewer Enterotoxigenic colonies in the early stage. (2) Find a convenient method to enumerate the number percentage of the ETEC present in the population. (3) To follow the numbers of LT-producing bacteria in the stools of patients from the acute phase of the disease through convalescence.

2. Background:

In 1967 the first report of enterotoxigenic *E.coli* isolated from young animals with diarrhoea (1, 4). These *E.coli* produced an enterotoxin similar to cholera enterotoxin which caused fluid accumulation in ligated ileal segments of adult rabbits and some other animals. Various studies done in the past 10 years have clearly indicated these *Escherichia coli* causes diarrhoeal disease either by invasion of the intestinal mucosa or by the elaboration of

enterotoxin (2, 3, 4). It was subsequently determined that the enterotoxigenic strains of *Escherichia coli* elaborate one or both of two plasmid mediated enterotoxins - 1) High molecular weight heat labile enterotoxin (LT) similar to cholera toxin which acts by the stimulation of adenylate cyclase and; 2) a low molecular weight heat stable enterotoxin (ST) which acts by the stimulation of guanylate cyclase (5, 6). Various methods have been tried out by different workers to test the LT and ST produced by *E.coli*. Of these Rabbit ileal loop, Infant rabbit model, Rabbit skin test, Rat epidydymal fat cell assay and the Y_1 adrenal cell and Chinese Hamster Ovary Cell Assay have been used for testing heat labile cholera toxin. The Infant mouse assay have been used for detecting the ST produced by *E.coli*. A comparative report of these assays on *E.coli* and cholera toxin has been described by Sack (2).

All the methods described above uses the culture filtrate of the overnight grown cultures of the *E.coli* in different culture medium. These filtrates are then tested on various animal models.

Most recently Dallas *et al.*, (7, 8) has characterized the *Escherichia coli* heat labile toxin gene and also shown the sequence homology of heat labile enterotoxin genes of *Escherichia coli*. They have found that the LT probe labelled with 32_p deoxynucleotide can be hyberidized with

DNA from Enterotoxigenic *E.coli* strains grown on nitrocellulose filters. This method allows the detection of the toxigenic strains from the isolation plate and thus a large number of colonies can be grown on the filters for examination. It is presumed that this technique will be of value in the field studies in the detection of toxigenic strains from stools of contacts or carriers of toxigenic *E.coli* where a small number of toxigenic bacteria may be present. Other bacteria which may produce LT or ST can also be studied by this method.

B. Specific Aim:

The specific aim of this study is to find a quicker less costly and convenient direct method for the detection of labile toxin and stable toxin from *E.coli* and other gram negative bacteria isolated from stool specimens from patients as well as carriers or contacts.

C. Methods and Procedures:

The stool samples are streaked onto a nitrocellulose filter paper placed onto a MacConkey's Agar plate or an appropriate dilution of faeces or water are passed through special membrane filters and the filters placed on the surface of a MacConkey's Agar plate. The plates incubated

for 37°C for 18-24 hours. The colonies grow on the surface of the filters. The filters are lifted and placed on a filter paper soaked with 0.5N Sod-hydroxide to lyse the cultures and soaked with 3 changes of 1M Tris buffer and one change with buffer + 1.5 MNaCl. The cells are fixed *in situ* and then hybridized with a specifically prepared P_{32} labelled DNA probe that is absolutely specific either for the *E.coli* LT genes or ST genes. Following hybridization the filters are washed, dried and then radiographed against a piece of X-ray film.

The LT^+ or ST^+ colonies appear as deep black spots on the film and can be enumerated.

D. Significance:

The proposed research work will give us a convenient and quicker method for the detection of enterotoxigenic *E.coli* directly from the isolation plate. This method will help to study the follow-up of the ETEC patients during days of hospitalisation as well as follow the household contacts including food, water supplies etc., for the presence of ETEC.

E. Facilities Required:

1. Office Space

No extra office space will be required.

2. Laboratory Space

Laboratory space will be provided in Room No. 109 where a portion of the bench space fitted with wash basin will be provided. In Matlab the same sort of bench space will be provided. The P_{32} probe will be shipped from New England Nuclear, U.S.A. by Prof. Falkow and a part will be hand carried by one of the investigators. The half life of P_{32} is 14.4 days and it decays out later without causing any problems.

3. Hospital Resources

For the follow-up of the clinical and serologically positive ETEC patients during the course of their illness patients will be kept for 4-5 days in the ward and daily R/S or stool will be collected. A total of 50 patients will be studied in Dacca in the ward and 15-20 of them will be followed-up on day 1, 3, 7, 14. In Matlab 10-15 Index cases will be choser for hospital and family members will be followed-up for ! weeks.

4. Animal Resources

1200 infant mouse will be needed to test 600 *E.coli* strains for ST from 200 suspected ETEC positive cases and contacts in the family study. (Each culture will have 2 single colonies and a pool of 5 colonies of *E.coli*). These animals will be required in March, 1980 at the rate of 600 per week.

5. Logistic Support

For the follow-up of the household contacts for each case 1 day follow-up sample will be taken. A total of 30 families will be followed-up (15-20) at Dacca and 10-15 at Matlab. One boat for 2 hours per day will be needed for 30 days.

6. Major Item of Equipment - None.

7. Other Specialised Requirements - None.

F. Collaborative Arrangement:

This is a collaborative research programme between the Department of Microbiology, University of Washington, Seatle and the ICDDR,B. One of the investigators will be

coming to Dacca from Seatle for 2-3 months starting last week of January, 1980. The work will be done under the supervision of Prof. Stanley Falkow of the Department of Microbiology, University of Washington. The arrangement will be made by Prof. Falkow for the shipment P_{32} labelled DNA probe to Dacca during the period of experiment. Arrangements to be made for cost of travel, from Seatle-Dacca-back and transportation and accommodation at Dacca and Matlab for Steeve Mosley one of the investigators. Prof. Stanley Falkow of the Department of Microbiology, University of Washington will act as a consultant for the study.

SECTION III - BUDGET

A. Detailed Budget:

1. Personnel Services

Name	Position	% effort Nos. of days	Salary Annual	Total Cost
Dr. S. Falkow	Consultant			
Mr. Steeve Mosley	Pre-Doc Fellow	3 months	-	-
Mr.Md.Imdadul Huq	Investigator	50% 3 months	Tk. 81,384	20,346
Mr. A. Alim		50% 1 month	40,644	1,694
Mr. Abdul Haque		50% 2 months	27,936	2,328
Laboratory Attendant		25% 3 months	12,336	771
Dr. Abu Eusuf		10% 1 month	44,316	369
Field Assistant, Matlab		1 month	20,316	1,693
Nurses		50% 2 months	20,556	1,720

2. Supplies and Materials

	Tk.
Media	2,000.00
Chemical and biologicals	800.00
Glass and plastic ware	6,500.00
Nitrocellulose filter 800 sheets	5,000.00
X-ray films	3,000.00
Office supplies and stationery	1,000.00
Miscellaneous	2,000.00

3. Equipments - None.

4. Patient Hospitalisation

30 patients for 5 days

30 x 150 = Tk. 4,500.00

5. Out-patient Care - None.

6. ICDDR,B Transport - 3.00/mile

Land Transport

2 trips to Dacca - Matlab - Dacca Tk. 900.00

Water Transport

Speedboat - 1 hour each day for

30 days Tk. 3,150.00

7. Travel & Transportation of Person

Air-ticket for Steeve Mosley

Seatle - Dacca - Seatle - $ 2200.00

Guest House cost - 35 days $ 700.00

Matlab stay - 35 days $ 700.00

8. Transportation of Things

Transportation of P_{32} labelled DNA probe - To be arranged by Prof. Falkow.

9. Rent, Communication and Utilities

Shipment of specimens	-	Tk. 3,500.00

10. Printing and Reproductions

Printing of forms	-	Tk. 1,500.00

11. Other Contractual Service

None

12. Construction

None

B. Budget Summary

Category	Taka	Dollar
1. Personnel	28,921.00	
2. Supplies	20,300.00	
3. Equipment	None	
4. Patient Hospitalisation	4,500.00	
5. Out-patients care	None	
6. Transport ICDDR,B	4,050.00	
7. Travel of Persons		3,600.00
8. Transportation of things	None	
9. Rent communication	3,500.00	
10. Printing and reproduction	1,500.00	
11. Other contractual service	None	
12. Construction	None	
TOTAL =	62,771.00	3,600.00

REFERENCES

1. Smith H.W., Hall S. 1967. J. Pathol. Bacteriol. 93:531-43.

2. Sack R.B. 1975. Human diarrheal disease caused by Enterotoxigenic Escherichia coli. Ann Rev. Microbiol. 29:333-353.

3. Dupont H.L., Formal S.B., Hornick R.B., Snider M.J., Libonati J.B., Sheahan D.G., La Breck E.H., Kalas J.P. 1971. Pathogenesis of E.coli Diarrhea. N. Eng. J. Med. 285:1-9.

4. Evans D.J., Evans D.G., Gorbach S.L. 1973. Production of Vascular permeability factor by Enterotoxigenic E.coli isolated from man. Infect. Immun. 8:725-730.

5. Banwell J.G., Sherr H. 1973. Effect of bacterial enterotoxin on the gastrointestinal tract. Gastroenterology 65:467-497.

6. Richard K.L., Douglas S.D. 1978. Pathophysiological effects of Vibrio cholerae and enterotoxigenic E.coli and their exotoxins in encaryotic cells. Microbiol. Rev. 42:592-613.

7. Dallas W.S., Gill D.M. and Falkow S. Characterisation of the Escherichia coli heat labile toxin gene. Abstract, 79th A.S.M. meeting. D-41. Session 90.

8. Dallas W.S., Moseley S.L. and Falkow S. Sequence homology of heat labile enterotoxin gene of Escherichia coli. Abstract 79th A.S.M. meeting. D-41, Session 90.