2 VG

Date ____________

ETHICAL REVIEW COMMITTEE, ICDDR,B.

incipal Investigator DR. M.I. HUQ

Trainee Investigator (if any) ____________

plication No. 83-006

Supporting Agency (if Non-ICDDR,B) ____________

tle of Study Detection Enterotoxigenic oli from stool culture samples by ridization with specific 32p labelled probe.

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

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

Source of Population:
(a) Ill subjects — (Yes) No
(b) Non-ill subjects — Yes No
(c) Minors or persons under guardianship — Yes No

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)

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)

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 Committee:
   ___ 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 Cttee. for review.

Only patients stool will be cultured for E. coli

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

[signature]
Principal Investigator

[signature]
Trainee

83-006
3/2/83

SECTION I - RESEARCH PROTOCOL

1. Title : Detection Enterotoxigenic E. coli from stool culture and environmental samples by hybridization with specific 32p labelled DNA probe.

2. Principal Investigator : Dr. M.I. Huq

Co-Investigators : Dr. P. Eschevierra
Dr. R.I. Glass

3. Starting Date : April 1, 1983

4. Completion Date : September 30, 1983

5. Total Direct Cost : US$ 9,401.00

6. Scientific Programme Head :

This protocol has been approved by the Disease Transmission Working Group.

Signature of Scientific Programme Head : [signature]

Date : 4/6/1982

7. Abstract Summary:

The proposed study of the extension of the protocol 80-001 for a period of six months starting August 1982.

It has been observed by Mosely et al (1) that the DNA encoding heat labile enterotoxin (LT) genes labelled with 32p deoxy nucleotides 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 spot. 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.

The results of the pilot study done under the above pilot protocol showed that by using colony hybridization technique for the detection of ETEC all the 31 LT producing test strains producing LT & ST or LT only could be detected. In that study using ST probe available we could detect 12 of 17 strains producing ST only and and 3 of 26 strains producing LT & ST. Since then a good ST probe has been developed by S. Falkow's group and we want to test this probe along with the LT probe in the study of E. coli from hospitalized and outdoor (Tc) from Dhaka and Matlab. 60(sixty) patients from the hospitals will be followed for day 1,3,7,14 and the family contacts will be swabbed on those dates. All the results will be compared by testing the strains by conventional bio-assay methods such as CHO cells for LT and infant mouse assay for ST.

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. Objectives:

The overall objective of this extended protocol is 1) To find out a reliable method for the detection of ST producing *E. coli* from patients and contacts. 2) To follow the number of ST producing *E. coli* in the stool of patients from the acute phase of disease through convalscence.

2. Background:

Current methods for the assay of Enterotoxins produced by the toxigenic *E. coli* are the biologic and immunologic assay system. The heat labile toxin is assayed by various methods (1-6) such as using CHO cells and Y_1 adrenal cells GM_1 ganglioside ELISA assay, ileal loop assay and skin assay. The heat stable toxin can only be assayed by using the infant mouse. All these methods detect the toxin itself produced by the organisms. A newer and more simpler method was worked out for the detection of enterotoxigenic Escherichia-coli by DNA colony hybridization method in which method the genes encoding the enterotoxins are detected rather than the toxin themselves (7). Radiolebelled fragments of DNA probing the heat labile or heat stable were used as hybridizstion probes for homologous DNA sequences in *E. coli* colonies grown and lysed on special nitrocellulose filters.

The methods in principle is that the strains of E. coli to be tested were inoculated into a nitrocellulose filter that had been over laid onto a suitable solid medium (MacConkey's medium in this case). After growth of the bacteria the colonies were lysed, and the DNA was denatured in situ on the nitrocellulose filter. The filter was then incubated with the radioactive probe DNA under conditions that allow duplex formation with homologous DNA sequence on the filters. They can then be exposed to X-ray film after proper washing. Enterotoxigenic strains were detected by exposure of the film over areas of the filter where the probe had annealed, an event indicaing the presence of DNA sequence homologous to toxin gene. The hybridizstion method also detected the presence of enterotoxigenic E. coli in bacterial growth in directly spotted stools from patients with acute diarrhoea.

The results with the pilot study showed that the LT probe detected all of the 31 E. coli strains producing ST and LT or only LT while the ST probe detected 12 of 17 strains producing only ST and 3 or 26 strains producing only ST and LT. The results showed the inefficacy of using ST probe. However, with further research work a better and workable ST probe has been made and in a small study done in may 1981 on about 75 patients we have found better results with ST probe: the probe picked more positive from regularly used infant mouse assay (8). A parallel study was done by one of the Investigator in Bangkok and the results were very satisfactory (9).

Though we could do about 110 hospitalized patients the follow up study in Dacca could be done on only 16 patients with the original LT probe. As ST constitutes majority of the toxin obtained we are proposing to continue the family study in Dacca and Matlab to see the a) superiority of hybridization method in the diagnosis of both ST & LT in the field over conventional methods b) suitability of the method to be used for the detection of C. plasmids in other enteropathogens.

B. <u>SPECIFIC AIMS</u>

The specific aim of the study is to find a better method for the detection of ST as well as LT from *E. coli* and other gram negative bacteria isolated from patients.

C. <u>METHODS AND PROCEDURES</u>

Two groups of patients will be studied :

<u>Group 1</u>: This group of patients numbering 60 will be followed for 3 days in the ward and approximately 15-20 of them will then be followed on day 7 and 14 in their houses. This group will include patients showing following creiteria on admission. a) Acute watery diarrhoea with moderate dehydration. b) Absence of motile vibrios in the stool under dark field microscopy. c) Absence of blood or pus cells in the stool specimen. d) Older than 5 years of age (to exclude rotavirus patient). Stool or R/S from these patients will be plated onto MacConkey and 2 lactose fermenting colonies and a pool of 5 colonies will be tested for toxigenicity

by standard assay as well as by colony hybridization technique on duplicate nitrocellulose filters. The growth on the filter will be precessed further. The family contacts of the patients will be swabbed on days 3,7 & 14. If possible 15-20 patients from Matlab hospital will be taken as index and the patient and its family members will be followed on days 1, 3, 7, 14 for the detection of ETEC.

Group-2: The second group will include 300 out-door patients from Dhaka & Matlab from whom the R/swab will be directly spotted on the nitrocellulose filter on MacConkey plates. After overnight incubation the growth on the filter will be processed in the following way along with those filters from group 1.

The nitrocellulose filters with growth of *E. coli* on it are lifted and placed on a filter paper soaked with 0.5N sod-hydroxide to lyse the cultures and soaked in 3 changes of IM Tris buffer PH 7.0 and one change with buffer+1.5 M Nacl. The filters are air dried and baked overnight at 65°C. The cells are hybridized with a specifically prepared P^{32} labelled DNA probe that are absolutely specific either for the *E. coli* LT genes or ST genes. Following hybridization the filters are washed, dried and radiographed against a piece of X-ray film.

The LT^{+} or ST^{+} colonies appear as deep black spot on the film and can be emmerated.

D. SIGNIFICANCE

The proposed study will lead us to a convenient and quicker method of detecting stable toxin and labil toxin producing *E. coli* from the stool

of diarrhoeal patients. This method will save us from the use of infant mouse (which have limitation in supply) and the cell line assays and will detect a few colonies present in the early stage of attack or at convalscent stage or carriers.

E. FACILITIES REQUIRED

1. Office Space - No Office Space will be required.
2. Laboratory Space:

Laboratory Space will be provided in Room 109 where this work was done before. In Matlab a portion of the bench space available be used for making the blots only. The P_{32} labelled DNA probe will be shipped from either New England nuclear Boston or from Dr. P. Escheviera from Bangkok through some collaborative arrangement. The half life of P_{32} is 14.4 days and it decays out later without causing any hazard. Necessary permission and badges have been obtained from Bangladesh Atomic Energy Commission for handling Radioactive materials.

3. Hospital Resources:

For the selection of patients the clinically positive E. coli patients will be cultured and kept in the hospital for 48 hours more so that stool may be cultured for second and third day before releasing the patients. A total of 40 patients will be studied in Dacca and 15-20 of them will be followed in day 7 & 14. In Matlab 30 cases will be chosen and of them 15 will be followed in their houses for themselves as well as for family members for 2 weeks.

4. Animal Resources:

1400 infant mouse will be needed to test 700 strains isolated from these cases contacts. These animal will be required in 3 batches for test.

5. Logistic Support:

For the follow up of patients in their houses on day 7 & 14 the services of field-worker will be obtained. In Matlab one boat for 1-2 hour per day will be needed for 20-30 days .

6. Major Item of Equipment: none

F. COLLABORATIVE ARRANGEMENTS:

This is a collaborative research programme between Dept. of Microbiology, University of Stanford and Dr. P. Echeverria of Armed Forces Research Institute of Medical Sciences, Bangkok. Prof. S. Falkow of University of Stanford will act as Consultant for the study. Dr. P. Echeverria will come to Dacca to set up the technique. Arrangements will be made for shipment of P_{32} labelled DNA probe either from Bangkok or from Boston, U.S.A. Initially the filter blots will be tested in Bangkok till a supply can be obtained in Dacca.

REFERENCES

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

NAME	POSITION	% OR NO. OF DAY	ANNUAL SALARY	PROJECT REQUIREMENT TAKA	DOLLAR
Prof. S. Falkow	Consultant	-	-	-	-
Dr. P. Echevierra	Vistg. Prof.	1 month	-	-	-
Dr. M.I. Huq	Scientist	10% 6 months	$ 53,740	-	2,687
Mr. Q.S. Ahmed	Sr. Res. Off.	25% 6 months	Tk.75,180	9,400	-
Mr. A. Alim	Sr. Res. Off.	25% 6 months	Tk.75,980	9,500	-
Dr. P. Bardhan	Physician	15% 4 months	Tk.81,060	4,050	-
Dr. A. Baqui	Physician	15% 2 months	Tk.65,940	3,300	-
To be named	Field Assistant Dacca	15% 3 months	Tk.25,000	1,000	-
To be named	Field Assistant Matlab	10% 2 months	Tk.25,000	500	-
To be named	Nurse	25% 4 months	Tk.42,480	3,540	-
				31,290	2,687

2. SUPPLIES AND MATERIALS

Item		Taka	Dollar
Media		2,000	-
Glasswaires, Petridishes		1,500	-
Nitrocellulose Filter		-	800
X-ray Film		-	300
ST & LT assay	700 x 14	9,800	
		13,300	1,100

3. EQUIPMENT - None

	PROJECT REQUIREMENT	
	TAKA	DOLLAR
4. PATIENT HOSPITALIZATION		
50 patient for 2 days (50 x 2 x 150)	15,000	-
5. OUTPATIENT CARE - None		
6. ICDDR,B TRANSPORT		
Land Transport 6 Trips (Dacca-Matlab-Dacca)	2,700	-
ICDDR,B Vehicle - 500 miles x 4	2,000	-
Speed Boat (30 hours)	3,000	-
	7,700	-
7. TRAVEL AND TRANSPORATION OF PERSONS		
Air-Ticket	-	1,500
8. TRANSPORTATION OF THINGS		
P_{32} labelled DNA	-	500
9. RENT, COMMUNICATION AND UTILITIES		
Guest House Charge & per diem	-	1,200
10. PRINTING AND REPRODUCTION	1,000	-
11. OTHER CONTRACTUAL SERVICES - None		
12. CONSTRUCTION, RENOVATION AND ALTERATION - None		

B. BUDGET SUMMARY

			TAKA	DOLLAR
1.	Personnel Services	-	31,290.00	2,687.00
2.	Supplies and Materials	-	13,300.00	1,100.00
3.	Equipment	-	-	-
4.	Patient Hospitalization	-	15,000.00	-
5.	Outpatient Care	-	-	-
6.	ICDDR,B Transport	-	7,700.00	-
7.	Travel and Transportation of Persons	-	-	1,500.00
8.	Transportation of Things	-	-	500.00
9.	Rent, Communication and Utilities	-	-	1,200.00
10.	Printing and Reproduction	-	1,000.00	-
11.	Other Contractual Services	-	-	-
12.	Constraction, Renovation and Alteration	-	-	-
		Sub-Total :	68,290.00	6,987.00

Grand Total : US$ 10,401.00

Conversion US$ 1.00 = Tk. 20.00

Personnel cost $ 4,251.50

Other Cost $ 6,149.50