ETHICAL REVIEW COMMI[illegible] ICDDR,B Library Date
Dacca—12

ıcipal Investigator DR. IVAN CIZNAR
ication No 84-031
le of Study USE OF BIOTINYLATED DNA
BES FOR DETECTION OF ENTEROTOXIGENIC
COLI

Trainee Investigator (if any) 22
Supporting Agency (if Non-ICDDR,B)
Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

le 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 NA
(c) Minors or persons under guardianship Yes No NA

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

re 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 Stool cultures will be used in this study.

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

Ivan Ciznar 12.6.1984
Principal Investigator

19 JUL 1984

Trainee

84-031
Rec'd 17/7/84

SECTION-I - RESEARCH PROTOCOL

1. TITLE : Use of biotinylated DNA Probes for detection of enterotoxigenic E. coli

2. PRINCIPAL INVESTIGATOR : Dr. Ivan Ciznar

   CO-INVESTIGATORS : Dr. T. C. Butler
   Dr. M. I. Huq
   Dr. C. Schuster (guest scientist)

3. STARTING DATE : September 1984

4. COMPLETION DATE : November 1984

5. TOTAL DIRECT COST : US$ 4,854

6. SCIENTIFIC PROGRAM HEAD : Dr. T. C. Butler

This protocol has been approved by the HOST DEFENSE WORKING GROUP

Signature of the Program Head : T. C. Butler

Date : June 12, 1984

7. ABSTRACT SUMMARY

One hundred fresh stool isolates of E. coli from diarrhoeal patients at ICDDR,B will be tested for LT and ST by standard assays. These isolates will also be tested with biotinylated DNA probes made by Dr. Schuster from clones of LT, ST-I, and ST-II. The sensitivities and specificities of the new probes will be assessed. The potential advantages of these non-radioactive DNA probes are their stability at 4°C for 12 months, their great sensitivity, their speed of detection in 4 hr, and avoiding the hazards of exposure to radioactivity.

8. REVIEWS

A. Research Involving Human Subjects: _____

B. Research Review Committee : _____

C. Director : _____

SECTION-II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives

a) To compare the sensitivities and specificities of new biotinylated DNA probes encoding LT, ST-I, and ST-II against the standard assays for LT and ST.

b) To consider whether these non-radioactive probes are more suitable for diagnostic use in developing countries than the radioactive probes.

2. Background

Enterotoxigenic E. coli (ETEC) is one of the most common causes of diarrhoea in Bangladesh. The currently used methods of detection are cumbersome: LT is detected in culture supernatants by observing morphological changes in tissue cultured Y-1 adrenal cells or Chinese hamster ovary cells and ST is detected in culture supernatants after inoculation into infant mice.

Hybridization of radioactive DNA (P-32) probes with E. coli has been tested as a means of detecting ETEC and has been evaluated in Bangladesh. Although this method showed promising results, the short half-life of P32 requires new batches of isotopes and probes every 2 weeks. Thus, there is a need for a non-radioactive method of DNA probes. One approach has been to incorporate biotin into DNA and to use either the chemical reaction with avidin to detect it or to use anti-biotin fluorescent antibody to detect it (1-2). This labeling of DNA is accomplished by attaching a stable non-radioactive biotin

molecule to dUTP. The biotinylated dUTP is incorporated into specifiec DNA and forms a normal unpreturbed helix upon hybridization. Detection of the biotinated DNA probe is visualized by a fluorescent antibody utilizing goat anti-biotin as the primary antibody. The advantages of this system are:

1. Biotinylated DNA probes are stable for 12 months at $4^{o}C$, therefore, assuring continuity, reproductibility, and reliability in future experiments.

2. Visualization can be accomplished within hours.

3. Picogram quantities of DNA can be detected.

4. Replacement of expensive p^{32}, along with its accompanying radioactive hazard and waste disposal problems.

This system is manufactured by ENZO BIO-PROBE SYSTEM and comes complete with a nick translation kit and a detection kit.

3. Rationale

Detection of diarrhoeal pathogens in developing countries requires rapid, reliable, and economical methods. ETEC are common causes of diarrhoea but diagnostic methods are slow, cumbersome, and expensive. Radioactive DNA probes are promising but requires frequent resupply because of the short half-life. The biotinylated probes offer advantages and should be field-tested.

B. SPECIFIC AIMS

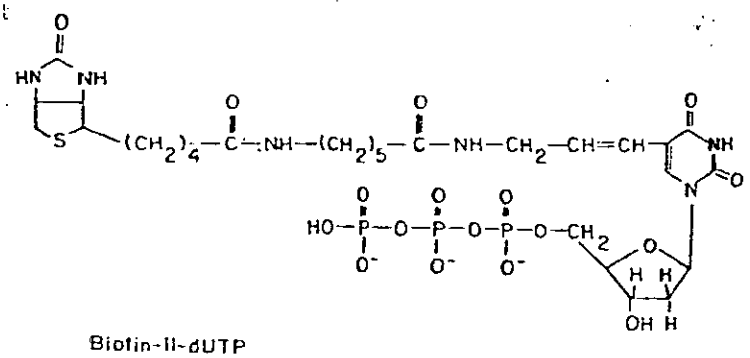
1. To obtain 100 strains of E. coli from diarrhoeal patients and test them with biotinylated probes for LT, ST-I, and ST-II. Sensitivities and specificities will be examined against the CHO test for LT and infant mouse for ST and compared with that of P^{32} DNA probes.

2. The practical matters of using these probes in developing countries will be considered and weighed against advantages of alternative methods.

C. METHODS OF PROCEDURE

1. Strains: In 100 stool cultures at ICDDR,B showing moderate or heavy growth of E. coli, 2 colonies per plate will be picked. They will be tested for LT in the Y-1 adrenal cell and/or CHO cell assays and for ST in the infant mouse assays as performed routinely at ICDDR,B.

2. Biotinylated probes: Dr. C. Schuster from Case Western Reserve University will bring the probes to Dhaka and work for a month in the lab to set up the methods. Briefly, biotin is covalently attached to deoxyuridine triphosphate (dUTP) via the C-5 position of the pyrimidine ring through an allylamine spacer arm. The biotinylated dUTP has an eleven atom spacer arm (Bio-II-dUTP). This arm length has been shown to be most effective in the applications described below.



Biotin-II-dUTP

(2'-DEOXYURIDINE TRIPHOSPHATE 5-ALLYLAMINE-BIOTIN)

The Bio-II-dUTP substitutes for thymidine triphosphate (TTP) in a standard nick translation reaction catalyzed by E. coli DNA polymerase 1. Biotinylated DNA probes hybridize at the same rate and to the same extent as non-biotinylated DNA probes. Detection of Bio-Probes may be performed using either fluorescent antibody or by enzymatic method. The fluorescent visualization utilizes an IgG fraction goat antibiotin and an FITC labeled rabbit anti-goat. This is applied for the visualization of specific DNA or RNA sequences in fixed cells of tissues following insitu hybridization. Detection, upon completion of hybridization, requires less than two hours. Detection may also be done enzymatically using a soluble complex of biotinylated horseradish peroxidase and streptavidin (SA). SA, a biotin binding protein produced by Streptomyces avidinii, is similar to egg white avidin but SA does not bind to DNA nonspecifically. It can be used for either insitu detection or visualization on nitrocellulose paper. This procedure will be used for purpose of this protocol.

3. Data analysis: The 100 strains of E. coli will be tabulated for their results in tests for ETEC and probe results. Sensitivities and specificities will be calculated.

D. SIGNIFICANCE

New diagnostic methods, like biotinylated DNA probes for LT and ST, may offer advantages over the currently used bioassays and over the P^{32} DNA probes. If they are shown to be promising, they could become useful tools for both clinical and epidemiological studies.

E. FACILITIES REQUIRED

The Microbiology Branch and Animal House have adequate facilities to support this pilot study.

F. COLLABORATIVE ARRANGEMENTS

Dr. Schuster of the Microbiology Department at Case Wester Reserve University will obtain leave for a month to travel to Bangladesh. He will make the probes and bring them with him. He will teach ICDDR,B scientists these techniques.

REFERENCES

1. Langer PR, Waldrop AA, and Ward DC. Enzymatic synthesis of biotin-labeled polynucleotidies: novel nucleic acid affinity probes. Proc Natl Acad Sa 78:6633, 1981

2. Singer RH, Ward DC. Actin gene expression visualized in chicken muscle tissue culture by using insitu hybridization with a biotinylated nucleotide analog. Proc Natl Acad Sci 79:7331, 1982

SECTION-III - BUDGET

A. DETAILED BUDGET

PERSONNEL SERVICES

Name	Designation	% effort	Taka	Dollar
Dr. I. Ciznar	Pr. Investigator	20% 1 month		1,170
Dr. T. C. Butler	Consultant	-		
Dr. C. Schuster	Guest Scientist	-		
Dr. M. I. Huq	Consultant	-		

SUPPLIES AND MATERIALS

			Cost/Test		
A.	Stool cultures	100	@ 10.00	1,000	
	LT test	200	@ 21.50	4,300	
	ST test	200	@ 21.50	4,300	
B.	Supplies for Probes			5,000	

EQUIPMENT	None		
PATIENT HOSPITALIZATION	None		
OPD CARE	None		
ICDDR,B TRANSPORT	None		
TRAVEL	Dr. Schuster round-trip Cleveland-Dhaka		2,200
	Dr. Schuster guesthouse 30 days at $30		900
TRANSPORTATION OF THINGS	None		
RENT AND COMMUNICATION	None		
PRINTING AND REPRODUCTION	None		
CONSTRUCTION	None		
OTHERS	None		
GRAND TOTAL		14,600	4,270

Conversion rate: US$ 1 = Taka 25
Tk.14,600 = US$ 584 584

TOTAL: 4,854

B. BUDGET SUMMARY

			US DOLLARS
1.	PERSONNEL SERVICES	...	1,170
2.	SUPPLIES AND MATERIALS	...	584
3.	EQUIPMENT	...	0
4.	PATIENT HOSPITILIZATION	...	0
5.	OPD CARE	...	0
6.	ICDDR,B TRANSPORT	...	0
7.	TRAVEL	...	3,100
8.	TRANSPORTATION OF THINGS	...	0
9.	RENT AND COMMUNICATION	...	0
10.	PRINTING AND REPRODUCTION	...	0
11.	CONSTRUCTION	...	0
12.	OTHERS	...	0
	TOTAL		4,854