Attachment 1. Date Nov 6, 1980

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. T. Maeda, Mr. M. I. Huq, Dr. L. Glass Trainee Investigator (if any) 300

Application No. 80-045 Supporting Agency (if Non-ICDDR,B) _____

Title of Study ELEK TEST FOR Project status:
Ewki LT Toxin - ; Field Evaluation
( ) 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).

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)

not applicable

5. Will signed consent form be required:
(a) From subjects Yes (No)
(b) From parent or guardian (if subjects are minors) Yes (No)
} NA

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) Pilot
✓ 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.

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

Principal Investigator Trainee

80-045
Received
4-12-80

SECTION 1 - RESEARCH PROPOSAL

1. Title: Evaluation of a Modified Elek Test for Detection of Heat Labile LT Toxin of Enterotoxigenic E. coli in Field Laboratories

2. Principle Investigator: Dr. Takeshi Honda, Mrs. S. Qudsiya Akhtar

   Co-Investigator: Dr. Roger Glass

3. Starting Date: Dec 12, 1980

4. Completion Date: April 30, 1981

5. Total Direct Cost:

6. Availability of Funds:

7. Scientific Program Head:

This protocol has been approved by the DTWG

Signature of Scientific Program Head: H Banaly

Date: 24/11/80

8. Abstract Summary:

Testing E. coli for LT toxin has important clinical and epidemiologic application in the field but current methods require sophisticated tests and techniques. Recently, modification of the classic Elek test has been developed which would be applicable for field use since it is cheap, requires no special equipment and can be performed by an unskilled technician. We propose to bring this test to Dacca for field trial. For one month, all specimens tested for LT by CHO cell assay (approximately 500) will be run simultaneously by the new Elek test. Similarly, 300 selected stock strains of known toxin type will be tested using the Elek test. The validity (i.e. sensitivity and specificity) of the new test will be compared with CHO cell assay and false positives or negatives will be examined to determine the source of error. A cost benefit analysis of several LT test methods will be performed and a simple laboratory manual for use of the Elek test will be prepared.

9. Review:

(a) Ethical Review Committee _____

(b) Research Review Committee _____

(c) Director _____

(d) BMRC: _____

(e) Controller /Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: To field test a new Elek method for the detection of LT toxin of enterotoxigenic E. coli. To evaluate the sensitivity and specificity of this test compared to the CHO cell assay and to determine its relative costs and benefits compared to other tests of LT toxin.

2. Background & Rationale: Enterotoxigenic E. coli produces two distinct enterotoxins: one is heat labile (LT), high molecular weight and antigenic, and the other is heat-stable (ST), low molecular weight and non-antigenic. Both have been considered to be responsible for diarrhea in man and cattle. For detection of LT, various assay methods have been developed including the ileal loop test, vascular permeability test, CHO cell assay, Y-1 adrenal cell assay, passive immune hemolysis, reverse passive hemagglutination, staphylococcal coagglutination method, radio immunoassay, and ganglioside GM1 enzyme-linked immunosorbent assay. Many of these assay methods are unsuitable for routine clinical purposes because they need special materials and techniques such as large numbers of animals, stocks of special tissue culture cells, and radioisotopes. To develop a simple and reproducible assay method which could be widely used in clinical laboratories, a modification of the classic Elek test has been made for detection of LT produced by enterotoxigenic E. coli.

Initial study of this test in Osaka showed that it had good specificity and sensitivity in 166 specimens tested by simultaneous CHO cell assay. The test has several distinctive advantage for the field laboratory: it is 1) relatively inexpensive 2) can be performed without complicated equipment on petri dishes and 3) requires a minimum of technical skill.

Full details of the test are included in the attached manuscript to appear in the Journal of Clinical Microbiology, Jan 1981, entitled A Modified Elek Test for Detection of Heat Labile Enterotoxin of Enterotoxigenic E. coli by T. Honda et al.

B. SPECIFIC AIMS

1. To compare the sensitivity and specificity of the Elek test for LT toxin with the CHO cell assay.

2. To compare the relative costs and benefits of the Elek test with other tests of LT toxin.

3. To prepare a simple manual for field workers interested in performing the Elek test.

C. METHODS AND PROCEDURES

1. Time Period: The study will begin in mid December and run for 6 weeks with a final analysis and written up done in 6 months.

2. Source of Samples: All specimens of E. coli sent for LT testing for 4-6 weeks will be tested simultaneously using the Elek test. Likewise, 300 stock ETEC strains of known toxin type from the lab will be retested with the Elek test. Results that are discrepant will be retested using both methods to identify the reason for the difference.

3. Modified Elek Test: Strains to be examined are inoculated onto agar plates and incubated for about 40 hours at 37°C. A paper disc 6mm in diameter soaked in 25µl of polymyxin B solution (10,000 IU/ml) is placed on a colony formed on the plate and incubated for several hours at 37°C. After incubation, 50µl of purified antiserum against cholera enterotoxin diluted 4 fold containing 1% Na N_3 is placed in a 4 mm well made 5 mm from the colony to be tested. A precipitin line occurs against 2.5 µg of purified cholera enterotoxin on the agar and is read as positive.

4. Analysis: The sensitivity and specificity of the Elek test will be compared with the CHO cell assay using standard methods. The relative costs, advantages, special reagents, special skills and equipment for Elek, CHO, Y-1 adrenal cell, Passive Immune Hemolysis, Radioimmunoassay, Elisa, and Staphylococcal coagglutination methods for testing ETEC - LT toxin will be compared and discussed.

5. Training: Several laboratory technicians will be trained to use

the Elek test and a simple field mannual will be prepared.

D. SIGNIFICANCE

If the Elek test is found to be simple to perform and reliable, it would be very useful for field laboratories without cell culture techniques, radioisotope or elisa facilities. WHO has a specific interest in simple diagnostic methods for diarrheal disease and this test might be considered among these simple diagnostics.

E. FACILITIES REQUIRED

1. Office space - No additional space required.

2. Laboratory Space - One desk top area (2x10) for 6 weeks.

3. Hospital resources - None

4. Animal resources - None

5. Logistic Support - Coordination of testing with regularly scheduled LT testing will be easy to facilitate.

6. Major items of equipment:

The single antiserum required for use in this test is not now commercially available. For this work and for future work with this reagent at ICDDR,B arrangements will be made to supply this from the Institute of Microbiology in Osaka. If this test proves

itself under field conditions, reagents would become available either through an international mechanism such as WHO or through a private enterprise.

F. COLLABORATIVE AGREEMENTS

This study will be performed in collaboration with Dr. Takeshi Honda, Department of Bacteriology and Serology, Research Institute for Microbiol Diseases, Osaka University.

Dr. Honda will bring with him supplies for the Elek tests and will make reagent available to ICDDR,B by mutual agreement when these studies are complete.

G. FOR REFERENCES AND FURTHER BACKGROUND, SEE ATTACHED MANUSCRIPT.

ABSTRACT SUMMARY

Testing E. coli for LT toxin has important clinical and epidemiologic application in the field but current methods require sophisticated tests and techniques. Recently, modification of the classic Elek test has been developed which would be applicable for field use since it is cheap, requires no special equipment and can be performed by an unskilled technician. We propose to bring this test to Dacca for field trial. For one month, all specimens tested for LT by CHO cell assay (approximately 500) will be run simultaneously by the new Elek test. Similarly, 300 selected stock strains of known toxin type will be tested using the Elek test. The validity (i.e. sensitivity and specificity) of the new test will be compared with CHO cell assay and false positives or negatives will be examined to determine the source of error. A cost benefit analysis of several LT test methods will be performed and a simple laboratory manual for use of the Elek test will be prepared.

This is a laboratory based study requiring only bacteria isolates and stock cultures, no patient information. Patients will not be involved, patient information will not be collected, and there should be no risks, interviews or problems of confidentiality.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	No of Days	Project Requirements Taka	Dollar
Dr. T. Honda	Investigator	100%	-	-
Mrs. S. Q . Akhtar	Co-Investigator	20%	3000	
Dr. Glass	Co-Investigator	5%		$300
	Research Assistant	50%	4030	

2. SUPPLIES AND MATERIALS

300 Mackonkey plates @2.50/plate	600
Retest 100 LT isolates @ 3/test	300

3. EQUIPMENT - None

4. PATIENTS HOSPITALIZATION - None

5. OUTPATIENT CARE - None

6. ICDDR,B TRANSPORT - None

7. TRAVEL AND TRANSPORTATION OF PERSONS -

Dr. Honda - travel Osaka - Dacca return	$1300
Lodging - Guest House - 40days @$20/day	800

8. TRANSPORTATION OF THINGS - None

9. RENT,COMMUNICATION AND UTILITIES - None

10. PRINTING AND REPRODUCTION - None

11. OTHER CONTRACTUAL SERVICES - None

12. CONSTRUCTION, RENOVATION, ALTERATIONS - None

B. BUDGET SUMMARY

CATEGORY	Taka	Dollars
1. Personnel	7030	350
2. Supplies and Materials	900	-
3. Equipment	-	-
4. Hospitalization	-	-
5. Outpatients	-	-
6. ICDDR,B Transport	-	-
7. Travel - Persons	-	2100
8. Transportation - Things	-	-
9. Rent Communication	-	-
10. Printing Reproduction	-	-
11. Contractual Services	-	-
12. Construction	-	-
Total:	7930	2450
	$(511.61)	
Grand Total :	$2961.61	