60

REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS Date 18 Oct 1977
CRL

Principal Investigator MICHAEL MERSON Trainee investigator(if any) _____

Application No 77-031 Supporting Agency(if Non-CRL) _____

Title of study ENTEROTOXIGENIC AND ENTEROPATHOGENIC COLI - SEROTYPES, ANTIBIOTIC SENSITIVITY AND DISEASE

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):

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 possibly 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

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 Board for review.

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

Michael D. Merson
Principal Investigator

_____
Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human

Received 26/10/77-031

SECTION 1 - RESEARCH PROTOCOL

1) Title: Enterotoxigenic and Enteropathogenic Escherichia coli: Serotypes, Antibiotic Sensitivity and Disease.

2) Principle Investigator: Michael H. Merson
Collaborating Investigator: Bernard Rowe, Division of Enteric Pathogens, Central Public Health Laboratories, Colindale, London.

3) Starting Date: October 24, 1977.

4) Completion Date: March 1, 1979.

5) Total Direct Cost:

6) Abstract Summary:

Stools from 1248 persons admitted in a 1 year period to the CRL Hospital with acute watery diarrhea will be examined for recognized enteric pathogens, including enterotoxigenic E. coli (ETEC), the classical enteropathogenic E. coli (EPEC), rotavirus and parvovirus (Norwalk Agent). Clinical features of illness associated with these organisms will be described. To better determine the importance of ETEC and EPEC as etiologic agents of diarrheal disease, the incidence of these organisms in this population will be compared to the incidence in persons of the same age admitted to another Dacca hospital with non-gastrointestinal illnesses.

The serotypes of ETEC isolated from approximately 380 patients in the study will be compared with serotypes of non-enterotoxigenic E. coli (non-ETEC) isolated from 862 patients with diarrhea of another etiology and 386 persons admitted to another Dacca hospital with non-gastrointestinal illnesses. The efficacy of a newly prepared pools of antisera against specific O groups believed to be most commonly associated with ETEC strains will be determined. Antibiotic sensitivity of ETEC and non-ETEC isolates will be compared.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: To determine (1) serotypes, seasonal occurrence, antibiotic sensitivity and etiologic significance of ETEC in Dacca. (2) serotype incidence, seasonal occurrence, antibiotic sensitivity and etiologic significance of EPEC in Dacca. (3) clinical features of disease associated with ETEC, EPEC, rotavirus and parvovirus (Norwalk Agent).

2. Background:

Enterotoxigenic E. coli - Since the initial reports of Gorbach et al (1) and Sack et al (2) in 1971 from Calcutta, studies conducted in many different geographic locations (3-14) have demonstrated the importance of ETEC as a diarrheal pathogen. Three of these studies documented these organisms to be responsible for cholera-like diarrhea in hospitalized adults in urban (5, 8) and rural (6) Bangladesh. In 2 as yet unpublished studies conducted in 1976 Black et al isolated these organisms from stools of 24% of children visiting the CRL hospital OPD with watery diarrhea in January and February, while Merson et al found them in 62% of male adults admitted to the CRL hospital in October - December with severe, acute, watery, non-cholera diarrhea. This latter stud also showed that diagnosis of ETEC could be made in 92% of ETEC infected hospitalized cases by testing only 1 isolated colony from the stool culture of each patient for toxigenicity. Testing of 2 colonies increased the diagnostic yield to 95%. This finding was in contrast to that of Black et al who found that in outpatients with less severe diarrhea diagnosis was made in only 45% of cases with testing of 1 colony and 55% with testing of 2 colonies for toxigenicity.

Strains of ETEC can produce heat-labile enterotoxin (LT only strains), heat stable enterotoxin (ST only strains), or both (LT-ST strains). The

study by Merson et al showed that in hospitalized adults in Bangladesh (a) LT-ST strains are most commonly and LT only strains least commonly isolated (b) patients infected with LT-ST strains have longer duration of diarrhea and greater stool volume and fluid requirement than those infected with ST only strains and (c) ST only strains have more antibiotic resistance than LT-ST strains. Insufficient number of LT only cases occurred in that study to compare the clinical course of LT only disease and the antibiotic resistance of LT only organisms with that of the other 2 groups.

The property of toxin production by E. coli is plasmid mediated (15, 16) and was originally believed to be unrelated to serotype. However, evidence has begun to accumulate that indicates that the property of enterotoxigenicity may be restricted to certain O groups (11, 17). Data from the study done by Merson et al suggests that this restriction appears to exist in Dacca, Bangladesh for LT-ST strains, but perhaps not for ST only or LT only strains (table 1). However, this latter study was done over a 3 month period in only adult patients and thus these results require confirmation. Documentation of any such restriction is critical to the development of serologic pools which would allow quick and simple diagnosis of ETEC disease. Two such pools have been prepared by Dr. Bernard Rowe at the Central Public Health Laboratory, London for field testing by laboratories worldwide. The usefulness of these pools requires additional information on the serotypes of non-ETEC strains. In this regard it has been shown that in healthy individuals resident strains of E. coli consisting of 1 or 2 serotypes are normally present (18, 19) and can be detected from analysis of 5 colonies.

Enteropathogenic E. coli - In the early 1950's the concept evolved that certain serogroups of E. coli, commonly designated as enteropathogenic,

were inherent enteric pathogens. The data supporting this concept was obtained primarily from outbreaks of diarrhea in children less than 2 years of age in the United States and Europe (20). For the most part investigators limited these serogroups to up to 15 0 groups (18, 26, 44, 55, 86, 111, 112, 114, 119, 124-128, 142) and believed these organisms caused disease primarily in children (21).

In the late 1950's and ealy 1960's evidence began to accrue that questioned the inherent virulence of these organisms, particularly in sporadic cases of diarrheal disease (22). This issue remained unresolved, although most pediatricians and microbiologists advocated serotyping of E. coli to look for these strains and attributed illness to them when they were isolated. With the recent discovery of ETEC, studies of EPEC have been again initiated worldwide with the objective of defining the importance of these organisms as pathogens. Although some evidence has recently been presented to validate these organisms as etiologic agents (23) and a possible pathogenic mechanism has been proposed (24), the question of their significance remains controversial. It is clear that organisms of at least one of the EPEC serogroups (0128) (table 1) are not infrequently enterotoxigenic (25). To date there has been only one study of the incidence of EPEC in Bangladesh, (I. Huq, unpublished). The results of this study were not definitive, although they suggested these organisms were etiologically significant in children.

5. Rationale: Many cases of diarrhea in infants and children in Bangladesh remain undiagnosed. Since diarrheal illness is the leading cause of mortality in this age group, it is important to define the responsible agents so that appropriate intervention measures can be implemented. For this reason a study to determine the relative importance and associated illness of ETEC and EPEC is warranted. As part of this evaluation it will be

necessary to define the incidence of other etiologic agents of diarrheal disease, including 2 newly recognized ones, rotavirus and parvovirus (Norwalk Agent).

ETEC has been shown to be an important cause of diarrhea in Bangladesh; precise definition of the serogroups associated most commonly with toxin production will be very helpful in laboratory diagnosis of diarrheal illnesses caused by these organisms. The relatively high incidence of disease caused by these organisms allows us the unique ability to define any relationship between toxin type and clinical severity and/ or antibiotic resistance patterns:

B. SPECIFIC AIMS

1. To determine the most common serotypes of the 3 toxin types of ETEC.
2. To determine the incidence of these serotypes in persons with diarrhea of another (or unknown) etiology and persons without diarrheal disease.
3. To evaluate the utility of a newly prepared antisera against specific O groups in identifying ETEC.
4. To compare the clinical spectrum of disease caused by the 3 toxin types of ETEC.
5. To compare the antibiotic sensitivity patterns of the three ETEC types.
6. To compare the incidence of EPEC in persons with and without diarrheal disease.
7. To describe the clinical features of illness associated with isolation of EPEC and compare them with clinical features of other diarrheal diseases including viral gastroenteritis.

C. METHODS OF PROCEDURE

1. Time Period - The study will be carried out for one year (approximately October 24, 1977 - October 24, 1978). Additional 6 months will be required for data analysis.

2. CRL Hospital Study

a) Population - All persons who present to the CRL hospital during this time period who fit the following definition will be eligible for inclusion in the study:

Male or female:

History of acute, watery diarrhea with onset within 36 hours of admission;

No history of antibiotic usage within 48 hours prior to admission;

Moderate or severe dehydration (on physical exam loss of at least 5% body weight);

No concomitant illness including severe malnutrition;

Admission darkfield examination of stool negative for Vibrio cholerae. (N.B. For hospital surveillance purposes persons initially selected for study who are darkfield positive will be cultured for V. cholerae).

Persons fitting this case definition will be stratified into 3 age groups: <2 years, 2-9 years, ≥10 years. For each age group 8 persons will be selected for study each week (i.e. 1-2 persons per day in each age group) using a random selection procedure.

b) Bacteriology Procedures

Isolation Procedures - Rectal swabs will be obtained from all study persons and plated on Monsur's, TCBS, SS and MacConkey agars. One swab also will be innoculated into 5cc Phosphate buffered Saline (PBS) for rotavirus and parvovirus (see Virology Procedures). The agar plates will be examined for Vibrios, Salmonellae and Shigellae. After 18-24 hours incubation at 37° two lactose-positive (LP) colonies that are the predominant colony type and are typical of E. coli will be selected from each MacConkey plate and inoculated to nutrient agar slants for further study as described below. In addition 1 lactose-

negative (LN) colony will be selected for the same analysis from plates in which there is 2+ or greater growth of these organisms and these organisms are found not to be shigellae or salmonellae.

Identification of enterotoxigenic E. coli

Slide agglutination - These 2 (or 3 if a LN is included) colonies from each patient will be tested for slide agglutination in 2 pools supplied by Dr. Bernard Rowe, Central Public Health Laboratory, London. These pools contain antisera against 0 groups that are thought to possibly represent a great majority of ETEC 0 groups (Pool I-0 Groups 6, 27, 78, 148, 159; Pool II-0 Groups 8, 15, 20, 25, 63, 115).

Toxin Testing - The same colonies will be tested for LT production by the Chinese Hamster Ovary (CHO) Cell Method (26) and for ST by the infant mouse assay (27) as modified by Morris et al (28). Each isolate will be grown in 13 x 50 mm tubes containing 2 ml trypticase soy broth with 0.6% yeast extract on a roller drum (22 revol/min) for 18-24 hours at 37°C as recommended by Wachsmuth et al (29).

Identification of enteropathogenic E. coli - These colonies will be tested for direct slide agglutination in 3 pools containing antisera against the 0 groups believed associated with EPEC disease. These pools will be provided by Dr. Rowe (Pool I -0Groups 26, 55, 111, 119 126; Pool II - 0 Groups 86,114,125,127,128: Pool III 0 Groups 18ac, 44 112ac, 124, 142.

Species Identification - Biochemical confirmation tests and 0, H and K (if possible) serotype determination of E. coli will be carried out by Dr. Rowe on the following isolates:

1. One or more isolates (at least one LP and one LN) from each patient who has E. coli that are toxin positive.
2. One or more isolates (at least one LP and one LN) from each patient who has E. coli that are toxin negative, ETEC pool

positive.

3. One or more isolates (at least one LP and one LN) from each patient who has E. coli that are EPEC pool positive.
4. One or more isolates (at least one LP and one LN) patients whose E. coli are toxin negative, ETEC pool negative, EPEC pool negative.

Antibiotic Sensitivity - The antibiotic sensitivity pattern of 75 ETEC isolates of each toxin type, 75 EPEC isolates, and 75 non ETEC, non EPEC isolates will be determined using the Kirby-Bauer method (31). Antibiotics to be tested will include triple sulfa, streptomycin, tetracycline, ampicillin, chloramphenicol, cephalothin, kanamycin, gentamicin, naladixic acid, and trimethoprim - sulfamethoxazole. An attempt will be made to select strains of the same serogroup from each of these types of organisms.

c) Virology Procedures - Swabs collected in PBS will be examined for rotavirus by the ELISA assay (32) at CRL and for parvovirus by a new radio-immune assay at the U.S. National Institutes of Health.

d) Clinical Assessment - Clinical feature of all male study patients (4 per week in each of the 3 age groups) will be systemically studied. Upon admission they will be queried using a standard form about gastrointestinal symptoms, illness duration and severity, and history of cholera vaccination and will receive a complete physical examination. Admission and discharge weight and height will be determined.

On admission serum will be obtained for measurement of specific gravity, electrolytes, BUN, osmolarity, protein and acute serologic determinations (this will only be performed if blood can be drawn from the antecubital vein; otherwise a fingerstick exam will

be done for specific gravity and acute serology). Four hours after admission a CBC and repeat specific gravity test will be done from a finger stick specimen. Stool will be obtained on admission by rectal catheter for determination of stool electrolytes and osmolarity, fecal leukocyte counts, and parasites. An aliquot of stool will be saved for future diagnostic studies. Nasal washings will be obtained from every other patient in the less than 2 year age group from November, 1977 - February, 1978 and examined for rotavirus by the ELISA assay.

During the first four hours after admission, all patients will receive replacement fluid intravenously using the standard "Dacca Solution". Patients will receive additional electrolyte fluid to match stool losses as determined every four hours. Intravenous electrolyte fluid will be given only. Additional drinking water will be allowed ad lib. A standard diet will be given as tolerated after 12 hours except milk will be excluded for 48 hours. Antibiotic therapy will not be given unless clinically indicated.

All study patients will remain hospitalized at least 24 hours after the end of the last four hour period in which a liquid stool is passed. Cessation of diarrhea will be defined as the absence of liquid stool for 2 four hour intervals. While in hospital the following parameters will be measured in 4 hour intervals:

(1) stool volume;

(2) duration of diarrhea, vomiting, fever, or other signs or symptoms.

(3) IV fluid requirement;

(4) urine output.

All persons will be asked to return 10-14 days after admission for the purpose of obtaining a convalescent serology by venopuncture from antecubital vein or by fingerstick. Taka payment will be given for travel expenses.

It will be important in the data analysis to compare the clinical features of patients in each etiologic group with those of cholera. I am assuming that such data is being collected by Dr. Sirajul Islam in Protocol No. 77-018. If additional cholera cases are needed a clinical assessment will be made of darkfield-positive patients. Females included in the study will intitally be given standard hospital management and will not receive further study. When a study ward is established in which they can be studied, they will undergo the same observations as described for male study patients.

e) Serology Procedures - As described acute and convalescent sera will be obtained from study patients. This sera will be divided into 2 aliquots. One aliquot will be used for measurement of antibody titers in patients in whom an etiologic diagnosis is not found from the bacteriologic and virologic studies. This will include measurements of antibody to E. coli labile toxin by the CHO assay and to rotavirus by the ELISA assay (33). The second aliquot will be stored for subsequent antibody determinations to known etiologic agents for which an antibody test is not presently available (e.g. ST toxin, parvovirus) and to agents which will be subsequently discovered.

3. Dacca Medical College Study (control group)

a) Population: All persons admitted to the Dacca Medical College medical, surgical and pediatric wards during the same time period and fitting the following definition will be eligible for study:

a) Male or female;

No history of any gastrointestinal illness within 1 week of hospitalization;

No history of antibiotic usage within 1 week of admission;

Duration of hospitalization less than 24 hours;

No serious protein calorie malnutrition;

Living in Dacca or vicinity.

From this population 1248 persons will be selected for study as a control for each person admitted into the CRL hospital study. This control will be matched for age group and sex and selected approximately 1-2 days after admission of the CRL case.

b) Bacteriology Procedures

Isolation

The same procedures used in the CRL hospital study for identification of vibrios, shigella, salmonella, rotavirus, parvovirus and for selection and storage of five LP organisms will be followed.

Identification of enterotoxigenic E. coli strain

In 386 patients two of the 5 LP isolates will be tested for slide agglutination in Dr. Rowe's ETEC pools and for production of LT and ST by the CHO and infant mouse assays as described in the CRL hospital study. The patients who will be tested will be selected according to the projected age distribution of all ETEC positive patients in the CRL hospital study (see Figure 1).

Identification of enteropathogenic E. coli

All five LP isolates from all 1248 patients will be tested for direct slide agglutination in Dr. Rowe's EPEC pools.

Species Identification

Biochemical confirmation tests and O and H serotyping of E. coli will be done by Dr. Rowe on the following isolates:

(1) One or more isolates from each patient who has E. coli that are toxin positive.

(2) One or more isolates from each patient who has E. coli that are toxin negative, ETEC pool positive.

(3) One or more isolates from each patient who has E. coli that are EPEC pool positive.

(4) One or more isolates from each patient who has E. coli that are toxin negative, ETEC pool negative, EPEC pool negative, (This group will include a vast majority of the patients; the precise numbers of E. coli isolates that will be serotyped will be determined in consultation with Dr. Rowe).

Antibiotic Sensitivity - The antibiotic sensitivity pattern will be determined for a sample of 75 non ETEC- non EPEC isolates as described in the CRL hospital study.

c) Virology Procedures - Methods for identification of rotavirus and parvovirus will be as described in the CRL hospital study.

4. Data Analysis

From this study data will be obtained on the following:

a) The serotypes of ETEC in Dacca for each of the 3 toxin types.

b) The serotypes of non-ETEC in stools of patients with diarrhea of another (or unknown) etiology (Control group 1) and in patients without gastrointestinal illness (Control group 2).

c) The sensitivity, specificity, and predictive value of Dr. Rowe's ETEC pools.

d) The carrier rate of ETEC, EPEC, rotavirus and parvovirus in the "normal" population in Dacca.

e) Antibiotic sensitivity of ETEC and non-ETEC strains which will enable us to determine any existing association between antibiotic resistance and toxin type for organisms of the same serotype.

f) Incidence, serotypes, and antibiotic sensitivity of EPEC in patients with severe diarrhea.

g) Etiologic significance of ETEC and EPEC as a cause of diarrhea in Dacca.

h) Seasonal incidence and age distribution of ETEC, EPEC, rotavirus, and parvovirus in hospitalized persons with severe diarrhea.

i) Clinical course (e.g. stool duration, stool volume, fluid requirement, stool electrolytes, etc.) of illness caused by ETEC of three toxin types, EPEC, rotavirus and parvovirus.

D. SIGNIFICANCE

This will provide information on the value of serotyping in identifying ETEC and EPEC. In the case of ETEC we will learn what serotypes, if any, are associated most often with toxin production and whether the presently available pools to identify these organisms are adequate in their sensitivity and specificity. Since identification of toxin-producing organisms presently requires relatively sophisticated procedures, a demonstration of the usefulness of a simple method, i.e. serotyping, for diagnosis will have great significance to countries such as Bangladesh where these organisms are a frequent cause of severe diarrheal disease. We will also learn from this study whether there is any relationship between the properties of toxin production and antibiotic resistance; this may lead to a clearer understanding of the compatibility of plasmids in bacterial cells. We will learn whether there is any difference between the clinical severity of illness associated

with the 3 types of toxin producing strains; this will be important for planning of future studies of antibiotic therapy in E. coli diarrhea. In the case of the enteropathogenic E. coli we will learn some information to help decide whether there is benefit in routinely looking for these organisms in stools of persons with diarrhea. This diagnostic procedure is presently being carried out worldwide despite considerable controversy as to whether these organisms are truly responsible for sporadic diarrheal disease. We will also learn if there is a unique clinical syndrome associated with these strains as compared to the clinical syndrome associated with ETEC and rotavirus infection. The study will also provide valubale information on the relative frequency of all currently known diarrheal pathogens in Dacca. Although data will be obtained from only the most ill patients, it will indicate what importance should be placed on vaccine development for prevention of disease caused by such agents as rotavirus and parvovirus.

E. FACILITIES REQUIRED

Office space - no additional space required.

Laboratory Space - Half a Revco freezer will be needed for storage of frozen specimens.

Hospital Resources - A study ward will be needed for study of 24 patients/week for one year. A procedure will need to be set up to allow darkfield examinations to be done before patients are admitted to the study ward.

Animal Resources - 4000 infant mice will be needed over a 1-year period 4-6 mice per patient).

Logistical Support - none.

Equipment - none.

F. COLLABORATIVE ARRANGEMENTS

With the present protocol design ETEC pools will be needed to screen 3,268 isolates and EPEC pools to screen 8,736 isolates. A minimum of about 1300

organisms will require serotyping. Dr. Bernard Rowe, Division of Enteric Pathogens, Central Public Health Laboratories, Colindale, London, will supply the necessary pools and perform all required serotyping.

The ELISA assay for rotavirus antigen detection will be set up at CRL by Dr. Bob Yolken, NIAID, NIH in December, 1977. Parvovirus antigen detection will be done by Harry Greenberg, NIAID, NIH, at the NIH in Bethesda.

TABLE 1

O Group and Toxin type of E.coli Strains Isolated from 109 Cases of Diarrhea Dacca, Bangladesh, 1976

O Group	Toxin Type		
	LT-ST	ST only	LT only
O4		1	
O6	9		
O7		1	
O8	19(1)	1	1
O15	1		
O20	1	3(2)	
O25	2		
O29		2	
O34		2	
O48			1
O63	2	2	
O78	20(3)	5	
O85	2	1	
O96			1
O114		1	1
O115	11	2	
O123		1	
O126	1	1	
O128		5	
O148		1	
O159			1
OX2			1
Neg O1-O163	1	3	
Rough		2	

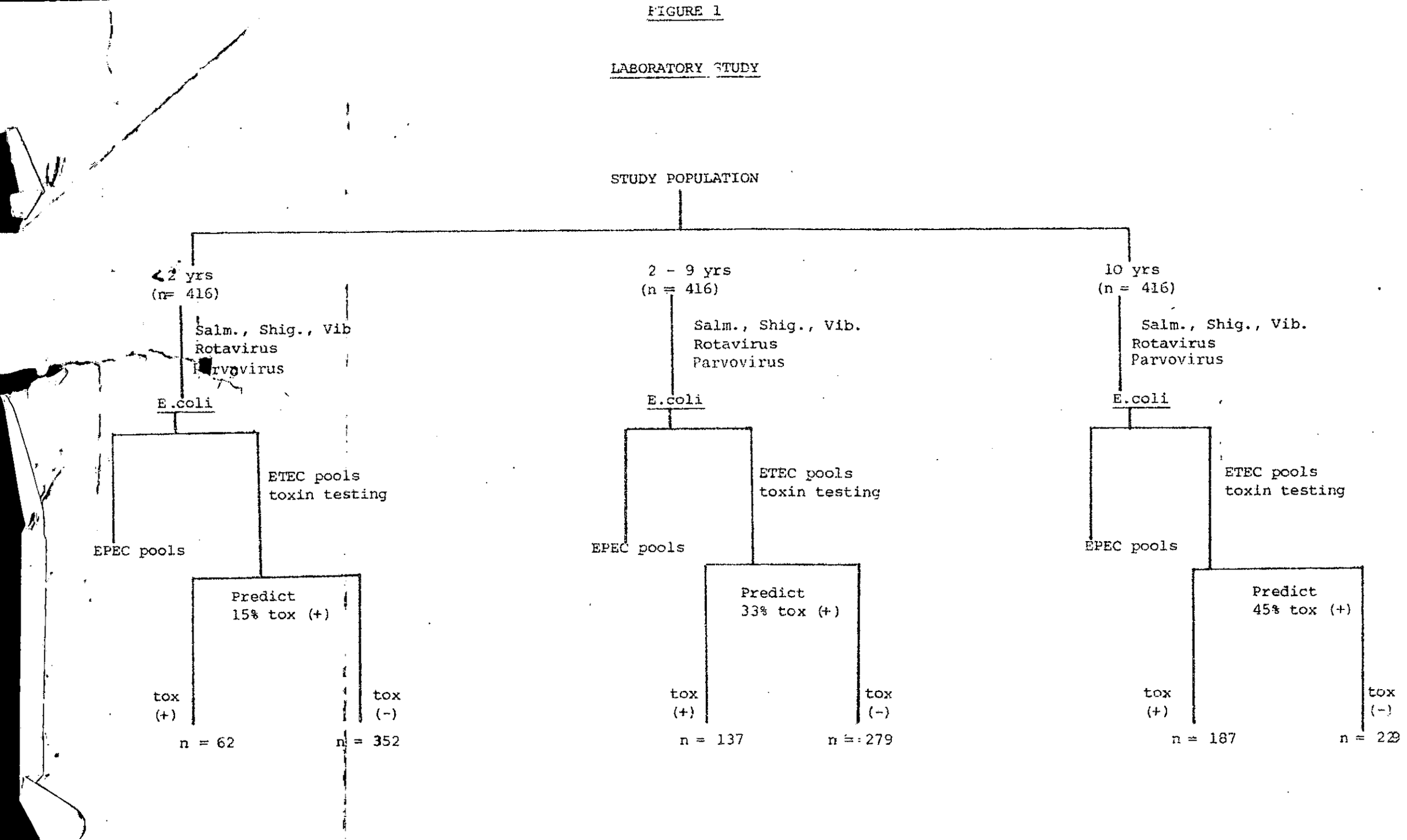
(1) Includes one O8:O60

(2) Includes three O20:O153

(3) Includes three O78:O44 and one O78:O13

FIGURE 1

LABORATORY STUDY



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ABSTRACT SUMMARY

This study is designed to determine the etiology of watery diarrhea in 1248 persons who visit the CRL hospital appearing moderately to severely dehydrated. Diagnostic studies will include those that will identify toxin production (ETEC) and enteropathogenic (EPEC) Escherichia coli, rotavirus and parvovirus. These 4 pathogens are not routinely looked for at CRL because they require more sophisticated diagnostic tests.

The study will particularly focus on the ETEC and EPEC. In the case of ETEC the specific serotypes and clinical picture of the three toxin types will be determined. For EPEC an attempt will also be made to define a unique clinical syndrome associated with these organisms. In both instances the frequency of isolation of these organisms by serotype in a diarrheal population will be compared with the frequency in a control population (Dacca Medical College - DMC).

1. Since the study seeks to define the associated illness and laboratory features of these organisms in all age groups, patients of all ages must be studied.
2. There are no significant risks to this study. Only rectal swabs, stool specimens (via rectal catheter) and blood (via fingerstick or antecubital vein) will be collected.
3. There are no risks; therefore this section is not applicable.
4. Confidentiality of the data collected will be ensured. Patient names will not be used in analysis or publication of the data. The original data will be locked in a file in the custody of the principle investigator who will restrict its use. In all laboratory studies only the hospital numbers will be used to identify patients.
5. A signed consent form will not be used since the study risks are minimal. For CRL patients only routine diagnostic procedures will be done - rectal swab, stool collection, and blood drawing; standard therapy will be given.

For the DMC participants only a rectal swab will be obtained.

For both groups information on what diagnostic procedures will be performed will be provided verbally and verbal consent obtained. For minors consent will be obtained from the authorized legal guardian or parent of the child. The verbal statement made will include a) the nature and purpose of each procedure, b) the physical risks, c) the benefits derived, d) the right to refuse to participate and e) confidentiality of data.

6. The only interview performed will be the routine taking of a medical history from ill persons.

7. Individual patients with diarrhea will benefit in that the cause of their illness will be obtained. Control persons will learn whether they are carriers of pathogenic organisms.

The potential benefit to society from the study will be a better understanding of the relative importance of etiologic agents as a cause of diarrhea. From this information the relative importance of such intervention measures as vaccine can be assessed. The study will also provide information that will improve methods of diagnosing ETEC diarrhea.

8. The study requires use of Matlab Hospital records and patient serum and stool

Statement to be read to Subjects when Verbal Consent is obtained.

A. CRL Study - The doctors at the Cholera Hospital are trying to find out what is the cause of your diarrhea. To find out we will do a rectal swab, obtain a stool specimen from your rectum, and draw a blood specimen. You will be provided all appropriate medicine for your illness. You can ask any questions you want and you are not required to take part in this study. Information collected will not be given to anyone except you, and doctors will combine it with information from other persons treated here.

B DMC Study - The doctors at the Cholera Hospital are trying to find out the cause of diarrhea in patients coming to the Hospital. They have found that certain bacteria can cause some of these cases and they are trying to find out whether these bacteria can be found in stools of persons without diarrhea. We would thus like to take a rectal swab from you to look for these bacteria. This procedure will not interfere with the care you are getting at this hospital. You can ask any questions you want and you are not required to take part in this study. Information collected will not be given to anyone other than yourself, and the doctors will combine it with information from other persons.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% or # of days	Annual Salary	Project Requirement Taka	Project Requirement $
Michael Merson	Investigator	20%	$ 42,000	-	8,400
Ward Physician	Co-investigator	100%	Tk 29,712	29,712	-
Imdadul Huq	Co-investigator	10%	Tk 99,034	9,903	-
Bob Black	Co-investigator	10%	$ 46,000	-	4,600
M.U. Khan	Co-investigator	5%	Tk 69,429	3,471	-
To be named	Sr. ward nurse	100%	Tk 15,837	15,837	-
To be named	Sr. ward nurse	50%	Tk 15,837	7,918	-
To be named	Ward nurse	50%	Tk 15,837	7,918	-
To be named	Cleaner	365d	Tk 5,950	5,950	-
To be named	Cleaner	150d	Tk 5,950	2,975	-
Golam Kibriya	Sr. Research Assistant	50d	Tk 63,437	10,570	-
To be named	Bact. Sr. Research Tech.	180d	Tk 30,800	15,400	-
To be named	Bact. Sr. Research Tech.	180d	Tk 18,480	9,240	-
To be named	Bact. Glasswasher	30d	Tk 17,171	1,431	-
Dr. N-Mahmood	Head, Animal Services Branch	30d	Tk 68,856	5,738	-
To be named	Veterinarian	30d	Tk 24,209	2,017	-
To be named	Biochem. Research Tech.	100d	Tk 15,837	7,918	-
To be named	Biochem.Lab Tech.	180d	Tk 11,180	5,590	-
Ansurradin Ahmed	Head, Immunology Branch	5%	Tk 71,776	3,589	-
To be named	Immunology Research Tech.	60d	Tk 15,837	2,640	-
To be named	Comm.Serv. Branch F.A.	365d	Tk 11,180	11,180	-
To be named	Comm.Serv. Branch F.A.	365d	Tk 11,180	11,180	-
To be named	Pathology Sr. Lab. Tech.	60d	Tk 11,180	1,863	-
			Sub total	172,040	13,000

2. SUPPLIES AND MATERIALS

Items	Unit Cost	Amount Required	Project Requirement Taka	$
Rectal swabs for SSV	Tk 7.6	2,496	18,970	-
Stock Vials	Tk 0.5	18,000	9,000	-
CHO assay	Tk 3.0	5,000	15,000	-
ST assay	Tk 3.1	5,000	15,500	-
Antib. Sens. Tests	Tk 8.5	300	2,550	-
2 dram vials (virus)	Tk 0.8	2,500	2,000	-
1 dram vials (stool)	Tk 0.6	1,250	750	-
1 dram vials (nasal wash)	Tk 0.6	250	150	-
Rectal Catheters	Tk 13.5	50	675	-
Glass Slides	$ .03	200	-	6
ELISA assay - stool/nasal wash	Tk 3.0	2,750	8,250	-
Disposable Syringes - 10cc	$ .07	3,000	-	210
Needles	$ .03	1,750	-	53
Test tubes	$ .03	200	-	6
Natelson tubes	$ .13	500	-	65
1 dram vials (serum storage)	Tk 0.6	2.000	1,200	-
Microcentrifuge tubes	$ .06	500	-	30
Stool electrolytes	Tk 2.5	1,250	3,125	-
Serum electrolytes, BUN osmol, protein P4	Tk 7.0	1,250	8,750	-
Plasma CBC, SG	Tk 3.5	1,250	4,375	-
Stool M/E	Tk 2.0	1,250	2,500	-
Urinalysis	Tk 4.0	1,250	5,000	-
IBM Cards	$ .001	2,500	-	2
Misc. books, paper	-	-	-	50
		Sub total	97,795	422

3. EQUIPMENT

Item	Unit Cost	Amount Required	Project Requirement Taka	$
Darkfield microscope condenser	$ 150	1	-	150
			Sub total	150

4. PATIENT HOSPITALIZATION

1250 hospitalizations for an average of 3 days total hospitalization = Tk506,250.

This total amount is not being included in the total project budget as these patients would have received this care without this protocol. I am budgeting 1/6 of this amount to the project as discharge may be delayed up to 1 day in some patients.

Budgeted expense = Tk 84,375.

5. OUTPATIENT CARE

Reimbursement for visit for convalescent blood

10 Tk/person = Tk 13,000

6. CRL TRANSPORT

CRL/Dacca Medical College/CRL

10 miles/day x 312 = 3120 miles = Tk 4,368

Other Dacca Transport - 5000 miles = Tk 7,000

Total 11,368

7. TRAVEL AND TRANSPORTATION OF PERSONS

Local travel - none

International travel - Meeting Travel = $2000

14 days per diem (36/day) = $ 504

Total 2504

8. TRANSPORTATION OF THINGS

Shipment of 9000 vials to London $3000

Shipment of 2000 vials to NIH $1000

Total 4000

9. RENT, COMMUNICATION, UTILITIES

Postage Tk 500

Telephone $ 100

10. PRINTING AND REPRODUCTION

Forms, record sheets	$100
Publication Cost	$400

11. OTHER CONTRACTUAL SERVICES

None.

12. CONSTRUCTION

None.

B. BUDGET SUMMARY

		Taka	Dollars
1.	Personnel	172,040	13,000
2.	Supplies	97,795	422
3.	Equipment	-	150
4.	Hospitalization	84,375	-
5.	Outpatients	13,000	-
6.	CRL Transport	11,368	-
7.	Travel - Persons	-	2,504
8.	Transportation - Things	-	4,000
9.	Rent/Communication	500	100
10.	Printing/Reproduction	-	500
11.	Contractual Services	-	-
12.	Construction	-	-
	Total	379,078	20,676
	Total Dollars		45,132

Conversion Rate $1.00 = Tk 15.5

NAG / E coli Studies

ADMISSION SHEET

DRAFT

PATIENT NAME _____

PATIENT NUMBER ____ ____ ____ ____ ____

DATE OF ADMISSION ____ ____ ____ ____ ____
day month year

TIME OF ADMISSION ____ ____ ____ ____ hours

AGE ____ ____ year ____ ____ months SEX: 1-Male 2-Female

DURATION OF DIARRHEA: ____ ____ hours before admission

TIME SINCE LAST STOOL PASSED: ~~____ hrs ____ mins~~ ~~hours~~ before admission

CHARACTER OF STOOL: 1-Watery: Yes No 2-Mucoid: Yes No 4-Bloody: Yes No

VOMITING: 1-Abdominal pain: Yes No 2-Muscle cramps: Yes No 4-Chills: Yes No

1-Fever: Yes No 2-Vomiting: Yes No

Duration of vomiting: ~~____ hrs ____ mins~~ ~~hours~~ before admission

LAST URINE PASSED: ~~____ hrs ____ mins~~ ~~hours~~ before admission

ANTIBIOTICS TAKEN FOR PRESENT ILLNESS: 1-No 2-Yes

What antibiotic(s) were taken _____

How many times were they taken _____

VACCINATED AGAINST CHOLERA: 1-Yes 2-No 3-Don't know

When was last vaccination: _____

ADMISSION WEIGHT: ____ ____ . ____ kg

SKIN TURGOR: 1-Good 2-Fair 3-Poor EYES SUNKEN: 1-Yes 2-No

WASHERWOMAN HANDS: 1-Yes 2-No MENTAL STATUS: 1-Clear 2-Not clear

MUCOUS MEMBRANES: 1-Dry 2-Moist

DEHYDRATION: 1-Severe 2-Moderate/Severe 3-Moderate

REHYDRATION FLUID REQUIREMENT (ml): _____ I.V.

~~(Circle first three letters)~~

CHARACTER OF STOOL: 1-Watery 2-Liquid 3-Soft 4-Formed 5-Dysentery

COLOR OF STOOL (Circle no more than 2): 1-Rice watery 2-Creamy 4-Red

1-Yellow 2-Brown 4-Green

Hours from Adm.	Temp	Pulse	BP	R	Stool per period	Cumm. Stool	Stool Guaiac	Urine per Period	Vomit per Period	I.V. per period	P.O. Oral Fluid per period	P.O. Other per period	Cumulative
0													
4													
8													
12													
16													
20													
24													
28													
32													
36													
40													
44													
48													
52													
56													
60													
64													

CLINICAL LABORATORY RESULTS

[illegible]: BUN ___ ___ mg % Creatinine ___ ___ mg %

Osmolality ___ ___ ___ mOsm/L Sp. Gr. 1.0 ___ ___

CO_2 ___ ___ ___ mEq/L Na ___ ___ ___ mEq/L

K ___ ___ ___ mEq/L Cl ___ ___ ___ mEq/L

Protein ___ ___ . ___ gm % pH ___ . ___

[illegible] Osmolality ___ ___ ___ mOsm/L

[illegible]:

CO_2 ___ ___ ___ mEq/L Na ___ ___ ___ mEq/L

K ___ ___ ___ mEq/L Cl ___ ___ ___ mEq/L

[illegible]: ___ ___ rbc/hpf ___ ___ f.l./hpf ___ ___ pus c./hpf

Amoeba: 0-Neg 1-Cysts 2-Trophs 3-Trophs + rbc

(Following parasites: 0-Neg 1-4 = 1-4+)

Giardia___ Trichomonas___ Tricluris___ Ascaris___

Hookworm___ F. buski___ Other___(_____)

[illegible]: Hct ___ ___ Sp Gr. 1.0 ___ ___

WBC ___ ___, ___ 00/cc

Diff: Polys ___ ___ Bands ___ ___ Lymps ___ ___

Monos ___ ___ Eos ___ ___ Baso ___ ___

[illegible]: Sp. Gr. 1.0 ___ ___ URINALYSIS: 1-Normal 2-Abnormal

[illegible] BACTERIOLOGY OBTAINED ___ NASAL WASHING OBTAINED ___

DISCHARGE ASSESSMENT

[illegible] OUTPUT DURING FIRST 24 HR: ___ ___ ___ ___ ___ ml

[illegible] NORMAL STOOL: ___ ___ ___ ___ ___ ml

[illegible] (0r) OF: Diarrhea___ ___ Vomiting___ ___ Fever___ ___

[illegible] FLUID GIVEN: I.V. ___ ___ ___ ___ ___ ml

P.O. (Oral fluid) ___ ___ ___ ___ ___ ml

P.O. (Other) ___ ___ ___ ___ ___ ml

[illegible] WEIGHT ___ ___ . ___ kg HEIGHT ___ ___ ___ cm

[illegible] FOR CONVALESCENT SEROLOGY FOLLOWUP _____

(Is address form complete)