Attachment 1. (FACE SHEET) 82. Library

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 22/07/97

Principal Investigator Wasif Ali Khan

Trainee Investigator (if any)

Application No. 97-011

Supporting Agency (if Non-ICDDR,B)

Title of Study Shiga toxin ELISA: proposal for evaluation of premier EHEC for the diagnosis of S. dysenteriae I infection and for determination of sequential shiga toxin excretion.

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 STOOL (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 Committee:
   - NA 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.

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

Wasif Ali Khan

Principal Investigator

Trainee

the general public). The kit is entirely self-contained. No additional reagents are required.

Head of Program: George Fuchs, M.D.

Application for project grant

Small Study Category

Principal Investigator: Wasif Ali Khan, MB, BS

Co-Investigators: Mohammed Abdus Salam, MB, BS
Monira Begum, MSc.
Ujjwal Dhar, MB, BS
Edward T. Ryan, M.D.

Collaborators/Consultants: Nigel Rollins, MB, BS
Asst. Prof., Dept. Ped.,
University of Natal, South Africa

David Acheson, M.D.
Asst. Prof., Dept. Medicine,
Tufts University School of Medicine; Boston, USA

Michael Bennish, M.D.
Asst. Prof., Dept. Medicine/Pediatrics,
Tufts University School of Medicine; Boston, USA

Title of Project: Shiga toxin ELISA: Proposal for Evaluation of Premier EHEC for the diagnosis of *Shigella dysenteriae* type 1 infections, and for determination of sequential Shiga toxin excretion.

THIS PROTOCOL IS FOR VALIDATION OF A FULLY LICENSED, COMMERCIALLY AVAILABLE ELISA KIT AMONG THE PATIENT POPULATION OF THE ICDDR,B

Starting date: August/September, 1997

Date of completion: Enrollment completion (one hundred stool samples; estimated 1-2 months)

Total budget: US$1500

Funding Source: Meridian Diagnostic Inc.

The only equipment required for this study protocol is an approved, fully licensed, commercially available enzyme linked immunoabsorbant assay (ELISA) plate-kit that has been donated by the manufacturer (Meridian Diagnostics, Inc.; Cincinnati, OH, USA) without stipulation. There is no commercial affiliation. The investigators have complete control over the conduct, manner and details of the study protocol, and retain full authority over reporting any scientific findings in an appropriate forum/forums (including if, when, where and why). The kit is already fully licensed and commercially available. Should scientific publication result, the manufacturer would have the ability to reference that publication in standard scientific fashion (as would any member of the general public). The kit is entirely self-contained. No additional reagents are required.

Head of Program: George Fuchs, M.D.

Abstract:

Shigellosis is an important cause of dysentary that results in significant morbidity and mortality worldwide. There are a number of species of *Shigella* that can cause shigellosis. Strains of *S. dysenteriae* type 1 secret the potent Shiga toxin, are associated with the most severe disease, and result in the highest mortality during and after infection. *S dysenteriae* is becoming increasingly resistant to antimicrobial agents. Expensive, broad-spectrum antibiotics are often required for appropriate therapy of *S. dysenteriae* and some data even suggest that the initiation of antimicrobial agents may be associated with the possible development of the serious *S. dysenteriae* type 1 Shiga toxin-associated complication of Heomlytic Uremic Syndrome (HUS). The other Shigella species (including *S. flexneri, S. boydii* and *S. sonnei*) display less antimicrobial resistance and are not commonly associated with the development of HUS. At present, evaluation of a patient with a dysenteric stool often includes a stool culture (the results of which are not available for at least 24 hours) and possibly microscopy (to exclude amebiasis). These evaluations are labor intensive and results may not available for mlultiple hours or even days. The results of these evaluations (specifically culture data) are therefore not helpful at the time of clinical decision regarding initiation of appropriate antibiotics. A recently developed ELISA kit has been approved for the detection of strains of *Escherichia coli* that produce Shiga toxin (Enterohemorrhagic *E. coli*; EHEC). This ELISA kit is based upon the detection of Shiga toxin, which is the same toxin produced by *S. dysenteriae* type 1. We propose to assess the utility of this ELISA kit to rapidly evaluate patients with dysenteric stools in an attempt to quickly identify individuals infected with Shiga toxin-producing strains of *S. dysenteriae* type 1 and to thus allow for the initiation of an appropriate and targeted antimicrobial agent. We propose a dual evaluation. The first part of the study would validate the functionality of the approved kit using stools from fifty Surveillance Study patients. We would also examine the stools of fifty individuals with dysentery with the ELISA kit, microscopy and selected culturing. Stool samples from fifty Surveillance Patients would also be analyzed with the EHEC kit. These evaluations are directed at ascertaining the utility of the kit for rapid qualitative analysis of stool samples. The second component of the study would involve the serial semi-quantitative analysis of the amount of Shiga toxin present in stool after the initiation of antibiotics. This component of the study would involve 15 patients and is directed at preliminarily evaluating the possible association of antimicrobial agents, Shiga toxin and HUS. Only stool specimens will be required from study participants. The study is non-invasive. There is no risk to study participants. The ELISA kit is NOT experimental. It is fully approved, licensed and is already commercially available for the evaluation of stool for the presence of Shiga toxin.

General Aim: Rapid evaluation of dysenteric stools for the presence of Shiga toxic.

Specific Aim: Evaluation using the commercially available Premier EHEC ELISA kit.

Significance: Identification of patients at risk for severe shigellosis and the possible development of HUS; and to allow for the rapid ascertainment of microbiological data to assist in the choice of an appropriate antimicrobial agent.

Ethical implications:

This study is non-invasive. The study involves the evaluation of stool samples from individuals presenting to the ICDDR,B with diarrhea. There is no risk to study participants. The ELISA kit is fully licensed, approved and commercially available for the evaluation of stool samples for Shiga toxin. Patients tested may benefit because they will have a diagnosis established 48 hours before that diagnosis established by traditional culture methods, thus allowing for initial therapy to be based upon more specific information than simple clinical findings. If this diagnostic test is effective, developing countries where *S. dysenteriae* type 1 is endemic may benefit because therapy can be more appropriately targeted, thus assuring that the broader spectrum, newer, often more expensive agents for treatment of *S. dysenteriae* type 1 infections are used only when they are required. By reducing the unnecessary use of these drugs, the development of antimicrobial resistance may also be slowed.

PRELIMINARY DATA

Using EHEC Premier Kits that we have purchased, we tested a number of stool samples (ten) from pediatric

Background, research plan and bibliography:

BACKGROUND

Enterohemorrhagic *Escherichia coli* infections have become an increasingly important public health problem in industrialized countries, and have received enormous publicity and attention in both the lay press and in the scientific literature. The majority of morbidity and mortality associated with Shigatoxin producing organisms, however, still occurs from infections with *Shigella dysenteriae* type 1, the organism in which Shiga toxin was first identified. *S. dysenteriae* type 1 is responsible for over two million cases of severe dysentery in the world every year, and an estimated 250,000 deaths (1,2). Many of these deaths happen during very dramatic epidemics, such as that which occurred in the Rwandan refugee camps in Goma, zaire, in 1994, when an estimated 30,000 persons died from *S. dysenteriae* type 1 infection (3).

Although in epidemic situations the diagnosis of *S. dysenteriae* type 1 can be easily made once it has been established that *S. dysenteriae* type 1 is the cause of the epidemic, the diagnosis of *S. dysenteriae* type 1 in endemic situations is not as easy. *S. dysenteriae* type 1 is now endemic in most of central and southern Africa, and in all of south Asia. In these countries, other species of *Shigella* and *Entamoeba histolytica* are also endemic, and also cause the dysentery syndrome. It is not possible on a clinical basis to determine which patient is infected with *S. dysenteriae* type 1, and which is infected with another species of *Shigella* or *E. histolytica.*

In these settings, determining the cause of infection is important because of the differing antimicrobial susceptibility patterns of *S. dysenteriae* type 1 and other species of *Shigella* (4), and the need for an entirely different class of agents to treat *E. histolytica* infection. *S. dysenteriae* is much more likely to be multiply-resistant, and to require treatment with a newer, more expensive agent than either other species of *Shigella* or *E. histolytica.* In the field setting, treatment must be given to the patient when they are the first seen, as facilities for culture diagnosis are not available. Even when they are, they are of little utility, as the 24-48 hours required for the results of cultures to become available is an impractical period for the initiation of treatment. Because of the severity of *S. dysenteriae* type 1 infection, therapy needs to be initiated when the patient is first seen, and thus there is a need for a rapid diagnostic method. It is possible that the Premier EHEC may serve such a role if it proves to be a sensitive determinant of *S. dysenteriae* 1 infection.

A separate issue is the impact of antimicrobial treatment on the pathogenesis of the hemolytic-uremic syndrome (HUS). It has been postulated that treatment of infections caused by Shiga toxin producing organisms puts the patient at increased risk of developing HUS, presumably by liberating toxin from previously intact organisms. The basis for the belief that antimicrobial treatment can be harmful is largely epidemiologic - the association in case-control studies of prior antimicrobial therapy with the development of HUS. These studies are flawed, however, by the initially more severe disease in patients who go on to develop HUS, and thus the greater likelihood that these patients will initially receive an antimicrobial agent. There has of yet been no in-vivo demonstration that antimicrobial therapy liberates toxin in appreciable amounts.

OBJECTIVES

1. To determine the sensitivity and specificity of Premier EHEC for the rapid diagnosis of *S. dysenteriae* type 1 infection in patients with dysentery.

2. To determine the effect of antimicrobial therapy on the sequential excretion of Shiga toxin in patients with *S. dysenteriae* type 1 infection.

PRELIMINARY DATA

Using EHEC Premier Kits that we have purchased, we tested a number of stool samples (ten) from pediatric

of toxin-encoding DNA, and standard cytoxicity assays. We will attempt to neutralize all samples that test positive in the cytotoxicity assay using specific antisera to Shiga toxin.

patients with dysentery at the King Edward VII Hospital at Durban, South Africa. Results to date are as follows:

Infecting Organism	# of Patients	+ on ELISA	- on ELISA	Indeterminate	Comments
S. dysenteriae type 1	3	3	0	0	
S. flexneri	3	0	3	0	
Culture negative	4	1	3	0	The one patient who had a + ELISA had a brother admitted with culture-confirmed *S. dysenteriae* 1 infection the next day

Sample processed were tested on the day that the patient was admitted. Samples were run in duplicate. Two positive *S. dysenteriae* type 1 samples were further diluted. Both were positive at 1/10, but negative at 1/100.

PROPOSED STUDY

Rapid Diagnosis

To more definitively determine the utility of the Premier EHEC Kit in the rapid diagnosis of *S. dysenteriae* type 1 infection, we propose to study 200 patients with frank dysentery. One-hundred of these patients would be enrolled at the International Centre for Diarrhoeal Disease Research, Bangladesh, in Dhaka, Bangladesh, and 100 at the King Edward VII Hospital, University of Natal, Durban, South Africa. All will be patients that have not received a potentially effective antimicrobial agent for their illness to increase the likelihood that the culture will yield the true pathogen, rather than be falsely negative.

Samples for this portion of the study will be run at the time the patient is admitted, and according to the methods recommended in the kit. This will give the best approximation of how the kit will function in actual use. The objective here is rapid diagnosis, and thus samples will not be frozen.

We estimate that of these 200 samples processed, at least 100 will come from patients infected with *S. dysenteriae* type 1. We thus should have sufficient numbers of patients to adequately evaluate the sensitivity and specificity of the assay for detecting *S. dysenteriae* type 1 infection. Samples will be tested both for spectrophotometric reading, and for visual color change. The latter will be the most useful indicator of the presence of *S. dysenteriae* type 1 infection under field conditions. It is possible, based upon the limited number of samples run to date, that patients with dysentery but without *S. dysenteriae* type 1 infection will have positive samples on ELISA. The spectrophotometric readings obtained in this study could define OD reading cutoffs for the diagnosis of *S. dysenteriae* type 1 infection different than the ones currently used for the diagnosis of EHEC infection.

At the ICDDR,B stool validation of the commercially available kit would be performed on dysenteric stool samples (bloody mucoid stool). All dysenteric stool specimens will also cultured and microscopically examined. Analysis of the Surveillance Study and dysenteric cohorts will allow for comparison of ELISA toxin results and culture results. Samples will then be divided into four groups: 1) *S. dysenteriae* type 1 culture-positive and ELISA toxin positive; 2) *S. dysenteriae* type 1 culture positive and ELISA toxin negative; 3) culture negative for *S. dysenteriae* type 1 and ELISA toxin positive; 4) culture negative for *S. dysenteriae* type 1 and ELISA toxin negative. Representative frozen samples from all four groups will be evaluated in Boston, USA for the presence of toxin using PCR identification of toxin-encoding DNA, and standard cytoxicity assays. We will attempt to neutralize all samples that test positive in the cytotoxicity assay using specific antisera to Shiga toxin.

Boston Dr. David Acheson, Assistant Professor, Department of Medicine, Tufts University School of Medicine, USA.

Quantitative Excretion Before and After Antimicrobial Therapy

The purpose of this portion of the study is to determine if initiating antibiotic therapy in patients who have *S. dysenteriae* results in an increase in the levels of free Shiga toxin in the stool.

In this part of the study, we propose to study 15 patients from each study site (30 total) with culture confirmed *S. dysenteriae* type 1 infection. Analysis at the ICDDR,B would be performed on stool samples from a frozen stool "library" that has already been collected as part of the fully approved and completed study "Randomized double-blind study to compare the safety and efficacy of ciprofloxacin suspension to pivmecillinam tablets in the treatment of childhood shigellosis" (RRC # 90-019). This study was recently completed at the ICDDR,B. All study participants/guardians gave informed consent for stool samples to be collected at various time points before and after initiation of antibiotics for research purposes related to shigellosis. A copy of the consent form is attached. We have received permission from the Principal Investigator of that study (Dr. Mohammed Abdus Salam, Chief Physician, ICDDR,B) to analyze these stool specimens that were collected over a 120 hour time period from study participants.

Samples for this part of the study would be frozen and run in batch. Fro stools that are not liquid, a BB size sample would (after thawing) be mixed with 200 microliters of diluent, vortexed for one minute, and then processed as per the kit protocol. All samples would be run in five dilutions (1/5, 1/10, 1/20, 1/40, 1/80) to ensure that the results are on the linear portion of a standard curve developed using known concentrations of Shiga toxin.

STUDY SITE

This study would be conducted at the International Centre for Diarrhoeal Disease Research, Bangladesh, in Dhaka, Bangladesh, and at the King Edward VII Hospital of the University of Natal, in Durban, South Africa. Both centers see large numbers of patients with dysentery, and *S. dysenteriae* type 1 is endemic at both sites. Both sites are equipped to carry out the ELISA testing required.

SIGNIFICANCE

If Premier EHEC is effective in determining which patients with dysentery have *S. dysenteriae* type 1 infection, it will be a potentially useful tool in determining the provision of appropriate therapy to patients with *S. dysenteriae*. It can also be useful for epidemiologic studies in defining which patients are infected with *S. dysenteriae* type 1. The use of ELISA kit for this purpose would be much simpler, and less expensive, then trying to culture samples.

If this study shows that antimicrobial treatment markedly increases the concentration of toxin in stool, then our current approach of treating all patients with *S. dysenteriae* type 1 infection with an antimicrobial will have to be reevaluated.

STUDY INVESTIGATORS

Dhaka: Dr. Wasif Ali Khan, Medical Officer, ICDDR,B
Monira Begum, MSc, CSD, Microbiology, ICDDR,B
Ujjwal Dhar, MB, BS, CSD; Medical Officer, ICDDR,B
Dr. Mohammed Abdus Salam, Chief Physician, ICDDR,B.
Dr. Edward T. Tyan, Visiting Fellow at ICDDR,B, Instructor, Harvard Medical School, Boston, USA.

Durban: Dr. Nigel Rollins, Assistant Professor, Depairtment of Pediatrics, University of Natal., South Africa.

Boston: Dr. David Acheson, Assistant Professor, Department of Medicine, Tufts University, School of Medicine, USA.

Study Coordinator: Dr. Michael Bennish, Associate Professor, Departments of Medicine and Pediatrics, Tufts University School of Medicine, USA.

REFERENCES

1. Bennish ML, Wojtyniak B. Mortality due to shigellosis: community and hospital data. Rev Infect Dis 1991;13(Suppl 14): 245-51.

2. Keusch GT, Bennish ML. Shigellosis In: Brachman PS, ed. Bacterial infections of humans. Epidemiology and control. New York: Plenum Medical Book Company, 1991. 593-620.

3. Goma Epidemiology Group. Public health impact of Rwandan refugee crisis: what happened in Goma, Zaire, in July 1994? Lancet 1995;345:339-44.

4. Bennish ML, Levy S. Antimicrobial resistance of enteric pathogens. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, eds. Infections of the gastrointestinal tract. New York: Raven Press, 1995:1499-523.

Curricula vitae: (please see attached).

Flow chart:

1-3 months:

Daily ELISA evaluation of aliquots of dysenteric and surveillance stool samples.

Specific tasks of each investigator:

Drs. Wasif, Ujjwal, Salam and Ms. Begum would be responsible for sample collection and ELISA analysis in Dhaka, Bangladesh. Ms. Begum would also oversee microbiological culturing and analysis. Dr. Rollins would be responsible for data acquisition in South Africa. Drs. Acheson and Bennish would be responsible for molecular strain analysis in Boston, USA. Dr. Bennish would also be the study coordinator between the two sites.

Budget : US$ 1500; The kit is entirely self-contained. No additional reagents are required.

Budget breakdown:

Stool M/E	: US$	300
Stool culture and isolation	: US$	1000
Xerox and memograph	: US$	100
Publication	: US$	100
Total	: US$	1500