

Date 27/4/88

ETHICAL REVIEW COMMITTEE, ICDDR,B.

DM2

Principal Investigator CONSTANCE SCHULTSZ Trainee Investigator (if any)

Application No. 88-013 Supporting Agency (if Non-ICDDR,B) USAID

Title of Study Secretory IgA antibody in saliva of children with shigellosis Project status:

(X) 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	<input checked="" type="radio"/>	No
(b) Non-ill subjects	<input checked="" type="radio"/>	No
(c) Minors or persons under guardianship	<input checked="" type="radio"/>	No
Does the study involve:		
(a) Physical risks to the subjects	<input checked="" type="radio"/>	No
(b) Social Risks	<input checked="" type="radio"/>	No
(c) Psychological risks to subjects	<input checked="" type="radio"/>	No
(d) Discomfort to subjects	<input checked="" type="radio"/>	No
(e) Invasion of privacy	<input checked="" type="radio"/>	No
(f) Disclosure of information damaging to subject or others	<input checked="" type="radio"/>	No
Does the study involve:		
(a) Use of records, (hospital, medical, death, birth or other)	<input checked="" type="radio"/>	No
(b) Use of fetal tissue or abortus	<input checked="" type="radio"/>	No
(c) Use of organs or body fluids	<input checked="" type="radio"/>	No
Are subjects clearly informed about:		
(a) Nature and purposes of study	<input checked="" type="radio"/>	No
(b) Procedures to be followed including alternatives used	<input checked="" type="radio"/>	No
(c) Physical risks	<input checked="" type="radio"/>	No NA
(d) Sensitive questions	<input checked="" type="radio"/>	No NA
(e) Benefits to be derived	<input checked="" type="radio"/>	No
(f) Right to refuse to participate or to withdraw from study	<input checked="" type="radio"/>	No
(g) Confidential handling of data	<input checked="" type="radio"/>	No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure	<input checked="" type="radio"/>	No NA

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 Ctee. for review.

(PTO)

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

Constance Schultsz

Principal Investigator

Trainee

REF

QW 138.5.S4

S 386s

1988

88-013

27/4/88

SECTION I - RESEARCH PROTOCOL

1. TITLE : Secretory IgA antibody in saliva of children with shigellosis
2. PRINCIPAL INVESTIGATOR : Ms. Constance Schultsz
- COINVESTIGATOR : Dr. Firdausi Qadri
Dr. Faruque Ahmed
3. STARTING DATE : May 1988
4. COMPLETION DATE : May 1989
5. TOTAL DIRECT COST : US\$ 13,400
(Funding source: USAID)
6. ASSOCIATE DIRECTOR : Dr. Ivan Ciznar

This protocol has been approved by the Laboratory Sciences Division.

Signature of Associate Director :

Date :

Ivan Ciznar
April 12, 1988

7. ABSTRACT SUMMARY

This study is meant to investigate the secretory IgA antibody response during shigellosis in children. Saliva samples from patients will be collected on day of diagnosis, day 7 and day 14 of illness. Saliva from children of a local school and from children who just arrived in Bangladesh from non-endemic areas, both without any history of recent diarrhoea, will be used as control.

The antibody response will be analyzed by ELISA and Western-blot, using outer membrane proteins (OMPs) and lipopolysaccharide (LPS), prepared from *Shigella dysenteriae*

type 1 and *Shigella flexneri* strains. *Shigella* toxin extracted from a *Shigella dysenteriae* type 1 strain will also be used in ELISA and Western-blot analysis. Unstimulated saliva samples will be used, after heating at 56°C and centrifugation. It is expected that these analyses will enable us to observe a rise in the IgA antibody titer in saliva during *Shigella* infection.

8. REVIEW

- a) Ethical Review Committee _____
- b) Research Review Committee _____
- c) Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective

To determine secretory IgA antibody response to *Shigella* infection in saliva from children.

2. Background

Vaccine development against shigellosis is one of the highest priorities in the WHO, Diarrhoeal Disease Control program (16). Since ORS alone is not sufficient for the treatment of shigellosis and resistance to antibiotics is expanding rapidly, immunoprophylaxis is one area of research attracting attention and gaining importance.

Development of an effective vaccine against shigellosis will substantially benefit from the knowledge of the immune response of the human body to pathogens. In addition, a knowledge of the immunogenic components of the pathogen is required. In intestinal infections, the local mucosal immune response is of great importance since it is the first step in defense against a pathogen. However, it is difficult to study this immune response without the use of invasive methods. As an alternative to this, saliva may be used to study the local humoral intestinal immunity.

Antigenic stimulation of lymphocytes residing in the Peyer's patches in the small intestine leads to proliferation and migration of the cells into the regional lymph nodes, the lymph and the circulation. The antigenically stimulated mature lymphocytes return to the small intestinal mucosa or settle in the various exocrine glands and differentiate into IgA secreting plasma cells. It is because of this mechanism of migration and homing of lymphocytes that the IgA excreted by the salivary glands is of similar specificity to the IgA secreted by the plasma cells which have returned to the intestinal mucosa (1,11). Various studies showed evidence that transport of circulating monomeric IgA into the secreting glands is minimal (11). It is for this reason that by measuring the salivary IgA levels in patients, one can evaluate the local humoral immune response in the small intestine.

Mellander *et al.* (9,10) have shown that infants can respond to exposure to *E. coli* antigens with production of salivary secretory IgA antibodies. Mestecky *et al.* (12) have immunized volunteers orally with capsulated and killed *Streptococcus mutans* which induced a significant rise in the secretory IgA titer in the saliva.

In Bangladesh, Islam *et al.* have detected salivary IgA antibodies against *Giardia lamblia* in a cohort study (4). There are no reports, however, regarding the presence of salivary IgA antibodies in patients with shigellosis (2). Several animal studies, focused on the local immune response after stimulation by *Shigella* spp., have shown an increase in local antibody production in the gut (5,8).

In shigellosis, various components of the bacteria may be responsible for generating an immune response. Of these, the outer membrane proteins (OMPs), lipopolysaccharides (LPS) and the *Shigella*-toxin appear to be important and are being studied so as to understand their immunogenic properties (8,13). Since secretory IgA in saliva represents the intestinal secretory immune response, it should be possible to investigate the immunogenicity of OMP, LPS and *Shigella*-toxin by measuring the salivary IgA levels against each of these bacterial components.

3. Rationale

The mucosal immune system provides the body with a major barrier against nutritional, as well as microbiological antigens. The importance of the mucosal immune system can be seen in patients with deficiency in secretory IgA. These patients suffer from recurrent respiratory and intestinal infections.

(3). Understanding the various factors involved in mucosal immunity is of a great importance for development of an effective vaccine. This study aims to give insight into the production of salivary IgA antibodies during shigellosis and to assess whether salivary IgA can be used as an indicator of the intestinal immune response.

In developing a method to estimate the amount of IgA antibodies in saliva during shigellosis, one may find a non-invasive procedure for the measurement of antibody response. Thus, this study might also be of relevance in its clinical and epidemiological application.

B. SPECIFIC AIMS

- a) To set up enzyme-linked assays for measuring IgA antibodies in saliva against OMPs, LPS and toxin obtained from *S. dysenteriae* type 1 and *S. flexneri* antigens.
- b) To observe a rise in the secretory IgA antibody titer in saliva during the course of a *Shigella* infection.

C. MATERIALS AND METHODS

a) Patients saliva

Unstimulated saliva from 10 children with *S. dysenteriae* type 1 infection and 10 children with

S. flexneri infection (bacteriologically confirmed), aged 2-12 years, will be collected on the day of diagnosis (2nd day of hospitalization) and on day 7 and day 14 of illness. Maximal duration of diarrhoea before attending the hospital will be 4 days. In case of breastfeeding, saliva will be collected 2 hours after feeding to prevent contamination with secretory IgA in breast milk. In order to obtain a statistically satisfactory number of children, it will be necessary to collect samples from children attending both the Dhaka and the Matlab treatment centres.

b) Control saliva

Saliva from 10 children in the same age group as the test group will be used. Only children without a history of recent *Shigella* infection will be included. These children will be found in a local social welfare school (Samaj Unnayan School, Siddeswari, Dhaka).

Further saliva of 10 children who have recently arrived in Bangladesh from a non-endemic area and without any history of shigellosis will be used as control (American International School, Dhaka).

c) Collection and treatment of saliva

Unstimulated saliva samples will be collected either by suction with a dropper, or by spitting into a beaker. The samples will be heated at 56°C for 15 min to

prevent enzymatic degradation (4), centrifuged at 5,000 X g and stored in aliquots at -70°C.

d) Stool samples

Stool samples from the patients group will be examined for *Salmonella*, *Shigella*, *V. cholera* and *E. coli* ST/LT on admission as is normal procedure in the ICDDR,B treatment centres. Stool samples will also be collected on day 7 and/or day 14 in case of persistent or recurrent diarrhoea only.

e) Analysis of antibody in saliva

i) Antigen

OMP (13) and LPS (15) for ELISA and Western-blotting will be prepared using strains *S. dysenteriae* type 1 No. 26406 and *S. flexneri* 2a No. 611. In case of different *Shigella flexneri* serotypes, homologous strains will be used. These strains are at present stored at -70°C. These bacteria will be subcultured and grown at 37°C with shaking in Trypticase Soy Broth containing 0.6% Yeast Extract. For preparation of *Shigella* toxin for ELISA and Western-blotting, *S. dysenteriae* type 1 strain No. 26406 will be used, using methods developed in the Immunology laboratory.

ii) ELISA

Microtitre plates (polystyrene) will be coated with OMP, LPS and *Shigella* toxin. After washing, serially diluted saliva samples will be added. Anti-human IgA conjugated to horseradish peroxidase will be used to determine the IgA content of the saliva. For visualization, 4-Chloro-1-Naphtol will be used as substrate and colour development will be measured at 540nm (6,13).

iii) Western-blotting

OMPs, LPS and *Shigella*-toxin, separated by 13.5% SDS-polyacrylamide gel electrophoresis (7), will be blotted onto nitrocellulose sheets in the transblot apparatus of Bio-Rad (14). Saliva samples will be used to probe specific antigens. Rabbit anti-human IgA linked to horseradish peroxidase will be used to detect recognition using 4-Chloro-1-Naphtol as substrate.

g) Statistical analysis

In order to measure the significance value of the obtained data the Student's t-test or other appropriate statistical evaluations will be carried out.

REFERENCES

1. Bienenstock, J. and Befus, A.D. (1980) Mucosal Immunology, review, *Immunology*, 41:249-269.
2. Mezz, Medline (1966 to April 1988); bibliography on antibody (IgA, IgG, or IgM) in saliva.
3. Harrison's Principles of Internal Medicine. (1987) 11th edition, McGraw-Hill Book Company, USA.
4. Islam, A. et al. (1983) *Giardia lamblia* infections in a cohort of Bangladeshi mothers and infants followed for one year. *J. Pediat.*, 103:996-1000.
5. Keren, D.F. et al. (1986) Secretory Immunoglobulin A response following peroral priming and challenge with *Shigella flexneri* lacking the 140-Megadalton virulence plasmid. *Inf. Immun.*, 54:920-923.
6. Keren, D.F. et al. (1985) Effect of antigen form on local immunoglobulin A memory response of intestinal secretions to *Shigella flexneri*. *Inf. Immun.*, 47:123-128.
7. Laemmli, K.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227:680-685.
8. Levine, M.M. et al. (1983) New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. *Microb. Rev.*, 47:510-550.

9. Mellander, L. et al. (1984) Appearance of secretory IgM and IgA antibodies to *Escherichia coli* in saliva during early infancy and childhood. *J. Pediat.*, 104:564-568.
10. Mellander, L. et al. (1985) Secretory IgA antibody response against *Escherichia coli* antigens in infants in relation to exposure. *J. Pediat.*, 107:430-433.
11. Mestecky, J. (1987) The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J. Clin. Immun.*, 7:265-276.
12. Mestecky, J. et al. (1978) Selective induction of an immune response in human external secretions by ingestion of bacterial antigen. *J. Clin. Invest.*, 61:731-737.
13. Oaks, E.V. et al. (1986) Serum immune response to *Shigella* protein antigens in Rhesus monkeys and humans infected with *Shigella* spp. *Inf. Immun.*, 53:57-63.
14. Towbin, H. et al. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose: procedure and some applications. *Proc. Natl. Acad. Sci.*, 76:4350-4354.
15. Westphal, O. et al. Bacterial lipopolysaccharides: Extraction with phenol water and further applications of the procedure. In: Whister, R.L. (ed), *Methods in Carbohydrate Chemistry*, Vol. 5, p.83-91, Academic Press, Inc., New York.

16. WHO, Diarrhoeal Diseases Control Programme, Development of Vaccines Against Shigellosis. Summary of a meeting held at the National Institute of Cholera and Enteric Diseases, Calcutta, India, May 1986.

ABSTRACT SUMMARY

This study is meant to investigate the secretory IgA antibody response during shigellosis in children. By measuring the salivary IgA levels in patients, one can evaluate the local immune response in the small intestine.

Saliva will be analyzed using ELISA and Western-blot.

It is expected that knowledge of the local immune system can give a valuable contribution to the development of an effective vaccine.

- 1) Since the majority of infections occurs in children, it is of more relevance to study the immune response in this high-risk group.
- 2) There are no risks involved.
- 3) N.A.
- 4) Patients will be identified by a study number, data will be handled with proper care.
- 5) All guardians will be asked to sign a consent form.
- 6) N.A.
- 7) No personal benefit for the patient; society can benefit if this study gives a relevant contribution to the development of a *Shigella* vaccine, as well as to the development of non-invasive methods for establishing a rapid diagnosis.
- 8) Saliva will be collected.

CONSENT FORM

At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), research is carried out for a better understanding how to prevent people from being attacked by blood dysentery. We are studying the defense mechanism of the body against the bacteria which cause blood dysentery. We sincerely ask for your cooperation in this study, hoping that it will help us to gain a better insight into the processes involved.

If you kindly agree to participate in this study, you may expect the following:

- a) We will collect some saliva from your child on the day of admission and day 7 and day 14 of illness. Your child will be asked to spit into a beaker or we will use a glass dropper.
- b) On the same days we will collect a stool sample from your child, using a rectal swab, in case there has been recurrent diarrhoea.
- c) We will visit your house to collect the samples in case you have already left the hospital before the completion of the study.
- d) Your medical records will be kept confidential.
- e) You do not have to participate in the study. Your decision to join or not to join the study will not in any way affect your medical treatment while you are in the hospital. Once you enter the study, you are free to leave the study without any risk of loosing your medical care.
- f) We will be ready to answer any of your questions concerning the study.

If you agree to participate in this study, please sign your name here.

Signature (left thumb impression)
of patient or guardian

Signature of investigator

Date

"સત્તું ગણ"

(ICDR,B)

આર્ગાન્ડિક ઉપરાયક ગવેષણા કેન્દ્ર, વાયામેન એ રણ આયાશ્ટેર કાર્યક્રમ થેકે બિલાબે રહેલા ગાંધી શાસ્ત્ર સે વાગ્યારે ગવેષણા ચલાયેલું હોય છે। આદરા રણ આયાશ્ટેર કાર્યક્રમ વે બાંકટેરિયા ડાર વિરાસ્તે પરીક્રમાર હે એકિજા રાયાની ડા તુલાદે ચેટો કરાયિ, એટે ગવેષણા કરીયા આદરા આનંદાર પ્રોત્સાહિક સહયોગીઓ કાર્યાના કરાયિ, શા આયામેર એટે ગવેષણાર વિષયકે ગણીય તથે તુલાદે સાધારણ કરાયિ ।

બધી આપની એટે કાર્યક્રમ અંશ ગ્રહણ ઇચ્છાક હો જવે નિરૂપિષિત વિષયગુમોદે આનંદારે સત્તું ગણી ખિંચે : -

- ૧। આદરા આનંદાર નિયુર મુખ થેકે કિંદુ લાદા (બુદ્ધ) નિવ હે દિવ સે તર્ફ હો એંધ અનુભૂતાર સ્નેહ એંધ ચહૂર્ય પિંદે આનંદાર નિયુરે એકટી ગાંધી મુખ ફેલાયે હો અથવા એકટી ડ્રગાર કાર્યાના કરાયા હોય ।
- ૨। સેએ એકટી દ્વિનૃમુદોદે આનંદાર નિયુર મળ (ગોત્રધાના) રેકટાસ સોધાર કાર્યાના કરાયે નાનું કરાયા હોય એટે બધી ડાયુરિયા ચલાયે થાકે ।
- ૩। બધી આપની કાર્યક્રમ અનંદાનું રેખે બાઢી ચલે યાય હો આદરા આનંદાર બાઢી થેકે હળ (ગોત્રધાના) નંત્રાય કરુબ ।
- ૪। આનંદાર સરદ તાનુસરી મરી પર ગોધુમ રાખા હોય ।
- ૫। આનંદાર એટે કાર્યક્રમ યોગદાન કરા આનંદાર યોગદાન કરા વા યા કરા કોન અને આનંદાર હાસપાઠામેર સુયોગ સુબિધાર ગાંધીને બાધાનું હો ના, બધી આપની એટે કાર્યક્રમ એકબાર અંધગ્રહણ કરાયે, હથાપિઓ હે કોન સદ્ગુરી આપની એટે કાર્યક્રમ થેકે અન્યાન્ય ખેટે પારોય એટે આનંદાર હાસપાઠામેર સુબિધા ખેટે સદ્ગુરી હોય ના ।
- ૬। આદરા એટે કાર્યક્રમ સન્નાંગુલીર હે કોન ધરણે એટેર ઉત્તર દિંદે કૈરી આયિ ।

આપની એટે ગવેષણા કાર્યક્રમ બાઢી હોય આયામેર એટે સત્તું ગણી સાથે સાથે તુલાંગુલીર હાપ નિવ ।

સ્વાહારાંગુલીર વાદ હાતેર તુલાંગુલીર હાપ

CONSENT FORM

At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), research is carried out for a better understanding how to prevent people from being attacked by blood dysentery. We are studying the defense mechanism of the body against the bacteria which cause blood dysentery. We sincerely ask for your cooperation in this study, hoping that it will help us to gain a better insight into the processes involved.

If you kindly agree to participate in this study, you may expect the following:

- a) We will collect some saliva from your child on the day of admission and day 7 and day 14 of illness. Your child will be asked to spit into a beaker or we will use a glass dropper.
- b) On the same days we will collect a stool sample from your child, using a rectal swab, in case there has been recurrent diarrhoea.
- c) In case you have left the hospital before the completion of the study, we would like you to visit the hospital on day 7 and/or day 14 of illness in order to collect the samples.
- d) Your medical records will be kept confidential.
- e) You do not have to participate in the study. Your decision to join or not to join the study will not in any way affect your medical treatment while you are in the hospital. Once you enter the study, you are free to leave the study without any risk of loosing your medical care.
- f) We will be ready to answer any of your questions concerning the study.

If you agree to participate in this study, please sign your name here.

Signature (left thumb impression)
of patient or guardian

Signature of investigator

Date

"সমুচ্চি পত্র"

(ICDDR,B)

আনুষাঙ্গিক উদ্যোগ গবেষণা কেন্দ্র, বাংলাদেশ এ উক্ত আবাসিকের আন্তর্ম্ময় থেকে বিভাবে রক্ষা পাওয়া যাব সে কানুনের গবেষণা চলছে। আমরা উক্ত আবাসিকের কানুন হে কানকটেরিয়া কানুন বিভাসের পর্যায়ে এই প্রতিক্রিয়া কানুনে জা বৃক্ষতে চেষ্টা করছি, এই গবেষণা কর্মে আমরা আগনীয় বৈকাণ্ঠিক সহকোগীকা কানুন করছি, যা আমাদের এই গবেষণার বিষয়কে পত্তীর ভাবে বৃক্ষতে পাহাড় করবে।

যদি আগবি এই কার্যকলাভ অংশ প্রয়োগ ইঙ্গুক হব তবে বিশ্বমিলিক বিষয়গুলোতে আগবাকে সমুচ্ছি দিকে হবে :-

- ১। আমরা আগনীয় পিশুর মুখ থেকে কিন্তু মালা (বৃক্ষ) নিব হে নিব সে উচি হবে এবং অসুস্থচার সপুর এবং চুর্ণ নিবে আগনীয় পিশুকে একটি পাত্র বৃক্ষ কলতে হবে অথবা একটি ত্রিপাত্র কানুন করা হবে।
- ২। সেই একই দিবগুলোতে আগনীয় পিশুর মল (গোক্রামা) রেক্টাল সোন্দাব কানুন করে সংশ্লিষ্ট করা হবে যদি জাহরিয়া চলতে থাকে।
- ৩। এক্ষেত্রে আগনীয় বাঢ়ী থেকে আমরা অসুস্থচার এবং এবং ১৪মিনিমে বৃক্ষ সংশ্লিষ্ট করব যদি আগবি কার্যকলাভ অসমাপ্ত রেখে হাসপাতাল হেতু বাঢ়ী চলে থান।
- ৪। আগনীয় সকল তাত্ত্বকী বর্ষী প্রতি গোপন রাখা হবে।
- ৫। আগনীয় এই কার্যকলাভ বৈগোপন করা আবশ্যিক নহ, আগনীয় বৈগোপন করা বা যা করা কোন ক্ষেত্রে আগনীয় হাসপাতালের সুবিধাৰ পাওয়াতে বাধাপ্রয়োগ হবে না, যদি আগবি এই কার্যকলাভ একবার অংশগ্রহণ কৰবে, তখাপি হে কোন সহজই আগবি এই কার্যকলাভ থেকে অকাইতি পেতে পারেন এতে আগনীয় হাসপাতালের সুবিধা পেতে কোন অসুবিধাৰ মুক্তি হবে না।
- ৬। আমরা এই কার্যকলাভ সন্তুষ্টে হে কোব ধৱণের প্রয়োগ উক্তর দিকে তৈরী আছি।

আগবি এই গবেষণা কার্যকলাভ রাজি হলে আমাদের এই সমুচ্ছি পত্রে সই অথবা বাস হাতের বৃক্ষাংগুলির কান নিব।

সুকর্ময়োগীর বাস হাতের বৃক্ষাংগুলির আপ

To the parents of

At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka research is carried out on all aspects of diarrhoeal diseases. Studies are carried out on the causes and treatment of diarrhoea, socio-economic aspects involved and also vaccine development.

We are planning to study the immune response of the body against the bacteria *Shigella* which cause bacillary dysentery. For this purpose, we will use saliva samples from children with shigellosis. We also need "control" saliva samples from healthy children without any history of bacillary dysentery and from non-endemic parts of the world.

We, therefore, would like to ask for your cooperation by giving us permission to collect some saliva (1-2 ml) from your child. The saliva will be collected at the American International School. We will call the child down from class for five minutes and he/she will just have to spit into a beaker. Besides this, we would like you to fill in the attached questionnaire.

All data will be handled with utmost discretion.

In case you have any further inquiries, please contact us at ICDDR,B (phone 600171-8, extension 243/286, Constance Schultz).

Thanking you for your cooperation.

Constance Schultz
Research Fellow

Dr. Firdausi Qadri
Scientist

Dr. Ivan Ciznar
Associate Director
Laboratory Sciences
Division

QUESTIONNAIRE

1. Are you willing to help us in this study by permitting your child to participate?

YES

NO

If "yes", please answer the following questions:

2. Did your child have diarrhoea after arrival in Bangladesh?

YES

NO

3. In case of diarrhoea, was a stool sample examined?

YES

NO

Diagnosis _____

4. Were there any family members who contracted diarrhoea after arrival in Bangladesh?

YES

NO

5. In case of diarrhoea, were stool samples examined?

YES

NO

Diagnosis _____

6. Where did your child live in the previous years, before coming to Bangladesh?

DATA FORM

NAME : .

AGE : .

I.C. No. : .

STUDY No. : PS

ON ADMISSION : _____ (DATE)

- DURATION OF DIARRHOEA : _____ DAYS (MAX. 4 DAYS)

- ASPECT OF STOOL : _____

- GRADE OF DEHYDRATION : MILD [] MODERATE [] SEVERE []

- DIAGNOSIS : *S. dysenteriae* 1 [] ✓

S. flexneri []

- TREATMENT : _____

- SALIVA SAMPLE COLLECTED : YES [] NO []

DAY 7 : _____ (DATE)

- SALIVA SAMPLE COLLECTED : YES [] NO []

- RECTAL SWAB COLLECTED : YES [] NO []

RESULT : _____

DAY 14 : _____ (DATE)

- SALIVA SAMPLE COLLECTED : YES [] NO []

- RECTAL SWAB COLLECTED : YES [] NO []

RESULT : _____

ICDDR,B
1988 BUDGET PROPOSAL
 (In US \$)

PARTICULARS

Division Name: LABORATORY SCIENCES DIVISION

Protocol/Branch name: Secretory IgA antibody in saliva of children with shigellosis.
 Immunology & Bacterial Genetics Department

Name of P.I./Branch Head/Division Head: Ms. Constance Schultsz/Dr. Ivan Ciznar

Budget Code: Starting Date: May 1988

Protocol No: Completion Date: May 1989

Donor Name: USAID Grant Amount:

Column A : Column B : Column C

EXPENSE CATEGORY

Actual	Estimated	Proposed
Jan.-June	Whole Yr.	1988
1987	1987	

A/C No.	Description	Refer Page	Column A	Column B	Column C
3100	Local Salaries	2			5,100
3200	Intl. Salaries	8			
3300	Consultants	14			
3500	Travel Local	15			
3600	Travel Intl.	16			
3700	Supplies & Mat.	18			4,450
4000	Other Costs	19			300
4800	Inter Deptl. Ser.	21			3,550
Total Direct Operating Cost					13,400
0300	Capital Expenditure Refer Page 22				
TOTAL DIRECT COST					13,400


 Branch Head

Associate Director

Reviewed by Budget & Finance

Budget Code: _____

PERSONNEL REQUIREMENT-(LOCAL STAFF) 1988

	No. of Positions	No. of Man Months	\$ Amount
1. Direct Project/Protocol/Branch Staff at 1.1.1988 Sourced from Page 3			
add:			
· New Recruitments Sourced from Page 4	1	12	4,224
· Manpower allocated from other areas Sourced from Page 5	2	4	876
(i) Sub-Total	3	16	5,100
less:			
· Separations Sourced from Page 6			
· Manpower allocated to other areas Sourced from Page 7			
(ii) Sub-Total			
(i)-(ii) TOTAL	3	16	* 5,100

*AGREES WITH
PAGE 1
A/C NO. 3100
COLUMN C

Budget Code:

NEW RECRUITMENT-(LOCAL STAFF)-1988

** See Annexure-A for rates

* AGREES WITH PAGE 2 ROW B

Budget Code

MANPOWER-ALLOCATED-FROM-OTHER-AREA-(LOCAL-STAFF)-1988

TOTAL : * 2 : XXXXXXXX : * 4 : XXXXXXXXXX : * 876

** See Annexure-C for average rate/month

* AGREES WITH PAGE 2 ROW C

Budget Code: _____

SUPPLIES AND MATERIALS-1988

A/C Code:	Item Description	\$ Amount
3701: <u>Drugs</u> (used for medication in the hospitals and field stations)		
3702: <u>Glassware</u> (Bottle, beaker, cylinder, petridish, aluminium seal, slides, stopper, tube etc.)		300
3703: <u>Hospital supplies</u> (bandage, gauze, blade, bowl, catheter, cotton, needle, syringe, solution, leukoplast, towel etc.)		
3704: <u>Stationery and office supplies</u> (Battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)		200
3705: <u>Chemicals and media</u> (Acid, reagent, dextrose, sodium, bactoagar etc.)		1,250
3706: <u>Materials for uniform</u> (Cloth, button etc. required for making uniforms)		
3707: <u>Fuel, oil and lubricants</u> (Diesel, mobil, petrol, kerosene etc.)		
3708: <u>Laboratory supplies</u> (Aluminium foil, bag, blade, brush, cap, container, film X-Ray etc.)		50
3709: <u>Housekeeping supplies</u> (Aerosol, battery, wiping cloth, duster, lock and key etc.)		50
3710: <u>Janitorial supplies</u> (Bleaching powder, brush, detol, detergent, insecticide, soap etc.)		
Total (balance c/f)		1,850

(Contd. to page No. 18)

Budget Code: -----

SUPPLIES AND MATERIALS-1988

(Contd. from Page No. 17)

A/C :	Item Description	\$ Amount
	Page total from page No.17 (balance b/f):	1,850
3711:	<u>Tools and spares</u> (Automobile spares, tyres, tubes, battery, stores required for maintenance services etc.)	
3712:	<u>Non-stock supplies</u> (Materials not normally kept in stock and purchased only against specific requisitions)	2,000
	Sub-Total	3,850
3713:	<u>Freight and other charges</u> Add 30% to above sub-total for imports.	600
	TOTAL	* 4,450
	*AGREES WITH	
	PAGE 1	
	A/C 3700	
	COLUMN C	

Note: For rates please contact Supply Ext.260.
Add 10% for inflation

Budget87.18

Budget Code: _____

OTHER COST-1988

A/C	Code	Accounts Description	\$ Amount
3800		<u>Repairs_and_maintenance</u> (Maintenance and repairs of vehicles, equipments, furniture and building)	
3900		<u>Rent_communication_and_utilities</u> (Postage, telephone, telegram, electricity etc.)	100
4100		<u>Bank_charges</u>	
4200		<u>Legal_and_professional_expenses</u> (Professional membership fee, legal fee, audit fee etc.)	
4300		<u>Printing_and_publication</u> (Printing of forms, books, journals, reprints etc.)	100
4400		<u>Hospitality_&_donation</u> (Guest house accommodation, donations, hospital food, lunch, refreshment etc.)	100
4500		<u>Service_charges</u> (Porter, labour, washing, laundry and other misc. exp.)	
4600		<u>Staff_development_and_training</u> (Training course fee, training materials, stipend, scholarship, subsistence paid to the staff)	
		TOTAL	* 300

*AGREES WITH
PAGE 1
A/C No. 4000
COLUMN C
=====

Budget Code: _____

****INTERDEPARTMENTAL SERVICES-1988**

A/C Code	Service Area	\$ Amount
4801	Computer	
4802	Transport Dhaka	500
4803	Transport Matlab	500
4804	Water transport-Matlab	500
4805	Transport Teknaf	
4806	Xerox and mimeograph	100
4807	Pathology	
4808	Microbiology tests	
4809	Biochemistry	
4810	X-Ray	
4811	I.V. fluid	
4812	Media	
4813	Patient hospitalisation study	
4814	Animal research	
4815	Medical illustration	400
4817	Telex	
4818	Out patient care	
4819	Maintenance charges	
4820	Vehicle maintenance charges	
4821	Library service charges	50
4822	Staff Clinic Charges - Dhaka	
	Page total (balance o/f)	2,050

(Contd. to page No. 21)