LSD 2001

Attachment I

Date:

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Firdausi Qadri

Application No. 2001-027

Title of Study: Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I and Part II

Trainee Investigator (if any): Mr. Tawfiqur R. Bhuiyan

Supporting Agency (if Non-ICDDR,B) Sida-SAREC

Project Status: RRC approved

[✓] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the 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) Minor or persons under guardianship — ~~Yes~~ No

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes ~~No~~
   - (b) Social risk — 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 or other) — ~~Yes~~ No
   - (b) Use of fetal tissue or abortus — Yes ~~No~~
   - (c) Use of organs or body fluids — ~~Yes~~ No

4. Are Subjects Clearly Informed About:
   - (a) Nature and purposes of the study — ~~Yes~~ No
   - (b) Procedures to be followed including alternatives used — ~~Yes~~ No
   - (c) Physical risk — ~~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 parents or guardian (if subjects are minor) — ~~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 with 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. Example 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 Committee for review

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

Firdausi Qadri
Principal Investigator

F. Qadri for Tawfiqur R. Bhuiyan
Trainee

ICDDR,B: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL

Protocol No.: 2001-027

FOR OFFICE USE ONLY

RRC Approval: Yes/ Date: 23/12/01

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: **Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I-Studies to facilitate ETEC vaccine efficacy trials. Part II- Cholera and ETEC vaccine studies.**

Theme: *(Check all that apply)*

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation✓
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health✓
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: ETEC, Cholera, novel antigen

Principal Investigators: **Division:** **Phone:**
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Professor Ann-Mari Svennerholm (Goteborg University, Sweden),

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Mahakhali 1212

Co-Principal Investigator(s):
Professor David A. Sack, ICDDR,B
Professor Jan Holmgren. Goteborg University, Sweden

Co-Investigator(s):Dr. A.K. Siddique, Dr. Tanvir Ahmed, Dr. G.B. Nair, , Dr. K. Zaman, Dr. M. A. Salam, Dr. A. S.G. Faruque Dr. Rob Breiman, Dr. K. Z. Hasan, Professor Wim Gaastra (Sweden).

Student Investigator/Intern: Mr. Tawfiqur Rahman Bhuiyan (Postgraduate student for studies leading to PhD)

Collaborating Institute(s): Department of Microbiology and Immunology, Goteborg University, Goteborg, Sweden

Population: Inclusion of special groups (*Check all that apply*):

Gender
- ☐ Male✓
- ☐ Females ✓

Age
- ☐ 0 – 5 years✓
- ☐ 5 – 9 years✓
- ☐ 10 – 19 years✓
- ☐ 20 +✓
- ☐ > 65
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify _____)
- ☐ Animal

Project / study Site (*Check all the apply*):
- ☐ Dhaka Hospital✓
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh - Mirpur field station✓
- ☐ Outside Bangladesh
  Name of country:
- ☐ Multi centre trial
  (Name other countries involved)

Approval of the Project by the Associate Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Name of the Associate Director	Signature	Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

1. **Signature of PI** **Date:**

ICDDR,B

2. **Signature of PI** **Date:**

Goteborg University,Sweden

Principal Investigator: Last, first, middle _Qadri Firdausi_____

Approval of the Project by the Associate Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Dr. G.B. Nair		Oct 7, 2001
Name of the Associate Director	Signature	Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Date:

1. **Signature of PI** Firdausi Qadri

ICDDR,B

2. **Signature of PI**

Göteborg University, Sweden Prof. Ann-Mari Svennerholm Leg.läk.

ABSTRACT SUMMARY

Acute diarrhoeal diseases are a major health problem in developing countries. Infection with Enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* are the most common causes of acute watery diarrhoea among children in developing countries and it also contributes to malnutrition. Between 15-20% of the diarrhoeal stools screened from patients in the 2% routine surveillance system of ICDDR,B are positive for these two bacterial pathogens. These infection however could be prevented by safe and immunogenic vaccines that are protective in children. One such vaccine undergoing evaluation is the oral inactivated ETEC vaccine consisting of recombinant cholera toxin B subunit and a mixture of formalin-inactivated whole cell ETEC bacteria expressing colonization factors (CF-BS-ETEC vaccine). This vaccine is being tested in a number of countries in phase-I-III in adults and children. Studies carried out in Bangladeshi children in the 18 months to 10 year range has shown that the vaccine is safe and immunogenic (Qadri et al., manuscript submitted). However, in younger Bangladeshi children, 6-17 months of age a lowered, one-fourth dose of the vaccine has been found to be safe and is the dose that will be used for children in studies in Bangladesh.

A key vaccine for protection from cholera is the oral inactivated whole cell cholera vaccine containing the recombinant B subunit of cholera toxin (WCO1-BS). It has been extensively field tested in both adults and children in Bangladesh and other countries of the world and is also licensed in some countries.

To make the inactivated oral ETEC and cholera vaccines applicable for use in children in developing countries like Bangladesh, further studies need to be carried out. Important among these are studies to prepare for a Phase III vaccine trial of the CF-BS-ETEC vaccine in Bangladesh, which include studies on optimal vaccine formulation and epidemiological and immunological studies on ETEC patients. Finally studies are also needed on the freeze dried formulation of the cholera vaccine so that it can be used more easily and effectively in developing countries. Since both ETEC and *V. cholerae* are the major bacterial pathogens causing acute watery diarrhoea, a combination vaccine to protect against both would be ideal for developing countries like Bangladesh and is planned here.

For the different component of the studies we plan to enroll and follow newborn infants for 2 years, children from 6 months of age to 17 years and adults up to 45 years of age. Healthy study subjects for the vaccine studies and patients with ETEC diarrhoea will be enrolled in the study. The study is planned in phases for a period of three years, so that results of one component will complement that of the next phase and lead to effective and efficient running of the project, evaluation of data and dissemination of information.

Clarification of points as required in the attached list are as follows:

1. Children in the 6 month to 5 years of age will be included since both ETEC diarrhoea and cholera are major cause of morbidity and mortality and a vaccine is needed urgently for them. However adults will also be studied where safety and immunogenicity studies on vaccines have to be first conducted in them before proceeding to children. In addition patients with ETEC diarrhoea, both children and adults will be studied. Only children whose guardians or parents have given voluntary consent to participate in this study will be enrolled. Children with ETEC diarrhoea who are 6-17 years of age will only be enrolled in the study if they agree to participate. Their parents/guardians will give verbal and written consent to participate in the study.

2. Methods used have very little chance of physical risk.
3. Since only blood will be collected there is little risk other than a minimum discomfort at puncture site. After feeding the oral ETEC and cholera vaccines the children will be kept under observation for 30 minutes and will also be monitored for side-effects for 3 consecutive days.
4. The interview records will be kept in a locker under supervision. Only the investigators will have access to it.
5. Informed consent will be obtained from the guardian or parent of the child. The potential risks and the procedures to be carried out are mentioned in the consent form.
6. The interview will be conducted in the homes of the children for obtaining history prior to enrollment in the different aspects of the study. Where necessary however, interviews will be carried out in the field site office. Monitoring the side-effects of the vaccine in the children will be carried out in subsequent home visits. In the case of adults who will be vaccinated, they will be interviewed 7 days after intake of each dose of the vaccine when they return to the field site. Approximately 30 minutes to 1 hour will be required for the interview.
7. The results of the studies on the ETEC and cholera trials and the studies on the children and adults with ETEC diarrhea will benefit children in any endemic country especially Bangladeshi children.
8. The activity will require collection of stool and small amounts of blood from the children and adults who will be enrolled in the study.

Table of Contents

___ Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (**TYPE TEXT WITHIN THE SPACE PROVIDED**).

Principal Investigator: Firdausi Qadri (ICDDR,B) and Professor Ann-Mari Svennerholm (Goteborg University,Sweden)

Project Name: **Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh**:

Part I-Studies to facilitate ETEC vaccine efficacy trials.

Part II- Cholera and ETEC vaccine studies

Total Budget . US$ 925,801 Beginning Date ASAP -2002 Ending Date 31/12/2004, 3 year duration

Acute diarrhoeal diseases are a major health problem in developing countries and ETEC and *V. cholerae* are the major bacterial pathogens contributing to the disease burden. Infection with ETEC is one of the most common causes of diarrhoea among children in developing countries and it also contributes to malnutrition. On an average, approximately 15% and 20% of the diarrhoeal stools screened from children up to 5 years of age in the 2% routine surveillance system of ICDDR,B are positive for ETEC and *V. cholerae* O1 respectively.

It is believed that these infections can be controlled and prevented by safe and immunogenic vaccines that are protective in children. The oral inactivated whole cell cholera vaccine containing the recombinant B subunit of cholera toxin (WC01-BS) has been extensively field tested in both adults and children (Clemens et al. 1990, Concha et al. 1995, Legros et al. 1999) and is also licensed in some countries. However studies are needed to facilitate its use in developing countries by freeze dried formulations of the vaccine so that it can be used more easily. Another vaccine undergoing evaluation is the oral inactivated ETEC vaccine consisting of mixture of formalin-inactivated whole cell ETEC bacteria expressing colonization factors (CF) plus BS (the CF-BS-ETEC vaccine). The vaccine has been tested in a number of countries in phase-I to II safety and immunogenicity trials in adults (Savarino et al. 1999,Qadri et al. 2000). These initial vaccine trials have been extended to larger field based studies in Egypt and Bangladesh in younger children and infants (Savarino et al. and Qadri et al. ongoing studies). Phase I-III studies in Egypt and Phase I-II studies carried out in Bangladeshi children in descending age groups from 9 years to 18 months of age have shown that the vaccine is safe and immunogenic (Qadri et al. 2000, Qadri et al., unpublished results). However since both ETEC and *V. cholerae* are the major bacterial pathogens causing acute watery diarrhoea, a combination vaccine to protect against both would be most useful in these settings. The main objective of this proposal include the following:

A. Conduct studies to prepare for a Phase III vaccine trial of the CF-BS-ETEC vaccine in Bangladesh. This includes to determine (1) optimal age at which to start vaccination. (2) to assess the need for buffer for the vaccination in the young children, 6-17 month of age (3) to carry out epidemiological studies to assess variations of ETEC infections and virulence factors and to see if protection is related to immune responses against CFs and other virulence antigens. Efforts are being made to monitor the prevalence of ETEC, emergence of new colonization factors and virulence antigens and study the protection afforded by natural ETEC infection so as to better understand the suitability of the ETEC vaccine in Bangladesh. To achieve this, studies will be carried out in a cohort of children 0-2 years of age, to follow the infection/ re-infection pattern and the natural protection that is attained by prior exposure.

B. Evaluate a combined CF-BS-ETEC and WC01-BS cholera vaccine in children.

C. Evaluate the dried formulation of the oral inactivated whole cell WC01-BS cholera vaccine.

D. Study the expression of virulence factors in ETEC during infection *in vivo* as compared with after culture *in vitro*. ETEC freshly isolated from stools of patients will be studied for novel and *in vivo* expressed antigens as well as the mRNA for these antigens to determine if some factors are expressed in the gut but lost on subculture. The mucosal immune response to these antigens will be tested to find out if these factor (s) are indeed immunogenic and contribute to the immune response.

INVESTIGATORS

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1..Dr. Firdausi Qadri, LSD	Immunological/immune response studies in subjects and vaccine	PI, ICDDR,B
2..Professor Ann-Mari Svennerholm	Immunologist/Development of vaccine & vaccine related work	PI.in Sweden
3.ProfessorD.A.Sack	Microbiologist/ Epidemiologist	Advice and support
4. Professor Jan Holmgren	Immunologist/Development of vaccine & vaccine related work	Expert advice
5..Dr. A.K. Siddique	Epidemiologist	Advice and support
6..Professor Wim Gaastra	Molecular Biologist	Expert. in Mol .biol
7..Dr.Tanvir Ahmed, LSD	Physician	Clinical support
8. Dr. K. Z. Hasan	Epidemiologist	Advice & support
9..Dr. ASG Faruque CSD	Epidemiologist	Advice & support
10..Dr. K.Zaman PHSD	Epidemiologist	Advice & support
11..Dr. Rob Brieman PIDVS. HSRD	Epidemiologist	Advice & support
12. Dr. M.A.Salam.	Physician	Advice & support
13. Dr. G.B. Nair	Microbiologist	Advice & support

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Part I

1.The CF-BS-ETEC vaccine in the appropriate formulation is safe and immunogenic in young children up to 3 years of age in Bangladesh.

2. ETEC infections appear early in life and a first infection modifies the severity of the later ETEC episodes by the presence of protective antibodies containing homologous and cross-reacting colonization factors.

3. ETEC may express novel antigens *in vivo* in the gut which are lost on subculture but are immunogenic in the host.

Part II

1. A combined formulation of oral inactivated CF-BS-ETEC and the WCO1-BS cholera vaccine is comparable in safety and immunogenicity as the individual vaccines alone in children.

2. A dried formulation of the oral whole cell cholera plus cholera toxin B-subunit vaccine (WCO1-BS) is safe and immunogenic in Bangladeshi volunteers as a means to its practical application in developing countries.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

Part I: Studies to prepare for an ETEC vaccine efficacy trial in Bangladesh

The main goal is to carry out studies needed to prepare for a Phase III trial of the oral inactivated whole cell enterotoxigenic *Escherichia coli* (ETEC) vaccine in Bangladeshi children. Since the majority of ETEC cases occur during the first 2- 3 years after birth, this would include the following epidemiological, immunological, buffer formulating as well as safety and immunogenicity studies.

1. Evaluate the requirement and suitability of different formulations of buffers which may be used to administer the ETEC vaccine in the appropriate dose to the children (6-17 months).

2. Prospectively analyze the incidence of ETEC infection in a cohort study in an urban setting in Bangladesh and determine the incidence related to age in children 0-2 years of age. Analyze the incidence of ETEC infections both toxin types and colonization factors and immunological response to homologous and cross-reacting CFs.

3. Study if there is *in vivo* expression of virulence factors in ETEC during infection as compared with after culture *in vitro* to identify possible novel antigens. Study the messenger RNA as well as the *in vivo* expression of novel antigens in ETEC freshly isolated from patients and determine if there are immune responses against such *in vivo* specific antigens.

Part II. Studies on Cholera and ETEC vaccines

To study the safety and immunogenicity of a combination of the CF-BS-ETEC vaccine and WCO1-BS vaccine in Bangladeshi children for wider protection from acute watery diarrhoea caused by the two most common bacterial pathogens.

1. To study the safety and immunogenicity of the combined cholera and ETEC vaccine in adults (18-45 years) before proceeding to children.

2. To study if the combined vaccine is safe and immunogenic for children 3 years to 18 months of age.

3. Study the safety and immunogenicity of the combined vaccine in which a ¼ dose of the ETEC vaccine is combined with one full dose of the cholera vaccine for children- 6-17 months of age in Bangladesh children.

4. Study the safety and immunogenicity of a dried formulation of the WCO1-BS vaccine in adult (18-45 years) Bangladeshi volunteers.

Background of the project including preliminary observations and previous studies carried out so far.

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

Studies on the CF-BS-ETEC Vaccine - Background and studies carried out so far

The pathogenicity of ETEC is due to their ability to colonize the small intestine and to produce one or both of two types of toxins, the heat stable enterotoxin (ST) (Field *et al.*, 1978) and/or the heat labile enterotoxin (LT) (Svennerholm and Holmgren, 1978). These bacteria also possess a variety of surface located adhesins that anchor them to the intestinal mucosal receptors (Gaastra and de Graaf, 1982; Gaastra and Svennerholm 1996). This anchorage enables the bacteria to resist the flushing action of the intestinal peristalsis and the toxins are delivered close to the enterocytes. The adhesins are termed colonization factors (CFs) and may be fimbrial, fibrillar or non-fimbrial in structure. Some 20 or more adhesins have been identified on human ETEC strains (Gaastra and Svennerholm, 1996).

Epidemiological studies carried out in various geographical areas show a large variation in the prevalence of CFs in ETEC isolated in the different regions (Gaastra and Svennerholm, 1996). However, the 7 best characterized CFs are CFA/I, CS1, CS2, CS3 (CFA/II components), CS4, CS5 and CS6 (CFA/IV components). These have been found in 50-80% of clinical ETEC isolates worldwide. In some areas CFA/I and CFA/IV are common (Lopez-Vidal *et al.*, 1990; Gothefors *et al.*, 1994), whereas in others CFA/II may be more prevalent (Wolf *et al.*, 1993; Levine *et al.*, 1993). In a prospective study carried out in Bangladesh, we have found that CFA/IV, CFA/I and CFA/II are the most common, although other CFs are also present (Qadri et al. 2000).

Epidemiological and immunological studies to understand the development of natural immunity to ETEC

The peak of incidence of ETEC diarrhoea in endemic countries occurs in the first year of life, with a declining incidence with age thereafter. Of the 7-8 episodes of diarrhoea annually in children, in the developing world, at least 2-4 attacks may be due to ETEC (Black, 1993). In a prospective study recently conducted in ICDDR,B, the highest incidence of ETEC was in children less that 5 years of age. About 74% of ETEC were isolated from diarrhoeal children less than 5 years of age, of whom 90% were below 3 years of age (Qadri *et al.*, 2000). It was observed that the incidence of ETEC diarrhoea increased from 3 months of age and peaking between 6 months to 2 years of age after which a decrease in the incidence was observed. The pattern of CFs expressed on ETEC from three different diarrhea surveillance sites of ICDDR,B in Bangladesh (PI. Dr. A.K Siddique and Dr. R.B. Sack) as well as Matlab (PI. Dr. A.S.G. Faruque) is also continuing and appears to be similar to the profile seen in ETEC isolated from patients in the Dhaka hospital. The CFs most commonly isolated are those present on the oral ETEC vaccine being tested which is composed of six CFs including CFA/I, CS1- CS5.

Although several studies (Cravioto 1990, Paniagua et al. 1997) have been carried out to determine the prevalence or incidence of ETEC infection and its colonization factors, only a few studies have analyzed the situation over time or have related the incidence of ETEC with the immune response. The aim of the present study is to prospectively analyze the incidence of ETEC infection and the colonization factors expressed on ETEC in relation

to age in an urban slum in Dhaka, Bangladesh. The follow up design for the first 2 years of life will also make it possible to examine the development of natural immunity to homologous CF of the infecting ETEC strain and to the cross-reacting CF antigens. ETEC is known to express over 20 different CFs; the most common and the best characterized ones are CFA/I, CS1,CS2,CS3, CS4, CS5, CS6. These seven CFs have been found in 50-80% of clinical ETEC isolates in epidemiological studies (Giron 1995). Although it has been claimed that the CFs are separate antigens, cross-reacting epitopes shared by some of the CFs have been demonstrated. Thus, partial antigenic homology exists between the subunits of certain CFs, namely CFA/I, CS1, CS2, CS4, and CS17 in Western blot analyses (McConnell et al. 1989). In addition it has been demonstrated that monoclonal antibodies raised against the fimbrial subunits of CFA/I (Rudin et al. 1994), as well as the sera of patients infected with CFA/1-expressing strains cross-reacted immunologically with CS1, CS2, CS4, CS17, and CS14 (Rudin et al 1997). A systematic study needs to be carried out to understand the protection against reinfection and the contribution of cross reactivating antigens in the protection. For this purpose infants will be followed up in an urban slum of Dhaka city to determine the infection-reinfection pattern due to ETEC over the first 2 years of life. Such a study has not been carried out earlier and only epidemiological retrospective studies have been carried out to study the incidence of ETEC infections and relate it to the development of protection. We hope that as a result of this design we will be able to directly link the immune factors that lead to protective immunity in residents in an ETEC endemic country.

Vaccines to protect against ETEC infection

For ETEC, which are noninvasive, antibodies that can be induced locally in the gut are believed to be protective (Levine, 1990). Both antibacterial (mainly directed against CFs) and antitoxic antibodies act synergistically to provide protection (Ahren and Svennerholm, 1982). Protective immunity is dependent on the stimulation of the mucosal immune system and generation of secretory IgA antibodies in the gut-associated lymphoid tissue. Studies in animal models and human volunteers suggest that primary ETEC infections can protect against re-infections (Levine *et al.*, 1984; Svennerholm *et al.*, 1990). Natural ETEC infection also appears to be protective. This is evident since the highest incidence of the disease is in the age group of 6-36 months and the rate of illness decreases with age, suggesting the development of protective immunity (Black *et al.*, 1986).

ETEC infection is spread through water or food contaminated by faeces from infected persons. The disease can be effectively prevented if clean water and food and good sanitary conditions are made available in the developing countries. However, since this is not possible to accomplish in the near future, the other alternative for the prevention of the disease would be the availability of a vaccine that is cheap and effective. The vaccine should be easily administered to infants and children, who are the most vulnerable to infections.

Since ETEC causes considerable morbidity and mortality the development of a safe and effective vaccine has been given a high priority (WHO, 1999). An ideal ETEC vaccine should be given orally and contain a combination of CFs and the heat-labile toxin antigen derived antigen. The oral inactivated CF-BS-ETEC vaccine containing recombinant cholera toxin B subunit (BS) and a mixture of formalin-killed ETEC strains expressing CFs (CF-BS-ETEC vaccine). The killed vaccine is the only vaccine that is undergoing extensive studies in adults in both developed and developing countries and that has reached the stage of phase-III clinical trials. The vaccine has been found to be safe and immunogenic in adult Swedish volunteers (Wenneras *et al.*,1992; Ahren *et al.*, 1993). Phase III trials of protective efficacy are ongoing in travellers from the USA going to Latin America and those from Europe going to Kenya (WHO, 1999). It has been tested in adult Bangladeshi volunteers and found to be safe and immunogenic, giving rise to ASCs in the circulation and in the gut as well as systemic and local antibodies in the gut (Qadri *et al.*, 2000; Wenneras *et al.*, 1999). The vaccine has also been found to be safe and immunogenic in children 3-9 years of age (Qadri *et al.*, 2000). The vaccine is also undergoing testing in other countries, including Egypt (Savarino *et al.*, 1998) and a phase-II study has been completed in children up to 2 years of age (Savarino *et al.*,1999). In addition phase III studies are being completed in children 6-18 months of age. Studies are being conducted in children down to 2 months of age. In addition safety and immunogenicity studies of combination of other vaccines (DPT, OPV, HepB) with the CF-BS-ETEC vaccine is being carried out in children 2-6 months of

age.

The studies carried out so far in Sweden, USA, Egypt and Bangladesh have found the ETEC vaccine to be safe. In these studies, adverse events were noted in about 2-6% of recipients, which included nausea, mild abdominal cramps, 1-2 loose stools per day during the 3 day observation period. These effects were seen in the group given vaccine and placebo.

In Bangladesh, a double blinded randomized placebo controlled ETEC vaccine study has been conducted in children aged from 3 years to 6 months of age in the Mirpur slum area in Dhaka (1999-2001, Funded by USAID). The study has been unblinded recently in children 18 months to 3 years of age where both the vaccine appears to be safe (Qadri et al. in manuscript). The children responded with significant IgA antibody secreting cell and plasma antibody responses to the different CFs and BS components of the vaccine which was significantly higher than that seen in the placebo recipient.

When the study was carried out in children 6 to 17 months of age, adverse events of vomiting was observed in some children. As a result a dose finding study has been conducted where a ½ dose has been compared with a ¼ dose of the vaccine in children 6 months to 12 years of age. In children 6-17 months of age, the ¼ dose of the vaccine has been found to be safe and immunogenic (Qadri et al. unpublished information). Following this a double blinded randomized study has now been completed in the children where the reduced ¼ dose of the vaccine/placebo has been tested in the children 6-17 months of age). Preliminary analyses shows that the reduced dose of the vaccine did not give rise to adverse events and reactogenicity in the children but was immunogenic (Qadri et al. ongoing studies). Dose finding studies have also been carried out in adults in Sweden where a lowered dose has been tested and compared to the full dose (Jertborn et al. 2001). The lowered dose gave rise to mucosal and systemic IgA antibody responses in the Swedish volunteers. In the study that we plan to carry out in 6-17 month old children in Bangladesh, we will only test a ¼ dose of the ETEC vaccine since it has been found to without adverse effects.

Studies of the appropriate buffer for formulation of the ETEC vaccine in children 6-17 months of age.

In addition to the studies on the ETEC and cholera vaccines we would also like to evaluate the suitability of the buffer that is optimal for formulation of the inactivated ETEC vaccine for children. The buffer is only needed to protect the B subunit of the toxin. It is not clear if acid neutralization is required in young infants and if so how much is needed to neutralize the gastric acidity. Information available in Bangladeshi children suggests that the gastric pH is higher in younger children and if so less buffer will be needed for the immunizations (Bardhan et al. personal communication). At present we use less amount of the bicarbonate buffer for the children as compared to the adults for formulation of the ETEC vaccine. In the case of the children, the amount is decreased based on the age. We will also compare the suitability of the sodium bicarbonate buffer that we use at present to CeraVacx which contains rice syrup solids, sodium bicarbonate and citric acid (Sack *et al.* 1997) and to the response in a group of children who will receive the vaccine which has not been formulated in any buffer.

Combined ETEC and cholera vaccine to protect against acute watery diarrhoea

Both *V. cholerae* and ETEC together account for the majority of cases of acute watery diarrhoea in children and adults. Taken together with rotavirus, the three comprise the major causes of diarrhoea in infants and children . It may be expected that a vaccine to prevent these three diarrhoeal infections would reduce about 70% of the episodes of diarrhoea in children which would resulting in a substantial decrease in morbidity and mortality in the vulnerable age group. A combined vaccine to protect against at least the most frequently encountered common bacterial pathogens, ETEC and *V. cholerae* O1 would be very useful in the developing country scenario where infants and children suffer from repeated episode of diarrhoea every year.

It has been estimated that ETEC infections alone causes 400 million episodes of diarrhoea and 300,000-700,000 deaths annually in children less than five years of age (WHO, 1999). The clinical symptoms of the disease are watery diarrhoea often accompanied with abdominal cramps, malaise, and low grade fever. It may last from 3-7 days and the disease spectrum can range from mild diarrhoea to dehydrating cholera like symptoms which are seen in about 5% of cases (Black, 1986). It has been estimated that of the 7-8 episodes of diarrhoea that occur annually in children in the developing world, at least 2-4 attacks may be due to ETEC (Black, 1993). As a result, ETEC diarrhoea may contribute to growth retardation and death in these children.

Cholera is a major health problem in developing countries and in 1998 a marked increase of nearly 100% was seen on all continents (WHO, 2001). About 293,121 cases and 10,586 deaths due to El Tor *V. cholerae* O1 were reported to WHO in 1998. International agencies, including the WHO, have attached a high priority to the control and prevention of cholera through vaccination since it appears to be the most appropriate short term strategy for the prevention of the disease in Asia, Africa as well as Latin American countries where it has spread.

The concept of combination vaccines to minimize number of immunizations and injections especially for children and infants has already been used for diptheria, tetanus and pertusis (DTP) with *Haemophilus* b polysaccharide-protein conjugates (DTP-Hib). Combination vaccines against enteric infections include potentially cholera, ETEC, Shigella, rotavirus and other diseases and may become available in the longer term. A phase II study is ongoing in Egypt in which the concomitant administration of TOPV-DPT-HepB plus ETEC vaccine is being compared with the TOPV-DPT-HepB vaccines (Savarino et al. personal communication). The study is being conducted in children 2-6 months of age. The two vaccines that appear very feasible to combine now are the cholera and ETEC vaccines since both these oral killed have reached Phase III or Phase IV levels of testing. The evaluation of the combinations however needs to be carried out with caution since it may influence reactogenicity and immunogenicity. It is felt that the requirements should not be made solely on assumptions based on the properties of the individual components. The study planned in this project is therefore to see if the safety or the immunogenicity of the individual vaccines are not reduced as a result of the combination in both children and adults.

Dried formulation of the WCO1-BS cholera vaccine

Although the licensed liquid oral killed whole cell–B subunit cholera vaccine (WCO1-BS) has proved to be both safe and efficacious in both adults and children worldwide, a dry formulation of the same or analogous vaccine which would maximize thermal stability and field distribution and storage of vaccine would be a significant further improvement of this vaccine for broad use in public health control programs. The dried formulation incorporates the needed components including vaccine and buffers which can be conveniently distributed at room temperature. The dried formulation with the needed antigens, buffers and carbohydrates have been prepared and packaged in unit dose sachets for convenient distribution. Preliminary safety and immunogenicity studies have been carried out with the dried WCO1-BS vaccine (Sack et al. manuscript submitted). Comparisons of the dried and liquid formulations have been carried out in adult North American volunteers (Sack *et. al.* personal communication, Chang and Sack, 2001). It was tested in Phase I studies in volunteers who were given two doses of the vaccine 14 days apart. When compared in 12 volunteers, the dried formulation did not result in any adverse event or gastrointestinal disorders of vomiting, diarrhea etc. when observed for a 7 day period after immunization with each dose of the vaccine (Sack et al. personal communication). The dried cholera vaccine induced increases in antibody responses in serum to cholera toxin. Antibodies of the IgA and IgG isotypes were induced in the antibody in lymphocyte supernatant assay where vaccine associated mucosal cells were stimulated (Chang and Sack, 2001, Chang PhD dissertation, manuscript submitted). Vibriocidal antibodies were also induced after immunization. Thus the dried formulation resulted in significant increases in immune responses, both systemic antibodies as well as mucosal immune responses.In the present study, we plan to carry out safety and immunogenicity studies of the dried vaccine. This evaluation will help understand whether the vaccine after drying retains its immunogenicity. To understand this better we will also study the liquid formulation of the cholera vaccine to assess how well the potency is retained. This will serve as a bridging study for large field based studies which will be carried out later on where we would like to test the effectiveness of the dried vaccine in cholera endemic areas of Bangladesh.

Expression of virulence factors on ETEC during infection *in vivo* as compared with after culture *in vitro*

The colonization factors of ETEC are important antigens contributing to antibacterial immunity. The virulence determinants of pathogenic bacteria are not necessarily constitutively expressed (Taylor et al. 1987, Maurelli 1989) and so the identification of protective immunity cannot only rely on organisms that have been grown *in vitro*. The stimuli, which induce expression, are under investigation, but appear to include temperature, pH, osmolarity and the presence of certain amino acids. Thus antibodies from a ETEC infected patient may detect antigens on *in vivo* grown bacteria which are different from those expressed on *in vitro* grown bacteria. A need exists to understand the physiology of pathogens during infection, the pathogenic mechanisms they employ at each stage of infection, and the genes or antigens that are expressed or down regulated during this process. Two of the important adhesins in cholera, the mannose sensitive hemagglutinin (MSHA) (Jonson et al. unpublished report) and the toxin-coregulated pilus (TCP) (Jonson et al. 1992) have been detected in bacteria isolated from the feces. These have also been shown in *in vitro* bacteria.

In addition to detecting *in vivo* antigens which are not labile and may be lost due to inactivation in the stools, it is now also possible to study the mRNA for the antigens which are more conserved using molecular biology techniques including RNAase protection assays. For this purpose Quantitative Competitive Reverse Transcription PCR (QCRT-PCR) will be used to detect gene expression for antigens using *in vivo* preparations of stools positive for ETEC. The QCRT-PCR is a very useful technique for direct analysis of bacteria from stool samples. This technique will be useful for comparing gene expression for virulence markers in *in vivo* and *in vitro* grown bacteria. The stool samples from patients positive for the pathogens will be used for this analysis. This may be accomplished by studying bacteria isolated directly from the stool without subculture *in vitro* conditions to determine immune responses against putative surface antigens in serum and intestinal specimens. To study the expression of these antigens in the human intestinal environment different techniques including PCR, immunoblotting and electron microscopic techniques will be utilized. This will help to understand if the known adhesion antigens and colonization factors are expressed *in vivo*, if previously not recognized adhesion factors or outer membrane proteins are expressed in vivo but not in vitro and if the *in vivo* bacteria are different from *in vitro* grown bacteria in recognizing mucosal and systemic antibodies from patients. This is a continuation of an already ongoing study to compare the expression of LT and ST and different CFs on ETEC strains recovered directly from the stool (without any subculture) from ETEC patients and corresponding strains cultured under optimal virulence factor expression conditions *in vitro*. Determinations (with quantification) of the different virulence factors will be done both phenotypically (GM1-ELISA and immunoblotting) and based on mRNA expression (QC-RTPCR) to evaluate if specified virulence factors are upregulated (or downregulated) *in vivo*. There will also be a search for possible *in vivo*-specific antigens using non-absorbed and absorbed patient sera tested against the *in vivo* and *in vitro*-grown homologous ETEC strain.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

1. Epidemiological and immunological studies to understand the development of natural immunity to ETEC.

Do frequent ETEC infections give rise to protection to homologous CF expressing ETEC and to cross-reacting antigens?

To understand this better we will follow children from birth up to 2 years of age and study the ETEC infection and re-infection pattern and study the development of mucosal and systemic antibody response to the CF antigens.

A follow-up community based study will be performed in an area of Dhaka city. For this purpose, specimens collected from a cohort of children residing in the Mirpur slum area will be followed up from birth up to 24 months of age).

The children will be recruited as newborns in the first 9 months of the study and each child will be monitored for 2 years. For the purpose of this study, diarrhea will be defined as a change in the child's stool pattern, characterized by an increase in frequency to at least 3 liquid stools per 24 h (WHO,1990). For and exclusively breast-fed infant, however, the clinical history from the mother and her perception about her infants' diarrhoea will be taken into consideration. This is because exclusively breast infants normally pass "pasty" stools ~5-6 times per 24 hour (WHO, 1990). A new diarrheal episode will be said to have commenced when they are preceded by 48 h without diarrhea and to end when the child is free of diarrheal symptoms for at least 24 h (WHO 1988). Stool samples will be tested for ETEC as well as *V. cholerae* O1/O139.

Time Frame:- Enrollment of newborns and completion of sample collection expected within 32 months from start (Appendix 1-2,4-6) and data analyses completed by the end of 3 years.

Sample size: Since the incidence of ETEC is around 20% in hospital based studies (Qadri et al. 2000), the incidence in the community should be higher (Black et al 1986). If we want to detect a true annual incidence of 25% at the 5% level of significance with power 90%, the sample size required will be 169, with 20% loss to follow up, a sample size is 212 (Lwanga and Lemeshow .1991). We plan to study about 220 children.

Surveillance and sampling: The following activities will be carried out in the field:
Pregnant woman will be identified by the female research assistant and based on their willingness to participate in the study, the EDD based on LMP will be recorded for a tentative date for the enrollment of the newborn into the study.

The inclusion criteria for the newborn will include the following (i) weight >2000g at birth. This will be recorded by the study nurse using a portable weighing scale (Seca) after the birth of the baby (ii) no apparent congenital abnormalities. (iii) has not been enrolled in any other study. The enrollment will be carried out actively with trained field research assistants and health workers of the newborn from birth. We would like to complete the enrollment over the first 9 months of the study period. History will be recorded at enrollment which will include filling in

forms with information regarding the epidemiological and socioeconomic conditions of each child's family (appendix 12a, dummy forms, these will be pre-tested prior to use in the study). The nutritional status of the children and other data pertaining to their well being will be assessed at intervals of 4 months when the child parent/guardian will be requested to bring the child to the field site (CRF form for surveillance for ETEC; Appendix 12c, dummy forms shown). In addition, the homes will be visited every week by the field assistant to record any incidence of diarrhoea.The parent/guardian of the child will be requested to visit the field station with the child when there is incidence of diarrhea and a detailed clinical evaluation carried out (appendix 12b, dummy forms shown). In case of diarrhea that needs hospital treatment, the child will be given be referred to the ICDDR,B hospital. In case of other illness the child will be referred to the pediatric hospital. Stool samples will be collected monthly (±5 days) from each child at up to 24 months of age, if they do not concur with an episode. The stools will be analyzed for ETEC and *V. cholerae* O1/0139. In addition stools will be collected at each diarrheal episode. Cord blood will be collected at birth. Finger prick blood samples (0.5 ml) will be collected at intervals of 4 months from each child at the field station. At each contact, the parents or any relations at home will be asked about diarrheal episodes. Thus it is expected that about a total of 5280 monthly stool samples, 1760 diarrheal stool samples (calculating 4 diarrheal episodes/child/ year; Zahid KH, personal communication) and 220 cord blood samples and 1320 finger prick blood samples will be collected from the study subjects. A flow chart showing the plans for the collection of samples is given (Appendices 3-6). All stool and blood samples will be transported to ICDDR,B in the cold on the day of collection for the processing and for laboratory tests.

2. ETEC and cholera vaccine studies

ETEC and *V. cholerae* are the two most common bacterial pathogens causing acute watery diarrhea in children. The aim is to design a combination vaccine which will protect against these two infections. To do so we plan to carry out a series of studies, some in parallel and some in sequence, one after the other with the ultimate goal to over the next 3-5 years have a vaccine that will be ready for protective efficacy and effectiveness studies in developing countries like Bangladesh. To achieve this, we will initiate studies to determine the optimum dose and formulation of the ETEC vaccine in young children, study the safety and immunogenicity of the combined ETEC/cholera vaccine in adults and older children and finally move with the combined vaccine in the right dosage and buffer formulation to the younger children. At the same time if the results of the immunogenicity of the dried cholera vaccine are found to be satisfactory it will be used for effectiveness studies in Bangladesh.

The different components of the vaccine studies are planned as follows:

i. The optimum buffer for formulation of the ¼ dose of the ETEC vaccine in children 6-17 months of age.

We would like to evaluate the suitability of the buffer which will be used to formulate the ¼ dose of the ETEC vaccine for these children. In addition we will also determine if the ETEC vaccine can be used without any buffer in these children. At present we use about 400 mg of the bicarbonate buffer for formulation of 1full dose or ¼ dose of the ETEC vaccine. We plan to decrease the amount further and test the immunogenicity.

We would also like to compare the immunogenicity of the ETEC vaccine after formulation in two different buffers in children 6-17 months of age. We will also formulate the ETEC vaccine in CeraVacx which contains rice syrup solids, sodium bicarbonate and citric acid (Sack at al. 1997). Finally we will study the response in a group of children who will receive the vaccine which has not been formulated in any buffer. The results of these studies will be used to formulate the ETEC vaccine in the most appropriate buffer.

We plan to study three groups of children (n=45/group)$_{15}$who will be given the 1/4th dose of the CF-BS-ETEC

vaccine formulated without any buffer, in bicarbonate-citric acid buffer (Recip AB, Stockholm) or in a rice syrup based electrolyte buffer, CeraVacx (Cera Products Inc., Columbia, Maryland). In addition two groups of children will be given the vaccine reconstituted with lower amounts of the bicarbonate buffer than the regular amount of 400 mg. This will be amounts of 200 mg and 100 mg each.

Sample size: 45 children will be studied in each of the five groups (n=45x5=225).

Time frame: Within 1-12 months of the initiation of the study.

ii. ETEC/cholera vaccine in adults

The ETEC and cholera vaccines and the combination vaccine are mainly intended for children. However before progressing to children, adult groups will be first vaccinated to test the expected responder rates for the immunological assays. For this purpose we will study 3 groups of adults, one group will be given the combination vaccine containing one full dose of the ETEC and one the full dose of the cholera vaccine, and one group the ETEC vaccine, and the third group the cholera vaccine.

iii. Dried formulation of the cholera vaccine in adults

Safety and immunogenicity data are needed for the dried formulation before proceeding to field based effectiveness studies in Bangladesh. The results obtained from the study will be helpful in understanding if the drying of the vaccine results in loss of properties that are inherent in the liquid vaccine.

Sample size: Studies on the liquid and dried formulation of the WCO1-BS cholera vaccine will be carried out in groups of 70 adults each (Appendix 2). This number is based on a responder rate of 70% for vibriocidal antibodies, based on studies of the liquid formulation carried out in Bangladesh (Jertborn et al. 1986) and has been estimated using ±12% precision and 95% confidence limit and a 20% loss to follow-up. We will use the same assumption for the dried vaccine.

Studies on the ETEC vaccine in Bangladeshi adults have shown that 90% respond with IgA antibodies to CFA/I and CTB (Qadri et al. 2000). A sample size of 44 would be sufficient using ±10% precision and 95% confidence limit. Therefore 70 individuals will be studied each in the combined vaccine CF-BS-ETEC + WCO1(rCTB for combined vaccine 1mg, not 2 mg), the dried vaccine and the liquid cholera vaccine. For the ETEC vaccine, 44 individuals will be studied (Appendix 2).

Time frame: We hope to initiate studies in the adults on the dried formulation of the cholera vaccine at sequentially in the order:

testing of the dried and liquid formulation of the cholera vaccine- first 12 months

testing of the combined ETEC/cholera and ETEC vaccine- 13 to 18 months of the study

iv. ETEC/ cholera vaccine in children (18 month to 5 years of age)

Based on results obtained in the adults above and if the combined vaccine has been found to be immunogenic, we will progress to children 18 months to 5 years of age.

Sample size: Studies have shown that about 71% of Bangladeshi children respond to the oral WCO1 vaccine (Qadri et al. manuscript in preparation). To estimate the sample size with ± 12% precision and 95% confidence limit, and with 20% calculated for loss of follow-up we will require 69 volunteers in this age group. Similarly studies on the ETEC vaccine in Bangladeshi children have shown that 90% respond (Qadri et al. 2000). A sample size of 44 has been estimated. Thus 69 volunteers will be given the WCO1 vaccine, and the combined vaccine each and 44 the ETEC vaccine. A total of 182 study subjects will be immunized.

Time frame: From around the 21st to the 26th month of the study.

v. Combined ETEC and cholera vaccine in children 6-17 months of age

Finally, if all the vaccine studies progress as planned, we will study the combined vaccine in children 6-17 months of age. In this age group of children only the appropriate dose of the ETEC vaccine which has found to be the most safe and immunogenic will be tested (Qadri et al. ongoing studies).

Sample size: 70 children will be immunized with the combined ETEC/cholera vaccine and cholera vaccine each (appendix 3).

Time frame- We hope to initiate these final studies from around 29 – 34th month of the study period.

Overall design for the vaccine studies:

The vaccine studies will be carried out at the Mirpur field site. For both the cholera and ETEC vaccines, two doses will be given 14 days apart. The reactogenicity will be monitored for 3 days after intake of each dose of the vaccine. In the case of the children 6 months to 5 years of age, active surveillance will be carried out by the field staff in the homes by visits every day for 3 days after intake of the vaccine. For the adults it will be by recall when the volunteers return to the field site, 7 days after intake of each dose of the vaccine for giving stool and blood samples. Since the ETEC and cholera vaccines have generally been found to be generally safe, passive surveillance has been used in most studies in the case of both adults and children carried out so far. We are however using more precaution by carrying out active surveillance for the children.
Blood and stool samples will be collected prior to immunization and 7 days after intake of each dose of the vaccines in volumes indicated for the immunological assays (Appendix 6). In the case of the adults they will not be allowed to eat or drink one hour before and after intake of the vaccine. In case of children, 6months to 3 years of age.o the period will be ½ an hour before and after the vaccination since it may be difficult to keep infants and small children without food or drink for longer.

Sequential progress of the vaccine studies: The vaccine studies will be carried out in a planned and organized manner as has been shown in Appendix 1. Since the results of one component of the vaccine study is related to rest of the study, we plan to complete each part and report to the RRC/ERC and then move to the next phase. Some of the studies will be run in parallel. We hope that by doing this we will be able to maintain a smooth flow and momentum in the study and keep the committees aware of the progress and if necessary make changes as and when thought necessary. In addition this will help us analyze the data and make it available for dissemination which will be helpful in progressing to Phase III or Phase IV studies of the different components. By this mechanism we will not have to wait for a duration of 3 years before initiating other related studies.

Selection of study subjects for vaccine studies:

Healthy adults (age range 18-45 years) and children (5 years to 6 months of age) both males and females who have not enrolled in any other research study including that being conducted by ICDDR,B will be recruited from the urban slums of Mirpur.

Before entering into the study, subjects in the different age groups will be screened from the slums and history recorded and their consent to participate in the study will be obtained (Appendix 7a-e,11 a-d). We have previously observed that 60% of the families agree to participate in the study. The volunteers prior to enrollment in the study will be carefully examined by a physician. A detailed history of the volunteer of previous immunizations, nutritional status, recent illness (as outlined below) that can compromise the immune system, family background etc. will be taken (Appendix 11 a-d). The volunteers should be free from any chronic disease or any recent illness that may compromise the immune system. Children will be excluded from enrollment if interviewers find: (a) a

history of chronic gastrointestinal disorder, (b) diarrhoeal illness in the past 2 weeks (diarrhoea being defined as passing of ≥ 3 loose or liquid stools in 24 hours (c) febrile illness in the preceding week, or (d) other serious chronic illness (e) had antibiotic treatment at least 7 days prior to the enrollment in the study (f) children suffering from third degree of protein energy malnutrition (PEM). Adult volunteers, who have diarrhoeal illness over the last 6 weeks will not be enrolled in the study. Pregnant women or those with a positive urine chorionic gonadotropin test 2 days before inclusion in the study will be excluded.

The nutritional status of the children to be recruited for the vaccine studies will be monitored using anthropometrical measurements (weight-for-age, and weight-for-length/height). Children below -2SD for weight for height/length of the NCHS median will not be enrolled. We plan to recruit children in the 6 month -5 year range for immunization.

Baseline evaluation of stools:

A routine stool microscopic examination and culture for *V. cholerae* O1/O139 and ETEC will be carried out four days prior to vaccination. Children whose stools are positive for these pathogens will not be vaccinated since this will interfere in the evaluation of the response to the vaccine When immunization for the second dose of the vaccine will be carried out (14 days after the first dose) children who have fever or diarrhoea will be excluded from the study.

Vaccine, administration and allocation: Both the ETEC and cholera vaccine are produced by the Swedish Bacteriological Laboratories (SBL Vaccin AB Stockholm, Sweden) One dose of the ETEC vaccine contains 1.0 mg BS plus $2x10^{10}$ formalin inactivated bacteria each of 5 strains of ETEC expressing 6 colonization factors. The oral inactivated cholera vaccine consists of killed bacteria prepared from individual batches of O1 El Tor and classical *V. cholerae* to include both LPS and protein antigens and also contains recombinant BS. One dose of vaccine contains 1.25×10^{11} CFU of the killed whole cell vaccine plus 1 mg of BS developed in Sweden and also produced by SBL.

Informed written consent will be taken from the adults or from the parent/guardian in the case of the children for the immunization and for the permission to draw blood and obtain stool samples (Appendices 7a-e).

Immediately before use individual doses of the liquid form of the vaccine will be mixed with the reconstituted bicarbonate buffer. For children 18 months -5 years of age , the vaccine (either the individual ETEC and cholera vaccine or the combined vaccine) will be dissolved in 30 ml (total 7 teaspoon) of a raspberry flavored bicarbonate buffer [the buffer is prepared by adding 100 ml water to a sachet of bicarbonate buffer; Recip AB, Stockholm of which 30 ml will be used to prepare the vaccine dose.

The dried cholera vaccine will be available in unit dose sachets. This vaccine will be prepared from the same lot of vaccine as the liquid formulation. To prepare the dry formulation, 3 ml of one dose of the liquid vaccine will be mixed with 150 ml of a syrup of the CeraVacx buffer (from one buffer sachet; each sachet of CeraVacx buffer contains 2 g of sodium bicarbonate, 0.5 g of trisodium citrate and 7 g of rice syrup solids).The suspension will then be spray dried. One dose of the dried vaccine will contain about 10 g of dry powder, which will be dissolved in 150 ml of water at the time of immunization and ingested orally.

For children 6-17 months of age, a lower dose of the ETEC vaccine ($1/4^{h}$ dose), full dose of the cholera vaccine or the combined vaccine (1/4 dose ETEC and full dose cholera) will be administered in 15 ml of the buffer (400 mg , prepared by adding 75 ml water to a sachet of the the bicarbonate buffer (2.8 g) of which 15 ml will be used). For making the formulation for 200 mg and 100 mg buffer, lowered volumes (7.5 and 3.75 ml respectively) of the bicarbonate buffer will be used. In case of decreasing amounts of bicarbonate formulation it will be administered in a total volume of 15 ml of buffer.

This is the procedure followed for immunization of children in the different age groups in our recent studies (Qadri *et al.* ongoing studies) and has been found suitable since in most cases the total dose of the vaccine was ingested. In case of the studies on the effect of different buffers and amounts on immunization, CeraVacx will be also be administered in 15 ml.

For the combined vaccine formulation, one dose of the whole cell cholera vaccine (WC-O1 vaccine without the rCTB component) will be mixed with one dose of the CF-BS-ETEC vaccine. One dose of the combined vaccine will therefore contain 1 mg of the recombinant cholera toxin B subunit and not 2 mg.

Sample collection and laboratory analysis:

Samples from vaccines that will be collected at 3 time points will be tested for antibody responses. Serum samples collected from vaccines at the Preimmune (day 0) and post-vaccination days (day 7 and day 21) will be tested for antibody responses. Sera from vaccinees receiving the CF-BS-ETEC vaccine or the combined vaccine will be tested for the IgA antibody responses to different antigens including CFA/I, CS1,CS2,CS3,CS4,CS5 and CTB. In addition in samples obtained from children 6-17 months of age IgM antibody responses to the above antigens will be tested. Using fecal extracts from the vaccinees at the 3 time-points, IgA antibody responses to CFA/1 and rCTB will only be meausured. These procedures are already set-up in the laborotary of the investigators (Qadri et al. 2000).

To test the immunogencity of the cholera vaccine and the combined cholera-ETEC vaccine we will test the vibriocidal antibody response at the 3 time points mentioned above. In addition the IgA and IgG antibody response to rCTB will be measured in those being immunized with the cholera or the ETEC or the combined vaccine.

Safety Monitoring Committee

A data safety and monitoring committee will be setup for this project. Dr. Iqbal Kabir (CSD) will be the Chairperson of the committee. Other members include Dr. Shafiqul Sarker (CSD), Dr. Nigar Shahid (PHSD) and Dr. Rashidul Haque (LSD). The members are in no way involved with the project.

Safety endpoint and safety evaluation

The primary end point for evaluating safety will be defined as the occurrence of any of the following diarrhoea, vomiting or abdominal cramps of at least moderate grade. Diarrhea will be defined as three or more loose or liquid stools in any 24-h period. A measured oral or rectal temperature above 38°C will be considered a fever.

3. Study of in vivo expressed novel antigens on ETEC isolated from patients (6 months to 45 years of age)

Do ETEC express important antigens *in vivo* which are lost on subculture?

To answer this we will study adults and children infected with ETEC.

Patients with acute watery diarrhea due to ETEC attending the CRSC of ICDDR,B will be recruited for this purpose. We will collect stools from these patients at the acute stage of infection and by differential centrifugation separate bacteria and store them at –70C and also prepare them for electron and immunoelectron microscopy. Acute watery stools of rice watery consistency will be collected from the CRSC and processed after a dark field assay for *V. cholerae* has been found negative. The samples will be processed immediately after collection and frozen. After confirmation of ETEC by dot blot immunoassay for the CFs the patient will be recruited into the study. This will be on the second day of hospitalization. If the sample is negative for CFs, the patient will not be enrolled and the sample discarded. For electron microscopy the samples separated from stool by differential centrifugation of stool will be applied on formvar coated Ni-grids and stained with ammonium molybdate or reacted with specific antibodies and also sera from patient (Helander et al. 1997). The electron microscopic analysis will be carried out at Goteborg University. For the separation of RNA from the samples, guanidine isothiocyanate containing reagents (RNAlater buffer) will be used and samples stored at –70°C. The samples will be analyzed by quantitative PCR using primers specific for the different CF antigens using procedures that have been optimized recently at Goteborg University and which will be set at ICDDR,B (Gaastra W, personal communication).

Sample size: Previous studies have shown that over 90% of patients respond with increases of IgA antibodies to homologous CF antigens.). A sample size of 44 would be sufficient using 90% assumption, ±10% precision and 95% confidence limit including 20% loss to follow-up visits.

Sample collection: Forty four patients from whom the stool samples will be positive for ETEC will be enrolled in the study. Stool samples will be collected for separation of *in vivo* bacteria. Blood and fecal samples will be collected from the patients for testing for immune responses to the in vivo expressed antigen. In addition the patients will be requested to come for follow-up at days 7 and 21 for collection of convalescent samples (both blood and feces). The amount of blood collected from the patients will be 5 ml for adults, 3 ml for children 18 months to 5 years of age and 1.5 ml for younger children. About 5 gm of stool will be collected at each time point. Informed written consent will be obtained from the patients or their guardians as is needed (Appendix 8a,8c). In the case of patients 6 –17 years of age, assent will be taken from them and written consent from the parents/guardians (Appendix 8b)

Time frame: For the 3 year duration of the study to complete recruitment of patients.

Overall laboratory tests for the project

Vaccinees, patients and other study subjects:

Stools will be cultured on Mackonkey agar and tested for colonization factor expression by dot blot immunoassay techniques and for the enterotoxins LT and ST (Svennerholm and Holmgren, 1978; Svennerholm *et al.*, 1986). Stools will also be plated on TTGA (taurocholate- tellurite-gelatin agar) and gelatin agar overnight (Gelatin agar) (Difco, Detroit, MI). Suspected vibrio colonies will be identified by slide agglutination using monoclonal antibodies (Qadri et al. 1994).

Serum separated from blood will be used to test for the antibody response to the cholera and ETEC vaccine specific antigens in the volunteers as necessary. To test the response to the cholera vaccine, vibriocidal antibodies and cholera toxin specific antibodies in the IgA and IgG isotypes will be measured (Jertborn et al. 1986). For the response to the BS-CF-ETEC vaccine, response to the different CF antigens in the IgA isotype and to CTB in the IgA and IgG isotype will be measured (Qadri et al 2000).

Using sera from the children in the birth cohort, antibody response to homologous and cross-reacting CF antigens will be assayed by ELISA (Ahren et al 1995). The sera and fecal specimens obtained from the children at 4 montly intervals will be tested for antibody responses. The samples will be tested to study the antibody response to CF of the infecting ETEC strain (homologous CF antigen with which the child may be infected, prior to the collection of that sample as well as to cross-reacting one. For example a child infected with CFA/I will be tested to CS2 and CS4 antigen in the IgA, IgM and IgG isotypes. Again a child infected with CS5 will be tested to CS14 as well as to CFA/I (cross-reacting antigen). We will also test samples from all children to at least 4 antigens CFA/I, CS1, CS2,CS5 in the IgA isotype over the 2 year period to record the development of antibodies. All this will be related to the incidence of ETEC infection and will help better understand the protection that is afforded.

Using specimens obtained from the patients with ETEC infection, immunoblot analyses, electron microscopy, PCR, etc. will be carried out. These will be carried out using *in vivo* as well as *in vitro* cultured bacteria obtained at the acute stage of disease as antigen and with sera and fecal samples obtained from the patients over the course of the infection (Appendix 5).

Field site for studies planned in the project:

Adults and children and newborn babies from the urban slums in the Mirpur, Dhaka city area will be recruited in the study. We have been conducting other studies at this site (Protocols 99033, 98009). A field station has been set up for the past 4 years for carrying out ETEC and cholera vaccine studies. Mirpur is about 6 km northwest of ICDDR,B. The area has been used for field studies since 1987. It is about 10 sq km in radius and divided into 14 sections. We have previously carried out studies in sections 10, 11 and 12. Each section is again divided into camps. A population of about 300,000 is estimated in these three sections. It is believed that there will be an adequate number of adults as well as infants and children in the different age groups to meet the requirement for the study. We plan to carry out the vaccinations and the community based study for monitoring ETEC infections at this field site. Subjects will only be recruited into the study if they have not been enrolled in any other protocol being carried out by ICDDR,B or other institutions which require vaccine or nutritional interventions for the duration of this study. Studies in patients for the detection of novel antigens will be carried out in the CRSC at ICDDR,B where patients will be screened for ETEC.

Facilities Available

Existing field, hospital, laboratory and office facilities are adequate and are outlined

Recruitment of volunteers and field site for study:

The volunteers will be recruited from the urban slums in Mirpur in Dhaka for the study. The project office will be around the same area which is at present being used for other studies ongoing in ICDDR,B (projects: 98009, 99033).

Clinical and field facilities:

The clinicians in the study will coordinate the clinical component of the study in the ICDDR,B hospital and in the Mirpur field site. Clinical facility needed in the study will be provided by the study physicians and other clinicians at the Clinical Research and Service Centre of the ICDDR,B hospital in Dhaka.

Laboratory facilities:

Existing laboratory facilities are adequate.

Data Analysis:

Methods to ensure data quality

To ensure the quality of data, the study site will be visited by the investigators for random checks. In addition a 5% sample of study subjects will be re-interviewed within one day to re-assess the original interview.

Data management; To maintain consistency the data forms will be reviewed by the investigators. This will be carried out toensure the completeness and accuracy of the data. If necessary additional home visits will be made to recheck entries or fill up missing entries. After editing, data will be entered in an appropriate data entry program that will be suitable for the study.

The data will be entered into a computer and a data base created. Data will be analyzed using an appropriate statistical programme, EpiInfo version 2000 or SPSS. Descriptive statistics will be generated using EpiInfo and SPSS for Windows (Version 10.00) or SigmaStat.

Statistical analysis will be carried out using appropriate parametric and non parametric tests. Overall rates of adverse reaction and seroconversion will be compared by χ^2 test with Yates correction or by the Fisher exact test . Seroconversion for the vibriocidal response will be defined as ≥4 fold rise in reciprocal serum vibriocical titer from pre to post vaccination. If necessary vibriocidal antibody titers will be transformed to logarithms to better approximate normality.

For the CF and CTB specific responses a ≥2 fold response from baseline to post vaccination will be considered a positive response. Differences in the geometric mean titers (GMTs) of the antibody will be compared by the Mann-Whitney test or the Wilcoxon Rank Sum Test or the Student's t-test as found appropriate. Incidence of diarrhoea will be expressed as number of episodes per child per week.

Itemized tasks for investigators at ICDDR,B

Dr. Firdausi Qadri and co-investigators at ICDDR,B will be involved directly in the vaccine and patient studies and the epidemiological studies in the field. These will include the following:

Responsible for coordinating vaccine studies and studies related to immunological studies patients in Bangladesh.

Supervise work in the laboratory and coordinate specimen collection from volunteers and patients and from the cohort in the urban slums.

Coordinate laboratory techniques between Sweden and Bangladesh.

Analyze data. Evaluate results and write reports and manuscripts.

Plan and initiate studies to progress to children on the CF-BS-ETEC studies in children 6 months to 3 years of age for protective efficacy Phase III studies.

Dr. D. A. Sack- Advice and support mainly in the vaccine related studies.

Dr. Tanvir Ahmed (Study Physician)
Volunteer and patient enrollment, clinical management, follow up of toxicity surveillance of vaccine. Coordinate field based studies with laboratory at ICDDR,B.
Study Physician at field site
Dr.Tanvir Ahmed will oversee the activities at the field site and will also supervise the activities of the epidemiological study in the field. In addition another physician will be responsible for these activities and the follow up of the infants, collection of blood, stool and coordinate other activities.

Dr. K .Zaman
Support in epidemiological and field based studies including vaccines
Dr. M. A. Salam
Clinical support and advice both in the hospital and in the field based study.
Dr. K.Z.Hasan
Support in the epidemiological and the birth cohort component.
Dr. A.K. Siddique
Support in epidemiological and field based studies including vaccines.
Dr. A.S.G. Faruque
Coordinate collection of clinical specimens from patients at ICDDR,B. Assistance in statistical analysis.

Dr. G.B. Nair
Advice and support mainly in the microbiological analysis

Dr. Rob Brieman
Support in epidemiological and field based studies including vaccines

Mr. Tawfiqur Rahman Bhuiyan (Postgraduate student for studies leading to PhD)

This trainee (supported by Sida-SAREC) will benefit from the proposed project :
He will be involved in postgraduate studies and will also be working on this project. Mr. Tawfiqur Rahman has been working as research officer in the laboratory for microbiological, molecular biological, immunological. histochemical and immunohistochemical studies. He will be enrolled in the Goteborg University and will carry out laboratory based studies both in Goteborg University and the Immunology section of ICDDR,B. His post-graduate studies will be focused on the study in the ETEC patients on understanding the expression of novel antigen *in vivo.* Funds available in Dhaka but that available at Goteborg University will be used for this purpose.

Research Officer-3
Blood: Separation of serum and storage at –70°C/-20°C. study of antibody response in patients.
Stool: Collection and extraction of fecal antibodies and filtration. Fractionation and storage in presence of protease inhibitors at –70°C.
ELISA and vibriocidal assays for study of immunogenicity of the cholera and ETEC vaccine.
Bacterial isolates: Microbiological and molecular biological studies on the vaccine strain and other isolates.
Microbiological and immunological help in the detection of ETEC and *V.cholerae*

Other personnel shown in the budget will help in the smooth functioning of the project in the different areas as necessary.

Itemized tasks for the Swedish investigators (Goteborg University)

Prof. Ann-Mari Svennerholm, the PI of the study in the collaborating institute in Sweden will help coordinate studies in Bangladesh with that carried out in other countries in terms of comparison of results, vaccines and reagents and will give scientific and academic feedback.

Professor Jan Holmgren will give scientific and academic feedback and coordinate the cholera vaccine studies in Bangladesh.
Professor Wim Gaastra and the other investigators from Goteborg University will help with expertise on molecular techniques for identifying CFs and mRNA for CFs and other virulence antigens.

Research Technician at Goteborg University
Carry out immunological and molecular biological assays for the study of in vivo specific antigen. Prepare CF antigens and other reagents for use in the vaccine study at ICDDR,B. Quality control tests will be carried out for the antigens that will be prepared.

The Swedish collaborating Institute and the different investigators will however not be directly involved in the volunteer and vaccine studies or in patient enrollment, recruitment but will support the study by:

a. Supplying purified colonization factor antigens, rCTB and other purified antigens needed for the ELISA and other immunoassays.
b. Carrying out quality control of serological assays including quality control of strains from time to time to confirm and when needed.

c. Transferring new techniques to the ICDDR,B laboratory as and when appropriate.
d. Giving advice and feedback on latest information and in joint publication of manuscripts and reports.

Responsibility of work for the Co-Investigators and personnel involved in the study:

(Percentage of effort of each investigator is shown in parenthesis)

Birth cohort (Field studies and laboratory)

Dr. K.H. Zahid- (20%)
Dr. K. Zaman- (5%)
Study Physician(1)
(to be named)- (75%)
Research Officer (1)- (100%)
Dr. Tanvir Ahmed (25%)
Study Nurse (1)- (100%)
Field Research Assistant (3)- (100%)
Health Worker (4)- (100%)
Laboratory Attendant (1)- (100%)
Data Management officer-(75%)

Vaccine studies

Dr. David A. Sack (overall)- (Effort – as required)
Dr. A.K. Siddique (dried and liquid formulation of cholera vaccine, bridging study)- (5%)
Dr. Rob Breiman- (as and when required)
Professor Jan Holmgren- (overall) -(as and when required)
Dr. M.A. Salam- Effort (2% or as needed)
Dr. K. Zaman- Effort (5%)
Dr. Tanvir Ahmed (75%)
Research Officer (1)- (100%)
Field Research Assistant (4)- (100%)
Senior Laboratory Attendant (2)- (100%)

ETEC- patient study (Clinical, Immunology, Microbiology)

Dr. A.S.G. Faruque- (5%)
Dr. G.B. Nair- Effort (2%)
Dr. M.A. Salam- Effort-(2%)
Dr. Wim Gaastra- (20%)
Study Physician (25%)
Research Technician (Sweden)-Effort- (50%)
Research Officer (also involved in post-graduate studies)- (100%)
Senior Laboratory Attendant (1)- (100%)

***PIs and other investigators will be involved with specific tasks with all aspects of the study.**

Ethical Assurance for Protection of Human Rights

The study will only be initiated after it has been approved by the Research Review Committee (RRC) and the Ethical Review Committtee (ERC) of ICDDR,B. In addition permission from the Directorate of Drug administration of Bangladesh will be obtained for carrying out the ETEC and cholera vaccine studies. We have

obtained approval from these committees in our previous studies on patients and vaccinees both related to both ETEC and cholera. We have ethical clearance for ongoing studies on ETEC and cholera patients as well as for recruiting volunteers for the CF-BS- ETEC vaccine which is funded by Sida-SAREC (Projects: 96014, 98032) and the USAID funded ETEC Phase II vaccine study in children (Project: 99033). In addition we have obtained similar approval from the ERC for carrying out studies on the WCO1-BS cholera vaccine in adults (Project: 94019, funded by the European Union) or in children (Project: 98001, funded by Thrasher). In all these studies prior approval was obtained from the ERC.

Before enrollment signed informed consent is obtained from the adults and from the guardians of the children. The consent form is written in Bangla in a language and format that is easily understood by the study subject of even little or no educational background. The consent form is read out to the subject or to the guardian/parent of the study subject if he/she is unable to read. Signed consent or the left thumb impression will obtained from the study subjects or from the parents for participation of the children in the study. Consent will be taken both for participation in the study and for drawing blood and collecting fecal samples. This will be done in the case of patients as well as volunteers for the vaccine studies (consent forms in appendix 6-8).

Risk analysis

The study is planned for a total period of 3 years. Previous studies (from 1993 onwards) mainly carried out by the Sida-SAREC funded projects have led to exchange of technological expertise in the Immunology laboratory such that the planned study will hopefully be carried out without any delay and as planned. We will however be dependent on the supply of CF-BS-ETEC vaccine, the dried and liquid WC-O1-BS vaccine and the combined vaccine from the Swedish Bacteriological laboratories in Sweden. The study will therefore be dependent on the availability of the relevant vaccines to us at ICDDR,B. The time frame of the study is however planned on the expectation that all the vaccines that will be used in the study will be available from period between 2002-2004 from the Swedish Bacteriological Laboratories.

Use of Animals

Not applicable for ICDDR,B, but needed for Goteborg University.

Dissemination and Use of Findings

The information obtained from this study will help facilitate the Phase III field-testing of the CF-BS-ETEC vaccine. In addition information will be obtained on the immunogenicity of the dried WCO1-BS cholera vaccine which will be helpful in future evaluation of its effectiveness in Bangladesh. The information of the combined ETEC and cholera vaccine will be helpful for future studies. We hope that this study will lead to a better understanding of the components needed in an ETEC vaccine, the development of protective immunity to ETEC infections and an understanding of the capacity of the inactivated vaccines to elicit a immune response which is appropriate for protection.

Collaborative Arrangements

This is a collaborative study between the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and the Department of Microbiology and Immunology, Goteborg University, Goteborg, Sweden.

Reply to reviewer's comments;

Reviewer 1.

The suggestion to include the results of the dose finding studies that have been carried out in children has now included in the background section (page11, para 3). The missing references have been listed in the bibliography now.

Reviewer 2.

1. Regarding the effort on the CF-BS-ETEC vaccine and the CF-BS-ETEC-WCO1 vaccine

The study in Part I on the CF-BS-ETEC vaccine is designed such that we can move to a phase III trial soon. This vaccine is under evaluation in children and adults in Phase III trials and our aim would be to move in that direction as soon as possible.

We do hope to move to a protective efficacy trial too with the combined cholera-ETEC vaccine study in the near future. However the studies, which will be initiated in this project, will help to tell us how safe and immunogenic the combined vaccine is in the different age groups and based on the results we can plan further studies. The combined vaccine is being studied the first time and therefore it has to be followed up in the different age groups. The project has been designed to give emphasis on two components that is the ETEC vaccine, formulation, dosage etc from which we will move to a Phase III efficacy trial and the combined cholera-ETEC vaccine study both of which are important measures by which we hope to protect children from the two major causes of acute watery diarrhea.

2. Justification of elimination of placebo groups

In regard to the elimination of placebo groups, it can be mentioned that our previous studies and that of others with either the cholera or ETEC vaccine have shown that the reactogenicity is low and similar in placebo or vaccinee. In the case of immunogenicity the vaccine is always significantly more immunogenic than placebo. Therefore, in the study we are planning not to pursue with a placebo group. However, in phase III studies this will be included.

3. Details on the timing of blood collection in vaccinees

The suggestion to include the details on the timing of blood collection and laboratory analysis has now been included in the "Research Design and Methods" section (page19) under the heading of "Sample collection and laboratory analysis". The following is added now:

Sample collection and laboratory analysis:

Samples from vaccinees that will be collected at 3 time points will be tested for antibody responses. Serum samples collected from vaccinees at the Preimmune (day 0) and post-vaccination days (day 7 and day 21) will be tested for antibody responses. Sera from vaccinees receiving the CF-BS-ETEC vaccine or the combined vaccine will be tested for the IgA antibody responses to different antigens including CFA/I, CS1, CS2, CS3, CS4, CS5 and CTB. In addition in samples obtained from children 6-17 months of age IgM antibody responses to the above antigens will be tested. Using fecal extracts from the vaccinees at the 3 time-points, IgA antibody responses to CFA/I and rCTB will only be measured. These procedures are already set-up in the laboratory of the investigators (Qadri et al. 2000).

To test the immunogenicity of the cholera vaccine and the combined cholera-ETEC vaccine we will test the vibriocidal antibody response at the 3 time points mentioned above. In addition the IgA and IgG antibody response to rCTB will be measured in those being immunized with the cholera or the ETEC or the combined vaccine.

4.Comment on "the Phase III ETEC vaccine study"

We are planning to carry out the double-blinded placebo controlled Phase III, CF-BS-ETEC vaccine studies in about 5000 children, 6 months to 3 years of age. This is based on our past and ongoing prevalence studies, which have shown that ETEC infections are in the highest in frequency in this age group. Thus the present project will help to answer some important questions that are needed to be answered for the Phase III study we plan. However these are only tentative plans but we hope that the study being reviewed that will be carried out in Mirpur will be able to complement the large efficacy study which we plan in the near future.

5. Definition of diarrhoeal episode

The suggestion of including the definition of diarrhoea episode "Research design and methods" section (page14) under the heading of "1. Epidemiological and immunological studies to understand the development of natural immunity to ETEC." The following is added now:

Diarrhea will be defined as a change in the child's stool pattern, characterized by an increase in frequency to at least 3 liquid stools per 24 h (WHO, 1990). For an exclusively breast-fed infant, however, the clinical history from the mother and her perception about her infants' diarrhoea will be taken into consideration. This is because exclusively breast infants normally pass "pasty" stools ~5-6 times per 24 hour (WHO, 1990). A new diarrhoeal episode will be said to have commenced when they are preceded by 48 h without diarrhea and to end when the child is free of diarrhoeal symptoms for at least 24 h (WHO 1988).

6. Exclusion of children with cholera and ETEC infections.

We plan to do this to exclude background infection in the recent past. We have been carrying out the culture for ETEC for the detection of LT/ST in a similar design for other ongoing studies and in our experience it takes us 2-3 days to complete these assays. However based on the comment, we now plan to take samples 4 days prior to immunization (under the heading of Baseline evaluation of stools, page 18, line 1)

7. For the combined vaccine formulation,

One dose of the whole cell cholera vaccine (WC-O1 vaccine without the rCTB component) will be mixed with one dose of the ETEC vaccine. One dose of the combined vaccine will therefore contain 1 mg of the recombinant cholera toxin B subunit and not 2 mg.

This has been inserted in the text now (Section "Research Design and Methods" P 16, last paragraph, line 3)

8. Relation of incidence of ETEC diarrhea with the immune response

This has been inserted in the text now (Section: Research Design and Methods, Subheadings: Overall laboratory tests for the project; Vaccinees, patients and other study subjects: Page 20, paragraph 3)

"The sera and fecal specimens obtained from the children at monthly intervals will be tested for antibody responses. The samples will be tested to study the antibody response to CF of the infecting ETEC strain (homologous CF antigen with which the child may be infected, prior to the collection of that sample as well as to cross-reacting one. For example a child infected with CFA/I will be tested to CS2 and CS4 antigen in the IgA, IgM and IgG isotypes. Again a child infected with CS5 will be tested to CS14 as well as to CFA/I (cross-reacting antigen). We will also test samples from all children to at least 4 antigens CFA/I, CS1, CS2, CS5 in the IgA isotype over the 2year period to record the development of antibodies. All this will be related to the incidence of ETEC infection and will help better understand the protection that is afforded".

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Wenneras C, Svennerholm AM, Ahren C, Czerkinsky C. Antibody-secreting cells in human peripheral blood after oral immunization with an inactivated enterotoxigenic Escherichia coli vaccine. Infect Immun. 1992 Jul;60(7):2605-11.

Wolf, M.K.,D.N.Taylor, *et al.* (1993). "Characterization of enterotoxigenic Escherichia coli isolated from U.S troops deployed to the Middle East." J Clin Microbiol 31(4):851-6.

World Health Organization. 1990. Manual for the treatment of diarrhoea: for use by physicians and other senior healthworkers (WHO/CDD/SER/80-2, Rev. 2). World Health Organization, Geneva.

World Health Organization. 1988. Persistent diarrhoea in children in developing countries: Memorandum from a WHO meeting. Bulletin of the World Health Organiazation, 66(6): 709-717 (1988).

Biography of the Principal Investigator

CURRICULUM VITAE OF APPLICANT (1 page maximum*)

A. Family name: Qadri — Date of birth: 31-03-51

First name(s) Firdausi — Nationality: Bangladeshi

B. Education (subjects, university or school, degree, year)

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	FIELD OF STUDY	YEAR(s)
University of Dhaka, Bangladesh,	BS,	Biochemistry,	1975
University of Dhaka, Bangladesh,	Masters,	Biochemistry,	1977
Liverpool University, UK ,	Ph.D,	Immunology,	1980

C. Present and most recent positions held (type of position, institution/authority, dates)

PROFESSIONAL EXPERIENCE

1995 onwards : Senior Scientist, Immunology Section, ICDDR,B.
1993-1995 : Scientist, Immunology Section, ICDDR,B.
1988-1993 : Associate Scientist, Immunology Section, ICDDR,B.
1986-1988 : Research Fellow, Immunology Section, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).
1981-1988 : Assistant Professor, Department of Biochemistry, University of Dhaka.
1977-1980 : Doctoral student

D. Recent publications: list only the five most important and relevant publications over the last five years (papers in press or submitted for publication are also acceptable).
Please give full bibliographic references: author(s), title, journal, volume, page numbers, year

SELECTED PUBLICATIONS

1. Qadri F, Wenneras C, Bardhan PK, Albert MJ, Sack RB, Svennerholm A-M. Safety and immunogenicity of an oral, inactivated enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine in Bangladeshi adults and children. Vaccine. 2000, 18, 2704-2712.
1. Qadri F, Giron JA, Helander A, Begum YA, Asaduzzaman M, Xicohtencatl-Cortes, Negrete E, Albert MJ. Human antibody response to longus type IV pilus and study of its prevalence among enterotoxigenic Escherichia coli in Bangladesh using monoclonal antibodies. Journal of Infectious Diseases. 2000, 181, 2071-2074
2. Qadri F. Swadesh Kumar Das, A.S.G. Faruque, George J. Fuchs, M. John Albert, R. Bradley Sack and Ann-Mari vennerholm. Prevalence of toxin types and colonization factors in enterotoxigenic Escherichia coli isolated during a two year period from diarrheal patients in Bangladesh J. Clin Micr. 38, 2000, 27-31.
3. Qadri F, Makela H, Holmgren J, Albert MJ, Mannoor K, Kantele A, Saha D, Salam MA, Kantele JM. Enteric infections in an endemic area induce a circulating antibody-secreting cell response with homing potentials to both mucosal and systemic tissues. J Infect Dis 1998 177:1594-1599.
4. Qadri F, Wenneras C, Albert MJ, Hossain J, Mannoor K, Begum YA, Mohi G, Salam MA, Sack RB, Svennerholm A-M. Comparison of immune responses in patients infected with *Vibrio cholerae* O139 and O1. Infect Immun 1997; 65:3571-3576.
5. Qadri F., Firoz A, MD. M. Karim, C. Wenneras, Y. A. Begum, M. J. Albert, J. R. McGhee. Lipopolysaccharide and cholera toxin specific subclass distribution of B cell responses in cholera. Clinical and Diagnostic Laboratory Immunology. 1999, 6, 812-81.

BIOGRAPHICAL SKETCH

Provide the following information for all key personnel.
Use this form or follow this format for each person.

NAME:	POSITION TITLE:
David A. Sack	Director, ICDDRB,B, Centre for Health and Population Researchand Professor, Department of International Health, Johns Hopkins University

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
Lewis and Clark College, Portland Oregon	BS	1961-65	Natural Science
University of Oregon Medical School, Portland, Oregon	MD	1964-68	Medicine
University of Iowa School of Medicine, Iowa City, Iowa		1968-70 & 1971-73	Internship & Residency in Internal Medicine
Johns Hopkins University School of Medicine, Baltimore		1974-75	Fellowship, Infectious Diseases

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

PROFESSIONAL EXPERIENCE

1999	Director, ICDDRB, Centre for Health and Population Research, Dhaka, Bangladesh
1994-1999	Head of the Johns Hopkins Vaccine Testing Unit, and Professor, Department of International Health, (with joint appointment in Department of Epidemiology) The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205. Joint appointment: Department of Medicine, Division of Infectious Diseases, The Johns Hopkins School of Medicine
1993-1994	Coordinator for Control of Diarrheal Disease Projects for BASICS, Rosslyn VA
1991-92	Medical Officer for USAID sponsored PRITECH project, with emphasis on assistance with cholera control
1985-1994	Associate Professor, Department of International Health, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205
1984-1987	Associate Director of ICDDR,B and Head of Division of Epidemiology and Laboratory Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh
1982-1985	Associate Professor, Division of Geographic Medicine, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
1977-1980	Scientist, International Centre for Diarrhoeal Disease Research, Bangladesh (formerly Cholera Research Laboratory) Dhaka, Bangladesh
1977-1982	Assistant Professor, Division of Geographic Medicine and Division of Infectious Disease, The Johns Hopkins University School of Medicine, Baltimore, Maryland
1976	Instructor, Infectious Disease Division, The Johns Hopkins University School of Medicine, Baltimore, Maryland
1971	Volunteer Physician, Luluabourg (Kananga), Zaire, Africa
1969-1971	Public Health Service, Director Indian Health Center, Lame Deer, Montana

ADVISORY PANELS

1985-1989	Member, World Health Organization Scientific Working Group on Immunology and Vaccine Development for the WHO Global Program on Diarrhoeal Diseases, Geneva
1990	Organizing Committee of an international symposium in Gothenburg Sweden on May 28-29, 1990: "New vaccines against enteric infections: Prospects for public health benefits in developing countries." Advisor to Virus Research Institute on vaccine development, Cambridge, Massachusetts
1992-1994	Head, Task force on Cereal Based ORS for the International Child Health Foundation
1993-1996	Member, Data safety and monitoring committee for the U.S. Army oral *E. coli* vaccine trials in Egypt,
1995-present	Boston
1999-present	Member, Data safety and monitoring committee for the ARIVAC trial of pneumococcal vaccine in Philippines

1. Donta ST, Sack DA, Wallace RB, DuPont HL, Sack RB. Tissue culture assay of antibodies to heat-labile *Escherichia coli* enterotoxins. N Engl J Med. 291:117-121, 1974.
2. Sack DA, Merson MH, Wells JG, Sack RB, Morris GK. Diarrhea associated with heat-stable enterotoxin-producing strains of *Escherichia coli*. Lancet. ii:239-241, 1975.
3. Sack DA, Kaminsky DC, Sack RB, Itotia JN, Arthur RR, Kapikian AZ, Orskov F, Orskov I. Prophylactic doxycycline for travellers' diarrhea: Results of a prospective double-blind study of Peace Corps Volunteers in Kenya. N Engl J Med. 298:758-763, 1978.
4. Sack DA, Chowdhury AMAK, Eusof A, Ali MA, Merson MH, Islam S, Black RE, Brown KH. Oral hydration in rotavirus diarrhea. A double blind comparison of sucrose with glucose electrolyte solutions. Lancet. ii:280-283, 1978.
5. Sack DA, Islam S, Brown KH, Islam A, Kabir AKMI, Chowdhury AMAK, Ali MA. Oral therapy in children with cholera: A comparison of sucrose and glucose electrolyte solution. J Pediat. 96:20-25, 1980.
6. Sack DA, Stephensen CB. Liberation of hydrogen from gastric acid following administration of oral magnesium. Dig Dis Sci. 30: 1127-1133, 1985.
7. Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Stanton BF, Kay BA, Khan MU, Yunus M, Atkinson W, Svennerholm A-M, Holmgren J. Field trial of oral cholera vaccines in Bangladesh. Lancet. ii: 124-127, 1986.
8. Sack DA, Chowdhury KA, Huq A, Kay BA, Sayeed S. Epidemiology of Aeromonas and Plesiomonas diarrhea. J Diarrheal Dis Res. 6:107-112, 1988.
9. Sack DA, Freij L, Holmgren J. Prospects for public health benefits in developing countries from new vaccines against enteric infections. J Infect Dis. 163:503-6, 1991.
10. Sack DA, Hoque ATMS, Huq A, Etheridge M. Hypothesis: Natural infection with *P. shigelloides* protects developing-country residents against *S. sonnei* infection. Lancet 343:1413-1415, 1994.
11. Sack DA, Clemens JD, Huda S, Harris JR, Khan MR, Chakraborty J, Yunus M, Gomes J, Siddique O, Ahmed F, Kay BA, Van Loon FPL, Rao MR, Svennerholm A-M, Holmgren J. Antibody responses following immunization with killed oral cholera vaccines during the 1985 vaccine field trial in Bangladesh. J Infect Dis. 164:407-11, 1991.
12. Bernstein DI, Davidson B, Glass RI, Rodgers G, Sack DA for the U.S. Rotavirus vaccine efficacy group. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in U.S. children. JAMA 273:1191-6, 1995.
13. Sack DA, Shimko J, Sack RB, Gomes G, MacLeod K, O'Sullivan, D, Spriggs D. Comparison of Alternative Buffers With Peru-15, a New Live, Oral Cholera Vaccine in Outpatient Volunteers. Infect Immun 65:2107-2111, 1997.
14. Sack DA, Sack RB, Shimko J, Gomes G, O'Sullivan D, Metcalfe K, Spriggs D. Evaluation of Peru-15, a new live oral vaccine for cholera, in volunteers. J. Infect Dis 176:201-5, 1997.
15. Clemens JD, Naficy A, Kay BA, VanLoon F, Sack DA. Breast-feeding and the risk of life threatening enterotoxigenic *E. coli* diarrhea in Bangladeshi infants and children. Pediatrics 100:1997.
16. Sack DA, Tacket CO, Cohen MB, Sack RB, Losonsky GA, Shimko J, Nataro JP, Edelman R, Levin MM, Gianella RA, Schiff G, Lang D. Validation of a Volunteer Model of Cholera Using Frozen Bacteria as the Challenge. Infect Immun 66:1968-72, 1998.
17. Sack DA, Lastovica AJ, Chang AH, Pazzaglia G. A microtiter assay for detecting *Campylobacter spp* and *Helicobacter pylori* with surface gangliosides which bind cholera toxin.. J Clin Microb 36:2043-2045, 1998.
18. Castaneda E, Chinchilla M, Sack DA, Svennerholm AM. Utilizacion de placas de ELISA de alta y de baja avidez en la determination de anticuerpos contra la toxina de colera. Biomedica. 18:147-152, 1998.
19. Wagatsuma Y, Aryeetey ME, Sack DA, Morrow RH, Hatz C, Kojima S. Resolution and resurgence of schistosoma haematobium-induced pathology after community-based chemotherapy in Ghana, as detected by ultrasound. J Infect Dis. 179:1515-22, 1999.
20. Bernstein DI, Sack DA, Rothstein E, Reisinger K, Smith VE, O'Sullivan D, Spriggs DR, Ward RL. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. Lancet. 354:287-90, 1999.
21. Faruque SM, Siddique AK, Saha MN, Asadulghani Rahman MM, Zaman K, Albert MJ, Sack DA, Sack RB. Molecular characterization of a new ribotype of Vibrio cholerae O139 Bengal associated with an outbreak of cholera in Bangladesh. J Clin Microbiol. 37:1313-8, 1999.

Principal Investigator: Last, first, middle _Qadri Firdausi

CURRICULUM VITAE

Ann-Mari, Larsdotter Svennerholm. M.D., Ph.D., Professor

13 December 1947, Uppsala, Sweden

Swedish

Education and degrees:

1975	Doctor of Medicine (Science) (Ph.D.)
1975	Assistant Professor (Docent) in Medical Microbiology (Göteborg University)
1977	Medical Licentiate (M.D.)
1975-88	Different positions as researcher and assistant and associate professor, Göteborg University.
1988 -	Professor of Infections and Immunity, Göteborg University.

Research education:

- Main supervisor for 14 PhD students
- C:a 15 post-docs and 6 visiting professors
- 4 PhD students and 4 post docs at present

Offices and National and International Committees; Member of:

The Scientific Working Group for Immunology, Microbiology and Vaccine Development of WHO 89-90.

The Board for the National Bacteriol. Lab. in Sweden (SBL), 92-93.

The Board for the Swedish Institute for Infectious Disease Control (SMI), 93-97.

The Board of the Technology Link Foundation, "Teknikbrostiftelsen i Göteborg", 1994-; Chairman of Board 2001 -

The Committee for Equality between sexes, Göteborg University, 1994-; Chairman of Committee 1999-.

The Medical Working Group, Board of Strategic Funds in Sweden ("Stiftelsen för Strategisk Forskning"), 95-96.

The Board of the Swedish Medical Research Council (MFR), 95-98.

The Board of the Working Group for Vaccine Research and Development of the WHO Global Programme for Vaccines and Immunization, 1997-.

The Royal Society of Arts and Sciences in Göteborg, 1998-.

The Swedish Agency for Research Cooperation with Developing Countries (SAREC) expert group for Health 1999-.

Vice rector, Göteborg University, 1999-.

Medical Faculty; Member of:

The Evaluation Committee for PhD thesis, 89-96; Chairman of the Committee 94-96.

The Committee for Recruitment of Professors to the Faculty, 1994-.

The Committee for Appointing Professors to the Medical Faculty (Tjänsteförslagsnämnderna) 94-99; Chairman of the Committee for Appointing Associate Professors, 95-99.

The Board for Gastrointestinal Research, Göteborg University, 93-96.

The Board of the Medical Faculty ("Fakultetsnämnden"), 1997-.

Miscellaneous:

Invited lecturer or Chairman at > 60 international symposia and congresses.

Invited expert/rapporteur at several international WHO-meetings.

In the organizing committee for several international meetings and symposia and coeditor for proceedings from such meetings.

Frequently invited to write book chapters and review papers in the field of enteric vaccines, mucosal immunology etc.

Frequent reviewer of international grant applications and scientific papers concerning gastro-intestinal infections, vaccines, mucosal immunology etc. (WHO, NIH, ASM journals etc.).

Research activities:

Basic studies on mechanisms of disease and immunity in enterotoxin-induced diarrheal diseases.

Development of oral cholera and *E. coli* (ETEC) vaccines, including clinical trials.

Basic studies of mucosal immune responses in humans and animals.

Studies of pathogenic and immune mechanisms in *Helicobacter pylori* infections, including work on the development of a therapeutic vaccine against such infections.

Involved in numerous international collaborative projects, e. g. projects together with scientists in developed countries: in the United States at Walter Reed (WRAIR), Washington; Johns Hopkins, Baltimore; NIH, Washington; Baylor College, Houston; CDC, Atlanta etc., and in Europe: University of Utrecht, Holland; Colindale, London, UK; Institute of International Health, Bergen, Norway; Statens Seruminstitut, Copenhagen, Denmark; Astra Research Center, Boston etc., and in developing countries: at ICDDR,B, Dhaka, Bangladesh; Instituto de la Nutrición, Mexico City; Instituto Nacional de Microbiologia, Buenos Aires; Instituto Nacional de Salud, Bogotà; University of Alexandria and NAMRU-3, Cairo, Egypt; University of Rio de Janeiro, Brazil; National Institute of Hygiene, Hanoi, Vietnam; AFRIMS, Bangkok, Thailand etc.

Publications:

More than 300 scientific papers (published and accepted papers and review articles) in microbiology, immunology, infectious diseases, vaccinology and biotechnology.

Curriculum vitae

Jan Roland HOLMGREN, M.D., Ph.D.

Since 1981 Professor of Medical Microbiology and Head of the Department of Medical Microbiology and Immunology, Gothenburg University (GU), Sweden

Born on March 25, 1944 in Borås, Sweden.

Married to Professor Ann-Mari Svennerholm, 3 children.

EDUCATION/DEGREES

1965 Medical candidate (M.B.), GU
1969 Doctor of Medicine (Science) (Ph.D.), GU
1970 Docent in medical microbiology and immunology, GU
1973 Medical Licentiate (legislation as physician/ M.D)

EMPLOYMENTS

Current:

1981- to date Professor of Medical Microbiology and Head/Chairman (Prefect) of the Department of Medical Microbiology and Immunology, GU

Previous:

1966-69	University assistant (instructor), Department of Bacteriology, GU
1969-71	Substitute positions as docent and assoc. professor, Department of Bacteriology, GU
1971-74	Researcher (Assist. Professor) Swedish Medical Research Council
1974-80	Senior researcher (Assoc Professor) in "Immunology of infectious diseases"), Swedish Medical Research Council

BOARD MEMBERSHIPS AND INSTITUTIONAL ACTIVITIES

Board of Directors

Current:

- Knut and Alice Wallenberg Foundation 1995-
- International Vaccine Institute (Seoul) 2000-
- Nio Meter Liv – Stiftelsen för mag-tarmforskning 1997- ; Chairman 1997-99.
- Companies:
  - Got-A-Gene AB 1994-
  - CanAg AB 1997-

Previous:

- International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) 1979-1983
- Swedish Agency for Research Cooperation with Developing Countries (SAREC), 1979-1988, 1994-96
- Swedish International Development and Co-operation Agency's -Sida's Research Board 1996-98
- Swedish Medical Research Council, 1983-1989
- World Health Organization's Diarrhoeal Disease Control Programme, 1986-1991
- Medical Faculty Board, Göteborg University, 1987-96
- BioVäst Biotechnology Foundation, Sweden 1988-1993
- Astra Research Centre, India 1990-97
- WHO's Global Vaccination Program (SAGE) 1996-99
- Company boards: Stena Diagnostics 1983-87 Syntello Vaccine Development AB (now Maxim Pharmaceutical Inc.) 1987-93

Other Boards etc.

Current:

- International Vaccine Institute (IVI), Seoul: Chairman Scientific Advisory Board, 1999- & DOMI vaccine program 2000-
- Boards of Swedish Functional Genomics Programs SWEGENE (Göteborg-Lund) and Consortium North (Stockholm, Uppsala, Umeå, Linköping), 2000-
- Company Scientific Advisory Boards : Active Biotech AB 1998- ; CanAg AB 2000- .

Previous:

- **Study section board (prioritation committee) for Microbiology and Immunology, Swedish Medical Research Council (SMRC/MFR) 1979-1989, Chairman 1983-1989**
- Various ad hoc study section boards of SMCR/MFR (Laboratory medicine, glyco-biology and protein chemistry etc.) 1983 –94
- Study section board for Health and Nutrition of Swedish Agency for Research Cooperation with Developing Countries (SAREC), 1978- 1988; Chairman 1983-88
- WHO's Steering Committee for Bacterial Enteric Infections, Diarrhoeal Disease Control Programme, 1980-1985
- WHO's Steering Committee for Transdisease Vaccinology, 1988-96. Chairman 1993-96
- Academic Advisory Board, Mexico's National Institutes of Public Health, 1990-96
- WHO's Steering Committee for Enteric Vaccines 1991-96
- Children's Vaccine Initiative's Technical Task Force 1996-99
- Various scientific advisory boards of pharmaceutical/biotech companies: e.g. Stena Diagnostics AB 1983-87; Baxter Diagnostics (USA) 1987-90; Applied Immune Sciences Inc. (USA) 1990-94 ; MicroProbe Inc. (USA) 1994-96; EVAX (Germany) 1998-2000

Selected other appointments/offices

- Scientific consultant to the Swedish National Bacteriological Laboratory, Stockholm 1973-1993.
- Member of International Scientific Review Meeting leading to the establishment of the International Diarrhoeal Disease Centre, Bangladesh, 1978.
- Founder of the Swedish Medical Research Council Planning Group for Infectious Diarrhoeal Diseases 1979. Chairman 1979-1981. Member 1979- to date.
- Member of WHO Advisory Group on Diarrhoeal Diseases 1979- to date.
- Member Swedish Molecular Biology Committee (KOMBI) 1984-1989.
- Member of Selection Committee of ICDDR,B 1979-1983.
- Member of ICDDR,B Director Search Committee 1979, 1982,1985.
- Chairman Swedish Medical Research Council Steering Committee for pertussis vaccine trials 1985-1992.

- Chairman Election Committee for Microbiology Section of Swedish Medical Association 1978-85.
- Chairman Swedish Medical Research Council Group on AIDS research 1985-1989.
- Member SAREC International Steering Group for "AIDS and Tropical Diseases Program" 1988- 95
- Member of the Fernström Medical Prize Committee, University of Göteborg 1991-96
- Chairman Näringslivsnämnden (Committee for Industrial Interactions) of the Medical Faculty, University of Göteborg 1990-96.
- Task Force Oral Microsphere Program, PCD-WHO, 1991-94.
- Scientific Expert Committee on AIDS Vaccine Research, Swedish Technical Development Foundation (NUTEK) 1993-95.
- Member of Scientific Working Groups of various WHO Programs: Immunity and Vaccine Development 1980; Invasive Diarrhoea Pathogens 1982; Drug Development and Management of Acute Diarrhoeas 1982; Cholera 1984; Basic Vaccinology 1987; Live Vectors 1989.
- Member of selection jury for the 1985 Artois-Baillet Latour Health Prize 1985 (Belgium).

Special expert (sakkunnig) for positions as:

- Professor of Medical Microbiology, University of Linköping 1983 (O Stendahl);
- Professor of Bacteriology, University of Lund 1986 (T Wadström);
- Swedish Medical Research Council, Special Research Positions in:
  - Microbial Pathogenesis/Immunology 1985 (C Svanborg-Edén);
  - Regulation of the immune system 1986 (E-L Larsson),
  - Molecular Immunology 1988 (E Severinsson);
  - HIV infections and AIDS 1989 (E-M Fenyö);
  - Experimental rheumatology 1990 (R Holmdahl);
  - Autoimmunity 1991 (L Hammarström)
- Professor and Head of Department of Microbiology (Virology),Oslo University 1989 (M Degré)
- Professor of Medical Microbiology, Oslo University, 1991 (T Bergan)
- Professor of Molecular Biology and Head of Molecular Biology Program, National Institutes of Public Health, Helsinki, Finland, 1991 (Leena Peltola)
- Professor of Microbiology, University of Uppsala 1994 (Akusjärvi)
- Professorships in:
  - Microbiology (Lund University) 1996 (Gunnar Lindahl);
  - Immunology (Stockholm University) 1998 (Marita Troye-Blomberg);
  - Molecular biotechnology (Royal Technical University, Stockholm) 1998 (Stefan Ståhl)

EDITORSHIP/ASSOC. EDITOR of SCIENTIFIC JOURNALS

Current:

- Microbial Pathogenesis 1986-
- Vaccine 1991-
- Scandinavian Journal of Immunology 1995-
- Clinical Microbiology and Infection 1996-
- Journal of International Health, Nutrition and Population, 2000-

Previous:

- Medical Biology 1974-1986
- Current Microbiology 1979-1980
- International Journal of Diarrhoeal Diseases 1983-
- Infection and Immunity 1992-97

SCIENTIFIC HONOURS/AWARDS

- The Royal Swedish Academy of Science Prize in Medicine for 1977 (Hilda and Alfred Erikssons pris)
- The Anders Jahre Prize II in Medicine for 1982 (Norway)
- The Swedish Medical Society Prize (Söderbergska Priset) for 1994
- The Louis Jeantet Prize for Medicine for 1994 (Switzerland)

- Elected member Royal Technical Science Academy (IVA) 1994.

- **Organizer or co-organizer of >20 international scientific conferences**
- **Invited honorary speaker or keynote speaker at >30 international conferences**
- **Invited speaker and/or chairman at >200 international conferences/symposia**

Selected honorory lectures etc.

- Key note speaker International Conference on Enteric Infections, Brugge 1981.
- Quadrennial lecture International Congress of Gastroenterology, Stockholm 1982.
- The Robert Koch Centennial Cholera Lecture, Berlin 1985.
- Key-note speaker Nobel Conference on Vaccines and Drugs against Diarrhea Stockholm 1985.
- McLaughlin Visiting Professor Lecture, Galveston, Texas 1987.
- The Sir Arthur Hurst Lecture to the British Society of Gastroenterology, 1989.
- Prize Winner's Award Lecture at the Swedish Medical Association's Annual Meeting 1997

PUBLICATIONS

Editor of Books

- Nobel Symposium "Cholera and related diarrheal disease", Karger 1980;
- Nobel Conference "Acute enteric infections", Elsevier 1981;
- "Tumor marker antigens", Studentlitteratur/ Chartwell Bratt 1985.
- Nobel Conference "Development of vaccines and drugs against diarrhea", Studentlitteratur/ Chartwell Bratt 1986.

Scientific papers

Author or co-author of near 500 scientific publications in microbiology, immunology, infectious diseases and biotechnology

- Ca 300 peer-reviewed original papers
- Ca 180 reviews, book chapters etc.

Appendix 1
Time frame[1] of different components of the study

	Months																																			
Birth Cohort[2] Study	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32				
ETEC and Cholera vaccine study[2]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Dried and Liquid cholera Vaccine study in -- adults	1	2	3	4	5	6	7	8	9	10	11	12	*																							
Optimum Buffer+ ETEC vaccine-- 6-17 months (1/4 dose)	1	2	3	4	5	6	7	8	9	10	11	12	*																							
Combined ETEC & cholera vaccine -- adults													13	14	15	16	17	18	*																	
Combined ETEC & cholera vaccine -- 18 mo. to 5 yrs.																					21	22	23	24	25	26	*									
Combined ETEC & cholera vaccine -- 6 mo. to 17 mo.																													29	30	31	32	33	34	*	
In vivo study[2]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

[1]The time period for recruitment and completion of planned study in study subjects is shown.

[2]The birth cohort, vaccine and *in vivo* study in patients will be carried out in parallel for the whole duration of the 3 years period of the study.

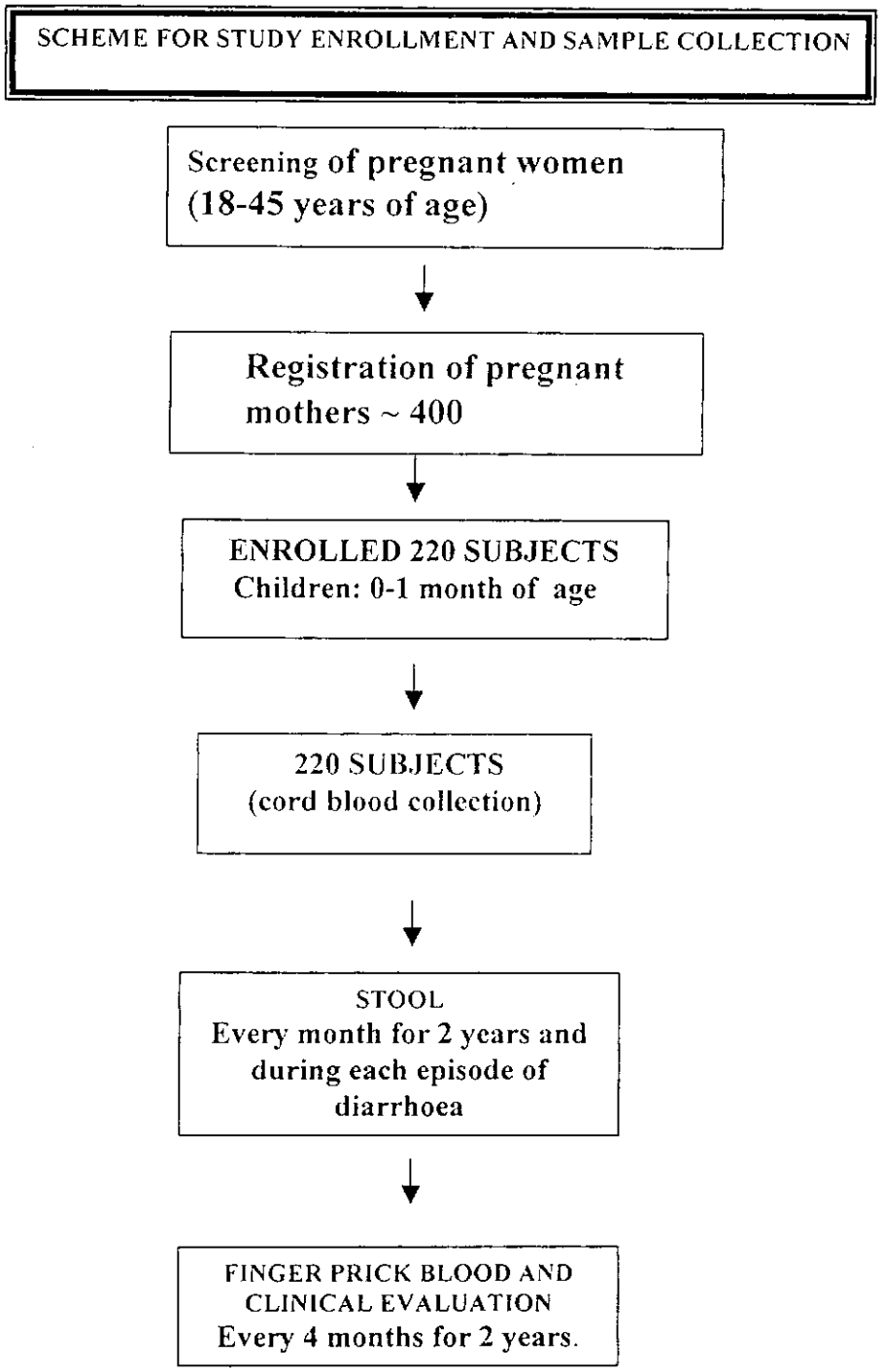
*The vaccine study will be carried out sequentially as shown above and after completion of each phase report will be submitted to RRC/ERC before starting the phase.

Appendix 2
Sample size of study subjects

Age group	Vaccine	No of study subjects	Sample size based on 95% confidence interval and assumption:
Adults (18-45 years):	Combined ETEC + cholera vaccine	70	Assumption: 70% (Lowest acceptable limit: 58%)
	Dried formulation of cholera vaccine	70	Assumption: 70% (Lowest acceptable limit: 58%)
	Liquid formulation of cholera vaccine	70	Assumption: 70% (Lowest acceptable limit: 58%)
	ETEC vaccine	44	
Children (18 months-5 years):	ETEC vaccine	44	Assumption: 90% (Lowest acceptable limit: 80%)
	Cholera vaccine	69	Assumption: 71% (Lowest acceptable limit: 59%)
	Combined ETEC + cholera vaccine	69	Assumption: 71% (Lowest acceptable limit: 59%)
Children(6-17 months):	Cholera vaccine	70	Assumption: 70% (Lowest acceptable limit: 58%)
	Combined ETEC (1/4 dose)÷ cholera vaccine	70	Assumption: 70% (Lowest acceptable limit: 58%)
	ETEC vaccine with different buffer formulations	225	Assumption: 90% (Lowest acceptable limit: 80%)
Infant (from 0 month)	ETEC cohort study	220	Assumption : 25% (Lowest acceptable limit): 20%
Patients (Children and adults)	Novel antigens in ETEC	44	Assumption : 90% (Lowest acceptable limit): 80%
Total study subjects: = 1065			

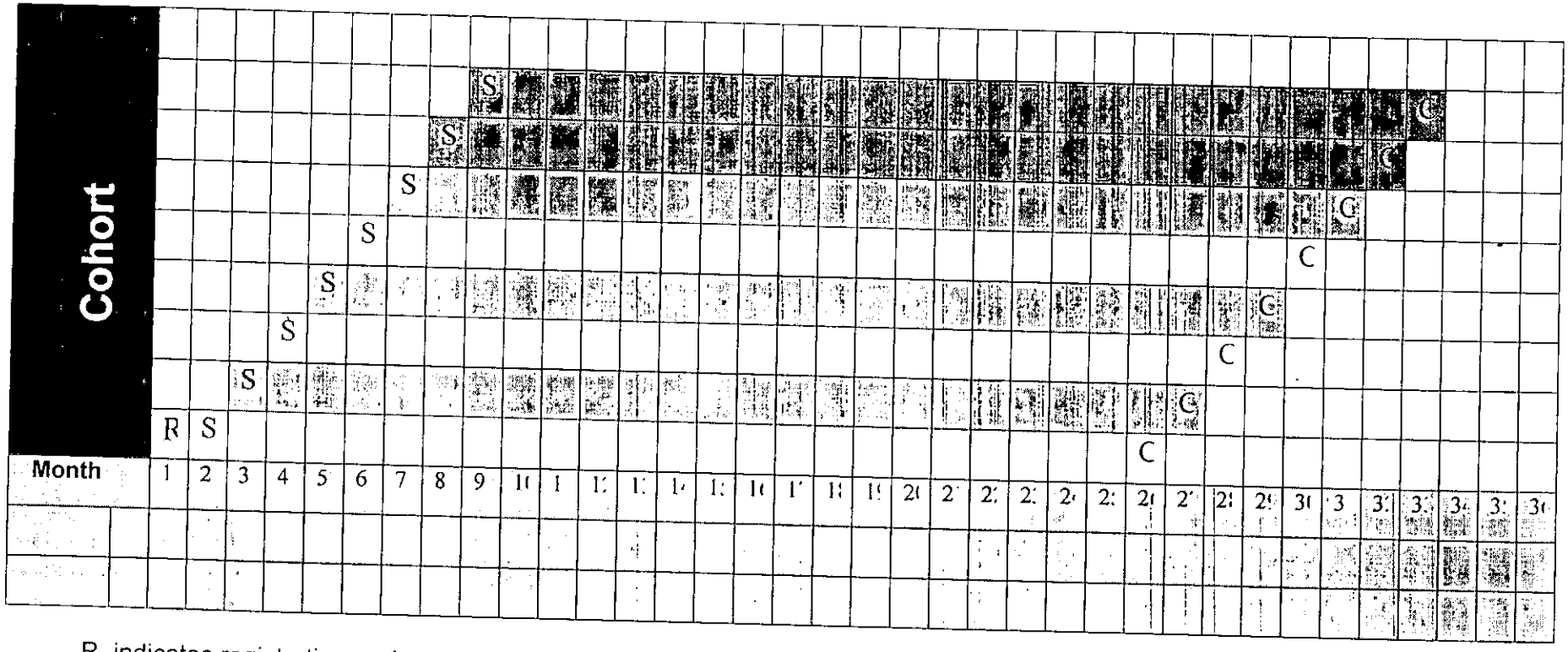
Appendix 3

Study plan in the birth cohort



Dummy forms 12a-c have been attached with the project. These will be pre-tested prior to use for the study.

Appendix 4
Birth Cohort Study



R, indicates registration and enrollment of pregnant women,
S, indicates start of the enrollment sample collection and C, the completion

Appendix- 5

Collection of stool and blood samples from the children in the birth cohort

Sample	Number	Sample collection days	Volume/amount to be collected
Cord blood	220	At delivery	5 ml
Finger prick	220x 6=1320	4 monthly	0.5 ml
Stool for microbiology (monthly* and immunology* (4 monthly)	220x24=5280	Monthly	Stool 5 gm
Diarrheal stools**	4x220x2=1760	At diarrheal episode	Stool- 5 gm

*Stool samples that will be collected at intervals of 4 months, at the same time point that blood will be collected will only be used for immunological assays. That is every 4th stool will be used for microbiological as well as immunological assays. **Based on an incidence of 4 episodes of diarrhoea per child per year.

Appendix- 6

Plans for collection of stool and blood for the Project

Study group	Number	Sample collection days	Samples to be collected
Adult ETEC and cholera vaccinees (18-45 years)	254	Day 0, day 7, day 21	Blood- 5 ml Stool- about 5gm
Children- ETEC and cholera vaccinees (18 months to 5 years)	182	Day 0, day 7, day 21	Blood- 3 ml Stool- about 5 gm
Children, ETEC and cholera vaccinees (6 months to 17 months)	140	Day 0, day 7, day 21	Blood- 1.5 ml Stool- about 5 gm
Children, ETEC vaccinees for optimization of buffer (6 months to 17 months)	225	Day 0, day 7, day 21	Blood- 1.5 ml Stool- about 5 gm
Children and adults (6 months to 45 years)-patients with ETEC For study of in vivo expressed antigens	44	Acute stage-day 2, day 7, day 21	Blood- 1.5 ml for children up 18 months, 3.0 ml for 18 mo. to 5 yrs. And 5.0 ml for adults Stool- about 5 gm* at acute stage at convalescence
Birth Cohort (0 to 2 years)	220	From 1-2 weeks of age to up to 2 years of age. Stool- monthly and diarrheal Blood- 4 monthly	Blood- 5 ml (cord blood) Blood- 0.5 ml (finger prick blood) Stool- about 5 gm

*Stool from the patients at the acute stage to be used for isolation of ETEC and for in vivo bacteria and for the antibody assays.

Detailed Budget for Sida-SAREC proposal- Fqadri, ICDDR,B-Part I-ETEC studies

Personnel	Position	Rate	Effort %	Salary	1st Year		2nd Year		3rd Year		TOTAL	
					US$	SEK	US$	SEK	US$	SEK	US$	SEK
FQadri	Sen.Scient	9747	5%	P5	5,848	60,351	6,140	63,369	6,447	66,537	18,436	190,258
T.Ahmed	Medical Officer	625	50%	NOA	3,909	40,341	4,104	42,358	4,310	44,476	12,323	127,175
Y. A.Begum	Sen.ScienOff	625	30%	NOA	2,346	24,211	2,463	25,421	2,586	26,692	7,396	76,324
	Res.Officer x1	333	100%	GS5	3,996	41,239	4,196	43,301	4,406	45,466	12,597	130,005
	Sen.Lab.AttendX1	179	100%	GS2	2,148	22,167	2,255	23,276	2,368	24,440	6,772	69,883
	Field Res AssisX2	179	100%	GS2	4,296	44,335	4,511	46,551	4,736	48,879	13,543	139,765
	Health WorkerX2	67	100%		1608	16,595	1,688	17,424	1,773	18,296	5,069	52,314
sub total					**24,151**	**249,238**	**25,359**	**261,700**	**26,626**	**274,785**	**76,136**	**785,724**
Local travel					1,500	15,480	1,200	12,384	1,500	15,480	4,200	46,440
International					1,500	15,480	1,700	17,544	3,000	30,960	6,200	63,984
sub total					**3,000**	**30,960**	**2,900**	**29,928**	**4,500**	**46,440**	**10,400**	**107,328**
Supplies and materials												
Immunoreagents,plastics,					8,000	82,560	8,000	82,560	6,500	67,080	22,500	232,200
Supplies from Goteborg for ICDDR,B					8,000	82,560	8,000	82,560	6,500	67,080	22,500	232,200
Medical supplies					2000	20,640	2000	20,640	3000	30,960	18,000	72,240
Food charges etc for volunteers					2000	20,640	2000	20,640	2000	20,640	12,000	61,920
Subtotal					**20,000**	**206,400**	**20,000**	**206,400**	**18,000**	**185,760**	**58,000**	**598,560**
Other contractual services					**1000**	**10,320**	**2000**	**20,640**	**1000**	**10,320**	**4000**	**41,280**
Repair/maintainence					**3000**	**30,960**	**6000**	**61,920**	**6000**	**61,920**	**15000**	**154,800**
Rent,comm,field office, utilities												
electricity, publication												
staff dev, training/workshop	Total											
printing, publication												

Interdepartmental charges (Computer, Pathol tests, microbiological test, biochimistry test, X-ray, biochimistry and nutrition, transport, xerox, mimeographs, etc)		**5,000**	**51,600**	**6,000**	**61,920**	**5,000**	**51,600**	**16,000**	**165,120**
Capital Equipment parts etc		**1000**	**10,320**	**2000**	**20,640**	**2000**	**20,640**	**5000**	**51,600**
TOTAL DIRECT COST		57,151	589,798	64,259	663,148	63,126	651,465	184,536	1,904,412
INDIRECT COST (25%)		14,288	147,450	16,065	165,787	15,782	162,866	46,134	476,103
TOTAL PROJECT COST(ICDDRB)	**Total 3 years**	**71,439**	**737,248**	**80,323**	**828,935**	**78,908**	**814,332**	**230,670**	**2,380,515**

Detailed Budget for Sida-SAREC proposal- A-M Svennerholm- Goteborg University Part I-ETEC studies

			1st Year		2nd Year		3rd Year		TOTAL	
Personnel	Position	Effort % Salary	US$	SEK	US$	SEK	US$	SEK	US$	SEK
	Technician	25%	9,000	92,880	9,000	92,880	9,000	92,880	27,000	278,640
CONSUMABLES										
Mol. Biological reagents,immunoreagents, antibodies, tissue cult materials, plastics etc.			12,000	123,840	12,000	123,840	11,000	113,520	35,000	361,200
Direct Cost			**21,000**	**216,720**	**21,000**	**216,720**	**20,000**	**206,400**	**62,000**	**639,840**
Indirect Cost (30%)			**6,300**	**65,016**	**6,300**	**65,016**	**6,000**	**61,920**	**18,600**	**191,952**
TOTAL BUDGET (part 1 Goteborg) =			**27,300**	**281,736**	**27,300**	**281,736**	**26,000**	**268,320**	**80,600**	**831,792**

Please note, the funds below from the ICDDR,B consumable component will be kept in Goteborg for purchasing reagents for ICDDR,B

8000	**82,560**	**8,000**	**82,560**	**6,500**	**67,080**	**22,500**	**232,200**

Total funds needed for study for ICDDR,B and Goteborg University (ETEC studies-PartI)

Total funds needed for study for ICDDR,B and Goteborg University (ETEC studies-PartI)

	1st Year		2nd Year		3rd Year		TOTAL	
	US$	SEK	US$	SEK	US$	SEK	US$	SEK
ICDDR,B	71,439	737,248	80,323	828,935	78,908	814,332	230,670	2,380,515
Goteborg University	27,300	281,736	27,300	281,736	26,000	268,320	80,600	831,792
Total Sida-SAREC	98,739	1,018,984	107,623	1,110,671	104,908	1,082,652	311,270	3,212,307

{Please note, the funds below are included in the budget above in the ICDDR,B component for purchasing reagents for ICDDR,B

	8,000	82,560	8,000	82,560	6,500	67,080	22,500	232,200

Budget-Sida-SAREC proposal-Fqadri PI, ICDDR,B-Part II ETEC and cholera vaccine study

Personnel	Position	Rate	Effort %	Salary	1st Year US$	1st Year SEK	2nd Year US$	2nd Year SEK	3rd Year US$	3rd Year SEK	TOTAL US$	TOTAL SEK
FQadri	Sen.Scient	9747	5%	P5	5,848	60,351	6,140	63,369	6,447	66,537	18,436	190,258
T.Ahmed	Medical Officer	625	50%	NOA	3,909	40,341	4,104	42,358	4,310	44,476	12,323	127,175
Y. A.Begur	Sen.ScienOff	625	20%	NOA	1,564	16,140	1,642	16,948	1,724	17,795	4,931	50,883
	Res.Officer x1	333	100%	GS5	3,996	41,239	4,196	43,301	4,406	45,466	12,597	130,005
	Sen.Lab.Attend	179	100%	GS2	2,148	22,167	2,255	23,276	2,368	24,440	6,772	69,883
	Field Res Assis	179	100%	GS2	4,296	44,335	4,511	46,551	4,736	48,879	13,543	139,765
	Health WorkerX	67	100%		1608	16,595	1,688	17,424	1,773	18,296	5,069	52,314
sub total					**23,369**	**241,168**	**24,537**	**253,226**	**25,764**	**265,888**	**73,671**	**760,282**
Local travel					1,300	13,416	1,400	14,448	2,000	20,640	4,700	48,504
International					2,000	20,640	2,200	22,704	3,500	36,120	7,700	79,464
sub total					**3,300**	**34,056**	**3,600**	**37,152**	**5,500**	**56,760**	**12,400**	**127,968**
Supplies and materials												
Immunoreagents,plastics,					7,500	77,400	8,000	82,560	6,500	67,080	22,000	227,040
Supplies and materials to be bought at Goteborg for ICDDR,B					7,500	77,400	8,000	82,560	6,500	67,080	22,000	227,040
Medical supplies					2,000	20,640	2,000	20,640	3,000	30,960	18,000	72,240
Food charges etc for volunteers					2,000	20,640	2,000	20,640	2,000	20,640	12,000	61,920
Subtotal					**19,000**	**196,080**	**20,000**	**206,400**	**18,000**	**185,760**	**57,000**	**588,240**
Other contractual services					**1,000**	**10,320**	**2,000**	**20,640**	**1,000**	**10,320**	**4,000**	**41,280**
Repair/maintainence					**4,000**	**41,280**	**6,000**	**61,920**	**6,000**	**61,920**	**16,000**	**165,120**
Rent,comm,field office, utilities												
electricity, publication												
staff dev, training/workshop Total												
printing, publication												

Interdepartmental charges (Computer, Pathol tests, microbiological test, biochimistry test, X-ray, biochimistry and nutrition, transport, xerox, mimeographs, etc)	**5,000**	**51,600**	**6,000**	**61,920**	**5,000**	**51,600**	**16,000**	**165,120**
Capital Equipment parts etc	**1000**	**10,320**	**2000**	**20,640**	**2000**	**20,640**	**5000**	**51,600**
DIRECT COSTS	56,669	584,824	64,137	661,898	63,264	652,888	184,071	1,899,610
Indirect Cost (25%)	14,167	146,206	16,034	165,475	15,816	163,222	46,018	474,903
TOTAL PROJECT COST (Part II) ICDDR,B	**70,836**	**731,030**	**80,172**	**827,373**	**79,080**	**816,110**	**230,088**	**2,374,513**

Budget-Sida-SAREC- A-M Svennerholm,Goteborg UniversityPart II-Cholera and ETEC vaccine studies

				1st Year		**2nd Year**		**3rd Year**		**TOTAL**	
Personnel	Position	Rate Effort %	Salary	US$	SEK	US$	SEK	US$	SEK	US$	SEK
	Technician	25%		9,000	92,880	9,000	92,880	9,000	92,880	**27,000**	**278,640**
CONSUMABLES											
Mol. Biological reagents,immunoreagents, antibodies, tissue cult materials, plastics etc for Goteborg				12,000	123,840	11,000	113,520	10,000	103,200	**33,000**	**340,560**
Direct Cost				**21,000**	**216,720**	**20,000**	**206,400**	**19,000**	**196,080**	**60,000**	**619,200**
Indirect Cost (30%)				**6,300**	**65,016**	**6,000**	**61,920**	**5,700**	**58,824**	**18,000**	**185,760**
Total Project Cost (part II Goteborg)				**27,300**	**281,736**	**26,000**	**268,320**	**24,700**	**254,904**	**78,000**	**804,960**

Please note, that the funds below for the ICDDR,B consumable component will be kept in Goteborg for purchasing reagents for ICDDI

	7500	**77,400**	**8000**	**82,560**	**6500**	**67,080**	237,360	227,040

Sida-SAREC ICDDR,B and Goteborg University (Cholera and ETEC-PartII)

	1st Year		2nd Year		3rd Year		TOTAL	
	US$	SEK	US$	SEK	US$	SEK	US$	SEK
ICDDR,B	70,836	731,030	80,172	827,373	79,080	816,110	230,088	2,374,513
Goteborg University	27,300	281,736	26,000	268,320	24,700	254,904	78,000	804,960
Total Sida-SAREC	**98,136**	**1,012,766**	**106,172**	**1,095,693**	**103,780**	**1,071,014**	**308,088**	**3,179,473**

Detailed Budget ETEC and Cholera vaccine studies proposal - Fqadri, ICDDR,B- additional supporting budget for Part I and PartII (other sources)

Personnel	Position	Rate	Effort %	1st Year	2nd Year	3rd Year	Total
				US$	US$	US$	US$
FQadri	Sen.Scient	9747	5%	5,848	6,140	6,447	18,436
A.Siddique	Sen.Scient	8174	5%	4,908	5,150	5,407	15,465
K. Z. Hasan	Asso. Scientist	1423	20%	3,416	3,586	3,766	10,768
M.A.Salam	Chief Physician	1730	2%	415	436	458	1,309
K. Zaman	Asso. Scientist	1439	5%	863	906	951	2,720
ASG Faruque	Scientist	1801	2%	432	454	477	1,363
	Medical Officer	659	100%	7,908	8,303	8,719	24,930
	Data Man. Off.	333	100%	3,996	4,196	4,406	12,597
	Res.Officer x1	333	100%	3,996	4,196	4,406	12,597
	Sen.Lab.AttendX1	214	100%	2,568	2,696	2,831	8,096
	Sen., Field Res Asst.X2	214	100%	5,136	5,393	5,662	16,191
	Field Res Asst.X1	179	100%	2,148	2,255	2,368	6,772
	Health WorkerX4	67	100%	3,216	3,377	3,546	10,138
	Study nurseX1	179	100%	2,148	2,255	2,368	6,772
Subtotal				**46,998**	**49,344**	**51,812**	**148,154**
Local travel				1,000	1,000	1,000	3,000
International				3,000	3,000	3,000	9,000
Subtotal				**4,000**	**4,000**	**4,000**	**12,000**
Supplies and materials							
Immunoreagents,plastics,				10,000	8,000	7,000	25,000
Medical supplies				4,000	4,000	4000	12,000
Food charges etc for volunteers				2000	2000	2000	6,000
Subtotal				**16,000**	**14,000**	**13,000**	**43,000**
Other contractual services				**1000**	**1000**	**1000**	**3000**

Repair/maintainence Rent,comm,field office, utilities electricity, publication staff dev, training/workshop printing, publication		**5000**	**5000**	**5000**	**15000**
Interdepartmental charges (Computer, Pathol tests, microbiological test, biochimistry test, X-ray, biochimistry and nutrition, transport, xerox, mimeographs, etc)		**3,000**	**3,000**	**3,000**	**9,000**
Capital Equipment(computer, printers and parts etc)		**5000**	**5000**	**5000**	**15000**
TOTAL DIRECT COST		80,998	81,344	82,812	245,154
INDIRECT COST (25%)		20,250	20,336	20,703	61,289
TOTAL PROJECT COST(ICDDRB)	**Total 3 years**	**101,248**	**101,680**	**103,515**	**306,443**

Principal Investigator: Last, first, middle _Qadri Firdausi

Vaccine: Adult

Appendix 7a

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh

CONSENT FORM (ADULT)

IMMUNIZATION AND COLLECTION OF BLOOD AND STOOL

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Diarrhoeal diseases are major health problem, and germs called *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including those caused by *Vibrio cholerae* and ETEC using effective vaccines.

We are conducting a study in your community to assess how healthy adults (18-45 years of age) respond to one of the following vaccines listed below:

1. Liquid formulation of cholera vaccine

2. Dried formulation of cholera vaccine

3. ETEC vaccine

4. ETEC/cholera combined vaccine

The vaccines to be used in this study have been tested in adults and children, and have been found to be safe. Studies carried out in Sweden and Egypt in adults found the ETEC vaccine to be safe during the 3-day observation period. An oral vaccine to prevent cholera has been previously tested in various countries of the world including Bangladesh and found safe.

We request you to participate in this study. For the purpose of this study, we would ask you to drink this vaccine twice, fourteen days apart. To determine the response to the vaccines, we will draw 5.0 ml (about one teaspoonful) of blood from a vein on your forearm, and collect small sample of your stool (5 grams) at the beginning of the study, and 7 and 21 days later. Other than momentary pain due to needle prick, a very small chance of bruising at the site of insertion of the needles, and rare chance of infection collection of this amount of blood will not cause any harm to you. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.

You may not directly benefit from participating in this study; however, results of this study will improve our knowledge about these vaccines and may thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available. We will carefully observe occurrence of any untoward reaction of the vaccine, and will provide best possible treatment at no cost to you. We will support your travel cost to attend this field office, compensate for your wage loss, if any.

You are free to accept or reject our proposal to enroll you in this study; and you may also withdraw yourself from the study at any time. If you agree to our proposal of enrolling you in this study, please indicate that by putting your signature or your left thumb impression at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the subject

Date: _____

_____
Signature of the investigator

Date: _____

_____
Signature of witness

Date: _____

পরিশিষ্ট - ৭ এ

ডায়ারিয়া টীকা : প্রাপ্ত-বয়স্ক স্বেচ্ছাসেবক

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

ডায়ারিয়া একটি প্রধান স্বাস্থ্য-সমস্যা এবং *ভিব্রিও কলেরী* ও এন্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক বিভিন্ন ধরনের জীবাণু বাংলাদেশের শিশু ও প্রাপ্ত-বয়স্কদের মধ্যে ডায়ারিয়ার প্রাদুর্ভাবের একটি প্রধান কারন। অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া বিশেষ করে *ভিব্রিও কলেরী* এবং ইটেক জীবাণু দ্বারা সংঘটিত ডায়ারিয়া টীকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আমরা এই এলাকায় আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি) এর মাধ্যমে সুস্থ প্রাপ্ত-বয়স্কদের (১৮-৪৫ বৎসর বয়স্ক) উপর নিম্নবর্নিত কলেরা এবং ইটেক টীকা আলাদা বা একসাথে প্রয়োগ করে এদের প্রভাব নিয়ে গবেষণা করব।

<> ১. তরল কলেরা টীকা
<> ২. পাউডার কলেরা টীকা
<> ৩. ইটেক টীকা
<> ৪. ইটেক এবং কলেরা যৌথ টীকা

পূর্ববর্তী গবেষণা থেকে জানা গেছে যে, এই গবেষণায় ব্যবহৃত ইটেক টীকা শিশু ও প্রাপ্ত-বয়স্কদের জন্য নিরাপদ। সুইডেন ও মিসরে গবেষণায় দেখা গেছে ইটেক টীকা খাওয়ানোর ৩-দিন পর্যবেক্ষণ কালের সময়ে প্রাপ্ত-বয়স্কদের জন্য এই টীকা সম্পূর্ন নিরাপদ। বাংলাদেশ সহ পৃথিবীর বিভিন্ন দেশের পূর্ববর্তী গবেষণায় মুখে খাওয়ানোর কলেরা টীকা নিরাপদ বলে প্রতীয়মান হয়েছে।

আমরা এই গবেষণায় অংশগ্রহণের জন্য আপনার সম্মতি চাচ্ছি। এতে গবেষণায় অংশগ্রহণ করলে আমরা আপনাকে ১৪ দিনের ব্যবধানে ২ বার এই টীকা খাওয়াবো। এই টীকার রোগ প্রতিষেধক ক্ষমতা দেখার জন্য আমরা আপনার হাতের শিরা হতে তিনবার ৫ মি. লি. (১ চা চামচ পরিমান) রক্ত ও পায়খানার (৫ গ্রাম) নমুনা সংগ্রহ করবো। এই রক্ত ও পায়খানার নমুনা টীকা খাওয়ানোর পূর্বে এবং গবেষণায় অংশগ্রহণের ৭ম ও ২১তম দিনে নেয়া হবে। রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে আপনার আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো।

যদিও এই গবেষণায় অংশগ্রহণের জন্য আপনার সরাসরি উপকার হবে না তথাপি দেশ, জাতি ও মানবতার বৃহত্তর স্বার্থে এর গবেষণালব্ধ ফল
এই টীকা সম্পর্কে আমাদের 'ানের পরিধি বাড়াতে সাহায্য করবে। আপনারা চাইলে আমরা এই গবেষণা ও টীকা সম্পর্কিত যে কোন প্রশ্নের উত্তর দিতে প্রস্তুত এবং এমনকি সম্ভব হলে গবেষণালব্ধ ফলাফল জানাতে পারবো।
গবেষণা চলাকালীন সময় টীকা খাওয়ানোর পর আপনার কোন রকম অসুবিধা দেখা দিলে আমরা সর্বোচ্চ সতর্কতা অবলম্বন করবো এবং এর জন্য কোন চিকিৎসার প্রয়োজন হলে তা বিনামূল্যে প্রদান করবো। এছাড়া গবেষণা চলাকালীন সময়ে আপনার দৈনন্দিন কাজের কোন ক্ষতি হলে ক্ষতিপূরনের ব্যবস্থা এবং যাতায়াতের ভাড়া প্রদান করবো।

একমাত্র আপনিই এ গবেষণায় আপনার অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন।

আপনি এ গবেষণায় আপনার অন্তর্ভূক্তির ব্যাপারে আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলের টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

পিতা/মাতা/অভিভাবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Appendix 7b

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh
CONSENT FORM (CHILDREN)
IMMUNIZATION AND COLLECTION OF BLOOD AND STOOL

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Diarrhoeal diseases are major health problem, and germs called *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including those caused by *Vibrio cholerae* and ETEC by using effective vaccines.
We are conducting a study at this community to observe how healthy children (5 years to 18 months of age) respond to the oral administration of killed *V. cholerae* and inactivated ETEC vaccine administered either alone or in combination. Children participating in the study would be required to drink one of the following vaccines:

1. Cholera vaccine **2. ETEC vaccine** **3. Combined ETEC and cholera vaccine**

The vaccines to be used in this study have been tested in adults and children, and have been found to be safe. Studies carried out in Sweden and Egypt have found the ETEC vaccine to be safe, and adverse events were noted in only about 2-6% of the recipients, which include nausea, mild abdominal cramps, and 1-2 loose stools per day during the 3-day observation period. An oral vaccine to prevent cholera has been previously tested in various countries of the world including Bangladesh, and was found safe.
We request your permission to enroll your child in this study. For the purpose of this study, we would ask your child to drink one of the above-mentioned vaccines twice, fourteen days apart. To determine the response to the vaccines, we will draw 3.0 ml (about half teaspoonful, from children 18 months to 5 years of age) of blood from a vein in her/his forearm and also a small sample of her/his stool (about 5 grams) at the beginning of the study, and 7 and 21 days later. Other than momentary pain due to needle stick, a very small chance of bruising at the site of insertion of the needles, and rare chance of infections drawing of such amount of blood will not cause any other harm to your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.
Your child may not directly benefit from participating in the study; however, results of this study will improve our knowledge about these vaccines and may thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available. We will carefully observe occurrence of any untoward effect of the vaccine, and will provide best possible treatment at no cost to your child in the event any such events. We will support your travel cost to attend this field office, and will also compensate for your wage loss, if any.
You are free to accept or reject our proposal to enroll your child in this study, and you would also be able to withdraw your child from the study at any time. If you agree to our proposal of enrolling child in our study, please indicate that by putting your signature or your left thumb impression at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of witness

Date: _____

_____
Signature of the investigator

Date: _____

পরিশিষ্ট - ৭ বি

ডায়ারিয়া টীকা : শিশু (১৮ মাস থেকে ৫ বৎসর)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

ডায়ারিয়া একটি প্রধান স্বাস্থ্য-সমস্যা এবং *ভিব্রিও কলেরী* ও এন্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক বিভিন্ন ধরনের জীবাণু বাংলাদেশের শিশু ও প্রাপ্ত-বয়স্কদের মধ্যে ডায়ারিয়ার প্রাদুর্ভাবের একটি প্রধান কারন। অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া বিশেষ করে *ভিব্রিও কলেরী* এবং ইটেক জীবাণু দ্বারা সংঘটিত ডায়ারিয়া টীকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আমরা এই এলাকায় সুস্থ শিশুদের (১৮ মাস থেকে ৫ বৎসর) উপর নিম্নবর্নিত কলেরা এবং ইটেক টীকা আলাদা বা একসাথে প্রয়োগ করে এদের প্রভাব নিয়ে গবেষণা করব। শিশুরা এই গবেষণায় অংশগ্রহন করলে নিম্নবর্নিত যে কোন একটি টীকা পান করতে হবে।

<> ১. কলেরা টীকা <> ২ ইটেক টীকা <> ২. ইটেক এবং কলেরা যৌথ টীকা

পূর্ববর্তী গবেষণা থেকে জানা গেছে যে, এই গবেষণায় ব্যবহৃত ইটেক টীকা শিশু ও প্রাপ্ত-বয়স্কদের জন্য নিরাপদ। সুইডেন ও মিসরে গবেষণায় দেখা গেছে ইটেক টীকা খাওয়ানোর ৩-দিনের মধ্যে মাত্র ২-৬% টীকা গ্রহনকারীদের বমি ভাব, অল্প পেট মোচড়ানো কিংবা দুই একবার পাতলা পায়খানা হয়েছে। বাংলাদেশ সহ পৃথিবীর বিভিন্ন দেশের পূর্ববর্তী গবেষণা থেকে জানা গেছে যে, মুখে খাওয়ানোর কলেরা টীকা সম্পূর্ণ নিরাপদ।

আমরা আপনার শিশুকে এই গবেষণায় অংশগ্রহণের জন্য আপনার সম্মতি চাচ্ছি। এতে গবেষণায় অংশগ্রহণ করলে আমরা আপনার শিশুকে ১৪ দিনের ব্যবধানে ২ বার এই টীকা খাওয়াবো। এই টীকার রোগ প্রতিষেধক ক্ষমতা দেখার জন্য আমরা আপনার শিশুর হাতের শিরা হতে তিনবার ৩ মি.লি (১/২ চা চামচ পরিমান) রক্ত ও পায়খানার (৫ গ্রাম) নমুনা সংগ্রহ করবো। এই রক্ত ও পায়খানার নমুনা টীকা খাওয়ানোর পূর্বে এবং গবেষণায় অংশগ্রহণের ৭ম ও ২১তম দিনে নেয়া হবে। রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে শিশুর আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো।

যদিও এই গবেষণায় অংশগ্রহণের জন্য আপনার সরাসরি উপকার হবে না তথাপি দেশ, জাতি ও মানবতার বৃহত্তর স্বার্থে এর গবেষণালব্ধ ফল এই টীকা সম্পর্কে আমাদের জ্ঞানের পরিধি বাড়াতে সাহায্য করবে। আপনারা চাইলে আমরা এই গবেষণা ও টীকা সম্পর্কিত যে কোন প্রশ্নের উত্তর দিতে প্রস্তুত এবং এমনকি সম্ভব হলে গবেষণালব্ধ ফলাফল জানাতে পারবো।
গবেষণা চলাকালীন সময় টীকা খাওয়ানোর পর আপনার শিশুর কোন রকম অসুবিধা দেখা দিলে আমরা সর্বোচ্চ সতর্কতা অবলম্বন করবো এবং এর জন্য কোন চিকিৎসার প্রয়োজন হলে তা বিনামূল্যে প্রদান করবো। এছাড়া গবেষণা চলাকালীন সময়ে আপনার দৈনন্দিন কাজের কোন ক্ষতি হলে ক্ষতিপূরনের ব্যবস্থা এবং যাতায়াতের ভাড়া প্রদান করবো।

একমাত্র আপনিই এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন। আপনি এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলের টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

পিতা/মাতা/অভিভাবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Vaccine: children
1/4 dose ETEC vaccine, change in buffer

Appendix 7c

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh
CONSENT FORM (CHILDREN, 6-17 month of age)
IMMUNIZATION AND COLLECTION OF BLOOD AND STOOL

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Diarrhoeal diseases are major health problem, and germs called *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases caused by *Vibrio cholerae* and ETEC by using effective vaccines.

We are conducting a study in your community to observe how healthy children aged 6 to 17 months respond to oral inactivated ETEC vaccine. The vaccine to be used in this study has been tested in adults and children in Bangladesh and in other countries.

Studies carried out in Sweden and Egypt have observed the ETEC vaccine to be safe, and untoward events were noted only in 2-6% of the recipients, which include nausea, mild abdominal cramps, and 1-2 loose stools per day during the 3-day observation period. We have recently completed a study using the same ETEC vaccine in children 6 months to 17 months of age in Bangladesh, and observed higher rates of vomiting. We then used 1/4th dose of the vaccine in same age group of children and found that to be safe in about 170 children that have been studied. We would now like to change the buffer solution in which we will mix the vaccine in order to determine the best formulation of the vaccine.

We request your permission to enroll your child in our study. For the purpose of this study, we would ask your child to drink either the ¼ dose of the ETEC vaccine formulated in different buffers, fourteen days apart. To determine the response to the vaccines, we will draw 1.5 ml (about 1/4th of a teaspoonful) from a vein of your child's forearm and a sample of stool (5 grams) at the beginning of the study, and 7 and 21 days later. Other than momentary pain due to needle stick, a very small chance of bruising at the site of insertion of the needles and rare chance of infection, drawing of such amount of blood will not cause any other harm your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.

Your child may not directly benefit from participating in the study; however, results of this study will improve our knowledge about the vaccine and may thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available. We will carefully observe occurrence of untoward effects of the vaccine, and provide best possible treatment at no cost to your child in the event of such events. We will support your travel cost to attend this field office, and will also compensate for your wage loss, if any.

You are free to accept or reject our proposal to enroll your child in this study, and you would also be able to withdraw your child from the study at any time. If you agree to our proposal of enrolling your child in this study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____ _____

Signature/LTI of the guardian Signature of the investigator

Date: _____ Date: _____

_____

Signature of witness

Date: _____

পরিশিষ্ট - ৭ সি

ডায়ারিয়া টীকা : শিশু (৬ মাস থেকে ১৭মাস)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

ডায়ারিয়া একটি প্রধান স্বাস্থ্য-সমস্যা এবং *ভিব্রিও কলেরী* ও এন্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক বিভিন্ন ধরনের জীবাণু বাংলাদেশের শিশু ও প্রাপ্ত-বয়স্কদের মধ্যে ডায়ারিয়ার প্রাদুর্ভাবের একটি প্রধান কারন । অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া বিশেষ করে *ভিব্রিও কলেরী* এবং ইটেক জীবাণু দ্বারা সংঘটিত ডায়ারিয়া টীকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আপনার এলাকায় আমরা একটি গবেষণা চালাচ্ছি যার মাধ্যমে আমরা দেখব যে সুস্থ শিশুদের প্রতিরোধ ক্ষমতা মুখে খাবার নিষ্ক্রিয় ইটেক জীবাণু হতে প্রস্তুত টীকার বিরুদ্ধে কেমন । বাংলাদেশ সহ পৃথিবীর বিভিন্ন দেশে শিশু ও প্রাপ্ত-বয়স্কদের উপর এই টীকাটি পরীক্ষা করে দেখা হয়েছে ।

সুইডেন ও মিসরে গবেষণায় দেখা গেছে যে ইটেক টীকা নিরাপদ এবং টীকা খাওয়ানোর ৩-দিনের মধ্যে মাত্র ২-৬% টীকা গ্রহনকারীদের বমি ভাব, অল্প পেট মোচড়ানো কিংবা দুই একবার পাতলা পায়খানা জাতীয় পার্শ্বপ্রতিক্রিয়া হয়েছে । অতি সম্প্রতি বাংলাদেশে এই একই খাবার টীকা ব্যবহার করে একটি গবেষণা পরিচালনা করা হয় যাতে অধিক হারে বমির লক্ষণ পাওয়া যায় । এজন্য এরপর আমরা ঐ টীকার এক-চতুর্থাংশ মাত্রা একই বয়সী শিশুদের খাওয়াই যা প্রায় ১৭০ জন শিশুতেই নিরাপদ বলে প্রমানিত হয় । এবার আমরা এই টীকার দ্রাবক পরিবর্তন করবো যাতে টীকাটি মিশ্রিত করা হয় এবং এর সর্বোত্তম প্রস্তুত প্রক্রিয়া নির্ণয় করব ।

আমরা আপনার শিশুকে এই গবেষণায় অংশগ্রহণের জন্য আপনার সম্মতি চাচ্ছি। এতে গবেষণায় অংশগ্রহণ করলে আমরা আপনার শিশুকে ১৪ দিনের ব্যবধানে ২ বার বিভিন্ন দ্রাবকে প্রস্তুত এক চতুর্থাংশ মাত্রার এই টীকা খাওয়াবো । এই টীকার রোগ প্রতিষেধক ক্ষমতা দেখার জন্য আমরা আপনার শিশুর হাতের শিরা হতে তিনবার ১.৫ মি.লি (১/৪ চা চামচ পরিমান) রক্ত ও পায়খানার (৫ গ্রাম) নমুনা সংগ্রহ করবো । এই রক্ত ও পায়খানার নমুনা টীকা খাওয়ানোর পূর্বে এবং গবেষণায় অংশগ্রহণের ৭ম ও ২১তম দিনে নেয়া হবে । রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে শিশুর আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো ।

যদিও এই গবেষণায় অংশগ্রহণের জন্য আপনার সরাসরি উপকার হবে না তথাপি দেশ, জাতি ও মানবতার বৃহত্তর স্বার্থে এর গবেষণালব্ধ ফল এই টীকা সম্পর্কে আমাদের জ্ঞানের পরিধি বাড়াতে সাহায্য করবে) আপনারা চাইলে আমরা এই গবেষণা ও টীকা সম্পর্কিত যে কোন প্রশ্নের উত্তর দিতে প্রস্তুত এবং এমনকি সম্ভব হলে গবেষণালব্ধ ফলাফল জানাতে পারবো ।
গবেষণা চলাকালীন সময় টীকা খাওয়ানোর পর আপনার শিশুর কোন রকম অসুবিধা দেখা দিলে আমরা সর্ব্বোচ্চ সতর্কতা অবলম্বন করবো এবং এর জন্য কোন চিকিৎসার প্রয়োজন হলে তা বিনামূল্যে প্রদান করবো । এছাড়া গবেষণা চলাকালীন সময়ে আপনার দৈনন্দিন কাজের কোন ক্ষতি হলে ক্ষতিপূরনের ব্যবস্থা এবং যাতায়াতের ভাড়া প্রদান করবো ।

একমাত্র আপনিই এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন। আপনি এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলের টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

পিতা/মাতা/অভিভাবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Principal Investigator: Last, first, middle _Qadri Firdausi

Vaccine: children 6-17 month. Combined vaccine

Appendix 7d

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh

CONSENT FORM (CHILDREN 6-17 months of age)

IMMUNIZATION AND COLLECTION OF BLOOD AND STOOL

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Diarrhoeal diseases are major health problem, and germs called *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including *Vibrio cholerae* and ETEC diarrhoea by using effective vaccines.

We are conducting a study in your community to observe how healthy children aged 6 months to 17 months respond to a oral killed cholera vaccine and an inactivated ETEC vaccine administered when administered separately and together. The vaccines to be used in this study have been tested in adults and children, and have been found to be safe.

Studies carried out in Sweden and Egypt have found the ETEC vaccine to be safe, and adverse events were noted in 2-6% of the recipients, which include nausea, mild abdominal cramps, and 1-2 loose stools per day during the 3-day observation period. We have recently completed a study on ETEC vaccine in children 6 months to 36 months of age in Bangladesh, and found vomiting to be a problem. We thought that use of a higher dose was perhaps responsible for vomiting, and thus in a subsequent study we administered this vaccine in $1/4^{th}$ dose and found that to be safe in this age group of children. An oral cholera vaccine has been tested in various countries of the world including Bangladesh and found safe. We would like to test a combined vaccine containing the $1/4^{th}$ dose of the ETEC vaccine and one dose of the cholera vaccine in children aged 6 months to 17 months.

We request your permission to enroll your child in our study. For the purpose of this study, we would ask you child to drink this vaccine twice, fourteen days apart. To determine the response to the vaccine, 1.5 ml (about ¼ th teaspoonful) of blood from a vein of your child's forearm and collect stool samples (5 grams) at the beginning of the study, and 7 and 21 days later. Other than momentary pain due to needle stick, a very small chance of bruising at the site of insertion of the needles, and rare chance of infection drawing of this amount of blood will not cause any other harm to your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.

Your child may not directly benefit from participating in this study; however, results of this study will improve our knowledge about these vaccines and may thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available.

We carefully monitor occurrence of any untoward effects of the vaccines, and provide best possible treatment at no cost to your child if any such events occur to your child. We will support your travel cost to attend this field office, and will also compensate for your wage loss, if any.

You are free to accept or reject our proposal to enroll your child in this study, and you would also be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment in this study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____ _____

Signature/LTI of the guardian Signature of the investigator

Date: _____ Date: _____

_____

Signature of witness

Date: _____

পরিশিষ্ট - ৭ ডি

ডায়ারিয়া টীকা : শিশু (৬ মাস থেকে ১৭মাস)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

ডায়ারিয়া একটি প্রধান স্বাস্থ্য-সমস্যা এবং *ভিব্রিও কলেরী* ও এন্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক বিভিন্ন ধরনের জীবাণু বাংলাদেশের শিশু ও প্রাপ্ত-বয়স্কদের মধ্যে ডায়ারিয়ার প্রাদুর্ভাবের একটি প্রধান কারন । অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া বিশেষ করে *ভিব্রিও কলেরী* এবং ইটেক জীবাণু দ্বারা সংঘটিত ডায়ারিয়া টীকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আমরা এই এলাকায় সুস্থ শিশুদের (৬ মাস থেকে ১৭ মাস বয়স্ক) মুখে খাবার কলেরা (মৃত কলেরা জীবানু থেকে তৈরী) এবং ইটেক টীকা (নিষ্ক্রিয় ইটেক জীবানু থেকে তৈরী) আলাদা বা একসাথে প্রয়োগ করে এদের প্রভাব নিয়ে গবেষণা করব । এই গবেষনায় ব্যবহৃত টীকাগুলো শিশু ও প্রাপ্ত-বয়স্কদের পরীক্ষা করে দেখা হয়েছে এবং এইসব টীকা নিরাপদ বলে প্রতীয়মান হয়েছে।

সুইডেন ও মিসরে গবেষণায় দেখা গেছে যে ইটেক টীকা নিরাপদ এবং টীকা খাওয়ানোর ৩-দিনের মধ্যে মাত্র ২-৬% টীকা গ্রহনকারীদের বমি ভাব, অল্প পেট মোচড়ানো কিংবা দুই একবার পাতলা পায়খানা জাতীয় পার্শ্বপ্রতিক্রিয়া হয়েছে । অতি সম্প্রতি বাংলাদেশে ৬ মাস থেকে ৩৬মাস বয়স্ক শিশুদের এই একই খাবার ইটেক টীকা ব্যবহার করে একটি গবেষণা পরিচালনা করা হয় যাতে অধিক হারে বমির লক্ষণ পাওয়া যায় । আমরা ধারনা করছি যে টীকার অতিরিক্ত মাত্রার জন্য এই বমি হয়েছে এবং পরবর্তি গবেষনায় দেখা গেছে যে ইটেক টীকার এক-চতুর্থাংশ মাত্রা একই বয়সী শিশুদের জন্য নিরাপদ । বাংলাদেশ সহ পৃথিবীর বিভিন্ন দেশের পূর্ববর্তী গবেষণা থেকে জানা গেছে যে, মুখে খাওয়ানোর কলেরা টীকা নিরাপদ । আমরা ৬ মাস থেকে ১৭ মাস বয়স্ক শিশুদের উপর মুখে খাওয়ানোর ইটেক টীকার এক-চতুর্থাংশ মাত্রা এবং এক মাত্রার কলেরা টীকা যৌথ ভাবে পরীক্ষা করে দেখব ।

আমরা আপনার শিশুকে এই গবেষণায় অংশগ্রহণের জন্য আপনার সম্মতি চাচ্ছি। এতে গবেষণায় অংশগ্রহণ করলে আমরা আপনার শিশুকে ১৪ দিনের ব্যবধানে ২ বার এক চতুর্থাংশ মাত্রার এই টীকা খাওয়াবো । এই টীকার রোগ প্রতিষেধক ক্ষমতা দেখার আমরা আপনার শিশুর হাতের শিরা হতে তিনবার ১.৫ মি.লি (১/৪ চামচ পরিমান) রক্ত ও পায়খানার (৫ গ্রাম) নমুনা সংগ্রহ করবো । এই রক্ত ও পায়খানার নমুনা টীকা খাওয়ানোর পূর্বে এবং গবেষণায় অংশগ্রহণের ৭ম ও ২১তম দিনে নেয়া হবে । রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে শিশুর আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো ।

দিও এই গবেষণায় অংশগ্রহণের জন্য আপনার সরাসরি উপকার হবে না তথাপি দেশ, জাতি ও মানবতার বৃহত্তর স্বার্থে এর গবেষণালব্ধ ফল এই টীকা সম্পর্কে আমাদের জ্ঞানের পরিধি বাড়াতে সাহায্য করবে । আপনারা চাইলে আমরা এই গবেষণা ও টীকা সম্পর্কিত যে কোন প্রশ্নের উত্তর দিতে প্রস্তুত এবং এমনকি সম্ভব হলে গবেষণালব্ধ ফলাফল জানাতে পারবো ।
গবেষণা চলাকালীন সময় টীকা খাওয়ানোর পর আপনার শিশুর কোন রকম অসুবিধা দেখা দিলে আমরা সর্বোচ্চ সতর্কতা অবলম্বন করবো এবং এর জন্য কোন চিকিৎসার প্রয়োজন হলে তা বিনামূল্যে প্রদান করবো । এছাড়া গবেষণা চলাকালীন সময়ে আপনার দৈনন্দিন কাজের কোন ক্ষতি হলে ক্ষতিপূরনের ব্যবস্থা এবং যাতায়াতের ভাড়া প্রদান করবো ।

একমাত্র আপনিই এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন। আপনি এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলের টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

পিতা/মাতা/অভিভাবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Principal Investigator: Last, first, middle _Qadri Firdausi

Novel antigen: adults 18-45 years of age

Appendix 8a

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh
CONSENT FORM (ADULTs)
COLLECTION OF BLOOD AND STOOL SAMPLES

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

You are suffering from a diarrhoeal disease very much like cholera but is caused by a germ named enterotoxigenic *Escherichia coli* (ETEC). ETEC diarrhoea is an important health problem in Bangladesh. We are conducting a study at this hospital to observe natural response of the human body to ETEC diarrhoea. We request your participation in this study.
Other than a closer observation and the usual good care and treatment provided by this hospital, you will not receive any other benefit through participation in this study. If you agree to enroll in our study the following will be done:

We will keep you admitted until completion of the study procedures or longer if required for your treatment. We will ask you questions related to illness, perform your thorough physical examination at enrollment to the study and at least once daily, and record and use the information for this study.
To determine your response to this infection, for special test of this study we will draw 5.0 ml (about one teaspoon) of blood from a vein of your forearm at the beginning of the study, and 5 and 19 days later, and will also collect stool samples (5 grams) three times- at the same study days. Other than momentary pain and a very small chance of bruising at the site of insertion of the needles, drawing of such amount of blood will not cause any harm to you. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.
There will be no direct benefit to you as a result of participation in this study, however, results of this study will improve our knowledge about these infections and thus benefit the society.
All information obtained from you will be stored in a secure place, and none other than the investigators of this study and the Ethical Review Committee of this Centre would have access to such information. Your name and identity will not be used at the time of analysis of data or in publishing the results of this study. We would be happy to answer your questions related to illness and our study, and to provide you with the results of your laboratory tests as and when they become available; however, we would like to inform you that results of some of the tests would become available only at the end of this study. You are the only one to decide for or against participation of you in our study, and you would also be able to withdraw your consent at any time during the study. You would receive the usual good treatment of this hospital if you do not enroll yourself in this study, and also if you withdraw your consent during the study.

You are free to accept or reject our proposal to enroll in this study; even after enrollment you will be able to withdraw yourself from the study at any time. If you agree to our proposal of enrollment in this study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____ Signature/LTI of the subject

Date: _____

_____ Signature of witness

Date: _____

_____ Signature of the investigator

Date: _____

পরিশিষ্ট ৮ এ

নভেল অ্যান্টিজেন : প্রাপ্ত-বয়স্ক

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

আপনি ডায়ারিয়া রোগে ভুগছেন যা *ভিব্রিও কলেরী* জীবাণু দ্বারা সংঘটিত কলেরা হতে পারে অথবা কলেরার ন্যায় এন্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক জীবাণু দ্বারা সংঘটিত ইটেক ডায়ারিয়া হতে পারে । ইটেক ডায়ারিয়া বাংলাদেশের একটি গুরুত্বপূর্ন স্বাস্থ্য সমস্যা । এ রোগজীবাণুর সংক্রমনে আমাদের শরীরে স্বাভাবিক কি ধরনের প্রতিক্রিয়া হয় তা দেখার জন্য আমরা এ হাসপাতালে একটা গবেষনা পরিচালনা করবো । আমরা এ গবেষণায় আপনার অংশগ্রহনের মাধ্যমে আমাদের প্রচেষ্টায় সহযোগিতা করার জন্যে আপনাকে অনুরোধ করছি। গবেষণায় অংশ গ্রহনের কারনে এ হাসপাতালের প্রচলিত সুচিকিৎসা ও নিবিড় পর্যবেক্ষণ ছাড়া আপনার বাড়তি কোন লাভের সম্ভাবনা নেই।

এ গবেষণায় আপনি অংশগ্রহন করলেঃ

আমরা গবেষণার বিভিন্ন প্রক্রিয়া সম্পূর্ন না হওয়া পর্যন্ত এবং চিকিৎসার প্রয়োজনে আরোও বেশী সময়ের জন্যে আপনাকে হাসপাতালে ভর্ত্তি রাখবো। আমরা আপনার অসুখের ব্যাপারে আপনাকে কিছু প্রশ্ন করবো, ভর্ত্তির সময় ও দিনে অন্ততঃ একবার তার সম্পূর্ন শারিরীক পরীক্ষা করবো এবং প্রাপ্ত তথ্যাদি গবেষণায় প্রয়োজনে ব্যবহার করবো।

গবেষণার বিশেষ পরীক্ষার জন্যে ভর্ত্তির দিন এবং তার ৫ ও ১৯ দিন পর মোট তিন দিন, আমরা আপনার বাহুর শিরা হতে ৫.০ মিঃলিঃ (১ চা-চামচ) রক্ত এবং মলের সামান্য নমুনা (প্রায় ৫.০ গ্রাম) সংগ্রহ করবো। রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে আপনার আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো।

এ গবেষণায় অংশগ্রহনের ফলে আপনার সরাসরি উপকার হয়তো হবে না , কিন্তু গবেষণায় প্রাপ্ত ফলাফল সংক্রামক রোগ সম্পর্কে সাহায্য করবে এবং তা সমাজের উপকারে আসবে ।
আপনার সমস্ত তথ্যাদি গোপনে সংরক্ষন করা হবে, এবং তা এ গবেষণার গবেষক ও এ কেন্দ্রের 'নৈতিকতা পর্যালোচনা কমিটি' ছাড়া আর কেউই জানতে পারবেনা। আপনি চাইলে আমরা আপনার অসুখের ব্যাপারে ও তার বিভিন্ন পরীক্ষার ফলাফল, আমাদের জানা সাপেক্ষে, আপনাকে জানাবো; তবে আমরা আপনাকে জানাতে চাই যে গবেষণার কিছু কিছু বিশেষ পরীক্ষার ফলাফল আমরা শুধুমাত্র গবেষণার শেষ পর্যায়েই জানতে পারবো। একমাত্র আপনিই এ গবেষণায় আপনার অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন। এ গবেষণায় অংশগ্রহন না করলে এমনকি অংশগ্রহনের পর সম্মতি প্রত্যাহার করলেও আপনি এ হাসপাতালের প্রচলিত সুচিকিৎসা পাবেন।

আপনি এ গবেষণায় আপনার অন্তর্ভূক্তির আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

স্বেচ্ছাসেবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Principal Investigator: Last, first, middle _Qadri Firdausi

Novel antigen : children 6- 17 years

Appendix 8b

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh
CONSENT FORM (CHILDREN, 6-17 years of age)
COLLECTION OF BLOOD AND STOOL SAMPLES

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Your child is suffering from a diarrhoeal very much like cholera but is caused by a different germ named enterotoxigenic *Escherichia coli* (ETEC). ETEC diarrhoea is an important health problem of children in Bangladesh. We are conducting a study at this hospital to observe the natural response of human body to ETEC diarrhoea, we request you to allow your child to take part in this study and also for your child to agree to participate.

Other than the more closer observation and the usual good care and treatment provided by this hospital, your child would not receive any other benefit through participation in this study. If you allow us to enroll your child in our study the followings will be done:

We will keep her/him admitted until completion of the study procedures at the initial stage of the disease or longer if required for her/his treatment. We will ask you questions related to illness of your child, perform her/his thorough physical examination at enrollment to the study and record and use the information for this study.

To determine your child's response to this infection, as to carry out the special test of this study we will draw 3.0 ml (about half of a teaspoonful) from a vein of your child's forearm at the beginning of the study, and 5 and 19 days later, and will also collect stool samples (about 5 grams) on these days. Other than momentary pain and a very small chance of bruising at the site of insertion of the needles, drawing of such amount of blood will not cause any harm to you or your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.

There will be no direct benefit to your child as a result of participation in this study, however, results of this study will improve our knowledge about these infections and thus benefit the society.

All information obtained from your child will be stored in a secure place, and none other than the investigators of this study and the Ethical Review Committee of this Centre would have access to such information. The name and identity of your child will not be used at the time of analysis of data or in publishing the results of this study. We would be happy to answer your or your child's questions related to illness and our study, and to provide you with the results of her/his laboratory tests as and when they become available; however, we would like to inform you that results of some of the tests would become available only at the end of this study. You and your child are the only ones to decide for or against participation in our study, and would also be able to withdraw the consent at any time during the study. Your child would receive the usual good treatment of this hospital if you do not enroll your child in this study, and also if the consent is withdrawn during the study.

You and your child are free to accept or reject our proposal to enroll into this study; even after enrollment will be able to withdraw from the study at any time. If you and your child agree to our proposal of enrollment in this study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of witness

Date: _____

_____
Signature of the investigator

Date: _____

পরিশিষ্ট ৮ বি

নভেল অ্যান্টিজেন : ৬-১৭ বৎসর বয়স্ক শিশু

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

আপনার শিশু ডায়ারিয়া রোগে ভুগছে যা *ভিব্রিও কলেরী* জীবাণু দ্বারা সংঘটিত কলেরা হতে পারে অথবা কলেরার ন্যায় এস্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক জীবাণু দ্বারা সংঘটিত ইটেক ডায়ারিয়া হতে পারে । ইটেক ডায়ারিয়া বাংলাদেশের একটি গুরুত্বপূর্ন স্বাস্থ্য সমস্যা । এ রোগজীবাণুর সংক্রমনে আমাদের শরীরে স্বাভাবিক কি ধরনের প্রতিক্রিয়া হয় তা দেখার জন্য আমরা এ হাসপাতালে একটা গবেষনা পরিচালনা করবো । আমরা এ গবেষণায় আপনার শিশুর অংশগ্রহনের মাধ্যমে আমাদের প্রচেষ্টায় সহযোগিতা করার জন্যে আপনাকে অনুরোধ করছি। গবেষণায় অংশ গ্রহনের কারনে এ হাসপাতালের প্রচলিত সুচিকিৎসা ও নিবিড় পর্যবেক্ষণ ছাড়া আপনার শিশুর বাড়তি কোন লাভের সম্ভাবনা নেই।

এ গবেষণায় আপানর শিশু অংশগ্রহন করলেঃ

আমরা গবেষণার বিভিন্ন প্রক্রিয়া সম্পূর্ন না হওয়া পর্যন্ত এবং তার চিকিৎসার প্রয়োজনে আরোও বেশী সময়ের জন্যে আপনার শিশুকে হাসপাতালে ভর্ত্তি রাখবো। আমরা শিশুর অসুখের ব্যাপারে আপনাকে কিছু প্রশ্ন করবো, ভর্ত্তির সময় ও দিনে অন্ততঃ একবার তার সম্পূর্ন শারীরীক পরীক্ষা করবো এবং প্রাপ্ত তথ্যাদি গবেষণায় প্রয়োজনে ব্যবহার করবো।

গবেষণার বিশেষ পরীক্ষার জন্যে ভর্ত্তির দিন এবং তার ৫ ও ১৯ দিন পর মোট তিন দিন, আমরা আপনার শিশুর বাহুর শিরা হতে ৩.০ মিঃলিঃ (১/২ চা-চামচ) রক্ত এবং মলের সামান্য নমুনা (প্রায় ৫.০ গ্রাম) সংগ্রহ করবো। রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে শিশুর আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো।

এ গবেষণায় অংশগ্রহনের ফলে আপনার শিশুর সরাসরি উপকার হয়তো হবে না , কিন্তু গবেষণায় প্রাপ্ত ফলাফল সংক্রামক রোগ সম্পর্কে সাহায্য করবে এবং তা সমাজের উপকারে আসবে ।

আপনার শিশুর সমস্ত তথ্যাদি গোপনে সংরক্ষন করা হবে, এবং তা এ গবেষণার গবেষক ও এ কেন্দ্রের 'নৈতিকতা পর্যালোচনা কমিটি' ছাড়া আর কেউই জানতে পারবেনা। আপনি চাইলে আমরা আপনার শিশুর অসুখের ব্যাপারে ও তার বিভিন্ন পরীক্ষার ফলাফল, আমাদের জানা সাপেক্ষে, আপনাকে জানাবো; তবে আমরা আপনাকে জানাতে চাই যে গবেষণার কিছু কিছু বিশেষ পরীক্ষার ফলাফল আমরা শুধুমাত্র গবেষণার শেষ পর্যায়েই জানতে পারবো। আপনি এবং আপনার শিশু এ গবেষণায় অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি বা আপনার শিশু সম্মতি প্রত্যাহার করতে পারবেন। এ গবেষণায় অংশগ্রহন না করলে এমনকি অংশগ্রহনের পর সম্মতি প্রত্যাহার করলেও আপনার শিশু এ হাসপাতালের প্রচলিত সুচিকিৎসা পাবেন।

আপনি এ গবেষণায় আপনার অন্তর্ভূক্তির আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

স্বেচ্ছাসেবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Principal Investigator: Last, first, middle _Qadri Firdausi

Novel antigen: children 6 months- 5 years

Appendix 8c

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh

CONSENT FORM (CHILDREN)

COLLECTION OF BLOOD AND STOOL SAMPLES

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Your child is suffering from a diarrhoeal very much like cholera but is caused by a different germ named enterotoxigenic *Escherichia coli* (ETEC). ETEC diarrhoea is an important health problem of children in Bangladesh. We are conducting a study at this hospital to observe natural response of human body to ETEC diarrhoea, we request you to allow your child to participate in this study.

Other than the more closer observation and the usual good care and treatment provided by this hospital, your child would not receive any other benefit through participation in this study. If you allow us to enroll your child in our study the followings will be done:

We will keep her/him admitted until completion of the study procedures at the initial stage of the disease or longer if required for her/his treatment. We will ask you questions related to illness of your child, perform her/his thorough physical examination at enrollment to the study and record and use the information for this study.

To determine your child's response to this infection, as to carry out the special test of this study we will draw 3.0 ml (about half of a teaspoonful, for those aged 18 months to 5 years) or 1.5 ml of blood (about one fourth of a teaspoonful, those 6-17 months of age) from a vein of your child's forearm at the beginning of the study, and 5 and 19 days later, and will also collect stool samples (about 5 grams) on these days. Other than momentary pain and a very small chance of bruising at the site of insertion of the needles, drawing of such amount of blood will not cause any harm to you or your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood.

There will be no direct benefit to your child as a result of participation in this study, however, results of this study will improve our knowledge about these infections and thus benefit the society.

All information obtained from your child will be stored in a secure place, and none other than the investigators of this study and the Ethical Review Committee of this Centre would have access to such information. The name and identity of your child will not be used at the time of analysis of data or in publishing the results of this study. We would be happy to answer your questions related to illness of your child and our study, and to provide you with the results of her/his laboratory tests as and when they become available; however, we would like to inform you that results of some of the tests would become available only at the end of this study. You are the only one to decide for or against participation of your child in our study, and you would also be able to withdraw your consent at any time during the study. Your child would receive the usual good treatment of this hospital if you do not enroll your child in this study, and also if you withdraw your consent during the study.

You are free to accept or reject our proposal to enroll your child in this study; even after enrollment you will be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment in this study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____ Signature/LTI of the guardian

Date: _____

_____ Signature of witness

_____ Signature of the investigator

Date: _____

পরিশিষ্ট ৮ সি

নভেল অ্যান্টিজেন : ৬ মাস-৫ বৎসর বয়স্ক শিশু

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

আপনার শিশু ডায়ারিয়া রোগে ভুগছে যা *ভিব্রিও কলেরী* জীবাণু দ্বারা সংঘটিত কলেরা হতে পারে অথবা কলেরার ন্যায় এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক জীবাণু দ্বারা সংঘটিত ইটেক ডায়ারিয়া হতে পারে । ইটেক ডায়ারিয়া বাংলাদেশের একটি গুরুত্বপূর্ন স্বাস্থ্য সমস্যা । এ রোগজীবাণুর সংক্রমনে আমাদের শরীরে স্বাভাবিক কি ধরনের প্রতিক্রিয়া হয় তা দেখার জন্য আমরা এ হাসপাতালে একটা গবেষনা পরিচালনা করবো । আমরা এ গবেষণায় আপনার শিশুর অংশগ্রহনের মাধ্যমে আমাদের প্রচেষ্টায় সহযোগিতা করার জন্যে আপনাকে অনুরোধ করছি। গবেষণায় অংশ গ্রহনের কারনে এ হাসপাতালের প্রচলিত সুচিকিৎসা ও নিবিড় পর্যবেক্ষণ ছাড়া আপনার শিশুর বাড়তি কোন লাভের সম্ভাবনা নেই।

এ গবেষণায় আপানর শিশু অংশগ্রহন করলেঃ

আমরা গবেষণার বিভিন্ন প্রক্রিয়া সম্পূর্ন না হওয়া পর্যন্ত এবং তার চিকিৎসার প্রয়োজনে আরোও বেশী সময়ের জন্যে আপনার শিশুকে হাসপাতালে ভর্ত্তি রাখবো। আমরা শিশুর অসুখের ব্যাপারে আপনাকে কিছু প্রশ্ন করবো, ভর্ত্তির সময় ও দিনে অন্ততঃ একবার তার সম্পূর্ন শারিরীক পরীক্ষা করবো এবং প্রাপ্ত তথ্যাদি গবেষণায় প্রয়োজনে ব্যবহার করবো।

গবেষণার বিশেষ পরীক্ষার জন্যে ভর্ত্তির দিন এবং তার ৫ ও ১৯ দিন পর মোট তিন দিন, আমরা আপনার শিশুর বাহুর শিরা হতে ৩.০ মিঃলিঃ (১/২ চা-চামচ , ১৮ মাস-৫ বৎসর বয়স্কদের) অথবা ১.৫ মিঃলিঃ (১/৪ চা-চামচ , ৬ মাস-১৭ মাস বয়স্কদের) রক্ত এবং মলের সামান্য নমুনা (প্রায় ৫.০ গ্রাম) সংগ্রহ করবো। রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে শিশুর আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জন্যে আমরা শুধুমাত্র একবার-ব্যবহার উপযোগী, জীবাণু-মুক্ত সিরিন্জ ও সূঁচ ব্যবহার করবো ও অন্যান্য সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো।

এ গবেষণায় অংশগ্রহনের ফলে আপনার শিশুর সরাসরি উপকার হয়তো হবে না , কিন্তু গবেষণায় প্রাপ্ত ফলাফল সংক্রামক রোগ সম্পর্কে সাহায্য করবে এবং তা সমাজের উপকারে আসবে ।

আপনার শিশুর সমস্ত তথ্যাদি গোপনে সংরক্ষন করা হবে, এবং তা এ গবেষণার গবেষক ও এ কেন্দ্রের 'নীতি পর্যালোচনা কমিটি' ছাড়া আর কেউই জানতে পারবেনা। আপনি চাইলে আমরা আপনার শিশুর অসুখের ব্যাপারে ও তার বিভিন্ন পরীক্ষার ফলাফল, আমাদের জানা সাপেক্ষে, আপনাকে জানাবো; তবে আমরা আপনাকে জানাতে চাই যে গবেষণার কিছু কিছু বিশেষ পরীক্ষার ফলাফল আমরা শুধুমাত্র গবেষণার শেষ পর্যায়েই জানতে পারবো। আপনি আপনার শিশুর এ গবেষণায় অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার শিশু সম্মতি প্রত্যাহার করতে পারবেন। এ গবেষণায় অংশগ্রহন না করলে এমনকি অংশগ্রহনের পর সম্মতি প্রত্যাহার করলেও আপনার শিশু এ হাসপাতালের প্রচলিত সুচিকিৎসা পাবেন।

আপনি এ গবেষণায় আপনার অন্তর্ভূক্তির আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা টিপসই দিন।

আপনার সহযোগিতার জন্যে ধনাবাদ।

স্বেচ্ছাসেবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Birth cohort study: children

Appendix 9

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh:
CONSENT FORM (CHILDREN)
COLLECTION OF BLOOD AND STOOL SAMPLES

Camp ID # / Subject ID # : |___|___| / |___|___|___|___| **Subject Name:** |_____|

Diarrhoeal diseases are major health problem in Bangladesh like any other developing country and your child is at a risk of suffering from a diarrhoeal disease which is like cholera but is caused by a different germ named enterotoxigenic *Escherichia coli* (ETEC). Cholera and ETEC diarrhoea are both important health problems of children as well as adults in Bangladesh. We are conducting a study at this community to observe natural response of human body to ETEC diarrhoea. We request that you allow your newborn child to participate in this study.

To determine your child's response to this infection, we will follow up your child for 2 years. During this period we will collect cord blood from the child immediately after birth, we will also draw 0.5 ml (7-8 drops) of blood for six times by finger prick at intervals of every 4 (four) months. We would also like to collect stool samples (5 grams) at the beginning of the study that is within the first 14 days and every month after that for 2 years and also during the episodes of each diarrhoea. Other than momentary pain, drawing of such amount of blood will not cause any harm to your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile lancet for drawing blood.

All information obtained from you or your child will be stored in a secure place, and none other than the investigators of this study and the Ethical Review Committee of this Centre will have access to such information. The name and identity of your child will not be used at the time of analysis of data or in publishing the results of this study. We would be happy to answer your questions related to the study, and to provide you with the results of laboratory tests as and when they become available; however, we would like to inform you that results of some of the tests would become available only at the end of this study. You are the only one to decide for or against participation of your child in our study, and you would also be able to withdraw your consent at any time during the study. There will be no direct benefit to your child as a result of participation in this study, however, results of this study will improve our knowledge about these infections and thus benefit the society.
You are free to accept or reject our proposal to enroll your child in this study; even after enrollment you will be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment in this study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of witness

Date: _____

_____
Signature of the investigator

Date: _____

পরিশিষ্ট -৯

কোহোর্ট স্টাডি : শিশু

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (আই,সি,ডি,ডি,আর,বি)

গবেষণা প্রকল্পের নামঃ বাংলাদেশে পাতলা পায়খানার (ডায়ারিয়ার) বিরুদ্ধে ডায়ারিয়া টীকার কার্যকারিতার যথপোযুক্ততা

অন্যান্য উন্নয়নশীল দেশের মত ডায়ারিয়া রোগ সমূহ একটি প্রধান স্বাস্থ্য-সমস্যা । আপনার শিশু কলেরার ন্যায় যা এন্ট্যারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ইটেক) নামক জীবাণু দ্বারা আক্রান্ত হতে পারে । কলেরা এবং ইটেক ডায়ারিয়া বাংলাদেশের শিশু ও প্রাপ্ত-বয়স্কদের মধ্যে ডায়ারিয়ার প্রাদুর্ভাবের একটি প্রধান কারন। আমরা এই এলাকার সুস্থ শিশুদের উপরে একটা গবেষনা পরিচালিত করবো যার মাধ্যমে ইটেক ডায়ারিয়ারয় আমাদের শরীরে স্বাভাবিক কি ধরনের প্রতিক্রিয়া হয় তা দেখব । আমরা এই গবেষণায় তার অংশগ্রহনের মাধ্যমে আমাদের প্রচেষ্টায় সহযোগিতা করার জনো আপনাকে অনুরোধ করছি।

এই রোগজীবাণুর সংক্রমনে শরীরের স্বাভাবিক প্রতিক্রিয়া দেখার জন্য আমরা আপনার শিশুকে ২ বৎসর নিবিড় পর্যবেক্ষণ করবো এবং গবেষণার বিশেষ পরীক্ষার জনো জন্মের পরপরই নাভিমূলের শিরা থেকে একবার এবং প্রতি চার মাস অন্তর মোট ছয়বার আংগুলের ডগা হতে ০.৫ মিঃলিঃ (৭/৮ ফোঁটা) রক্তের নমূনা সংগ্রহ করবো। এছাড়াও আপনার শিশুর কাছ থেকে আমরা জন্মের ১৪ দিনের মধ্যে একবার, প্রতি মাস অন্তর মোট ২ বৎসর এবং প্রতি ডায়রিয়া চলাকালীন সময়ে সামান্য পরিমান (৫ গ্রাম) মলের নমূনা সংগ্রহ করবো । রক্ত সংগ্রহের সময় সূঁচের আঘাতজনিত তাৎক্ষনিক সামান্য ব্যথা ও তার আশেপাশের ত্বকের রংয়ের পরিবর্ত্তনের সামান্য সম্ভাবনা, এবং রোগজীবাণুর সংক্রমনের ক্ষীণ সম্ভাবনা ছাড়া এ পরিমানে রক্ত নেবার কারনে শিশুর আর কোন ক্ষতির সম্ভাবনা নেই। রক্ত সংগ্রহের জনো আমরা শুধুমাত্র একবার ব্যবহার উপযোগী জীবাণু -মুক্ত সূঁচ ব্যবহার করবো ও অন্যানা সমস্যা প্রতিরোধের ব্যাপারে যত্নবান থাকবো।

আপনার শিশুর সমস্ত তথ্যাদি গোপনে সংরক্ষন করা হবে, এবং তা এ গবেষণার গবেষক ও এ কেন্দ্রের 'নীতি পর্যালোচনা কমিটি' ছাড়া আর কেউই জানতে পারবেনা। আপনি চাইলে আমরা শিশুর অসুখের ব্যাপারে ও তার বিভিন্ন পরীক্ষার ফলাফল, আমাদের জানা সাপেক্ষে, আপনাকে জানাবো; তবে আমরা আপনাকে জানাতে চাই যে গবেষণার কিছু কিছু বিশেষ পরীক্ষার ফলাফল আমরা শুধুমাত্র গবেষণার শেষ পর্যায়েই জানতে পারবো। এ গবেষণায় অংশগ্রহনের ফলে আপনার শিশুর সরাসরি উপকার হয়তো হবে না , কিন্তু গবেষণায় প্রাপ্ত ফলাফল সংক্রামক রোগ সম্পর্কে সাহায্য করবে এবং তা সমাজের উপকারে আসবে ।, একমাত্র আপনিই এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির ব্যাপারে সিদ্ধান্ত দেবার অধিকারী, এবং গবেষণা চলাকালীন যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন।

আপনি এ গবেষণায় আপনার শিশুর অন্তর্ভূক্তির আমাদের প্রস্তাবে রাজী থাকলে অনুগ্রহ করে নীচের নির্দ্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা টিপসই দিন।

আপনার সহযোগিতার জন্যে ধন্যবাদ।

পিতা/মাতা/অভিভাবকের স্বাক্ষর/টিপসই তারিখঃ

গবেষকের স্বাক্ষর তারিখঃ

সাক্ষীর স্বাক্ষর তারিখঃ

Principal Investigator: Last, first, middle _Qadri Firdausi

Appendix 10

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH BANGLADESH, (ICDDR,B)

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh:

Study Subject Selection Form: For expression of novel antigen in patients

Study Subject ID # : |__|__| / |__|__|__|__| Subject Name: |_____|

Basic data: Day = 0 (Day of enrollment)

1. Date of enrollment [ ][ ] [ ][ ] [ ][ ] (D D M M Y Y)
2. Patient No. [ ][ ][ ][ ][ ][ ][ ]
3. Patient's ID [ ][ ][ ][ ][ ]
4. Gender [1=Male] [1=Female]
5. Responder in case [1= Subiect] [2= Mother] [3 =Father] [4= Other]
6. Date of birth (Bengali/Arabic calendar) [ ][ ] [ ][ ] [ ][ ] (D D M M Y Y)
7. Date (English calendar) [ ][ ] [ ][ ] [ ][ ] (D D M M Y Y)
8. Age in years [ ][ ]
9. Age in months [ ][ ][ ]
10. Height (cm) [ ][ ][ ][ ]
11. Weight (kg) [ ][ ] [ ]
12. W/H (% of NCHS median) [ ][ ] [ ]
13. MUAC (cm) [ ][ ] [ ]
14. Oedema [1= Present] [2= Absent]
15. Presence of illness [1=No] [2=Fever] [3= Diarrhoea] [4=Others]
16. Education of mother/ female adult subject [1=Illiterate] [2=Class I - IV] [3=Class V - VIII] [4=Class IX - X] [5=> Class X]
17. Profession of mother/ female adult subject [1=Housewife] [2=Industrv worker] [3=Dailv worker] [4=others]
18. Education of father/ male adult subject [1= Illiterate] [2= Class I - IV] [3= Class V - VIII] [4= Class IX - X] [5=> Class X]
19. Profession of father/ male adult subject [1=Rickshaw puller] [2=Industrv worker] [3=Dailv worker]

20. Family income (Taka) [1= 1000-2000] [2= 2001-3000] [3= 3001-4000] [4= > 40011]

21. Housing [1= Thatched] [2=Tin shed] [3=Building]

22. Latrines [1= Sanitary but public] [2= Sanitary, personal] [3= Open]

23. Water source [1= Tap water] [2=Tube well] [3=Pond] [4= Others]

24. Exclusive Breast Feeding child subject [1=Yes] [2=No] [9 = not applicable]

25. Duration (months) of exclusive breastfeeding (Code 99 if not applicable) [ ][ ]

26. Breast Feeding Practice among child subject [1=Yes] [2=No] [3= Occasional] [4 = Continuing after 9 months] [9 = not applicable]

27. Duration of breastfeeding (Code 99 if not applicable) [ ][ ]

28. Presence of illness [1 = No] [2 = Fever] [3 = Diarrhoea] [4 = Others]

Clinical examination: Day = [ ][ ]
General Examination:

29. Date [ ][ ] [ ][ ] [ ][ ]
D D M M Y Y

30 Appearance [1=Normal] [2=Irritable] [3=Lethargic]

31 Pulse (rate/min) [ ][ ][ ]

32. Respiration (rate/min) [ ][ ][ ]

33. Vomiting [1 = No] [2= Yes]

33. Dehydration: [1 = No] [2= some] [3= Sev]

34. Temperature (°C) [ ][ ] . [ ]

35. Pallor [1 = No] [2= Mild] [3= Mod] [4= Severe]

36. Skin Condition: [1 = Normal] [2= Rash] [3= Other]

Principal Investigator: Last, first, middle _Qadri Firdausi

Systemic Examination:

37. Abdominal distension: 1 = No | 2= Mod | 3= Severe

38. Abdominal tenderness: 1 = No | 2= Localized | 3= Generalized

39. Lungs (auscultation): 1 = Normal | 2= Bronchial breath | 3= Rhonchi | 4= Rales

40. Heart (auscultation): 1= Normal | 2= Abnormal:

41. Movement 1= Normal | 2 = Restricted:

Stool Examination:

40. Microbiology 1= No pathogen | 2= ETEC | 3 = Others

Blood Sample:

42. Collection 1= Yes | 2= No

Appendix 11a

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH BANGLADESH, (ICDDR,B)

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Screening form

Study Subject ID # |___|___|___|___| **Name of the study subject** |_____|

Basic data

1. **Date of interview** |___|___|___|
   DD MM YY

2. Camp colde /Family No. |__|__| / |__|__|__|__|

3. Screening No. |__|__| / |__|__|__|__|

4. Study subject's ID |__|__| / |__|__|__|__|

5. Respondent
   1= Mother 2=Father 3= Other

6. Sex
   1=Male 2=Female

7. Date of birth (Bengali/Arabic calendar) |___|___|___| DD MM YY

8. Date of birth (English calendar) |___|___|___| DD MM YY

9. Age in years |__|__|

10. Age in months |__|__|

[If day is unknown write 15. Month and year must be written. You can write according to Bengali/Arabic calender at the time of interview, and then translate into English later.]

11. Height (cm) |___|___|___|.|___|

11. Weight (kg) |__|__|.|__|

12. % of NCHS (W/A) |__|__|.|__|

13. % of NCHS (W/H) |__|__|.|__|

14. Oedema (Present) 1= Yes 2=No

15. Presence of illness 1=No 2=Fever 3= Diarrhoea 4=others

Previous immunization

16. DPT 1=No 2= 1 dose 3=2 dose 4= 3 dose

17. OPV 1=No 2= 1 dose 3=2 dose 4= 3 dose

18. Measles 1=No 2= Yes

19. BCG 1=No 2= Yes

Appendix 11 b

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH BANGLADESH, (ICDDR,B)

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Study subject vaccination and clinical evaluation form

Study child's name |_____| Name of the examiner |_____|

20. Day of vaccination |__|__|
21. Date |__|__|__| DD MM YY
22. Vaccine lot No. |_____|
23. Date of vaccination |__|__|__| DD MM YY
24. Type of vaccination |__| 1 = Primary 2 = Secondary
25 Presence of illness
1 = No 2 = Fever 3 = Diarrhoea 4 = Others

General Examination

26. Pulse (rate/min) |__|__|__|
27. Respiration (rate/min) |__|__|__|
28. Temperature (°C) 1 = Normal 2 = Raised
29. Pallor 1=No 2=mild 3=moderate 4=severe
30. Skin Condition: 1 = Normal 2 = Rash 3 = Others

Systemic Examination

31. Abdominal distension: 1=No 2=Moderate 3=Severe
32. Abdominal tenderness: 1=No 2=Yes if tender 3= localized 4= generalized
33. Lungs(auscultation): 1=Clear 2=Added sound: if added sound 3=Ronchi 4=Rales
34. Heart(auscultation): 1=Normal 2=Added sound
35. Appearance: 1=Normal 2=Irritable 3=Lethargic
36. Movement 1=Normal 2=Restricted
38. Stool test for ETEC 1 = Negative 2 = Positive
39. Blood Sample: . Collection 1 = Yes 2 = No if yes
40. Date of collection |__|__|__| DD MM YY

Appendix 11c

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Post vaccination adverse event follow up form Surveillance After Vaccination

63. **Primary vaccination**

64. Date |___|___|___|
DD MM YY

65 Day 1/ Day 2/ Day 3

66. Completeness of dosing 1 = Yes 2 = No

67 Amount ingested: 1=100% 2=≥ 90% 3=<90%

LOCAL REACTION (68 - 73)

68. Abdominal pain 1= No 2=Mild 3= moderate 4=severe 69. Distension 1=No 2= moderate 3= severe

70. Vomiting 1=No 2= Yes If yes, .71. Number/day |___| / day

72. Diarrhoea [(> 3) watery or loose stool] 1=No 2= Yes If yes, 73. Number/day |___| / day

SYSTEMIC REACTION (74 – 81)

74. Fever (Axillary temp>37.8°C) 1= No 2= Yes 75. If yes, temperature (°C) |___|___|.|___|

76. Loss of appetite/ Poor feeding 1= No 2= Yes 77. Irritability 1= No 2= Yes

78. Nausea 1= No 2= Yes 79. Dizziness 1= No 2= Yes

80. Exanthema 1= No 2= Yes 81. Other 1= No 2= Yes

82. Relation to vaccine: 1 = None 2 = Possible 3 = Probable 4 = Highly probable

83. Therapy required 1= No 2= Yes

84. Outcome 1 = resolved 2 = improved 3 = unchanged 4= worse

Appendix 11d

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh:
Severe adverse event form
Surveillance After Vaccination

Random # **Investigator** **Patients Initials** **Inpatient Number**

.....

Were there any adverse events associated with administration, whether or not they were considered vaccine-related?
If yes, please complete all sections below (circle appropriate number) 1=No & 2=Yes.

Adverse events, specify: Please list one event per line	**Serious Including Life-threat** 1=No 2=Yes	**Start Date** dd mm yy	**Stop Date** dd mm yy	**Severity** 1=Mild 2=Moderate 3=Severe	**Relation to Study Vaccine** 1=None 2=Remote 3=Possible 4=Probable	**Therapy Required** 1=No 2=Yes (specify below) 5=Highly Probable	**Outcome** 1=Resolved 2=Improved 3=Unchanged 4=worsened 5=Follow up not done	
	1 2			1 2 3	1 2 3 4 5	1 2	1 2 3 4 5	
	1 2			1 2 3	1 2 3 4 5	1 2	1 2 3 4 5	
	1 2			1 2 3	1 2 3 4 5	1 2	1 2 3 4 5	

Further details of adverse events and classification of above entries (specify dates and include information on concomitant medications and concurrent conditions)

Date..... Investigator's signature.....

Appendix 11d (contd)
Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Adverse Events

Report all adverse events which occurred from the beginning of the study up to the end

Serious/Life-threatening adverse events are the following :

- death,
- life-threatening events,
- hospitalization or prolongation of hospitalization,
- disability,
- congenital anomaly,
- occurrence of malignancy.

Adverse event severity definitions :

Mild : Adverse event usually transient in nature and generally not interfering with normal activities.
Moderate : Adverse event causing sufficient discomfort to interfere with normal activities.
Severe : Adverse event which is incapacitating and prevents normal activities.

In case of occurrence of adverse events, even if treatment is discontinued, the observation will be considered as complete only when all of the information mentioned above is collected.

Relation to vaccine - Definition :

None: No relation to administration of the study vaccine, i.e. existence of a clear alternative explanation, an unreasonable temporal relationship between the vaccine and the event, non-plausibility.

Remote: A clinical including a laboratory test abnormality, with a temporal relationship to vaccine administration which makes a causal relationship improbable, and in which other drugs, chemical or underlying disease could provide explanations.

Possible: A clinical including a laboratory test abnormality, with a reasonable time sequence in relation to administration of the study vaccine, but which could also be explained by concurrent disease or other drugs or chemicals.

Probable: A clinical event, including a test abnormality, with a reasonable time sequence in relation to administration of the study vaccine, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which shows a satisfactory clinical course following withdrawal.

Highly Probable: A report suggesting an adverse reaction which cannot be evaluated because information is insufficient or contradictory, and which can neither be supplemented nor verified. Evaluation of the relationship enabling classification between the above items should nevertheless be attempted, with the outcome of the event being taken into consideration.

Dummy forms (to be pre-tested prior to use in study)

Appendix 12a

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH BANGLADESH, (ICDDR,B)

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: ETEC infection in a cohort study in an urban setting in Bangladesh : Screening form

Name of pregnant mother |_____| **Study ID** |_____|

Basic data

1. **Date of interview** |___|___|___|
   DD MM YY

2. Camp code /. |___|___| / Family No. |___|___|___|___|

3. Screening No. |___|___| / |___|___|___|___|

4. Study subject's ID |___|___| / |___|___|___|___|

5. Respondent
   1= Mother 2=Father 3= Other

7. Sex
   1=Male 2=Female

7. Date of birth |___|___|___| 8. Date of birth |___|___|___|
   (Bengali/Arabic calendar) DD MM YY (English calendar) DD MM YY

9. Age in days |___|___| 10. Age in months |___|___|

[If day is unknown write 15. Month and year must be written. You can write according to Bengali/Arabic calender at the time of interview, and then translated into English later.]

11. Length (cm) |___|___|___|.|___| 11. Weight (kg) |___|___|.|___|

13. % of NCHS (W/A) |___|___|.|___| 13. % of NCHS (W/L) |___|___|.|___|

14. Presence of illness 1=No 2=Fever 3= Diarrhoea 4=others

Principal Investigator: Last, first, middle _Qadri Firdausi_____

Dummy forms (to be pre-tested prior to use)

Appendix 12b

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: ETEC infection in a cohort study in an urban setting in Bangladesh

Diarrhoeal stool surveillance form

Month of |_____| Year of |_____| Camp code | | |

Name of Child:_____ Child's ID#: | | | | |

Age in days |_____| months:_____ Date of enrollment: _____

Date	Stool Freq	Stool consi	No. of loose/liquid stool in last 24 hrs	Mucus in stool	Blood in stool	Stool collected	Vomiting	Fever (> 37^0 C)	Treatment
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Principal Investigator: Last, first, middle _Qadri Firdausi_____

Dummy forms (to be pre-tested prior to use)

Appendix 12c

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh:
ETEC infection in a cohort study in an urban setting in Bangladesh
Clinical evaluation form: every three month and during episodes of diarrhoea

Study subject's Mother'sName:_____ Father's Name : _____

Study subject's name----------(to be entered at later period if not available)

VARIABLES

1) Child's ID Number ---------- Camp code [ ][ ] [ ][ ][ ][ ]

2) No. of Diarrhoea ..... [ ][ ]

3) Date ..... [ ][ ][ ][ ][ ][ ]
I. DD MM YY

4) Age (months)..... [ ][ ]

5) Sex ..... [ ]

6) Use of replacement fluid before arrival ..... [ ]

7) Chemotherapy before arrival ..... [ ][ ]

8) How many members of your family had ..... [ ]
Diarrhoea in the past 7 days

9) Age of last intake of vitamin-A Capsule ..... [ ][ ]

10) Temperature ..... [ ]

11) Duration of diarrhoea before arrival ..... [ ]

12) Consistency of stool ..... [ ]

13) Stool contents ..... [ ]

14) Number of the stools in the last 24 hours ..... [ ][ ]

15) Abdominal pain ..... [ ]

16) Vomiting in the last 24 hours ..... [ ][ ]

17) History of cough with diarrhoea ..... [ ]

18) History of convulsion ..... [ ]

19) Other diseases (specify) .....

PHYSICAL EXAMINATION

20) General condition .....

21) Radial pulse .....

22) Respiration .....

23) Clinical assessment of hydration .....

24) Convulsion .....

25) Vitamin A deficiency .....

26) Ear = Otitis media .....

27) Sore mouth .....

28) Lungs .....

29) Abdomen .....

30) Liver and spleen .....

31) Rectum prolapse .....

32) Extrimities .....

33) Diagnosis .....

34) Outcome .....

TREATMENT

35) Rehydration method used .....

36) Chemotherapy after arrival .....

37) Weight in KG .....

38) Height in Cm .....

39) Arm circumference

Numerical scoring system for severity of diarrhoea (Modified Ruuska *et. al*)

40) Duration of diarrhea (days=points) |_____|

1 – 4 days = 1 point
5 days = 2 points
≥ 6 days = 3 points.

41) Maximum no. of diarrhea/24 hours |_____|

1 – 3 = 1 point
4 – 5 = 2 points
≥ 6 = 3 points

42) Duration of vomiting (days) |_____|

1 day = 1 point
2 days = 2 points
≥ 3 days = 3 points.

43) Maximum no. of vomiting episode/24 hours |_____|

1 = 1 point
2 – 4 = 2 points
≥ 5 = 3 points

44) Fever (from January 2000)

upto 37 °C = 0
37.1 – 37.9 °C = 1 |_____|
38 – 38.4 °C = 2
38.5 – 38.9 °C = 3
≥ 39 °C = 4

45) Dehydration (Expected or recent wt. – Current wt.) X 100
Expected wt.

None = 0 point |_____| - |_____| *x 100*
1-5% = 2 points ---------- = |_____| % |_____|
≥ 6% = 3 points |_____|

46) Treatment

None = 0 point |_____|

Polyclinical/
Rehydration = 1 point
Hospitalization = 2 points

47) Ruuska total points |_____|

Budget justification:

The budget should reflect all planned activities and the costs required. Please write your justification and explain the necessity of all items listed in budget. Also, provide any calculations that you have used to obtain costs for items in the budget.

The budget includes costs for personnel, field site activities for volunteer recruitment, hospitalization charges for patient studies, service charges for clinical microbiology and pathology etc, costs for immunological and molecular microbiology reagents and supplies.

Travel

The PI and/or Co-PIs and other investigators will need to travel for carrying out collaborative work in the different laboratories, for presentation of results at meetings etc.

Justification for indirect costs:

A minimum of 25% overhead for institutional costs is required for using ICDDR,B facilities and include among others finance, procurement, personnel, as well as rent and communication utilities. For Goteborg University 30% overhead has been included which is based on the institutional cost of the University

Other Support (Dr. F. Qadri):

1. Study of specific and innate mechanisms of the immune response in acute watery diarrhoea due to *Vibrio cholerae* and enterotoxigenic *Escherichia coli*: Studies in patients and vaccinees (January1999-2001). Funding Sida-SAREC (ending 2001)
2. Epidemiology and ecology of *Vibrio cholerae* infection in Bangladesh (July 1996 -2001). Funding- NIH
3. Phase II safety and immunogenicity studies of the enterotoxigenic *E. coli* (ETEC) vaccine in Bangladeshi children (January 2000-2001).Funding USAID
4. Immune response to V. cholerae in Bangladesh (January 2001-2005)

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

Memorandum

3 February 2002

To : Dr. Firdausi Qadri
Laboratory Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Approval of protocol # 2001-027**

Thank you for memo of 27 January 2002 attaching the modified version of the your protocol # 2001-027 entitled "Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I- studies to facilitate ETEC vaccine efficacy trials. Part II-Cholera and ETEC vaccine studies" incorporating the observations of the Committee made in its meeting held on 9th January 2002. The modified version of the protocol is hereby approved.

You shall conduct the study according to the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of the ERC Guidelines. You shall also submit report(s) as required under the ERC Guidelines. Relevant excerpt of the ERC Guidelines is attached for your information and guidance.

I wish you all the success in running the above mentioned study.

Copy: Associate Director
Laboratory Sciences Division

DRAFT

Memorandum

4 February 2002

To : Dr. Firdausi Qadri
Laboratory Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Approval of protocol # 2001-027**

Thank you for memo of 27 January 2002 attaching the modified version of the your protocol # 2001-027 entitled "Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I- studies to facilitate ETEC vaccine efficacy trials. Part II-Cholera and ETEC vaccine studies" incorporating the observations of the Committee made in its meeting held on 9th January 2002. The modified version of the protocol is hereby approved.

You shall conduct the study according to the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of the ERC Guidelines. You shall also submit report(s) as required under the ERC Guidelines. Relevant excerpt of the ERC Guidelines is attached for your information and guidance.

I wish you all the success in running the above mentioned study.

Copy: Associate Director
Laboratory Sciences Division

Memorandum

22 January 2002

To : Dr. Firdausi Qadri
Laboratory Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Approval of protocol # 2001-027**

Thank you for memo of ~~00~~ 21 January 2002 attaching the modified version of the your protocol # 2001-027 entitled "Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I- studies to facilitate ETEC vaccine efficacy trials. Part II-Cholera and ETEC vaccine studies" incorporating the observations of the Committee made in its meeting held on 9th January 2002. The modified version of the protocol is hereby approved.

You shall conduct the study according to the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance of the applicable provision of the ERC Guidelines. You shall also submit report(s) as ~~stipulated in~~ the ERC Guidelines. Relevant excerpt of the Guidelines is attached for your information and guidance.

I wish you all the success in running the above mentioned study.

Copy: Associate Director
Laboratory Sciences Division

DRAFT

Proposed addition to the memo according ERC approval

You shall conduct the research according to the protocol approved by the ERC; and shall be responsible for protecting the rights and welfare of the subjects and compliance of the applicable provisions of the ERC Guidelines. You shall also submit report(s) as stipulated in the ERC Guidelines. Relevant excerpt of the Guidelines is attached for your information and guidance.

wonder if this could be done in 1st person i.e. "I" in place of "you". That constitute an undertaking as well.

To
Chairman
Ethical Review Committee

Since this will be part of the memo from ERC chair to the PI, this will be an advice from ERC chair to the PI. Hence 'second person' will be appropriate.

Thank you.

Bejay
15/1

15/12/09

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH

INTERIM/FINAL

SUMMARY COMPLETION FORM FOR PROTOCOLS

Title: Studies on intestinal ion transport with high potassium and low sodium containing electrolyte solution in malnourished rabbits during diarrhoea induced by *Escherichia coli*.

Investigator(s): Sufia Islam

Protocol No. : 99-008 **Budget Code :** 63361

Findings (Abstract):

Background: Currently, there is no experimental model to measure responses of the kidneys, and small-and large intestine to dehydration, malnutrition, and infection.

Objective: The objective of the study was to develop an experimental model of dehydration, malnutrition and infection that could help in measuring kidney, small and large intestinal response to dehydration, malnutrition and infection. Focus was made on the small intestine response to dehydration, nutritional status, and composition of rehydration solution.

Methods: Malnourished model of rabbits was developed by 50% food restriction by pair feeding with control groups. Well-nourished and malnourished rabbits were dehydrated by water deprivation for 46 hours or infected by *RDEC-1*. Biochemical indicators of dehydration, *in vivo* and *in vitro* intestinal water and ion transports, and the effect of malnutrition on mucosal structure and function were studied. In a first series of experiments, 9 male New Zealand white rabbits were dehydrated by water deprivation (D). The malnourished group (MD) and well-nourished, control groups (CD) were dehydrated by water deprivation. Another group of malnourished rabbits was infected with *RDEC-1* for the development of diarrhoea.

Results: Compare to control (C) animals, the dehydrated (D) animals lost 12% body weight along with 87% reduction in urine volume, increased urine osmolality (1287 ± 45mOsm), and 94% increase in BUN. In the colon of D animals, short-circuit current (Isc) and net sodium trans-epithelial flux (J^{Na}_{Net}) were increased, while electrical trans-epithelial conductance (G) was decreased. In the jejunum, net *in vivo* absorption of water, sodium and potassium were increased. *In vitro*, Isc, J^{Na}_{Net} and G were increased. All these differences between C and D groups were significant at $P<0.01$. Compare to control (CD) animals, the malnourished and dehydrated (MD) animals displayed almost identical body weight loss curves; decrease in urinary volume, and increase in the urine osmolality and BUN were not different in both groups. There was no significant difference in brush border enzyme activities and the intestinal permeability between the malnourished and control groups infected with *RDEC-1*. Comparison of ReSoMaL to WHO solution indicated no difference in water absorption, but a significant decrease in sodium and an increase in potassium absorption ($P<0.01$) in MD group compared to CD group.

Significance: This study indicates that ReSoMaL infusion in the jejunum is more appropriate than WHO-solution to correct for the deficit observed in malnourished and dehydrated children.

Policy Implications: Results of this study are expected to improve management of diarrhoea in severely malnourished children through identification of more efficient formulation of ORS such as ReSoMaL that contains higher amount of potassium and lower amount of sodium compared to the standard WHO ORS.

Dissemination plans: An abstract has been submitted in upcoming AGA Meeting, San Francisco, California, USA. Part of the findings was presented in the WCPGHN, Boston, USA in 1999.

Date: 6.12.2001 **Signature of the P.I.** Sufia Islam

International Centre for Diarrhoeal Disease Research, Bangladesh
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Cable : Cholera Dhaka

The protocol may be considered for ethical clearance as required. Information has been incorporated by PI. Thanks 29/1/02

Date : 27/1/02

To : Professor Mahmudur Rahman
Chairperson, ERC

From : Firdausi Qadri Firdausi Qadri
LSD

Subject: Resubmission of Protocol # 2001-027 entitled "Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I-studies to facilitate ETEC vaccine efficacy trial. Part II- Cholera and ETEC vaccine studies"

Based on the comments made by the Committee, the protocol is being resubmitted with changes and insertions suggested.

a. The oral inactivated, WCO1-BS cholera vaccine is a licensed and safe vaccine when tested in both adults and children worldwide. Based on this the dried formulation would be expected to be safe. Comparisons have been carried out on the safety and immunogenicity in adult North American volunteers (Sack *et. al.* personal communication, Chang and Sack, 2001). When compared in 12 North American volunteers, the dried formulation as compared to the liquid formulation did not result in any adverse event or gastrointestinal disorders of vomiting, diarrhea etc. when observed for a 7 day period after immunization with each dose of the vaccine (Sack et al. personal communication). The dried cholera vaccine induced increases in antibody responses in serum to cholera toxin. Antibodies of the IgA and IgG isotypes were induced in the antibody in lymphocyte supernatant assay where vaccine associated mucosal cells were stimulated (Chang and Sack, 2001, Chang PhD dissertation, manuscript submitted). Vibriocidal antibodies were also induced. Thus the dried formulation resulted in significant increases in immune responses, both systemic antibodies as well mucosal immune responses.
This information has been inserted in the text (page 12).

b. The reconstitution and concentration of ingredients has been insert in the protocol (page 18).
We hope we have now been able to address the comments made by the committee suitably .
A copy of the revised protocol is being resubmitted for further consideration.

Thank you.

26.01.2002

To,
Chairman
ERC, ICDDR,B
Dhaka.

From : Prof. J. Ashraful Haq
Department of Microbiology, BIRDEM

Comments on the modified protocol titled "Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part-I –Studies to facilitate ETEC vaccine efficacy trials. Part-II- Cholera and ETEC vaccine studies"

a. The PI has mentioned that data on safety of the dried cholera vaccine has been included in page-12. In fact, The PI has not incorporated the safety data of dried cholera vaccine except mentioning that the said vaccine was tried on North American volunteers. No data has been mentioned on the number of subjects, safety parameters monitored, and the results.

PI has mentioned that the procedure for reconstitution of dried cholera vaccine has been incorporated in page-18. The constituents (ingredients) and their concentration have not been given. The ingredients of the buffer and their concentration need to be clearly mentioned and incorporated in the protocol.

Therefore, the observation made by the ERC on above two points has not been satisfactorily addressed. So, the protocol may only be recommended for ethical clearance subject to satisfactory incorporation above information.

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To
Dr. Jalaluddin Ashraful Haq
Member, ERC

For your review and comments, please. ERC chair is out of town today
Thank you.

Beijing
17/1/2002

Date : 17/1/02

To : Professor Mahmudur Rahman
Chairperson, ERC

From : Firdausi Qadri F. Qadri
LSD

Subject: Resubmission of Protocol # 2001-027 entitled "Studies to evaluate vaccines against watery diarrhoea suitable for use in Bangladesh: Part I-studies to facilitate ETEC vaccine efficacy trial. Part II- Cholera and ETEC vaccine studies"

Changes have been made in the protocol as was suggested as follows:

a). Data on the safety and immunogenicity of the dried vaccine has been included in the protocol (Page 12). The procedure for reconstitution of the dried vaccine has now been included in the "Vaccine administration and allocation section" (Page 18).

b. The combined vaccine will contain only 1 mg of the recombinant cholera toxin B subunit. It will contain the CF-BS-ETEC vaccine which contains 1 mg of rCTB and the WCO1 vaccine (that is without rCTB). This has been clarified on page 16 and 18 of the protocol.

c. We will obtain approval from the Directorate of Drug Administration,GoB for testing the dried cholera vaccine in Bangladesh.

d. The consent form (8a) has been corrected as suggested. The translation of the "dried" has been translated in Bangla as more appropriately as "powder" in the consent form (7a).

We hope we have been able to address the comments suitably.

A copy of the protocol is being resubmitted for further consideration.

Thank you.