International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

*Memorandum*

LSD
2004

6 January 2005

To : Dr. Firdausi Qadri
Principal Investigator of research protocol # 2004-046
Laboratory Sciences Division (LSD).

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

Sub : Approval of research protocol # 2004-046

Thank you for your memo dated January 3, 2005 and enclosed modified version of your research protocol # 2004-046 titled "Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines". The ERC considered the protocol in its meetings held on December 15 and 29, 2004. The Committee observed your satisfactory addressing of all issues in the modified version of the protocol, and accorded its approval for implementation of the research protocol. However, you would be required to observe the following terms and conditions in implementing the research protocol:

As the Principal Investigator, the ultimate responsibility for scientific, and ethical conduct including the protection of the rights and welfare of study participants vest upon you. You shall also be responsible for ensuring the competence, integrity and ethical conduct of the investigators and other staff directly involved in this research protocol.

You shall conduct the study in accordance with the ERC-approved protocol and shall fully comply with any subsequent determinations by the ERC.

You shall obtain prior approval from the Research Review Committee and the ERC for any modification in the approved research protocol and/or approved consent form(s), except in case of emergency to safeguard/eliminate apparent immediate hazards to study participants. Such changes must be immediately reported to the ERC Chairman.

You shall recruit/enroll participants for this study strictly adhering to the criteria mentioned in the research protocol.

You shall obtain legally effective informed consent (i.e. consent should be free from coercion or undue influence) from the selected study participants or their legally responsible representative, as approved in the protocol, using the approved consent form prior to their enrollment in this study. Before obtaining consent, all prospective study participants must be adequately informed about the purpose(s) of the study, its methods and procedures, and also what would be done if they agree and also if they do not agree to

participate in the study. They must be informed that their participation in the study is voluntary and that they can withdraw their participation any time without any prejudice. Signed consent forms should be preserved for a period of at least five years following official termination of the study.

You shall promptly report the occurrence of any Adverse Event or Serious Adverse Event or unanticipated problems of potential risk to study participants or others to the ERC in writing within 24 hours of such occurrences.

Any significant new findings, developing during the course of this study that might affect the risks and benefits and thus influence either participation in the study or continuation of participation should be reported in writing to the participants and the ERC.

Data/samples should be collected and interviews should be conducted, as specified in the ERC-approved protocol, and confidentiality must be maintained. Data/samples must be protected by reasonable security, safeguarding against risks such as their loss or unauthorized access, destructions, used by others, and modification or disclosure of data. Data/samples should not be disclosed, made available or use for purposes other than those specified in the protocol, and shall be preserved for a period, as specified under Centre's policies/practices.

You shall obtain permission of the Directorate of Drug Administration, Government of the People's Republic of Bangladesh, for using the vaccine for the study.

The ERC shall constitute a Data Safety Monitoring Board (DSMB) for over sighting the implementation of the study.

You shall promptly and fully comply with the decision of the ERC to suspend or withdraw its approval for the research protocol.

You shall report progress of research to the ERC for continuing review of the implementation of the research protocol as stipulated in the ERC Guidelines. Relevant excerpt of ERC Guidelines and '*Annual/Completion Report for Research Protocol involving Human Subjects*' are attached for your information and guidance.

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

Copy: Acting Director, LSD

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Cable : Cholera Dhaka

# *Memorandum*

2 January 2005

To : Dr. Firdausi Qadri
Principal Investigator of research protocol # 2004-046
Laboratory Sciences Division (LSD).

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

Sub : Research protocol # 2004-046

Thank you for the modified version of your research protocol # 2004-046 titled "Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines" addressing the issues raised by the Committee made in its special meeting held on December 15, 2004. The modified version of your research protocol was reviewed by the Committee in its meeting held on December 29, 2004. After review and discussion, the Committee made following observations on the modified version of your research protocol:

a) Water is not considered as buffer; it is rather dissolving fluid. As such correction should be made in specific aims and other places of the protocol. Further, the words 'without any buffer' (p12) should be replaced by the words 'with water'; and the words 'buffers to be tested (p24) should be replaced by the words 'vaccine dissolving fluid'.

b) The word 'temporary' should be inserted before the word 'withholding' (specific aim # 2).

c) The duration of withholding of breastfeeding (p13) should be three hours instead of 2 hours.

d) The statement 'some of the studies will be run in parallel' should be further elaborated since the PI plans to run the study sequentially.

e) The Bangla and English version of consent forms should be consistent.

You are, therefore, advised to revise the protocol addressing above issues and submit the modified version of the protocol for consideration of the Chair.

Thank you once again.

Copy: Director, LSD

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Cable : Cholera Dhaka

OK.

5/1/05

Date : 3/1/05

To : Chairperson, ERC

From : P.I. Dr. Firdausi Qadri F. Qadri

Subject: Resubmission of project entitled "Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines (Protocol no. 2004-046).

We are resubmitting the protocol after having modified it based on suggestions made by the committee. The changes made are as follows:

a) Water as dissolving fluid for formulation of the vaccines
We have inserted the term in pages 12 and 24, as well as in other sections (pages 8, 11).

b) The word "temporary" has been inserted before the word "withholding" in relevant areas.
c) The duration of withholding breast milk has been corrected.

d) The order in which the study will be carried out has been described in the text (page 11)and elsewhere.

e) The Bangla and English version of the consent forms have been edited to make them more consistent.

A copy of the modified protocol in which the changes have been highlighted is being resubmitted for further consideration.

Thank you

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OK.

5/1/05

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To : Chairperson, ERC

From : P.I. Dr. Firdausi Qadri F. Qadri

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A copy of the modified protocol in which the changes have been highlighted is being resubmitted for further consideration.

Thank you

Attachment I

Date:

(FACE SHEET)

# ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Firdausi Qadri

Trainee Investigator (if any): ___

Application No. **2004-046**

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

Title of Study: Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines.

Project Status: RRC approved

[ X ] 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
   - X Protocol (Required)
   - X Abstract Summary (Required)
   - X 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
   - NA Informed consent form for subjects
   - X Informed consent form for parent or guardian
   - X Procedure for maintaining confidentiality
   - X 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

_____
Trainee

# Abstract Summary

Since natural cholera and ETEC infections may induce protection against further disease, it is believed that these infections can be controlled and prevented by safe and immunogenic vaccines that are protective in children. Vaccine trials have been extended to larger field based studies in different countries of the world in children and infants. The efficacy and immunogenicity of enteric vaccines however has generally been found to be lower in developed than in developing countries. There are a number of factors that may contribute to such differences. The main objective of this proposal is to identify different factors/administration regimens that may improve the immunogenicity of the oral inactivated cholera and ETEC vaccines in young children and infants that may be further evaluated in future vaccine trials in Bangladesh and other developing countries. These include determination of the optimal dissolving fluid for the formulation of the vaccine, the effect of breast milk and the effect of zinc that is appropriate for vaccination of young children.

There are a number of factors that may contribute to such differences in the immunogenicity of vaccines. The factors that can effect of the efficacy of vaccination on young children include the type of fluid used for formulation of oral ETEC and cholera vaccine which may influence "take rates" in children in Bangladesh. Other factors that may influence effectiveness of vaccines is breast feeding. Studies have suggested that breast feeding of newborn and young infants may adversely influence the response to vaccination. This effect was more pronounced in developing versus developed countries. We therefore would like to study the effect of each of these different factors for their contribution to the immunogenicity of oral cholera and/or ETEC vaccines. The main objective of this proposal is to identify different factors/administration regimens that may improve the immunogenicity of the oral inactivated cholera and ETEC vaccines in young children and infants that may be further evaluated in future vaccine trials in Bangladesh and other developing countries. These include (1) to assess the optimal dissolving fluid appropriate for vaccine formulation for young children. (2) effect of breast milk and (3) the effect of zinc on the immunogenicity of the vaccines. For this purpose we will study a total of 1140 children. We will first determine the best formulation for the vaccines and then use this finding for the study of the effect of breast feeding and zinc. Of the total children, 570 will be studied to see the effects of the above factors on the immunogenicity of the ETEC vaccine and 570 for the cholera vaccine. Only children 6 months to two years of age will be studied. Blood (3 ml) and stool samples (5 gm) will be collected from the children at three time points during the course of the study.

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

1. Only 6-24 month old children will be enrolled in the study. Only participants whose parents/guardians give voluntary consent will be enrolled in the study.
2. Methods used have very little no chance of physical risk and the two killed vaccines have been tested in children using the dose and length of intervention has not found to be of any harm.
3. Since blood will be collected, there is little risk, other than physical discomfort or bruising at the site of puncture. We will use aseptic precautions and use disposable, sterile syringe and needles for drawing blood.
4. The interview records and other information from the study will be kept in a locker under supervision. Only investigators will have access to it.
5. Informed consent will be obtained from the guardian or parent of the child. The potential risk 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 and weekly in the different aspects of the study. Where necessary however interview will be carried out at the field site office. Monitoring the side-effects of the study agent in the children will be carried out in subsequent home visits. Approximately 30 minutes will be required for the interview.
7. The results of the vaccine study are relevant to either laboratory or clinical sciences and to health services. A dissemination plan will be made for projection of results from this study.
8. The activity will require collection of stool and blood from the children who will be enrolled in the study. In addition hospital records may be used to assess infections and illnesses over the course of the study period.

Principal Investigator: Last, first, middle _Qadri Firdausi

**Centre for Health & Population Research** **RRC APPLICATION FORM**

**RESEARCH PROTOCOL NUMBER: Protocol No.: 2004-046**

**FOR OFFICE USE ONLY**

RRC Approval: ☒Yes / ☐No Date: 23/11/04

ERC Approval: ☐Yes / ☐No Date:

AEEC Approval: ☐Yes / ☐No Date:

**Project Title: Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines.**

**Short protocol title (in 50 characters): Enhance immunogenicity of cholera & ETEC vaccines**

**Theme:** ***(Check all that apply)***

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

Key words: Immunogenicty, ETEC vaccine, cholera vaccine

**Relevance of the protocol:**

Since natural cholera and ETEC infections may induce protection against further disease, It is believed that these infections can be controlled and prevented by safe and immunogenic vaccines that are protective in children. Vaccine trials have been extended to larger field based studies in different countries of the world in children and infants. The efficacy and immunogenicity of enteric vaccines however has generally been found to be lower in developed than in developing countries. There are a number of factors that may contribute to such differences. The main objective of this proposal is to identify different factors/administration regimens that may improve the immunogenicity of the oral inactivated cholera and ETEC vaccines in young children and infants that may be further evaluated in future vaccine trials in Bangladesh and other developing countries. These include determination of the optimal vaccine dissolving fluid for formulation, effect of breast milk and the effect of zinc on the immunogenicity of the ETEC and cholera vaccines in order to determine the most appropriate strategy for vaccination of young children.

**Centre's priority: Code 19. Define the need for selected vaccines**

**Programmes-**

- ☒ Child Health Programme
- ☐ Nutrition Programme
- ☒ Programme on Infectious Diseases & Vaccine Science
- ☐ Poverty and Health Programme
- ☐ Health and Family Planning Systems Programme
- ☐ Population Programme
- ☐ Reproductive Health Programme
- ☐ HIV/AIDS Programme

*Revised on: 2nd March 2004*

Principal Investigator: Last, first, middle _Qadri Firdausi_____

| Principal Investigator: | Division: | Phone: |
|---|---|---|
| (should be a Centre's staff) | | |
| Dr. Firdausi Qadri (ICDDR,B) | LSD | 8802-8811751-60 Extn. 2431 |
| Professor Ann-Mari Svennerholm ( Goteborg University, Sweden), | | |


**Address:** Immunology Section, LSD, ICDDR,B
Mohakhali 1212

Email: fqadri@icddrb.org


**Co-Principal Investigator(s): Professor David A. Sack, ICDDR,B**
**Internal**

**Co-Principal Investigator(s): Professor Jan Holmgren, Goteborg University, Sweden**
**External**
**(Please provide full official address and Gender)**

**Co-Investigator(s): Dr. M. A. Salam, Dr. Mohiul Islam Chowdhury,**
**Internal**

**Co-Investigator(s):**
**External**
**(Please provide full official address and Gender)**

**Student Investigator/Intern:**
**External**
**(Please provide full address of educational institution and Gender)**

**Student Investigator/Intern:**
**Internal (Centre's staff)**

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

**Please provide full address**

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

Gender
- ☒ Male
- ☒ Females

Age
- ☒ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 – 64 years
- ☐ 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
- ☐ Mirsarai
- ☐ Patyia
- ☒ Other areas in Bangladesh -Mirpur field station
- ☐ Outside Bangladesh

Principal Investigator: Last, first, middle _Qadri Firdausi_____

- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar

name of country: _____

☐ Multi centre trial
(Name other countries involved)
_____

**Type of Study** *(Check all that apply)*:

- ☐ Case Control study
- ☒ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- ☐ Others

**Targeted Population** *(Check all that apply)*:

- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

**Consent Process** *(Check all that apply)*:

- ☒ Written
- ☐ Oral
- ☐ None
- ☒ Bengali language
- ☒ English language

**Proposed Sample size:**

Total sample size: _____1140_____ ☐

Sub-group _____ETEC-_____570_____ ☐ _____ ☐

_____Cholera_____570_____ ☐ _____ ☐

**Determination of Risk: Does the Research Involve** *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☒ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☒ Existing data available via public archives/source
- ☒ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

**Yes/No**

☒ ☐ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ☒ Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ☒ a. place the subject at risk of criminal or civil liability?

☐ ☒ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Principal Investigator: Last, first, middle _Qadri Firdausi_____

**Do you consider this research** *(Check one)*:

☐ greater than minimal risk ☒ no more than minimal risk

☐ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

**Yes/No**

☒ ☐ Is the proposal funded?

If yes, sponsor Name: __Sida-SAREC_____

**Yes/No**

☐ ☒ Is the proposal being submitted for funding ?If yes, name of funding agency: (1) (2) Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder)with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above? No*IF YES, submit a written statement of disclosure to the Executive Director.*

| **Dates of Proposed Period of Support** | **Cost Required for the Budget Period ($)** | | | | | |
|---|---|---|---|---|---|---|
| *(Day, Month, Year - DD/MM/YY)* | **Direct Cost** | | **Indirect Cost** | | **Total Cost** | |
| | US$ | SEK | US$ | SEK | US$ | SEK |
| Beginning date 01/01/2005 Year-1; ICDDR,B : | 47,266 | 352,000 | 11,816 | 88,000 | 59,082 | 440,000 |
| Goteborg University : | 18,592 | 138,462 | 5,578 | 41,539 | 24,170 | 180,000 |
| Total Sida-SAREC : | 65,858 | 490,462 | 17,394 | 129,539 | 83,252 | 620,001 |
| Year-2 ICDDR,B : | 47,802 | 355,998 | 11,951 | 89,000 | 59,753 | 444,998 |
| Goteborg University : | 13,428 | 100,002 | 4,028 | 30,001 | 17,456 | 130,003 |
| Total Sida-SAREC : | 61,230 | 456,000 | 15,979 | 119,001 | 77,209 | 575,001 |
| End date 31/12/2007 Year-3 ICDDR,B : | 56,933 | 423,996 | 14,233 | 105,999 | 71,166 | 529,995 |
| Goteborg University : | 22,724 | 169,231 | 6,817 | 50,769 | 29,541 | 220,000 |
| Total Sida-SAREC : | 79,657 | 593,227 | 21,050 | 156,768 | 100,707 | 749,995 |

**TOTAL: funds for 3 years (detailed budget attached)** **US$ 261,168 SEK 1.944, 996**

Principal Investigator: Last, first, middle _Qadri Firdausi_____

**Approval of the Project by the Division 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 Division 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.

**Signature of PI**

**Date:**

**Name of Contact Person** (if applicable)

# Table of Contents



[X] Check here if appendix is included✓

Principal Investigator: Last, first, middle _Qadri Firdausi_____

**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 (Gpteborg University,Sweden)

**Project Name:** Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

Beginning Date ASAP -2005 Ending Date 31/12/2007

Acute diarrhoeal diseases by two bacterial pathogens pathogens, *V. cholerae* O1and enterotoxigenic Escherichia coli (ETEC) as well as rotavirus contribute to over 60% of of the diarrhoeal disease burden in children in Bangladesh. On an average, approximately 18% and 20% of hospitalized diarrhoeal patients screened from children up to 5 years of age due to ETEC and *V. cholerae* O1, respectively. If effective vaccines could be provided for these pathogens a large of the disease burden could be decreased. The development of candidate vaccines for Bangladesh and developing countries need to be optimized with respect to dosage, supplementation of nutrients, adjuvants etc and issues relevant to better utilization of vaccines so that an optimum efficacy is provided. Thus, issues related to the composition of the candidate vaccines not only need attention but equally important are nutritional (and environmental factors) that may contribute to decrease efficacy of vaccines.

Since natural cholera and ETEC infections may induce protection against further disease, 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 (WCO1-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 now licensed in Europe as well as in many countries e.g. in Latin America. The oral inactivated ETEC vaccine consisting of mixture of formalin-inactivated whole cell ETEC bacteria expressing prevalent colonization factors (CF) plus BS (the CF-BS-ETEC vaccine) is another vaccine that has been evaluated in different countries of the world. The vaccine has been tested in a number of countries in phase-I to III 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 (Svennerholm and Savarino 2004). 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, in a recent phase III trial with active surveillance in 6-18 mo children in Egypt the vaccine was not protective. For enteric vaccines in children, the efficacy and immunogenicity has generally been found to be lower in developed than in developing countries. This has been found to be the case for the cholera (Clemens et al. 1990), rotavirus (Rennels MB 1996) as well as the ETEC vaccine (Svennerholm and Savarino, 2004). There are a number of factors that may contribute to such differences. Other important factors that may influence efficacy of vaccination of young children include the type of buffer or formulating fluid used for co-administration of oral vaccines; these may affect ETEC and cholera vaccine "take rates" in children in Bangladesh. Another factor that may influence the immunogenicity is the influence that breast feeding may have on the immunogenicity and efficacy of vaccines. Studies have suggested that breast feeding of newborn and young infants may adversely influence the response to vaccination (Domok et al. 1974). This effect was more pronounced in developing versus developed countries. Breast feeding has also been shown to interfere with the serum immune responses to rotavirus vaccine although this effect could be overcome by administering three doses of the oral RRV-TV rotavirus vaccine rather than one dose (Rennels et al. 1996). This effect has, however, not been studied for bacterial enteric vaccines. Thus, dosing of vaccines may have to be adjusted when given to breast fed children. Another factor that may affect the immunogenicty is the effect of zinc. Studies have shown that zinc enhances the immune response to the cholera vaccine in participants > 2 years of age (Albert and Qadri et al. 2003, Qadri et al. 2004, Karlsen et al. 2003) and calcium was found to inhibit the adhesion of ETEC to the gut (Ingeborg et al. 2003). We therefore would like to study the effect of each of these different factors for their contribution to the immunogenicity of oral cholera and/or ETEC vaccines. The main objective of this proposal is to identify different factors/administration regimens that may improve the immunogenicity of the oral inactivated cholera and ETEC vaccines in young children and infants that may be further evaluated in future vaccine trials in Bangladesh and other developing countries. These include determination of the (1) optimal formulation of the vaccine for young children (2) effect of breast milk on the immunogenicity of the vaccines (3) the effect of zinc on vaccination.

Principal Investigator: Last, first, middle _Qadri Firdausi_____

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. Professor D.A.Sack | Microbiologist/ Epidemiologist | Advice and support |
| 4. Professor Jan Holmgren | Immunologist/Development of vaccine & vaccine related work | Expert advice |
| 5. Dr. Mohiul Islam Choudhury | Coordinating physician | |
| 6. Dr. M.A. Salam | Physician | 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.

The immunogenicity of the cholera and/or ETEC vaccine is enhanced in young children if:

1. optimum buffers or dissolving fluid are used to give better "take rates" of the cholera and ETEC vaccines
2. breast milk is withheld for the first 2 hours before and one hour after oral immunization.
3. zinc is added in adjunct with vaccination

## 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).

The overall aim is to carry out studies that will help improve the immunogenicity of the cholera and ETEC vaccines in infants and young children . The aims are to study the effect of :

1. different buffers or water on the immunogenicity of the vaccines
2. breast milk (temporary withholding) when administering vaccine
3. zinc on the immunogenicity of the vaccines

The main goal is to carry out studies to improve the immunogenicity of the oral inactivated whole cell cholera and enterotoxigenic *Escherichia coli* (ETEC) vaccine in young Bangladeshi children and infants. This will include the testing of the different buffer formulations or water only as dissolving fluid on the immunogenicity of the vaccines. This will be followed by studying the effect of breast feeding and zinc on the vaccines that will be prepared in the most appropriate formulation.

## 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).**

**Vaccines to protect against cholera and ETEC infection**

The control of diarrhoeal diseases has made progress over the past decade. However even then about 2.5 million children die each year from diseases that could be prevented. *V. cholerae* and ETEC together account for the majority of cases of bacterial causes of acute watery diarrhoea in children in Bangladesh. Taken together with rotavirus, the three pathogens 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 less than 3 years, which would result in a substantial decrease in morbidity and mortality in this vulnerable age group. Vaccines to protect against rotavirus diarrhoea has progressed to Phase III efficacy studies and a number of candidate vaccines are being evaluated (Svennerholm AM and Steele D. 2004). Vaccines to protect against the most frequently encountered common bacterial pathogen worldwide, i.e. ETEC, would be very useful in the developing country scenario where infants and children suffer from repeated episodes of diarrhoea every year.

It has been estimated that ETEC infections alone causes 400 million episodes of diarrhoea and around 400,000 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 which is probably a gross underestimate due to poor reporting from many countries. 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.

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, e.g. 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). This killed vaccine is the only vaccine that has been subjected to 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, Jertborn et al 1986). Phase III trials of protective efficacy in travelers have shown that the vaccine provides significant protection against significant symptoms, e.g interfering with daily activity, in travelers from the USA going to Latin America (Sack DA et al, 2002). The vaccine has been tested in adult Bangladeshi volunteers as well as children down to 18 months of age 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, 2003; Wenneras *et al.*, 1999). The vaccine has also undergone testing in other countries, including Egypt (Savarino *et al.*, 1999) and phase I-III studies have been completed in children down to 6 months of age (Savarino *et al.*,1999). The whole cell ETEC vaccine contains five strains of ETEC amounting to $5x10^{11}$ CFU per vaccine dose. Although it was found to be safe and without adverse effects in travelers, adults and older children there seems to be some reactogenicity in children less than 17 months of age (Qadri et al. in manuscript, Svennerholm and Savarino, 2004). In studies carried out in Bangladesh children 6 to 17 months of age, adverse events of vomiting was observed in some children. As a result a dose finding study was conducted in which a full, a half and a quarter dose of the vaccine were compared in children 6 months to 12 years of age. In children 6-17 months of age, a quarter dose of the vaccine was found to be safe and immunogenic (Qadri et al. submitted for publication). In Egypt infants there was also some adverse events in the form of vomiting as compared to in older age groups.

In an initial pilot study the CF-BS- ETEC vaccine was shown to confer 82% protective efficacy ($p<0.05$) against ETEC disease in European travelers going to 20 different countries in Africa, Asia and Latin America (Wiedermann et al 2000). However, the number of cases fulfilling the inclusion criteria was low. In a large placebo controlled trial in nearly 700 American travelers going to Mexico and Guatemala the ETEC vaccine was shown to be effective (protective efficacy 77%) against non-mild ETEC diarrheal illness, i.e. disease that interfered with the travelers' daily activities (Sack D.A et al. 2002, Svennerholm and Savarino 2004). However, in a recent pediatric study in rural Egypt the vaccine did not confer significant protection in the 6-18 months old children tested (Savarino et al, to be published).

There are a number of factors that may explain why enteric vaccines have lower efficacies in children in developing countries than in older children and adults in developed as well as in developing countries(Clemens et al. 1985, Richie et al. 2000, Savarino et al. 2004). Pre-existing malnutrition can lead to more severe enteric infections, possibly due to the immunocompromised nature of the host that also predisposes individual to greater bacterial load in the mucosal surfaces of the gut than the well nourished child (Mathur R et al. 1985). In a study in India, diarrhoeal illness including that caused by ETEC was found to be more severe in children with malnutrition. These factors may also be the reason for the poor efficacy of enteric vaccines in infants and young children.

Micronutrient deficiency such as vitamin A and zinc is quite common in developing countries and generally increases the morbidity due to diarrhoeal illnesses, although the effect on the morbidity of ETEC diarrhoea has not yet been studied (Rahman et al. 2001, Raqib et al. 2004). It has been estimated that in Bangladesh over 40 % of children under 5 years of age may have zinc deficiency (Qadri et al. 2004). Supplementation with zinc increases the adaptive immune responses to cholera vaccination in children and adults (Albert et al. 2003, Qadri et al.,2004, Karlsen et al. 2003) and in children with shigellosis (Raqib et al. 2004). The effect of micronutrient deficiency in the immune response in cholera and ETEC vaccine field has not been studied, but is an area that needs attention.

Repeated diarrhoeal episodes including those caused by other infectious disease may be an important cause predisposing the child to malnutrition (Mata 1992, Black, 1984).

Although breast feeding reduces overall diarrhea and mortality (VanDerslice J et al. 1994, Guigliani ER, 2000) it may have an antagonistic effect on enteric vaccines. Thus, in the case of oral polio and rotavirus vaccines (Domork et al. 1974, Rennels et al. 1996) there appears to be a lowered immunogenicity in the presence of breast milk .. This effect may be counteracted by giving more doses of the oral vaccines so that the overall efficacy of the vaccines remain unchanged. Since calcium has been shown to inhibit binding of enteric pathogens including ETEC CFs in animal models as well as in humans (Ingeborg MJ et al. 2003) it is possible that breast feeding may lower the immunogenicty of a CF containing vaccine due to less antigen exposed to the antigen-presenting cells.

Based on this background we would like to try to enhance the immunogenicity of the ETEC vaccine, and also of the oral inactivated BS-WC cholera vaccine and the CF-BS-ETEC vaccinees, in infants and young children by evaluating different strategies as discussed above.

We would also like to identify a optimal buffering of the inactivated cholera and ETEC vaccines for use in young children. The buffer is needed to protect the B subunit of the cholera toxin that is added to the whole cell ETEC and cholera vaccines to elicit antitoxic immunity. It is not clear if acid neutralization is required in young infants and if so how much is needed to neutralize the gastric acidity and what is the optimum formulation. 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 bicarbonate buffer for the children as compared to the adults (150-100 ml of adults compared to 20 ml for infants). In the case of the children, the amount is decreased based on the age. A less hypertonic buffer of lower osmolarity such as CeraVacx (316 mmol/liter) may be better than a more hypertonic buffer like the standard sodium bicarbonate buffers (513 mmol/liter) that have used for such vaccine studies (Sack *et al.* 1997). To address these issues we will compare the immunogenicity of the vaccines when given in the sodium bicarbonate- citric acid buffer that we use at present, when given in a buffer, CeraVacx which contains rice syrup solids together with sodium bicarbonate and citric acid (Sack *et al.* 1997) or when the vaccines are formulated in water only.

## 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).**

ETEC and *V. cholerae* are the two most common bacterial pathogens causing acute watery diarrhea in children in Bangladesh. The aim is to design and optimize vaccines which will protect against these two infections in young children and infants. 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 young children in developing countries like Bangladesh. To achieve this, we will in the first six months initiate studies to determine the optimal formulating liquid for the oral vaccine in young children to determine the suitable liquid composition for the cholera and ETEC vaccines. For this part we will first carry out studies on the cholera vaccine and then on the ETEC vaccine. Having done this we will determine the effect of breast milk (+/-) and zinc (+/-) on the vaccination. These two components will be run in parallel. At first the studies on the cholera vaccine will be carried out and this will be followed by the study on the ETEC vaccine. We hope that with these results we will finally proceed with a modified cholera and ETEC vaccine /formulation

for efficacy studies.

The different components of the vaccine studies are planned and will initiate with the cholera vaccine followed by the ETEC vaccine as follows:

**Sample size**: The sample size has been calculated in the different interventions, using a one sided hypothesis and confidence of approximately 95%, a minimum detectable difference in mean (MDD) of 50% improvement over baseline, and with 15% for dropout adjustment.
As the whole cell cholera vaccine with standard buffer in subjects aged 2-5 years of age 36% respond with vibriocidal antibodies (Albert et al. 2003, Qadri et al.2004) the sample size of each group in the two buffer components and in the formulation in water will be 102. As our earlier work on ETEC vaccine in Bangladesh we have found on average 55% percent of children in the 6 months to 2 years of age respond with CF specific antibodies (Qadri et al. in manuscript), the sample size of the each group of the breast feeding component will be 57; Using the results obtained on zinc supplementation on the whole cell cholera vaccine in subjects aged 2-5 years of age (Albert et al. 2003, Qadri et al.2004), 36% respond with vibriocidal antibodies before and a 66% responder frequency is seen after the intervention, the sample size of each group on the zinc supplementation component will be 75.

**1. The optimum buffer for formulation of the vaccines in children**

We would like to evaluate the suitability of the buffer which will be used to formulate the cholera or ETEC vaccine for use in young children. In addition we will also determine if the vaccines can be used without any buffer that is in water in these children. At present we use about 400 mg of the bicarbonate buffer for formulation of the ETEC vaccine for the young children. We plan to evaluate if this amount can be omitted with retained immunogenicity of the vaccines.

We would also like to compare the immunogenicity of the ETEC and/or cholera vaccine after formulation in two different buffers in children. We will formulate the cholera and/or ETEC vaccine in CeraVacx which contains rice syrup solids, sodium bicarbonate and citric acid (Sack DA 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 cholera and ETEC vaccine in the most appropriate dissolving liquid.

Based on this we plan to study three groups of children who will be given the cholera or ETEC vaccine formulated (A) in water (B) in bicarbonate-citric acid buffer (Recip AB, Stockholm) or (C) in a rice syrup based electrolyte buffer, CeraVacx (Cera Products Inc., Columbia, Maryland). One group in both vaccine groups will be given vaccine without any buffer, that is when dissolved in water. The best formulation will be used in further studies described below to study other interventions.

**Sample size:** Approximately children will be studied in each of three groups (n=102x3= 306; 102x3=306).

**Time frame:** Within first 6 months of the initiation of the study.

**2. Effect of breast feeding on vaccination**
Breast fed infants aged between 6 month -2 years will be given the cholera and subsequently the ETEC vaccine. The children will be withheld from breast-feeding for a period of about 3 hours before ingestion of the vaccine and one hour after. In two weeks time a second dose will be given and the same protocol used. Age matched children will be allowed to breast feed within an hour of taking the oral vaccine. Only breast milk will be withheld and children will be allowed to drink
Age matched control children will be given breast milk without limitations.
Sample size- About 228 children will be studied (57x2=114 for cholera vaccine; 57x2=114for ETEC vaccine).

**Time frame:** Within 6 months -2 years of the initiation of the study.

**3. Effect of zinc on the immunogenicity of ETEC and cholera vaccine in young children**
We would like to study the effect of zinc on the immunogenicty of oral cholera and ETEC vaccine. Our recent studies have shown zinc enhances the antibacterial antibody responses in toddlers (2-5 years of age) given the oral killed cholera or the ETEC vaccine. We hope that it will also have an effect of improving the immunogenicty in younger children 6-24 months of age who are given the cholera or ETEC vaccine. For this purpose we will supplement children 6 months -2 years of age with daily zinc sulfate (10 mg/day) for two weeks prior to immunization and for the duration of the study. Two doses of the oral killed vaccine will be given two weeks apart and studies continued until a week after the second dose. Thus the study will be undertaken for a period of about 5 weeks in total. A comparison group will be given the vaccine two weeks apart without any zinc supplementation.

Sample size- A total number of 300 children will be studied (75x2= 150 for ETEC vaccine related; 75x2= 150 for cholera related groups for 2 vaccines, with and without zinc).
**Time frame:** Within 6 month –2 years of the initiation of the study.

**Overall design for the vaccine studies:**

The vaccine studies will be carried out at the Mirpur field site in urban Dhaka. 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 2 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. Since the ETEC and cholera vaccines have generally been found to be safe at the doses being used, 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-1-7). They will not be allowed to eat or drink one hour before and after intake of the vaccine except where specified where the duration will be longer. Only when the effect of breast feeding on the immunogenicity of the cholera and ETEC vaccine will be studied the children will be withheld from breast feeding for 3 hours prior to and one hour afterwards. They can drink water during this period.

**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 2. 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 studies of the different components.

## Selection of study subjects for vaccine studies:

Healthy children (6 months to 2 years of age) both males and females who have not enrolled in any other research study including that are 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 4a-4c). We have

previously observed that at least 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-4a-c). 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). 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. Children who have received zinc in the past two months will also not be recruited.

We plan to recruit children in the 6 month -2 year range for immunization.

**Baseline evaluation of samples:**

**Stool examination:** 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.

**Zinc**: Serum zinc concentrations will be determined at baseline only. Venous blood collected in trace element free vacutainers will be placed in ice until arrival at the ICDDR,B laboratory on the same day. Serum will be assayed by flame atomic absorption spectrophotometry and zinc deficiency will be defined as a serum zinc concentration <60 ug/dl (31).

**Vaccine, administration and allocation:** Both the cholera and ETEC 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 (Dukoral) 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 $1.25 \times 10^{11}$ CFU of the killed whole cell vaccine plus 1 mg of BS developed in Sweden and also produced by SBL. New batches of ETEC vaccine containing 1.0 mg of BS plus about $10^{11}$ formalin inactivated bacteria expressing colonization factors will be prepared for the study. For all children a quarter dose of the CF-BS-ETEC vaccine will be tested since some adverse events of vomiting was observed with the higher dose (Qadri et al. in manuscript).

Informed written consent will be taken from the parent/guardian for the children for the immunization and for the permission to draw blood and obtain stool samples (Appendices).

Immediately before use individual doses of the liquid form of the vaccine will be mixed with the reconstituted bicarbonate or CeraVacx buffer. For children, the vaccine (either the individual ETEC or cholera vaccine) will be dissolved in 20 ml (total 4 teaspoon) of a raspberry flavored bicarbonate buffer-citric acid buffer [the buffer is prepared by adding 100 ml water to a sachet of bicarbonate buffer (2 g bicarbonate; 750 mg of citric acid; Recip AB, Stockholm) which will be used to prepare the vaccine dose. The vaccines will be mixed with 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 in 150 ml volume). For the children, 20 ml of either buffer will be used for the formulation. This is the procedure followed for immunization of children in the youngest 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. A third group will be given the vaccines in 20 ml of water.

For studies of zinc on the immunogenicity of the ETEC and/or cholera vaccine, children 6 months-2 years of age

will be tested. The children will be given zinc or no supplement 10 days prior to immunization. All children will receive vitamin A supplement as is necessary for the different age groups (e.g. 200,000 IU for 1-2 year old children every 6 months as is the procedure in Bangladesh). The intake of the micronutrients will be initiated 10 days before the first dose and will continue up to 7 days after the second dose of the vaccine (that is for a total of 32 days as shown in appendix 7).

Both the cholera and ETEC vaccine have been extensively tested in Bangladesh and other countries of the world. We will however obtain permission from the Bangladesh Drug Administration before importing and using the vaccine described in this project.

**Sample collection and laboratory analysis:**

**Coding of samples**

Although the study is an open design, the clinician in the field will code the samples before sending them to the laboratory so that the laboratory personnel will be blinded when the analyses are carried out. The laboratory will only know if the samples have to be analyzed for the cholera or the ETEC vaccine immunological responses.

Samples from vaccines that will be collected at 3 time points will be tested for antibody responses. From blood PBMC will be separated for preparation of antibody in lymphocyte supernatants (ALS, Qadri et al. 2003). ALS and 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. ALS and sera from vaccinees receiving the CF-BS-ETEC vaccine or the cholera vaccine will be tested for the IgA antibody responses to different antigens including (for ETEC CFA/I, CS1,CS2,CS3,CS4,CS5, for cholera vaccine to LPS and to CTB for both). Using fecal extracts from the vaccinees at the 3 time-points, IgA antibody responses to CFA/I, V. cholerae O1 LPS and CTB will be meausured. These procedures are already set-up in the laboratory of the investigators (Qadri et al. 2000,2003). Further to test the immunogencity of the cholera vaccine we will test the vibriocidal antibody response at the 3 time points mentioned above as well as the response to LPS (O1 Ogawa and Inaba) using sera. In addition, the IgA and IgG antibody responses to rCTB will be measured in those being immunized with the cholera or the ETEC vaccine.

The serum zinc level will also be assessed at the baseline in all the children since it may have an effect on the response to the intervention(s) and the vaccination.

**Safety Monitoring Committee**

Dr. N.H Alam (CSD) will serve as the Chairperson of the committee. Other members include Dr. Ashraf (CSD), Dr. Debashish (CSD) and Dr. Sayera Banu (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.

## Overall laboratory tests for the project

**Vaccinees:**

Stools will be cultured on Mackonkey agar and tested for colonization factors of ETEC by dot blot immunoassay

techniques and for the enterotoxins LT and ST (Svennerholm and Wiklund, 1983; Svennerholm *et al.*, 1986, Qadri et al. 2000). Stools will also be plated on TTGA (taurocholate- tellurite-gelatin agar) and gelatin agar overnight (Gelatin agar) (Difco, Detroit, MI). Suspected *V. cholerae* O1 and O139 colonies will be identified by slide agglutination using monoclonal antibodies (Qadri et al. 1995).

Plasma 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). Antibody in lymphocyte supernatants will be collected from cultured PBMC (Qadri et al. 2003).

**Field site for studies planned in the project:**

Children 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, 98009,2000-027, 2000-017). A field station has been set up for the past 8 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 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.

## Facilities Available

Existing field, hospital, laboratory and office facilities are adequate and are outlined. The purified antigens used for the study will be supplied by the collaborating laboratory at Goteborg University Sweden.

## 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, 2001-027,2000-027).

## 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

All samples will be coded before reaching the laboratory and blinded until immunological analyses have been completed.

Data management; To maintain consistency the data forms will be reviewed by the investigators. This will be carried out to ensure the completeness and accuracy of the data. 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 $\chi^2$ test with Yates correction or by the Fisher exact test . Seroconversion for the vibriocidal response will be defined as $\geq$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, LPS and CTB specific responses in ALS and serum samples, a $\geq$2 fold response from baseline to post vaccination will be considered a positive response. The increase in responses due to any intervention should be at least 2 fold or higher increase from that seen in the vaccinees in whom any intervention has not been carried out. We will therefore expect to see a 4 fold higher increase in the intervention group. 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.

# Itemized tasks for investigators at ICDDR,B

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

Responsible for coordinating vaccine studies and the immunological component in Bangladesh.

Supervise work in the laboratory and coordinate specimen collection from volunteers 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 ETEC studies in children for protective efficacy Phase III studies.

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

Dr. Mohiul Islam Chowdhury (Study Physician at field site)

Enrollment of participants and clinical management, follow up of toxicity surveillance of vaccine. Coordinate field based studies with laboratory at ICDDR,B.

Dr. M. A. Salam

Clinical support and advice both in the hospital and in the field based study.

Research Officer-

Blood: Separation of plasma and storage at –70°C/-20°C. study of antibody responsed.

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, reagents, techniques and will give scientific and academic feedback.

Professor Jan Holmgren will give scientific and academic feedback and coordinate the vaccine studies in Bangladesh.

Research Technician at Goteborg University

Carry out immunological assays. 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 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. Working on improvement of ETEC vaccine, in particular increase the amount of CFs on the whole cell components without increasing bacterial numbers.
e. Giving advice and feedback on latest information and in jointly prepare manuscripts and reports.

**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 obtained ethical clearance for ongoing studies on ETEC and cholera patients as well

as for recruiting volunteers for the CF-BS- ETEC vaccine which was 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) and for the live oral cholera vaccine Peru 15 (Project:2000-027). In all these studies prior approval was obtained from the ERC.

Before enrollment signed informed consent is obtained from the guardians of the children. The consent form will be 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 for the vaccine studies (consent forms in appendix-4a-4c,Part IA).

**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 WC-O1-BS 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 2005-2007 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 whole cell cholera vaccine which will be helpful in future evaluation of its effectiveness in Bangladesh. The information of the 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.

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## Collaborative Arrangements

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

**Appendix-1**

**INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH**

Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

# Plans for collection of stool and blood from the vaccinees

| Sample | Number | Sample collection days | Volume/amount to be collected |
|---|---|---|---|
| Blood (6mo-2 years) | 1140 x3=3420 | Day 0, 7, 21 | 1.5 ml for 6 mo to 1 year<br>3.0 ml for over 1 year |
| Stool (6mo-2 years) | 1140 x3=3420 | Day 0, 7, 21 | Stool 5 gm |

# Appendix 2

## Studies on the Cholera and ETEC vaccine in children

| Age group | Vaccine | No of study subjects▪ | Timeline |
|---|---|---|---|
| Children (6months -2 years)<br><br>Formulation of vaccine[1] | 1. Cholera vaccine<br>↓<br>2. ETEC vaccine | 306<br><br>306 | First 6 months |
| Children (6months -2 years)<br><br>Breast Feeding temporary restriction | 1. Cholera vaccine<br>↓<br>2. ETEC vaccine | 114<br><br>114 | From 6 month to 2 years of initiation of study |
| Children (6months-2 years):<br><br>Zinc supplementation | 1. Cholera vaccine<br>↓<br>2. ETEC vaccine | 150<br><br>150 | From 6 month to 2 years of initiation of study |
| Total children | Cholera and ETEC | 1140 | Within 2 years of start of study |

[1]The first phase of the study will be carried out on the testing of the cholera vaccine in the different buffer formulations and in water. The second phase of this component will be on the ETEC vaccine. After the optimum formulations has been found, it will be used to test the effect of the different interventions, that is zinc and breast feeding on the immunogenicity of the vaccines.
The studies on cholera vaccine will be followed by studies on the ETEC vaccine.

For the study, 102 children will be studied in the each of the two buffer components and 102 for the formulation in water, 57 children will be studied in breast feeding; 75 children in the zinc; and for each group in the ETEC vaccine and the cholera vaccine studies. The field component will be completed within the first two years. The laboratory analyses will be carried out as each component has been completed and will continue up to the 3rd year. Data analyses will be carried out as each field and laboratory component has been completed.

## Appendix 3

| Vaccine dissolving fluid* | Composition of dissolving fluid | Volume to be used for vaccine formulation |
|---|---|---|
| Bicarbonate-citric acid buffer, RecipAB[1] | 2.0 g NaHCO3, 725 mg citric acid per 100 ml volume in water | 20 ml |
| Rice syrup based electrolyte, CeraVacx[2] | 2 g NaHCO3, 500 mg sodium citrate, 7 g of rice syrup per 150 ml volume in water | 20 ml |
| *Water | Water | 20 |

[1]RecipAB is produced by Swedish Bacteriological Laboratories in Sweden. [2]CeraVacx is produced by Cera Products Inc. Columbia, Maryland. *Groups of children will also be given the vaccine that has been formulated in water to compare the immunogenicty in these participants with those that are receiving the buffered formulations.

Principal Investigator: Last, first, middle _Qadri Firdausi_____

APPENDIX 4a

**International Centre for Diarrhoeal Disease Research, Bangladesh**

# Voluntary Consent Form

**Title of the Research Project:** Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

**Principal Investigators:**
Dr. Firdausi Qadri; Immunology Section, Laboratory Sciences Division, ICDDR,B; Tel: 8811751-60 Extn. 2431
**Professor Ann-Mari Svennerholm ( Goteborg University, Sweden)**

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

## CONSENT TO PARTICIPATE IN A RESEARCH STUDY
## ICDDRB: Center for Health and Population Research

Diarrhoeal diseases are major health problem of Bangladesh, and germs called *Vibrio cholerae* and *Enterotoxigenic escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children 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 (6months-2 Years of age) respond to the oral administration of killed *V. cholerae* and inactivated ETEC vaccine. The vaccine dissolving fluid mentioned below will be used for formulation of the Cholera and ETEC vaccine.

**a. Bicarbonate buffer b. Ceravacx buffer c. Water**

Children participating in the study required to drink one of the following vaccines formulation with one of the dissolving fluids mentioned above :

**1. Cholera vaccine** **2. ETEC vaccine**

As your child is within 6 months to 2 years of age, so, we would like to explain what will be required of your child and yourself if you agree to participate in this study. We will then ask for your consent (permission) to participate.

**PROCEDURES:** If you consent to allow your child to participate in this study, you or your child will be asked to do the following:

1. For the purpose of this study, we would ask your child to drink one of the vaccines twice, fourteen days apart, formulated with anyone of the buffer mentioned above which will be selected by randomization.
2. To determine the response to the vaccines, we will draw 3.0 ml (about half teaspoonful, from children 6 months - 2 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

3. 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.

4. For each blood collection schedule., baseline information collection and immunization you need to bring your

child at the field site i.e. a maximum of 6 times during the whole study period.

5. The entire study will require 21days and a maximum of about 9 ml blood in 3 blood samples will be collected (about 2 teaspoons).

## ALTERNATIVES FOR NOT PARTICIPATING IN THE RESEARCH

To not participate in the study.

## RISKS

There are no major risks associated with your childs participation in this study. Risks of removing blood from a vein in your child's arm include some discomfort, bruising, dizziness and rarely infection. The amount of blood that will be taken will not affect your child's health in any way The vaccines to be used in this study have been tested in 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.

## BENEFITS

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 which will be useful to public health professionals for establishing programs and policies.

## CONFIDENTIALITY

The information obtained from this study will be shared within ICDDR,B. Names and personal information will be kept confidential to protect your and your child's identity, however, absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

## COST/COMPENSATION

There will be no cost to you to participate in this study. You will receive monetary compensation for the transportation costs to and from the center.. This compensation will be provided to you on the day you come to the center for that purpose even if you decide to withdraw before completing the study.

## EMERGENCY CARE AND TREATMENT FOR INJURY

If you are injured as a direct result of research procedures, you will receive reasonably necessary medical treatment at no cost. ICDDR,B does not provide any other form of compensation for injury.

## RIGHT TO REFUSE OR WITHDRAW

Participation in this study is voluntary.. You are not required to join this study. If you decide to participate, you are free to change your mind about being in the study and quit at any time.

## QUESTIONS

If you have any questions, please ask us. If you have additional questions later, Dr. Firdausi Qadri, Dr. M. A. Salam, Dr. Mohiul Islam Chowdhury or one of their assistants will answer them. Dr. Firdausi Qadri and Dr. Mohiul Islam Chowdhury can be reached at Laboratory Sciences Division, ICDDR,B. Mohakhali, Dhaka, Bangladesh, Tel: 8811751-60 Extn. 2413. Dr. M. A. Salam; can be reached at Clinical Sciences Division, ICDDR,B. Mohakhali, Dhaka-1212, Tel: 8811751-60 Extn. 2300 or 988-2399.

**CONSENT:** YOUR WRITTEN CONSENT BELOW, WILL INDICATE THAT YOU HAVE DECIDED TO VOLUNTEER AS A RESEARCH SUBJECT AND THAT YOU HAVE UNDERSTOOD THE INFORMATION PROVIDED ABOVE.

Principal Investigator: Last, first, middle _Qadri Firdausi_____

Name of participant (print): _____

Signature or thumbprint of parents/guardian : _____ Date _____

Name of investigator (print): _____

Signature of investigator: _____ Date _____

Name of witness(print): _____

Signature of witness: _____

Date _____

Note- We will give you a dated and signed copy of this consent form to keep with you. If you would like to know anything more about the rights of the study participant please contact Mr. Bijoy Saha, at ICDDR,B, Mohakhali, Dhaka, phone no. 8811751-60;extn. 2115

Principal Investigator: Last, first, middle _Qadri Firdausi_____

APPENDIX 4b

**International Centre for Diarrhoeal Disease Research, Bangladesh**

# Voluntary Consent Form

**Title of the Research Project:** Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

**Principal Investigators:**
Dr. Firdausi Qadri; Immunology Section, Laboratory Sciences Division, ICDDR,B; Tel: 8811751-60 Extn. 2431
**Professor Ann-Mari Svennerholm ( Goteborg University, Sweden)**

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

## CONSENT TO PARTICIPATE IN A RESEARCH STUDY
## ICDDRB: Center for Health and Population Research

Diarrhoeal diseases are major health problem of Bangladesh, and germs called *Vibrio cholerae* and *Enterotoxigenic Escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children 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 (6months-2 Years of age) respond to the oral administration of killed *V. cholerae* and inactivated ETEC vaccine in addition with zinc supplementation.

**1. Cholera vaccine** **2. ETEC vaccine**

Children in two groups participating in the study would be required to drink one of the above vaccines:

As your child is within 6 months to 2 years of age, so, we would like to explain what will be required of your child and yourself if you agree to participate in this study. We will then ask for your consent (permission) to participate.

**PROCEDURES:** If you consent to allow your child to participate in this study, you or your child will be asked to do the following:

1.For the purpose of this study, we would ask your child to drink one of the above-mentioned vaccines twice, fourteen days apart. In addition 10 mg of zinc in 5 ml of syrup (1 teaspoon) or placebo may be given daily to your child which will be selected by randomization. Starting from 10 days prior to the first vaccine dose and up to 21 days later that is for a total period of 32 days.

2.To determine the response to the vaccines, we will draw 3.0 ml (about half teaspoonful, from children 6 months - 2 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 day before immunization and 7 and 21 days later of 1st immunization.

3. 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.

4. For each blood collection schedule,, baseline information collection and immunization you need to bring your child at the field site i.e. a maximum of 6 times during the whole study period.

5.The entire study will require 32 days and a maximum of about 9 ml blood in 3 blood samples will be collected (about 2 teaspoons).

**ALTERNATIVES FOR NOT PARTICIPATING IN THE RESEARCH**

To not participate in the study.

**RISKS**

There are no major risks associated with your child's participation in this study. Risks of removing blood from a vein in your child's arm include some discomfort, bruising, dizziness and rarely infection. The amount of blood that will be taken will not affect your child's health in any way The vaccines to be used in this study have been tested in 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. Consuming 10 mg zinc per day for about five weeks (i.e. twice the recommended dose) will not associated with any adverse health outcomes.

**BENEFITS**

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 which will be useful to public health professionals for establishing programs and policies.

**CONFIDENTIALITY**

The information obtained from this study will be shared within ICDDR,B. Names and personal information will be kept confidential to protect your and your child's identity, however, absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

**COST/COMPENSATION**

There will be no cost to you to participate in this study. You will receive monetary compensation for the transportation costs to and from the center.. This compensation will be provided to you on the day you come to the center for that purpose even if you decide to withdraw before completing the study.

**EMERGENCY CARE AND TREATMENT FOR INJURY**

If you are injured as a direct result of research procedures, you will receive reasonably necessary medical treatment at no cost. ICDDR,B does not provide any other form of compensation for injury.

**RIGHT TO REFUSE OR WITHDRAW**

Participation in this study is voluntary. You are not required to join this study. If you decide to participate, you are free to change your mind about being in the study and quit at any time.

**QUESTIONS**

If you have any questions, please ask us. If you have additional questions later, Dr. Firdausi Qadri, Dr. M. A. Salam, Dr. Mohiul Islam Chowdhury or one of their assistants will answer them. Dr. Firdausi Qadri and Dr. Mohiul Islam Chowdhury can be reached at Laboratory Sciences Division, ICDDR,B. Mohakhali, Dhaka, Bangladesh, Tel: 8811751-60

Principal Investigator: Last, first, middle _Qadri Firdausi_____
Extn. 2413. Dr. M. A. Salam; can be reached at Clinical Sciences Division, ICDDR,B. Mohakhali, Dhaka-1212, Tel: 8811751-60 Extn. 2300 or 988-2399.

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**CONSENT:** YOUR WRITTEN CONSENT BELOW, WILL INDICATE THAT YOU HAVE DECIDED TO VOLUNTEER AS A RESEARCH SUBJECT AND THAT YOU HAVE UNDERSTOOD THE INFORMATION PROVIDED ABOVE.

Name of participant (print): _____

Signature or thumbprint of parents/guardian : _____ Date _____

Name of investigator (print): _____

Signature of investigator: _____ Date _____

Name of witness(print): _____

Signature of witness: _____
Date _____

Note- We will give you a dated and signed copy of this consent form to keep with you. If you would like to know anything more about the rights of the study participant please contact Mr. Bijoy Saha, at ICDDR,B, Mohakhali, Dhaka, phone no. 8811751-60;extn. 211

Principal Investigator: Last, first, middle _Qadri Firdausi_____

APPENDIX 4c

**International Centre for Diarrhoeal Disease Research, Bangladesh**

# Voluntary Consent Form

**Title of the Research Project:** Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

**Principal Investigators:**
Dr. Firdausi Qadri; Immunology Section, Laboratory Sciences Division, ICDDR,B; Tel: 8811751-60 Extn. 2431
**Professor Ann-Mari Svennerholm ( Goteborg University, Sweden)**

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## CONSENT TO PARTICIPATE IN A RESEARCH STUDY
## ICDDRB: Center for Health and Population Research

Diarrhoeal diseases are major health problem of Bangladesh, and germs called *Vibrio cholerae* and *Enterotoxigenic . Escherichia coli* (ETEC) are responsible for many cases of diarrhoea among children 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 (6months-2 Years of age) respond to the oral administration of killed *V. cholerae* and inactivated ETEC vaccine.

Children participating in the study will be divided into two groups:

**I).Breast fed as usual** **II). Restriction from Breast feeding** (3 hours before and 1 hour after ingestion of the vaccine)

Children participating in the study required to drink one of the following vaccines:

**1. Cholera vaccine** **2. ETEC vaccine**

As your child is within 6 months to 2 years of age, so, we would like to explain what will be required of your child and yourself if you agree to participate in this study. We will then ask for your consent (permission) to participate.

**PROCEDURES:** If you consent to allow your child to participate in this study, you or your child will be asked to do the following:

1.For the purpose of this study, we would ask your child to drink one of the above-mentioned vaccines twice, fourteen days apart. The children may be withheld from breast-feeding for a period of 3 hours before and 1 hour after ingestion of the vaccine which will be selected by randomization.

2.To determine the response to the vaccines, we will draw 3.0 ml (about half teaspoonful, from children 6months-2.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

3. 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.

4. For each blood collection schedule, baseline information collection and immunization you need to bring your child at the field site i.e. a maximum of 6 times during the whole study period.

5.The entire study will require 21days and a maximum of about 9 ml blood in 3 blood samples will be collected (about 2 teaspoons).

**ALTERNATIVES FOR NOT PARTICIPATING IN THE RESEARCH**

To not participate in the study.

**RISKS**

There are no major risks associated with your child's participation in this study. Risks of removing blood from a vein in your child's arm include some discomfort, bruising, dizziness and rarely infection. The amount of blood that will be taken will not affect your child's health in any way The vaccines to be used in this study have been tested in 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.

**BENEFITS**

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 which will be useful to public health professionals for establishing programs and policies.

**CONFIDENTIALITY**

The information obtained from this study will be shared within ICDDR,B. Names and personal information will be kept confidential to protect your and your child's identity, however, absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

**COST/COMPENSATION**

There will be no cost to you to participate in this study. You will receive monetary compensation for the transportation costs to and from the center.. This compensation will be provided to you on the day you come to the center for that purpose even if you decide to withdraw before completing the study.

**EMERGENCY CARE AND TREATMENT FOR INJURY**

If you are injured as a direct result of research procedures, you will receive reasonably necessary medical treatment at no cost. ICDDR,B does not provide any other form of compensation for injury.

**RIGHT TO REFUSE OR WITHDRAW**

Participation in this study is voluntary. You are not required to join this study. If you decide to participate, you are free to change your mind about being in the study and quit at any time.

**QUESTIONS**

If you have any questions, please ask us. If you have additional questions later, Dr. Firdausi Qadri, Dr. M. A. Salam, Dr. Mohiul Islam Chowdhury or one of their assistants will answer them. Dr. Firdausi Qadri and Dr. Mohiul Islam Chowdhury can be reached at Laboratory Sciences Division, ICDDR,B. Mohakhali, Dhaka, Bangladesh, Tel: 8811751-60 Extn. 2413. Dr. M. A. Salam: can be reached at Clinical Sciences Division, ICDDR,B. Mohakhali, Dhaka-1212, Tel:

Principal Investigator: Last, first, middle _Qadri Firdausi_____
8811751-60 Extn. 2300 or 988-2399.

**CONSENT:** YOUR WRITTEN CONSENT BELOW, WILL INDICATE THAT YOU HAVE DECIDED TO VOLUNTEER AS A RESEARCH SUBJECT AND THAT YOU HAVE UNDERSTOOD THE INFORMATION PROVIDED ABOVE.

Name of participant (print): _____

Signature or thumbprint of parents/guardian : _____ Date _____

Name of investigator (print): _____

Signature of investigator: _____ Date _____

Name of witness(print): _____

Signature of witness: _____
Date _____

Note- We will give you a dated and signed copy of this consent form to keep with you. If you would like to know anything more about the rights of the study participant please contact Mr. Bijoy Saha, at ICDDR,B, Mohakhali, Dhaka, phone no. 8811751-60:extn. 2115

Principal Investigator: Last, first, middle _Qadri Firdausi_____

# Appendix 5a

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

Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines:

## 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 5 b

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

Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines:

**Study subject vaccination and clinical evaluation form**

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

20. Day of vaccination |___|___|

21. Date |___|___|___| 22. Vaccine lot No. |_____|
DD MM YY

23. Date of vaccination |___|___|___| 24. Type of vaccination |__| 1 = Primary 2 = Secondary
DD MM YY

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

Principal Investigator: Last, first, middle _Qadri Firdausi_____

# Appendix 5c

**INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH**

Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines:

## 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

Principal Investigator: Last, first, middle _Qadri Firdausi_____

# Appendix 5d

**INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH**

Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

## adverse event form

## Surveillance After Vaccination

**Random # Number** **Investigator** **Initials of participants** **Inpatient**

.....

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 5d (contd)

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

## 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.

## Appendix-6

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

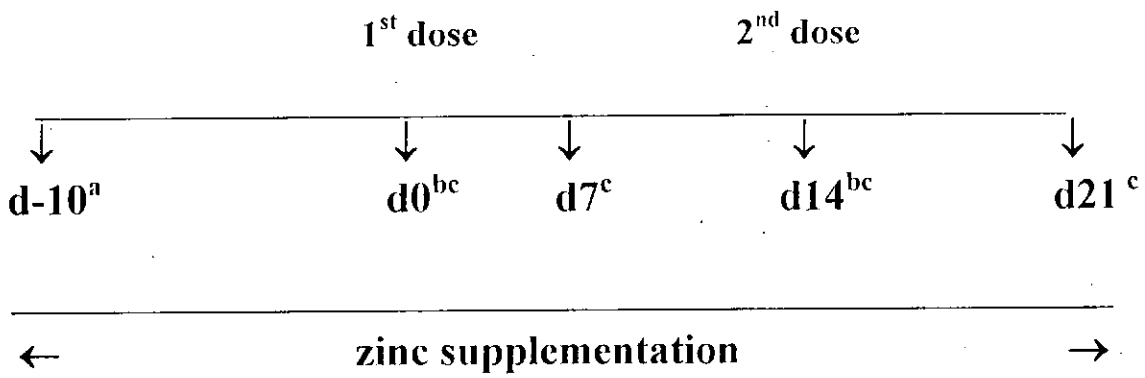
Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines

### Different volumes of buffer in the different age groups to be used for formulation of the ETEC

| Age group | Buffer[a] volume |
|---|---|
| 6 mo -2 years | 20 ml as full strength |
| 6 mo- 2 years | 20 ml of water |

[a]Bicarbonate- citric acid (RecipAB), Ceravacx or water only will be used for formulation of vaccine

## Appendix-7




[a]Zinc supplementation started at d-10 (10 days prior to first vaccine dose)
[b]2 doses of vaccine at day 0 and day 14
[c]Blood draw and stool samples collected at day 0, day 7 and day 21

## পরিশিষ্ট - 4a

গবেষনা (STUDY)

### "গবেষনায় অংশ গ্রহন করার জন্য সম্মতি পত্র"
### আন্তর্জাতিক উদারাময় গবেষনা কেন্দ্র, বাংলাদেশ (আই. সি. ডি. ডি. আর, বি)

**গবেষনার নাম:** ভিব্রিওকলেরী ও এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ই,টি,ই,সি)টিকার রোগ প্রতিরোধ ক্ষমতা বাড়ানোর জন্য গবেষনা

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**গবেষকদের নাম, বিভাগ, ফোন নং :**

(১) ডঃ ফেরদৌসী কাদরী ; ইমিউনোলোজী; এল. এস. ডি; আই, সি, ডি, ডি, আর, বি । ফোন নং-৮৮১১৭৫১-৬০/২৪৩১

(২) প্রফেসর এ্যানমেরী সেভেনারহোম (গোটেবর্গ ইউনিভার্সিটি, সুইডেন)।

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**সূচনা:**

ডায়রিয়া বাংলাদেশের একটি প্রধান স্বাস্থ্য সমস্যা এবং ভিব্রিওকলেরী ও এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ই,টি,ই,সি) নামক জীবানু বাংলাদেশী শিশুদের মধ্যে ডায়রিয়ার প্রাদুর্ভাবের প্রধান কারন গুলির মধ্যে অন্যতম। অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া বিশেষ করে ভিব্রিও কলেরী এবং ই,টি,ই,সি জীবানু দ্বারা সংঘটিত ডায়রিয়া টিকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আমরা এই এলাকায় আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র বাংলাদেশ (আই সি ডি ডি আর, বি)এর মাধ্যমে সুস্থ শিশুদের (৬ মাস থেকে ২বৎসর) উপর নিম্নবর্ণিত বাফার অথবা পানির সহিত কলেরা অথবা ই,টি,ই,সি টিকা প্রয়োগ করে এদের কার্যকারীতা নিয়ে গবেষনা করব।

১. বাইকার্বনেট বাফার ২. সেরাভেক্স বাফার ৩. পানি

গবেষনায় অংশগ্রহনকারী শিশুদের উপরোল্লিখিত যে কোন একটি বাফার অথবা পানির সহিত মিশিয়ে নিম্নের যে কোন একটি টিকা খেতে হবে।

১. কলেরা টিকা ২. ই,টি,ই,সি টিকা

যেহেতু আপনার শিশু ৬ মাস থেকে ২ বৎসরের মধ্যে সেহেতু আপনি যদি আপনার শিশুকে আমাদের গবেষনায় অংশগ্রহন করাতে ইচ্ছুক থাকেন তাহলে আপনার এবং আপনার শিশুর কি কি করতে হবে তা আমরা ব্যখ্যা করব। এর পর আমরা আপনার শিশুকে আমাদের গবেষনায় দেওয়ার জন্য আপনার সম্মতি চাইব।

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**উদ্দেশ্য :**

আপনার শিশুকে একটি গবেষনায় অংশগ্রহন করার জন্য আহবান করা হচ্ছে।

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**পদ্ধতি:**

১। আপনি যদি স্বেচ্ছায় আপনার শিশুকে এই গবেষনায় অংশগ্রহন করাতে চান তবে আমরা আপনাকে আপনার পরিবারের সদস্যদের শিক্ষা, পেশা এবং আয় ইত্যাদি সম্পর্কিত কিছু প্রশ্ন করব। এরপর আমরা দৈবচয়ন পদ্ধতিতে আপনার শিশুকে ১৪দিনের ব্যবধানে উপরে উল্লেখিত যেকোন একটি বাফার যে কোন একটি টিকার সাথে মিশিয়ে ২ বার খাওয়ানো হবে।

২। টিকার কার্যকারীতা পরীক্ষা করার জন্য আমরা গবেষনার শুরুতে এবং গবেষনার ৭ম ও ২১ তম দিনে আপনার শিশুর বাহুর শিরার থেকে সরু সুঁইয়ের মাধ্যমে ৩মিঃ লিঃ (১/২ চামচের একটু বেশী) রক্তের নমুনা সংগ্রহ করব এবং একই দিনে আমরা আপনার শিশুর মলের(প্রায় ৫ গ্রাম) নমুনাও সংগ্রহ করব।

৩। আপনার শিশুকে টিকা খাওয়ানোর পর ৩ দিন পর্যন্ত প্রতিদিন আমাদের মাঠ কর্মীরা আপনার শিশুকে দেখার জন্য বাড়ী যাবে।

৪। প্রতিবার রক্ত সংগ্রহ করার সময়, টিকা খাওয়ানোর সময়, এবং প্রাথমিক তথ্য সংগ্রহ করার জন্য সর্বমোট ৬ বার আপনার শিশুকে আমাদের মাঠ কেন্দ্রে নিয়ে আসতে হবে।

৫। পুরো গবেষনা সমাপ্ত হতে ২১ দিন সময় লাগবে, এবং ৩ বারে সর্বমোট ৯ মিঃ লিঃ(প্রায় ২ চা চামচ) রক্ত সংগ্রহ করা হবে।

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**গবেষনায় অংশ গ্রহন না করার বিকল্প :**

এই গবেষনায় অংশ গ্রহন না করা।

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**ঝুঁকি সমূহ :**

এই গবেষনায় অংশ গ্রহনের ফলে আপনার শিশুকে বিশেষ কোন ঝুঁকির সম্মুখীন হতে হবে না। শিরা থেকে রক্ত নেওয়ার সময় সামান্য ব্যথা, ক্ষত সৃষ্টি, মাথা ঘুরানো বা জীবানুর সংক্রমন হওয়ার ক্ষীন সম্ভাবনা থাকে। যেটুকু রক্ত আপনার শিশুর শরীর থেকে নেয়া হবে তাতে তার স্বাস্থ্যের কোন ক্ষতি হবে না। গবেষনায় ব্যবহারকৃত ই,টি,ই,সি টিকা সুইডেন এবং মিসরে পরীক্ষায় দেখা গেছে যে টিকাটি নিরাপদ এবং টিকা খাওয়ানোর পর ৩ দিনের মধ্যে শুধুমাত্র ২-৬% ক্ষেত্রে বমি বমি ভাব, মৃদু পেট মোচড়ানো এবং ১-২ বার পাতলা পায়খানা ছাড়া

অংশগ্রহনকারীর অনুস্বাক্ষর----------

তেমন কোন পার্শ্ব প্রতিক্রিয়া দেখা যায়নি। মুখে খাওয়ানোর কলেরা টিকাও বাংলাদেশ সহ বিভিন্ন দেশে পরীক্ষা করা হয়েছে এবং সেটিও নিরাপদ টিকা বলে প্রমানিত হয়েছে।

**উপকারীতা/লাভঃ**

এই গবেষনায় আপনার শিশুর সরাসরি কোন উপকারে আসবে না। তবে আমাদের এই গবেষনার ফলাফল সামগ্রিকভাবে সমাজের সবার উপকারে আসবে। জনস্বাস্থ্য বিশেষজ্ঞরা এই গবেষনালব্ধ তথ্যকে কাজে লাগিয়ে বিশ্বের বিভিন্ন স্থানে প্রয়োজনীয় পদক্ষেপ নিতে পারবেন।

**গোপনীয়তাঃ**

এই গবেষনায় প্রাপ্ত তথ্য শুধুমাত্র আই, সি, ডি, ডি, আর, বি তে রক্ষিত থাকবে। আপনার শিশুর নাম ও অন্যান্য ব্যক্তিগত তথ্য সমূহ গোপন রাখা হবে এবং ফলাফল প্রকাশের সময় আপনার শিশুর পরিচিতি ব্যবহার করা হবে না। তবে পরিচয় গোপন রাখার পরিপূর্ন নিশ্চয়তা দেয়া সম্ভব নয়, কারন গবেষনায় প্রাপ্ত তথ্য প্রয়োজনে আদালতে সাক্ষ্য প্রমানের জন্য ব্যবহৃত হতে পারে।

**ক্ষতিপুরনঃ**

এই গবেষনার জন্য আমরা আপনার কাছে সময় চাইব। গবেষনায় অংশ গ্রহনের জন্য আপনাকে কোন খরচ বহন করতে হবে না। এই হাসপাতালে শিশুর সাথে আসার জন্য আপনাকে যাতায়াতের খরচ দেয়া হবে। যাতায়াতের এই খরচ আপনি যে দিন শিশুকে আমাদের স্বাস্থ্যকেন্দ্রে নিয়ে আসবেন শুধুমাত্র সেই দিনের জন্য দেয়া হবে। যদি গবেষনা কার্যক্রম শেষ হওয়ার পূর্বে আপনি নিজেকে প্রত্যাহার করার সিদ্ধান্ত নেন তাহলে ও আপনি যে দিন শিশুকে উক্ত গবেষনার কাজে আমাদের স্বাস্থ্যকেন্দ্রে নিয়ে আসবেন সেই দিনের জন্য আপনাকে যাতায়াতের খরচ দেয়া হবে।

**জরুরী পরিচর্যা ও অসুস্থতার কারন জনিত চিকিৎসাঃ**

যদি গবেষনা প্রক্রিয়ায় আপনার শিশুর কোন শারীরিক ক্ষতি হয় তবে আপনাকে বিনামূল্যে যথাসাধ্য চিকিৎসার ব্যবস্থা করা হবে। আই, সি, ডি, ডি, আর, বি এ ছাড়া অন্য কোন ধরনের ক্ষতিপুরন দিতে অপারগ।

**গবেষনায় অংশগ্রহন প্রত্যাহার করার অধিকারঃ**

এই গবেষনায় সম্পূর্ন স্বেচ্ছায় অংশগ্রহন করার জন্য আপনাকে আহবান করা হচ্ছে। গবেষনা কার্যক্রম শুরু হবার পরও আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন সেজন্য আপনাকে কোন ক্ষতিপূরন দিতে হবে না

**প্রশ্নোত্তরঃ**

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডঃ ফেরদৌসী কাদরী, ডাঃ এম এ সালাম, ডাঃ মহিউল ইসলাম চৌধুরী বা গবেষনা কর্মীরা দিবেন। ডঃ ফেরদৌসী কাদরী, ডাঃ মহিউল ইসলাম চৌধুরী ঠিকানাঃ ইমিউনোলোজী এল. এস. ডি. আই, সি, ডি, ডি, আর, বি, মহাখালী, ঢাকা, বাংলাদেশ, ফোন নং-৮৮১১৭৫১-৬০/ ২৪১৩। ডাঃ এম এ সালাম ঠিকানাঃ সি, এস, ডি, আই, সি, ডি, ডি, আর, বি মহাখালী, ঢাকা, বাংলাদেশ, ফোন নং-৮৮১১৭৫১-৬০/২৩০০ অথবা ৯৮৮২৩৯৯।

অংশগ্রহনকারীর অনুস্বাক্ষর----------

**সম্মতি:**

আপনার নীচের স্বাক্ষর এই অর্থ বহন করে যে, আপনি স্বেচ্ছায় আপনার শিশুকে গবেষনায় অংশগ্রহন করতে সম্মত হয়েছেন এবং আপনি উপরে বর্নিত তথ্য সমূহ এবং গবেষনায় অংগ্রহনকারী হিসাবে আপনার অধিকার সম্পর্কে অবগত আছেন।

অংশগ্রহনকারীর নাম----------

অংশগ্রহনকারীর অভিভাবকের স্বাক্ষর/ বৃদ্ধাঙ্গুলির ছাপ---------- তারিখ----------

গবেষকের নাম ----------

গবেষকের স্বাক্ষর ---------- তারিখ----------

স্বাক্ষীর নাম ----------

স্বাক্ষীর স্বাক্ষর ---------- তারিখ ----------

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষনের জন্য দেওয়া হবে। আপনি গবেষনা কাজে অংশগ্রহনকারী ব্যক্তির অধিকার সম্পর্কিত আরো তথ্য জানতে চাইলে জনাব বিজয় সাহা, আই, সি, ডি, ডি, আর, বি, মহাখালী ঢাকা, ফোন নং-৮৮১১৭৫১-৬০/২১১৫ যোগাযোগ করুন।

## পরিশিষ্ট - 4b

গবেষনা (STUDY)

# "গবেষনায় অংশ গ্রহন করার জন্য সম্মতি পত্র"

## আন্তর্জাতিক উদারাময় গবেষনা কেন্দ্র, বাংলাদেশ (আই. সি. ডি. ডি. আর, বি)

**গবেষনার নাম:** ভিব্রিওকলেরী ও এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ই,টি,ই,সি)টিকার রোগ প্রতিরোধ ক্ষমতা বাড়ানোর জন্য গবেষনা

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**গবেষকদের নাম, বিভাগ, ফোন নং :**

(১) ডঃ ফেরদৌসী কাদরী ; ইমিউনোলোজী; এল. এস. ডি; আই, সি, ডি, ডি, আর, বি । ফোন নং-৮৮১১৭৫১-৬০/২৪৩১

(২) প্রফেসর এ্যানমেরী সেভেনারহোম (গোটেবর্গ ইউনিভার্সিটি, সুইডেন)।

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**সূচনা:**

ডায়রিয়া বাংলাদেশের একটি প্রধান স্বাস্থ্য সমস্যা এবং ভিব্রিওকলেরী ও এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ই,টি,ই,সি) নামক জীবানু বাংলাদেশী শিশুদের মধ্যে ডায়রিয়ার প্রাদুর্ভাবের প্রধান কারন গুলির মধ্যে অন্যতম। অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া বিশেষ করে ভিব্রিও কলেরী এবং ই,টি,ই,সি জীবানু দ্বারা সংঘটিত ডায়রিয়া টিকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আমরা এই এলাকায় আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র বাংলাদেশ (আই সি ডি ডি আর, বি)এর মাধ্যমে সুস্থ শিশুদের (৬ মাস থেকে ২বৎসর) উপর নিম্নবর্ণিত কলেরা অথবা ই,টি,ই,সি টিকা খাওয়ানোর পাশাপাশি জিংক প্রয়োগ করে এদের কার্যকারীতা নিয়ে গবেষনা করব।

গবেষনায় অংশগ্রহনকারী শিশুদের নিম্নের যে কোন একটি টিকা খেতে হবে।

১. কলেরা টিকা ২. ই,টি,ই,সি টিকা

যেহেতু আপনার শিশু ৬ মাস থেকে ২ বৎসরের মধ্যে সেহেতু আপনি যদি আপনার শিশুকে আমাদের গবেষনায় অংশগ্রহন করাতে ইচ্ছুক থাকেন তাহলে আপনার এবং আপনার শিশুর কি কি করতে হবে তা আমরা ব্যখ্যা করব। এর পর আমরা আপনার শিশুকে আমাদের গবেষনায় দেওয়ার জন্য আপনার সম্মতি চাইব।

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**উদ্দেশ্য :**

আপনার শিশুকে একটি গবেষনায় অংশগ্রহন করার জন্য আহবান করা হচ্ছে।

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**পদ্ধতি:**

১। আপনি যদি স্বেচ্ছায় আপনার শিশুকে এই গবেষনায় অংশগ্রহন করাতে চান তবে আমরা আপনাকে আপনার পরিবারের সদস্যদের শিক্ষা, পেশা এবং আয় ইত্যাদি সম্পর্কিত কিছু প্রশ্ন করব। এরপর আমরা আপনার শিশুকে ১৪দিনের ব্যবধানে উপরে উল্লেখিত যেকোন একটি টিকা খাওয়াবো। এ ছাড়া শিশুকে প্রথম টিকা খাওয়ানোর ১০ দিন পূর্ব হতে ২১ দিন পর পর্যন্ত প্রতিদিন ১০ মি.গ্রা.(৫ মি.লি.) জিংক খাওয়ানো হবে।

২। টিকার কার্যকারীতা পরীক্ষা করার জন্য আমরা গবেষনার শুরুতে এবং গবেষনার ৭ম ও ২১ তম দিনে আপনার শিশুর বাহুর শিরার থেকে একটি সরু সূঁইয়ের মাধ্যমে ৩মিঃ লিঃ (১/২ চামচের একটু বেশী) রক্তের নমুনা সংগ্রহ করব এবং একই দিনে আমরা আপনার শিশুর মলের(প্রায় ৫ গ্রাম) নমুনাও সংগ্রহ করব।

৩। আপনার শিশুকে টিকা খাওয়ানোর পর ৩ দিন পর্যন্ত প্রতিদিন আমাদেও মাঠ কর্মীরা আপনার শিশুকে দেখার জন্য বাড়ী যাবে।

৪। প্রতিবার রক্ত সংগ্রহ করার সময়, টিকা খাওয়ানোর সময়, এবং প্রাথমিক তথ্য সংগ্রহ করার জন্য সর্বমোট ৬ বার আপনার শিশুকে আমাদের মাঠ কেন্দ্রে নিয়ে আসতে হবে।

৫। পুরো গবেষনা সমাপ্ত হতে ৩২ দিন সময় লাগবে, এবং ৩ বারে সর্বমোট ৯ মিঃ লিঃ(প্রায় ২ চা চামচ) রক্ত সংগ্রহ করা হবে।

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**গবেষনায় অংশ গ্রহন না করার বিকল্প :**

এই গবেষনায় অংশ গ্রহন না করা।

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**ঝুঁকি সমূহ :**

এই গবেষনায় অংশ গ্রহনের ফলে আপনার শিশুকে বিশেষ কোন ঝুঁকির সম্মুখীন হতে হবে না। শিরা থেকে রক্ত নেওয়ার সময় সামান্য ব্যথা, ক্ষত সৃষ্টি, মাথা ঘুরানো বা জীবানুর সংক্রমন হওয়ার ক্ষীন সম্ভাবনা থাকে। যেটুকু রক্ত আপনার শিশুর শরীর থেকে নেয়া হবে তাতে তার স্বাস্থ্যের কোন ক্ষতি হবে না। গবেষনায় ব্যবহারকৃত ই,টি,ই,সি টিকা সুইডেন এবং মিসরে পরীক্ষায় দেখা গেছে যে টিকাটি নিরাপদ

অংশগ্রহনকারীর অনুস্বাক্ষর----------

এবং টিকা খাওয়ানোর পর ৩ দিনের মধ্যে শুধুমাত্র ২-৬% ক্ষেত্রে বমি বমি ভাব, মৃদু পেট মোচড়ানো এবং ১-২ বার পাতলা পায়খানা ছাড়া তেমন কোন পার্শ্ব প্রতিক্রিয়া দেখা যায়নি। মুখে খাওয়ানোর কলেরা টিকাও বাংলাদেশ সহ বিভিন্ন দেশে পরীক্ষা করা হয়েছে এবং সেটিও নিরাপদ টিকা বলে প্রমানিত হয়েছে। দৈনিক ১০ মি. গ্রা. জিংক (অর্থ্যাৎ নির্দেশিত মাত্রার দ্বিগুন) খাওয়ার কোন পার্শ্ব প্রতিক্রিয়া নেই। পূর্ববর্তী গবেষনা সমূহে দেখা গেছে যে, নিয়মিত জিংক খাওয়ানোর সহিত স্বাস্থ্যর ক্ষতিকর প্রভাবের কোন সম্পর্ক নেই।

**উপকারীতা/লাভঃ**

এই গবেষনায় আপনার শিশুর সরাসরি কোন উপকারে আসবে না। তবে আমাদের এই গবেষনার ফলাফল সামগ্রিকভাবে সমাজের সবার উপকারে আসবে। জনস্বাস্থ্য বিশেষজ্ঞরা এই গবেষনালব্ধ তথ্যকে কাজে লাগিয়ে বিশ্বের বিভিন্ন স্থানে প্রয়োজনীয় পদক্ষেপ নিতে পারবেন।

**গোপনীয়তাঃ**

এই গবেষনায় প্রাপ্ত তথ্য শুধুমাত্র আই, সি, ডি, ডি, আর, বি তে রক্ষিত থাকবে। আপনার শিশুর নাম ও অন্যান্য ব্যক্তিগত তথ্য সমূহ গোপন রাখা হবে এবং ফলাফল প্রকাশের সময় আপনার শিশুর পরিচিতি ব্যবহার করা হবে না। তবে পরিচয় গোপন রাখার পরিপূর্ন নিশ্চয়তা দেয়া সম্ভব নয়, কারন গবেষনায় প্রাপ্ত তথ্য প্রয়োজনে আদালতে সাক্ষ্য প্রমানের জন্য ব্যবহৃত হতে পারে।

**ক্ষতিপুরনঃ**

এই গবেষনার জন্য আমরা আপনার কাছে সময় চাইব। গবেষনায় অংশ গ্রহনের জন্য আপনাকে কোন খরচ বহন করতে হবে না। এই হাসপাতালে শিশুর সাথে আসার জন্য আপনাকে যাতায়াতের খরচ দেয়া হবে। যাতায়াতের এই খরচ আপনি যে দিন শিশুকে আমাদের স্বাস্থ্যকেন্দ্রে নিয়ে আসবেন শুধুমাত্র সেই দিনের জন্য দেয়া হবে। যদি গবেষনা কার্যক্রম শেষ হওয়ার পূর্বে আপনি নিজেকে প্রত্যাহার করার সিদ্ধান্ত নেন তাহলে ও আপনি যে দিন শিশুকে উক্ত গবেষনার কাজে আমাদের স্বাস্থ্যকেন্দ্রে নিয়ে আসবেন সেই দিনের জন্য আপনাকে যাতায়াতের খরচ দেয়া হবে।

**জরুরী পরিচর্যা ও অসুস্থতার কারন জনিত চিকিৎসাঃ**

যদি গবেষনা প্রক্রিয়ায় আপনার শিশুর কোন শারীরিক ক্ষতি হয় তবে আপনাকে বিনামূল্যে যথাসাধ্য চিকিৎসার ব্যবস্থা করা হবে। আই, সি, ডি, ডি, আর, বি এ ছাড়া অন্য কোন ধরনের ক্ষতিপুরন দিতে অপারগ।

**গবেষনায় অংশগ্রহন প্রত্যাহার করার অধিকারঃ**

এই গবেষনায় সম্পূর্ন স্বেচ্ছায় অংশগ্রহন করার জন্য আপনাকে আহবান করা হচ্ছে। গবেষনা কার্যক্রম শুরু হবার পরও আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন সেজন্য আপনাকে কোন ক্ষতিপূরন দিতে হবে না

**প্রশ্নোত্তরঃ**

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডঃ ফেরদৌসী কাদরী, ডাঃ এম এ সালাম, ডাঃ মহিউল ইসলাম চৌধুরী বা গবেষনা কর্মীরা দিবেন। ডঃ ফেরদৌসী কাদরী, ডাঃ মহিউল ইসলাম চৌধুরী ঠিকানাঃ ইমিউনোলোজী এল. এস. ডি. আই, সি, ডি, ডি, আর, বি, মহাখালী, ঢাকা, বাংলাদেশ, ফোন নং-৮৮১১৭৫১-৬০/ ২৪১৩। ডাঃ এম এ সালাম ঠিকানাঃ সি, এস, ডি, আই, সি, ডি, ডি, আর, বি মহাখালী, ঢাকা, বাংলাদেশ, ফোন নং-৮৮১১৭৫১-৬০/২৩০০ অথবা ৯৮৮২৩৯৯।

অংশগ্রহনকারীর অনুস্বাক্ষর----------

সম্মতি:

আপনার নীচের স্বাক্ষর এই অর্থ বহন করে যে, আপনি স্বেচ্ছায় আপনার শিশুকে গবেষনায় অংশগ্রহন করতে সম্মত হয়েছেন এবং আপনি উপরে বর্নিত তথ্য সমূহ এবং গবেষনায় অংগ্রহনকারী হিসাবে আপনার অধিকার সম্পর্কে অবগত আছেন।

অংশগ্রহনকারীর নাম----------

অংশগ্রহনকারীর অভিভাবকের স্বাক্ষর/ বৃদ্ধাঙ্গুলির ছাপ---------- তারিখ----------

গবেষকের নাম ----------

গবেষকের স্বাক্ষর ---------- তারিখ----------

স্বাক্ষীর নাম ----------

স্বাক্ষীর স্বাক্ষর ---------- তারিখ ----------

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষনের জন্য দেওয়া হবে। আপনি গবেষনা কাজে অংশগ্রহনকারী ব্যক্তির অধিকার সর্ম্পকিত আরো তথ্য জানতে চাইলে জনাব বিজয় সাহা, আই, সি, ডি, ডি, আর, বি, মহাখালী ঢাকা, ফোন নং-৮৮১১৭৫১-৬০/২১১৫ যোগাযোগ করুন।

## পরিশিষ্ট - 4c

গবেষনা (STUDY)

### "গবেষনায় অংশ গ্রহন করার জন্য সম্মতি পত্র"
### আন্তর্জাতিক উদারাময় গবেষনা কেন্দ্র, বাংলাদেশ (আই. সি. ডি. ডি. আর, বি)

**গবেষনার নাম:** ভিব্রিওকলেরী ও এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ই,টি,ই,সি)টিকার রোগ প্রতিরোধ ক্ষমতা বাড়ানোর গবেষনা

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**গবেষকদের নাম, বিভাগ, ফোন নং :**

(১) ডঃ ফেরদৌসী কাদরী ; ইমিউনোলোজী ; এল. এস. ডি ; আই, সি, ডি, ডি, আর, বি । ফোন নং-৮৮১১৭৫১-৬০/২৪৩১

(২) প্রফেসর এ্যানমেরী সেভেনারহোম (গোটেবর্গ ইউনিভার্সিটি, সুইডেন)।

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**সূচনা:**

ডায়রিয়া বাংলাদেশের একটি প্রধান স্বাস্থ্য সমস্যা এবং ভিব্রিওকলেরী ও এন্টারোটক্সিজেনিক *ইশেরিচিয়া কোলাই* (ই,টি,ই,সি) নামক জীবানু বাংলাদেশী শিশুদের মধ্যে ডায়রিয়ার প্রাদুর্ভাবের প্রধান কারন গুলির মধ্যে অন্যতম। অন্যান্য অনেক রোগের মত কিছু কিছু ডায়রিয়া, বিশেষ করে ভিব্রিও কলেরী এবং ই,টি,ই,সি জীবানু দ্বারা সংঘটিত ডায়রিয়া, টিকার মাধ্যমে প্রতিরোধ করা যেতে পারে।

আমরা এই এলাকায় আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র বাংলাদেশ (আই সি ডি ডি আর, বি)এর মাধ্যমে সুস্থ শিশুদের (৬ মাস থেকে ২বৎসর) উপর নিম্নবর্ণিত কলেরা অথবা ই,টি,ই,সি টিকা প্রয়োগ করে এদের কার্যকারীতা নিয়ে গবেষনা করব।

গবেষনায় অংশগ্রহনকারী শিশুদের দুভাগে ভাগ করা হয়েছে।

১) বুকের দুধ পান করবে ২) বুকের দুধ পান করা থেকে সাময়িক (টিকা খাওয়ানোর ৩ ঘন্টা পূর্বে এবং ১ ঘন্টা পরে ) বিরত থাকবে

গবেষনায় অংশগ্রহনকারী শিশুদের নিম্নের যে কোন একটি টিকা খেতে হবে।

১. কলেরা টিকা ২. ই,টি,ই,সি টিকা

যেহেতু আপনার শিশু ৬ মাস থেকে ২বৎসরের মধ্যে সেহেতু আপনি যদি আপনার শিশুকে আমাদের গবেষনায় অংশগ্রহন করাতে ইচ্ছুক থাকেন তাহলে আপনার এবং আপনার শিশুর কি কি করতে হবে তা আমরা ব্যখ্যা করব। এর পর আমরা আপনার শিশুকে আমাদের গবেষনায় দেওয়ার জন্য আপনার সম্মতি চাইব।

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**উদ্দেশ্য :**

আপনার শিশুকে একটি গবেষনায় অংশগ্রহন করার জন্য আহবান করা হচ্ছে।

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**পদ্ধতি:**

১। আপনি যদি স্বেচ্ছায় আপনার শিশুকে এই গবেষনায় অংশগ্রহন করাতে চান তবে আমরা আপনাকে আপনার পরিবারের সদস্যদের শিক্ষা, পেশা এবং আয় ইত্যাদি সম্পর্কিত কিছু প্রশ্ন করব। এরপর আমরা আপনার শিশুকে ১৪দিনের ব্যবধানে উপরে উল্লেখিত যেকোন একটি টিকা খাওয়াবো। দৈবচয়ন পদ্ধতিতে শিশুকে টিকা খাওয়ানোর ৩ ঘন্টা পূর্বে এবং ১ ঘন্টা পরে বুকের দুধ করা হতে বিরত রাখার জন্য বলা হতে পারে।

২। টিকার কার্যকারীতা পরীক্ষা করার জন্য আমরা গবেষনার শুরুতে এবং গবেষনার ৭ম ও ২১ তম দিনে আপনার শিশুর বাহুর শিরার থেকে সরু সুঁইয়ের মাধ্যমে ৩মিঃ লিঃ (১/২ চামচের একটু বেশী) রক্তের নমুনা সংগ্রহ করব এবং একই দিনে আমরা আপনার শিশুর মলের(প্রায় ৫ গ্রাম) নমুনাও সংগ্রহ করব।

৩। আপনার শিশুকে টিকা খাওয়ানোর পর ৩ দিন পর্যন্ত প্রতিদিন আমাদের মাঠ কর্মীরা আপনার শিশুকে দেখার জন্য বাড়ী যাবে।

৪। প্রতিবার রক্ত সংগ্রহ করার সময়, টিকা খাওয়ানোর সময়, এবং প্রাথমিক তথ্য সংগ্রহ করার জন্য সর্বমোট ৬ বার আপনার শিশুকে আমাদের মাঠ কেন্দ্রে নিয়ে আসতে হবে।

৫। পুরো গবেষনা সমাপ্ত হতে ২১ দিন সময় লাগবে, এবং ৩ বারে সর্বমোট ৯ মিঃ লিঃ(প্রায় ২ চা চামচ) রক্ত সংগ্রহ করা হবে।

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**গবেষনায় অংশ গ্রহন না করার বিকল্প :**

এই গবেষনায় অংশ গ্রহন না করা।

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**ঝুঁকি সমূহ :**

এই গবেসনায় অংশ গ্রহনের ফলে আপনার শিশুকে বিশেস কোন ঝুঁকির সম্মুখীন হতে হবে না। শিরা থেকে রক্ত নেওয়ার সময় সামান্য ব্যথা, ক্ষত সৃষ্টি, মাথা ঘুরানো বা জীবানুর সংক্রমন হওয়ার ক্ষীন সম্ভাবনা থাকে। যেটুকু রক্ত আপনার শিশুর শরীর থেকে নেয়া হবে

তাতে তার স্বাস্থ্যের কোন ক্ষতি হবে না। গবেষনায় ব্যবহারকৃত ই,টি,ই,সি টিকা সুইডেন এবং মিসরে পরীক্ষায় দেখা গেছে যে টিকাটি নিরাপদ এবং টিকা খাওয়ানোর পর ৩ দিনের মধ্যে শুধুমাত্র ২-৬% ক্ষেত্রে বমি বমি ভাব, মৃদু পেট মোচড়ানো এবং ১-২ বার পাতলা পায়খানা ছাড়া তেমন কোন পার্শ্ব প্রতিক্রিয়া দেখা যায়নি। মুখে খাওয়ানোর কলেরা টিকাও বাংলাদেশ সহ বিভিন্ন দেশে পরীক্ষা করা হয়েছে এবং সেটিও নিরাপদ টিকা বলে প্রমানিত হয়েছে।

**উপকারীতা/লাভঃ**

এই গবেষনায় আপনার শিশুর সরাসরি কোন উপকারে আসবে না। তবে আমাদের এই গবেষনার ফলাফল সামগ্রিকভাবে সমাজের সবার উপকারে আসবে। জনস্বাস্থ্য বিশেষজ্ঞরা এই গবেষনালব্ধ তথ্যকে কাজে লাগিয়ে বিশ্বের বিভিন্ন স্থানে প্রয়োজনীয় পদক্ষেপ নিতে পারবেন।

**গোপনীয়তাঃ**

এই গবেষনায় প্রাপ্ত তথ্য শুধুমাত্র আই, সি, ডি, ডি, আর, বি তে রক্ষিত থাকবে। আপনার শিশুর নাম ও অন্যান্য ব্যক্তিগত তথ্য সমূহ গোপন রাখা হবে এবং ফলাফল প্রকাশের সময় আপনার শিশুর পরিচিতি ব্যবহার করা হবে না। তবে পরিচয় গোপন রাখার পরিপূর্ন নিশ্চয়তা দেয়া সম্ভব নয়, কারন গবেষনায় প্রাপ্ত তথ্য প্রয়োজনে আদালতে সাক্ষ্য প্রমানের জন্য ব্যবহৃত হতে পারে।

**ক্ষতিপুরনঃ**

এই গবেষনার জন্য আমরা আপনার কাছে সময় চাইব। গবেষনায় অংশ গ্রহনের জন্য আপনাকে কোন খরচ বহন করতে হবে না। এই হাসপাতালে শিশুর সাথে আসার জন্য আপনাকে যাতায়াতের খরচ দেয়া হবে। যাতায়াতের এই খরচ আপনি যে দিন শিশুকে আমাদের স্বাস্থ্যকেন্দ্রে নিয়ে আসবেন শুধুমাত্র সেই দিনের জন্য দেয়া হবে। যদি গবেষনা কার্যক্রম শেষ হওয়ার পূর্বে আপনি নিজেকে প্রত্যাহার করার সিদ্ধান্ত নেন তাহলে ও আপনি যে দিন শিশুকে উক্ত গবেষনার কাজে আমাদের স্বাস্থ্যকেন্দ্রে নিয়ে আসবেন সেই দিনের জন্য আপনাকে যাতায়াতের খরচ দেয়া হবে।

**জরুরী পরিচর্যা ও অসুস্থতার কারন জনিত চিকিৎসাঃ**

যদি গবেষনা প্রক্রিয়ায় আপনার শিশুর কোন শারীরিক ক্ষতি হয় তবে আপনাকে বিনামূল্যে যথাসাধ্য চিকিৎসার ব্যবস্থা করা হবে। আই, সি, ডি, ডি, আর, বি এ ছাড়া অন্য কোন ধরনের ক্ষতিপুরন দিতে অপারগ।

**গবেষনায় অংশগ্রহন প্রত্যাহার করার অধিকারঃ**

এই গবেষনায় সম্পূর্ন স্বেচ্ছায় অংশগ্রহন করার জন্য আপনাকে আহবান করা হচ্ছে। গবেষনা কার্যক্রম শুরু হবার পরও আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন সেজন্য আপনাকে কোন ক্ষতিপূরন দিতে হবে না

**প্রশ্নোত্তরঃ**

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডঃ ফেরদৌসী কাদরী, ডাঃ এম এ সালাম, ডাঃ মহিউল ইসলাম চৌধুরী বা গবেষনা কর্মীরা দিবেন। ডঃ ফেরদৌসী কাদরী, ডাঃ মহিউল ইসলাম চৌধুরী ঠিকানাঃ ইমিউনোলোজী এল. এস. ডি. আই, সি, ডি, ডি, আর, বি, মহাখালী, ঢাকা, বাংলাদেশ, ফোন নং-৮৮১১৭৫১-৬০/ ২৪১৩। ডাঃ এম এ সালাম ঠিকানাঃ সি, এস, ডি, আই, সি, ডি, ডি, আর, বি মহাখালী, ঢাকা, বাংলাদেশ, ফোন নং-৮৮১১৭৫১-৬০/২৩০০ অথবা ৯৮৮২৩৯৯।

অংশগ্রহনকারীর অনুস্বাক্ষর----------

**সম্মতি:**

আপনার নীচের স্বাক্ষর এই অর্থ বহন করে যে, আপনি স্বেচ্ছায় আপনার শিশুকে গবেষনায় অংশগ্রহন করতে সম্মত হয়েছেন এবং আপনি উপরে বর্নিত তথ্য সমূহ এবং গবেষনায় অংগ্রহনকারী হিসাবে আপনার অধিকার সম্পর্কে অবগত আছেন।

অংশগ্রহনকারীর নাম----------

অংশগ্রহনকারীর অভিভাবকের স্বাক্ষর/ বৃদ্ধাঙ্গুলির ছাপ---------- তারিখ----------

গবেষকের নাম ----------

গবেষকের স্বাক্ষর ---------- তারিখ----------

স্বাক্ষীর নাম ----------

স্বাক্ষীর স্বাক্ষর ---------- তারিখ ----------

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষনের জন্য দেওয়া হবে। আপনি গবেষনা কাজে অংশগ্রহনকারী ব্যক্তির অধিকার সর্ম্পকিত আরো তথ্য জানতে চাইলে জনাব বিজয় সাহা, আই, সি, ডি, ডি, আর, বি, মহাখালী ঢাকা, ফোন নং-৮৮১১৭৫১-৬০/২১১৫ যোগাযোগ করুন।

| Personnel | Position | Rate | Effort % | Salary | 1st Year US$ | SEK | 2nd Year US$ | SEK | 3rd Year US$ | SEK | TOTAL US$ | SEK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Please note, the funds below from the ICDDR,B consumable component will be kept in Goteborg for purchasing reagents for ICDDR,B | | | | | 2000 | 14,895 | 1,200 | 8,937 | 1,800 | 13,405 | 5,000 | 37,237 |

## Total funds needed for study for ICDDR,B and Goteborg University

Total funds needed for study for ICDDR,B and Goteborg University

| | 1st Year US$ | SEK | 2nd Year US$ | SEK | 3rd Year US$ | SEK | TOTAL US$ | SEK |
|---|---|---|---|---|---|---|---|---|
| ICDDR,B | 59,082 | 440,000 | 59,753 | 444,998 | 71,166 | 529,995 | 190,001 | 1,414,993 |
| Goteborg University | 24,170 | 180,000 | 17,456 | 130,003 | 29,541 | 220,000 | 71,167 | 530,003 |
| Total Sida-SAREC | 83,252 | 620,001 | 77,209 | 575,001 | 100,707 | 749,995 | 261,168 | 1,944,995 |
| {Please note, the funds below are included in the budget above in the ICDDR,B component for purchasing reagents for ICDDR,B | 2,000 | 14,895 | 1,200 | 8,937 | 1,800 | 13,405 | 5,000 | 37,237 |

## A.Detailed Budget for Sida-SAREC proposal- Fqadri, ICDDR,B ETEC/Cholera Vaccine studies

| Personnel | Position | Rate | Effort % | Salary | 1st Year | | 2nd Year | | 3rd Year | | TOTAL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | US$ | SEK | US$ | SEK | US$ | SEK | US$ | SEK |
| FQadri | Sen.Scient | 9821 | 5.10..% | P5 | 5,893 | 43,887 | 12,257 | 91,282 | 12,747 | 94,933 | 30,897 | 230,101 |
| M.A. Salam | Director-CSD | 11526 | Eff-5%,Sal-0% | D1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M.I. Chow | Medical Officer | 739 | 50% | NOA | 4,434 | 33,021 | 4,655 | 34,672 | 4,888 | 36,406 | 13,978 | 104,100 |
| To be named | Res.Officer | 410 | 100% | GS5 | 4,920 | 36,641 | 5,166 | 38,473 | 5,424 | 40,395 | 15,510 | 115,510 |
| To be named | Research Asst. | 311 | 100% | GS3 | 3,732 | 27,793 | 3,919 | 29,183 | 4,115 | 30,642 | 11,765 | 87,618 |
| Parveen | Lab Tech. | 302 | 100% | GS3 | 3,624 | 26,989 | 3,805 | 28,338 | 3,995 | 29,755 | 11,425 | 85,083 |
| Jahir | Lab attendant | 210 | 100% | GS1 | 2,520 | 18,767 | 2,646 | 19,706 | 2,778 | 20,691 | 7,944 | 59,164 |
| To be named | Attendant.X2 | 122 | 100% | | 2,928 | 21,806 | 3,074 | 22,896 | 3,228 | 24,041 | 9,231 | 68,742 |
| To be named | FRAx2 | 292 | 100% | GS3 | 7,008 | 52,191 | 7,358 | 54,800 | 7,726 | 57,540 | 22,093 | 164,531 |
| **sub total** | | | | | **35,059** | **261,095** | **42,881** | **319,350** | **44,903** | **334,405** | **122,843** | **914,849** |
| Local travel | | | | | 1,000 | 7,447 | 200 | 1,489 | 800 | 5,958 | 2,000 | 14,895 |
| International | | | | | 1,500 | 11,171 | 1,000 | 7,447 | 2,000 | 14,895 | 4,500 | 33,513 |
| **sub total** | | | | | **2,500** | **18,618** | **1,200** | **8,937** | **2,800** | **20,852** | 6,500 | 48,407 |
| Supplies and materials | | | | | | | | | | | | |
| Immunoreagents,plastics, | | | | | 2,107 | 15,688 | 500 | 3,724 | 2,000 | 14,895 | 4,607 | 34,308 |
| Supplies from Goteborg for.ICDDR,B | | | | | 2,000 | 14,895 | 1,200 | 8,937 | 1,800 | 13,405 | 5,000 | 37,237 |
| Medical supplies | | | | | 500 | 3,724 | 200 | 1,489 | 800 | 5,958 | 1,500 | 11,171 |
| Food charges etc for volunteers | | | | | 1000 | 7,447 | 421 | 3,135 | 500 | 3,724 | 1,921 | 14,306 |
| Subtotal | | | | | 5,607 | 41,753 | 2,321 | 17,285 | 5,100 | 37,981 | 13,028 | 97,019 |
| Repair/maintainence | | | | | 1000 | 7,447 | 500 | 3,724 | 1500 | 11,171 | 3,000 | 22,342 |
| Rent,comm,field office, utilities | | | | | | | | | | | | |
| electricity, publication | | | | | | | | | | | | |
| staff dev, training/workshop | | | | | | | | | | | | |
| printing, publication | | | | | | | | | | | | |

## A.Detailed Budget for Sida-SAREC proposal- Fqadri, ICDDR,B ETEC/Cholera Vaccine studies

| Personnel | Position | Rate | Effort % | Salary | 1st Year | | 2nd Year | | 3rd Year | | TOTAL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | US$ | SEK | US$ | SEK | US$ | SEK | US$ | SEK |
| FQadri | Sen.Scient | 9821 | 5,10..% | P5 | 5,893 | 43,887 | 12,257 | 91,282 | 12,747 | 94,933 | 30,897 | 230,101 |
| M.A. Salam | Director-CSD | 11526 | Eff-5%,Sal-0% | D1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M.I. Chow | Medical Officer | 739 | 50% | NOA | 4,434 | 33,021 | 4,656 | 34,672 | 4,888 | 36,406 | 13,978 | 104,100 |
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| To be named | FRAx2 | 292 | 100% | GS3 | 7,008 | 52,191 | 7,358 | 54,800 | 7,726 | 57,540 | 22,093 | 164,531 |
| sub total | | | | | 35,059 | 261,095 | 42,881 | 319,350 | 44,903 | 334,405 | 122,843 | 914,849 |
| Local travel | | | | | 1,000 | 7,447 | 200 | 1,489 | 800 | 5,958 | 2,000 | 14,895 |
| International | | | | | 1,500 | 11,171 | 1,000 | 7,447 | 2,000 | 14,895 | 4,500 | 33,513 |
| **sub total** | | | | | **2,500** | **18,618** | **1,200** | **8,937** | **2,800** | **20,852** | 6,500 | 48,407 |
| Supplies and materials | | | | | | | | | | | | |
| Immunoreagents,plastics, | | | | | 2,107 | 15,688 | 500 | 3,724 | 2,000 | 14,895 | 4,607 | 34,306 |
| Supplies from Goteborg for.ICDDR,B | | | | | 2,000 | 14,895 | 1,200 | 8,937 | 1,800 | 13,405 | 5,000 | 37,237 |
| Medical supplies | | | | | 500 | 3,724 | 200 | 1,489 | 800 | 5,958 | 1,500 | 11,171 |
| Food charges etc for volunteers | | | | | 1000 | 7,447 | 421 | 3,135 | 500 | 3,724 | 1,921 | 14,306 |
| Subtotal | | | | | 5,607 | 41,753 | 2,321 | 17,285 | 5,100 | 37,981 | 13,028 | 97,020 |
| Repair/maintainence | | | | | 1000 | 7,447 | 500 | 3,724 | 1500 | 11,171 | 3,000 | 22,342 |
| Rent,comm,field office, utilities | | | | | | | | | | | | |
| electricity, publication | | | | | | | | | | | | |
| staff dev, training/workshop | | | | | | | | | | | | |
| printing, publication | | | | | | | | | | | | |

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# *Memorandum*

23 November 2004

To : Dr. Firdausi Qadri
Principal Investigator of research protocol # 2004-046
Laboratory Sciences Division (LSD)

From: David A. Sack, MD
Chairman
Research Review Committee (RRC)

Sub : Approval of research protocol # 2004-046

Thank you for your memo dated November 21, 2004 with the modified version of your research protocol # 2004-046 titled "Studies to enhance and improve immunogenicity of cholera and enterotoxigenic *E. coli* (ETEC) vaccines". The issues that were raised by the RRC in its meeting held on November 9, 2004 on your research protocol have been addressed in the modified version of the protocol to the satisfaction of the Committee. Accordingly, the Committee approved the research protocol to proceed subject to the approval of the ERC.

**Terms of approval**

The research protocol is approved as submitted for 3-year period from the date of starting the activities of the protocol. You should, therefore, notify the Committee Coordination Secretariat of the start date of the protocol.

This approval is only valid whilst you hold a position at ICDDR,B; and in the event of your departure from the Centre, a new Principal Investigator will be designated for the research protocol.

This approval shall remain valid for starting the protocol for a period up to 2 years from the date of the approval of the ERC, after two years, you shall have to seek approval (revalidation) of the RRC/ERC before starting the protocol. The RRC/ERC approval shall automatically deemed to be revoked after three years if the protocol is not started.

You should notify the RRC and the ERC immediately of any serious or unexpected adverse effects on participants or unforeseen events that might affect continued acceptability of the protocol.

Any changes to the research protocol require the submission and approval of an amendment/addendum. Substantial variations may require a new protocol.

Continued approval of this protocol is dependent on your periodically updating the Centre's database for the protocol to show the progress; and a final report/completion report should be submitted at the conclusion of the protocol.

You shall submit a report for time extension of the protocol (in prescribed form) if you are unable to complete the protocol activities within the time mentioned in the protocol.

The RRC should be notified if the project is discontinued before the expected date of completion. The report form is available at the Committee Coordination Secretariat and on the Centre's intranet.

You are responsible for systematic storage and retention of the original data pertaining to the research protocol; and the ownership of data after certain period shall be determined as per Centre's rules and regulations.

I wish you all the success in conducting the research protocol.

Thank you once again.

Copy: Director, LSD