ICDDR,B Library

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 15 December 1983
12.1.84

Principal Investigator JOHN CLEMENS Trainee Investigator (if any)

Application No. 84-001 Supporting Agency (if Non-ICDDR,B)

Title of Study Cholera Vaccine Trial

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects — Yes (No)
(b) Non-ill subjects — (Yes) No
(c) Minors or persons under guardianship — (Yes) No

Does the study involve:
(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — (Yes) No
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids — (Yes) No

Are subjects clearly informed about:
(a) Nature and purposes of study — (Yes) No
(b) Procedures to be followed including alternatives used — (Yes) No
(c) Physical risks — (Yes) No
(d) Sensitive questions — Yes (No)
(e) Benefits to be derived — (Yes) No
(f) Right to refuse to participate or to withdraw from study — (Yes) No
(g) Confidential handling of data — (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will signed consent form be required:
(a) From subjects — (Yes) No
(b) From parent or guardian (if subjects are minors) — (Yes) No
Also Verbal Consent in some Cases

6. Will precautions be taken to protect anonymity of subjects — (Yes) No

7. Check documents being submitted herewith to Committee:

___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
✓ Informed consent form for subjects
✓ Informed consent form for parent or guardian
✓ Procedure for maintaining confidentiality
___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

Principal Investigator

AM 1.06

Trainee

ttachment 1a.

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Cttee will not consider any application which does not include an abstract summary. The abstract should summarize the purpose of the study, the methods and procedures to be used, by addressing each of the following items. If an item is not applicable, please note accordingly:

1. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and an assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.

5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

   (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course of action.

   (c) If there is a potential risk to the subject or privacy of the individual is involved in any particular procedure include a statement in the consent form stating whether or not compensation and/or treatment will be available.

6. If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

ie statement to the subject should include information specified in items 2, 3, 4, c) and 7, as well as indicating the approximate time required for participation in the tivity.

ttachment 1a.

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

he Cttee will not consider any application which does not include an abstract summary. he abstract should summarize the purpose of the study, the methods and procedures to e used, by addressing each of the following items. If an item is not applicable, lease note accordingly:

- Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

- Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

- Describe procedures for protecting against or minimizing potential risks and an assessment of their likely effectiveness.

- Include a description of the methods for safeguarding confidentiality or protecting anonymity.

- When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

  (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

  (b) If information is to be withheld from a subject, justify this course of action.

  (c) If there is a potential risk to the subject or privacy of the individual is involved in any particular procedure include a statement in the consent form stating whether or not compensation and/or treatment will be available.

- If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

- Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned work. Indicate how the benefits outweigh the risks.

- State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

ie statement to the subject should include information specified in items 2, 3, 4, c) and 7, as well as indicating the approximate time required for participation in the tivity.

SECTION I - RESEARCH PROTOCOL

1. Title: Field Trial of Oral B-Subunit/Whole-cell Cholera Vaccine

2. Principal Investigator: John D. Clemens

Co-Investigators: J. Chakraborty. M.I. Huq, M.U. Khan, B. Stanton, and M. Yunus

Consultants: W.B. Greenough, S. Walter

3. Starting Date: January 1, 1984

4. Completion Date: July 1, 1985 (earliest)

5. Total Direct Cost: US$ 986,951.78

6. Scientific Program Head:

This protocol has been approved by the Disease Transmission Working Group.

Signature of Scientific Program Head: K. M. S. Aziz

Date: 10/1/84

7. Abstract Summary:

Prior to the ~~1984~~ 1985 fall cholera season, all children aged 2-14 years and all non-pregnant women aged >14 years living in selected portions of the Matlab field study area will be given either oral B-subunit/whole-cell cholera vaccine or a placebo vitamin preparation in a randomized, double-blinded fashion. Prior to this administration, the vaccine lot for the trial will have been demonstrated to be safe and immunogenic in a pretrial conducted during ~~March-May, 1984~~ July - Sept. 1985. The vaccine and placebo agents will be given with a solution designed to raise gastric pH to a level of ≥4.0 for 30 minutes. The vaccine will be given in three doses, and the doses will be separated by intervals of 4 weeks. In the first dose, 2 mg

B-subunit/$2x10^{11}$ killed whole-cells will be given, and in the second and third doses 1 mg B-subunit/$1x10^{11}$ killed whole-cells will be given. The efficacy of the vaccine in preventing cholera and/or decreasing the severity of illness will be assessed by comparing the vaccine and placebo groups for the incidence of cholera cases who are treated at the Matlab Hospital and the Nayergaon treatment center, or who identified during intenseive surveillance studies in the field. The efficacy of the vaccine against heat-labile (LT) enterotoxin-producing Escherichia coli will be determined by performing stool cultures of cases visiting Matlab Hospital and Nayergaon treatment center. Serosurveys and breast milk surveys will be conducted at various times during the trial to determine the height and duration of antibacterial and antitoxin responses to the vaccine and to assess the relation between these vaccine-induced responses and clinical protection against diarrhoea dur to cholera and LT - E. coli.

8. Reviews:

(a) Research Involving Human Subjects: _____

(b) Research Review Committee: _____

(c) Director: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: To test the efficacy of oral B subunit/whole cell cholera vaccine in preventing clinical cholera in Matlab Bazar Thana.

2. Background: The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), formerly the Cholera Research Laboratory (CRL), has conducted controlled cholera vaccine trials since 1962 in its Vaccine Trial Surveillance (VTS) area in Matlab Bazar Thana, a rural area in Comilla District in Bangladesh. Between 1963 and 1970, four field trials were conducted using killed bacterial vaccines (1-4). Results of these trials confirmed the efficacy of immunization with these products but this protection was found to be of short duration. With the discovery in the late 1960's that cholera diarrhoea was caused by an action of an enterotoxin produced in vivo (5) attention was directed toward development of an immunizing toxoid. In 1974 the first field trial of a toxoid was conducted at CRL; the toxoid tested was treated with glutaraldehyde and contained protamine and aluminum phosphate adjuvants. Protection was observed against Inaba cholera for a short duration (approximately 3 months) and only in children 5-14 years of age (6). Relatively high levels of serum antitoxin produced by the vaccine did not ameliorate the clinical severity of disease (7).

Because of the limited efficacy of vaccines delivered by the parenteral route, interest has turned to the development of oral vaccines

which could stimulate local immunity in the intestine. The concept that mucosal immunity may be critically important in vaccine efficacy has been reinforced by animal experiments indicating that anti-bacterial and antitoxin antibodies in the gut act synergistically to provide protection (8). Accordingly, a combined vaccine consisting of B-subunit (mucosal receptor attachment fragment) of the cholera toxin and killed whole cholera cells has been developed and tested in North America, Europe, and Bangladesh. The B-subunit portion of the vaccine has been produced by Institut Merieux (Lyon, France) and the killed whole cell portion has been produced by the National Bacteriological Laboratory (Stockholm, Sweden). Experiments in humans with the combined B-subunit/whole-cell (BS/WC) oral vaccine indicate that the oral route is superior to the parenteral route in stimulating the formation of secretory IgA (SIgA) antibody in the gut mucosa and local immunologic memory (9). Moreover, when compared with clinical cholera in humans, the combined oral vaccine produced both equivalent acute phase mucosal SIgA responses and equivalent local immunologic memory upon re-challenge with cholera antigens (9).

In view of the cogent evidence that the oral BS/WC vaccine offers a promising new approach to the control of cholera, an informal consultation of WHO experts, convened in Washington, D.C. June 10-11, 1983, concluded:

"The group agrees that there is sound justification for the under-taking of a field trial with the B-subunit/whole-cell vaccine at

ICDDR,B..... and recommends that a proposal to this effect be submitted for consideration by the appropriate authorities at ICDDR,B, the Bangladesh Medical Research Council, and the Government of Bangladesh" (10).

The ICDDR,B proposes to conduct a field trial of the BS/WC vaccine. Included in this trial will be an evaluation of a vaccine preparation containing both a portion of the toxin and whole cells against clinical and asymptomatic cholera. In addition, since heat-labile (LT) toxin of Escherichia coli and cholera toxin are structurally and antigenically similar (11), the efficacy of the B-subunit portion of the toxin in protecting against diarrhoea caused by strains of E. coli that produce LT toxin will be studied. Such protection has been observed in animals (12).

The Matlab field study area of ICDDR,B includes approximately 180,000 persons living in 160 villages. In this area the ICDDR,B maintains an accurate registration system for vital events through the efforts of its field staff (composed of approximately 65 field assistants and supervisory personnel) and operates ambulance boat stations to facilitate transport of diarrhoeal patients to a central treatment center (Matlab Hospital). These combined abilities and facilities make ICDDR,B an ideal place to study vaccine efficacy and safety. In addition, since the last vaccine field trial, cholera

has continued to be a frequent cause of diarrhoea in patients treated at the Matlab Hospital (13), and recent studies have demonstrated that LT-producing E. coli is a frequently isolated pathogen in patients seen at the Hospital (14).

3. Rationale: Cholera is an important cause of morbidity and mortality in Bangladesh and other developing countries. While the ultimate control of cholera is through establishment of proper sanitation and waste disposal measures to provide safe water and food, these measures cannot be presently achieved in many developing countries because of their cost and the long-established water usage and sewage disposal patterns of persons living in them. Thus, development of a vaccine to prevent cases and ameliorate severity of disease at present provides the best opportunity to decrease the impact on society of the high mortality and high morbidity from cholera. If this vaccine could also provide protection against diarrhoea caused by LT-producing ETEC, another major cause of diarrhoea in developing countries, its importance as a preventive health measure would be even more significant.

B. SPECIFIC AIMS

1. Primary: To determine the protective efficacy of BS/WC vaccine in preventing clinically severe cholera and to assess the duration of protection.

2. Secondary:

a. To assess the protective efficacy and the duration of efficacy of BS/WC vaccine against symptomatic but mild cholera, and against asymptomatic cholera.

b. To evaluate the efficacy and duration of effect of BS/WC vaccine against diarrhoea caused by LT - producing ETEC and non-cholera vibric organisms.

c. To correlate the level of serum vibriocidal and antitoxin titers induced by BS/WC vaccine with protection conferred by the vaccine against cholera and ETEC.

d. To examine whether vaccine-induced immunity in mothers confers protection against cholera and ETEC in unimmunized breast-feeding children

C. RESULTS OF EARLIER STUDIES

1. Vaccine Safety:

Initial studies in Sweden and Bangladesh demonstrated that the vaccine given orally caused no detectable side-effects. In the studies in Sweden, 10 adult volunteers received one or two doses of vaccine containing 0,5 mg of B-subunit and 5×10^{10} organisms of the heat-killed classical Inaba and Ogawa vaccine components. In no instance were any local or systemic side-effects observed within one month after immunization (15). In subsequent studies, 30 adult Swedes and 16 Bangladeshi women were given two doses of vaccine each containing 0,5-2.5 mg of B-subunit and 5×10^{10} of the heat-killed classical

Inaba and Ogawa vibrios. Surveillance for side reactions was performed for 10 days after each immunization by a physician or a health worker; in no instance did these oral immunizations give rise to any detectable side-effects (15).

In more recent studies in Bangladesh, one or two doses (given one day apart) of 1 mg or 5 mg of B-subunit alone were given orally to about 1,200 family contacts (aged 1-70 years) of patients with cholera to evaluate its possible effectiveness as a toxin-receptor blocking agent. No local or systemic side-effects were observed (16).

2. Mucosal Immunogenicity and Serum Antibodies in Volunteers:

At the ICDDR,B and in Sweden, mucosal immunogenicity studies have provided information on the ability of the BS/WC vaccine to stimulate mucosal antibody in the intestine of volunteers. The whole-cell portion of the vaccine used in these studies (9) consisted of the heat-killed classical Inaba and Ogawa vaccine components (2.5×10^{10} organisms of each per oral dose) of the presently proposed vaccine. Three groups (I-III) of 8-9 healthy Bengali women were given 2 oral or 2 intramuscular doses of vaccine 28 days apart and were compared with one group (9 persons) of cholera convalescents given a single oral vaccination. Five minutes before the oral immunization 100 cc of 0.1 m $NaHCO_3$ solution was given to neutralize gastric acidity; the oral vaccine was then administered in 100 cc of the same solution. Intestinal lavage was performed and fluid specimens were examined 0, 3, 9, and 28 days after each immunization, or (for cholera convalescents) 9 and

28 days after the onset of disease. Antitoxin and antibacterial antibodies in the lavage fluids were measured by an ELISA test using purified cholera toxin and LPS, respectively, as solid phase antigens. Total IgA was also determined by ELISA to permit the expression of all titers in relation to total IgA.

The results showed that a single peroral administration of 2,5 mg of B subunit induced a significant increase in antitoxin titer in most recipients. Two immunizations with a 0,5 mg dose of B subunit also induced a significant mucosal antitoxin response in most instances. Although intramuscular injections also induced a rise in the antitoxin IgA titer in many cases, the duration of the response was significantly shorter than after oral administration. The study thus showed the ability of B-subunit, administered orally, to stimulate intestinal mucosal antitoxin responses in Bangladeshi adults.

Measurements of the intestinal mucosal antibacterial antibody response revealed that although clinical cholera induced a substantially increased titer of intestinal IgA antibody to V. cholerae LPS in most recipients, increases occurred less frequently and were of lesser magnitude following a single oral or intramuscular vaccination with whole-cell vaccine. However, following the second oral administration of the whole cell vaccine, intestinal antibacterial resonses were induced that were comparable to those following disease in 12 out of 13 vaccinees. Both the magnitude and the duration of the mucosal

response attained after two oral administrations were superior to those obtained by the 2-dose parenteral immunization regime.

As its final objective, this study explored the extent to which immunization could induce (or, in the naturally-primed Bangladeshi volunteers, perhaps boost) immunological memory for a mucosal response. Oral immunization with combined vaccine appeared to be as effective as clinical cholera in preparing the intestine for a local IgA antibody response to restimulation by cholera antigens. Both the antitoxin and the anti-LPS responses were seen within 3 days after the second immunizations, which was earlier than in the case of the initial vaccinations. As regards the mucosal IgA antitoxin, a rapid response was also seen in many volunteers who had been vaccinated with B subunit 15 months earlier, suggesting that antitoxic memory was long-lasting. (9)

The SIgA antitoxin and anti-LPS antibody responses in intestinal lavage specimens following one or two immunizations with similar doses of combined vaccine were also studied in adult Swedish volunteers (15). Significant titer increases of both antibodies were observed in most vaccinees, but they were about 50% lower than those observed in Bangladeshi volunteers.

3. Challenge Studies:

Studies of vaccine efficacy were recently performed at the Center for Vaccine Development in Baltimore, USA (17). Nineteen adult volunteers

received 3 oral doses of combined vaccine at 2-week intervals. Each dose of vaccine consisted of 5 mg of B-subunit and 5×10^{10} heat-killed classical Inaba, 5×10^{10} heat-killed classical Ogawa and 1×10^{11} formalin-treated El Tor Inaba organisms - i.e. the first three components of the whole-cell vaccine proposed for the field trial. Three hours prior to ingesting vaccine, the volunteers ingested 300 mg of cimetidine; one minute before vaccination, they took 2 g sodium bicarbonate in 150 ml of distilled water. Antibody responses in jejunal fluid and serum were measured after each immunization. Five weeks after the last immunization, the susceptibility of 11 vaccinees to oral challenge with 2×10^6 live El Tor V. cholerae organisms (strain N16961) administered with 2 g sodium bicarbonate was compared with that of unimmunized control volunteers.

No notable adverse reactions were observed in any vaccine recipients. The results of the challenge studies showed that the vaccine gave significant protection against an ID_{100} challenge dose with live vibrios: 4 of 11 vaccinated persons as compared with 7 of 7 controls developed diarrhoeal illness after challenge (64% vaccine efficacy, $p = 0,01$). The vaccination afforded complete protection against severe disease; no vaccinee had diarrhoea exceeding 1 litre, while 4 of 7 controls had diarrhoeal stools of 2 litres or more. The vaccine had no effect on the rate of excretion of V. cholerae organisms, though the quantity of excreted organisms was slightly less in the vaccinated group.

D. METHODS OF PROCEDURE:

1. Nature of the Antigens:

Purified B-subunit of cholera toxin will be prepared by Institut Merieux, France, in accordance with European Pharmacopy recommendations for human vaccine, using the procedure previously described (18). After sterile filtering, B-subunit will be diluted to a final concentration of 1 mg per ml with phosphate buffered saline. Prior to use the vaccine lot will be tested for safety in mice and guinea pigs with oral, intra-peritoneal, and intravenous administration of up to 5 ml doses according to the requirements of the European Pharmacopy, and will be ascertained to have no toxic activity in rabbit skin (19) and small bowel loops (20) when tested in amounts 10,000 times higher than the minimal effective dose of holotoxin in the respective assays. The B-subunit will also be demonstrated to be pure by SDS gel electrophoresis (21) immunodiffusion, and ELISA tests using serum to V. cholerae concentrated culture filtrates (22).

In vitro tests of B-subunit antigenic integrity will include GM1 - binding capacity assessments to assure that binding of the B-subunit is similar to holotoxin (23), and protective immunogenicity in rabbits (24).

Killed whole cells in the vaccine also produced by Institut Merieux will consist of the following: a) heat-killed V. cholerae classical Inaba, strain Cairo 48 (2.5×10^{10} organisms); b) heat-killed

V. cholerae classical Ogawa, strain Cairo 50 (2.5×10^{10} organisms); c) formalin-killed V. cholerae El Tor Inaba, strain Phil 6973 (2.5×10^{10} organisms); and d) formalin-killed V. cholerae classical Ogawa, strain Cairo 50 (2.5×10^{10} organisms). The cells will be mixed with 8 ml phosphate-buffered saline and 1 mg thiomersal. Because the cells will be washed before inclusion in the vaccine, there will be no formalin contained in the vaccine.

The whole-cell and B-subunit preparation will be combined by Institut Merieux into single 9 cc ampules containing 1 mg B-subunit and 1×10^{11} killed whole-cells and will be maintained in a $4^{\circ}C$ cold chain.

An identically appearing, smelling, and tasting solution, consisting of vitamin C together with same vehicle solution as that used for the vaccine, will be prepared by Institut Merieux. The vaccine and placebo ampules will be letter coded (one set of letters for the vaccine and another set of letters for the placebo). The letter code will be kept jointly by Institut Merieux, The National Bacteriological Laboratory, and WHO headquarters, and will not be divulged to anyone involved in the execution of the trial at ICDDR,B. Both the vaccine and the placebo agents will be administered with an acid-neutralizing agent capable of raising gastric pH from 1.5 to ≥ 4 for the duration of gastric transit of the vaccine (about 20-30 minutes for most subjects). Below a Ph 4, GM1 ganglioside binding data demonstrate a reduction in B-subunit binding and/or antigenic integrity.

Neutralization will be accomplished by mixing the liquid vaccine in a flaovoured sodium bicarbonate plus citric acid solution before ingestion. The exact dose of the neutralizing solution will be selected within the range known to be safe for oral intake and will be made on the basis of data from immunogenicity studies to be carried out at ICDDR,B during December 1983 and January, 1984.

2. Pre-test:

Although extensive information is available to document the safety and immunogenicity of the BS/WC vaccine (vide supra) it will be necessary to field test the vaccine lot to be used in the field trial prior to the execution of the trial. The specific goals of the pre-test will be to ascertain that the vaccine lot is free of serious side-effects and that it is immunogenic.

a. Location:

Three villages (V19, V65 and V66) in the "Comparison Area" of the Matlab field study area will be chosen. These villages will not be included in the subsequent field trial.

b. Recruitment of Subjects and Ascertainment of Eligibility:

Approximately 1000 subjects from 3 villages will be recruited for the pre-test. Eligibility requirements for participation will include: a) age 2-14 years, or >14 years and female; b) absence of pregnancy; c) willingness to participate, (see informed consent form, Appendix A).

c. Allocation of the Vaccine and Placebo Agents:

Computerized random allocation, using census tapes updated as of ~~January~~ June, 1984, will assign vaccine and placebo agents, and the assignments will be distributed to the female village workers (FVW) who normally work in the villages designated for the pre-test. Each resident on the census list will have an assignment corresponding to the letters of the vaccine and placebo ampules. If 800 of the recruited subjects ~~agree~~ agree to participate, with equal numbers in each of the two compared groups, the pre-trial will be able to detect rare adverse outcomes (occurring in 2% of the population) with adequate statistical power (≥90).

d. Field Personnel:

As in the main field trial, administration of the vaccine and placebo will be implemented by three-person teams consisting of the FVW, who will administer the assigned agents; a recorder, who will be in charge of maintaining the allocation lists and noting which persons participate- and a porter, who will be responsible for transmportation of vaccination materials.

Three doctor teams, each consisting of one doctor and three field assistants, will be responsible for examination of pre-test subjects and for detection of adverse vaccine reactions. Field technicians who are responsible for obtaining fingertip blood specimens from a sample of the pre-test population will complete the roster of field personnel.

e. Schedule:

A vaccine schedule identical to that for the field trial will be tested. The volunteers will receive an initial dose of 2 mg B-subunit + 2 z 10^{11} likked whole-cells, (2 ampules of vaccine or placebo) followed at 4 week intervals by 2 doses of 1 mg B-subunit + 1 x 10^{11} killed whole cells (1 ampule of vaccine or placebo on each occasion). The placebo agent will be the same preparation chosen for the field trial. Both groups will receive acid-neutralizing regimens with each dose. The vaccine and placebo ampules will be colour-coded (using a different scheme from that for the trial) so that double-blinding can be maintained.

The field test will begin ~~March 1~~ July 1, 1984, with second and third rounds of vaccination beginning at 4 week intervals subsequent to this date (Table 1). We anticipate that each field vaccinating team will be able to administer vaccine or placebo to 50 patients per day. Since three vaccination teams will be in operation - one for each village - vaccination for each village should be completed within 7 days for each round of vaccination. One doctor team assigned to each village will examine each participant the day of vaccination and for two consecutive days thereafter. Prior to administration of vaccine or placebo, 500 subjects will be randomly selected for 100 lambda finger-prick blood specimens. Blood will also be collected just prior to the second and third rounds of vaccination, and two weeks after the third round of vaccination. Data from these specimens will permit assessment of vaccine immunogenicity.

f. Detection and Care of Adverse Reactions:

A standardized form (Figure 1) will be used to detect and grade potential adverse reactions. A physician will evaluate each reaction and administer appropriate care, referring any serious reaction to Matlab Hospital.

g. Serological Studies:

Each blood specimen will be evaluated for IgG antitoxin antibodies using ELISA and for vibriocidal antibodies using microtiter techniques. Both evaluations have been well standardized in past ICDDR,B investigation of the vaccine.

h. Cholera Cases:

Once the pre-trial has begun, all cases of cholera visiting Matlab Hospital from any of the three pre-test villages will be identified. Optimal care will be given to these cases, and a standard form will be used to describe the clinical features of the cases.

i. Data Analysis:

The vaccine and placebo groups will be compared with respect to the frequency of adverse reactions using chi-square analysis to assess statistical significance. Based on previous experience, we expect the vaccine lot to induce $\geq$2 fold rises of IgG antitoxin in approximately 60% of persons and $\geq$2 fold rises of vibriocidal antibodies in 50% of persons. It should be noted that these responses are less

frequent than mucosal IgA antibody responses to toxin and to V. cholerae cells, but are nonetheless specific indicators of response in relation to mucosal immunity. We will test departures from these levels of serum responses with chi-square assessments of statistical significance.

To preserve confidentiality, all data forms will be kept in locked filing cabinets at Matlab and at ICDDR,B. Subjects will not be identified by name, but only by study number, and no subject will be individually identified in any report of the findings.

3. Methods of Procedure for the Field Trial

a. General Approach

If the results of the pre-test demonstrate satisfactory immunogenicity and minimal toxicity of the vaccine lot, a field trial of the vaccine will be initiated on ~~July 1, 1984~~ January 8, 1988. Volunteers will receive either the BS/WC vaccine or placebo on 3 successive occasions, separated by four-week intervals. Approximately 35,000 persons who are aged 2-14 years or who are >14 years and female will be allocated to each of the two groups. This schedule will permit completion of the three rounds of vaccination before the fall cholera epidemic season. Follow-up for cholera will be accomplished by both passive and active surveillance mechanisms. All cases of bacteriologically proven cholera will be recorded and optimal clinical care will be provided.

b. Antigens

The vaccine and placebo will be the oral antigens described earlier in section D1. Although evidence to date suggests that the vaccine

is not temperature-sensitive within the range of ambient temperatures encountered in Bangladesh (15), we will maintain a cold chain at 4°C for the vaccine prior to administration. The vaccine and placebo agents will be dispensed in lettered vials (one set of letters for the vaccine and another set of letters for placebo), using different letter codes from those employed in the pre-test) and the identities of the different letters will be kept secretly by Institut Merieux, The National Bacteriology Laboratory of Sweden, and WHO. Thus, workers at ICDDR,B will have no knowledge of the identities of the different lettered ampules, ensuring complete objectivity in the conduct of the trial. The letter-code will not be broken until the first analysis of the results, one year after vaccination begins.

c. Eligibility:

Eligibility for the trial will require a) that participants be listed in the Demographic Surveillance System census as full-time residents of those areas of Matlab which are selected for the trial; b) that participants be 2-14 years of age, or, if older than 14, that participants be female; c) that participants not be pregrant; and d) that subejcts verbally volunteer their willingness to participate in the trial irrespective of whether they receive vaccine or placebo (see informed consent statement,Appendix B). Younger children will not be included because of their low risk of cholera and adult males will not be included because of their frequent unavailability during the progress of long-term studies.

d. Sample Size:

Based on cholera surveillance data in Matlab over the past sixteen years, we estimate that the incidence of clinical cholera requiring clinical care will be at least one case per 1000 person-years for the groups eligible for the trial. If we assume: a) that the vaccine will confer at least 70% protection with 2 doses and at least 25% protection with a single dose; b) that the dropout rate between each round of vaccination will be 30% (i.e., that 70% of those receiving the first dose will receive two or more doses); and c) that 80% power will be required to detect vaccine protection, then 35,260 subjects will be needed in each of the two compared groups. Since 80% of the potentially eligible population is expected to volunteer for the trial and since 65% of the general population will be potentially eligible according to the criteria outlined earlier, an area of 135,616 people (total population) will be required for the field trial. This population can be assembled readily from the entire "Treatment" region (90,000 population) and a portion of the "Comparison" region (90,000 population) of the Matlab field study area. To maximize the probability that cholera cases from the trial population in the "Comparison" area will go to Matlab Hospital for therapy, the Amirabad portion of the "Comparison" area will be chosen for the trial, since these villages are proximate to the Matlab Hospital and have demonstrably high rates of utilization of Matlab Hospital.

e. Recruitment of Subjects:

A census for those portions of the Matlab study area to be used for the trial will be updated in ~~early~~ mid 1984, and will identify potentially eligible subjects for the trial. The method of recruitment will take advantage of the existing system of FVWs working in the Matlab field study area. In the "Treatment" portion of the field study area, the workers make fortnightly rounds to each home in their assigned area, and in the "Comparison" area the workers make weekly visits to their assigned homes. During the two visits prior to the initiation of the trial, the workers will announce the trial to potentially eligible subjects and answer any questions. Analogous efforts to recruit volunteers will occur during the first vaccination round. Consent for participation will be obtained after detailed explanation in Bengali and will be obtained verbally (see Appendix B).

f. Allocation of the Vaccine and Placebo:

A computerized random-number program will allocate the two sets of letters, corresponding to the different letters of the vaccine and placebo ampules, to every member of the population in the vaccine trial area. Randomization will be based on the terminal two digits of each person's demographic surveillance registration number. Each FVW will receive a computerized list of her assigned population with the appropriately letter next to each name. Thus,

assignment will take place before agreement to participate has been obtained, but double-blinding will obviate any biases that could result from selective participation conditional upon knowledge of vaccine assignment.

g. Acquisition of Baseline Information:

Even with randomized allocation, it will be necessary to document the comparibility of the vaccine and placebo groups with respect to several baseline variables known to influence the risk of cholera. Baseline information about age, gender, socio-economic status, and family size will be obtained for each participant by linkage with existing Matlab Demographic Surveillance System census tapes. Past histories of hospitalized cholera or cholera vaccination will be acquired for each participant by linkage with existing computer tapes from Matlab Hospital and from past cholera vaccine trials. Comparability with respect to nutritional status will be ascertained by obtaining the following nutritional measurements from a sample of 1000 children prior to vaccination: age, height, weight, and mid-arm circumference. This survey will be accomplished by special teams of field workers who will be given special training in taking these anthropometric measurements and will take place in four randomly sampled villages in the "Treatment" portion of the field study area. Finally, for each recruited subject, the recorder member of the vaccination team will fill out a specially prepared vaccine trial card, noting the following

information: name, registration number, date and letter of received ampule, and, if recruitment was not successful, reasons for non-participation.

h. Vaccine and Placebo Administration:

Three-person teams composed in the same fashion as for the pre-trial will administer vaccine and placebo. As in the pre-trial vaccination will take place in three rounds, beginning July 1, 1984, with each round separated by four weeks. In the first round, two apmules of vaccine (or placebo) will be given with the selected acid-neutralizing regimen. Thus, 2 mg B-subunit and 2×10^{11} killed whole cells will be given during the first round. In the subsequent two rounds, one apule of vaccine or placebo will be given. Accordingly, during each of the second and third rounds vaccine recipients will receive 1 mg B-subunit and 1×10^{11} killed whole-cells. We anticipate that in each round coverage of the population can be accomplished within 7-10 days (assuming coverage of 50 persons per day by each team). The recorder and porter will accompany the FVW on her remaining visits during the month so that any subjects who were not present on the initial visit for the round or who decide to participate after initially refusing will have the opportunity to volunteer. Although no specific attempts will be made to recruit young children (<2 years) of adult men, subjects from these two groups will be given the opportunity to participate according to the randomized assignments

for the trial if a specific desire to volunteer is expressed. To summarize, the schedule of the three rounds of vaccination will be:

Round	Date Initiated	Date Terminated
I	January 8, 1985 ~~July 4, 1984~~	Feb. 4, 1985 ~~July 28, 1984~~
II	Feb. 5, 1985 ~~July 29, 1984~~	March 4, 1985 ~~August 25, 1984~~
III	March 5, 1985 ~~August 26, 1984~~	April 1, 1985 ~~September 22, 1984~~

The vaccine and placebo will be given in cups. The recorder will write the recipient's name, the date of vaccination, and the letter of the ampule on a piece of tape already attacked to the cup and the cup will be given to the recipient. Correct receipt of the vaccine will be checked by visiting 1000 randomly selected households and comparing the letters of cups with the assigned letters on the computerized allocation lists.

Although pre-trial testing should make the possibility of any serious adverse reaction negligible, special physician coverage and a speed boat ambulance available 24 hours a day will handle patients with serious untoward reactions, and such reactions will be carefully monotored during the course of the trial.

i. Surveillance for Cholera:

Surveillance for cholera will begin at the onset of the first

round of vaccination and will be implemented with both passive and active systems.

1. Passive Sureveillance:

All patients presenting with diarrhoea to the Matlab Hospital and to the Nayergaon treatment center will be identified through computerized census lists as to whether they reside in the vaccine trial area. An assistant will be on duty at both locations specifically for this purpose. At Matlab Hospital, each patient thus identified will have stool cultures for evaluation of V. cholerae and non-cholera vibrios, as well as for the assessment of toxigenic (LT) E. coli (vide infra). At Nayergaon treatment center, where bacteriologic facilities are not available, stool samples will be cultured on carrier media and transferred to Matlab Hospital for bacteriologic evaluation. At both sites, acute phase sera will be obtained by fingerprick specimens (100 lambda) and will be frozen for subsequent evaluation for cholera serologies (vide infra).

2. Active Surveillance:

a. Fatal Diarrhoea Episodes:

All diarrhoea-related fatalities not occurring at a treatment center will be identified by bari volunteers (one woman residing in each bari of the "Treatment" portion of the field study area and already involved in the administration of oral rehydration

solution) or by existing Demographic Surveillance System procedures, which routinely acquire cause-specific mortality information. A special team will be sent to acquire rectal swabs from the deceased, swabs of clothing that the deceased wore during the fatal diarrhoeal illness, and rectal cultures from each family contact of the deceased. The swabs will be placed in transport media and taken to Matlab Hospital for bacteriological evaluation.

b. Morbidity Surveillance:

1. Comprehensive Surveillance:

As noted earlier, bari volunteers already conducted a diarrhoea-monitoring system in the "Treatment" portion of the Matlab field study area. During the autumn and spring cholera epidemics, special field teams will visit each bari in the treatment area twice weekly. At each visit, the team will be informed by the volunteer which persons had diarrhoea since the last visit. All subjects so identified will be visited to obtain simple demographic and clinical information, and to obtain a rectal swab. The swabs will be transported back to Matlab Hospital for bacteriologic evaluation.

2. Sentinal Case Surveillance:

To detect mild and asymptomatic cases of cholera, special teams will visit the house of each cholera case

identified through passive surveillance at Matlab Hospital and Nayergaon treatment center. The visit will occur immediately upon notification from the bacteriology laboratory that a cholera case has occurred in the vaccine trial population. Rectal swabs will be obtained from each member of the house (defined as all persons eating from the same pot) who is present and who is willing to comply with daily rectal swabs for 6 consecutive days. A fingertip blood specimen will also be obtained on day 1 from all contacts who volunteer for this investigation.

j. Case Evaluation and Classification:

All cases presenting to the ICDDR,B treatment facilities from the vaccine trial population will be interviewed by a physician who will inquire about duration and frequency of diarrhoea, examine the patient and note signs of dehydration, and obtain admission (and later discharge) weights and plasma specific gravity measurements as a quantitative guide to the state of hydration. Based on initial clinical assessments, the patient will be treated with intravenous or oral fluid. Patients who are judged to have lost 10% or more of their body weight will be initially treated with intravenous fluid. The clinical course of inpatients will be followed; observations will include duration and volume of stool, requirement for oral and intravenous fluids, and other appropriate clinical observations. Antibiotics are acceptable adjuncts to

therapy and will be administered as appropriate. Based on the severity of the diarrhoea and the resulting state of hydration, cases detected at the treatment facilities will thus be classified as severe, mdoerate or mild.

Cases detected in the field through active diarrhoea surveillance via sentenel case follow-up and comprehensive surveillance will be evaluated by a questionnaire administered by non-medical field staff. The questionnaire will inquire about diarrhoea and will attempt to elicit an estimate of severity. Anyone with significant diarrhoea will be referred for treatment at the Matlab Hospital by ambulance boat. Persons without diarrhoea who have V. cholerae recovered from a rectal swab will be classified as asymptomatic.

k. Serologic Field Studies:

To permit assessment of the relationship between vaccine-induced seroconversion and clinical vaccine efficacy, serological field studies will be performed. 100 lambda of fingerstick blood will be obtained during the week prior to the first round of vaccination from 4000 subjects chosen from the anticipated field trial population. One-half (2000) of these subjects will be rebled just prior to the second vaccination round, and the other half will be rebled 23 weeks after the completion of the third round. Since cholera is expected to peak in incidence during late November, a

random sample of 2000 persons from the initial 4000 persons studied at baseline will be bled at a time coinciding with the peak. The remaining 2000 subjects will be bled 6 months after the end of the third round to coincide with the smaller epidemic of cholera expected to occur in the spring. (See Appendix C for written informed consent statement).

As noted earlier, acute phase sera will be drawn from all members of the field trial population presenting to the Matlab Hospital with clinical cholera, and all family contacts of sentinel cases will have fingertip sera drawn on day 1 of their follow-up.

Microtiter vibriocidal titers and IgG antitoxin titers (with ELISA) will be assessed on all serological specimens (25,26).

1. Breast Milk Studies:

To evaluate the role of B-subunit/whole-cell vaccine in inducing immunity against cholera and ETEC diarrhoea via breast milk, we will perform several assessments. First, 250 randomly sampled breast-feeding women will be asked to participate in a study in which standard anthropometric measurements will be taken and in which breast milk and fingerstick blood samples will be sampled serially before vaccination, just prior to the second vaccination, and 23 weeks after completion of the third vaccination. (See Appendix D for informed consent form). These samples will

allow estimation of vaccination-induced breast antibodies.

All children aged 0-4 treated for watery diarrhoea at Matlab Hospital or the Nayergaon treatment center will be evaluated for cholera and diarrhoea due to LT toxigenic E. coli, as noted below. If such children are breast-feeding at the time of admission, note will be made of the letters of the ampules received (if any) by both the mother and the child in the field trial. During admission, anthropometric measurements will also be taken for each mother-child pair. The expected distribution of received ampules and of nutritional status will be ascertained in two mutually exclusive samples of lactating mothers in the vaccine trial area. The surveys, which will each randomly select 250 lactating mothers and which will be conducted during the fall and spring cholera epidemics, respectively, will gather information about age, ampule receipt, and nutritional status (via anthrometry) from the selected lactating mothers and their breast-fed children. (See Appendix E for informed consent form).

Microtiter vibriocidal, anti-lipopolysaccharide antitoxin titers (with ELISA) will be assessed on all blood specimens; ELISA will also be used to assay secretory IhA (SIgA) antitoxin and anti-lipopolysaccharide in relation to the total SIgA concentration of the breast milk specimens.

m. Bacteriologic Evaluarion:

For patients who present directly to Matlab Hospital, rectal swabs or fresh stool will be pated directly and after 6 hour enrichment in bile peptone broth on Monsur's agar. Plates will be examined at 18-24 hours for V. cholerae and isolates will be biochemically confirmed, serotyped, and biotyped. Stool will also be plated on MacConkey agar and 2 individual lactose positive colonies typical of E. coli and 5 pool will be selected and tested for production of LT using the Chinese hamster ovary cell assay.

For patients presenting to Nayergaon treatment center and for patients identified through field surveillance mechanisms, rectal swabs will first be inserted into transport media, and then will be processed by the above cultural methods.

n. Data Analysis

The protective efficacy (PE) of a vaccine is calculated as:

$$PE = \frac{\left[\text{Incidence of disease in controls}\right] - \left[\text{Incidence of disease in vaccines}\right]}{\left[\text{Incidence of disease in controls}\right]}$$

We will calculate protective efficacy of the vaccine for several cholera outcomes: bacteriologically-proven severe clinical cholera (defined as fatal cholera or cholera requiring

intravenous hydration); bacteriologically-proven symptomatic cholera (defined as cholera associated with at least 3 loose bowel movements per day); and bacteriologically-proven asymptomatic cholera. Statistical significance and 95% confidence intervals for these estimates will be assessed with conventional methods. If any baseline cholera risk factors are found to be unequally distributed between the vaccine and placebo populations, protective efficacy estimates that are adjusted for these inequalities will be evaluated with multiple logistic regression.

Analogous approaches will be used to estimate the protective efficacy of the cholera vaccine against symptomatic and asymptomatic infections with LT producing E. coli and with non-cholera vibrios.

The relationship between the level of serum titers of antibacterial and antitoxic antibodies and clinical protection against cholera and toxigenic (LT) E. coli will be ascertained by comparing the distribution of serological titers (both vibriocidal and anti-toxic) in acute phase sera from cholera and LT-ETEC cases with contemporaneous titers from the field trial population obtained during the serological field surveys. The existence of serial cohort serosurveys will also permit assessment of the relationship between sero-conversion and vaccine protection.

In a similar way, the relationship between maternal receipt of B-subunit/whole-cell vaccine and the occurrence of cholera

and LT-ETEC diarrhoea will be ascertained for breast-feeding children of vaccinated and non-vaccinated mothers. The level of vaccine protection for such children can be assessed from a case-control analysis in which the cases are breast-feeding children with the particular type of diarrhoea, and the controls are breast-feeding children without these types of diarrhoea whose mothers have been included in the field surveys of lactating women. The expression (1-Odds Ratio) for such analyses yields an estimate of protective efficacy for the vaccine, as defined above. Using multiple logistic regression, this odds ratio can be adjusted for maternal and child age, season at the time of sampling, receipt of the vaccine by the breast-feed child, and other potentially confounding variables. Such analyses will be performed two ways. In the first, only cases and controls whose mothers participated in the trial will be analysed. In the second, all sampled cases and controls will be evaluated. In addition, we will evaluate vaccine-induced immunity according to maternal and child nutritional status, to assess the modulating effects of these variables upon vaccine-induced breast immunity.

To preserve confidentiality, all data forms will be kept in locked filing cabinets at Matlab and at ICDDR,B. Subjects will not be identified by name, but only by study number, and no subject will be individually identified in any report of the findings.

E. SIGNIFICANCE:

The oral BS/WC vaccine represents an important advance in the development of cholera vaccines. The combined BS/WC product is an attempt to induce synergistic antitoxic and antibacterial immunity. The oral route of administration may well represent the most effective strategy for delivering antigens, and has obvious advantages in the public health implementation of large-scale vaccination programs. If found to be effective, the oral BS/WC vaccine could conceivably initiate a new era of oral vaccines effective against diarrhoeal illnesses.

REFERENCES

1. Oseasohn RO, Benenson AS, Fahimuddin M. Field trial of cholera vaccine in rural East Pakistan. First year of observation. Lancet i:450-452, 1965.

2. Benenson AS, Mosley WH, Fahimuddin M, Oseasohn RO. Cholera Vaccine field trials in East Pakistan. 2. Effectiveness in the field. Bull. WHO. 38:359-372, 1968.

3. Mosley WH, McCormack, Fahimuddin M, Aziz KMA, Rahaman ASMM, Chowdhury AKMA, Martin AR, Feeley JC, Phillips RA. Report of the 1966-1967 Cholera Vaccine field trial in rural East Pakistan. I. Study design and results of the first year of observation. Bull. WHO 40:177-185, 969.

4. Mosley WH, Woodward WE, Aziz KMA, Rahaman ASMM, Chowdhury AKMA, Ahmed A, Feeley JC. The 1968-1969 cholera va-cine field trial in rural East Pakistan. Effecti- ness of monovalent Ogawa and Inaba vaccines and a purified antigen with comparative results of serological and animal protection tests. J. Inf. Dis (Supp) 121:51-59, 1970.

5. Finkelstein RA, Lospalluto JJ. Production of highly purfied choleragen and choleragenoid. J. Inf. Dis. 121:563-572, 1970.

6. Curlin GT, Levine R, Aziz KMA, Rahaman ASMM, Verway WF. Field Trial of Cholera toxoid. Proceedings of the Eleventh Joint Conference on Cholera, New Orleans, La, November 1975, p.314-329.

7. Curlin GT, Levine R, Aziz KMA, Rahaman ASMM, Verway WF. Serological aspects of a cholera toxoid field trial in Bangladesh. Proceeding of the Twelfth Joint Conference on Cholera.

8. Svennerholm A, Holmgren J: Synergistic protective effect in rabbits of immunization with Vibrio cholerae lipopolysaccharide and toxin/ toxoid. Inf. Imm. 13:735-740, 1976.

9. Svennerholm A-M. Personal communication.

10. WHO Diarrhoeal Diseases Control Programme. Development and field testing of an oral cholera vaccine at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Report of an Informal Consultation, Washington, D.C., 10-11 June, 1983.

11. Smith NW, Sack RB. Immunologic cross reactions of enterotoxins from Escherichia coli and Vibrio cholerae. J. Infect. Dis. 127; 164-170, 1973.

12. Pierce NF. Protection against chllenge with Escherichia coli heat labile enterotoxin by immunization of rats with cholera toxin/toxoid. Inf. Immun. 18:338-341, 1977.

13. Glass R, Becker S, Huq I, Stoll B, Merson M, Lee J, Black R. Endemic cholera in rural Bangladesh, 1966-1980. Am J Epidemiol. 116:959-970, 1982.

14. Ryder RW, Sack DA, Kapikian AZ, McClaughlin JC, Chakraborty J, Rahman ASMM, Merson MH, Wells TG. Enterotoxigenic Escherichia coli and reovirus-like agent in rural Bangladesh. Lancet 1:659-662, 1976.

15. Holmgren J. Personal Communication.

16. Glass R. Personal Communication.

17. Levine M. Personal Communication.

18. Holmgren J., et al. Development of improved cholera vaccine based on subunit toxoid. Nature (London). 269:602-604, 1977.

19. Craig JP. A permeability factor (toxin) found in cholera stools and culture filtrates and its neutralization by convalescent sera. Nature (London). 207:614-616, 1965.

20. De SN, Chatterjee DN. An experimental study of the mechanism of action of V. cholerae on the intestinal mucous membrane. Journal of Path. and Bact. 45:554-562, 1953.

21. Lonnroth I, Holmgren J. Subunit structure of cholera toxin. Journal of Microbiology. 76:417 427, 1973.

22. Holmgren J, Svennerholm A-M. Enzyme-linked immunosorbent assays for cholera serology. Infection and immunity. 7:753 757, 1973.

23. Lonnroth I, Holmgren J. Protein reagnet modification of cholera toxin: Characterization of effects of antigenic, receptor-binding, and toxic properties. Journal of General Microbiology 91:263-277, 1975.

24. Svennerholm, A-M., et al. Intestinal antibody responses in humans after immunization with cholera B-subunit. Lancet. 1:305-308, 1982.

25. Benenson AS, Saad A, Mosley WH: Serological studies in cholera. 2. The vibriocidal antibody response of cholera patients determined by microtechnique. Bull. WHO 38:277-285, 1968.

26. Ahmed A, Mahmood A, Neogi KB, Curlin GT. Serological aspects of a field trial of cholera toxoid. 1. Description of a passive hemagglutination technique for estimation of cholera toxin, in preparation.

TABLE 1 SCHEDULE FOR VACCINE PRE-TEST AND TRIAL

	Events	Dates (1984)
1.	First dose of vaccine and first bleed	~~March 1 – March 8~~ July 1 – July 8
2.	Doctor examination	~~March 1 – March 10~~ July 1 – July 10
3.	Second dose of vaccine and second bleed	~~March 29 – April 5~~ July 29 – Aug. 5
4.	Doctor examination	~~March 29 – April 7~~ July 26 – Aug. 7
5.	Third dose of vaccine and third bleed	~~April 27 – May 4~~ Aug. 26 – Sept. 2
6.	Doctor examination	~~April 27 – May 6~~ Aug. 26 – Sept. 4
7.	Fourth bleed	~~May 20 – 22~~ Sept. 16 – 18
8.	Main trial begins	~~July 1, 1984~~ January 8, 1985

FIGURE 1: ADVERSE REACTION FORM FOR ORAL ADMINISTRATION
REACTION SURVEILLANCE FORM FOR ORAL ADMINISTRATION
OF B SUBUNIT ANTIGEN AND WHOLE CELL VACCINE

Colour of Ampoule: _____

Name _____ Code Number _____ Census Number _____

Date of Vaccination _____ Time _____

Primary Immunization ☐ Secondary Immunization ☐ Tertiary Immunization ☐

Preimmunization Temperature _____

Are there any objective and/or subjective symptoms of the following which can be referred to the oral intake of the ampoule? _____

Local Reactions	Day 0 (Imm. Day)	Day 1	Day 2	Late* Reactions
Abdominal Pain	☐	☐	☐	☐
Abdominal Cramps	☐	☐	☐	☐
Abdominal Distention	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhoea	☐	☐	☐	☐
Systemic Reactions				
Body Temperature	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Dizziness	☐	☐	☐	☐
Exanthema	☐	☐	☐	☐
Edema	☐	☐	☐	☐

Others** _____

* Date of reaction _____

** If yes, nature of symptoms _____

ABSTRACT SUMMARY

A vaccine trial will be conducted in the Matlab field study area to test the efficacy of an oral B-subunit/whole-cell vaccine in reducing the incidence and severity of clinical cholera. The eligible population will be vaccinated between ~~July - September, 1984~~ January - April, 1985, and their experience during the ensuing year will be monitored. Surveillance will include following cases treated at Matlab Hospital and at Nayergaon treatment center as well as cases detected with comprehensive and bari-contact cultures in the field. The serologic response to the vaccine will be observed in a cohort of 4000 subjects followed serially during the trial and in cholera cases who present themselves for treatment or who are detected in the field. The role of breast milk antibodies in conferring clinical protection against cholera will be immunologically assessed in serial observations of 250 breast-feeding mothers and epidemiologically assessed by contrasting breast-feeding mothers whose children present themselves for treatment with breast-feeding mothers randomly sampled in the field.

1. The two-cell trial will require giving the intial dose of the vaccine (2 mg B-subunit + 2 x 10^{11} killed whole-cells) to 70,520 persons; 35,260 vaccines and 35,260 controls. The subsequent two doses of vaccine (each dose consisting of 1 mg subunit + 1 x 10^{11} killed whole-cells) will be given at 4-week intervals to as many of the participants as are available. Children (non-infants) and adult women are at highest risk for contracting cholera and would thus benefit most from an effective vaccine. The total population from which 70,520 eligible volunteers may be recruited will be about 135,000 persons. This population can be assembled from the entire

"Treatment Area" (population 90,000) and a portion of this "Comparison Area" (population 90,000) of the Matlab field area.

2. The trial will be preceded by a pre-test in 800 volunteers from villages of the "Comparison Area" which will not be included in the trial. The volunteers will be given either vaccine or placebo according to the same schedule as that for the trial and will be observed for side-effects and for serum antibody responses to assure that the vaccine lot for the trial is safe and immunogenic.

3. To treate unanticipated side-effects that occur in the trial, physicians will be posted in several accessible locations in Matlab during the entire period of vaccine administration. Treatment will also be available at Matlab Hospital and at Nayergaon treatment center.

4. The vaccine and placebo ampules will be leeter-coded, and the identities of the letters will be held at Institut Merieux (Lyon, France), the National Bacteriology Laboratory (Stockholm, Sweden), and at WHO headquarters. The letter of the agent to be received by each participant will be indicated on computerized census lists. The ageents actually received by participants will be indicated on these lists and on separate registration cards. After administration of the vaccine and placebo is completed, this information will be computerized and will be printed out into a bound volume to be kept at ICDDR,B.

5. Informed consent will be obtained from each participant as in previous trials. Dependent children (age 2-14) will be included only if accompanied by a parent or guardian. A statement (attached) will be read to each adult female and parent/guardian of children. Every effort will be made to insure that each adult female or parent/guardian understands the tenets of informed consent. Verbal consent will be considered sufficient for participation. Males over the age of 14 who wish to be vaccinated will be allowed to give consent for themselves and will receive either vaccine or placebo according to a pre-arranged random method.

6. No special interview will take place.

7. Cholera is endemic in Matlab Thana and in other areas in Bangladesh and no adequate measures are currently available to prevent transmission of the disease. A safe, effective vaccine would offer one excellent preventive and control measure. The presently available cholera vaccines offer limited protection for a short duration. The vaccine to be tested in this trial represents a new approach in the development of cholera vaccines that offers a greater chance of protection. There is reason to expect that the B/subunit may have some effect against some E. coli diarrhoeas, which would also be of major importance. Should this vaccine be found to be highly effective, it would offer a significant advancement in our ability to prevent cholera worldwide and would provide valuable information on the development of immunity to cholera infection.

8. This study requires collection of fingertip-blood specimens and breast-milk specimens in certain subgroups of the volunteers.

9. The drugs testing laboratory of the Institute of Public Health, Mohakhali, Dhaka-12, will be approached for clearing the vaccine before testing it in the field.

BUDGET: PART I (PRETEST)

PERSONNEL SERVICES

Name	Position	% One FTE	Annual Salary	Project Requirement Taka	Dollar
Dr. J.D. Clemens	P. Investigator	33%	$ 63,970	-	21,323.33
Mr. J. Chakrabarty	Co-Investigator	25%	$ 5,754	-	1,438.50
Dr. M.U. Khan	" "	8%	$ 71,000	-	5,680.00
Dr. B. Stanton	" "	8%	$ 51,380	-	4,110.40
Dr. M. Yunus	" "	8%	$ 9,420	-	753.60
To be named	Supervisor (1)	25%	$ 6,100	-	1,525.00
To be named	Dhaka Coordinator	33%	$ 9,300	-	3,300.00
Serology Team:					
To be named	Health Asst. (2)	66%	$ 2,951	-	1,967 33
To be named	Porter (1)	33%	$ 650	-	216.33
Vaccine Team:					
To be named	Health Asst. (4)	132%	$ 2,951	-	3,895.32
To be named	Porter (2)	66%	$ 650	-	433.33
MD Teams:					
To be named	Physicians (2)	66%	$ 4,472	-	2,981.33
To be named	Health Asst. (2)	66%	$ 2,951	-	1,967.33
Data Staff:					
To be named	Health Asst. (1)	33%	$ 2,951	-	983.66
To be named	Coding Asst. (2)	40%	$ 1,988	-	795.20
To be named	Data Manager	33%	$ 4,070	-	1,356.66
To be named	Data Proc. Asst. (2)	40%	$ 2,468	-	987.20
Laboratory Personnel:					
To be named	Sr. Lab. Tech. (lmm) (1)	25%	$ 4,407	-	1,011.75
To be named	Lab. Tech. (lmm) (4)	100%	$ 2,694	-	2,694.00
Transport:					
To be named	Speedboat driver (2)	50%	$ 2,551	-	1,275.50
			Sub-total:	-	58,695.77

SUPPLIES AND MATERIALS:

Antitoxin and vibriocidal assays (8,000) @ $ 2.00/assay		-	16,000.00
Lancets and vials (8,000) @ $ 0.50/sample		-	4,000.00

	Project Requirement	
	Taka	Dollar
Vaccine administration cups (8,000) @ $ 0.10/ cup	-	800.00
Paper, Xeroxing, Office Supplies	-	100.00
IBM Tapes (1) - @ $ 12.00/tape	-	12.00
IBM Card (2,000) - @ $ 72.00/10,000 cards	-	14.40
Sub-total:	-	20,926.40

EQUIPMENT: - None

PATIENT HOSPITALIZATION:

100 perso (@ Tk. 150.00/day) Sub-total:	1,500.00	-

OUTPATIENT CARE:

100 visits (@ Tk. 75.00/visit) Sub-total:	7,500.00	-

ICDDR,B TRANPSORT:

Dhaka/Matlab/Dhaka - 25 round trips @ Tk. 550.00/trip	13,750.00	-
Speed boat - 360 hours (@ Tk. 350.00/hour)	126,000.00	-
Sub-total:	139,750.00	-

TRAVEL AND TRANSPORT OF PERSONNEL - None

TRANSPORT OF THINGS:

Dhaka/Matlab/Dhaka - 20 trips (@ Tk. 550.00/trip)	11,000.00	-

RENT, COMMUNICATION AND UTILITIES - None

PRINTING AND REPRODUCTION:

Forms and Record Sheets Sub-total:	-	250.00

OTHER COTRACTUAL SERVICES:

Computer Sercices (20 hours) @ Tk. 300.00/hour Sub-total:	6,000.00	-

CONSTRUCTION, RENOVATION, ALTERATIONS: - None

BUDGET : PART I (PRE-TEST)

BUDGET SUMMARY:

		Dollars	Taka
1.	Personnel Services	58,695.77	-
2.	Supplies and Materials	20,926.40	-
3.	Equipment	-	-
4.	Patient Hospitalization	-	1,500
5.	Outpatient Care	-	7,500
6.	ICDDR,B Transport	-	139,750
7.	Travel and Transport of Persons	-	-
8.	Transort of Things	-	11,000
9.	Rent, Communications, Utilities	-	-
10.	Printing and Reproduction	250.00	-
11.	Other Contractual Services	-	6,000
12.	Construction, Renovation, Alterations	-	-
		79,872.17	165,750

Dollar Equivalent = US$ 86,582.70

BUDGET: PART II (FIELD TRIAL)

PERSONNEL SERVICES

Name	Position	% One FTE		Annual Costs	Project Requirement Taka	Dollar
Dr. J. Clemens	P. Investigator	80%	$	63,970	-	51,176.00
Mr. J. Chakrabarty	Co-Investigator	60%	$	5,754	-	3,452.40
Dr. M.I. Huq	Co-Investigator	10%	$	67,600	-	6,760.00
Dr. M.U. Khan	Co-Investigator	20%	$	71,000	-	14,200.00
Dr. B. Stanton	Co-Investigator	20%	$	51,380	-	10,276.00
Dr. M. Yunus	Co-Investigator	20%	$	9,420	-	1,884.00
To be named	FRO Supervisor(2)	60%	$	6,100	-	3,660.00
To be named	Dhaka Coordinator	100%	$	9,900	-	9,900.00
Vaccine Team:						
To be named	C.H.W. (36)	1200%	$	658	-	7,896.00
To be named	Health Asst. (36)	1200%	$	2,951	-	35,412.00
To be named	Porters (30)	1000%	$	658	-	6,580.00
Surveillance Team:						
Comprehensive Surveillance:						
To be named	Health Asst. (30)	2000%	$	2,951	-	59,020.00
To be named	Porters (12)	800%	$	658	-	5,264.00
To be named	C. Boatman (30)	2000%	Tk.	9,125	182,500.00	
Sentinel Surveillance:						
To be named	H. Asst. (30)	2500%	$	2,951	-	73,775.00
To be named	Porter (2)	125%	$	658	-	822.50
To be named	C.H.W. (2)	125%	$	658	-	822.50
Passive Surveillance:						
To be named	H. Assistant (5)	500%	$	2,951	-	14,755.00
Medical Team						
To be named	Physician (2)	70%	$	4,472	-	3,130.40
To be named	H. Assistant (2)	70%	$	2,951	-	2,065.70
Sero-Studies:						
To be named	H. Assistant (12)	600%	$	2,951	-	17,706.00
To be named	Porter (6)	300%	$	658	-	1,974.00
To be named	C. Boatman (6)	300%	Tk.	9,125	27,375.00	-
Breast Milk Studies:						
To be named	H. Assistant (6)	150%	$	2,951	-	4,426.50
To be named	Porter (3)	75%	$	658	-	493.50
To be named	C. Boatman(3)	75%	Tk.	9,125	6,843.75	-
Nutrition Studies:						
To be named	H. Assistant (2)	100%	$	2,951	-	2,951.00
To be named	Porter (2)	100%	$	658	-	658.00

	Position	% One FTE	Annual Costs	Project Requirement Taka	Project Requirement Dollar
Data Staff:					
To be named	H. Asst. (3)	250%	$ 2,951	-	7,377.50
To be named	Coding Asst. (3)	300%	$ 1,988	-	5,964.00
To be named	Data Manager (1)	80%	$ 4,070	-	3,256.00
S. Walter	Statistical Consultant - 3 consultations			-	12,000.00
D. Leon	Programmer	20%	$ 43,700	-	8,740.00
To be named	Data Processing Asst. (4)	200%	$ 2,468	-	4,963.00
Lab. Personnel:					
To be named	Sr.Lab.Tech (Immun) (1)	50%	$ 4 047	-	2,023.50
	Lab.Tech(Immun)(3)	300%	$ 2,694	-	8,082.00
Transport:					
To be named	Speedboat Mechanic(1)	30%	$ 2,166	-	649.80
	Speedboat Drivers(15)	500%	$ 2,551	-	12,755.00
		Sub-Total:	216,718.75		404,871.30

SUPPLIES AND MATERIALS:

Cultures:

a. Passive surveillance(12,000 routine) @15T/Culture	180,000	-
b. Passive surveillance(6000 LT) @ 7.50T/culture	45,000	-
c. Sentinel Surveillance(10,500 routine) @15T/culture	157,500	-
d. Death Surveillance(1400 routine) @15T/culture	21,000	-
e. Comprehensive surveillance(60,000 routine) @15T/culture	900,000	-

Serological Studies:Assays:

a. Antitoxin and vibriocidal assays(26,600) @$2 per specimen	-	53,200

	Project Requirements	
	Taka	Dollars
Breast-milk Studies: Assays		
a. Antitoxin and Anti-LPS assays (750) @ $2 per specimen		1,500.00
Lancets and vials for serum and breast-milk studies (27,250) @ $0.50/sample		13,625.00
Administration cups (250,000) for vaccine @$0.10 per cup		25,000.00
Administration Materials: (Insulated cases, etc.)		1,200.00
Stationary and Other Miscellaneous Supplies	-	2,000.00
IBM Tape (5) @ $12/tape	-	60.00
IBM Cards (00,000) @ $72/10,000 Cards	-	5,760.00
Computerized volumes of census and vaccine allocations	-	500.00
Sub-total:	1,303,500	102,845.00
EQUIPMENT:		
Revcos (for serum and breast milk storage (14) @ $8150 per Revco		114,100.00
Refrigerators to store vaccine (40) @ $1520 per regrigerator		60,800.00
Filing Cabinet (5) @ 1600T/cabinet	8,000	-
Calculator (10) @ $25/Calculator	-	250.00
Weighing Machine (2) @ $200	-	400.00
Micro Computer	-	4.000.00
Length Boards:		
(4-vertical) @ $12.50	-	50.00
(4-lying (@ $12.50		50.00
Sub-total:	8,000	179,650.00
PATIENT HOSPITALIZATION:		
(1000) person-days) @ 150T/day	150,000	-
Sub-total:	150,000	
OUTPATIENT CARE:		
(500 visits) @ 75T/visit	37,500	-
Sub-total:	37,000	

		Project Requirements	
		Taka	Dollars
ICDDR,B TRANSPORT:			
Dhaka/Matlab/Dhaka 125 round trips @ 550T/trip		68,750	-
Speedboat(8364 running hours) @ 350T/hour		2,927,400	
Car (12,800 miles) @ 4.5T/mile		57,600	
	Sub-total:	3,053,750	
TRAVEL AND TRANSPORT OF PERSONS:			
Trips to International Meetings (4) @ $3,000 per trip		-	12,000
	Sub-total:		12,000
TRANSPORT OF THINGS:			
Dhaka/Matlab/Dhaka (50 trips) @ 550T/trip		27,500	-
Launch hire (21 @ 3500T/hire)		7,000	-
	Sub-total:	34,500	-
RENT, COMMUNICATION & UTILITIES:			
Postage		2,000	
	Sub-total:	2,000	-
PRINTING AND REPRODUCTION:			
Forms and record sheets		-	2,000
Publication, Figures, Reprints		-	2,000
	Sub-total:	-	4,000
OTHER CONTRACTUAL SERVICES:			
Computer services(200 hours) @ 300T/hour		60,000	-
	Sub-total:	60,000	-
CONSTRUCTION, RENOVATION, ALTERNATIONS:			
None			

BUDGET: PART II (FIELD TRIAL)

BUDGET SUMMARY:

		Dollars	Taka
1.	Personnel	404,871.30	216,718.75
2.	Supplies and Materials	102,845.00	1,303,500.00
3.	Equipment	179,650.00	8,000.00
4.	Patient Hospitalization	-	150,000.00
5.	Outpatient Care	-	37,500.00
6.	ICDDR,B Transport	-	3,053,750.00
7.	Travel and Transport of Persons	12,000.00	-
8.	Transport of Things	-	34,500.00
9.	Rent, Communications, and Utilities	-	2,000
10.	Printing and Reproduction	4,000.00	-
11.	Other Contractual Services	-	60,000.00
12.	Construction, Repair, Alterations	-	-
		703,366.30	4,865,968.75

Dollar Equivalent (@ 24.7T/Dollars) = $ 900,369.08

APPENDIX A

Written Consent Statement for Potential Participants in Pre-test

We wish to evaluate a new vaccine against cholera. Unlike past cholera vaccines, this vaccine is taken by mouth, not through an injection. It appears to be at least as effective as other cholera vaccines, and it has had no serious side-effects, when tested in many volunteers over the past three years. To be fully effective, this vaccine is given in three doses, each separated by about one month.

We will assign you/your child to receive either the vaccine or a vitamin preparation which is known to promote health.(*) Neither preparation is expected to cause any side-effects, but just in case, our doctors will visit you today and for the next two days to look after any such problems. Of course, our station at Nayergaon and our facilities at Matlab Hospital will also be available for treatment of any problem.

You/your child may decide whether or not you wish to participate. Please ask any questions that you have, and then inform me whether you give your consent to volunteer you/your child for participation. You/your child may refrain from participating in this program if you desire and the treatment that you normally receive will in no way be affected.

I fully understand the methods and basis for this study and agree to participate

Date: Signature _____

LTI _____

*For the subjects included in the serosurvey also read:
"A small quantity of fingertip blood will be taken today for examination before the second and third administered doses, and two weeks after the third administered dose."

APPENDIX B

Verbal Consent Statement to be Read to Potential Participants in Trial

We wish to evaluate a new vaccine against cholera. Unlike past cholera vaccines, this vaccine is taken by mouth, not through an injection. It appears to be at least as effective as other cholera vaccines, and it has had no serious side-effects, when tested in many volunteers over the past three years. To be fully effective, this vaccine is given in three doses, each separated by about one month.

You/your child will either receive the vaccine or a vitamin preparation known to promote health. Neither preparation is expected to cause any side-effects. In the unlikely event that you/your child subsequently experience any side-effect that you think may be related to the preparation that you/your child receive, we will have a physician stationed nearby, who you may see free of charge. Also, should you/your child experience diarrhoea despite having taken the preparation, we encourage you to attend Matlab Hospital or Nayergaon Treatment center, where we will be happy to treat you free of charge.

You/your child may decide whether or not you wish to participate. Please ask any questions that you have, and then inform me whether you give your consent to volunteer you/your child for participation. You/your child may refrain from participating in this program if you desire and the treatment that you normally receive will in no way be affected.

APPENDIX C

Written Consent Statement to be Read to Potential Participants in Serosurveys for Field Trial

In several days we will be offering you/your child the opportunity to participate in trial of a new vaccine against cholera. Unlike past cholera vaccines, this vaccine is taken by mouth, not through an injection. It appears to be at least as effective as other cholera vaccines, and it has had no serious side-effects when tested in many volunteers over the past three years. To be fully effective the vaccine must be given in 3 doses each separated by one month.

If you/your child choose to participate in the vaccine trial, you/your child will receive either the vaccine or a vitamin preparation which is known to promote health. If you think that you/your child will be participating when we return to give either the vaccine or the vitamin, we would now like to take a small quantity of fingertip blood. We will also take a repeat specimen in 1-2 months time, and a third specimen either in November or in March. These specimens will allow us to more fully evaluate the vaccine's strength in preventing cholera and other forms of diarrhoea.

If you/your child choose not to give a specimen, the treatment that you/your child normally receive from Matlab Hospital will in no way be affected and you may still participate in the vaccine trial.

I fully understand the methods and basis for this study and agree to participate.

Date: _____ Signature: _____

LTI: _____

Written Cosent Statement for Potential Participants in Breast-milk Studies

In several days we will be offering you the opportunity to participate in a trial of a new vaccine against cholera. Unlike past cholera vaccines, this new vaccine is taken by mouth, not through an injection. It is at least as effective as other cholera vaccines, and it has no known serious seide-effects.

If you decide to participate in the vaccine trial, you will either receive the vaccine or a vitamin preparation known to promote health. If you think that you will be participating when we return in several days to give you the vaccine or vitamin, we would now like to take a small quantity of breast-milk and a fingertip blood specimen for analysis. We will also take specimens in 4 weeks and 3 months. We would also like to measure your height, weight, and the skin-full thickness of your upper arm to better understand your state of nutrition. This will enable us to assess the effect of the vaccine in promoting health of your children through breast-feeding.

If you choose not to give specimens, the treatment that you receive from Matlab Hospital will in no way be affected, and you may still elect to participate in the trial.

I fully understood the methods and purpose of this study and agree to participate.

Date:_____ Signature:_____

LTI:_____

APPENDIX E

Written Consent Statement for Potential Participants in Surveys of Lactating Women and their Children

We understand that you are now feeding a child by breast. To understand whether breast-feeding may promote the protection against cholera in breast-fed children when the mother receives the vaccine, we would like to take some nutritional measurements of you and your child. Those measurements require no blood-drawing or other specimens, and only that you and your child permit us to take measurements of you/your child's height, weight, and arm size.

If you choose not to participate, the treatment that you receive from Matlab Hospital will in no way be affected.

I fully understand the methods and purpose of this study and agree to participate.

Date:_____ Signature:_____

LTI:_____

প্রাক-পরীক্ষায় সম্ভাব্য অংশগ্রহনকারীদের জন্যে লিখিত সম্মতি পত্র

আমরা কলেরা বিরোধী একটি নতুন টিকার মূল্যায়ন করতে চাই। আগেকার কলেরার টিকার সাথে এর পার্থক্য হচ্ছে যে, এই ঔষধ মুখে খেতে হয়, ইনজেকশনের মাধ্যমে দেওয়া হয় না। দেখা যাচ্ছে যে, এটি অন্যান্য কলেরার টিকার অন্ততঃপক্ষে সমান কার্যকরী, এবং গত তিন বছরে স্বেচ্ছাসেবীর উপর পরীক্ষায় এর কোন মারাত্মক পার্শ্বপ্রতিক্রিয়া দেখা যায়নি। সম্পূর্ণ কার্যকারিতার জন্যে এই টিকা এক মাসের ব্যবধানে তিনটি মাত্রায় দেওয়া হয়।

আপনাকে/আপনার শিশুকে তেরিশন দেওয়া হবে, অথবা দেওয়া হবে একটি ভিটামিন যুক্ত ঔষধ যা স্বাস্থ্যবর্ধক হিসাবে পরিচিত। (*) এ দু'এর কোনটিতেই পার্শ্বপ্রতিক্রিয়া সৃষ্টির আশংকা নেই, তবুও যদি কিছু হয় সেজন্যে আমাদের চিকিৎসকগন আজ এবং আগামী দু'দিন, এ ধরনের যে কোন সমস্যা সমাধান করতেই আপনাকে দেখে যাবেন। যে কোন সমস্যার চিকিৎসার জন্যে অবশ্যই আমাদের বায়েরগাঁও কেন্দ্র ও মতলব হাসপাতালের সুবিধে আপনার জন্যে রয়েছে।

আপনি/আপনার শিশু এতে অংশগ্রহণ করবেন কিনা সে ব্যাপারে সিদ্ধান্ত নিতে পারেন। আপনার যদি কোন প্রশ্ন থাকে, তাহলে আমাকে জিজ্ঞেস করুন, এবং তারপর আপনি/আপনার শিশু স্বেচ্ছাসেবী হিসাবে অংশগ্রহণ করছেন কি না তা আমাকে জানান। আপনি/আপনার শিশু ইচ্ছে করলে এই কর্মসূচীতে অংশ নাও নিতে পারেন, এবং তাতে আপনার স্বাভাবিক চিকিৎসার কোন ত্রুটি হবে না।

আমি এই পরীক্ষার পদ্ধতি এবং তিক্তি সম্পূর্ণ বুঝতে পেরেছি এবং এতে অংশগ্রহনে সম্মত হচ্ছি।

স্বাক্ষর----------

তারিখ---------- বাম বৃদ্ধাংগুলির ছাপ----------

---

*রক্ত পরীক্ষায় অনুর্ভুক্ত ব্যক্তির জন্যে আরও জ্ঞাতব্যঃ

"আজ, দ্বিতীয় ও তৃতীয় মাত্রা দেওয়ার আগে ও তৃতীয় মাত্রা দেওয়ার দু'সপ্তাহ পরে পরীক্ষার জন্যে আংগুলের অগ্রভাগ থেকে অল্প পরিমানে রক্ত নেওয়া হবে।