Attachment 1.
(FACE SHEET)

Date June 17, 1992

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. ZIA U. AHMED

Trainee Investigator (if any) 22

Application No. 92-019

Supporting Agency (if Non-ICDDR,B)

Title of Study :"Stability of killed-whole cell cholera vaccine containing the B-sub-unit of cholera toxin under heat-stress conditions"

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

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

1. Source of Population:
   (a) Ill subjects N/A Yes No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve:
   (a) Physical risks to the subjects N/A Yes No
   (b) Social Risks Yes No
   (c) Psychological risks to subjects Yes No
   (d) Discomfort to subjects Yes No
   (e) Invasion of privacy Yes No
   (f) Disclosure of information damaging to subject or others Yes No
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) N/A 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 study N/A 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 N/A Yes No
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects Yes No N/A
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.

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

Principal Investigator

Trainee

A-031961

92-019
17.6.92

APPLICATION FOR PROJECT GRANT

1. PRINCIPAL INVESTIGATOR : Zia Uddin Ahmed

2. OTHER INVESTIGATORS : R.B. Sack
A.S.M.H. Rahman
Ann-Mari Svennerholm,
(University of Goteborg)

3. PROJECT TITLE : Stability of killed-whole-cell cholera vaccine containing the B-subunit of cholera toxin under heat-stress conditions.

4. STARTING DATE : June 01, 1992

5. COMPLETION DATE : November 30, 1992

6. FUNDING SOURCE : SAREC

7. BUDGET REQUIRED : US $7018/00

8. HEAD OF DIVISION'S APPROVAL : R. Bradley Sack
R. B. Sack

9. AIM AND SIGNIFICANCE OF THE PROJECT:

Aim

The aim of the project is to examine the stability of an oral killed whole cell cholera vaccine containing the B-subunit of cholera toxin when stored under different environmental conditions.

Significance

An oral cholera vaccine consisting of killed whole cells and the B-subunit of the cholera toxin has been field-tested in Bangladesh with encouraging results (Clemens *et al.* 1986, 1991.) The present protocol will examine whether the vaccine would be stable under temperature-stress of the kind that it may be subjected to during field-use in the absence of a cold chain. If the vaccine exhibits an acceptable level of stability under temperature stress, a cold-chain may not be necessary during marketing and field-use of the vaccine, which will be highly advantageous in terms of ease of use and cost.

10. ETHICAL IMPLICATIONS : None

11. ABSTRACT SUMMARY:

A killed whole cell cholera vaccine containing recombinant B-subunit of cholera toxin (WC+RCTB) will be studied with respect to its stability when stored under different heat-stressed conditions. We use the expression "heat-stress" here in a general way, which does not reflect on the subject of biological responsiveness of living cells to changes in temperatures. At each temperature of 4°C, 20°C and 40°C, the vaccine will be maintained for 2 weeks, 4 weeks and 6 months. The vaccine will be assayed for LPS and B-subunit

content after storage under these conditions. Immunogenicity will be studied by immunizing rabbits subcutaneously and measuring anti-LPS and anti-CTB titers in sera of immunized animals. This vaccine is being considered for large-scale production in Bangladesh.

12. BACKGROUND, RESEARCH PLAN, BIBLIOGRAPHY

[A] BACKGROUND

An oral cholera vaccine comprising killed whole cells of *Vibrio cholerae* 01 (classical and El Tor biotypes, Ogawa and Inaba serotypes) and the B-subunit of cholera toxin was subjected to a large field trial in Bangladesh and evaluation of its efficacy monitored over a period of 3 years following vaccination (Clemens *et al.* 1986, 1990). Encouraging results were obtained as to the safety and protective efficacy of the vaccine. The vaccine was administered as a liquid containing the killed cells and the toxin subunit. It was maintained in the cold (+4^{o}C) from its production until its field-administration. Cold chain requirement by a vaccine is a potentially negative factor in mass use of a vaccine in the third world. It has not yet been tested whether the above cholera vaccine will be stable at elevated temperatures and if so, for how long.

We wish to examine heat-stability of the vaccine by subjecting the vaccine to different conditions of heat-

stress using different temperatures and different lengths of exposure to a particular temperature.

[B] RESEARCH PLAN

THE VACCINE

The vaccine will be the same as the whole-cell + B-subunit vaccine which was field tested, expect that the B-subunit in the present vaccine formulation is a product of recombinant DNA technology and as such referred to here as 'recombinant cholera toxin B-subunit' (RCTB). The vaccine is thus abbreviated as WC + RCTB oral cholera vaccine. The vaccine will be obtained from Prof. J. Holmgren of the University of Göteborg as bulk liquid lots of 300 ml containing 100 doses. Each dose of the vaccine will contain 1×10^{11} killed *Vibrio* cells and 1 mg of RCTB in a volume of 3 ml.

We wish to mention here that this is the vaccine on which a feasibility study as to its production and use in Bangladesh has been recently completed. The study indicates that prospects of this vaccine being produced in Bangladesh are good and the vaccine may therefore be locally used as a public health tool in programs of cholera prevention and control.

EXPERIMENTS

The vaccine will be maintained at temperatures 4°C (control), 20°C and 40°C for durations of 2 weeks, 4 weeks and 6 months at each temperature in single-dose aliquots in sterile sealed vials. At each time point, the vaccine vials will be used for the following experiments:

(i) Determination of sterility
(ii) Determination of LPS content of the vaccine,
(iii) Determination of the B-subunit content of the vccine,
(iv) Immunogenicity studies of the vaccine in rabbits by determining serum antibody.

Determination of LPS content

The amount of LPS will be quantitated by using an LPS-inhibition ELISA. A fixed quantity of pure LPS will be bound to microtiter wells in the solid phase. This will be reacted with a fixed quantity of anti-LPS MAb which has been previously mixed with the test vaccine cells in the liquid phase. The test cells will compete with the solid phase LPS for the available MAb combining-sites. The extent of inhibition of MAb binding to solid phase LPS will be proportional to the competing cell-bound LPS in the test specimen. LPS titer in the test specimen may then be expressed as reciprocal of the highest dilution of the test

sample that gave an inhibition equal to half that of a standard reference cell suspension.

Briefly, the procedure which is followed by the Swedish Bacteriological Laboratory (SBL) is as follows. A microtiter plate will be coated with a standard LPS preparation of *Vibrio cholerae* 01 (plate 1). In a separate microtiter plate (plate 2) the test sample will be placed in 3-fold dilutions in one row of the plate in a total volume of 60 µl. These wells will then receive a fixed quantity (in a 60 µl volume) of an anti-LPS MAb. The standard LPS coated plate 1 will be washed and each well of this plate will receive 100 µl aliquots from the corresponding wells of plate 2. After incubation at room temperature for 90 min, the plate will be washed and treated with an anti-mouse Ig-HRP conjugate and incubation continued for another 90 min. After washing, the plate will be developed with orthophenyl diamine (OPD) and read for $A_{450\ nm}$. A curve will be made by plotting absorbance values against log dilutions of the test sample. The sample dilution that inhibited 50% binding of anti-LPS MAb to the solid-phase-bound LPS will be determined. This 50% inhibitory titer of the test sample will be used to determine the amount of LPS present in the sample by comparing such values with that of a standard vaccine preparation.

Determination of B-subunit content

B-subunit content of the vaccine will be determined by single radial immunodiffusion (SRID) method of Mancini (1965) using a high-titer rabbit anti-CTB serum and known amounts of RCTB to generate a standard curve, relating CTB concentration to the diameter of the precipitin ring.

Protein profile of crude cell envelope preparation

A crude cell envelope protein extract will be prepared by the method described by Manning *et al.* (1982). The extracts will be examined by SDS-PAGE for evidence of any qualitative and quantitative changes in the profile during storage of the vaccine at elevated temperatures.

Immunogenicity of the vaccine

Five adult New Zealand white rabbits of approximately 2 kg. weight will be subcutanously immunized with each vaccine sample. A booster dose will be given 14 days later. Sera will be collected on day 0, 7, 14, 21 and 28. Anti-LPS and anti-CTB titers will be determined by ELISA.

Data analysis

Quantitation of LPS and RCTB: Determination of the quantity of LPS and RCTB will be carried out in true triplicates (using triplicate samples of the vaccine at each sampling time). Average of the values will be accepted as representing a correct estimate if individual values fall within standard deviations.

Immunogenicity and protective efficacy: Titers of antibodies against LPS and CTB will be determined in each group of animals and values expressed as mean ± standard error.

Comparison: Comparison of data sets obtained by using the vaccine samples will be made by using Fisher's exact test.

Collaboration

The vaccine and other reagents such as purified LPS, anti-LPS MAb and rabbit anti-CTB serum will be obtained from Drs. Holmgren and Ann-Mari Svennerholm, University of Göteborg.

[C] BIBLIOGRAPHY

Clemens JD, Sack DA, Harris JR, Chakraborty J *et al.* 1986, Lancet ii, 124-127.

Clemens JD, Sack DA, Harris JR, vanLoon F, *et al.* 1990. Lancet i, 270-273.

Cray WC Jr, Tokunaga E, Pierce NF. 1983. Successful colonization and immunization of adult rabbits by oral inoculation with *Vibrio cholerae* o1. Infect. Immun. 41, 735-741.

Mancini G, Garbenara AO, Heremans, VF. 1985. Immunochemical quantitation of antigen by single radial immunodiffusion. Immunochemistry 2, 235.

Manning PA, Imbesi F, Haynes DR. 1982. Cell envelope protein of *Vibrio cholerae*. FEMS Microbiol. Lett., 14, 159-166.

BUDGET

A. Personnel

Name	%involvement	Cost, $
Zia Uddin Ahmed	10%	-
R.B. Sack	5%	-
A.S.M.H. Rahman	10%	-
Trainee (6 months)	100%	1000

B. Materials and Supplies

Media, microtiter plates, reagents	1000

C. Animals

Three temperature-stress conditions	
Three sampling time	
Five rabbits per sampling	
(3 x 3 x 5 = 45 + 45 control = 90	
@ $16/animal	1440
Care-cost, $ 1.42/d/animal	
90 animals x 28 days x $1.42	3578
	7018/00

aa/zua/pr1(pgsarec1)

Title: Stability of killed-whole-cell cholera vaccine containing the B-subunit of cholera toxin under heat-stress conditions.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	✓		
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification [ ]

b) with qualification [ ]

- on technical grounds [✓]
- on level of financial support [ ]

I do not support the application [ ]

Name of Referee: Rudolf A. Karch

Signature: Rudolf A. Karch Date: 6/4/92

Position: Consulting Microbiologist

Institution: Centers for Disease Control
1600 Clifton Rd. N.E.
Atlanta, GA 30333 USA

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel thay are justified.

(Use additional pages if necessary)

Title: Stability of Killed-whole-cell cholera vaccine containing the B-subunit of Cholera Toxin under heat-stress conditions

PI: Dr. Zia Uddin Ahmed

Reviewer: Bradford A. Kay

A very practical, and needed protocal- Well thought out and well described. The budget is in keeping with the requirements of the protocal- My only suggestion is to assess the sterility of the vaccine in addition to determining the LPS, B-subunit and Immunogenicity. Contamination, as much as heat, would alter the vaccine component. I have no reservation in supporting the theoretical and scientific basis of this protocal.

Bradford A. Kay

For Dr. Zin

Title: Stability of killed-whole-cell cholera vaccine containing the B-subunit of cholera toxin under heat-stress conditions.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are nought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	See Comment		
Potential value of field of knowledge	✓		
		✓	

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification ☐

- on technical grounds ☑
- on level of financial support ☐

I do not support the application ☐

Name of Referee:

Signature: ..... Date: 12/6/92

Position: Deputy Director

Institution: CAMR, Porton
Salisbury
U.K.

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel thay are justified.

(Use additional pages if necessary)

Title: Stability of killed-whole-cell cholera vaccine containing the B-subunit of cholera toxin under heat-stress conditions.

PI: Zia Uddin Ahmed

Reviewer: .....

I see no scientific problems with the project, it is a simple straight-forward stability study employing standard methodologies.

The timescale is given as 1/6/92 to 30/11/92. Since the final [illegible] at 6 months and [illegible] at least 28 days, the study will have to overrun the allotted time.

Heat-stress studies of killed cholera vaccine

RESPONSE TO REVIEWERS' COMMENTS

Reviewer	Response
Dr. Bradford A. Kay	The suggestion that sterility of the vaccine be monitored during the study is now incorporated in the protocol.
Dr. J. Melling	The time-frame is extended to 6 months instead of 3 months because the immunogenicity studies in rabbits may be more time consuming than previously thought.

Zia Uddin Ahmed