


Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Qadri F, Wierzbina TF, Ali M, et al. Efficacy of a single-dose, inactivated oral cholera vaccine in Bangladesh. *N Engl J Med* 2016;374:1723-32. DOI: 10.1056/NEJMoa1510330

This supplement contains the following items:

1. Original protocol
2. Summary of changes (amendments)
3. Final protocol
4. Statistical analysis plan

		RRC APPLICATION FORM			
RESEARCH PROTOCOL Number: PR-12090 Version No. 1.0 Version date: 20-11-2012		FOR OFFICE USE ONLY			
		RRC Approval:	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	Date: 20/11/2012
		ERC Approval:	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Date:
		AEEC Approval:	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Date:
		External IRB Approval	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Date:
If, 'yes' Name of IRB: _____					
Protocol Title: An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shanchol) in Dhaka, Bangladesh					
Short title (in 50 characters including space): Single dose oral cholera vaccine study in Dhaka					
Originating Centre <input type="checkbox"/> Child and Adolescent Health <input type="checkbox"/> Chronic Diseases <input type="checkbox"/> Communicable Diseases <input type="checkbox"/> Equity and Health Systems <input type="checkbox"/> Food and Waterborne Diseases		<input type="checkbox"/> HIV/ AIDS <input type="checkbox"/> Nutrition and Food Security <input type="checkbox"/> Population, Urbanisation and Climate Change <input type="checkbox"/> Reproductive Health <input checked="" type="checkbox"/> Vaccine Sciences <input type="checkbox"/> Other (specify)			
SP2020 Research Priority Area (check all that apply) <input type="checkbox"/> Healthy Life Course <input type="checkbox"/> Mitigating Risk and Vulnerability		<input checked="" type="checkbox"/> Combating Priority Diseases <input type="checkbox"/> Equitable Health Systems			
Research Phase (4 Ds) <input checked="" type="checkbox"/> Discovery <input type="checkbox"/> Development		<input checked="" type="checkbox"/> Delivery <input checked="" type="checkbox"/> Evaluation of Delivery			
Anticipated Impact of Research (check all that apply) <input checked="" type="checkbox"/> Knowledge Production <input checked="" type="checkbox"/> Capacity Building		<input type="checkbox"/> Informing Policy <input checked="" type="checkbox"/> Health and Health Sector Benefits <input checked="" type="checkbox"/> Economic Benefits			
Does this Protocol use the Gender Framework: [please refer to Gender Analysis Tool with Guidance Document in the Intranet]			<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		
If 'no' is the response, its reason(s) in brief:					
Will this research specifically benefit the disadvantaged (economically, socially and/or otherwise)?			<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		
Does this Protocol use Behaviour Change Communication:			<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Key words: Cholera vaccine, single dose, randomized trial					
Principal Investigator (Should be icddr,b staff): Sex <input checked="" type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No. (if any), cell phone number, and Email address): Dr. Firdausi Qadri Employee ID- I00003; Centre for Vaccine Sciences, icddr,b, 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh.. Tel: 880 2 9841751 to 9841760, Ext. 2431; Fax: 8802- 8823116; e-mail: fqadri@icddr.org ;					
Co-Principal Investigator(s) Internal: Sex <input checked="" type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address): [if more than one, please copy and paste this row for additional Co-PIs] Dr.Fahima Chowdhury, Dr Amit Saha, Dr Iqbal Ansary Khan, Dr Ashrafur Islam Khan					

Co-Principal Investigator(s) - External: Sex <input type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address). [if more than one, please copy and paste this row for additional Co-PIs] Dr. Thomas Wierzba International Vaccine institute(IVI) SNU Research Park San 4-8 Nakseongdae-dong, Kwanak-gu, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803 Mail address: twierzba@ivi.int													
Co-Investigator(s) - Internal: Sex <input checked="" type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including phone no(s), Extension No (if any), cell phone number, and Email address): [if more than one, please copy and paste this row for additional Co-Is] Dr. Yasmin Ara Begum													
Co-Investigator(s) – External: Sex <input checked="" type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address): [if more than one, please copy and paste this row for additional Co-Is] Drs. Ajit Pal Singh (IVI), Binod Sah (IVI), Mohammad Ali (IVI), Ms Yang Hee Kim (IVI), Dr. Mahmudur Rahman (IEDCR & NIC) ,Dr. S.A.J. Md. Musa,(PHC), Dr. Baizid Khoorshid Riaz, Dr. Md. Tajul Islam A. Bari EPI & Surveillance , Dr.Md.Shamsuzzaman (EPI), Dr Sanjida Islam (DCC)													
Student Investigator(s) - Internal (Centre’s staff): Sex <input type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address):													
Student Investigator(s) - External: Sex <input type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address):													
Collaborating Institute(s): Please provide full official address Institution # 1 <table border="1"> <tr> <td>Country</td> <td>Seoul, South Korea</td> </tr> <tr> <td>Contact person</td> <td>Dr. Thomas Wierzba</td> </tr> <tr> <td>Department (including Division, Centre, Unit)</td> <td>Translational Research Division, International Vaccine Institute</td> </tr> <tr> <td>Institution (with official address)</td> <td>International Vaccine Institute</td> </tr> <tr> <td>Directorate (in case of GoB i.e. DGHS)</td> <td>International Vaccine Institute SNU Research Park San 4-8 Bongcheon 7-dong Kwanak, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803</td> </tr> </table>		Country	Seoul, South Korea	Contact person	Dr. Thomas Wierzba	Department (including Division, Centre, Unit)	Translational Research Division, International Vaccine Institute	Institution (with official address)	International Vaccine Institute	Directorate (in case of GoB i.e. DGHS)	International Vaccine Institute SNU Research Park San 4-8 Bongcheon 7-dong Kwanak, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803		
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Collaborating Institute(s): Please provide full official address Institution # 1 <table border="1"> <tr> <td>Country</td> <td>Bangladesh</td> </tr> <tr> <td>Contact person</td> <td>Dr. S.A.J. Md. Musa, Director (PHC) & Line Director MNC & AH</td> </tr> <tr> <td>Department (including Division, Centre, Unit)</td> <td>Primary Health Care</td> </tr> <tr> <td>Institution (with official address)</td> <td>Director (PHC), DGHS, Mohakhali, Dhaka. Tel: 9883137, Mob: 01819487770 Email : sajmusa@yahoo.com</td> </tr> <tr> <td>Directorate (in case of GoB i.e. DGHS)</td> <td>DGHS</td> </tr> <tr> <td>Ministry (in case of GoB)</td> <td>MoHFW</td> </tr> </table>		Country	Bangladesh	Contact person	Dr. S.A.J. Md. Musa, Director (PHC) & Line Director MNC & AH	Department (including Division, Centre, Unit)	Primary Health Care	Institution (with official address)	Director (PHC), DGHS, Mohakhali, Dhaka. Tel: 9883137, Mob: 01819487770 Email : sajmusa@yahoo.com	Directorate (in case of GoB i.e. DGHS)	DGHS	Ministry (in case of GoB)	MoHFW
Country	Bangladesh												
Contact person	Dr. S.A.J. Md. Musa, Director (PHC) & Line Director MNC & AH												
Department (including Division, Centre, Unit)	Primary Health Care												
Institution (with official address)	Director (PHC), DGHS, Mohakhali, Dhaka. Tel: 9883137, Mob: 01819487770 Email : sajmusa@yahoo.com												
Directorate (in case of GoB i.e. DGHS)	DGHS												
Ministry (in case of GoB)	MoHFW												

Institution # 2

Country	Bangladesh
Contact person	Professor Mahmudur Rahman Director, IEDCR & NIC
Department (including Division, Centre, Unit)	IEDCR
Institution (with official address)	Director, IEDCR & NIC DGHS, Mohakhali, Dhaka. Tel: 8821237 (Off), 8950828 (Res), Mob: 01711595139, mrahman57@hotmail.com
Directorate (in case of GoB i.e. DGHS)	DGHS
Ministry (in case of GoB)	MoHFW

Institution # 3

Country	Bangladesh
Contact person	Dr. Md. Tajul Islam A. Bari Programme Manager, EPI & Surveillance
Department (including Division, Centre, Unit)	EPI & Surveillance
Institution (with official address)	Programme Manager, EPI & Surveillance, DGHS, Mohakhali, Dhaka. Tel: 9880530 (Off), 8821910 (Off), 01711976956, Email: tajulepi@yahoo.com
Directorate (in case of GoB i.e. DGHS)	DGHS
Ministry (in case of GoB)	MoHFW

Institution # 4

Country	Bangladesh
Contact person	Dr Sanjida Islam , Project Officer
Department	Health Unit
Institution	Dhaka City Corporation Tel :9557055(Off), 01713092669
Ministry (in case of GoB)	Ministry of Local Government Division (LGRD), Urban primary health care services delivery project

Contribution by the Members of the Scientific Team

Member' Name	Contribution								
	Research idea/concept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/interpretation of results	Manuscript writing
Dr Firdausi Qadri	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr Iqbal Ansary Khan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr.Fahima Chowdhury	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr.Amit Saha	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr Ashraful Islam Khan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Prof Mahmudur Rahman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr Yasmin Ara Begum	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Md. K. Zaman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Md Atique Iqbal	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Dr. Thomas Wierzba	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Binod Sah	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Mohammad Ali	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Ajit Pal Singh	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Taufiqur Rahman Bhuyan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr Farhana Khanam	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Afroza Akter	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Muhammad Asaduzzaman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. Md. Arifuzzaman Khan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Md Murshed Alam	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Dr. S.A.J. Md. Musa	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dr. Md. Tajul Islam A. Bari	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dr Sanjida Islam	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dr. Nurun Nabi	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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

Sex

- Male
 Female


Age

- 0 – 4 years
 5 – 10 years
 11 – 17 years
 18 – 64 years
 65 +

- Pregnant Women
 Fetuses
 Prisoners
 Destitutes
 Service Providers
 Cognitively Impaired
 CSW
 Others :
 Animal

NOTE: It is icddr.b's policy to include men, women, and children in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Project/study Site (Check all that apply):	
<input type="checkbox"/> Chakaria <input checked="" type="checkbox"/> Dhaka Community, Mirpur <input checked="" type="checkbox"/> Dhaka Hospital <input type="checkbox"/> Kamalapur Field Site/HDSS <input type="checkbox"/> Matlab DSS Area <input type="checkbox"/> Matlab non-DSS Area <input type="checkbox"/> Matlab Hospital <input type="checkbox"/> Mirzapur	<input type="checkbox"/> Bandarban <input checked="" type="checkbox"/> Other areas in Bangladesh Name: Hospitals and Health facilities in Mirpur, Dhaka <hr/> <input type="checkbox"/> Outside Bangladesh Name the Country: _____ <hr/> <input type="checkbox"/> Multi Centre Trial (Name other countries involved): _____
Which of the Millennium Development Goal(s) this Proposal Relates to:	
<input type="checkbox"/> 1. Eradicate extreme poverty and hunger	<input type="checkbox"/> 2. Achieve universal primary education
<input type="checkbox"/> 3. Promote gender equality and empower women	<input checked="" type="checkbox"/> 4. Reduce child mortality
<input type="checkbox"/> 5. Improve maternal health	<input checked="" type="checkbox"/> 6. Combat HIV/AIDS, malaria and other diseases
<input type="checkbox"/> 7. Ensure environmental sustainability	<input type="checkbox"/> 8. Develop a global partnership for development
Type of Study (Check all that apply):	
<input type="checkbox"/> Case Control Study <input checked="" type="checkbox"/> Clinical Trial (Hospital/Clinic/Field)* <input type="checkbox"/> Community-based Trial/Intervention <input type="checkbox"/> Cross Sectional Survey <input type="checkbox"/> Family Follow-up Study <input type="checkbox"/> Longitudinal Study (cohort or follow-up) <input type="checkbox"/> Meta-analysis <input type="checkbox"/> Programme Evaluation	<input type="checkbox"/> Programme (Umbrella Project) <input type="checkbox"/> Prophylactic Trial <input type="checkbox"/> Record Review <input type="checkbox"/> Secondary Data Analysis <input checked="" type="checkbox"/> Surveillance/Monitoring <input type="checkbox"/> Systematic Review <input type="checkbox"/> Others:
<p>*Note: Following RRC and ERC approval of a clinical trial, the PI should provide relevant information about the research protocol to the IRB Secretariat (Research Administration Services) for uploading into websites (usually at the https://register.clinicaltrials.gov/), for its registration. In the event of modification of the initially approved protocol the PI shall, after securing approval of RRC and ERC, provide relevant new information to the IRB Secretariat for updating of the protocol in the website.</p> <p>ICMJE defined clinical trial as “Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome”.</p>	
Targeted Population (Check all that apply):	
<input checked="" type="checkbox"/> No ethnic selection (Bangladeshi) <input checked="" type="checkbox"/> Bangalee <input type="checkbox"/> Tribal group	<input type="checkbox"/> Expatriates <input type="checkbox"/> Immigrants <input type="checkbox"/> Refugee <input type="checkbox"/> Special group (specify):
Consent Process (Check all that apply):	
<input checked="" type="checkbox"/> Written <input checked="" type="checkbox"/> Oral <input type="checkbox"/> None	<input checked="" type="checkbox"/> Bengali Language <input type="checkbox"/> English Language
Biological Specimen	
a) Will the specimen be stored for future use?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable
b) If yes, how long the specimens will be preserved?	5 years
c) What types of tests will be carried out with the preserved specimens?	Microbiological, culture and sensitivity, immunological tests in subgroup of specimens

d) Will consent be obtained from study participants for use of the stored specimen for unrelated to this study without their re-consent?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/ countries?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Not applicable
f) Will the surplus/unused specimen be returned to icddr,b? If no, then the surplus/unused specimen must be destroyed.	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Not applicable
g) Who will be the custodian of the specimen at icddr,b?	Dr. Firdausi Qadri
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	Not Applicable
i) Who will be the owner(s) of the specimens?	icddr,b
j) Has a MoU been developed for the protocol covering the specimen collection, storage, use and ownership? If yes, please attach a copy. If no, it is mandatory to provide reason(s) why this is not applicable?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Not applicable
Risk Group of Infectious Agent and Use of Recombinant DNA	
a) Will specimens containing infectious agent be collected?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable
b) Will the study involve amplification by culture of infectious agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable
c) If response to questions (a) and/or (b) is positive, to which Risk Group (RG) does the agent(s) belong? (ref cdc link...)	<input type="checkbox"/> RG1 <input checked="" type="checkbox"/> RG2 <input type="checkbox"/> RG3 <input type="checkbox"/> RG4
d) Does the study involve experiments with recombinant DNA?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Not applicable
Does the study involve any biohazardous materials/agents or microorganisms of risk group 2, 3, or 4 (GR2, GR-3 or GR4)?	
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
If yes, I (the Principal Investigator) certify that standard icddr,b laboratory procedures will be followed for biosafety of the hazardous materials/agents or microorganisms in the conduction of the study.	
 Signature of the Principal Investigator	19/11/2012 Date
Proposed Sample Size: Sub-group (Name of subgroup (e.g. Men, Women) and Number	
Name	Number
(1) Vaccine arm	95,115
(2) Placebo Arm	95,115
Total sample size	190,230

Determination of Risk: Does the research involve (Check all that apply):

Is the information recorded in such a manner that study participants can be identified directly or through identifiers linked to them?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does the research deal with sensitive aspects of participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No

Do you consider this research (Check one):

<input type="checkbox"/> Greater than minimal risk	<input checked="" type="checkbox"/> No more than minimal risk	<input type="checkbox"/> Only part of the diagnostic test
----------------------------------------------------	---------------------------------------------------------------	-----------------------------------------------------------

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

Funding

Is the protocol funded?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
If the answer is yes, please provide sponsor(s)'s name	Bill and Melinda Gates foundation to IVI	

If fund has not been identified N/A

Is the proposal being submitted for funding?	<input type="checkbox"/> Yes	<input type="checkbox"/> No
If yes, name of the funding agency	1.	
	2.	

Dissemination plan [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is a yes)
Seminar for icddr,b scientists/ staff	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	For internal staffs to disseminate the findings
Internal publication	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	In Health and Science Bulletin of icddr,b
Working paper	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	For record keeping and dissemination
Sharing with GoB (e.g. DGHS/ Ministry, others)	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Through dissemination meetings where findings will be shared with GoB to assist in taking the vaccine to high risk population
Sharing with national NGOs	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Through dissemination meeting along with GoB
Presentation at national workshop/ seminar	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	We will share results with stakeholders in Bangladesh through meetings and seminar
	<input type="checkbox"/> No	<input type="checkbox"/> Yes	
Presentation at international workshop/ conference	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	We will present our findings at the different international conferences
Peer-reviewed publication	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	We will submit our manuscripts in peer reviewed journals
Sharing with international agencies	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Our data and findings will be shared with international agencies, particularly with WHO, UNICEF and GoB.
Sharing with donors	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Our data and findings will be shared
Policy brief	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes	

Conflict of interest

Do any of the participating investigators and/or member(s) of their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

No Yes (please submit a written statement of disclosure to the Executive Director, icddr,b)

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date : ASAP

End Date : 2 .5 years after study initiation

Cost Required for the Budget Period (\$)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1	1,891,752	252,898	2,144,650
Year-2	1,506,666	226,000	1,732,666
Year-3	690,664	103,600	794,264
Total	4,089,082	582,498	4,671,580

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the SUCHONA (Form # 2) if a grant is awarded as a result of this application.

Firdausi Qadri
Signature of PI

31/10/2012
Date

Approval of the Project by the Centre Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Centre level.

Firdausi Qadri
Name of the Centre Director

Firdausi Qadri
Signature

31/10/2012
Date of Approval

Definition of Abbreviations used

AE	Adverse Event
GOB	Government of Bangladesh
CHW	Community Health Worker
CI	Confidence Interval
CRA	Clinical Research Associate
CRFs	Case Report Forms
DSMB	Data Safety Monitoring Board
DSS	Demographic Surveillance System
ERC	Ethical Review Committee. The name of the ethical review committee at the icddr,b
GCP	Good Clinical Practices - a set of standards used to document the accuracy of the clinical data being collected
GIS	Geographical Information system
GMT	Geometric Mean Titre
icddr,b	The International Centre for Diarrhoeal Disease Research, Bangladesh – Sometimes referred to as “the Centre.”
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IEDCR	Institute of Epidemiology Disease Control and Research
IR	Incidence rate
IVI	International Vaccine Institute
NGO	Non Government Organization
NICED	National Institute of Cholera and Enteric Diseases
NIHE	National Institute of Hygiene and Epidemiology (Hanoi)
PI	Principal investigator
PID	Personal Identification
RRC	Research Review Committee
SAE	Serious Adverse Event
WHO	World Health Organization

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Check here if appendix is included

Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: Dr. Firdausi Qadri	
Research Protocol Title: An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shanchol) in Dhaka, Bangladesh	
Proposed start date: ASAP	Estimated end date: 2.5 years after initiation
<p>Background (brief):</p> <p>a. Burden: Bangladesh remains endemic for cholera, which peaks biannually with further increases seen during floods, cyclones or any natural disaster [1, 2]. It affects all age groups with the majority of fatal cases occurring in children [3-6] although the disease is seen in all age groups. Therefore, immunization against cholera remains an important public health tool for preventing the spread of cholera and for control of the disease [6].</p> <p>b. Knowledge gap: The current two-dose regimen of the internationally available oral cholera vaccines (OCV) create a logistical and programmatic challenge for use in national programs or in epidemics [7]. Post-disaster situations and the capabilities to respond in non- endemic countries can vary vastly in different disaster and economic settings, and an option to use single dose in areas where major population disruption occurs versus use of two doses at an interval of 2 weeks, can make a lot of difference on deciding for use of vaccine by policy makers. Getting the vaccine to the same people twice poses difficulties in the control of cholera in both endemic and epidemic settings. Studies performed in cholera-endemic country using the OCV, Shanchol induces significant vibriocidal responses even after a single dose as compared to two doses of vaccine. It is important to determine if a single dose vaccine will be protective in regions where cholera is endemic, e.g. Bangladesh.</p> <p>c. Relevance: Since vibriocidal antibody response is only an indirect serological correlate of protection, for killed cholera vaccine at present, an efficacy trial with placebo control would be required to confirm the usefulness of the single dose [8]. If the vaccine is found to be efficacious following a single dose, this will have profound implications on the use of the vaccine in areas with limited resources particularly in complex emergencies where a multiple dose regimen is difficult to deploy. A single-dose regimen of this vaccine will improve its “field ability” and allow the vaccine to be used even in outbreak control especially in difficult settings where the risk of cholera is extremely high and provisions for clean water and sanitation are not available [9]. The OCV production is in low supply, thus larger populations can be immunized against cholera, if a single dose is found to be efficacious. A single-dose schedule will also facilitate its inclusion in the EPI schedule and in national immunization programs as it may be given together with other vaccines in endemic settings where young children are at highest risk for the disease.</p> <p>Hypothesis (if any): The study hypothesis is to “decrease” the incidence rate (IR) of cholera among single dose recipients of the oral cholera vaccine to the incidence rate among placebo recipients with a pre-specified difference of 50%.</p> <p>Objectives: The primary objective of the study is to evaluate protective efficacy of a single dose regimen of a</p>	

bivalent, killed, whole cell oral cholera vaccine given to healthy, non-pregnant residents aged one year and over in Dhaka, Bangladesh, against culture-proven *V. cholerae* O1 diarrhea detected in all treatment settings serving the catchment population over a 12-month follow-up.

Methods:

The study design is a two-arm individually randomized double-blind placebo-controlled trial. Individuals living in each household under surveillance will serve as the units of randomization with 1:1 vaccinee-to-placebo ratio.

Outcome measures/variables: The primary outcome of the study is the proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with faecal excretion of *V. cholerae* O1 in the study treatment centres from 14 days to 12 months after dosing and whose identity is confirmed through home visit.

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: No Yes (describe below)

The study hypothesis is to “decrease” the incidence rate (IR) of cholera among single dose recipients of the oral cholera vaccine to the incidence rate among placebo recipients with a pre-specified difference of 50%.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

Primary objective of the study

The primary objective of the study is to evaluate protective efficacy of a single dose regimen of a bivalent, killed, whole cell oral cholera vaccine given to healthy, non-pregnant residents aged one year and over in Dhaka, Bangladesh, against culture-proven *V. cholerae* O1 diarrhea detected in all treatment settings serving the catchment population over a 12-month follow-up.

Secondary Objective

- To evaluate protective efficacy of a single dose regimen of a bivalent, killed, whole cell oral cholera vaccine over a 6-month of follow-up.
- To evaluate protective efficacy and safety of a single dose regimen of a bivalent, killed, whole cell oral cholera vaccine given to healthy, non-pregnant residents aged one year and above against culture-proven *V. cholerae* O1 diarrhea detected in all treatment settings serving the catchment population over a 24-months of follow-up.

- To evaluate protective efficacy of a single dose regimen of a bivalent, killed, whole cell-based oral cholera vaccine given to healthy, non-pregnant residents one year of age and above, during 12 and 24 months of follow-up against:
 - Culture-proven *V. cholerae* O1 diarrhea , detected in treatment centres
 - Culture-proven *V. cholerae* O1 diarrhea with severe dehydration detected in treatment centres
 - Culture-proven *V. cholerae* O139 diarrhea, detected in treatment centres
 - Culture-proven *V. cholerae* O139 diarrhea, with severe dehydration detected in treatment centres
 - Acute watery diarrhea detected in treatment centres
 - Acute watery diarrhea with severe dehydration detected in treatment centres
- To evaluate serum vibriocidal (to El Tor Inaba and Ogawa serogroup O1 and to serogroup O139 organisms) antibody responses to a single dose regimen of bivalent, killed, whole cell-based oral cholera vaccine in healthy, non-pregnant residents, aged one year and above in a subset of population (324 participants).
- To evaluate and compare the safety of a single dose of a bivalent, killed, whole cell oral cholera vaccine administered to healthy, non-pregnant residents aged one year and above.

Background of the Project including Preliminary Observations

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Cholera continues to be a serious public health problem worldwide. In 2010, a total of 237,000 cases including around 6,000 deaths were reported to the World Health Organization (WHO) from globally primarily in Africa, and Asia [6]. Compared to the 2007 figures, this represents 8% and 27% increase in the number of cases and deaths respectively. Moreover, when analysed by 5-year period, the global incidence and number of deaths due to cholera have shown an increasing trend in the last ten years. A cumulative total of 838,315 cases were notified to World Health Organization (WHO), compared with 676,651 cases between 2000 and 2004, representing a 24% increase in the number of cases reported for this most recent 5-year period [10]. The true figures are likely to be much higher due to underreporting as WHO estimates that only 5-10% of cholera cases are actually reported [11].

More recently, unprecedented outbreaks have been seen recently in many countries including Zimbabwe, Haiti, Pakistan, Nepal, Guinea, Cuba, Congo, and Sierra Leone. These cholera outbreaks cause undue suffering with high mortality and morbidity figures as well as economic and social

disruption. Regions in India and Bangladesh have long been recognized as the home-land of cholera where 6 of the 7 reported cholera pandemics had their origin [12]. Bangladesh remains endemic for cholera, which peaks biannually with further increases seen during floods and cyclones [1, 2, 13]. It affects all age groups although the majority of fatal cases occur in children [3-6]. Therefore, immunization against cholera remains an important public health tool for preventing the spread of cholera and for control of the disease [6].

Provision of safe water and food, establishment of adequate sanitation, and implementation of personal and community hygiene constitute the main public health interventions against cholera. These measures cannot be implemented fully in the near future in most cholera-endemic areas. A safe, effective, and affordable vaccine would be a useful tool for cholera prevention and control. A parenteral killed whole cell cholera vaccine, previously available for many years, is no longer recommended by WHO because of its limited efficacy and high rates of adverse reactions [8].

Considerable progress has been made during the last decade in the development of new generation oral vaccines against cholera. DukoralTM (Crucell/ SBL), a killed whole cell *V. cholerae* O1 with recombinant B-subunit (rBs-WC) containing vaccine is the first to be licensed internationally, has been available mostly in developed countries as a traveller's vaccine. This vaccine is licensed in over 50 countries including Bangladesh. Several mass vaccination programs have been carried out successfully with Dukoral including in Beira, Mozambique, in Indonesia after the Tsunami, in Madagascar, Sudan and Zanzibar. Overall over 500,000 people have been vaccinated with Dukoral in these mass vaccinations. Analyses of the herd protective effects of killed oral cholera vaccine trial showed that a greater than 90% reduction in cholera disease burden can be achieved having only moderate (~50% - 60%) level of coverage [14] and shown to be efficacious even in developing country settings [15, 16]. The WHO now recommends Dukoral for both endemic and epidemic cholera. However, two disadvantages limit broader use of Dukoral. First, its current price is prohibitively expensive, for example in Bangladesh it is sold for the equivalent of \$18 per dose. Second, Dukoral needs to be administered with a buffer which complicates large scale deployment. These pose logistical barrier for its public health use.

Another cholera vaccine available only in Vietnam, ORC-VaxTM, a bivalent (*V. cholerae* O1 and O139) killed whole cell oral cholera vaccine, has been in use since 1997 and more than 9 million doses have been given in the Vietnam's public health setting [17]. This vaccine, also given in two doses, was shown to be safe and effective in Vietnam [17, 18] and was targeted for internationalization by the IVI through ensuring it's WHO prequalification. However, upon evaluation by the IVI, the vaccine's manufacturing process does not comply with current Good Manufacturing Practices (cGMP) and WHO guideline and the Vietnamese National Regulatory Authority (NRA) is not recognized by the WHO. For

a vaccine to be purchased by UN agencies such as the UNICEF, the vaccine must be prequalified by the WHO. WHO prequalification is only possible if the vaccine is produced by a manufacturer located in a country with a WHO-recognized National Regulatory Authority.

This vaccine was therefore reformulated, its production technology improved to comply with international guidelines and its technology transferred to a manufacturer in India whose national regulatory authority [Drugs Controller General India (DCGI)] is prequalified by the WHO in 2011.

The technology for vaccine (a killed bivalent O1 and O139 whole-cell oral cholera vaccine Shanchol) manufacturing has been transferred by the IVI to Shantha Biotechnics in India (now owned by Sanofi) , a company with WHO-prequalified products. A large double-blind placebo controlled phase III trial by NICE and IVI has evaluated the efficacy of the vaccine produced by Shantha in preventing diarrhea from cholera in 70,000 people in Kolkata. An interim analysis of the phase III trial has been completed and has concluded that the vaccine was around 70% efficacious [19]. The vaccine was licensed in India in February 2009 and is now available for general use in the country. Advantages of the Shanchol vaccine include that its cost is lower (\$1.85 in India), and does not require administration with buffer thus making it more feasible for use in mass vaccination programs in resource poor settings. We have recently conducted a randomized placebo controlled study on the Shanchol vaccine in Mirpur in 330 participants in three age groups including adults followed by toddlers and infants. Participants were randomized to receive either 2 doses of the vaccine or placebo which were given 14 days apart. Primary end point was to see the occurrence of diarrhea, vomiting or abdominal cramps of at least moderate grade over the 3 days surveillance period as well as cholera vaccine specific antibody responses.

Summary of findings from previous clinical studies

Phase II clinical trials of whole cell bivalent vaccine in Vietnam [20] and India [21] and in Bangladesh [22] have shown that this vaccine is safe and immunogenic in both adults and children. Following these successful clinical trials, a phase III cluster-randomized, double-blind, placebo-controlled trial was initiated in July 2006 in Kolkata. Results from an interim analysis showed that the vaccine is safe, confers 67% protection among all participants aged 1 year and older in Kolkata, two years after receipt of the two-dose regimen [19]. A large feasibility study has also been carried out in Bangladesh which has shown that the Shanchol also been proven safe. Based on the results from studies in India, this modified vaccine was licensed in February 2009 to Shantha Biotechnics in India under the trade name Shanchol. Subsequent analysis demonstrated that the vaccine maintained the efficacy even after three years of administration [23]. In a phase II study conducted in Bangladesh to evaluate the safety and immunogenicity of Shanchol, showed the vaccine to be safe with good immune responses in

participants that were studied. Vibriocidal antibody responses in adults were 60%, 72% and 21% against *V. cholerae* O1 Inaba, *V. cholerae* O1 Ogawa and *V. cholerae* O139 respectively. In toddlers, responses were 84%, 75% and 64% and in younger children it was 74%, 78% and 54% against Inaba, Ogawa and O139 serotypes respectively [22]. Recently a large feasibility study is ongoing to determine the impact of two doses of Shanchol vaccine in a high risk population in Mirpur in Dhaka, Bangladesh (PR#10061; Clinical trial.gov ID: NCT01339845).

Justification for Single dose

The current multi-dose schedule has restricted the application of the oral cholera vaccine in situations where they are most needed. Based on the experiences of recent complex emergencies and in demonstration projects have suggested that the current two-dose regimen of the internationally available cholera vaccines may create some logistical and programmatic challenges [7]. Post-disaster situations and the capabilities to respond in non-endemic countries can vary vastly in different disaster and economic settings, and an option to use single dose vaccine in area where major population disruption has occurred versus use of two doses at an interval of 2 weeks, can make a lot of difference on deciding for use of vaccine by policy makers. Getting the vaccine to the same people twice poses difficulties in the control of cholera in both endemic and epidemic settings. Excellent immune responses after two doses of the vaccine seen in the earlier Phase II studies [20, 21]. In a study performed among 80 adults and 80 children in a cholera-endemic area in Kolkata, it was found that the modified Shanchol vaccine induces significant vibriocidal responses even after a single dose [24]. Both the GMF-rise of vibriocidal titers to *V. cholerae* O1 and the number who seroconverted were higher after the first dose as compared to after the second dose. Furthermore 7 subjects (6 individuals aged 15 years and older and one 4 year old) fulfilled the definition of seroconversion after one dose but not after two doses. Only 2 subjects, both aged 3 years with low baseline titers < 80, had higher post-dose 2 titers [24]. These findings differ from the previous studies using the older generation killed OCV, where higher titers were obtained after the second dose [25-27]. Since there is no serologic correlate of protection for cholera, serum vibriocidal response to *V. cholerae* O1 are used in clinical trials as markers for appropriate immune stimulation. Small intestinal mucosal IgA response measurement appears to be the only direct predictor of protection however this is impractical to employ in large scale clinical trials, therefore serum vibriocidal antibodies are used [8, 28]. One of the possible explanations to this observation was the fact that maybe the first dose of the vaccine was acting as a booster dose in an endemic population already exposed to cholera and having a baseline titre of antibodies.

Since there is a limited supply of the oral cholera vaccine, it is important to know how to accomplish the greatest public health benefit with this limited supply. Even if a single dose may be somewhat less

effective than two doses, the single dose strategy will have the potential to avert more cases than the two dose strategy since a larger number of susceptible people will be vaccinated. This will be useful for epidemics and outbreaks and for endemic cholera prevention.

Trial design justification

Since vibriocidal antibody response is not an appropriate serologic correlate of protection for killed cholera vaccine at present, an efficacy trial with placebo control would be required to confirm the usefulness of the single dose [8]. If the vaccine is found to be efficacious following a single dose, this will have profound implications on the use of the vaccine in areas with limited resources particularly in complex emergencies where a multiple dose regimen is difficult to deploy. A single-dose regimen of this vaccine will improve its “fieldability” and allow the vaccine to be used even in outbreak control especially in difficult settings where the risk of cholera is extremely high and provisions for clean water and sanitation are not available [9]. A single-dose schedule will also facilitate its inclusion in the EPI schedule and in national immunization strategies as it may be given together with other vaccines in endemic settings where young children are at highest risk for the disease. The proposal we plan has a double blind individual randomized, placebo controlled design. This design was selected as the most efficient, to provide efficacy data for single dose of Shanchol with minimum number of subject participation.

Summary of known and potential risks:

There is no comparative data on protection between a single dose and two doses of Shanchol. However, as of now, plan from the Government of Bangladesh will be based on the results of the feasibility studies being carried out and it is hoped that the country will be able to use Shanchol in public health programs in the next five years. So participants have negligible chance to receive the vaccine outside of this study. The potential risk to the participants will be minimal, since there is extensive documentation of the safety of the cholera vaccine to be used and all clinical and immunization procedures (oral vaccine administration or venous blood collection, rectal swab collection,) will be performed by adequately trained and experienced personnel under regular supervision. There is a very small risk of anal/rectal area skin abrasion while taking a swab from the rectal area. Additionally, there is also a small risk associated with phlebotomy for participants who are requested to give a blood sample. This may include pain, redness and, very rarely, local infection at the phlebotomy area.

Risk minimization and benefits

All personnel involved in taking biological samples are trained personnel, who will be provided with additional training to avoid or minimize the possibility of any unplanned side effects of these procedures. Sterile techniques and disposable sterile needles and syringes will be utilized to obtain

blood. All study records and data will be kept confidentially under lock and key and/or electronic password protection, as appropriate, for 5 years. Only senior study personnel will have access to these records. The direct benefit the participants may expect from participating in this study will be free laboratory examination and treatment for diarrheal diseases.

The main benefit of obtaining data on the efficacy of the single dose schedule of cholera vaccine will be that, if proven protective, the single dose schedule will greatly simplify vaccine delivery and will also result in substantial cost reduction and will be available for a larger population of people at risk of cholera. The single dose vaccine will be particularly useful in cholera epidemics and complex emergencies. All recipients of the vaccine will potentially benefit from the probable protective effects against cholera. The risks associated with the use of the vaccine or the placebo and various other study procedures proposed to be used in this trial are expected to be minimal to non-existent.

After completion or termination of study, all subjects in the placebo group will be provided with single dose of vaccine after completion of follow up duration for the study. Otherwise, if one dose regime of the vaccine is not found to be satisfactorily effective, all subjects will be offered two doses of vaccines as per the currently licensed regime. The trial will be conducted in compliance with protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

Trial Design

Description on type of study

The study design is a two-arm individually randomized double-blind placebo-controlled trial. Individuals living in each household under surveillance will serve as the units of randomization with 1:1 placebo-to-control ratio. A household is defined as a group of individuals residing in a geographically circumscribed area and recognizing the same household head.

Study population

Mirpur is a part of the Dhaka metropolitan area with an estimated population of over 2.5 million people. Different socio-economic groups of communities such as low income, middle class, and high income live in the area. The icddr,b hospitals treat more patients from Mirpur than from any other parts of

Dhaka. Mirpur is divided into 16 wards of the Dhaka City Corporation. We have selected wards 9, 10, 11, 12, 13, 15 and 41 considering a high incidence of cholera based on the number of patients seen with cholera at the icddr,b hospitals during the last few years. It was observed that most of the cholera patients were coming from overcrowded households with low income, poor sanitation, unsafe water use, sharing of source of water and poor living conditions. In preparation for this single dose trial we will commission a census of these high cholera incidence wards in Mirpur. These wards are separate from the other wards where the feasibility study of the oral cholera vaccine is being carried out (Figure 1) (Appendix 1E). First, the census team will create digital maps of buildings and other structures in the target wards using satellite derived map. The digitized maps will be updated by ground truthing. The census team will visit each building and ascertain whether or not people are living in the building. If people reside in the building the census team will assess whether the residential structures are overcrowded, have poor sanitation and drainage, unhealthy living conditions, share water among several families to assess high risk groups [29]. Based on this survey, the team will assess whether the people living in the building/structure are a high risk group or not. If the residence meets these criteria, the census team will collect verbal consent from the respondent and other information of the household (Appendix 1A). The supervisors will also subsequently check whether this assessment fulfils the requirement for defining them as high risk population. We will enumerate over 190,000 high risk residents from the target wards. The census data will be collected by various field teams, and then the data will be used for data entry and management at the end of each day of visit. For census update and disease surveillance, handheld systems will be used and will offer an advantage in being a portable method to digitize information. Implementation of the handheld system will permit direct data entry for cholera surveillance and follow up census updates. The census data to be collected by various field teams will be transferred to the main database at the end of each visit of the day. The proposed study sites are areas where no cholera vaccine trials are being performed. Prior to the start of the study, preparatory activities will be performed in the study site. These will include a baseline census of residents in the study area. Augmented passive surveillance of diarrhoea cases will be performed in these surveillance units to document the incidence of cholera in the community. The study participant will be encouraged to bring the study card with them when they visit the icddr,b hospital in Mohakhali and Mirpur, other health facilities in study area for diarrheal illness during and after vaccination (Appendix 1F). Cholera cases will be followed-up at home 7 days after presentation to verify the identity of the case and to record the outcome.

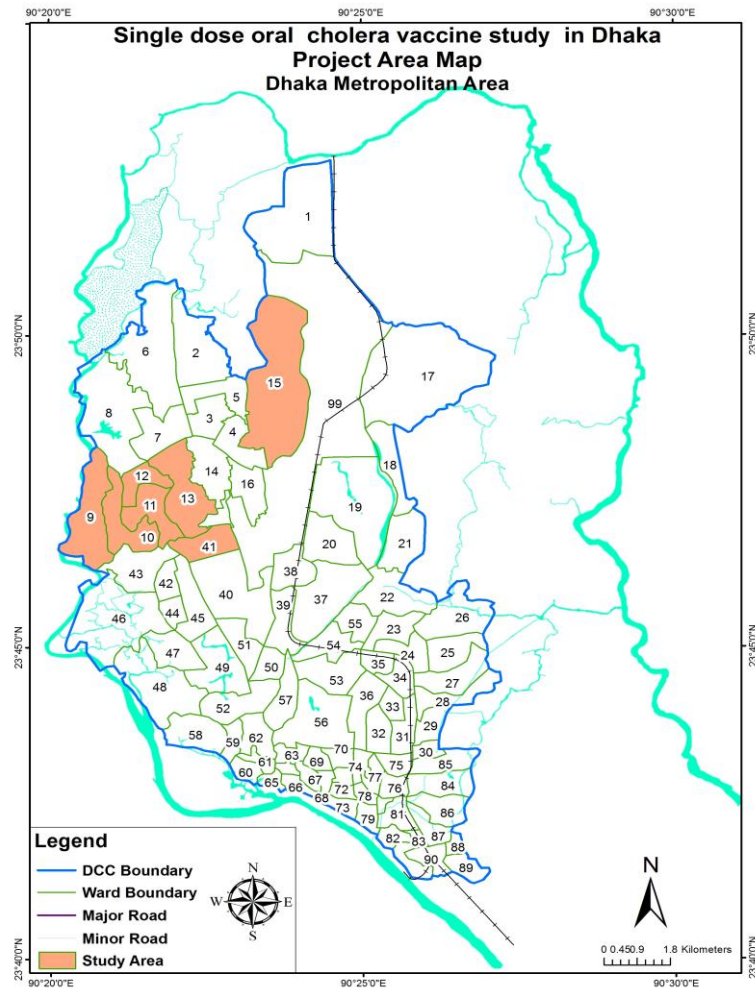


Figure 1. Study Area

Study Procedure and methodology

Pre dosing census of study population

The surveillance will be conducted with regular demographic updates by community health workers to ensure the time contribution of the population and to encourage individuals to visit project clinics/vaccination site. Subsequent census updates will be performed biannually using handheld devices. After the baseline census, a unique ID number will be assigned to each study subject. A closeout census will be conducted at the conclusion of the trial.

Each participant over the age of one year and non pregnant females living in communities randomized to receive vaccine or placebo. Pregnancy status will be enquired verbally for all married women of child bearing age during the census update as well as before vaccination to exclude them from the study. If she is unable to state, she will be asked for her last menstrual period (LMP). If LMP exceeds more than

four weeks and in doubtful cases, she will not be eligible for vaccination [19]. We will collect this data on pregnancy during census update, and again before vaccinating married women of child bearing age. The study participant who give birth during the study period can be identified by the ongoing census update.

Cholera vaccine delivery

We will use the killed whole cell oral cholera vaccine, Shanchol manufactured by Shantha Biotechnics, in Hyderabad, India for the study. The vaccine is registered in India and is prequalified by WHO. The company is going to supply the vaccine and for its import we will approach the Directorate General of Drug Administration for its use in the study. Vaccine will be transported from the manufacturer to a designated EPI cold room arranged for this study where it will be stored. We will identify vaccination centers within the intervention areas. These sites may include the government's EPI outposts, non-government facilities utilized during national immunization days, or other appropriate facilities which are easily accessible by the target population(Appendix 1C). Vaccine will be maintained at 2-8°C for the study. Shanchol vaccine is available in a single dose vial. During vaccination, vaccinators will shake the vial well to disperse the cellular contents and then open it to feed all its content to the recipient. Before vaccination consent will be taken from the adults and older children (1 year and above; Appendix 1Bi and 1B ii) and they can take the vaccine by themselves (those 5 years and above) but vaccinators will feed the vaccine contents to younger children when needed. After intake of the vaccine, the vaccinator will offer half a cup of water (~50 ml) to the vaccinees. Training will be provided to: (i) Vaccinators, managers and supervisors of this project, MOHFW, DCC and NGOs and (ii) Volunteers recruited for the study.

Interventions

Immunization with vaccine and Placebo

Name and description of products:

- a. *Bivalent oral killed cholera vaccine*: each dose of this vaccine contains
- | | |
|-----------------------------------------------------------------------|--------------------------------------------------|
| <i>V. cholerae</i> O1 Inaba El Tor strain Phil 6973 formalin killed | 600 Elisa units (EU) of Lipopolysaccharide (LPS) |
| <i>V. cholerae</i> O1 Ogawa classical strain Cairo 50 heat killed | 300 EU of LPS |
| <i>V. cholerae</i> O1 Ogawa classical strain Cairo 50 formalin killed | 300 EU of LPS |

<i>V. cholerae</i> O1 Inaba classical strain Cairo 48 heat killed	300 EU of LPS
<i>V. cholerae</i> O139 strain 4260B formalin killed	600 EU of LPS

b. Non Biological placebo: The composition of the placebo is as follows :

Ingredients	Per 1.5 ml dose
Starch	60mg
Red color[1mg/ml]	10 µl
Yellow color [1mg/ml]	5 µl
Xanthum Gum (1% solution)	300 µl
Water	Upto 1.5 ml

For this evaluation of clinical safety and immunogenicity, a “non-biological” will be used which is in nature and appearance is similar to vaccine. The placebo lacks any virulence characteristics and will be prepared under Good Manufacturing Practice conditions at Shantha Biotechnics. This ‘non biological’ placebo has been earlier used in a clinical study involving 330 subjects in Bangladesh and with no associated safety concerns [22]. Each dose of vaccine or placebo is 1.5 ml in volume. The vaccine and placebo will be dispensed in liquid form in identical vials.

Administration of vaccine or placebo

Vaccination will be done through vaccine fixed site vaccination outposts in the study area. After acquisition of informed consent and ascertainment of eligibility, consenting, eligible subjects will be entered into the trial. The agent to be received will be determined according to the randomization list. After shaking the vial properly, 1.5 ml will be poured into the mouth by the recipient, followed by intake of a small volume of water. We shall allow the vaccinees to take the vaccine by themselves for those who are 5 years and above in age. For small children, the vaccine will be given by the vaccinator. If it is judged that a dose is not successfully ingested (e.g., regurgitated or spat out), recipients will be offered a single replacement dose, using the same procedure. Each single-dose vial will be opened and used up according to the randomization list. At the time of the dosing, information about vaccine administration will be entered into the Vaccination Record Book. Vials used for administration of the vaccine and placebo will be disposed of after each dose, to prevent inadvertent administration of contaminating amounts of non-assigned agents.

Selection and Withdrawal of Subjects

Subject inclusion criteria:

All healthy, consenting, non-pregnant (as ascertained by history) residents of high risk group at least 1 year of age of the study area will be included in the trial.

Subject exclusion criteria:

The following will be excluded from the trial:

- Pregnant women (identified through verbal screening);
- Age less than 1 year
- History of intake of any cholera vaccine

Subjects may withdraw from the study at any point. The data collected for withdrawn subjects, in addition to standard questionnaire data, will include the reason for withdrawal. No additional follow-up is envisioned for withdrawn subjects.

Accountability procedures for the investigational product, including the comparator:

The study agents will be stored in a secure place in the EPI cold room. Comprehensive training of all study staff, and a detailed questionnaire will ensure and document that study protocol requirements are being followed. Vaccine and placebo will be stored according to cold chain requirements, and detailed inventory logs will be maintained. The investigator or the person in-charge of the product management will maintain records of the product delivery to the trial site, the inventory at the site, the dose given to each subject, and the return of unused doses to the manufacturer. All used and unused vials of vaccine and placebo will be accounted for destruction carried out.

Maintenance of treatment randomization codes and procedures for breaking codes:

The treatment randomization codes will be maintained by the vaccine manufacturer. Codes will not be broken until all serology results are available for analysis.

The identification of any data to be recorded directly on the CRFs (i.e. no prior written or electronic record of data), and to be considered to be the source data:

A description of the measures taken to minimize/avoid bias, including randomization & blinding:

Blinding will be achieved in this trial by masking the identity of the agents using codes for the vaccine and for the placebo. To maintain blinding, Shantha will code the agents in Hyderabad prior to shipment to the study site. The statistician will generate a randomization list which will be matched to the appropriate letter code indicating the agent to be received by all study participants. The sub-sample of individuals for collection of blood for serology will be randomly pre-selected by the same randomization list.

The identity of the codes will only be known to an IVI staff who is otherwise not involved in the study and the Shantha staff who will label the vaccine or placebo vials. The identity of the codes (in sealed envelopes) will be provided to the DSMB, and the on-site clinical monitor. Each sealed envelope will be labelled with a letter code on the outside and the identity (vaccine or placebo) on the inside.

The staff conducting the trial and the trial participants will be blinded as to the identity of the agents. The individuals will be randomized in blocks of 4 (2 for vaccine and 2 for placebo), which will be labelled as 8 different letter codes. The DSMB is responsible to un-blind the codes in the event of severe putative vaccine reactions. Otherwise, the codes will not be revealed until the end of the trial and until the computerized dataset has been frozen. If the intervention assignment is un-blinded, all study collaborators will be notified immediately. Interim analysis if and when required will be done with concurrence of DSMB, keeping the study team blinded to the codes.

A description of allocation of the investigational product, dosage form, packaging and labelling:

Each dose of vaccine or placebo is 1.5 ml. The vaccine and placebo will be dispensed in liquid form in identical vials. A single dose of either vaccine or placebo will be administered orally to participating subjects as per the randomization list. Allocation of vaccine will be done through individual randomization. All eligible members of either vaccine or placebo group, will be allocated according to the random allocation number. Using the data collected during the census and surveillance period, each individual will be randomly allocated to receive the vaccine or placebo.

Each vaccine or placebo vial will be letter/ number coded with one of two letters (e.g. L/ 1001 Labelling and coding of the vaccine and placebo will be done in Hyderabad at Shantha. The staff that will be responsible for affixing letter/number on each vial of the agents will not be involved in any other way in the conduct of the trial nor will be present at the study site.

Measurements

Census update

The census in the study population will be updated every six months. Data collectors will visit each house in the intervention areas and collect information on births, deaths and migrations (Appendix 1D).

Disease Surveillance

1. Surveillance at the icddr,b hospitals in Mohakhali and Mirpur

All patients admitted to the hospital with diarrhea will be included in routine hospital surveillance. A diarrhoeal visit is defined as a visit by a patient who has in the 24 h before presentation, three or more

loose or liquid stool, according to WHO criteria [30] [19]. The diarrhoeal disease surveillance for the project will be conducted at icddr,b hospitals at Mohakhali and Mirpur for the patients coming from Mirpur study area (wards 11,12 and 13). Clinical staff at each of the two hospitals will evaluate each patient at the hospital triage area and provide treatment as is the routine procedure.

If the patient has a study card, it will be scanned using a bar code scanner. The front desk staff will also verify and confirm his/her identity by asking name, age, family members, address etc. In case of unavailability of study card, there will be an option in SHEBA to search a particular patient identification (PID) number for the study participants; this search will be done on basic parameters such as name, age/date of birth, area of residence, police station, sex or village. SHEBA is an integrated icddr,b hospital data management system which records patient's history, treatment, management and related auxiliary data at icddr,b hospitals at Mohakhali and Mirpur treatment centre. The study requires treatment management data, during hospitalization of the patients from study area. Informed consent will be taken (Appendix 1Gi and 1Gii) and a study surveillance questionnaire will be used to obtain information related to the study (Appendix 1H). Basic Demographic Surveillance System (DSS) data of the study subjects will be replicated to SHEBA from study server. This process will run from SHEBA server at a regular interval. Data Management assistants at hospital registration desk will enter PID number from the survey area. Study participants will be admitted in the hospital and information collected using SHEBA. After 24 hours of discharge, the data from the patients triage information (Appendix 1H) along with other information including IV saline consumption, ORS intake, drug used etc. initial differential diagnosis and final diagnosis on discharge and any intra-hospital or external referrals will be populated separately through a scheduler in SHEBA server. A stool or rectal swab specimen will be collected as soon as possible and sent to the laboratory for culture and analysis. Fixed laboratory test requisition for the study will be raised by the SHEBA system to the study server. Microbiological results from laboratory will be sent back to the SHEBA database. A pull engine in study server will replicate the populated information from SHEBA to the study server. At the end of enrolment of study patients, the residence of culture confirmed cholera cases will be visited within a week by the health worker to confirm their identity and other information. Two episodes of diarrhea in a study participant will be separated by an interval of ≥ 7 days from the date of discharge of the previous visit as used in a previous cholera vaccine study [19].

2. Surveillance at other health facilities in the study area in Mirpur

It is assumed that the vast majority of severe diarrheal patients from Dhaka city seek care at the two icddr,b hospitals. However we will also include Governmental and non-governmental hospitals/clinics at Mirpur area which is visited by the study population for diarrheal treatment (Appendix 1F). We know from our own experience that treatment of diarrheal patients may be delayed due to the traffic problems

and therefore we will not encourage patients to be referred to the icddr,b Mohakhali hospital from these health facilities. Health staff of the facilities will be oriented/informed/motivated about the cholera vaccine study objectives and activities by the icddr,b clinicians and study investigators. Two staff members from each of these facilities will be directly responsible for dealing with the patients from the study sites and also be part of the field team. These persons will be specially trained in completing the questionnaires and also collecting specimens for microbiological analyses. These facilities will be also be under surveillance by the staff. One surveillance staff will be present at each health facility throughout the day to facilitate proper reporting of diarrheal cases from study area. Study patients will be identified by use of cholera cards. To ensure that we do not miss any study participant, all patients from the study wards will in addition have demographic and clinical data filled up on a structured questionnaire similar to the one used at the icddr,b hospitals (Appendix 1H). Data will be checked and verified and entered into the computerized database of the study. Stool specimens/rectal swab will be transported in Cary-Blair media to the laboratory at icddr,b within 8 h of specimen collection and these clinical and lab data will be entered into our database (Appendix 1I).

Laboratory Assessment: We will collect stool or rectal swab specimens from diarrheal patients coming from the Mirpur study area to the icddr,b hospital in Mohakhali and Mirpur and also from other selected health facilities frequented by the study population for diarrheal diseases. Specimens will be evaluated for *V. cholerae* O1 and O139 and also tested for ETEC, another important bacterial causes of acute dehydrating diarrhea [31-33]. For isolation of *V. cholerae*, specimens will be cultured on taurocholate-tellurite gelatin agar (TTGA). Specific monoclonal antibodies will be used to detect *V. cholerae* O1, Ogawa and Inaba serotypes, as well as the O139 serogroup [33, 34]. For microbiological evaluation, specimens will be also enriched in alkaline peptone water overnight and then cultured as above [33, 35]. For detection of ETEC, stools will be cultured overnight on MacConkey agar plates and lactose-fermenting colonies will be isolated and tested for the presence of heat labile toxin (LT) and heat stable toxin (ST) by multiplex PCR [36]. The microbiological data will be collated in the database to determine efficacy of the interventions being carried out in the study.

Follow-up visits - For those with laboratory-confirmed cholera, a follow-up visit 7 days after the initial presentation will be done to assess clinical progress and cholera-related disability (see Cholera Follow-up form). The follow up visit will also verify the identification of the patient.

Immunological assays using blood specimens from study participants:

Blood: Immunological analyses will be conducted in a small subgroup of patients as has carried out earlier in the feasibility study of oral cholera vaccine (icddr,b protocol # 10061). Venous blood (2ml) will be collected from 324 participants (vaccine and placebo; based on randomization list) prior to

immunization and 7 days after intake of study agent (day 0, day 7 and day 21). Sera separated from blood will be stored at -20°C for immunological analyses. Sera obtained will be used to carry out vibriocidal antibody assays as well as *V. cholerae* O1/O139 LPS specific IgA antibody assays.

Follow up for Immunogenicity

Since there is no certainty that all individuals in the geographic population will participate in the study, a list of 1000 participants will be randomly selected by stratified random sampling based on allocated agent (each code letter of the vaccine or placebo) and age group (less than 5 years, 5 to 15 years and over 15 years of age). Based on this list, the first 324 subjects who sign the informed consent for blood draw and study agent intake will be included (Appendix 1J). A blood draw form will be filled up (see Data Form/CRF) (Appendix 2A, 2B, 2C). These participants will be requested to provide about 2-5 ml of venous blood at the time of dosing, 7 days after and 21 days after, for testing of vibriocidal antibodies. The blood samples will be transmitted to the laboratory (within 6 hours of collection). At the laboratory, serum will be separated and stored at -20 degree C until testing for antibodies. Technicians unaware of the codes of the agents received by the trial participants will test in random order paired serum samples for vibriocidal antibody titres.

Serum vibriocidal antibodies to *V. cholerae* O1 (El Tor Inaba; strain T19479; El Tor Ogawa; strain X25049) will be evaluated by a microtiter assay. To measure vibriocidal antibodies to *V. cholerae* O139, the partly encapsulated vaccine strain CIRS 134 will be used [37]. The vibriocidal titre will be defined as the highest dilution causing 50% inhibition of bacterial growth. Two-fold serial dilutions of pre- and post-immunization specimens will be tested side-by-side in duplicates on each plate. Titres will be adjusted in relation to a reference serum specimen included in each test to compensate for variations between analyses on different occasions. The vibriocidal antibody titre ascribed to each sample will be the mean of the duplicated determinations, which will not be allowed to vary more than one two-fold dilution for either the reference or the test sera. The tests will be repeated if larger variations are observed. A four-fold or greater increase in titre between pre- and post-immunization sera will be taken to represent sero-conversion.

Serum vibriocidal antibody assay

The vibriocidal antibody assay is a bactericidal assay requiring the presence of complement-fixing antibody bound specifically to vibrios; this serum antibody response increases after clinical cholera or after vaccination. Serum vibriocidal antibodies to *V. cholerae* O1 Ogawa and Inaba strains [38] as well as to the *V. cholerae* O139 will be performed [37]. The serum samples from the volunteer prior to immunization and 7 and 21 days later will be tested for the vibriocidal antibody assay. To control for variations, test plates will contain pooled convalescent serum sample from patients with cholera as a positive control (pooled O1 Ogawa, O1 Inaba and O139 sera from our collection of specimens from

cholera patients) [33, 37, 39]. When progressing from one phase of study to another, laboratory pooled control specimens will be also be used [25]. This will validate the specificity of the assay further. An increase of vibriocidal antibody titer by 4-fold or higher between acute and convalescent sera will be considered a significant antibody response.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

Sample size calculation for Primary efficacy endpoint

The primary outcome of the study will be to evaluate the efficacy of the vaccine after a year following vaccination. However, it will also be useful to determine short term protection that is after 6 months after the intake of the vaccine by carrying out an interim analyses. Thus the sample size has been calculated based on this factor.

We assume that the single dose will give at least a short term protection, i.e., for six months per se. Therefore, we have planned to do an interim analysis at six months following vaccination. Thus, we aim to power this trial to measure the vaccine efficacy against culture-confirmed *V. cholerae* O1, during the 6 months of follow-up following vaccination. The following assumptions were made for calculation of the sample size required to demonstrate the efficacy of the single dose at 6-months of follow-up:

- Incidence = 1.20 cases/1000/year
- Vaccine Protective Efficacy = 50%
- Alpha = 0.05 (one-tailed)
- Power = 0.80
- Drop-out rate = 25% per year
- Participation rate = 75%

With these assumptions, we need at least 95,115 individuals in an arm of the study i.e., a total of 190,229 population will be required to conduct the trial. The sample size however if based on a one year follow up will be 95,067.

Sample size required for immunogenicity endpoint

The sample size is calculated with the assumption that it is important to evaluate whether the vaccine induces acceptable serum vibriocidal responses in relation to the placebo group. Based on an immunogenicity study of the whole-cell killed oral cholera vaccine in Kolkata (1 dose vs. 2 doses of Shanchol) [19], we make the following assumptions:

For serum vibriocidal responses, defined as >4-fold increases between baseline and post-dosing in either Inaba or Ogawa antibodies, for each age group (less than 5 years, 5 to <15 years and 15 years and above), we assume 1) the background rate of responses in the placebo group will be 3% after the first dose and 2) the true rate of vibriocidal responses in the vaccine groups is 50%. At $p < 0.05$ (1-tailed), 0.8 power, to exclude a difference of seroconversion among vaccine and placebo recipients of 25% and a 20% drop-out, a total of 54 subjects per group would be needed. We will require approximately 54 subjects per arm per age group, for a total of 324 subjects.

	< 5 years	5-<15 years	15 years and above
Vaccine group	54	54	54
Placebo group	54	54	54

Procedures for reporting any deviation from the original statistical plan:

Additional analysis may be required, and will be conducted if agreed among the participating institutions.

Direct Access to Source Data/Documents

The investigator/institutions will permit (by way of written agreement) trial-related monitoring, audits, IRB/IEC review, and regulatory inspection, providing direct access to source data/documents.

Quality Control and Quality Assurance Procedures

Study Monitoring and Source Data Verification

After appropriate ethical approval by an Institutional Review Board (IRB) is available (and the final protocol has been amended as required by IRB), a pre site initiation visit will be conducted by a designated study monitor. During this visit, the requirements of GCP, protocol procedures, and logistical issues will be discussed. The training of study staff will be carried out and documented.

Later a site initiation visit will be conducted before the first subject is enrolled in the study. The subjects cannot be enrolled until occurrence of such visit and its documentation.

After the study is initiated, the study monitor will be in regular contact with the site to obtain information on the performance of the study. These contacts will be scheduled to take place at regular intervals. Subsequent to start of recruitment, routine monitoring visits would occur after prior appointment with the investigators.

The investigator and his/her staff are obliged to devote a suitable amount of time and an appropriate place for the monitoring visits. During each visit, the monitor will review the Case Report Form (CRF) of each subject in the study with regard to completeness, thoroughness and compliance with the protocol. In addition, at a minimum, the original subject data (e.g., entry cards, index cards, original findings) will be reviewed to ensure that:

- subject informed consent is incorporated;
- inclusion/exclusion criteria are properly followed;
- the CRF data are consistent with the physician's original records, which also have to clearly indicate that the subject is included in a clinical study;
- all relevant clinical and laboratory findings and concomitant medication are documented in the CRFs;
- quantity and dosing schedule of concomitant medication is documented in the CRFs;
- quantity and dosing schedule of the Investigational/Comparator Product is in accordance with the protocol;
- all relevant information (e.g., any adverse event) has been recorded in the appropriate place in the CRFs;
- the Investigational/Comparator Product is being stored correctly, and its supply is being properly accounted for;
- Incorrect or illegible entries in the CRFs would be submitted to the investigator for correction.

The monitor will retrieve completed CRFs during the regularly held monitoring visits.

Auditing of the study will be carried when necessary with respect to study procedures, data entry, data management and related matters by the responsible regulatory authorities.

Confidentiality:

Subject confidentiality will be maintained at all times.

Management of the Single dose cholera vaccine project

The management structure of the cholera vaccine project is attached (Appendix 4,5). icddr,b will collaborate with several other organizations to accomplish the objectives of this project throughout its time period (Appendix 3). We will conduct advocacy meetings at national and service levels with officials and staff members of MOHFW, DCC, NGOs and local elites for sensitization and to support this project. These collaborations include partnerships with the Director General of Health Services of the Ministry of Health and Family Welfare of the Government of Bangladesh, the Expanded Program on Immunization of the Government of Bangladesh and the Dhaka City Corporation which is

responsible for providing immunization services within Dhaka city. The International Vaccine Institute is a collaborating institution and providing technical support to assist in conducting the study, in developing the data analysis plan, and in reviewing the division of the study population in the study areas. The International Vaccine Institute is also providing the vaccine.

The existing committees on Protocol #10061 (ICVB project; Introduction of cholera vaccine in Bangladesh) has 3 functional committees and these will be also used for the present study. The Advisory Committee on Introduction of Cholera Vaccine consisting of paediatricians, gastroenterologists, epidemiologists, expert on vaccines and health systems as well as other diarrheal disease experts from Bangladesh and international organizations including the WHO will oversee the progress and monitor the study. The committees will meet prior to the study as well as at regular intervals. The study will use facilities of the Government of Bangladesh which include the Directorate General of Health Services, Dhaka City Corporation. The planning and implementation Committee (PLIC) formed with members of the GoB, headed by the Director Primary Health Care (PHC), DGHS with members from EPI, CDC, DCC of the DGHS as well as the members from the core group will support the study. The resources of the administrative and professional services of the respective areas at EPI, DCC, DGHS and icddr,b will be used to facilitate the study. The Immunization related logistics including EPI cold storage space used by EPI and DCC will be used for the study. They will provide strategic leadership and coordination to the project. In addition to a staff of coordinators and consultants, the study will be overseen by a Steering Committee. Scientists from the IVI will be collaborating investigators in the study. The crisis communication committee of the ICVB project (Protocol #10061) which is headed by National Professor Dr. M. R. Khan will also oversee the activities of this committee and provide advice and support.

Data Analysis

be plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be ed by the investigators themselves or by other professionals. Specify what statistical software packages will d and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis e required to determine further course of the study.

Efficacy outcome for the study

Primary outcome of the study:

Proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with fecal excretion of *V. cholerae* O1 in the study treatment centres from 14 days to 12 months after dosing and whose identity is confirmed through home visit.

Secondary outcome of the study are as follows:

- Proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with fecal excretion of *V. cholerae* O1 in the study treatment centres from 14 days to 6 months after dosing and whose identity is confirmed through home visit.
- Proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with fecal excretion of *V. cholerae* O1 in the study treatment centres from 7 days to 12 months after dosing and whose identity is confirmed through home visit.
- Proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with fecal excretion of *V. cholerae* O139 in one of the study treatment centres from 14 days to 12 months after dosing.
- Proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with fecal excretion of *V. cholerae* O1 and with severe dehydration in one of the study treatment centres from 14 days to 1 year and 2 years after dosing.
- Proportion of participants detected with watery diarrhea in one of the study treatment centers from 14 days to 1 year and 2 years after receipt of the dose.
- Proportion of participants detected with watery diarrhea and with severe dehydration in one of the study treatment centres from 14 days to 1 year and 2 years after receipt of the dose
- Proportion of non-participating subjects who are detected with diarrhea with fecal excretion of *V. cholerae* O1 in one of the study treatment centres from 14 days to 1 year and 2 years after dosing of study participants.
- Proportion of non-participating subjects who are detected with watery diarrhea in a study treatment setting from 14 days to 1 year and 2 years after dosing of study participants
- Proportion of non-participating subjects who are detected with watery diarrhea with severe dehydration in a study treatment setting from 14 days to 1 year and 2 years after dosing of study participants.
- Geometric mean serum vibriocidal (to El Tor Inaba and Ogawa O1 serogroup organisms and to O139 serogroup organism) titers measured in sub group of participants at baseline and one week after receipt of either vaccine or placebo.
- Proportion of participants exhibiting 4-fold or greater rises in titers of serum vibriocidal antibodies (to El Tor Inaba and Ogawa O1 serogroup organisms and to O139 serogroup organisms), relative to baseline, one week after receipt of either vaccine or placebo in sub group of participants.

Efficacy assessment and endpoints

Ascertainment of vaccination:

Receipt of the cholera vaccine during the recruitment will be ascertained in the vaccination registry.

Assessment of Efficacy

The primary purpose of the analysis is to evaluate vaccine efficacy during 12 months of follow-up after receipt of one complete dose of an assigned agent.

A diarrheal visit is defined as: An inpatient or outpatient visit for care of diarrhea in which the patient described:

- 3 or more loose or liquid stools; or
- At least 1 bloody stool; or
- 1-2 or an indeterminate number of loose or liquid stools and exhibited at least some dehydration

Diarrhea episode definition: A diarrheal episode is defined as follows:

- All diarrheal visit(s) for which the date of onset for a diarrheal visit was less than or equal to 7 days from the date of discharge for the previous visit, constitute a single “diarrheal episode”.
- The onset of a diarrheal episode was defined as the day on which it was reported to have begun for the first visit of the episode.

Cholera Episode Definition: A cholera episode is defined as:

- A faecal specimen from at least one component visit which yields *V. cholerae* O1 in the icddr,b laboratory; and
- A diarrheal episode in which no component visit is described as bloody diarrhea; and
- An identity check performed 7 days after discharge for the visit in which *V. cholerae* O1 is isolated, confirmed that the person whose name was given at the treatment centre had indeed sought care for diarrhea on the date of presentation.

Study Endpoints

Primary endpoint for the study efficacy is the first-episodes of culture-confirmed *V. cholerae* O1 diarrhea detected in the study treatment centres from 14 days to 12 months after dosing among those who received 1 dose of vaccine or placebo.

Secondary endpoints for the efficacy are

- First-episode of culture-confirmed *V. cholerae* O1 diarrhea detected in the study treatment centres from 14 days to 6 months after dosing, respectively among those who received 1 dose of vaccine or placebo

- First-episodes of culture-confirmed *V. cholerae* O1 diarrhea detected in the study treatment centres from 7 days to 24 months after dosing, respectively among those who received 1 dose of vaccine or placebo
- First-episode of culture-confirmed *V. cholerae* O1 diarrhea with severe dehydration detected in the study treatment centres from 14 days to 12 and 24 months, respectively after dosing among those who received 1 dose of vaccine or placebo
- First-episode of culture-confirmed *V. cholerae* O139 diarrhea detected in the study treatment centres from 14 days to 12 and 24 months, respectively after dosing among those who received 1 dose of vaccine or placebo
- First-episode of culture-confirmed *V. cholerae* O139 diarrhea with severe dehydration detected in one of the study treatment centres from 14 days to 12, and 24 months, respectively after dosing among those who received 1 dose of vaccine or placebo
- First-episode of watery diarrhea detected in the study treatment centres from 14 days to 12 and 24 months, respectively after dosing among those who received 1 dose of vaccine or placebo
- First-episodes of watery diarrhea with severe dehydration detected in the study treatment centres from 14 days to 12 and 24 months, respectively after dosing among those who received 1 dose of vaccine or placebo

Secondary safety endpoints

- Persons who received 1 dose of vaccine or placebo and present for care of vaccine adverse effects at treatment settings or who die from the time of dosing until one month later (by type of complaint and by cause of death)
- Secondary immunogenicity endpoints
- Geometric mean serum vibriocidal (to El Tor Inaba and Ogawa O1 serogroup organisms and to O139 serogroup organisms) titers measured in participants at baseline and two weeks after receipt of either vaccine or placebo
- Persons exhibiting 4-fold or greater rises in titers of serum vibriocidal antibodies (to El Tor Inaba and Ogawa O1 serogroup organisms and to O139 serogroup organisms), relative to baseline, two weeks after receipt of either vaccine or placebo

Tertiary endpoints

- First-episodes of culture-confirmed *V. cholerae* O1 diarrhea detected in one of the study treatment centres from the time of dosing to 12 and 24 months, respectively among those who did not receive vaccine or placebo but were enumerated in the census as study population
- First-episodes of culture-confirmed *V. cholerae* O1 diarrhea with severe dehydration detected in one of the study treatment centres from the time of dosing to 12 and 24 months, respectively

among those who did not receive vaccine or placebo but were enumerated in the census as study population

- First-episodes of culture-confirmed *V. cholerae* O139 diarrhea detected in one of the study treatment centers from the time of dosing to 12 and 24 months, respectively among those who did not receive vaccine or placebo but were enumerated in the census as study population
- First-episodes of culture-confirmed *V. cholerae* O139 diarrhea with severe dehydration detected in one of the study treatment centres from the time of dosing to 12 and 24 months, respectively among those who did not receive vaccine or placebo but were enumerated in the census as study population
- First-episodes of watery diarrhea detected in one of the study treatment centres from the time of dosing to 12 and 24 months, respectively among those who did not receive vaccine or placebo but were enumerated in the census as study population
- First-episodes of watery diarrhea with severe dehydration detected in one of the study treatment centres from the time of dosing to 12 and 24 months, respectively among those who did not receive vaccine or placebo but were enumerated in the census as study population .

Analysis plan

Analyses of vaccine protection will use Cox proportional hazard regression models, verifying first that the proportionality assumption is satisfied for all independent variables. Hazard ratios (HRs) of the target outcome in the vaccine versus placebo groups will be estimated by exponentiation the coefficient for the vaccine variable in these models, and vaccine efficacy is estimated as $[(1 - HR) \times 100\%]$. Standard errors for the coefficients will be used to estimate P values and 95% confidence intervals for the HRs. Kaplan-Meier survival curves for the vaccine and placebo groups will be prepared for descriptive purposes. Simple analyses of vaccine impact will be performed. Final adjusted estimates will be obtained from models that included the variables found to be independently associated with the time to the event at $P < 0.1$ in a backward selection algorithm. To evaluate heterogeneity of vaccine protection among different subgroups, we will evaluate interaction terms between the vaccination and subgroup variables in these models.

Primary Analysis after 1 year of follow-up:

Since the primary analysis will be performed at 12 months following dosing, the P value does not need to be changed since the analysis at one year is not an interim analysis. All other analyses following dosing are considered secondary.

In addition we will estimate the protective impact of vaccination against culture-proven *V. cholerae* O139 diarrhoea episodes severe enough to require treatment in a health care facility; age-specific protective efficacy; indirect and age-specific indirect vaccine protection; overall and age-specific

overall vaccine protection; culture-proven *V. cholerae* associated with potentially life-threatening dehydration; episodes of acute watery diarrhoea severe enough to require treatment in a health care facility; episodes of acute watery diarrhoea irrespective of severity. Seroconversion in the vaccine and control groups will also be compared. Statistical methods for these secondary analyses will be the same as those used in the primary analyses.

Analysis at 6 months of follow-up:

Since the primary analysis will be performed at 12 months following dosing, the analysis at 6 month will be considered as an interim analysis. In the interim analysis of the trial, using the Haybittle-Peto rule, the threshold for significance in the primary analysis will be set at $P \leq 0.025$, and 97.5% CIs will be calculated [40]. However, since the sample size is calculated based on $\alpha=0.05$, we will perform the analysis if we have statistical power to do the interim analysis.

Analysis after 2 years of follow-up

Analysis at the second year of follow-up will be performed following the above mentioned plan.

Surveillance for 2 years

Surveillance will be continued to complete 2 years of follow-up. Blinding will be maintained until the end of the follow-up period. Analysis at 2 years of follow-up will still be performed in a blinded manner.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

A Data and Safety Monitoring Plan will be made for the study. It will provide the overall framework for the research protocol's data and safety monitoring. It is not necessary that the DSMP covers all possible aspects of each element. When designing an appropriate DSMP, the following will be kept in mind.

- a) All investigations require monitoring;
- b) The benefits of the investigation should outweigh the risks;
- c) The monitoring plan should commensurate with risk; and
- d) Monitoring should be with the size and complexity of the investigation.

Direct Access to Source Data/Documents

The investigator/institutions will permit (by way of written agreement) trial-related monitoring, audits, IRB/IEC review, and regulatory inspection, providing direct access to source data/documents.

Adverse event definition, assessment and reporting

Neither the bivalent, killed, oral cholera vaccine nor the placebo is known to cause any significant adverse reactions. However, as a precaution, procedures will be in place to detect adverse events in the trial participants as follows. All participants will be observed for 30 minutes immediately after receiving the study agent. Individuals with immediate adverse events will receive emergency treatment and the event will be recorded in the form. The adverse event or Serious adverse event form, as appropriate (Appendix 2D, 2E), will be completed for all adverse events that are reported within 14 days after vaccination whether they are considered vaccine-related or not. Any hospitalization or death in a vaccinee occurring within 14 days after immunization whether they are considered vaccine-related or not will be investigated immediately when they will visit our study hospitals and will be reported using the Adverse event and Serious adverse event Form. If any pregnant woman gets accidentally vaccinated, follow up will done for the pregnant woman vide pregnancy notification using the form ‘update event of the demographic and health events’ (appendix 1 D).

Safety Assessment, Monitoring and Reporting**Definition of Adverse event:**

An adverse event will be defined as an untoward medical event (diarrhea, vomiting, abdominal pain/cramps or any other local and systemic symptoms) with an onset up to 14 days after receipt of a dose which may or may not be associated with the vaccine. At the vaccination sessions after each dose, recipients will be asked to wait for half an hour at the site, where one staff member will be stationed to monitor any immediate adverse event following vaccination.

All vaccinees will be asked to consult the ‘AEFI Case Management Cell’ at the icddr,b hospital in Mirpur for any untoward effect after vaccination. Clinicians as well as study staff will be available for 24 hours during the AEFI surveillance period (from initiation of the vaccination program until 14 days later). The cell will at anytime have a clinicians as well as required staff as well as medication for management of AEFI after dosing at the Cell. Any adverse event will be monitored up to 14 days after vaccination. All AEs must be graded for intensity and relationship to study procedure.

Intensity of Event: For AEs, the following guidelines will be used to quantify intensity.

- Mild: Events require minimal or no treatment and do not interfere with the study participant’s daily activities.
- Moderate: Events result in a low level of inconvenience or concern with the vaccination. Moderate events may cause some interference with functioning.

Definition of Serious Adverse Event

A serious adverse event (experience) is any untoward medical occurrence that results in death, life-threatening. The term “life-threatening” in the definition of “serious” refers to an event in which the recipients will be at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death, if that were more severe.

A serious adverse event (SAE) is defined as an AE meeting one of the following conditions:

- Death during the period of protocol-defined surveillance
- Life-threatening event (defined as a study participant at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance. This will be related to hospitalization other than that related to management of diarrhea and that which is without complications.
- Results in congenital anomaly or birth defect, or malignancy
- Results in a persistent or significant disability/incapacity

Any other important medical event that may not result in death, be life threatening, may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the study participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Prudent medical judgment must be exercised to decide whether reporting is appropriate.

Data Safety Monitoring Board

The DSMB will be formed by the ERC of the icddr,b and will constitute members of the ERC as well as relevant experts in the field. The DSMB will hold meetings with the investigators of the study, at the initiation, and at regular intervals for study updates. Adverse events will be reported as per guideline to the DSMB.

Reporting Procedures:

The Adverse Event Following Immunization (AEFI), as followed by EPI, will be strengthened to identify, investigate and manage any adverse event. For this purpose local health workers and managers will be trained and given proper support from the study. Provision for preferential case management facility will be provided at the different health centres and compensation will be given when deemed necessary. The compensation will include transportation costs, medication costs and wage loss. The icddr,b hospital at Mirpur will be designated as the AEFI Case Management Cell where all reports will be submitted and a Central AEFI committee will oversee all reports (Appendix 2D,2E). Staff at all other health facilities in the study will report adverse events for 14 days after receipt of vaccine. Causal relationships between detected events and vaccination will be assessed by review of case report forms

by experienced clinicians and program personnel will investigate the case (Appendix 2D,2E). The AEFI monitoring will continue for completion of 14 days of evaluation for all groups after vaccination.

During census update, card distribution and vaccination sessions, participants will be informed to report any untoward effect to the icddr, hospital at Mirpur, Mohakhali as well as the other health facilities selected for the study. All AEFI information will be overseen by the Central AEFI committee. Adverse events will be reported as per guideline to the Data Safety Monitoring Board (DSMB) will be formed for the study.

Adverse Events

There will be adverse event reporting format at the site through which study staff will report any event (Appendix 2D). In addition to follow-up for adverse events noted within 14 days of each vaccination as described above, any other adverse or serious adverse event (SAE) that occurs from the beginning of the study up to the end will be reported using- Medications, Adverse Events, and Serious Adverse Event forms (Appendix 2D,2E,2F).

Serious Adverse Events

All SAEs occurring in the study to participants will be reported within 24 hours to the ERC and the DSMB as well as IVI and Shantha Biotechnics. The SAE form will always be completed as thoroughly as possible with all available details of the event, signed by the principal investigator. If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before submitting the report. If deemed necessary, the DSMB will have the authority to call a temporary suspension of the study, for careful review and assessment of the reported event(s). The study physician/medical officer will follow-up subjects with SAEs until the event has: resolved, subsided, stabilized, or disappeared or the event is otherwise explained, or the subject is lost to follow-up. The date of final disappearance of the adverse event will be documented. The study physician/medical officer will always provide an assessment of causality at the time of the initial report.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The Nuffield Council of Bioethics recommends that “wherever appropriate, participants in the control group should be offered a universal standard of care for the disease being studied. Where it is inappropriate to offer such a standard, the minimum that should be offered is the best intervention currently available as part of the national public health system” [41]. While some critics may argue that

the standard of care should be the “best method”, Wendler, et al suggested four conditions when the standard of care may be less than a universally accepted “best method” and these conditions include: a) scientific necessity; b) the relevance of the study for the host community should address important health issues of the communities participating in the studies; c) the need for the clinical trial to produce a fair level of benefit for the communities participating in the trial; and d) subject and host community non-maleficence i.e., the study participant must not be “prospectively worse off” than they would if the trial were not conducted [42]. This study fulfils all four criteria. There is a scientific necessity to perform the trial; the study is of relevance to the communities to be included in the trial as the population would have high rates of cholera (areas will be chosen based on previously reported cholera cases in the hospital surveillance being performed by icddr,b and the community will benefit from the surveillance activity that not only does diarrhoea surveillance but at the same time promote safe water and sanitation practices. Appropriate ethical and regulatory clearances will be obtained prior to the trial. Once the vaccine is approved for the single dose indication, provisions to make the cholera vaccine available to placebo recipients will be made.

The study will be conducted in compliance with the procedures outlined in this protocol and in accordance with the ethical guidelines and local regulatory requirements for the trial. The investigators’ responsibilities will follow the WHO guidelines for GCP. The privacy and confidentiality of all data and information collected from trial participants, including those derived from clinical and biological specimens will be ensured both during and after the conduct of the trial. Individuals will not be identified in any reports and publications based on the trial data.

Verbal and written informed consent will be obtained prior to intervention from eligible adult participants and the parents/guardians of participants aged <18 years; in addition, assent will be obtained from children aged 11-17 years of age (see Informed Consent form), Consent and assent will be documented by signature or thumbprint on the appropriate forms and noted down in the Vaccination Record. Participants and parents/guardian of the child will be informed of the study activities, and they will be encouraged to ask questions regarding the study. Signature (or thumbprint, if illiterate) of the participants and parents/guardian of the child will be obtained before their enrolment in the study, and dated prior to any study-related activity. A witness will also sign in the informed consent form in the event a participant, parents/guardian of a child participant is not literate. The informed consent form must be signed and dated by the study personnel who obtain the consent. In addition informed consent will be obtained from 324 participants who agree to take the vaccine for collection of blood at three time points during the study period.

If new information, not covered in the proposal, on the study products becomes available that may be relevant to the participants’ willingness to continue in the study, the investigator will inform that in a

timely manner and use a revised written informed consent form. The proposal will be revised and resubmitted to RRC and ERC for the amendment and will then be used for obtaining permission.

The expected duration of subject participation

The duration of follow-up for each subject would be 24 months after dosing.

A description of "stopping rules" or "discontinuation criteria" for individual subjects, parts of the trial and the entire trial

The study is planned for duration of 2 years follow up after vaccination. An interim analysis will be conducted at 6 months and primary analysis at 1 year of follow up. At the time of interim and primary analysis, the efficacy results will be submitted to the DSMB. For futility examination, reverse conditional power will be used. If the interim analysis shows unsatisfactory results in all age groups with enough statistical power, option to discontinue will be considered by sponsors in consultation with DSMB.

The trial may be stopped for ethical reasons at the recommendation of IRB/IEC of any of the partner institutions or for the safety reasons at the recommendations of the DSMB and collaborating institutes.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

NA

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

This project is a collaborative study of icddr,b with the Government of Bangladesh and the International Vaccine Institute in Korea as well as other International experts in the field of vaccine field.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

A large area based on updated GIS maps is available in high cholera prone field site at Mirpur urban area in Dhaka. Diarrheal hospitals of icddr,b in Mohakhali and Mirpur and existing health facilities as well as laboratory facilities are available for the study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

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Principal Investigator/Program Director (*Last, first, middle*): Dr. F.Qadri.
 Project Title: Randomized Placebo control trial by single dose Shanchol in Bangladesh

**DETAILED BUDGET FOR BUDGET PERIOD
 DIRECT COSTS ONLY**

		FROM: 01 Jan. 2013 THROUGH 30 Jun. 2015			
Cost Categories		Year 1	Year 2	Year 3	TOTAL US\$
PERSONNEL					
International personnel		122,700	127,608	66,356	316,664
Local Personnel		992,650	939,770	516,874	2,449,294
SUBTOTALS PERSONNEL		1,115,350	1,067,378	583,230	2,765,958
CONSULTANT COSTS					
Consultants		26,061	27,194	0	53,255
SUBTOTALS CONSULTANTS		26,061	27,194	0	53,255
EQUIPMENT (Itemize)					
EQUIPMENT		205,768	0	0	205,768
SUBTOTALS EQUIPMENT		205,768	0	0	205,768
SUPPLIES (Itemize by category)					
Furniture & Stationeries		18,000	12,500	11,500	42,000
SUBTOTALS SUPPLIES		18,000	12,500	11,500	42,000
TRAVEL					
Local Travel		45,200	38,500	16,250	99,950
International Travel			12,000	12,000	24,000
SUBTOTALS TRAVEL		45,200	50,500	28,250	123,950
Other costs					
Training cost, printing, Rent, specimen testing, GIS, vaccine delivery etc.		316,156	201,268	67,684	585,108
Hospital cost					
icddr, b, Mirpur treatment centre and other health facilities cost		165,217	147,826	0	313,043
SUBTOTALS OTHERS		481,373	349,094	67,684	898,151
SUBTOTAL DIRECT COSTS		1,891,752	1,506,666	690,664	4,089,082
Overhead @15%		252,898	226,000	103,600	582,498
TOTAL Budget		2,144,650	1,732,666	794,264	4,671,580


 M. Rahman Chowdhury
 Sr. Budget Co-ordinator

Budget Justifications

Budget for the study is needed for carrying out vaccine delivery in the selected field sites of Mirpur area. Surveillance of the diarrheal patients coming from the study sites will be carried out at the hospitals and health facilities in Mirpur and icddr,b hospitals and microbiological tests carried out at icddr,b. Other important costs are for GIS activity, advocacy meetings, local and international travel, transportation of vaccine, programme monitoring, training, data management etc.

Personnel:

Personnel comprise core component, data management, census, GIS activity, vaccine delivery, census update, passive surveillance and quality team. Total amount budgeted under the categories is US \$2,765,958 for 30 month period.

Consultant costs:

The category consists of 2 external consultants and support for infrastructure amounting US\$ 53,255 in 24 months.

Equipment:

Various capital equipments are required for the study for data collection and laboratory support. All these equipment would be procured in year 1 amounting to US\$ 205,768

Supplies:

Supplies comprise of stationeries and furniture amounting to US\$ 42,000 in the 30 month period.

Travel:

Local travel in all years will require US \$99,950 for supervisory visit and data collection. International travel required in year 2 and year 3 amounting to US\$ 24,000 for study seminar/ workshops/meetings at national and international locations.

Vaccine delivery:

Vaccine delivery in the Mirpur site will be required in year 1 amounting to US\$ 51,600

GIS:

GIS mapping and licensing will be required in initial stages through a subcontract with a GIS company.

Other costs:

Other costs comprising training, printing and office rent, immunogenicity study, specimen testing (324 subjects) etc. amounting US\$ 520,813 in the 30 month period.

Hospital costs:

The costs for icddr,b, Mirpur treatment centre and other health facilities are US\$ 313,043 in the first 24 months of the study.

Overhead costs:

Overhead costs is calculated at 25% amounting to cover direct overhead costs (15%) as well as built in cost.

Comments of External Reviewer 1

EVALUATION FORM

Title: **An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shanchol™) in Dhaka, Bangladesh**

Summary of Referee's Opinions:

Rank Score

	High	Medium	Low
Quality of project	X		
Adequacy of project design	X		
Suitability of methodology	X		
Feasibility within time period	X		
Appropriateness of budget	X		
Potential value of field of knowledge	X		

CONCLUSIONS

I support the project proposal

a) without qualification	X
b) with qualification	
c) on technical grounds	
d) on level of financial support	

I do not support the project proposal

--

Name of Referee: Robert H. Hall, Ph.D.

Signature:.....

.Date: October 27, 2012.....



Position & Institution: I have reviewed this project in my private capacity as an international expert with 27 years knowledge and experience with Cholera Vaccines. The attached comments do not constitute any endorsement by the National Institutes of Health and may not be used to signify any approval by the U.S. Federal Government. No honorarium.

Detailed Comments:

This is an important study. The investigators and collaborators are highly experienced in working on this scale and complexity. The organization and management plans are built on prior relevant experience and are appropriate.

Minor comments:

- 1> The Table of Contents is not complete (may be reviewer's software)
- 2> The pages are not numbered
- 3> Project summary: The case for using single dose for the benefit of Bangladesh is well made. There is also demand for single-dose efficacy data from numerous NGOs and Agencies. The particular humanitarian urgency for a scientific basis for decision-making regarding single dose cholera vaccine for complex emergencies and internally displaced persons is noted. Project summary also makes a good case for the study to enable improved fieldability, inclusion in EPI, and direct protection data rather than vibriocidal assay.
- 4> Hypothesis is apparent but not very clearly stated
- 5> Objectives and outcome measures/variables are appropriate
- 6> Primary and Secondary Objectives are highly appropriate
- 7> Background – although most likely very familiar to the IRB etc, the various OCV products are not very clearly laid out in their formulations and labeling limitations.
- 8> Justification for Single Dose – Substantial reluctance to use OCV due to the lack of single dose data has been apparent in policy fora and the published literature. In addition to benefitting Bangladesh, the proposed study will also be decisive in the policy-making arena in global health; and if successful could offer significant direct and indirect cost savings.
- 9> Study population: is the GIS-indexing suitable for determining herd protection?
- 10> Augmented passive surveillance of diarrhoea to document cholera incidence in the community: will this provide data or can it be extrapolated to a full season?
- 11> Study Procedure and Methodology: There is great confidence that this expert team will implement the study design effectively
- 12> Subject Exclusion criteria: Will women who give birth during the study period be identified (for example in the passive surveillance questionnaire?) Will they be included in a sub-group analysis?
- 13> Will there be a bias in enrollees lost to migration during the study period?
- 14> Management of the single-dose cholera vaccine project: this is a strong plan and a strong team.
- 15> Primary & secondary outcomes are appropriate and feasible.
- 16> Assessments of efficacy, study endpoints, data analysis, are all appropriate. Data safety monitoring plan, adverse event definition, assessment, reporting, ethical assurance, and "stopping rules" all appear to be appropriate.
- 17> References --- there appear to be some formatting issues, (perhaps related to his reviewer's software). There is more literature on the compelling need for single dose OCV data that could be added if necessary.
- 18> Budget: appropriate
- 19> Conclusion: this is a well-planned and important study for the benefit of Bangladeshis and others at heightened risk of cholera. The project is likely to succeed and to generate results of global significance to the cholera control field.

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 they are justified.

This is original and important work that will be widely cited. If the hypothesis is proved correct, there will be a significant positive impact on policy. The immediate beneficiaries will be Bangladeshis at heightened risk of cholera either through endemic exposure or due to epidemics caused by extreme climate events. There will also be major significance at the global level. There is a high degree of confidence that this team will conduct an excellent study. The only suggestion is to consider an assessment of herd protection.

Title: An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shanchol™) in Dhaka, Bangladesh

Reviewer: 

Robert H. Hall, Ph.D.....

Point by point response to the External reviewer 1

Detailed Comments:

This is an important study. The investigators and collaborators are highly experienced in working on this scale and complexity. The organization and management plans are built on prior relevant experience and are appropriate.

Minor comments:

1. **Comment: (regarding Table of content; page 10)** The Table of Contents is not complete (may be reviewer's software)

Response: The Table of Contents has been completed.

2. **Comment:** The pages are not numbered

Response: The pages have been numbered

3. **Comment: (regarding project summary; page 11)** The case for using single dose for the benefit of Bangladesh is well made. There is also demand for single-dose efficacy data from numerous NGOs and Agencies. The particular humanitarian urgency for a scientific basis for decision-making regarding single dose cholera vaccine for complex emergencies and internally displaced persons is noted. Project summary also makes a good case for the study to enable improved fieldability, inclusion in EPI, and direct protection data rather than vibriocidal assay.

Response: None needed

4. **Comment: (regarding Hypothesis; page 12)** Hypothesis is apparent but not very clearly stated

Response: We have made the hypothesis clearer (page 13)

5. **Comment: (regarding objectives; page 12)** Objectives and outcome measures/variables are appropriate.

Response: None needed

6. **Comment: (regarding objective; page 12)** Primary and Secondary Objectives are highly appropriate

Response: None needed

7. **Comment: (regarding background; page 14)** Background – although most likely very familiar to the IRB etc, the various OCV products are not very clearly laid out in their formulations and labeling limitations.

Response: We have now added the various OCV products in their formulation and labeling limitation (page 15, 16).

8. **Comment: (regarding justification of single dose; page 16)** Justification for Single Dose – Substantial reluctance to use OCV due to the lack of single dose data has been apparent in policy fora and the published literature. In addition to benefitting Bangladesh, the proposed study will also be decisive in the policy-making arena in global health; and if successful could offer significant direct and indirect cost savings.

Response: None needed

9. **Comment: (regarding justification of study population; page 19)** Study population: is the GIS-indexing suitable for determining herd protection?

Response: The GIS-indexing will be used to determine the household contacts within a distance-based neighborhood, and then the vaccine coverage will be computed within the household contacts, and then the herd protection will be evaluated by measuring the risk among placebo recipients living in high coverage neighborhood versus risk among placebo recipients living in low coverage neighborhood as it has been done elsewhere (Ali et al., 2005). This has now been inserted in the protocol (page).

10. **Comment: (regarding passive surveillance of study population; page 25)** Augmented passive surveillance of diarrhea to document cholera incidence in the community: will this provide data or can it be extrapolated to a full season?

Response: The passive surveillance data will not be extrapolated. The passive surveillance for incidence of cholera will provide the data to a full season and will be carried for over 2 years.

11. **Comment: (regarding Study Procedure and Methodology ; page 25)** Study Procedure and Methodology: There is great confidence that this expert team will implement the study design effectively

Response: Not needed

12. **Comment: (regarding Subject exclusion criteria ; page 23)** Subject Exclusion criteria: Will women who give birth during the study period be identified (for example in the passive surveillance questionnaire?) Will they be included in a sub-group analysis?

Response: The study participant who give birth during the study period can be identified by the ongoing census update. We however do not have any plan to analyze this subgroup but can do this through an addendum in future if needed. For the study recruitment we will purposively exclude enrolment of pregnant females in the study area by the criteria outlined in the proposal (page 23).

13. **Comment: (regarding Subject migration)** Will there be a bias in enrollees lost to migration during the study period?

Response: We have calculated 25% migration out (lost to follow up) per year in calculating our sample size. We hoped it will provide us an unbiased analysis result at the end of the study period.

14. Comment: (regarding Study management; page 23) Management of the single-dose cholera vaccine project: this is a strong plan and a strong team.

Response: None needed

15. Comment: (regarding outcomes; page 29) Primary & secondary outcomes are appropriate and feasible.

Response: None needed

16. Comment: (regarding study endpoint, efficacy ; page 35)

Assessments of efficacy, study endpoints, data analysis, are all appropriate. Data safety monitoring plan, adverse event definition, assessment, reporting, ethical assurance, and “stopping rules” all appear to be appropriate.

Response: None needed

17. Comment: (regarding reference ; page 43)

References --- there appear to be some formatting issues, (perhaps related to his reviewer’s software). There is more literature on the compelling need for single dose OCV data that could be added if necessary.

Response: We have formatted our reference in endnote manager now and few references have also been added (page 43).

18. Comment: (regarding budget ; page 47) Budget: appropriate

Response: None needed

19. Comments: (regarding conclusion) Conclusion: this is a well-planned and important study for the benefit of Bangladeshis and others at heightened risk of cholera. The project is likely to succeed and to generate results of global significance to the cholera control field.

Response: None needed

Comments from External Reviewer 2

EVALUATION FORM

Title: **An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shanchol™) in Dhaka, Bangladesh.**

Summary of Referee's Opinions:

Rank Score

	High	Medium	Low
Quality of project	X		
Adequacy of project design	X		
Suitability of methodology	X		
Feasibility within time period	X		
Appropriateness of budget			
Potential value of field of knowledge	X		

CONCLUSIONS

I support the project proposal

a) without qualification	X see suggestion below
b) with qualification	
c) on technical grounds	
d) on level of financial support	

I do not support the project proposal

Name of Referee:



Signature:.....

.Date: Oct 28, 2012.....

Position: Professor

Institution: Johns Hopkins University, Department of International Health

Detailed Comments: (Please use additional page if necessary.)

This is a very important study and is of very high quality. If I were to add to the rationale for the study, I would have framed it a bit differently, but this does not detract from the study design.

There could be several justifications for determining the efficacy of a single dose of Shanchol.

1. Limited vaccine supply. Since there is a limited supply, it is important to know how to accomplish the greatest public health benefit with this limited supply. Even if a single dose were somewhat less effective than two doses (e.g. 50% rather than 70%), the single dose strategy has the potential to avert more cases than the two dose strategy. To illustrate: if one had 200,000 doses of vaccine, it may be possible to give two doses to 100,000 people or alternatively, one could give a single dose to 200,000. If two doses induced 70% protective efficacy and if the rate of cholera in the placebo group was 2 per 1000, the vaccine would avert 140 cases, but if the single dose vaccine had an efficacy of 50% and was given to 200,000 people, the program would avert 200 cases. Thus, the single dose strategy with 50% efficacy would avert more cases than a two dose strategy with a 70% efficacy.
2. Documenting an early onset of protection. Some have argued that a two dose requirement suggests that the vaccine is not protective until after the second dose is given, in fact, because past studies have used a study design in which cases are counted for the primary analysis 14 days after the second dose is given. This time frame for past efficacy was interpreted (I feel wrongly interpreted) to suggest that the vaccine did not start protecting until 14 days after the final dose is given. From an immunological assessment, it seems highly likely that protection actually starts within 7 days of the first dose. To avoid this misinterpretation about 14 days, I suggest that the primary analysis be based on 7 days following the dose. There are likely to be very few cases during the period between 7 and 14 days, but the perception could be changed considerably. To be clear, documenting the onset of protection will not be possible with a field trial – it would only be possible with a volunteer challenge study – but it would be best if the field trial did not suggest the myth that protection starts only after 14 days following the date of vaccination.

Clearly, if this study documents efficacy with a single dose, it is clear that one can assume that protection starts much earlier than 14 days following the second dose.

3. If the single dose is found to be efficacious, it may still be wise to have programs giving more than a single dose since two doses may be more have more long lasting protection, or it may prime the immune system better. Thus, the system that is more effective may be one that incorporates booster doses, and this study cannot answer every question, but it will answer an important question.

The ethical issues surrounding a placebo controlled trial was thoroughly discussed and considered with the conclusion that it is appropriate to carry out such study in a setting such as the icddr,b where good treatment can be provided and is a readily available.

Point by point response to the External reviewer comment 2

Detailed Comments: (Please use additional page if necessary.) This is a very important study and is of very high quality. If I were to add to the rationale for the study, I would have framed it a bit differently, but this does not detract from the study design.

There could be several justifications for determining the efficacy of a single dose of Shanchol.

1. **Comment: (regarding vaccine supply)** Limited vaccine supply. Since there is a limited supply, it is important to know how to accomplish the greatest public health benefit with this limited supply. Even if a single dose were somewhat less effective than two doses (e.g. 50% rather than 70%), the single dose strategy has the potential to avert more cases than the two dose strategy. To illustrate: if one had 200,000 doses of vaccine, it may be possible to give two doses to 100,000 people or alternatively, one could give a single dose to 200,000. If two doses induced 70% protective efficacy and if the rate of cholera in the placebo group was 2 per 1000, the vaccine would avert 140 cases, but if the single dose vaccine had an efficacy of 50% and was given to 200,000 people, the program would avert 200 cases. Thus, the single dose strategy with 50% efficacy would avert more cases than a two dose strategy with a 70% efficacy.

Response: We appreciate the comment very much and have added this to the justification of the study (page 17).

2. **Comment: (regarding protection)** Documenting an early onset of protection. Some have argued that a two dose requirement suggests that the vaccine is not protective until after the second dose is given, in fact, because past studies have used a study design in which cases are

counted for the primary analysis 14 days after the second dose is given. This time frame for past efficacy was interpreted (I feel wrongly interpreted) to suggest that the vaccine did not start protecting until 14 days after the final dose is given. From an immunological assessment, it seems highly likely that protection actually starts within 7 days of the first dose. To avoid this misinterpretation about 14 days, I suggest that the primary analysis be based on 7 days following the dose. There are likely to be very few cases during the period between 7 and 14 days, but the perception could be changed considerably. To be clear, documenting the onset of protection will not be possible with a field trial it would only be possible with a volunteer challenge study but it would be best if the field trial did not suggest the myth that protection starts only after 14 days following the date of vaccination.

Clearly, if this study documents efficacy with a single dose, it is clear that one can assume that protection starts much earlier than 14 days following the second dose.

Response:. We will use the 14 day endpoint as the primary outcome. However, as secondary outcome we will measure efficacy after 7 days after vaccination and based on the results of the study we can disseminate the information obtained to change the strategy of vaccination.

3. **Comment: (regarding single dose vs. double dose)** If the single dose is found to be efficacious, it may still be wise to have programs giving more than a single dose since two doses may be more have more long lasting protection, or it may prime the immune system better. Thus, the system that is more effective may be one that incorporates booster doses, and this study cannot answer every question, but it will answer an important question. The ethical issues surrounding a placebo controlled trial was thoroughly discussed and considered with the conclusion that it is appropriate to carry out such study in a setting such as the icddr,b where good treatment can be provided and is a readily available.

Response: None needed


Check-List

**Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)**
[Please check all appropriate boxes]

<p>1. Has the proposal been reviewed, discussed and cleared by all listed investigators?</p> <p><input checked="" type="checkbox"/> Yes <input type="checkbox"/> No</p> <p>If the response is No, please clarify the reasons:</p>
<p>2. Has the proposal been peer-reviewed externally?</p> <p><input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> External Review Exempted</p> <p>If the response is 'No' or "External Review Exempted", please explain the reasons:</p> <p>If the response is "Yes", please indicate if all of their comments have been addressed?</p> <p><input checked="" type="checkbox"/> Yes (please attach)</p> <p><input type="checkbox"/> No (please indicate reason(s)):</p>
<p>3. Has the budget been reviewed and approved by icddr,b's Finance?</p> <p><input checked="" type="checkbox"/> Yes <input type="checkbox"/> No (reason): _____</p>
<p>4. Has the Ethics Certificate(s) been attached with the Protocol?</p> <p><input checked="" type="checkbox"/> Yes <input type="checkbox"/> No</p> <p>If the answer is 'No', please explain the reasons:</p>
<p><u>Firdausi Qadri</u> Signature of the Principal Investigator</p> <p>31/10/2012 Date</p>

SCVB PROTOCOL (12090) VERSION HISTORY LOG

Number	Description of approved protocol/addendum	Approval date		
		RRC	ERC	Version
SCVB Protocol		20 th NOV 2012	09 th DEC 2012	Version 1.0
1st Addendum Change in Pages- 37,38,39	We would like to extend the safety monitoring period after delivering vaccine/placebo to the study participant for a period of 28 days. This is to monitor the adverse event for a longer period.	Not required	27 th December 2012	Version 1.1
2nd Addendum Change in Pages- 28,45 Appendix- 1B(i),1B(ii),1G(i), 1G(ii),1J(i),1J(ii)	In the protocol, participant number has been corrected. Some additions have been made in the informed consent form for the safety of the patient and according to the GCP guidelines. We have added additional fund in the budget section.	Not required	8 th January 2013	Version 1.2
3rd Addendum Change in Pages- 2,5,7,9,13,14,20,23,2 4,25,26,28,29,30,32,3 5,39,40	Changes are made in the following sections of the protocol-Investigators' list, sample size, census population, study area,Budget,Primary and secondary objective evaluation period, budget, pregnancy follow up schedule, randomization procedure, immunogenicity study schedule, follow up visit after surveillance etc. Inclusion of new sections e.g Active follow up for safety, Auditing, Assessment of Causality and Assessment of severity of adverse events.	13 th October 2013	12 th November 2013	Version 2.0
4th Addendum Change in Pages- 2,5,22,24,25,29,40, 42	Changes are made in the following sections of the protocol-investigator and scientific team member list,Vaccination site number, composition of non biological placebo,Accountability procedures for the investigational product, including the comparator Immunological assays using blood specimens from study participants,Temperature scale and Ethical Assurance for Protection of Human rights	Not required	15 th December 2013	Version 3.0
		RRC	ERC	Version
5th Addendum (Appendix 6A, 6Bi, 6Bii, 6Biii 13), Page 24,	We will do verbal autopsy in our study area among vaccinated population to identify the number of death and the probable causes of death (Page- 30). For this purpose, we will use two questionnaires for verbal autopsy, one for child aged 1 year to 14 years and the other for person aged 15 years & above 2. We have inserted a change in the composition of placebo (page-24) in the protocol ‘ 3. Minor changes in informed consent and pregnancy questionnaires (Appendix 6A, 6Bi, 6Bii, 6Biii).	19th MAR 2014	17th April 2014	Version 4
6th addendum	Inclusion of external Co-Principal Investigator and Co-Investigator	1st April 2015	6th April 2015	Version 5

		RRC APPLICATION FORM	
RESEARCH PROTOCOL Number: PR-12090 Version No. 5.0 Version date: 01-04-2015		FOR OFFICE USE ONLY	
		RRC Approval:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No Date:20/11/2012
		ERC Approval:	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No Date:09/12/2012
		AEEC Approval:	<input type="checkbox"/> Yes <input type="checkbox"/> No Date:
		External IRB Approval	<input type="checkbox"/> Yes <input type="checkbox"/> No Date:
		If, 'yes' Name of IRB: _____	
Protocol Title: An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shancho1™) in Dhaka, Bangladesh			
Short title (in 50 characters including space): Single dose oral cholera vaccine study in Dhaka,Bangladesh			
Originating Centre <input type="checkbox"/> Child and Adolescent Health <input type="checkbox"/> Chronic Diseases <input type="checkbox"/> Communicable Diseases <input type="checkbox"/> Equity and Health Systems <input type="checkbox"/> Food and Waterborne Diseases		<input type="checkbox"/> HIV/ AIDS <input type="checkbox"/> Nutrition and Food Security <input type="checkbox"/> Population, Urbanisation and Climate Change <input type="checkbox"/> Reproductive Health <input checked="" type="checkbox"/> Vaccine Sciences <input type="checkbox"/> Other (specify)	
SP2020 Research Priority Area (check all that apply) <input type="checkbox"/> Healthy Life Course <input type="checkbox"/> Mitigating Risk and Vulnerability		<input checked="" type="checkbox"/> Combating Priority Diseases <input type="checkbox"/> Equitable Health Systems	
Research Phase (4 Ds) <input type="checkbox"/> Discovery <input type="checkbox"/> Development		<input checked="" type="checkbox"/> Delivery <input checked="" type="checkbox"/> Evaluation of Delivery	
Anticipated Impact of Research (check all that apply) <input checked="" type="checkbox"/> Knowledge Production <input checked="" type="checkbox"/> Capacity Building		<input type="checkbox"/> Informing Policy <input checked="" type="checkbox"/> Health and Health Sector Benefits <input checked="" type="checkbox"/> Economic Benefits	
Does this Protocol use the Gender Framework: [please refer to Gender Analysis Tool with Guidance Document in the Intranet]		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
If 'no' is the response, its reason(s) in brief:			
Will this research specifically benefit the disadvantaged (economically, socially and/or otherwise)?		<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	
Does this Protocol use Behaviour Change Communication:		<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
Key words: Cholera vaccine, single dose, randomized trial			
Principal Investigator (Should be icddr,b staff): Sex <input checked="" type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No. (if any), cell phone number, and Email address): Dr. Firdausi Qadri Employee ID- I00003; Centre for Vaccine Sciences, icddr,b, 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh.. Tel: 880 2 9841751 to 9841760, Ext. 2431; Fax: 8802- 8823116;e-mail: fqadri@icddr.org ;			

<p>Co-Principal Investigator(s) Internal: Sex <input checked="" type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address): [if more than one, please copy and paste this row for additional Co-PIs] Dr John D Clemens Address icddr,b, 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh.. Tel: 880 2 9841751 to 9841760, Ext. 3100; Fax: 8802- 8823116;e-mail: jclemens@icddrb.org Dr.Fahima Chowdhury(fchowdhury@icddrb.org), Dr Amit Saha(amit@icddrb.org), Dr Iqbal Ansary Khan(iak@icddrb.org), Dr Ashraful Islam Khan(ashrafk@icddrb.org) Address icddr,b, 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh.. Tel: 880 2 9841751 to 9841760, Ext. 3465; Fax: 8802- 8823116</p>											
<p>Co-Principal Investigator(s) - External: Sex <input type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address). [if more than one, please copy and paste this row for additional Co-PIs] Dr.LauraDigilio International Vaccine institute(IVI) SNU Research Park San 4-8 Nakseongdae-dong, Kwanak-gu, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803 Mail address:Laura.Digilio@IVI.INT Dr. Thomas F. Wierzba International Vaccine institute(IVI) SNU Research Park San 4-8 Nakseongdae-dong, Kwanak-gu, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803 Mail address: twierzba@ivi.int</p>											
<p>Co-Investigator(s) - Internal: Sex <input checked="" type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including phone no(s), Extension No (if any), cell phone number, and Email address): [if more than one, please copy and paste this row for additional Co-Is] Dr.Yasmin Ara Begum, Dr. Taufiqur Rahman Bhuiya,Dr. Dilruba Ahmed,Dr.Mohiul Islam Chowdhury</p>											
<p>Co-Investigator(s) – External: Sex <input checked="" type="checkbox"/> Female <input checked="" type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address): [if more than one, please copy and paste this row for additional Co-Is] Dr.Jerome Hahn Kim(IVI),Dr.Alejandro Cravioto (IVI),Ajit Pal Singh (IVI), Sachin N. Desai (IVI), Mohammad Ali, Dr. Mahmudur Rahman (IEDCR & NIC) ,Dr. S.A.J. Md. Musa,(PHC),Dr.BaizidKhoorshidRiaz, Dr. Md. Tajul Islam A. Bari EPI & Surveillance ,Dr.Md.Shamsuzzaman (EPI), Dr Sanjida Islam (DCC)</p>											
<p>Student Investigator(s) - Internal (Centre’s staff): Sex <input type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address):</p>											
<p>Student Investigator(s) - External: Sex <input type="checkbox"/> Female <input type="checkbox"/> Male Address (provide full official address, including land phone no(s), Extension No (if any), cell phone number, and Email address):</p>											
<p>Collaborating Institute(s): Please provide full official address</p>											
<p>Institution # 1</p> <table border="1"> <tr> <td>Country</td> <td>Seoul, South Korea</td> </tr> <tr> <td>Contact person</td> <td>Dr. Thomas F.Wierzba</td> </tr> <tr> <td>Department (including Division, Centre, Unit)</td> <td>Translational Research Division, International Vaccine Institute</td> </tr> <tr> <td>Institution (with official address)</td> <td>International Vaccine Institute</td> </tr> <tr> <td>Directorate (in case of GoB i.e. DGHS)</td> <td>International Vaccine Institute SNU Research Park San 4-8 Bongcheon 7-dong Kwanak, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803</td> </tr> </table>		Country	Seoul, South Korea	Contact person	Dr. Thomas F.Wierzba	Department (including Division, Centre, Unit)	Translational Research Division, International Vaccine Institute	Institution (with official address)	International Vaccine Institute	Directorate (in case of GoB i.e. DGHS)	International Vaccine Institute SNU Research Park San 4-8 Bongcheon 7-dong Kwanak, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803
Country	Seoul, South Korea										
Contact person	Dr. Thomas F.Wierzba										
Department (including Division, Centre, Unit)	Translational Research Division, International Vaccine Institute										
Institution (with official address)	International Vaccine Institute										
Directorate (in case of GoB i.e. DGHS)	International Vaccine Institute SNU Research Park San 4-8 Bongcheon 7-dong Kwanak, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803										

Collaborating Institute(s): Please provide full official address
Institution # 2

Country	Bangladesh
Contact person	Dr. S.A.J. Md. Musa, Director (PHC) & Line Director MNC & AH
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Directorate (in case of GoB i.e. DGHS)	DGHS
Ministry (in case of GoB)	MoHFW

Institution # 3

Country	Bangladesh
Contact person	Professor Mahmudur Rahman Director, IEDCR & NIC
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Institution (with official address)	Director, IEDCR & NIC DGHS, Mohakhali, Dhaka. Tel: 8821237 (Off), 8950828 (Res), Mob: 01711595139, mrahman57@hotmail.com
Directorate (in case of GoB i.e. DGHS)	DGHS
Ministry (in case of GoB)	MoHFW

Institution # 4

Country	Bangladesh
Contact person	Dr. Md. Tajul Islam A. Bari Programme Manager, EPI & Surveillance
Department (including Division, Centre, Unit)	EPI & Surveillance
Institution (with official address)	Programme Manager, EPI & Surveillance, DGHS, Mohakhali, Dhaka. Tel: 9880530 (Off), 8821910 (Off), 01711976956, Email: tajulepi@yahoo.com
Directorate (in case of GoB i.e. DGHS)	DGHS
Ministry (in case of GoB)	MoHFW

Institution # 5

Country	Bangladesh
Contact person	Dr Sanjida Islam , Project Officer
Department	Health Unit
Institution	Dhaka City Corporation Tel :9557055(Off), 01713092669
Ministry (in case of GoB)	Ministry of Local Government Division (LGRD), Urban primary health care services delivery project

Contribution by the Members of the Scientific Team

Member' Name	Contribution									
	Rese arch idea/ Con cept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/ interpretation of results	Manuscript writing	
Dr Firdausi Qadri	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr John D Clemens	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr Iqbal Ansary Khan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr.Fahima Chowdhury	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr.Amit Saha	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr Ashraful Islam Khan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Prof Mahmudur Rahman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr Yasmin Ara Begum	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr.Dilruba Ahmed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Md. K. Zaman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Md Atique Iqbal	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Dr Alejandro Cravioto	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Laura Digilio	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Thomas F.Wierzba	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Sachin N. Desai	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Mohammad Ali	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Jerome Hahn Kim	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr.Ajit Pal Singh	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr.Taufiqur Rahman Bhuyan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr.Mohiul Islam Chowdhury	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr FarhanaKhanam	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Afroza Akter	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Muhammad Asaduzzaman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. Md. Arifuzzaman Khan	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Md RasheduzzamanRashu	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	
Dr. S.A.J. Md. Musa	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Dr. Md. Tajul Islam A. Bari	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Dr Sanjida Islam	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Dr.Md Shamsuzzaman	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

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

Sex

- Male
 Female

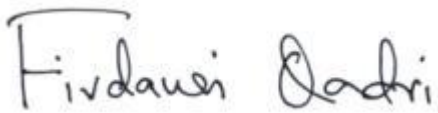
Age

- 0 – 4 years
 5 – 10 years
 11 – 17 years
 18 – 64 years
 65 +

- Pregnant Women
 Fetuses
 Prisoners
 Destitutes
 Service Providers
 Cognitively Impaired
 CSW
 Others :
 Animal

NOTE:It is icddr.b's policy to include men, women, and children in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Project/study Site (Check all that apply):	
<input type="checkbox"/> Chakaria <input checked="" type="checkbox"/> Dhaka Community, Mirpur <input checked="" type="checkbox"/> Dhaka Hospital <input type="checkbox"/> Kamalapur Field Site/HDSS <input type="checkbox"/> Matlab DSS Area <input type="checkbox"/> Matlab non-DSS Area <input type="checkbox"/> Matlab Hospital <input type="checkbox"/> Mirzapur	<input type="checkbox"/> Bandarban <input checked="" type="checkbox"/> Other areas in Bangladesh Name: Hospitals and Health facilities in Mirpur, Dhaka <hr/> <input type="checkbox"/> Outside Bangladesh Name the Country: _____ <hr/> <input type="checkbox"/> Multi Centre Trial (Name other countries involved): _____
Which of the Millennium Development Goal(s) this Proposal Relates to:	
<input type="checkbox"/> 1. Eradicate extreme poverty and hunger	<input type="checkbox"/> 2. Achieve universal primary education
<input type="checkbox"/> 3. Promote gender equality and empower women	<input checked="" type="checkbox"/> 4. Reduce child mortality
<input type="checkbox"/> 5. Improve maternal health	<input checked="" type="checkbox"/> 6. Combat HIV/AIDS, malaria and other diseases
<input type="checkbox"/> 7. Ensure environmental sustainability	<input type="checkbox"/> 8. Develop a global partnership for development
Type of Study (Check all that apply):	
<input type="checkbox"/> Case Control Study <input checked="" type="checkbox"/> Clinical Trial (Hospital/Clinic/Field)* <input type="checkbox"/> Community-based Trial/Intervention <input type="checkbox"/> Cross Sectional Survey <input type="checkbox"/> Family Follow-up Study <input type="checkbox"/> Longitudinal Study (cohort or follow-up) <input type="checkbox"/> Meta-analysis <input type="checkbox"/> Programme Evaluation	<input type="checkbox"/> Programme (Umbrella Project) <input type="checkbox"/> Prophylactic Trial <input type="checkbox"/> Record Review <input type="checkbox"/> Secondary Data Analysis <input checked="" type="checkbox"/> Surveillance/Monitoring <input type="checkbox"/> Systematic Review <input type="checkbox"/> Others:
<p>*Note: Following RRC and ERC approval of a clinical trial, the PI should provide relevant information about the research protocol to the IRB Secretariat (Research Administration Services) for uploading into websites (usually at the https://register.clinicaltrials.gov/), for its registration. In the event of modification of the initially approved protocol the PI shall, after securing approval of RRC and ERC, provide relevant new information to the IRB Secretariat for updating of the protocol in the website.</p> <p>ICMJE defined clinical trial as “Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome”.</p>	
Targeted Population (Check all that apply):	
<input checked="" type="checkbox"/> No ethnic selection (Bangladeshi) <input checked="" type="checkbox"/> Bangalee <input type="checkbox"/> Tribal group	<input type="checkbox"/> Expatriates <input type="checkbox"/> Immigrants <input type="checkbox"/> Refugee <input type="checkbox"/> Special group (specify):
Consent Process (Check all that apply):	
<input checked="" type="checkbox"/> Written <input checked="" type="checkbox"/> Oral <input type="checkbox"/> None	<input checked="" type="checkbox"/> Bengali Language <input type="checkbox"/> English Language
Biological Specimen	
a) Will the specimen be stored for future use?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable
b) If yes, how long the specimens will be preserved?	5 years
c) What types of tests will be carried out with the preserved specimens?	Microbiological, culture and sensitivity, immunological tests in subgroup of specimens

d) Will consent be obtained from study participants for use of the stored specimen for unrelated to this study without their re-consent?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable								
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/ countries?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Not applicable								
f) Will the surplus/unused specimen be returned to icddr,b? If no, then the surplus/unused specimen must be destroyed.	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Not applicable								
g) Who will be the custodian of the specimen at icddr,b?	Dr. Firdausi Qadri								
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	Not Applicable								
i) Who will be the owner(s) of the specimens?	icddr,b								
j) Has a MoU been developed for the protocol covering the specimen collection, storage, use and ownership? If yes, please attach a copy. If no, it is mandatory to provide reason(s) why this is not applicable?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Not applicable								
Risk Group of Infectious Agent and Use of Recombinant DNA									
a) Will specimens containing infectious agent be collected?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable								
b) Will the study involve amplification by culture of infectious agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not applicable								
c) If response to questions (a) and/or (b) is positive, to which Risk Group (RG) does the agent(s) belong?	<input type="checkbox"/> RG1 <input checked="" type="checkbox"/> RG2 <input type="checkbox"/> RG3 <input type="checkbox"/> RG4								
d) Does the study involve experiments with recombinant DNA?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Not applicable								
Does the study involve any biohazardous materials/agents or microorganisms of risk group 2, 3, o 4 (GR2, GR-3 or GR4)?									
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No									
If yes, I (the Principal Investigator) certify that standard icddr,b laboratory procedures will be followed for biosafety of the hazardous materials/agents or microorganisms in the conduction of the study.									
									
01/04/2015									
Signature of the Principal Investigator	Date								
Proposed Sample Size:									
Sub-group (Name of subgroup (e.g. Men, Women) and Number									
<table border="1"> <thead> <tr> <th>Name</th> <th>Number</th> </tr> </thead> <tbody> <tr> <td>(1) Vaccine arm</td> <td>102,219</td> </tr> <tr> <td>(2) Placebo Arm</td> <td>102,219</td> </tr> <tr> <td>Total sample size</td> <td>204,438</td> </tr> </tbody> </table>		Name	Number	(1) Vaccine arm	102,219	(2) Placebo Arm	102,219	Total sample size	204,438
Name	Number								
(1) Vaccine arm	102,219								
(2) Placebo Arm	102,219								
Total sample size	204,438								

Determination of Risk: Does the research involve (Check all that apply):

Is the information recorded in such a manner that study participants can be identified directly or through identifiers linked to them?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does the research deal with sensitive aspects of participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No

Do you consider this research (Check one):

<input type="checkbox"/> Greater than minimal risk	<input checked="" type="checkbox"/> No more than minimal risk	<input type="checkbox"/> Only part of the diagnostic test
----------------------------------------------------	---------------------------------------------------------------	-----------------------------------------------------------

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

Funding

Is the protocol funded?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
If the answer is yes, please provide sponsor(s)'s name	International Vaccine Institute, SNU Research Park San 4-8 Bongcheon 7-dong Kwanak, Seoul, Korea Telephone (82-2) 872-2801, Fax (82-2) 872-2803	

If fund has not been identified N/A

Is the proposal being submitted for funding?	<input type="checkbox"/> Yes	<input type="checkbox"/> No
If yes, name of the funding agency	1.	
	2.	

Dissemination plan [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is yes)
Seminar for icddr,b scientists/ staff	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	For internal staffs to disseminate the findings
Internal publication	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	In Health and Science Bulletin of icddr,b
Working paper	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	For record keeping and dissemination
Sharing with GoB (e.g. DGHS/ Ministry, others)	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Through dissemination meetings where findings will be shared with GoB to assist in taking the vaccine to high risk population
Sharing with national NGOs	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Through dissemination meeting along with GoB
Presentation at national workshop/ seminar	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	We will share results with stakeholders in Bangladesh through meetings and seminar
Presentation at international workshop/ conference	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	We will present our findings at the different international conferences
Peer-reviewed publication	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	We will submit our manuscripts in peer reviewed journals
Sharing with international agencies	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Our data and findings will be shared with international agencies, particularly with WHO, UNICEF and GoB.
Sharing with donors	<input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes	Our data and findings will be shared
Policy brief	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes	

Conflict of interest

Do any of the participating investigators and/or member(s) of their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

No Yes (please submit a written **statement of disclosure to the Executive Director, icddr,b**)

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date : ASAP

End Date : 2.5 years after study initiation

Cost Required for the Budget Period (\$)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1	2,515,075	353,696	2,868,771
Year-2	1,815,005	272,251	2,087,256
Year-3	768,024	115,204	883,228
Total	5,098,104	741,151	5,839,255

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the SUCHONA (Form # 2) if a grant is awarded as a result of this application.

Firdausi Qadri

01/04/2015

Signature of PI

Date

Approval of the Project by the Centre Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Centre level.

Dr.Firdausi Qadri

Firdausi Qadri

01/04/2015

Name of the Centre Director

Signature

Date of Approval

Definition of Abbreviations used

AE	Adverse Event
GOB	Government of Bangladesh
CHW	Community Health Worker
CI	Confidence Interval
CRA	Clinical Research Associate
CRFs	Case Report Forms
DSMB	Data Safety Monitoring Board
DSS	Demographic Surveillance System
EPI	Expanded Programme on Immunization
ERC icddr,b	Ethical Review Committee. The name of the ethical review committee at the icddr,b
GCP	Good Clinical Practices - a set of standards used to document the accuracy of the clinical data being collected
GIS	Geographical Information system
GMT	Geometric Mean Titre
icddr,b	The International Centre for Diarrhoeal Disease Research, Bangladesh – Sometimes referred to as “the Centre.”
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IEDCR	Institute of Epidemiology Disease Control and Research
IR	Incidence rate
IVI	International Vaccine Institute
NGO	Non Government Organization
NICED	National Institute of Cholera and Enteric Diseases
NIHE	National Institute of Hygiene and Epidemiology (Hanoi)
PI	Principal investigator
PID	Personal Identification
RRC	Research Review Committee
SAE	Serious Adverse Event
WHO	World Health Organization
SCVB	Single dose oral cholera vaccine study in Dhaka,Bangladesh

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Check here if appendix is included

Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: Dr. Firdausi Qadri	
Research Protocol Title: An individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of bivalent, killed, whole cell oral cholera vaccine (Shancho1™) in Dhaka, Bangladesh	
Proposed start date: ASAP	Estimated end date: 2.5 years after initiation
Background (brief):	
<p>a. Burden: Bangladesh remains endemic for cholera, which experiences biannual outbreaks with additional epidemics seen during times of floods, cyclones or any natural disaster [1, 2]. It affects all age groups with the majority of fatal cases occurring in children [3-6]. Therefore, immunization against cholera remains an important public health component in the prevention and control of the disease [6].</p> <p>b. Knowledge gap: The current two-dose regimen of the internationally available oral cholera vaccines (OCV) create a logistical and programmatic challenge for use in national programs or during epidemics [7]. Responses in post disaster settings and outbreaks in non-endemic countries can vary greatly based upon the nature of the disaster, the economic status of the country, and the infrastructural resources available. In a phase 3 randomized controlled trial Shancho1™ was shown to be 66% efficacious during the 3 years following dosage in endemic Kolkata, India [23]. When comparing one versus two doses in Kolkata, it was also shown that a significant vibriocidal responses were achieved after a single dose and these did not increase following the second dose [25]. It is important to determine if a single dose vaccine will be protective in regions where cholera is endemic, e.g. Bangladesh.</p> <p>c. Relevance: Since the serum vibriocidal antibody response is only an indirect serological correlate of protection, for killed cholera vaccines at present, an efficacy trial with a placebo control would be required to confirm the usefulness of a single dose [8]. If the vaccine is found to be efficacious following a single dose, this will have profound implications for the use of the vaccine in areas with limited resources particularly in complex emergencies where a multiple dose regimen is difficult to deploy. A single-dose regimen of this vaccine will improve its 'field ability' and allow the vaccine to be used for outbreak control, especially in difficult settings where the risk of cholera is extremely high and provisions for clean water and sanitation are not available [9]. With low OCV production rates, larger populations could be immunized against cholera if a single dose is found to be efficacious. A single-dose schedule could facilitate the inclusion of a global stockpile strategy.</p>	
Hypothesis (if any):	
The incidence rate of cholera among single dose recipients of the OCV, Shancho1™, will be at least 50% lower than that in placebo recipients.	
Objectives:	
The primary objective of the study is to evaluate the protective efficacy of a single dose regimen of the bivalent, killed, whole cell oral cholera vaccine Shancho1™ against culture-proven <i>V. cholerae</i> O1 diarrhea, detected in selected treatment settings serving the catchment populations over a 6 month follow up period. The vaccine will be given to healthy, non-pregnant residents	

aged one and above in Dhaka, Bangladesh.

Methods:

The study design is a two-arm individually randomized double-blind placebo-controlled trial.

Outcome measures/variables: The primary outcome of the study is the proportion of persons receiving 1 dose of vaccine or placebo (“participants”) who are detected with diarrhea with faecal excretion of *V. cholerae* O1 in the study treatment centres from 7 days to 6 months (180 days) after dosing and whose identity is confirmed through home visit.

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: No Yes (describe below)

The incidence rate of cholera among single dose recipients of the OCV Shanchol™ will be at least 50% lower than that in placebo recipients.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

Primary objective of the study

The primary objective of the study is to evaluate protective efficacy of a single dose regimen of the bivalent, killed, whole cell oral cholera vaccine Shanchol™, given to healthy, non-pregnant residents aged one and above in Dhaka, Bangladesh, against culture-proven *V. cholerae* O1 diarrhea which has been detected in all treatment settings serving the catchment population with onset of 7 days to 6 months (180 days) after dosing.

Secondary Objectives

- To evaluate the protective efficacy of a single dose regimen of a bivalent, killed, whole cell-based oral cholera vaccine Shanchol™, given to healthy, non-pregnant residents one year and older with onset of 7 days to 12 months, from 7 days to 18 months and from 7 days to 24 months after dosing, against culture-proven *V. cholerae* O1 diarrhea detected in treatment centres
- To evaluate protective efficacy of a single dose regimen of a bivalent, killed, whole cell-based oral cholera vaccine Shanchol™, given to healthy, non-pregnant residents one year and older over the period of 7 days to 12 months, from 7 days to 18 months and 7 days to 24 months after dosing against:
 - Culture-proven *V. cholerae* O1 diarrhea with severe dehydration detected in treatment centres

- Culture-proven *V. cholerae* O139 diarrhea, detected in treatment centres
- Culture-proven *V. cholerae* O139 diarrhea with severe dehydration detected in treatment centres
- To evaluate protective efficacy of the single dose regimen of Shanchol™ against acute watery diarrhea detected in treatment centres with onset of 7 days to 6 months, 7 days to 12 months, from 7 days to 18 months and 7 days to 24 months after dosing
- To evaluate protective efficacy of the single dose regimen of Shanchol™ against acute watery diarrhea with severe dehydration detected in treatment centres with onset of 7 days to 6 months, 7 days to 12 months, from 7 days to 18 months and 7 days to 24 months after dosing
- To evaluate serum vibriocidal (to El Tor Inaba and Ogawa serogroup O1 and to serogroup O139 organisms) antibody responses to a single dose regimen of the bivalent, killed, whole cell-based oral cholera vaccine Shanchol™ in healthy, non-pregnant residents, aged one year and older in a subset of population
- To evaluate the safety up to 28 days following a single dose of the bivalent, killed, whole cell oral cholera vaccine Shanchol™ administered to healthy, non-pregnant residents one year and older.

Background of the Project including Preliminary Observations

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Cholera continues to be a serious public health problem worldwide. In 2010, a total of 237,000 cases and around 6,000 deaths were reported to the World Health Organization (WHO) globally primarily in Africa, and Asia[6]. Compared to the 2007 figures, this represents 8% and 27% increase respectively. Moreover, when analysed by 5-year periods, the global incidence and number of deaths due to cholera have shown a rising trend in the last ten years. A cumulative total of 838,315 cases were notified to the World Health Organization (WHO), compared with 676,651 cases between 2000 and 2004, representing a 24% increase in the number of cases reported for this most recent 5-year period [10]. The true figures are likely to be much higher due to underreporting; the WHO estimates that only 5-10% of cholera cases are actually reported [11].

More recently, unprecedented outbreaks have been seen in many countries including Zimbabwe, Haiti, Pakistan, Nepal, Guinea, Cuba, Congo, and Sierra Leone. These cholera outbreaks cause undue suffering with high mortality and morbidity figures as well as economic and social disruption. Regions in India and Bangladesh have long been recognized as the homeland of cholera where 6 of the 7 reported cholera pandemics had their origin [12]. Bangladesh remains endemic for cholera, which peaks biannually with further increases seen during floods and cyclones [1, 2, 13]. It affects all age groups, although the majority of fatal cases occur in children [3-6]. Therefore, immunization against cholera remains an important public health tool for preventing and controlling the disease [6].

The provision of safe water and food, establishment of adequate sanitation, and implementation of personal and community hygiene constitute the main public health interventions against cholera. These measures cannot be fully implemented in the near future in most cholera endemic areas. A safe, effective, and affordable vaccine would be a useful tool for cholera prevention and control. A parenteral killed whole cell cholera vaccine, previously available for many years, is no longer recommended by WHO because of its limited efficacy and high rates of adverse reactions [8].

Considerable progress has been made during the last decade in the development of new generation oral vaccines against cholera. Dukoral™ (Crucell/ SBL), a killed whole cell *V. cholerae* O1 with recombinant B-subunit (rBS-WC), was the first to be licensed internationally and has been available mostly in developed countries as a traveller's vaccine. This vaccine is licensed in over 50 countries, including Bangladesh. Several mass vaccination programs have been carried out successfully with Dukoral, including in Beira, Mozambique, Indonesia after the tsunami, Madagascar, Sudan, and Zanzibar. Overall over 500,000 people have been vaccinated with Dukoral in these mass vaccinations.

Analyses of the herd protective effects of this vaccine showed that a greater than 90% reduction in cholera disease burden can be achieved having only moderate (~50% - 60%) level of coverage [14] and that the vaccine can be efficacious even in developing country settings [15, 16]. The WHO now recommends Dukoral for both endemic and epidemic cholera. However, two disadvantages limit the broader use of Dukoral. First, its current price is prohibitively expensive; for example in Bangladesh it is sold for the equivalent of \$18 per dose. Second, Dukoral needs to be administered with a buffer, which complicates large scale deployment. These pose a logistical barrier for its public health use.

Another cholera vaccine available only in Vietnam, ORC-Vax, is a bivalent (*V. cholerae* O1 and O139) killed whole cell oral cholera vaccine, and has been in use since 1997. More than 9 million doses of ORC-Vax have been given in Vietnam's public health setting[17]. This vaccine, also given in two doses, was shown to be safe and effective in Vietnam [17, 18] and was targeted for internationalization by IVI through ensuring its WHO prequalification. However, upon evaluation by the IVI, the vaccine's manufacturing process did not comply with current Good Manufacturing Practices (cGMP) and WHO guidelines. Additionally the Vietnamese National Regulatory Authority (NRA) is not recognized by the WHO. For a vaccine to be purchased by the UN agencies such as UNICEF, the vaccine must be prequalified by the WHO. WHO prequalification is only possible if the vaccine is produced by a manufacturer located in a country with a WHO-recognized National Regulatory Authority.

This vaccine was therefore reformulated, its production technology improved to comply with international guidelines, and its technology transferred to a manufacturer in India whose national regulatory authority [Drugs Controller General India (DCGI)] was approved; the vaccine was prequalified by the WHO in 2011.

The technology for vaccine (a killed bivalent O1 and O139 whole-cell oral cholera vaccine Shanchol™) manufacturing has been transferred to Shantha Biotechnics in India (now owned by Sanofi) by IVI. A large double-blind placebo controlled phase III trial by NICE and IVI has evaluated the efficacy of the vaccine produced by Shantha in preventing diarrhea from cholera in 70,000 people in Kolkata. An analysis of the phase III trial after three years concluded that the vaccine was 66% efficacious [19]. The vaccine was licensed in India in February 2009 and is now available for general use in the country. Advantages of the Shanchol™ vaccine include that its cost is lower (\$1.85 in the public health market), and does not require administration with buffer, thus making it more feasible for use in mass vaccination programs in resource poor settings. We have recently conducted a randomized placebo controlled study of the Shanchol™ vaccine in Mirpur in 330 participants in three age groups, including adults followed by toddlers and infants. Participants were randomized to receive either 2 doses of the vaccine or placebo, which were given 14 days apart. We evaluated the occurrence of

diarrhea, vomiting or abdominal cramps of at least moderate grade over the 28 days surveillance period as well as serum vibriocidal antibody responses (*vide infra*).

Summary of findings from previous clinical studies

Phase II clinical trials of the whole cell bivalent vaccine Shanchol™ in Vietnam [20] and India [21] and in Bangladesh [22] have shown that this vaccine is safe and immunogenic in both adults and children. Following these successful clinical trials, a phase III cluster-randomized, double-blind, placebo-controlled trial was initiated in July 2006 in Kolkata. Results from this trial showed that the vaccine is safe, it confers 66% protection among all participants aged 1 year and older in Kolkata, three years after receipt of the two-dose regimen [19]. A large feasibility study has also been carried out in Bangladesh, which has shown that the Shanchol™ vaccine is safe. Based on the results from studies in India, this modified vaccine was licensed in February 2009 to Shantha Biotechnics in India under the trade name Shanchol™. In a phase II study conducted in Bangladesh to evaluate the safety and immunogenicity of Shanchol™, the vaccine was shown to be safe with good immune responses in participants who were studied. Serum vibriocidal antibody responses seven days following the second dose of vaccine in adults were 60%, 72%, and 21% against *V. cholerae* O1 Inaba, *V. cholerae* O1 Ogawa and *V. cholerae* O139 respectively. Similarly, responses against Inaba, Ogawa, and O139 serotypes were 84%, 75%, and 64% and 74%, 78%, and 54% in toddlers and infants respectively. These responses were found to be similar seven days following the first dose of vaccine, possibly suggesting protection starting as early as 7 days post vaccination or even earlier [22]. Recently, a large feasibility study was started to determine the impact of two doses of the Shanchol™ vaccine in a high risk population in the Mirpur area of Dhaka, Bangladesh (PR#10061; Clinical trial.gov ID: NCT01339845).

Justification for Single dose

The current multi-dose schedules for both Dukoral and Shanchol™ have restricted the application of the oral cholera vaccine in situations where they are most needed. Based on the experiences of recent complex emergencies, and in demonstration projects, it has been suggested that the current two-dose regimen of the internationally available cholera vaccines may create some logistical and programmatic challenges [7]. Logistical challenges in post-disaster situations and in non-endemic settings can vary vastly, and an option to use a single dose vaccine in areas where major population disruption has occurred as opposed to the use of two doses at an interval of 2 weeks, can make the use of a vaccine more attractive. Getting a vaccine to the same people twice poses difficulties in the control of cholera in both endemic and epidemic settings. Since there is no serologic correlate of protection for cholera, serum vibriocidal response to *V. cholerae* O1 are used in clinical trials as markers for appropriate immune

stimulation [8, 23]. Excellent immune responses after two doses of the vaccine were seen in the earlier Phase II studies [20, 21]. In a study performed among 80 adults and 80 children in a cholera-endemic area in Kolkata, India it was found that Shanchol™ induces significant vibriocidal responses even after a single dose [24]. Both the GMF-rise of vibriocidal titers to *V. cholerae*O1 and the number who seroconverted were higher after the first dose compared to after the second dose. Furthermore, 7 participants (6 individuals aged 15 years and older and one 4 year old) fulfilled the definition of seroconversion after one dose, but not after two doses. Only 2 participants, both aged 3 years with low baseline titers < 80, had higher post-dose 2 titers [24]. These findings differ from the previous studies using the older generation killed OCV, where higher titers were obtained after the second dose [25-27]. One of the possible explanations for this observation was that the LPS content of Shanchol™ is substantially higher than that of Dukoral.

Since there is a limited supply of Shanchol™, it is important to know how to accomplish the greatest public health benefit with this limited supply. Even if a single dose may be somewhat less effective than two doses, the single dose strategy will have the potential to avert more cases than the two dose strategy, since a larger number of susceptible people will be vaccinated, and protection will begin sooner after the initiation of dosing. This study is designed to determine the protective efficacy and duration of protection offered by a single dose regimen and is not intended to replace the recommended 2 dose regimen in endemic areas. This will be useful for epidemics and outbreaks

Trial design justification

Since the serum vibriocidal antibody response is not an appropriate serologic correlate of protection for killed cholera vaccine, an efficacy trial with a placebo control is required to confirm the usefulness of a single dose [8]. If the vaccine is found to be efficacious following a single dose, this will have profound implications on the use of the vaccine in areas with limited resources, particularly in complex emergencies where a multiple dose regimen is difficult to deploy. A single-dose regimen of this vaccine will improve its “field ability” and allow the vaccine to be used even in outbreak control, especially in difficult settings where the risk of cholera is extremely high and provisions for clean water and sanitation are not available [9]. The proposal we plan has a double blind individually randomized, placebo controlled design. This design was selected as the most efficient approach to provide efficacy data for single dose of Shanchol™ with a minimum number of participants needed for a statistically meaningful result. Since an active control design would likely result in herd protection and subsequently would prevent the trial from answering the relevant question, a placebo controlled design is essential to establish that scientific necessity is met.

Summary of known and potential risks:

There is no comparative data on the protection conferred by one versus two dose Shanchol™. However, as of now, plans for use of the vaccine by the Government of Bangladesh will be based on the results of the feasibility studies being carried out; and it is hoped that the country will be able to use Shanchol™ in public health programs in the next five years. Since Shanchol™ is not licensed in Bangladesh; participants will be unable to access the vaccine unless they are part of the study cohort. The potential risk to the participants will be minimal, since there is extensive documentation of the safety of the cholera vaccine to be used and all clinical and immunization procedures (oral vaccine administration/venous blood collection/stool/rectal swab collection) will be performed by adequately trained and experienced personnel under regular supervision. There is a very small risk of anal/rectal area skin abrasion while taking a swab from the rectal area in patients presenting with diarrhea. Additionally, there is also a small risk associated with phlebotomy for participants who are requested to give a blood sample. This may include pain, redness and very rarely, local infection at the phlebotomy area.

Risk minimization and benefits

All personnel involved in taking biological samples are trained personnel, who will be provided with additional training to avoid or minimize the possibility of side effects during these procedures. Sterilization techniques and disposable sterile needles and syringes will be utilized to obtain blood. All study records and data will be kept confidentially under lock and key and/or electronic password protection, as appropriate, for 5 years. Only the senior study personnel will have access to these records. The direct benefit the participants may expect from participating in this study will be a free laboratory examination and treatment for diarrheal diseases.

The main benefit of obtaining data on the efficacy of the single dose schedule of cholera vaccine will be that, if proven protective, the single dose schedule will greatly simplify vaccine delivery, result in substantial cost reduction, and make the current limited supply of Shanchol™ available for a larger population of people at risk for cholera in outbreak setting. The single dose vaccine will be particularly useful in cholera epidemics and complex emergencies. All recipients of the vaccine will potentially benefit from the probable protective effects against cholera. The risks associated with the use of the vaccine or the placebo and various other study procedures proposed to be used in this trial are expected to be minimal to non-existent.

After completion of the study, all participants will be offered the standard 2 dose regimen of the oral cholera vaccine. The trial will be conducted in compliance with the study protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

Trial Design

Description on type of study

The study design is a two-arm individually randomized double-blind placebo-controlled trial (Single Dose Cholera Vaccine Study, Bangladesh-SCVB).

Study population

Mirpur is a part of the Dhaka metropolitan area with an estimated population of over 2.5 million people. Different socio-economic groups of communities, such as low, middle income, and high income, live in the area. The icddr, b hospitals treat more patients from Mirpur than from any other part of Dhaka. Mirpur is divided into 16 wards of the Dhaka City Corporation. We have selected 9 wards in Mirpur (7-13, 15 and 41) for the study. The selection has been based on the high number of cholera patients from these wards visiting the icddr, b hospitals over the last few years (2008-2010). It was observed that most of the cholera patients were living in overcrowded households with low income, poor sanitation, unsafe and shared water usage, and general poor living conditions. In preparation for this "Single Dose Cholera Vaccine Trial (SCVB)" trial we will commission a census of these high cholera incidence wards in Mirpur. These wards are separated from the other wards where the feasibility study of the oral cholera vaccine "Introduction of Cholera Vaccine in Bangladesh (ICVB)" is being carried out (Figure 1) by a 300 meter buffer zone. This buffer zone will help minimize contamination between two study areas. First, the census team will create geographic information system (GIS) database by digitizing buildings and other structures in the target wards using satellite derived images. The digitized buildings and structures will be verified for ground verification. The census team will visit each building and ascertain whether or not people are living in the building. If people reside in the building, the census team will assess whether the residential structures are overcrowded, have poor sanitation and drainage, unhealthy living conditions, and/or share water among several families in order to assess high risk groups [28].

Based on this survey, the team will assess whether the people living in each building/structure are a high risk group or not. If the residence meets these criteria, as described above, the census team will collect verbal consent from the respondent and other information about the household (Appendix 1Ai, 1Aii, 1Aiii, 1Aiv). The supervisors will also subsequently check whether this assessment fulfills the requirement for defining them as a high risk population. We will conduct a *de jure* census and will enumerate 324,178 high risk residents from the target wards.

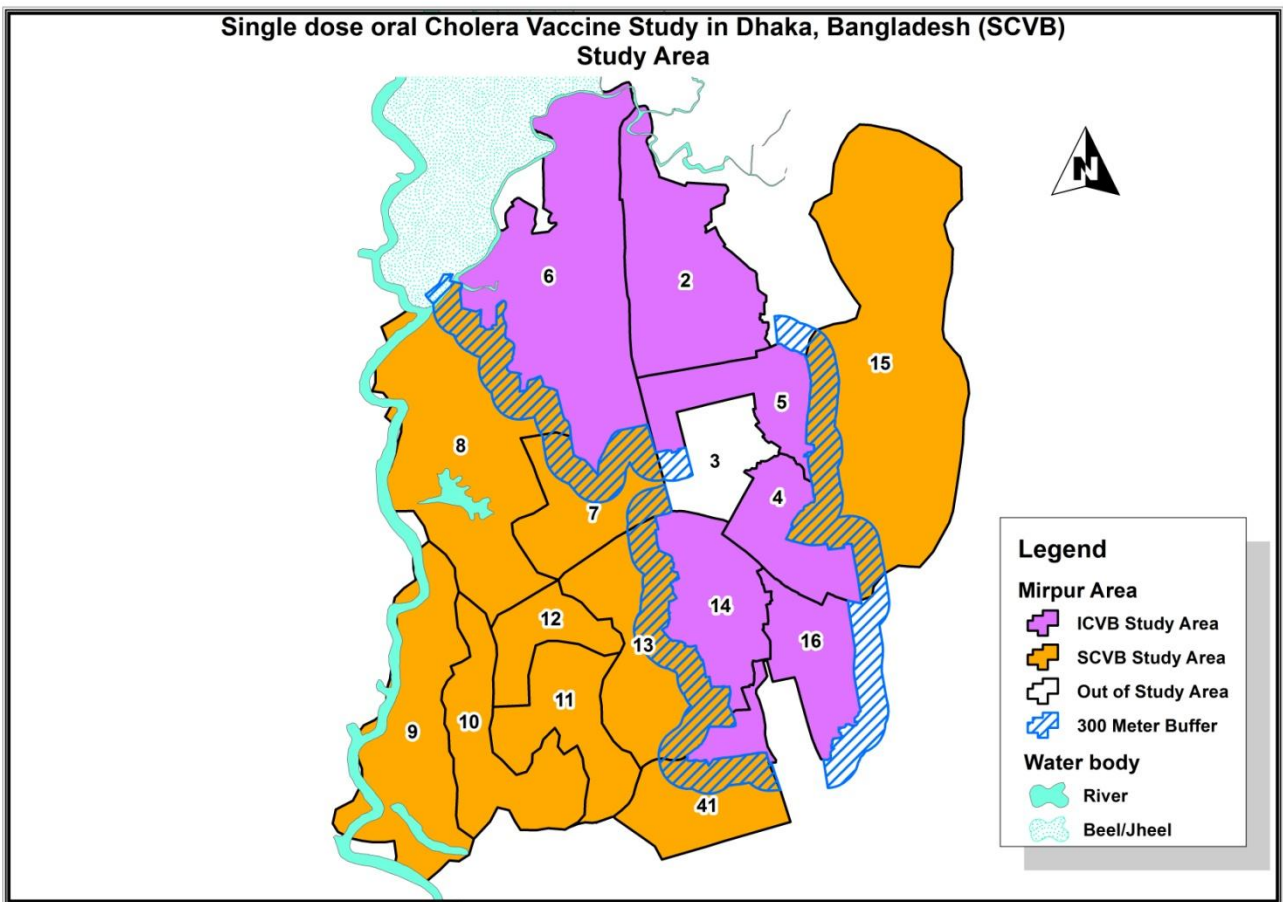


Figure 1. Study Area

Study Procedure and methodology

Census of study population

A paperless data collection system (direct data entry) will be used in the census survey and subsequent census updates will also be performed biannually using handheld devices, a Samsung tab ("TAB"), which will offer the advantage of being a portable method to digitize information. The demographic surveillance will be conducted by community health workers to update the population through vital demographic events including births, deaths, internal and external migrations.

During the demographic surveillance, the community health workers will encourage individuals to participate in the study and seek care for diarrhea at the icddr, b treatment centres (Mohakhali and Mirpur hospitals). After the baseline census, a unique ID number will be assigned to each study participant. The data will be collected by the field teams using the TAB and at the end of each day data will be transferred to a designated computer at the Mirpur field office using a data cable. The data will be processed for range and consistency checks. Necessary checks will be built in both for the direct data entry and the server database systems to ensure the reliability of the data. The data will then be transferred to the main data base ("SCVB server") based at the icddr, b Mohakhali hospital via the intranet. The SCVB baseline database and the updated census database will also be updated in each TAB so that the study personnel can use and check the information when necessary; also use it for identification of the study participants during the vaccination or follow up period as well as during the passive surveillance. Closeout census will be conducted at the end of the trial.

Delivery of study agent

We will use the killed whole cell oral cholera vaccine, Shanchol™ as well as an oral placebo for the study. The vaccine is manufactured by Shantha Biotechnics, in Hyderabad, India and registered in India and is prequalified by the WHO. Shanchol™ is available in a single dose vial. The study agents will be supplied by the company and imported by approaching the Directorate General of Drug Administration of Bangladesh. Both the vaccine and placebo will be transported from the manufacturer to a designated EPI cold room at Mohakhali, Dhaka arranged for this study where it will be stored. We will identify vaccination centers within the intervention areas. These sites may include the government's EPI outposts, non-government facilities utilized during national immunization days, or other appropriate facilities which are easily accessible by the target population (Appendix 1C). Study agents will be maintained at 2-8°C. Immunization will be carried to the study population in three phases. Vaccination teams composed of the required number of vaccinators, record keepers, consent takers, supervisors, and other support staff will be present at each vaccination site (approximately 30 sites). Each participant over

the age of one year and non pregnant females living in communities will be individually randomized to receive vaccine or placebo.

Pregnancy status will be enquired verbally for all married women of child bearing age during the census update as well as before vaccination to exclude them from the study. If she is uncertain about her pregnancy status, she will be asked for her last menstrual period (LMP). All married females will be asked about their last LMP, if this is more than four weeks prior, irregular, or unknown, will be considered ineligible for the study. Pregnancy status in married women will also be ascertained at least two months after vaccination by home visits of trained field staff. Those who will be confirmed for pregnancy at this time point will be followed up at 6 months as well as with further visits monthly to ascertain pregnancy outcome after delivery. A pregnancy followup questionnaire will be used after consent taking for these visits (Appendix 6A, 6 B).

Prior to vaccination, informed consent will be taken from the adults and guardians of minor participants (1-10 years) (Appendix 1Bi and 1B ii). Assent will be taken from older children (11-17 years). Training will be provided to: (i) Vaccinators, managers and supervisors of this project, MOHFW, DCC and NGOs and (ii) Volunteers recruited for the study.

Interventions

Each dose of vaccine or placebo will be 1.5 ml in volume. The study agents will be dispensed in liquid form in identical vials.

Name and description of products:

a. *Bivalent oral killed cholera vaccine*: each dose of this vaccine contains

<i>V. cholerae</i> O1 Inaba El Tor strain Phil 6973 formalin killed	600 ELISA units (EU) of Lipopolysaccharide (LPS)
<i>V. cholerae</i> O1 Ogawa classical strain Cairo 50 heat killed	300 EU of LPS
<i>V. cholerae</i> O1 Ogawa classical strain Cairo 50 formalin killed	300 EU of LPS
<i>V. cholerae</i> O1 Inaba classical strain Cairo 48 heat killed	300 EU of LPS
<i>V. cholerae</i> O139 strain 4260B formalin killed	600 EU of LPS
Preservative.	Thiomersal .02% (w/v)

b. *Non Biological* placebo: The composition of the placebo is as follows:

Ingredients	Per 1.5 ml dose
Starch	60mg
Ponceau 4R dye	0.019 mg
Brilliant blue	0.003mg
Tetrazine dye	0.02 mg
Xanthum gum	3 mg
Preservative	.002%(w/v)ofThiomersal (equivalent to .03 mg per 1.5 ml)
Water	Up to 1.5 ml

All the above ingredients are pharmaceutical grade.

For this evaluation of clinical safety and immunogenicity, a “non-biological” placebo, which is in nature and appearance similar to the vaccine, will be used. The placebo lacks any virulence characteristics and will be prepared under Good Manufacturing Practice conditions at Bilcare (Pune, India). The vaccine and placebo will be dispensed in liquid form in identical vials.

Administration of vaccine or placebo

The agent to be received by the participants will be determined by the randomization number on the vial. Vials will be consecutively numbered, but individually randomized into the vaccine or placebo arm. After shaking the vial properly, it will be opened and given to the participant. The contents will be poured into the mouth by the recipient, followed by intake of a small volume of water. We shall allow the participant to take the study agent by themselves for those who are 5 years and older. For younger children, the vaccine will be given by the vaccinator. At the time of the dosing, completeness of dosing will be observed and this information will be documented. Vials used for the administration of the vaccine and placebo will be disposed by incineration. The records of the vaccine and placebo usage and destruction will be maintained in the study file and also shared with the manufacturer for reconciliation.

Selection and Withdrawal of Participants

Inclusion criteria:

All healthy, consenting, non-pregnant (as ascertained by history) residents of a high risk group, at least 1 year of age, of the study area will be included in the trial.

Exclusion criteria:

The following will be excluded from the trial:

- Pregnant women (identified through verbal screening);
- Aged less than 1 year
- History of intake of any cholera vaccine

Participants may withdraw from the study at any point. The data collected for withdrawn participants, in addition to standard questionnaire data, will include the reason for withdrawal. No additional follow-up is envisioned for withdrawn participants.

Accountability procedures for the investigational product, including the comparator:

The study agents will be stored in a secure place in the EPI cold room in Mohakhali. Comprehensive training of all study staff, and a detailed questionnaire will ensure and document that the study protocol requirements are being followed. The vaccine and placebo will be stored according to cold chain requirements and detailed inventory logs will be maintained. The investigator or the person in-charge of the product management will maintain records of the product delivery to the trial site, the inventory at the site, the dose given to each participant, and the number of unused doses. All used and unused vials of the vaccine and placebo will be accounted for and destroyed locally at the end of the trial. Vaccine (from Shantha, Hyderabad India) and non biologic placebo (from Bilcare Pune, India) will be filled and labelled at Bilcare (Pune India). Final batch of study individually randomized vials will be shipped to icddr, b in Dhaka. All participating institutions in this process have been visited by IVI investigators to ensure strict procedure and protocol to ensure safety, sterility and blinding is maintained.

Maintenance of randomization number codes and procedures for breaking the codes:

In order to safeguard against bias, we will employ a randomization scheme. Blinding will be achieved in this trial by masking the identity of the agents by using randomization number codes for the vaccines and the placebos. After consenting and screening, eligible participants will receive investigational product in a sequential manner. The identity of the codes will be known to the statistician of IVI, who will otherwise not be involved in the study, the staff at Shantha, who will provide the study vaccine, and the staff at Bilcare, who will provide nonbiologic placebo and label all study agent vials before shipment to icddr, b. Study participants will be randomized to intervention or placebo group based on the randomization number of the study agent they will receive at the study site. Each vial will, in addition, contain a detachable sticker with the randomization number and study code on it. This will be used to label the vaccine register alongside the participant study ID during the vaccination in order to minimize transcription error in the vaccine register. The identity of the randomization numbers (in sealed envelopes) will be provided to the Data and Safety Monitoring Board (DSMB) and the on-site principal investigator. The safety monitor(s) of the study will have access to the list when needed.

Data and Safety Monitoring Board (DSMB)

The DSMB will be formed by the ERC of the icddr,b and will constitute members of the ERC as well as relevant experts in the field from Bangladesh and externally. The DSMB will hold meetings with the investigators of the study in the beginning and at regular intervals for study updates. Adverse events will be reported, to the DSMB. Members will visit the study site as needed. They will evaluate trial conduct and compliance with the protocol, the standard operating procedures, Good Clinical Practice, and the applicable regulatory requirements. The results of the study will be shared with DSMB for review. The DSMB will periodically review and evaluate the accumulated study data for safety, study-related adverse events, study conduct, completeness, timeliness, and protocol violation.

The DSMB will also be responsible for un-blinding the randomization number codes in the event of severe putative vaccine reactions. Otherwise, the codes will not be revealed until the end of the trial and until the computerized dataset has been frozen. If the intervention assignment is un-blinded, all study collaborators will be notified immediately.

Measurements

Census update

The census in the study population will be updated every six months after intervention with the study agents. During this process in-migrants within the study area will get a SCVB card with unique ID to ease the disease surveillance process. Data collectors will visit each house in the intervention areas and collect information on births, deaths, migrations (in/out), missed in previous census, change in marital status, pregnancies and other events (Appendix 1D).

Disease Surveillance

1. Surveillance at the icddr,b hospitals in Mohakhali and Mirpur

All patients from the study area presenting for care at the hospital with diarrhea will be included in routine hospital surveillance. A diarrhoeal visit is defined as a visit by a patient who has in the 24 h before presentation, three or more loose or liquid stool or at least 1 bloody loose stool or any number of loose stools with signs of dehydration present, as stated in criteria used at the icddr,b hospitals[29]. The assessment of dehydration status in diarrhea will be carried out by trained personnel.

The diarrheal disease surveillance for the project will be conducted at icddr,b hospitals at Mohakhali and Mirpur and other health facilities for patients coming from the Mirpur study area (wards 7-13, 15 and 41). Clinical staff at each of the two hospitals and other facilities will evaluate each patient at the hospital triage area and provide treatment as per the routine procedure. Internal clinical monitoring for quality control will also be carried out for hospitalized study patients using a disease surveillance questionnaire (Appendix 2B). Around 5% of the study patients will be further evaluated immediately after admission. A trained physician will function as the safety monitor for the study and will oversee

clinical procedures for patient enrolment and management during surveillance to monitor GCP practice and trial conduct.

The data from the Demographic Surveillance System (DSS) of the study will be replicated to the online integrated icddr,b data management system SHEBA from the SCVB study server. Replication will be done in 6 month cycles after completion of each round of the DSS update. The SHEBA system records patient history, treatment, management and related auxiliary data at the icddr,b hospitals at Mohakhali and Mirpur. This system will be used to identify patients from the study population. If a study participant brings the SCVB study card, it will be scanned using a bar code scanner at the hospital front desk. The hospital front desk staff will also verify and confirm his/her identity by address of residence, ward, name (similar), age (± 1 y for <5 y, ± 2 for 5-10 y, ± 3 for 11-20 y, ± 5 for 21-30 y, ± 10 for 31-50 y, and ± 20 for 50 y+), sex, mother/father's name, home district and mobile number. The information will be checked on the online census database available at the icddr,b hospital sites through SHEBA. In case of unavailability of the study card, there will be an option in the online hospital data management system (SHEBA) to search the ID of the patient. The search will be carried out by use of basic parameters mentioned above. In the ongoing study (PR10061) we have been able to identify 99% of participants. Informed consent and/or assent will be obtained (Appendix 2Ai, 2Aii) and a study surveillance questionnaire will be used to obtain information related to the study (Appendix 2B). 24 hours after discharge of the patient, the data on the hospital stay along with all other necessary clinical records including IV fluid consumption, ORS intake, and drugs used will be recorded (Appendix 2B). The initial differential and final diagnosis on discharge and any intra-hospital or external referrals will be populated separately through a scheduler in the SHEBA server. A stool or rectal swab specimen will also be collected as soon as possible from the study participants and sent to the laboratory to culture for *V. cholerae* O1/O139. Necessary laboratory test requisitions will be raised by the SHEBA system and delivered to the study server at the diagnosis unit of the SCVB study. Microbiological results from the laboratory will be sent back to the SHEBA database. A pull engine in the study server will replicate the populated information from SHEBA to the study server.

2. Surveillance at other health facilities in the study area in Mirpur

It is assumed that the vast majority of severe diarrheal patients from Dhaka city seek care at the two icddr,b hospitals. However we will also include Governmental and non-governmental hospitals/clinics in the Mirpur area which is visited by the study population for diarrheal treatment (expected 12-16 facilities). Health staff in these facilities will be oriented/informed/motivated about the cholera vaccine study objectives and activities by the icddr,b clinicians and study investigators. Study staff for each of these health facilities will be directly responsible for dealing with the patients coming from the study

area. These facilities will be under SCVB surveillance. All those involved in the surveillance will be trained in completing the questionnaires on the digital device (TAB). Census data will be available on the TAB for identification of the participant. Specimens will also be collected for microbiological analyses. One surveillance staff will be present at each health facility throughout the day to facilitate proper reporting of diarrheal cases from the particular area. Study patients will be identified by use of Study ID cards (SCVB card) as described above for the surveillance at the icddr,b hospitals (Appendix 2A,2B). Data will be checked and verified and entered into the computerized database of the study. Stool specimens/rectal swabs will be transported in Cary-Blair media to the laboratory at icddr,b within 12 h of specimen collection and the clinical and laboratory data will be entered into the SCVB database (Appendix 11).

Laboratory Assessment

We will collect the stool or rectal swab specimens from diarrheal patients coming from the Mirpur study area to the icddr,b hospital in Mohakhali and Mirpur and also from other selected health facilities frequented by the study population for diarrheal diseases. Specimens will be evaluated for *V. cholerae* O1 and O139 [30-32]. For isolation of *V. cholerae*, specimens will be cultured on taurocholate-tellurite gelatine agar (TTGA). Specific monoclonal antibodies will be used to detect *V. cholerae* O1, Ogawa and Inaba serotypes, as well as the O139 serogroup [32, 33]. For microbiological evaluation, specimens will be also enriched in bile peptone water overnight and then cultured as above [32, 34]. The microbiological data will be collated in the database to determine efficacy of the interventions being carried out in the study.

Follow-up visits

The residence of all participants who were culture confirmed for cholera will be visited within 14 days by a health worker to confirm the identity of the patient. An identity confirmation form will be used where the patient's demographic address and date of presentation will be recorded from the surveillance database. The interviewer will ask whether the person sought treatment for diarrhea at the clinic on the date mentioned in the form. This follow-up visit will also be carried out to assess clinical progress and cholera-related disability (Appendix 5).

Immunological assays using blood specimens from study participants:

Blood: Immunological analyses will be conducted in a small subgroup of patients as has carried out earlier in the feasibility study of oral cholera vaccine (icddr,b protocol # 10061). For this purpose, a

sample size of 324 participants is needed for an adequately powered sample. To account for unblocked unique number coding, we plan on enrolling 500 participants (less than 5 years, 5 to less than 15 years and 15 years of age or older; so as to obtain at least 108 in each age group) from the vaccination sites. Necessary consent and blood draw forms will be filled up (Appendix 3A-C). Venous blood (2-5ml) will be collected from these participants prior to immunization and 14 days after intake of the study agent (day 0 and day 14). Participants will be given a 2 day window for their 14 day blood draw (+/-2 days).

The blood samples will be transported to the laboratory (within 6 hours of collection). At the laboratory, plasma will be separated and stored at -20 degree C until tested. Technicians unaware of the codes of the agents received by the trial participants will test in random order paired plasma samples for vibriocidal antibody titres and different isotype of LPS specific antibodies (IgA, IgG and IgM) by ELISA.

Plasma vibriocidal antibodies to *V. cholerae* O1 (El Tor Inaba; strain T19479; El Tor Ogawa; strain X25049) will be evaluated by a microtiter assay. To measure vibriocidal antibodies to *V. cholerae* O139, the partly encapsulated vaccine strain CIRS 134 will be used [35]. The vibriocidal titre will be defined as the highest dilution causing 50% inhibition of bacterial growth. Two-fold serial dilutions of pre- and post-immunization specimens will be tested side-by-side in duplicates on each plate. Titres will be adjusted in relation to a reference serum specimen included in each test to compensate for variations between analyses on different occasions. The vibriocidal antibody titre ascribed to each sample will be the mean of the duplicated determinations, which will not be allowed to vary more than one two-fold dilution for either the reference or the test sera. The tests will be repeated if larger variations are observed. A four-fold or greater increase in titre between pre- and post-immunization specimens will be taken to represent seroconversion.

To control for variations, test plates will contain pooled convalescent serum sample from patients with cholera as a positive control (pooled O1 Ogawa, O1 Inaba and O139 sera from our collection of specimens from cholera patients) [32, 35, 36]. When progressing from one phase of the study to another, laboratory pooled control specimens will also be used [25]. This will validate the specificity of the assay further. An increase of vibriocidal antibody titer by 4-fold or higher between acute and convalescent sera will be considered a significant antibody response.

Active follow up for safety:

We will select 6,000 participants purposively from the study participants after verbal consent is obtained. These 6,000 participants will be actively followed once every two weeks for safety outcomes for a 28 day period after study agent administration. A questionnaire will be used for documenting and reporting solicited safety outcomes in this subset of the study population (Appendix 4A). Number of

adverse events and serious adverse events in this subgroup who received 1 dose of vaccine or placebo and present for care of vaccine adverse effects at treatment settings or who die from the time of dosing until 28 days after dosing to be captured every 2 weeks active surveillance via solicited questionnaires. For this purpose, trained medical personnel will visit homes of participants every two weeks to collect medical history and assess the safety of the study agent.

Verbal Autopsy:

We will carry out 'Verbal Autopsy' in our study area where deaths will be identified through a household search by home visits which will be conducted by study staff. Verbal autopsy will be conducted on death cases identified by this procedure. The aim of the 'Verbal Autopsy' is to identify the number of deaths and the probable causes of death in this time period after receiving the investigational product. Consent will be obtained from a relative of the dead participant (Appendix 13A). We will use adopted WHO verbal autopsy questionnaire[44] to conduct verbal autopsy, (Appendix 13B). After identification, trained medical professional will visit homes of the deceased to perform verbal autopsy.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

Sample size calculation for primary analysis

In this placebo-controlled individually randomized trial, the primary analysis is to evaluate efficacy of the vaccine after six months following vaccination. Even this trial design is to test the conventional null hypothesis of no difference, a criterion that a confidence limit for relative risk is assumed less than a specified value for monitoring the outcome at six month. Therefore, we wish to show that the ratio of risk in population 1 (treatment group) to risk in population 2 (placebo group) is less than specific relative risk rather than 1. The usual null value of relative risk (R_0) is, therefore, required to be excluded by the pre-specified value, say 0.10; thus, the R_0 becomes 0.90 (1.00-0.10).

The sample size, N , for this study is, therefore, calculated based on logarithmic transformation method described elsewhere (Blackwelder, 1993) using the following formula

$$N = (z_\alpha + z_\beta)^2 [q_1/kp_1 + q_2/(1-k)p_2] / (\log R_0 - \log R)^2$$

With the following assumptions:

- Incidence among placebo group, p_2 , = 0.56 cases/1000/six month
- Vaccine protective efficacy (1- R) = 50%
- Significance level (α) = 0.05 (one-tailed)
- Statistical power (1- β) = 0.80
- Proportion of the total sample from population 1 (k) = $n_1/N=0.50$, where $N=n_1+n_2$, n_1 and n_2 are the population sizes for vaccine and placebo arms, respectively.

The method with the above assumptions yielded 191,660 individuals required in the two arms of the study. Assuming 65% participation rate, attrition 25% annually (6.25% for the 6 month period) and excluding those <1

year (2%) and pregnant women (1%), a total of 324,178 population are required to be registered during population enumeration. Considering the attrition rate, at least 204,438 individuals are required to be vaccinated for this study.

Sample size required for immunogenicity endpoint

The sample size has been calculated with the assumption that it is important to evaluate whether the vaccine induces acceptable vibriocidal responses in relation to the placebo group. Based on an immunogenicity study of the whole-cell killed oral cholera vaccine in Kolkata (1 dose vs. 2 doses of Shanchol™) [19], we use the following assumptions:

For vibriocidal responses, defined as >4-fold increases between baseline and post-dosing in either Inaba or Ogawa antibodies, for each age group (less than 5 years, 5 to less than 15 years and 15 years or older), we assume 1) the background rate of responses in the placebo group will be 3% after the first dose and 2) the true rate of vibriocidal responses in the vaccine groups is 50%. At $p < 0.05$ (1-tailed), 0.8 power, to exclude a difference of seroconversion among vaccine and placebo recipients of 25% and a 20% drop-out, a total of 54 participants per group would be needed. We will require approximately 54 participants per arm per age group, for a total of 324 participants.

Procedures for reporting any deviation from the original statistical plan:

Additional analysis may be required, and will be conducted if agreed among by the investigators and will be initiated by the principal investigator.

Direct Access to Source Data/Documents

The investigator/institutions will permit (by way of written agreement) trial-related monitoring, audits, IRB/IEC review, and regulatory inspection, providing direct access to source data/documents.

Quality Control and Quality Assurance Procedures

Study Monitoring and Source Data Verification

After appropriate ethical approval by an Institutional Review Board (IRB) is available (and the final protocol has been amended as required by IRB), a pre-site initiation visit will be conducted by a designated study monitor. During this visit, the requirements of GCP, protocol procedures, and logistical issues will be discussed. The training of study staff will be carried out and documented.

Later a site initiation visit will be conducted before the first participant is enrolled in the study. The participants cannot be enrolled until occurrence of such a visit and its documentation.

After the study is initiated, the study monitor will be in regular contact with the site to obtain information on the performance of the study. These contacts will be scheduled to take place at regular

intervals. Subsequent to start of recruitment, routine monitoring visits, as per a monitoring plan, would occur after prior appointment with the investigators.

The investigator and his/her staff are obliged to devote a suitable amount of time and an appropriate place for the monitoring visits. During each visit, the monitor will review the Case Report Form (CRF) of each participant in the study with regard to completeness, thoroughness and compliance with the protocol. In addition, at a minimum, the original participant data (e.g., SCVB cards, Master list, informed consent, CRF's, different logs) will be reviewed to ensure that:

- informed consent of participant is incorporated;
- inclusion/exclusion criteria are properly followed;

the CRF data are consistent with the physician's original records, which also have to clearly indicate that the participant is included in a clinical study;

- all relevant clinical and laboratory findings and concomitant medication are documented in the CRFs;
- quantity and dosing schedule of concomitant medication is documented in the CRFs;
- quantity and dosing schedule of the Investigational/Comparator Product is in accordance with the protocol;
- all relevant information (e.g., any adverse event) has been recorded in the appropriate place in the CRFs;
- the Investigational/Comparator Product is being stored correctly, and its supply is being properly accounted for;
- Incorrect or illegible entries in the CRFs would be submitted to the investigator for correction.

The monitor will retrieve completed CRFs during the regularly held monitoring visits.

Auditing

In addition to the above outlined monitoring visits, the study site may be audited. This audit may be carried out by representatives of Shantha Biotech or an external independent auditor or by the responsible regulatory authority (ies). Such an audit would be done to review whether the data has been properly recorded in the interim or final report and whether the performance of the study is in accordance with the protocol and other relevant guidelines. Confidentiality of participants will be maintained at all times. The investigator will inform other partners if an audit has been requested by a regulatory authority.

Confidentiality:

Confidentiality of the participant will be maintained at all times.

Management of the Single dose cholera vaccine project

The management structure of the cholera vaccine project is attached (Appendix8). Co-investigators at the icddr,b will collaborate with several other organizations to accomplish the objectives of this project throughout its time period (Appendix7,10). We will conduct advocacy meetings at national and service levels with officials and staff members of MOHFW, DCC, NGOs and local elites for sensitization and to support this project. These collaborations include partnerships with the Director General of Health Services of the Ministry of Health and Family Welfare of the Government of Bangladesh, the Expanded Programme on Immunization of the Government of Bangladesh and the Dhaka City Corporation which is responsible for providing immunization services within Dhaka city. The International Vaccine Institute is a collaborating institution and will provide technical support. The study agent will be provided by the sponsoring institutions.

The existing Planning and Implementation Committee (PLIC) on Protocol #10061 (ICVB project; Introduction of cholera vaccine in Bangladesh) will be used for the present study. The committee will meet prior to the study as well as at regular intervals. The study will use facilities of the Government of Bangladesh, which includes the Directorate General of Health Services and Dhaka City Corporation. The PLIC is headed by the Director Primary Health Care (PHC), DGHS with members from GoB, EPI, CDC, and DCC of the DGHS as well as the members from the core group who will oversee the progress and monitor the study. The resources of the administrative and professional services of the respective areas at EPI, DCC, DGHS and icddr,b will be used to facilitate the study. The Immunization related logistics including EPI cold storage space used by EPI and DCC will be used for the study. They will provide strategic leadership and coordination to the project. Scientists from the IVI will be collaborating investigators in the study. The crisis communication committee of the ICVB project (Protocol #10061) which is headed by National Professor Dr. M. R. Khan will also oversee the activities of this committee and provide advice and support.

Data Analysis

<p>Plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the</p>

Efficacy assessment and endpoints

Ascertainment of vaccination:

Receipt of the cholera vaccine during the recruitment will be ascertained in the vaccination registry.

Assessment of Efficacy

The primary purpose of the analysis is to evaluate vaccine efficacy during 6months of follow-up after receipt of one complete dose of an assigned agent.

A diarrheal visit is defined as: An inpatient or outpatient visit for care of diarrhea in which the patient described:

- 3 or more loose or liquid stools; or
- At least 1 bloody loose or liquid stool; or
- 1-2 or an indeterminate number of loose or liquid stools and exhibited at least some dehydration

A diarrheal episode is defined as follows:

- All diarrheal visit(s) for which the date of onset for a diarrheal visit was less than or equal to 7 days from the date of discharge for the previous visit, constitute a single “diarrheal episode”.
- The onset of a diarrheal episode was defined as the day on which it was reported to have begun for the first visit of the episode.

A cholera episode is defined as:

- A faecal specimen from at least one component visit which yields *V. cholerae* O1/O139 in the icddr, b laboratory; and
- A diarrheal episode in which no component visit is described as bloody diarrhea; and
- An identity check performed within 10 days after discharge for the visit in which *V. cholerae* O1/O139 is isolated, confirmed that the person whose name was given at the treatment centre had indeed sought care for diarrhea on the date of presentation.

Study Endpoints

The primary endpoint for the study efficacy is the onset date of the first episode of culture-confirmed *V. cholerae* O1 diarrhea episodes detected in the study treatment centres with onsets of 7 days to 6 months after dosing among those who have received 1 dose of the vaccine or placebo.

Secondary endpoints for the efficacy are as follows:

- Date of onset for first episode of culture-confirmed *V. cholerae* O1 diarrhea detected in the study treatment centres from 7 days to 12, 18 and 24 months, respectively after dosing among those who have received 1 dose of vaccine or placebo

- Date of onset for first episode of culture-confirmed *V. cholerae* O1 diarrhea with severe dehydration detected in the study treatment centres from 7days to 6, 12,18 and 24 months, respectively after dosing among those who have received 1 dose of vaccine or placebo
- Date of onset for first episode of culture-confirmed *V. cholerae* O139 diarrhea detected in the study treatment centres from 7days to 6, 12,18and 24 months, respectively after dosing among those who have received 1 dose of vaccine or placebo.
- Date of onset for first episode of culture-confirmed *V. cholerae* O139 diarrhea with severe dehydration detected in one of the study treatment centres from 7days to 6, 12,18 and 24 months, respectively after dosing among those who have received 1 dose of vaccine or placebo.
- Date of onset for first episode of watery diarrhea detected in the study treatment centres from 7days to 6,12, 18 and 24 months, respectively after dosing among those who have received 1 dose of vaccine or placebo
- Date of onset for first episode of watery diarrhea with severe dehydration detected in the study treatment centres from 7days to,6, 12,18and 24 months, respectively after dosing among those who received 1 dose of vaccine or placebo
- Date of onset for first episode of diarrhea with fecal excretion of *V. cholerae*O1 in non-participating subjects, who are detected within one of the study treatment centres from 7 daysup to 6,12,18and 24 monthsafter dosing of study participants.
- Date of onset for first episode of diarrhea with severe dehydration and fecal excretion of *V. cholerae*O1 in non-participating subjects, who are detected within one of the study treatment centres from 7 daysup to 6,12,18and 24 monthsafter dosing of study participants.

Secondary safety endpoints

- Number of adverse events and serious adverse events in participants who received 1 dose of vaccine or placebo and present for care of vaccine adverse effects at treatment settings or who die from the time of dosing until 28 days later (passive surveillance by type of complaint and by cause of death).

Secondary immunogenicity endpoints

- Geometric mean serum vibriocidal (to El Tor Inaba and Ogawa O1 serogroup organisms and to O139 serogroup organisms) titers measured in participants at baseline (day0) and 14 days after receipt of either vaccine or placebo.
- Four-fold or greater rises in titers of serum vibriocidal antibodies (to El Tor Inaba and Ogawa O1 serogroup organisms and to O139 serogroup organisms), relative to baselineday 0 and 14 two days after receipt of either vaccine or placebo

Analysis plan

To analyze vaccine protection, we will use Cox proportional hazard regression models verifying first that the proportionality assumption is satisfied for all independent variables. Checking of the proportional hazard assumption will be performed by plotting the observed standardized score with simulated realizations for each covariate in the model, and the p-value will be derived from the Kolmogorov-type supremum test based on 1,000 simulations. If a variable does not satisfy the proportionality assumption, then we will exclude the variable from the model. However, the critical variables such as vaccination status and age will be kept in the model irrespective of the results of the test for proportionality assumption.

The outcome will be the time to event of the onset of the first episode of cholera. Hazard ratios (HRs) of the target outcome in the vaccine versus placebo groups will be estimated by exponentiating the coefficient for the vaccine variable (independent variable) in these models, and vaccine efficacy is estimated as $[(1 - \text{HR}) \times 100\%]$. Standard errors for the coefficients will be used to estimate P values and 95% confidence intervals for the HRs. Kaplan-Meier cumulative event free survival curves for patients assigned to vaccine and placebo groups will be prepared for descriptive purposes.

Simple analyses of vaccine impact will be performed. Final adjusted estimates will be obtained from models that included the covariates found to be independently associated with the time to the event at $P < 0.1$ in a backward selection algorithm. To evaluate heterogeneity of vaccine protection among different subgroups, we will evaluate interaction terms between the vaccination and subgroup variables in these models. The threshold for significance in the primary analysis will be set at $p < 0.05$ (one tailed) and 95% CIs (lower bound) will be calculated. In all other analyses, the threshold for significance will be set at $p < 0.05$ with two-tailed analysis.

Primary Analysis at 6 months of follow-up:

Primary analysis will be planned at 6 months following dosing. In addition, we will estimate the protective impact of vaccination against culture-proven *V. cholerae* O1 diarrhoea episodes severe enough to require treatment in a health care facility; age-specific protective efficacy; indirect and age-specific indirect vaccine protection using a GIS approach described elsewhere (Ali et al 2005); overall and age-specific overall and total vaccine protection; culture-proven *V. cholerae* associated with severe dehydration (the definition is in Appendix 12); and episodes of acute watery diarrhoea severe enough to require treatment in a health care facility; episodes of acute watery diarrhoea irrespective of severity. Seroconversion in the vaccine and control groups will also be compared. Statistical methods for these secondary analyses will be the same as those used in the primary analyses contingent on an adequate number of cases.

Analysis at 6,12,18 and 24 months of follow-up:

Primary analysis is to be performed at 6 months. The following available time points (12,18 and/or 24 months) will be for secondary analysis points.

Surveillance for 24 months

Surveillance will be continued to complete 2 years of follow-up. Blinding will be maintained until the end of the follow-up period. The analysis at the 2 years of follow-up point will still be performed in a blinded manner.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

A Data and Safety Monitoring Plan (DSMP) will be made for the study. It will provide the overall framework for the research protocol's data and safety monitoring. It is not necessary that the DSMP covers all possible aspects of each element. When designing an appropriate DSMP, the following will be kept in mind:

- a) All investigations require monitoring;
- b) The benefits of the investigation should outweigh the risks;
- c) The monitoring plan should commensurate with risk
- d) Monitoring should be performed accounting for the size and complexity of the investigation.

Direct Access to Source Data/Documents

The investigator/institutions will permit (by way of written agreement) trial-related monitoring, audits, IRB/IEC review, and regulatory inspection; providing direct access to source data/documents.

Assessment of Safety

All participants will be informed to contact the vaccination sites during vaccination period or Mirpur treatment centre if the participant requires medical care within 28 days of study agent dosing.

All adverse events which are observed actively or reported/volunteered by subject/guardian will be recorded with information about severity (i.e., whether mild, moderate or severe) and possible relation to the study vaccine by the clinical monitor as described below. The investigator will report adverse events, all abnormal findings, from laboratory and other specific examinations, which are clinically apparent, or in the investigator's opinion clinically significant (Appendices 3A,3C).

In case of any serious adverse events, the investigator should notify the local site IRB, DSMB, IVI and Shantha Biotech immediately by telephone/facsimile/email within 24 hrs and take appropriate measures to safeguard the participants. Every SAE must be reported, even if the Investigator considers that it is not related to the vaccine. The Completed serious adverse event(SAE) form should be submitted to local site IRB/IEC as well as to Shantha's Pharmacovigilance Department within 72 hours either by fax, to the following number: +91 40 2323 4133 (to the attention of Pharmacovigilance Department of Shantha) or by email to: sblpvd@shanthabiotech.co.in. The causality relationship of the SAE shall be established as mentioned above in the adverse event document section.

A follow-up SAE form must be completed and submitted to Shantha within 24 hours after the Investigator has become aware of any new relevant information concerning the SAE (e.g. outcome, precise description of medical history, results of the investigation).

Copies of documents (e.g., medical records, discharge summary, autopsy) may be requested by the Shantha's PV Department. The anonymity of the subject must always be respected when forwarding this information.

Participants with adverse events shall be followed up till the satisfactory resolution or stabilization with reasonable background of clinical & scientific judgment. Serious adverse events shall be followed up until their resolution.

Irrespective of the investigator's statutory obligations, the sponsor will report all pharmacovigilance data to the competent authorities and to all investigators involved, in accordance with requirements of the ICH Guidelines for Good Clinical Practice as well as local regulatory requirements.

Safety Assessment, Monitoring and Reporting

Neither the bivalent, killed, oral cholera vaccine nor the placebo is known to cause any significant adverse reactions. However, as a precaution, procedures will be in place to detect adverse events in the trial participants as follows: At the vaccination sessions after each dose, recipients will be requested to wait for half an hour at the site, where one staff member will be stationed to monitor any immediate adverse event following vaccination. Individuals with immediate adverse events will receive emergency treatment and the event will be recorded on the form. The adverse event or serious adverse event form will be reported using Medications, Adverse Events, and Serious Adverse Event forms (Appendix 4A, 4B, 4C) and will be reported within 28 days after vaccination whether they are considered vaccine-related or not.

Definition of Adverse event:

An adverse event will be defined as an untoward medical event (diarrhea, vomiting, abdominal pain/cramps or any other local and systemic symptoms) after receipt of a dose which may or may not be

associated with the vaccine. Adverse events will be monitored passively in all participants up to 28 days after vaccination.

Participants will be asked to consult the 'AEFI Case Management Cell' at the icddr,b hospital in Mirpur about any untoward effect after vaccination which will be used as the control room for AEFI management. Assigned physicians, as well as study nurses will be available for 24 hours during the AEFI surveillance period. In addition, support from the existing icddr,b hospital treatment facility will be obtained for AEFI case management. An AEFI committee will be formed for advice and to oversee all reports (Appendix 4A,4B,4C). The 6 member team will include physicians from within the icddr,b hospital and well as those from outside. Necessary medication and emergency services will be available for management of AEFI in the control room. Staff at all other health facilities in the study will report adverse events to the control room at the Mirpur icddr,b hospital. The control room will coordinate and supervise AEFI reporting and management from all the health facilities including the icddr,b Mohakhali hospital. Referral to other hospitals will be made when necessary (for non-diarrheal, acute severe illness requiring specialized care). Emergency ambulance services will also be available for transfer to referral hospitals. Provisions for a preferential case management facility will be provided at the different health centres and compensation will be given when deemed necessary. The compensation will include transportation costs, medication costs, and wage loss.

Active follow up for 28 days in a pre-specified subset of 6,000 study participants will also be carried out. Active surveillance for solicited AEs and all SAEs will be conducted once every two weeks during this month via contact with participants from this cohort. All documentation will be recorded on the previously described AE and SAE forms.

Assessment of Causality

Assessment of an adverse event's relationship to the study drug will be done by a team of medical doctors overseen by the safety monitor of the study and this will be part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is a treatment-emergent adverse event, the event should be reported. The relationship of the administration of the study agent to the serious adverse event will be assessed as follows:

- Very Likely/Certain:** A clinical event with a plausible time relationship to vaccine administration and which cannot be explained by concurrent disease or other drugs or chemicals.
- Probable:** A clinical event with a reasonable time relationship to vaccine administration; is unlikely to be attributed to concurrent disease or other drugs or chemicals.

- Possible:** A clinical event with a reasonable time relationship to vaccine administration, but which could also be explained by concurrent disease or other drugs or chemicals.
- Unlikely:** A clinical event whose time relationship to vaccine administration makes a causal connection improbable, but which could be plausibly explained by underlying disease or other drugs or chemicals
- Unrelated:** A clinical event with an incompatible time relationship and which could be explained by underlying disease or other drugs or chemicals.
- Unclassifiable:** A clinical event with insufficient information to permit assessment and identification of the cause.

Assessment of severity of adverse events

The intensity of the adverse event will be rated by the following adapted guidelines [40], applicable to the local context.

Illness or clinical adverse event (as defined according to applicable regulations)	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)
Fever (°F)	100.4 -101.2	101.3 – 102.1	≥102.2
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires IV hydration
Diarrhea	2 – 3 loose stools or < 400 gms/ 24 hours	4 – 9 stools within 24 hour	10 or more watery stools within 24 hours or requires IV hydration
Headache	No interference with activity	Some interference with activity or repeated use of non-narcotic pain reliever	Significant, prevents daily activity or repeated use of narcotic pain reliever
Fatigue	No interference with activity	Some interference with activity	Significant, prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant, prevents daily activity

Changes in the severity of an adverse event will be documented to allow an assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent will require documentation of onset and duration of each episode.

Definition of Serious Adverse Event

A serious adverse event (experience) is any untoward medical occurrence that results in death or is life-threatening. The term “life-threatening” in the definition of “serious” refers to an event in which the

recipients will be at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death, if that were more severe.

A serious adverse event (SAE) is defined as an AE meeting one of the following conditions:

- Death during the period of protocol-defined surveillance
- Life-threatening event (defined as a study participant at immediate risk of death at the time of the event)
- An event requiring hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance. In case of diarrhea, an SAE will be one that requires admission to an inpatient ward for >24 hours.
- Results in congenital anomaly or birth defect, or malignancy
- Results in a persistent or significant disability/incapacity

Any other important medical event that may not result in death or be life threatening, may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the study participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Prudent medical judgment must be exercised to decide whether reporting is appropriate.

Reporting Procedures of Serious Adverse Events

All SAEs that occur during the study will be reported by study PI or designee within 24 hours to the ERC and the DSMB as well as to IVI and ShanthaBiotechnics. The SAE form will always be completed as thoroughly as possible with all available details of the event, signed by the principal investigator. If the investigator does not have all the information regarding an SAE, he/she will not wait to receive additional information before submitting the report. If deemed necessary, the DSMB will have the authority to call a temporary suspension of the study, for careful review and assessment of the reported event(s). The study physicians/medical officers will follow-up the participants with SAEs until the event has: resolved, subsided, stabilized or disappeared or the event is otherwise explained, or the participant is lost to follow-up. The date of final disappearance of the adverse event will be documented. The study physician will always provide an assessment of causality at the time of the initial report.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The Nuffield Council of Bioethics recommends that “wherever appropriate, participants in the control group should be offered a universal standard of care for the disease being studied. Where it is inappropriate to offer such a standard, the minimum that should be offered is the best intervention currently available as part of the national public health system”[38][45]. While some critics may argue that the standard of care should be the “best method”, Wendler, et al suggested four conditions when the standard of care may be less than a universally accepted “best method” and these conditions include: a) scientific necessity; b) the relevance of the study for the host community should address important health issues of the communities participating in the studies; c) the need for the clinical trial to produce a fair level of benefit for the communities participating in the trial; and d) subject and host community non-maleficence i.e., the study participant must not be “prospectively worse off” than they would if the trial were not conducted [39]. This study fulfils all four criteria. There is a scientific necessity to perform the trial; the study is of relevance to the communities to be included in the trial as the population has high rates of cholera (areas will be chosen based on previously reported cholera cases in the hospital surveillance being performed by icddr, b) and the community will benefit from the surveillance activity that not only does diarrhoea surveillance but at the same time promotes safe water and sanitation practices. Medical care will be provided for any medical ailment or injury that occurs to any participant that is likely due to the study agent. Appropriate ethical and regulatory clearances will be obtained prior to the trial. Once the vaccine is approved for the single dose indication, provisions to make the cholera vaccine available to placebo recipients will be made.

The study will be conducted in compliance with the procedures outlined in this protocol and in accordance with the ethical guidelines and local regulatory requirements for the trial. The investigators’ responsibilities will follow the WHO guidelines for GCP. The privacy and confidentiality of all data and information collected from the trial participants, including those derived from clinical and biological specimens, will be ensured both during and after the conduct of the trial. Individuals will not be identified in any reports and publications based on the trial data.

Verbal and written informed consent will be obtained prior to intervention from eligible adult participants and the parents/guardians of participants aged <18 years; in addition, assent will be obtained from children aged 11-17 years of age (see Informed Consent form), Consent and assent will be documented by signature or thumbprint on the appropriate forms and noted down in the Vaccination Record. Participants and parents/guardian of the child will be informed of the study activities, and they will be encouraged to ask questions regarding the study. Signature (or thumbprint, if illiterate) of the participants and parents/guardian of the child will be obtained before their enrollment in the study and dated prior to any study-related activity. A witness will also sign in the informed consent form in the

event a participant, parents/guardian of a child participant, is not literate. The informed consent form must be signed and dated by the study personnel who obtain the consent. In addition informed consent will be obtained from 324 participants who agree to have blood taken at two time points following dosing during the study period. Verbal consent will be obtained from participating subjects who will be followed by active surveillance for safety and also from those who will be monitored for pregnancy outcomes.

If new information, not covered in the proposal, on the study product becomes available that may be relevant to the participants' willingness to continue in the study, the investigator will inform that in a timely manner and use a revised written informed consent form. The proposal will be revised and resubmitted to RRC and ERC for the amendment and will then be used for obtaining permission.

The expected duration of subject participation

The duration of follow-up for each participant will be 24 months after dosing.

A description of "stopping rules" or "discontinuation criteria" for individual participants, parts of the trial and the entire trial

The study is planned for the duration of 2 years of follow up after vaccination. Primary analysis will be conducted at 6 months, with secondary analyses conducted at 12, 18 and 24 months of follow up. Efficacy results will be submitted to the DSMB following primary and interim results.

The trial may be stopped for ethical reasons

at the recommendation of IRB of any of the partner institutions or for the safety reasons at the recommendations of the DSMB and collaborating institutes.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

NA

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

This project is a collaborative study of icddr, b, the International Vaccine Institute (IVI) in Korea, and Shantha Biotechnics in India as well as other international and national experts in the field of vaccines .

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

A large area based on updated GIS maps is available in high cholera prone field site at Mirpur urban area in Dhaka. Diarrheal hospitals of icddr, bin Mohakhali and Mirpur and existing health facilities as well as laboratory facilities are available for the study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the “standard” length.

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Principal Investigator/Program Director (*Last, first, middle*): Dr. A. Qadir.

An Individually randomized, placebo-controlled trial to measure the protection conferred by a single dose regimen of live-attenuated, killed, whole cell oral cholera vaccine (Shanchol) in Dhaka, Bangladesh.

**DETAILED BUDGET FOR BUDGET PERIOD
DIRECT COSTS ONLY**

Cost Categories	FROM 01 Jan. 2014 THROUGH 30 Jun. 2015			
	Year 1	Year 2	Year 3	TOTAL US\$
PERSONNEL				
International personnel	181,515	183,776	98,163	463,454
Local Personnel	1,390,098	1,173,277	550,263	3,113,582
SUBTOTALS PERSONNEL	1,571,613	1,361,997	648,426	3,582,036
CONSULTANT COSTS				
Consultants	67,440	70,288	0	137,728
SUBTOTALS CONSULTANTS	67,440	70,288	0	137,728
EQUIPMENT (itemize)				
EQUIPMENT	157,100	0	0	157,100
SUBTOTALS EQUIPMENT	157,100	0	0	157,100
SUPPLIES (itemize by category)				
Furniture & Stationeries	156,446	36,000	16,000	208,446
SUBTOTALS SUPPLIES	156,446	36,000	16,000	208,446
TRAVEL				
Local Travel	43,750	44,100	18,322	106,172
International Travel	23,400	34,320	18,876	76,596
SUBTOTALS TRAVEL	67,150	78,420	37,398	182,968
Other costs				
Training cost, printing, Rent, specimen testing, GIS vaccine delivery, good clinics practices etc.	345,325	193,300	66,200	604,825
Hospital cost				
icdsh, Mipur treatment centre and other health facilities cost	150,000	75,000	0	225,000
SUBTOTALS OTHERS	495,325	268,300	66,200	829,825
SUBTOTAL DIRECT COSTS	2,515,075	1,815,005	768,024	5,098,104
Overhead @15%	353,696	272,251	115,204	741,151
TOTAL Budget	2,868,771	2,087,256	883,228	5,839,255


M. Rahman Chowdhury
Sr Budget Co-ordinator

Budget Justifications

Budget for the study is needed for carrying out vaccine delivery in the selected field sites of Mirpur area. Surveillance of the diarrheal patients coming from the study sites will be carried out at the hospitals and health facilities in Mirpur and icddr,b hospitals and microbiological tests carried out at icddr,b. Other important costs are for GIS activity, advocacy meetings, local and international travel, transportation of vaccine, programme monitoring, training, data management etc.

Personnel:

Personnel comprise core component, data management, census, GIS activity, vaccine delivery, census update, passive surveillance and quality team. Total amount budgeted under the categories is US \$3,582,036 for 30 month period.

Consultant costs:

The category consists of 2 external consultants and support for infrastructure amounting US\$ 137,728 in 24 months.

Equipment:

Various capital equipments are required for the study for data collection and laboratory support. All these equipment would be procured in year 1 amounting to US\$ 157,100

Supplies:

Supplies comprise of stationeries and furniture amounting to US\$ 208,446 in the 30 month period.

Travel:

Local travel in all years will require US \$106,372 for supervisory visit and data collection. International travel required in year 2 and year 3 amounting to US\$ 76,596 for study seminar/ workshops/meetings at national and international locations.

Vaccine delivery:

Vaccine delivery in the Mirpur site will be required in year 1 amounting to US\$ 50,000

GIS:

GIS mapping and licensing will be required in initial stages through a subcontract with a GIS company.

Other costs:

Other costs comprising training, printing and office rent, immunogenicity study, specimen testing (324 subjects) etc. amounting US\$ 554,826 in the 30 month period.

Hospital costs:

The costs for icddr,b, Mirpur treatment centre and other health facilities are US\$ 225,000 in the first 24 months of the study.

Overhead costs:

Overhead costs is calculated at 25% amounting to cover direct overhead costs (15%) as well as built in cost.

Check-List

**Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)
[Please check all appropriate boxes]**

1. Has the proposal been reviewed, discussed and cleared by all listed investigators? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No If the response is No, please clarify the reasons:
2. Has the proposal been peer-reviewed externally? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> External Review Exempted If the response is "No" or "External Review Exempted", please explain the reasons: If the response is "Yes", please indicate if all of their comments have been addressed? <input checked="" type="checkbox"/> Yes (please attach) <input type="checkbox"/> No (please indicate reason(s)):
3. Has the budget been reviewed and approved by icddr,b's Finance? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No (reason): _____
4. Has the Ethics Certificate(s) been attached with the Protocol? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No If the answer is "No", please explain the reasons:
<p><u>Firdausi Qadri</u> Signature of the Principal Investigator</p> <p>31/10/2012 Date</p>



DATA ANALYSIS PLANS

Single dose oral cholera vaccine (SCVB)
study in Dhaka, Bangladesh

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1. OVERVIEW

Protocol: Single dose oral cholera vaccine study in Dhaka, Bangladesh (PR-12090)

Vaccine: Reformulated Bivalent (anti-O1, anti-O139), Killed, Whole-cell Oral Cholera Vaccine

Indication: Prevention of *V. cholerae* O1 and O139 diarrhea

Sponsor: Bill and Melinda Gates Foundation

Principal Investigator: Dr. Firdausi Qadri, icddr,b, Dhaka, Bangladesh

Co-PIs: Dr. John D Clemens,

Dr. Fahima Chowdhury, Dr. Amit Saha, Dr. Iqbal Ansary Khan, Dr. Ashraf Islam Khan (internal)

Dr. Thomas F. Wierzba, IVI (external)

Co-Investigators (internal): Dr. Yasmin Ara Begum, Dr. Taufiqur Rahman Bhuiyan, Dr. Muhammad Asaduzzaman

Co-Investigators (external): Dr. Alejandro Cravioto (IVI), Ajit Pal Singh (IVI), Sachin N. Desai (IVI),
Mohammad Ali (JHU), Dr. Mahmudur Rahman (IEDCR & NIC),
Dr. S.A.J. Md. Musa (PHC), Dr. Baizid Khorshid Riaz,
Dr. Md. Tajul Islam A. Bari EPI & Surveillance, Dr. Md. Shamsuzzaman
(EPI), Dr. Sanjida Islam (DCC)

2. SUMMARY OF THE ANALYTICAL PLANS

Objectives To evaluate protective efficacy of a single dose regimen of the bivalent, killed, whole cell oral cholera vaccine Shanchol™, given to healthy, non-pregnant residents aged one and above in Dhaka, Bangladesh, against culture-proven *V. cholerae* O1 diarrhea which has been detected in all treatment settings serving the catchment population with onset of 7 days to 6 months (180 days) after dosing

Design Two-arm individually randomized double-blind placebo-controlled trial

Subjects All enumerated healthy consenting subjects, residing in Wards 7-13, 15 and 41 of Mirpur, Dhaka, Bangladesh. Subjects will be included if they are permanent residents of the study site, not pregnant, and at least 1 year of age during vaccination.

Population size Approximately 350,000 subjects in the study area

Vaccination Period: January 10 to February 4, 2014

Agents Bivalent, killed, whole-cell oral cholera vaccine

End-points The primary endpoint for the study efficacy is the onset date of the first episode of culture-confirmed *V. cholerae* O1 diarrhea episodes detected in the study treatment centers with onsets of 7 days to 6 months (180 days) after dosing among those who have received one complete and correct dose of the vaccine or placebo.

Period of study 6 months post-receipt of either vaccine or placebo

3. POPULATION AND DEMOGRAPHIC EVENTS

A census was conducted by the project people during July 2012 to December 2013 to enumerate the study area population. The census captured the *de jure* population defined as persons who stated their residence in the study area was their regular residence. The population has been regularly updated thorough vital demographic events of the study area population. A close out census will be conducted after six months of follow-up.

Out-migrations (internal/external): Individuals who were present at zero time (defined in Chapter 5: Other definition),but migrated out any time after zero time.

Deaths: Individuals who were present at m but died any time after zero time.

For the analysis, we will follow the demographic date assignment flow chart (Appendix 5).

4. DEFINITION OF OUTCOMES

Diarrheal visit: A diarrheal visit is defined as a visit for care of diarrhea in which the patient described:

- 3 or more loose or liquid stools; or
- At least 1 bloody stool; or
- 1-2 or an indeterminate number of loose or liquid stools and exhibited at least some dehydration

Diarrhea episode: A diarrheal episode is defined as:

- All diarrheal visit(s) for which the date of onset for a diarrheal visit was less than or equal to 7 days from the date of discharge for the previous visit, constitute a single “diarrheal episode”.
- The onset of a diarrheal episode was defined as the day on which it was reported to have begun for the first visit of the episode.

Cholera episode: A cholera episode is defined as:

- A diarrheal episode in which no component visit was described as bloody diarrhea; and

- A fecal specimen from at least one component visit yielded *V. cholerae* 01 in the ICDDR,B laboratory; and
- An identity check performed after discharge for the visit in which *V. cholerae* 01 was isolated, confirmed that the person whose name was given at the treatment center had indeed sought care for diarrhea on the date of presentation

5. OTHER DEFINITIONS

Zero time: The date of dose for the dose recipients and the start date of the mass vaccination (January 10, 2014) for non-dose recipients.

Complete dose recipients: Individuals who drank and swallowed one complete dose (the full course).

Incomplete dose recipients: Individuals who swallowed any amount of dose but not completed the full course.

No-dose recipients: Individuals who were present at zero time but not dosed. During routine demographic updates, individuals were identified as entered into the population prior to dosing and therefore would have been eligible at the time of vaccination. These individuals are also considered as the no-dose recipients.

Two-dose recipients: Individuals found to have taken two doses based on the vaccine record book.

Lost to follow up: Individuals who died or moved out of the study area from the resident any time after zero time. Note that internal migration out demographic events will not be considered as lost to follow-up.

Duration of follow up period: The time between 7 and 180 days after the zero time.

Pre-dosing period: The period on or before the zero time. Events whose onsets occur in this period will not be considered in the analysis.

Post dose interval: The interval between the zero time and 7 days from zero time. Cholera events whose onsets occur in this period will not be considered in the analysis.

Post follow up period: The time after the completion of the follow up period. Events whose onsets occur in this period will not be considered in the analysis.

6. THE ANALYSES

6.1. Primary analysis (direct protection against severe and non-severe cholera)

This primary analysis will compare subjects according to the study agent actually received and will include only those subjects who satisfied the inclusion/exclusion criteria, followed the protocol, and received one complete dose.

Onset of follow-up for counting events: All cholera episodes will be included if they have onsets between 7 and 180 days after the zero time.

Direct protection is estimated by comparing the incidence of cholera among vaccine recipients and the incidence of the cholera among placebo recipients.

Numerator events for analysis: Numerator events will be cholera episodes (defined above) whose onsets are between 7 and 180 days after the zero time. Cholera cases that occur between the zero time and the beginning of follow-up (zero time +7 days) will not be considered as a cholera episode and will not be included in this analysis.

Denominator for analysis: Subjects in the denominator for analysis are those who completely ingested dose of the agent and were at least 12 months of age at zero time and were otherwise eligible and were noted as having received either oral cholera vaccine or placebo. In analyses of time-to-event, the person-time at risk begins at the zero time and continues until the date of death, date of out-migration, date of event under analysis, or to zero time +180 days for this analysis.

6.2. Secondary analysis (direct protection against severely dehydrated cholera)

A severely dehydrating cholera episode, is defined by the presence of at least two of the following signs and symptoms: sunken eyes, dry tongue, thirsty, irritable condition, less active than usual, skin-pinch goes back slowly, low volume of radial pulse along with inability to drink, or uncountable or absence of radial pulse.

Onset of follow-up for counting events: Severely dehydrated cholera episodes will be included if they have onsets between 7 and 180 days after the zero time.

Direct protection is estimated by comparing the incidence of severely dehydrated cholera among vaccine recipients and the incidence of the severely dehydrated cholera among placebo recipients.

Numerator events for analysis: Numerator events will be severely dehydrated cholera episodes (defined above) whose onsets are between 7 and 180 days after the zero time. Cases that occur between the zero time and the beginning of follow-up (zero time +7 days) will not be considered as an episode and will not be included in this analysis.

Denominator for analysis: Subjects in the denominator for analysis are those who completely ingested dose of the agent and were at least 12 months of age at zero time and were otherwise eligible and were noted as having received either oral cholera vaccine or placebo. In analyses of time-to-event, the person-time at risk begins at the zero time and continues until the date of death, date of out-migration, date of event under analysis, or to zero time +180 days for this analysis.

7. CANDIDATE VARIABLES FOR COVARIATES

Candidate variables for inclusion as covariates in the final model are listed below. In this instance, the variables that will have p value <0.10 will be selected. The candidate variables are as follows:

- Individual level
 - Age at zero time
 - Gender
 - Had diarrhea in past 6 months at baseline census
- Household level
 - Number of member in the household
 - House ownership
 - Months living in the area
 - Source of drinking water
 - Type of drinking water
 - Sanitation status
 - Hand washing practices
 - Per-capita household expenditure
 - % of vaccinees within 100m around the household
- Spatial variables
 - Distance to the nearest health facility
 - Distance from the nearest ICVB intervention clusters
 - Improved sanitation coverage within 100m around the household
 - Safe water coverage within 100m around the household
 - Population density within 100m around the household
 - Mobility rate within 100m around the household. This is to be calculated as $(\text{migration in} + \text{migration out}) / \text{zero time population all ages} * 100$

8. SUBGROUP ANALYSIS

Age-specific protective efficacy will be estimated by

Age group:

Age at zero time

- 1-4.9 years
- 5-14.9 years
- 15 years +

Duration of follow-up:

- Zero time to 90 days
- 91 days to 180 days from zero time

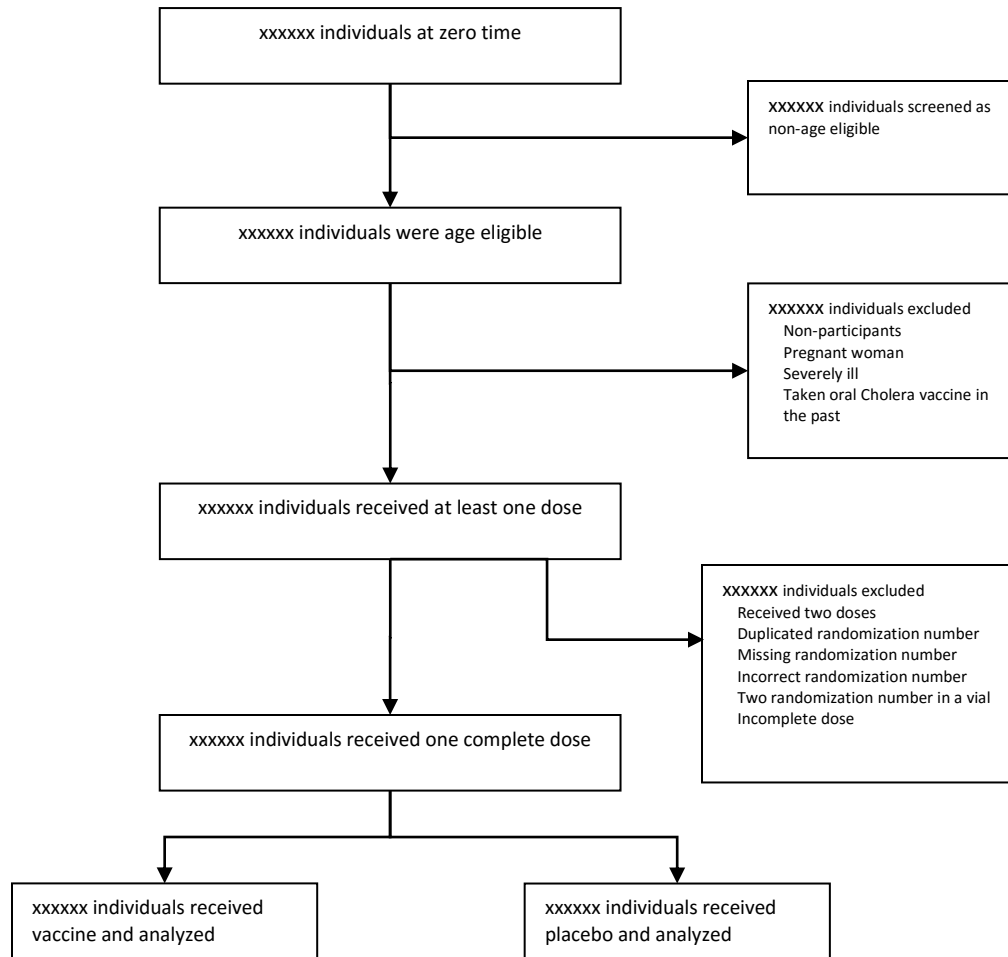
9. STATISTICAL METHODS

The primary analysis will address occurrence of cholera during six months of follow-up among vaccine and recipients. Cox proportional hazard regression models will be used, verifying first that the proportionality assumption is satisfied for all independent variables. Hazard ratios (HRs) of the target outcome in the vaccine recipients versus placebo recipients will be estimated by exponentiating the coefficient for the vaccine variable in these models, and vaccine protection will be estimated as $[(1 - HR) \times 100\%]$. Standard errors for the coefficients will be used to calculate one-tailed p values and 95% upper confidence intervals for the HRs.

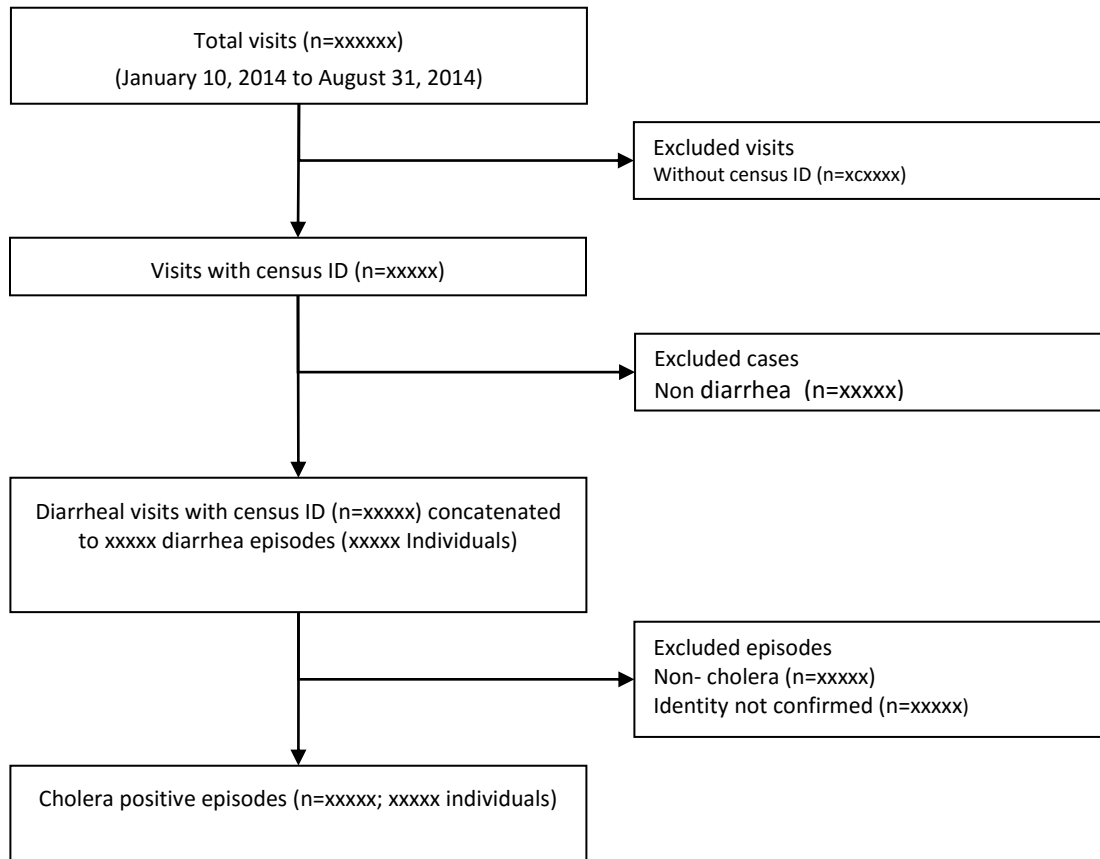
Final adjusted estimates will be obtained from models including the variables which will be found to be independently associated with the time to the event at p value $< .10$ in a backward selection algorithm.

To evaluate heterogeneity of vaccine protection among different subgroups, interaction terms between vaccination and subgroup variables in these models will be evaluated. P values and 95% confidence intervals for these analyses will be calculated as two-tailed.

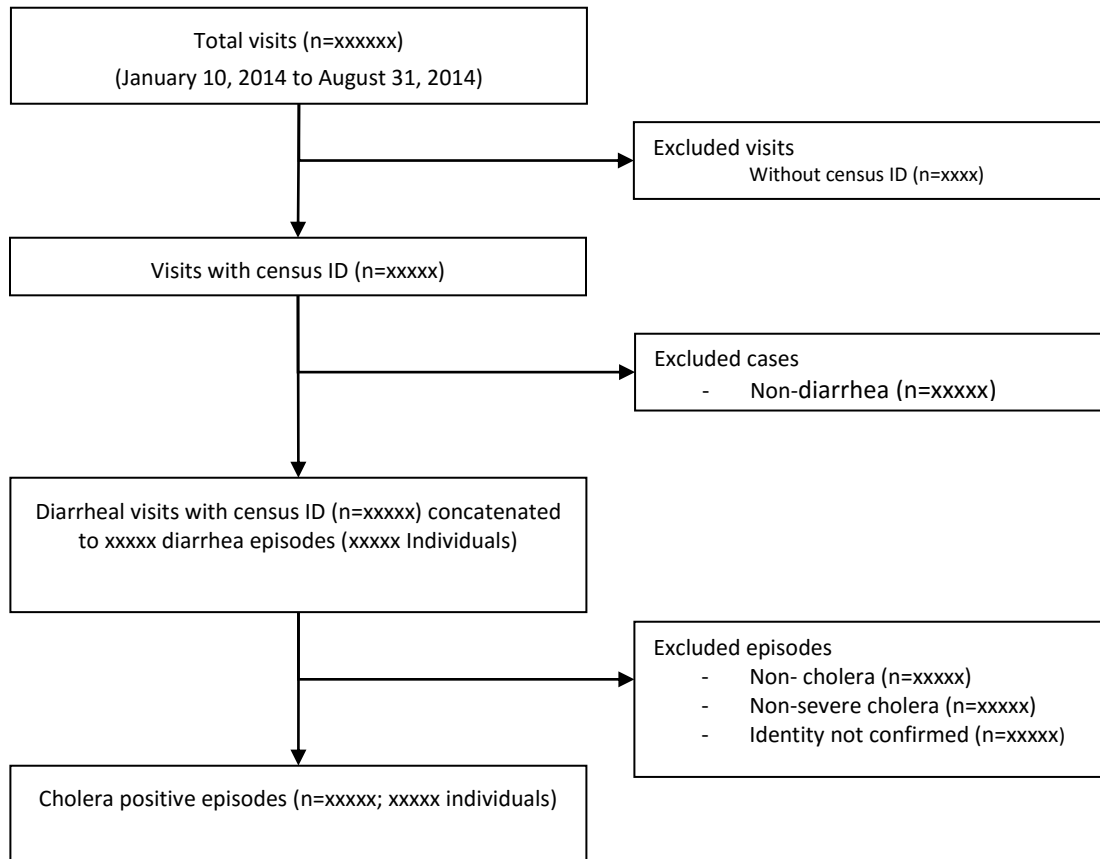
Appendix1. CONSORT for assembling population for the primary analysis



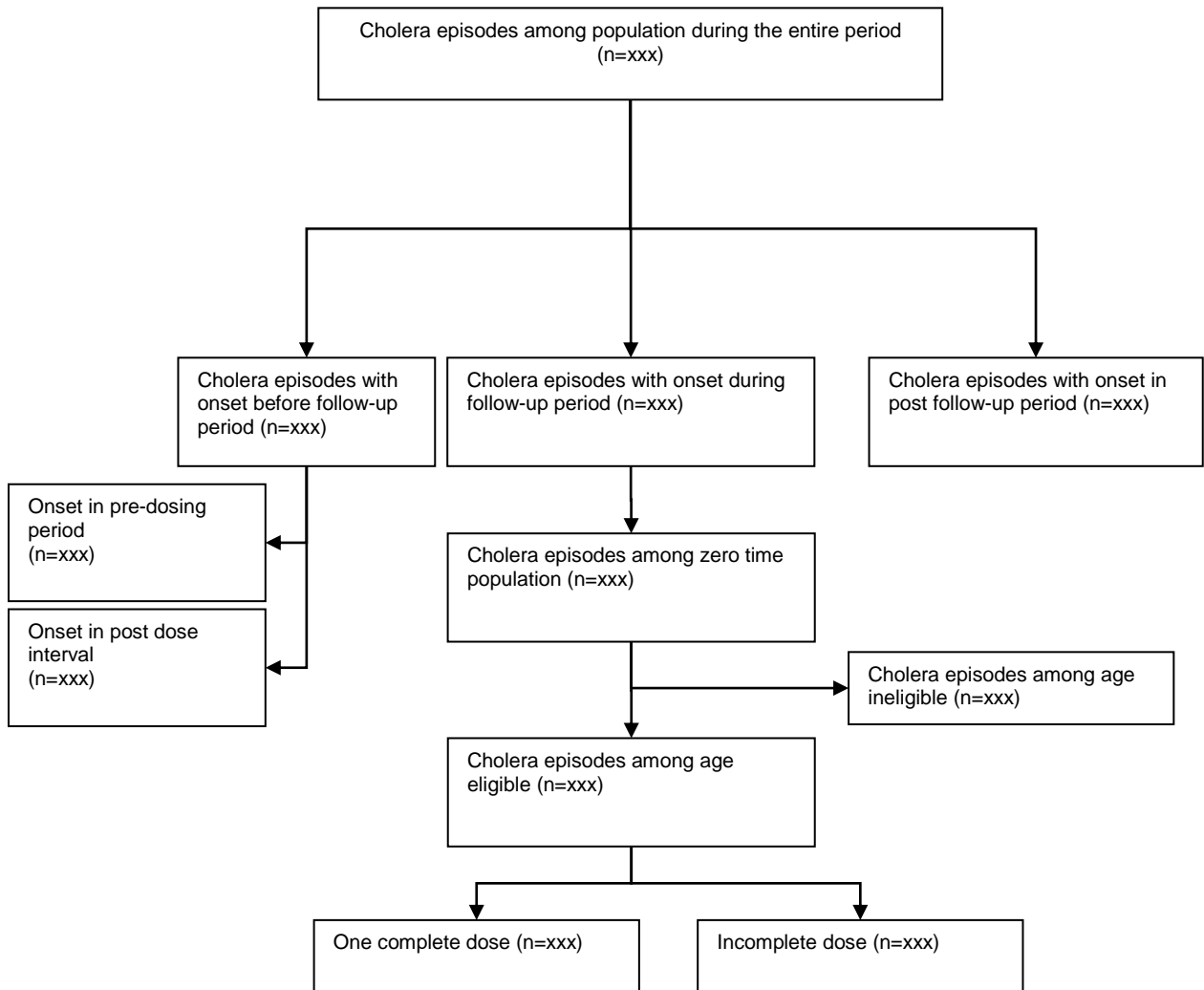
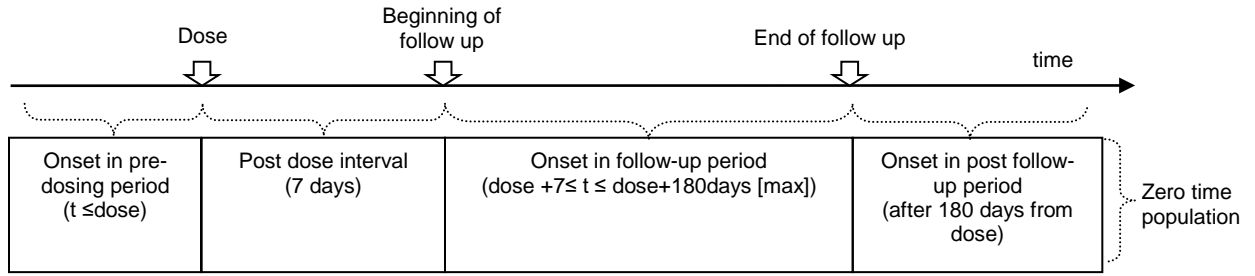
Appendix2. Episode classification (primary endpoint)



Appendix 3. Episode classification (secondary endpoint)

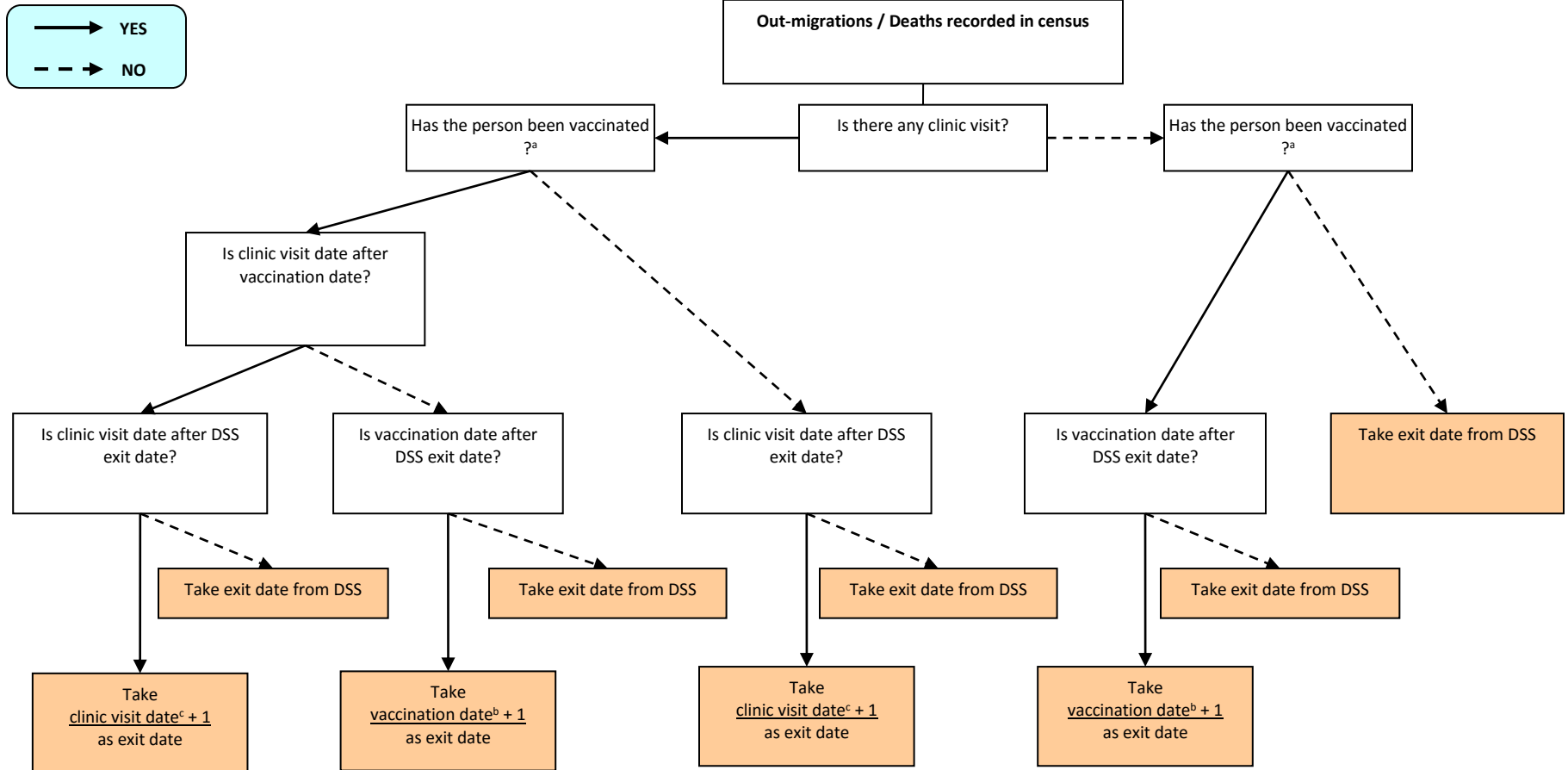


Appendix4. Case follow chart



Appendix 5. Demographic date assignment flowchart for SCVB project

Out-Migrations or Deaths



a – Vaccinated means anyone who received either vaccine or placebo.

b – Date of the last dose.

c- If the individual had multiple clinic visits, then take the last visit date.