icddr,b	**RRC APPLICATION FORM**

RESEARCH PROTOCOL **Number: PR-24059** **Version No. 1.3** **Version date: 15-07-2024**	**FOR OFFICE USE ONLY**			
	RRC Approval:	☒ Yes	☐ No	Date:13-06-2024
	ERC Approval:	☒ Yes	☐ No	Date:18-07-2024
	AEEC Approval:	☐ Yes	☐ No	Date:
	External IRB Approval	☐ Yes	☐ No	Date:
	Name of External IRB: _____			

Will the protocol be submitted for expedited review? ☐ Yes ☒ No

If yes, please check all that apply:

- ☐ Outbreak investigation
- ☐ Secondary Analysis
- ☐ Formative research
- ☐ Short surveys
- ☐ Pilot Study
- ☐ Student protocol
- ☐ Observational study
- ☐ Approved by external IRB (local or abroad) except Randomized Clinical Trial
- ☐ Others (explain the justifications):_____

***** Cover Letter to RRC/ERC Chairperson through SDD must be attached.***

Protocol Title:* (maximum 250 characters including space) **Evaluation of typhoid conjugate vaccine (TCV) effectiveness among Bangladeshi children using the test-negative design**

Short Title: (maximum **100** characters including space) **ZyVac-TCV Bangladesh study**

Key Words:* Bangladeshi population, conjugate vaccine, immunogenicity, safety, typhoid fever

Name of the Research Division Hosting the Protocol:*

- ☐ Health Systems and Population Studies Division (HSPSD)
- ☐ Nutrition Research Division (NRD)
- ☒ Infectious Diseases Division (IDD)
- ☐ Maternal and Child Health Division (MCHD)
- ☐ Other (specify) _____

Has the Protocol been Derived from an Activity:* ☒ No ☐ Yes (please provide following information):

Activity No. :
Activity Title:
PI:
Grant No.: Budget Code: Start Date: End Date:

icddr,b Strategic Priority/ Initiative (SP 2019-22):* (check all that apply)

☐ Reducing maternal, neonatal and child mortality and improving the well-being of women, children and adolescents ☐ Preventing and treating maternal and childhood malnutrition ☒ Detecting and controlling enteric and respiratory infections ☐ Detecting and controlling emerging and re-emerging infections ☐ Achieving universal health coverage	☐ Achieving gender equality and promoting sexual and reproductive health and rights ☐ Examining the health consequences of and adaptation to climate change ☐ Preventing and treating non-communicable diseases ☐ Others (specify) _____
Research Phase (4 Ds):* (check all that apply**)** ☒ Discovery ☒ Development	☐ Delivery ☐ Evaluation of Delivery
Anticipated Impact of Research:* (check all that apply and please provide details below**)** ☒ Knowledge Production ☒ Capacity Building	☒ Informing Policy ☒ Health and Health Sector Benefits ☒ Economic Benefits

Knowledge gap: Typhoid fever is a systemic illness caused by *Salmonella enterica* serotype Typhi (*Salmonella* Typhi) and Paratyphi A, B, C. It is estimated to affect more than 9 million people with 110,000 deaths worldwide annually, primarily in low- and middle-income countries in Asia and Africa. A multi-center study was conducted in Bangladesh, Nepal, and Malawi between 2016 and 2018. In Bangladesh, the overall incidence of typhoid fever was 161/100,000 person-years of observation with the highest incidence in children aged 5-9 years. The study has also reported 39% multidrug resistance (resistance to chloramphenicol, ampicillin, and cotrimoxazole) *Salmonella* Typhi strains from Bangladesh, which is a major public health concern for treating typhoid patients as well as for reducing typhoid burden. Current efforts to control typhoid fever recommend the joint delivery of effective vaccines against typhoid and improvements in water, sanitation, and hygiene. The World Health Organization (WHO) has recommended typhoid conjugate vaccines (TCVs) to be introduced in the routine and catch-up vaccination with TCVs in countries with a high burden of typhoid fever.

Here, we propose to conduct a prospective closed cohort, open-label, phase III effectiveness study to evaluate the effectiveness and immunogenicity of the Typhoid Vi Conjugate Vaccine I.P. manufactured by M/s. Zydus Lifesciences Limited.

Capacity building: The study will generate data on the effectiveness and immunogenicity of the Typhoid Vi Conjugate Vaccine I.P. manufactured by M/s. Zydus Lifesciences Limited named 'ZyVac® TCV'. It will increase the supply of vaccines and will meet the demand for vaccines in Bangladesh and the region.

Informing policy: The result will be disseminated to the Communicable Disease Control (CDC), Directorate General Health Services (DGHS), Government of Bangladesh (GoB), WHO, and the global community.

Health and health sector benefit: The study results will be used to inform the country decision-making for the introduction of typhoid vaccine into the routine expanded programme on immunization (EPI) in Bangladesh.

Economic benefits: Typhoid burden has negatively impacted the global economy. In the developing world, this impact is felt even greater. A safe and immunogenic vaccine against typhoid fever could reduce the burden of typhoid fever in Bangladesh and globally.

List of Abbreviations

AE	Adverse event
AEFI	Adverse events following vaccination
AR	Adverse reaction
ALS	Antibody in Lymphocyte Supernatant
ASC	Antibody secreting cell
BSL2	Biosafety level 2
CRF	Case report form
CI	Confidence interval
CDC	Communicable Disease Control
CTL	Cellular Technology Limited
DSMB	Data and Safety Monitoring Board
DGDA	Directorate General of Drug Administration
DGHS	Directorate General of Health Services
DSCC	Dhaka South City Corporation
ELISA	Enzyme-linked Immunosorbent Assay
ERC	Ethical Review Committee
EDC	Electronic data capturing
EPI	Expanded programme on immunization
ELISPOT	Enzyme-linked immunospot assay
FACS	Fluorescence-activated cell sorting
GAVI	Global Alliance for Vaccines and Immunisation
GOB	Government of Bangladesh
GMT	Geometric Mean Titre
GCP	Good Clinical Practice
GIS	Geographic Information System
GPS	Global Positioning System
HH	Household
icddr,b	International Centre for Diarrheal Disease Research, Bangladesh
ID	Identification number
ICF	Informed Consent Form
ICH	International Council on Harmonization
IRB	Institutional Review Boards
IMP	Investigational Medicinal Product
IgG	Immunoglobulin G

ISM	Independent safety monitor
IVI	International Vaccine Institute
LMP	Last menstrual period
MDR	Multidrug- resistant
MoU	Memorandum of understanding
MTA	Material transfer agreement
NIBSC	National Institute for Biological Standards and Control
pCRF	Paper Case report form
PI	Principal investigator
PCR	Polymerase chain reaction
RT-PCR	Real time polymerase chain reaction
RRC	Research Review Committee
SAE	Serious Adverse Event
SQL	Structured Query Language
SOP	Standard operating procedures
STRATAA	Strategic Typhoid alliance across Africa and Asia
TCV	Typhoid conjugate vaccine
TND	Test negative design
TT	Tetanus toxoid
TyVAC	Typhoid Vaccine Acceleration Consortium
WHO	World Health Organization
Vi-PS	Vi polysaccharide
Vi-TT	Vi polysaccharide conjugated to tetanus toxoid
Vi-rEPA	Vi-polysaccharide conjugated with a recombinant exoprotein A from *Pseudomonas aeruginosa*
VVM	Vaccine Vial Monitor
XDR	Extensively drug-resistant

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

Principal Investigator: Dr. Farhana Khanam

Research Protocol Title: Evaluation of typhoid conjugate vaccine (TCV) effectiveness among Bangladeshi children using the test-negative design

Proposed start date: 01-08-2024 Estimated end date: 31-05-2026

Background (brief):

a. Burden:

Typhoid fever is a systemic illness caused by the human-restricted pathogen *Salmonella enterica* serotypes Typhi (*Salmonella* Typhi) and Paratyphi A, B, C. It is estimated to affect more than 9 million people worldwide annually, with an estimated 110,000 deaths per annum, primarily in low- and middle-income countries in Asia and Africa. A multi-center study was conducted in Bangladesh, Nepal, and Malawi under the Strategic Typhoid Alliance across Africa and Asia (STRATAA) consortium. In Bangladesh, the overall incidence of typhoid fever was 161/100,000 person-years of observation with the highest incidence in children aged 5-9 years. The study has also reported 39% multidrug resistance (resistance to chloramphenicol, ampicillin, and cotrimoxazole) to *Salmonella* Typhi in Bangladesh which is a major concern for treating typhoid patients as well as for reducing the burden of typhoid fever. Another multicentre study was conducted in Bangladesh, Nepal, and Malawi under the Typhoid Vaccine Acceleration Consortium (TyVAC) using Typbar-TCV. The study result shows 85% (97·5% confidence interval [CI] 76 to 91, p<0·0001), 81.6% (95% CI, 58.8 to 91.8; P<0.001) , and 80.7% (95% CI, 64.2 to 89.6) effectiveness in Bangladesh, Nepal and Malawi respectively.

b. Knowledge gap:

The World Health Organization (WHO) has recommended typhoid conjugate vaccines (TCVs) to be introduced in the routine and catch-up vaccination with TCVs in countries with a high burden of typhoid fever. But, there is a global shortage of vaccine to introduce in the endemic settings. It highlights the uncertain pace of country introductions, and the challenges countries face to adopt the vaccine.

c. Relevance:

An effective vaccination program in the higher risk populations will be useful to apply the cost-effective control measures. So, the evaluation of new vaccine effectiveness will be crucial to reduce the demand uncertainty and the need to improve forecast accuracy as well as concerns to ensure industry can plan supply availability.

d. Hypothesis (if any):

The typhoid Vi-polysaccharide conjugate vaccine will be effective in preventing typhoid fever among Bangladeshi children.

e. Objectives:

Primary objective:

To evaluate the effectiveness of typhoid conjugate vaccine (TCV) against blood culture-confirmed typhoid fever using the test-negative design

- To evaluate the effectiveness of typhoid conjugate vaccine (TCV) against blood culture-confirmed typhoid fever stratified by age groups (6 months to <2 years, 2 to 4 years, 5 to 15 years)
- To evaluate the effectiveness of typhoid conjugate vaccine (TCV) against blood culture-confirmed typhoid fever stratified by the period of follow-up (first and second six months) after vaccination
- To determine the immunogenicity of TCV in a subset of participants, stratified by age groups (6 months to <2 years, 2 to 4 years, 5 to 15 years)

f. Methods:
This is a prospective closed cohort, open-label, phase III effectiveness study using a test-negative design of a typhoid conjugate vaccine, ZyVac® TCV (purified Vi capsular polysaccharide of *Salmonella* Typhi conjugated to tetanus toxoid as carrier protein), manufactured by Zydus Lifesciences Limited. The study will be conducted in a closed cohort population among children aged 6 months to 15 years residing in wards 5, 6, 7, 48, 49, 50, 63, 71, and 72 of Dhaka South City Corporation (DSCC). The targeted number of age-eligible children in the study area is ~92,000 among them ~60,000 will be vaccinated. A subset of the first 600 consenting participants will be selected by age strata (6 months to <2 years, 2-4 years, 5-15 years) for enrollment in the immunogenicity study with an additional three follow-up visits. Diary cards will be used to collect adverse events (AEs) following immunization (AEFI) data up to day 7 for a subset of active follow-up of the first 600 vaccinated participants. Participants not in this subset will be encouraged to go to the 'Adverse Event Monitoring Cell' at the Maniknagar field office. Data on serious adverse events (SAEs) will be reported for six months after vaccination. All study updates including AEs and SAEs will be reported to the data safety and monitoring board (DSMB) and sponsor. Passive surveillance for typhoid fever will be carried out in the Maniknagar field office and Mugda Medical College and Hospital in the catchment area among the age-eligible children.

g. Outcome measures/variables:

Primary outcome
Blood culture-confirmed typhoid fever

Secondary outcome
Anti-Vi-IgG antibody concentration
Blood culture-confirmed paratyphoid fever

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 typhoid Vi-polysaccharide conjugate vaccine will be effective in preventing typhoid fever among Bangladeshi children.

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:
To evaluate the effectiveness of typhoid conjugate vaccine (TCV) against blood culture-confirmed typhoid fever using the test-negative design

Secondary objective:

- To evaluate the effectiveness of typhoid conjugate vaccine (TCV) against blood culture-confirmed typhoid fever stratified by age groups (6 months to <2 years, 2 to 4 years, 5 to 15 years)
- To evaluate the effectiveness of typhoid conjugate vaccine (TCV) against blood culture-confirmed typhoid fever stratified by the period of follow-up (first and second six months) after vaccination
- To determine the immunogenicity of TCV in a subset of participants, stratified by age groups (6 months to <2 years, 2 to 4 years, 5 to 15 years)

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.

Typhoid fever is a systemic illness caused by the human-restricted pathogen *Salmonella enterica* serotypes Typhi (*Salmonella* Typhi) and Paratyphi A, B, C [1]. Patients with typhoid fever typically experience symptoms such as fever, malaise, abdominal pain, and alternating constipation and diarrhea. Untreated or improperly managed cases can lead to severe complications, including intestinal perforation, bleeding, and encephalopathy. Approximately 3% to 5% of patients may become long-term asymptomatic carriers without proper treatment. Among these carriers, about 90% are likely to develop gallstones or gallbladder disease. The carrier state of typhoid, whether accompanied by gallstone disease or not, is a risk factor for gallbladder cancer. The highest incidence and mortality from typhoid fever occur in the developing regions of South Asia, Southeast Asia, and Africa, largely due to inadequate sanitation and water supply [1-3]. Annually, it is estimated that typhoid fever affects over 9 million people and causes 110,000 deaths globally. The incidence rates of typhoid fever are categorized as low (less than 10 cases per 100,000 population per year), medium (10-100 cases per 100,000 population per year), high (more than 100 but less than 500 cases per 100,000 population per year), and very high (over 500 cases per 100,000 population per year). Central Africa reports the highest rates, followed by certain areas in Central, South, and Southeast Asia. A multi-center study by the Strategic Typhoid Alliance across Africa and Asia (STRATAA) consortium, conducted in Bangladesh, Nepal, and Malawi, found that in Bangladesh, the overall incidence rate was 161 cases per 100,000 person-years, with the highest rates in children aged 5-9 years. The study also highlighted a concerning 39% prevalence of multidrug-resistant *Salmonella* Typhi in Bangladesh, posing significant challenges for treatment and efforts to reduce the typhoid burden [4]. The diagnostic challenges in accurately identifying acute typhoid fever cases and asymptomatic carriers of *Salmonella* Typhi causes the rise of multidrug-resistant (MDR) strains (resistant to chloramphenicol, ampicillin, and cotrimoxazole) and

extensively drug-resistant (XDR) strains (additionally resistant to fluoroquinolones and third-generation cephalosporins), are exacerbating the complexity of typhoid fever management.

Historically, control of typhoid fever has been achieved through enhanced sanitation and infrastructure improvements, which have successfully eradicated the disease as a major public health concern in most developed nations. Despite this success, high-incidence regions face significant financial and logistical challenges in adopting these measures. Therefore, incorporating an effective vaccination program aimed at the most vulnerable populations could be a valuable and cost-efficient supplement to existing control strategies. Since typhoid-causing organisms are exclusive to humans, global eradication is feasible, and a potent vaccine could significantly advance this goal. In 1999, the World Health Organization (WHO) endorsed the use of new-generation vaccines for school-aged children in light of the persistent typhoid burden and the emergence of antibiotic-resistant strains. The WHO also advised immunization in regions with elevated typhoid rates or significant antibiotic resistance. Nonetheless, apart from specific areas in China, Vietnam, and India, this guidance has not been widely implemented in countries where typhoid is endemic [5].

The WHO recommended that endemic countries consider the programmatic use of vaccines, to target only the high-risk groups and populations for most countries with the strategies of school- or community-based vaccination or as appropriate depending on the local context such as disease incidence, age-specific disease burden, and percent of school enrollment population. Vaccination is also recommended for outbreak control [6]. In response to an outbreak of XDR *Salmonella* Typhi, that occurred in November 2016, in Hyderabad, Pakistan, the Aga Khan University Karachi in collaboration with the Ministry of health Sindh had initiated a mass immunization campaign with Typbar-TCV vaccine targeting children aged 6 months to 10 years [7]. Pakistan was the first country to introduce the Typhoid Conjugate Vaccine (TCV) into its routine immunization schedule in 2019 with GAVI Support. Following Pakistan, several other countries including Liberia, Malawi, Nepal, Zimbabwe (GAVI Supported), and Samoa (without GAVI support) have also incorporated TCV into their routine immunization programs [8, 9]. Currently, GAVI approved the vaccination campaign in Bangladesh to cover children aged 9 months to <16 years. Considering the global incidence, a huge bulk of WHO-prequalified vaccines is needed to control the disease. Different vaccine manufacturers step up to mitigate the short supply of the vaccine. Moreover, the 62nd ICC approved the TCV vaccine application, and the request was sent to the Gavi Secretariat on September 13, 2022. Since the TCV is going to be rolled out in Bangladesh with Gavi's support soon, it would be considered unethical to conduct a randomized controlled trial for the TCV. The test-negative design (TND) approach allows researchers to leverage existing data and infrastructure without the need for extensive experimental settings and additional resources. In India, there are four licensed vaccines for typhoid fever. The ZyVac® TCV is one of them which was manufactured by Zydus Lifesciences Limited, Ahmedabad, India. There are several preclinical and clinical trials conducted using this vaccine. Currently, the company has applied for WHO pre-qualification which requires an effectiveness study. The purpose of this study is to evaluate the effectiveness of the typhoid conjugate vaccine (TCV) among Bangladeshi children using the TND.

Currently available typhoid vaccines

Inactivated whole-cell vaccine

This vaccine consists of heat-phenol-inactivated whole-cell *Salmonella* Typhi, which is injected subcutaneously in two doses four weeks apart. It had an efficacy of 51-67% in controlled trials. It was associated with a high degree of reactogenicity, causing fever and systemic symptoms in 9-34% of recipients leading to school absence in 2-17% of cases [10]. Due to these side effects, it has largely dropped out of mainstream use, however, it is still used in several developing countries.

Vi Polysaccharide vaccine (Vi-PS)

Developed in the 1980s this vaccine consists of purified virulence factor (Vi antigen) capsular polysaccharide (Vi-PS) that forms the capsule of, and is specific to, *Salmonella* Typhi. It elicits a T-cell independent antibody response, which means it has poor immunological memory and repeat doses do not result in an additional boosting response [11, 12]. Similar to other polysaccharide vaccines, the Vi-PS vaccine is poorly immunogenic and not licensed for use in children under 2 years old, presumably due to the absence of specific splenic marginal zone B-cells that are needed to produce an immunological response to polysaccharides. In clinical trials, efficacies were of 64-72% [13-15]. Additionally, protection is short-lived, lasting only 2-3 years [16, 17].

Live attenuated oral vaccine (Ty21a)

Also developed in the 1980s, this is an attenuated strain of *Salmonella* Typhi (Ty21a) that has had many virulence genes mutated chemically, including the gene leading to failure to produce the Vi antigen. Ingestion of this strain induces local gut mucosal immunity as well as systemic antibody and cell mediated response [18, 19]. The strain is lyophilized and administered in either an oral enteric capsule or a liquid solution and requires 3-4 doses to induce effective protective immunity. Clinical trials performed in Chile and Indonesia demonstrated Ty21a vaccine had a protective efficacy of 67% and 53%, respectively [20, 21]. While the enteric-coated formulation is difficult to administer to young children, the alternative liquid formulation is better tolerated but may be less immunogenic in younger children [19, 22]. Ty21a vaccine is not licensed for children under the age of 6 years.

Vi-rEPA vaccine

This vaccine was developed by the US National Institute for Health (US NIH) in 1994 utilizing Vi-polysaccharide conjugated with a recombinant exoprotein A from *Pseudomonas aeruginosa* (rEPA) [23]. A two-dose schedule six weeks apart was shown to be highly immunogenic with a protective efficacy of 91.1% in children aged 2 to 5 years in a trial in Vietnam [24]. More recently, a study has demonstrated its immunogenicity in infants [25]. However, the licensure of Vi-rEPA has been delayed due to a lack of regulatory precedent for the use of rEPA carrier-based vaccines.

Vi antigen typhoid conjugate vaccine (Vi-TCV)

Typhoid conjugate vaccines consist of Vi polysaccharide antigen linked to different carrier proteins that can extend protection and allow immunization of infants and toddlers, which unconjugated Vi (T-independent antigen) cannot do successfully. Vi-TCV (Typbar-TCV) is a newly available vaccine developed by Bharat Biotech International Limited, India, consisting of 25µg of Vi polysaccharide antigen conjugated to a nontoxigenic tetanus toxoid carrier protein. Similar to other vaccines which are designed to protect against encapsulated bacterial pathogens and are conjugated to tetanus toxoid carrier proteins, Vi-TCV induces a T-cell dependent response. It can therefore produce an immunogenic response in infants under 2 years of age and has the potential to generate a durable immune response via induction of immunological memory. Vaccines should be stored at 2–8°C. This was first licensed in India in 2013 for use in individuals from 6 months of age up to 45 years of age. A Phase III randomized controlled trial comparing Vi-TCV with Vi-PS demonstrated seroconversion to anti-Vi IgG in the 6 month to 2 year age group [26]. Additionally, a comparison of the sub-groups receiving boosters of either vaccine at two years demonstrated significantly higher anti-Vi IgG titers in the Vi-TCV group compared to the Vi-PS group (mean titre of 1685.3 EU/ml [95% CI: 1468-1797] in Vi-TCV vs 445.6 EU/ml [95% CI: 323-615] in Vi-PS) [26]. Safety data from the same study demonstrated that Vi-TCV was well tolerated by all age groups and that there were no differences in the number or variety of adverse events reported between the vaccine arms [27, 28]. The multicentre study was conducted in Bangladesh, Nepal, and Malawi under the Typhoid Vaccine Acceleration Consortium (TyVAC) using Typbar-TCV. The study result shows 85% (97·5% CI 76 to 91, P <0·0001), 81.6% (95% CI, 58.8 to 91.8; P<0.001) and 80.7% (95% CI, 64.2 to 89.6, P<0.001) effectiveness in Bangladesh, Nepal and Malawi respectively [29-31].

Peda-Typh (BioMed)

Another Vi-TT vaccine, Peda-Typh (BioMed). It consists of Vi coupled to tetanus toxoid (TT). This vaccine has been licensed only in India for children over 3 months of age [32], but this has not been prequalified by the WHO and only

limited clinical data are available to document its safety and immunogenicity. Each dose of the vaccine contains 5µg of Vi polysaccharide of *Salmonella* Typhi conjugated to 5µg of tetanus toxoid.

Vi-CRM (TYPHIBEV Biological E)
Vi-CRM is a typhoid conjugate vaccine (TCV) manufactured by Biological E and developed in collaboration with the GSK Vaccines Institute for Global Health. It contains Vi polysaccharide from Citrobacter freundii, which is clinically indistinguishable from the Vi polysaccharide from *Salmonella* Typhi, conjugated to a variant of diphtheria toxin (CRM197) as the carrier protein. TYPHIBEV was prequalified by WHO in December 2020. Results from a Phase 2/3 study conducted in India demonstrated that the immune response profiles of TYPHIBEV are comparable to Typbar-TCV [33, 34]. This vaccine is likely to be the vaccine given during the national vaccination campaign in Bangladesh.

Results of clinical study of the trial vaccine, ZyVac® TCV

Phase II/III clinical trial: In this study, the seroconversion rate (proportion of subjects achieving ≥4-fold increase in anti-Vi IgG antibody titre) at 6 weeks post-vaccination in the subjects aged 6 months to 45 years (overall population), 6 months to < 18 years (pediatric cohort) and 18 to 45 years (adult cohort) was 94.8%, 93.1%, 96.6% respectively. The seroconversion rate reported with ZyVac® TCV was non-inferior to that reported with the comparator TCV. In the subjects who had received ZyVac® TCV, the pre-vaccination geometric mean titre (GMT) of anti-Vi IgG antibodies reported were 7.6 EU/ml, 5.7 EU/ml, and 10.0 EU/ml in the overall population, pediatric cohort, and adult cohort respectively, while the GMT of anti-Vi IgG antibodies reported at 6 weeks post-vaccination was 1121.0 EU/ml, 891.1 EU/ml and 1411.0 EU/ml in the overall population, pediatric cohort and adult cohort respectively. There was a significant increase in GMTs at 6 weeks post-vaccination as compared to pre-vaccination GMTs (P<0.0001). Both the pre-vaccination and post-vaccination GMTs reported in the subjects who had received ZyVac® TCV were comparable to the respective GMTs reported in the subjects who had received the comparator TCV in this study [35].

Phase IV clinical trial: In this study, the subjects who had received primary vaccination with TCV in the previous phase II/III clinical trial were followed up to 3 years of post vaccination. 77.2% of the subjects who had received ZyVac® TCV in the previous phase II/III clinical trial and enrolled in this study had anti-Vi IgG antibody titre above the protocol-defined cut-off titre of 10 IU/ml when assessed using the first WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (NIBSC code 16/138) which is equivalent to the proposed seroprotective cut-off titre of 2 µg/ml as derived from the studies of other TCV. The baseline GMT of anti-Vi IgG antibodies (3 years after primary vaccination) reported in this study in the subjects who had received ZyVac® TCV as a part of previous phase II/III clinical trial was 140.8 EU/ml. This baseline GMT was significantly higher as compared to pre-vaccination GMT reported for the same subjects in the previous phase II/III clinical trial (P<0.0001). A total of 17 subjects who had baseline anti-Vi IgG antibody below the proposed seroprotective cut-off titre were administered booster vaccination with ZyVac® TCV. All the subjects followed up at 10 days and 28 days after booster vaccination achieved seroconversion (≥4-fold increase in anti-Vi IgG antibody titre). The GMTs of antibodies reported at 10 days and 28 days after booster vaccination were 2306.9 EU/ml and 1900.5 EU/ml respectively. The GMTs of antibodies after booster vaccination were higher than those reported after primary vaccination in the previous phase II/III clinical trial [35].

Phase III clinical trial: The study was conducted in healthy adults aged 45 to 65 years, the seroconversion rate (proportion of subjects achieving ≥4-fold increase in anti-Vi IgG antibody titre) at 4 weeks post-vaccination was 94.1%. The seroconversion rate reported with ZyVac® TCV was non-inferior to that reported with the comparator TCV. In the subjects who had received ZyVac®TCV, the pre-vaccination GMT of anti-Vi IgG antibodies reported was 8.0 EU/ml while the GMT of anti-Vi IgG antibodies reported at 4 weeks post-vaccination was 1378.3 EU/ml. There was a significant increase in GMTs at 4 weeks post-vaccination as compared to pre-vaccination GMTs (P<0.0001). Both the

pre-vaccination and post-vaccination GMTs reported in the subjects who had received ZyVac® TCV were comparable to the respective GMTs reported in the subjects who had received the comparator TCV in this study [35].

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.

Study design

This will be a phase III effectiveness study of the typhoid conjugate vaccine (Vi-TCV) (ZyVac® TCV). The study will be conducted in a closed cohort population of children aged 6 months to 15 years residing in a selected geographical catchment area in Dhaka, Bangladesh using a test-negative design (TND). The TND is a particular type of case-control study including controls who present themselves with similar signs and symptoms (to those of the cases) in the same healthcare facilities. Individuals with the disease of interest are tested with a laboratory assay where test-positive and test-negative individuals are considered cases and controls, respectively.

Study area

The study will be conducted in wards 5, 6, 7, 48, 49, 50, 63, 71, and 72 of Dhaka South City Corporation, covering an area of ~10.33 km^2. No TCV campaigns have been conducted in this area. According to the Population and Housing Census 2022, the total population within the selected geographical catchment area is ~465,000 with households of ~122,000. The proportion of males is 54 percent and the average household size is ~3.8 people. Assuming ~20% of the population is from the 6-month to 15-year age group, the expected number of age-eligible children is ~92,000.

Baseline mapping

A map of the study area will be created based on open-source georeferenced satellite images (source: Esri, Maxar, Earthstar Geographics, and the GIS User Community). The satellite image of the study area will be printed by separating it into several small pieces and provided to the field team for data collection. In this process field team will visit the entire area according to the map and draw the structure/building, road, and other salient geographic features in the printed images. A unique geographical information system (GIS) identification number will be assigned for all the buildings/structures, and submitted to the GIS team to develop the digital vector data (point, line, and polygon feature). The digital data of structure/building (polygon feature) centroid x and y coordinates will be used to represent real-world coordinate values or GPS values.

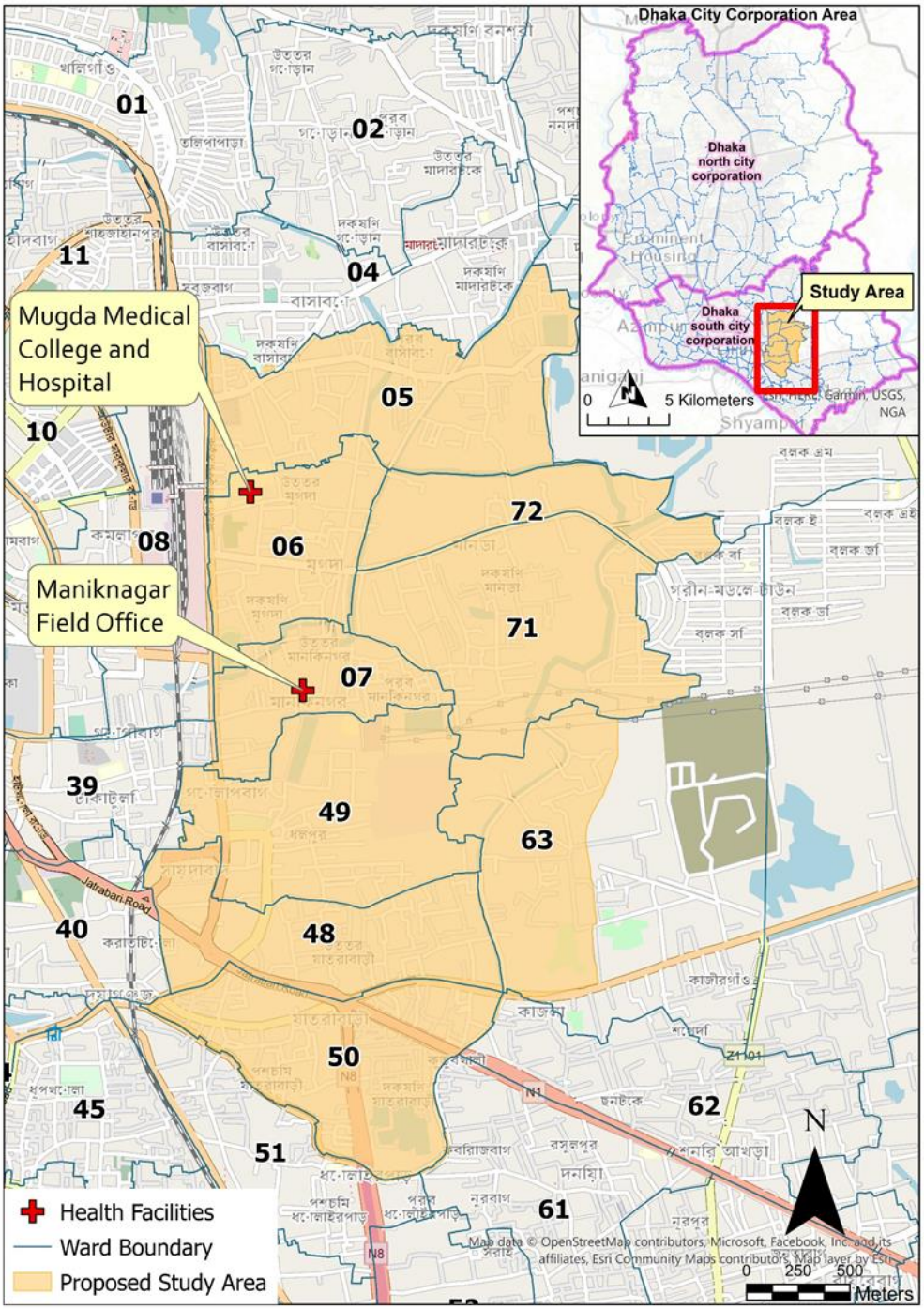


Figure: Study catchment area with health facilities

Baseline listing

All households in the study area will be assigned a unique identification number and all household members will be enumerated. Information about the age-eligible children including name, age, sex, date of birth, and parent's name will be obtained. In addition, the name of the HH head, address, and cell phone number of HHs will be collected. The previously assigned GIS ID will be incorporated into the baseline listing form and the newly identified structure will be updated in the GIS database. We will take photos of birth certificates or national immunization cards to confirm the age of participants if available. Verbal consent will be provided by either the head of the household or a key informant.

Participant card for potentially eligible children for vaccination

After preparing the baseline list, we will identify the age-eligible (6 months to 15 years) participants. Then we will prepare participant cards for all eligible children which will be provided before the vaccination. There will be a unique identification number on the participant card which will be used during vaccination. A master list of potential eligible participants will be prepared.

Vaccination

Inclusion criteria for vaccination:

The participant must satisfy all the following criteria to be eligible for enrolment:

- Participant was included in the baseline list of the study population
- Participants living within the study catchment area at the time of vaccination
- Parent/guardian is willing and competent to provide informed consent (if the participant is 11 to 15 years of age, assent will also be sought)
- Participants aged between 6 months to15 years (i.e. up to 15 years 364 days) at the time of vaccination
- Apparently healthy (no complaints of febrile illness) on the day of vaccination
- Parent/guardian confirms that their child will be willing and be able to comply with study requirements

Exclusion criteria for vaccination:

The participant will not be enrolled if any of the following criteria apply:

- Has knowingly received a typhoid vaccine in the past
- Known allergy to any vaccine in the past
- Medical or social reasons that will prevent the participant from conforming to the study requirements as judged by a medical professional
- Planning to move away from the catchment area within the next 12 months
- Pregnant at the time of vaccination, as confirmed by a urine test (urine pregnancy test will be done in girls who are married)
- Confirmed or suspected immunosuppressive or immunodeficiency disorder; or subjects on any immunosuppressive or immunostimulant therapy
- Subject participated in another clinical study in the past 3 months

Temporary exclusion criteria for vaccination:

Participants will be temporarily excluded from being vaccinated if, at the point of vaccination, any of the following apply:

- Receipt of any other vaccines in the last 30 days
- Current temperature of at least 38^{o}C or reported fever within 24 hours prior to vaccination
- Use of antipyretics within 4 hours prior to vaccination
- Unmarried girls between the ages of ≥12 to 15 years old whose first day of their last menstrual period (LMP) is more than 28 days ago or who do not know the date they last menstruated upon presentation

If these apply, the participant will be temporarily excluded from vaccination until the temporary exclusion criteria no longer apply. Please note, for children with fever above 38^{o}C this must be a minimum of 48 hours after the fever has resolved. Unmarried girls between the ages of ≥12 to 15 years whose first day of their LMP is more than 28 days or who do not know the date they last menstruated upon presentation will be asked to return after starting their next

menstruation for vaccination. A re-assessment will be conducted to ensure these temporary exclusion criteria no longer exist.

Verification of the potentially eligible children

All the households that have age-eligible children will be informed to visit the nearest vaccination centre. If a participant's birth certificate or national immunization cards were not provided during the baseline listing, we will notify their parents or guardians to bring them during vaccination. After arrival at the vaccination centre, the participant and vaccination ID will be verified with the listing database and the master list. Then the screening will be carried out according to inclusion/exclusion criteria.

Informed consent

The latest approved version of written informed consent/assent will be presented to the participant's parent/guardian in the local language detailing no less than the exact nature of the study, what it will involve for the participant, the implications and constraints of the protocol, the known side effects and any risks involved in taking part. It will be clearly stated that the participant's parent/guardian is free to withdraw their child from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal. The participant and their parents/guardians will be allowed as much time as they wish to consider the information, within the recruitment period, and the opportunity to question the investigator or other independent parties to decide whether they will participate in the study. Written informed consent/assent will then be obtained, with additional opt-in consent for blood specimen, using participants' parent/guardian's dated signature or thumbprint and dated signature of the person who presented and obtained the informed consent. Information sheets will be given to children aged 11 -15 years in a language that they can understand. The person obtaining consent will record informed assent on the consent form, for children aged 11 and older. The person who obtained the consent must be suitably qualified and experienced and have been authorized to do so by the Principal Investigator (PI). A copy of the signed informed consent will be given to the participant. The original signed form will be retained at the study site. A third party will act as a witness for the parent/guardian to attest that the information in the consent form and any other written information was accurately explained to, and understood by the parent/guardian and that informed consent was freely given by the parent/guardian. The witness will also sign and date the consent form.

Screening and eligibility assessment

An informed written consent will be obtained after confirming the participants by checking the database. After proper consenting process, a temperature will be taken and parents will be asked about antipyretic use and timings of previous vaccination to assess temporary exclusion criteria. A brief medical history will be taken and eligibility will be assessed against inclusion and exclusion criteria. Participants with temporary exclusion criteria will be informed and asked to return 48 hours after the fever has resolved or 30 days after the last vaccination was given for repeat assessment and re-consent. Unmarried girls between the ages of ≥12 to 15 years whose first day of their LMP is more than 28 days or who do not know the date they last menstruated upon presentation will be asked to return after starting their next menstruation, for vaccination. A member of the study team will collect/edit demographic information (including age and address) and participant contact details by screening case report form (CRF). After completion of the eligibility checking, a sticker will be allocated for the participant which includes the information of vaccination ID and temperature; these will be used in the vaccine accountability log.

Vaccination

The vaccination team will finally check the identification and then administer the vaccine. After vaccination, information will be recorded in the vaccination CRF. If the participant is included in the immunogenicity study, a blood sample will be taken by a suitably trained staff member before vaccination. After administration of the study vaccine, participants will be asked to wait at least 30 minutes for any immediate hypersensitivity reactions or any other adverse

events (AEs). The female participants of reproductive age should have been advised not to be pregnant within three months after the vaccination.

Investigational Medicinal Product (IMP)

Purified Vi capsular polysaccharide of *Salmonella* typhi conjugated to tetanus toxoid as carrier protein (Typhoid Vi Conjugate Vaccine I.P.). Trade name: ZyVac® TCV, Zydus Lifesciences Limited.
Each 0.5ml vaccine dose contains:

- Purified Vi-capsular polysaccharide of *S*.typhi 25 µg
- conjugated to tetanus toxoid (carrier protein) 16 to 50 µg
- 2-Phenoxyethanol (as preservative) 2.50 mg
- Isotonic buffer solution q.s.

The vaccine is packaged as a pre-filled 2.5ml 5-dose vial. It will be administered as an intramuscular injection preferably in the anterolateral aspect of the middle thigh for younger children, or the upper arm for older children with aseptic precautions.

Supply

The Vi-TCV vaccine (ZyVac® TCV) will be provided by Zydus Lifesciences Limited.

Storage

The Vi-TCV study vaccine will be stored at 2° to 8° C in a temperature monitored icddr,b cold room facilities, when not in use for daily activities. The vaccines will be stored in temperature-monitored refrigerators or cool boxes, when in use for daily activities. Each vial will be labeled with a "vaccine vial monitor"(VVM); a temperature-sensitive dot that provides an indication of the cumulative heat to which the vial has been exposed. It warns the end-user when exposure to heat is likely to have degraded the vaccine beyond an acceptable level and should not be used.

Special precautions for storage
Store at 2°C to 8°C.
Do not freeze. Discard if frozen.
Keep out of reach of children.
Shake gently before use.
Do not use the vaccine after the expiration date shown on the label

Accountability of the study vaccine

The vaccines will be shipped to a central storage facility in Bangladesh and passed through customs. It will then be transported and distributed to local vaccination sites whilst maintaining the cold chain (aiming for a temperature between 2°-8°C). The number of doses of study vaccines that are received, used, and wasted will be documented daily during the study and checked weekly.

Adverse events following immunization

After administration of the study vaccine, participants will be observed closely for at least 30 minutes for any immediate hypersensitivity reactions or any other adverse events (AEs). All details will be recorded in the CRF.

Adverse events follow up (Day 7, -1/+7):
A subset of 600 participants will be selected for active surveillance follow-up and the immunogenicity sub-study. A diary card will be given to each participant on the day of vaccination. Participants will be contacted by telephone or in-person after seven days of vaccination for follow-up and to record any adverse events following immunization (AEFI).

The selection of these participants will be age-stratified (6 months to <2 years, 2-4 years, and 5-15 years) with an allocation of 200 in each age stratum.
Parents of participants not in this subset will be encouraged to go to the 'adverse event monitoring cell' at the Maniknagar field office which will have a medical doctor and also available on-call 24 hours a day for adverse event monitoring throughout the whole vaccination period + 7 days.
Information will include:

- Verbal reconfirmation of consent for participation in the study.
- Report from parent/guardian of adverse events related to vaccination and use of medications following vaccination.
- After administration of TCV, solicited local AEs (pain, redness, swelling and induration) and solicited systemic AEs (fever, nausea, vomiting, diarrhea, headache1, malaise1, myalgia1, arthralgia1, abnormal crying2, loss of appetite2, drowsiness2 and irritability2 (1: applicable for subjects aged ≥2 years; 2: applicable for subjects <2 years of age)) will be recorded for 7 days after vaccination. Unsolicited (other) AEs will be recorded for 28 days after vaccination.
- Reiteration of contact details and instructions to attend the Maniknagar field office in case of fever (≥2 days and/or temperature ≥38^{o}C at presentation).

***Safety reporting*:**

Definitions

Below are the various categories of adverse events following immunization (AEFIs).

Adverse event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences that are not necessarily caused by or related to that product.
Adverse reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.
Serious adverse event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalization or prolongation of existing hospitalization • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardize the participant or require an intervention to prevent one of the above consequences.

	NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Causality

The relationship of each adverse event to the study medication must be determined by a medically qualified individual according to the following definitions:

Related: There is suspicion that there is a relationship between the study vaccine and the AE (without determining the extent of probability); there is a reasonable possibility that the study vaccine contributed to the AE

Not related: There is no suspicion that there is a relationship between the study vaccine and the AE; there are other more likely causes and administration of the study vaccine is not suspected to have contributed to the AE

Procedures for recording adverse events

From vaccination through day 7

After administration of TCV, solicited local AEs (pain, redness, swelling and induration), solicited systemic AEs (fever, nausea, vomiting, diarrhea, headache1, malaise1, myalgia1, arthralgia1, abnormal crying2, loss of appetite2, drowsiness2 and irritability2 (1: applicable for participants aged ≥2 years; 2: applicable for participants <2 years of age)) for 7 days after vaccination. Unsolicited (other) AEs will be recorded for 28 days after vaccination.

For injectable vaccines, the local signs and symptoms to be documented usually include, as a minimum, pain, redness, and swelling at the injection site in all age groups. Measuring devices of various types may be used to record the extent of redness and swelling. Fever will be documented using digital thermometers and axillary temperature will be determined. For subjective symptoms (for example, fatigue and myalgia) following scoring system will be considered.

The following scoring system will be used for grading of severity:

Parameter	Mild	Moderate	Severe	Potentially life threating
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Fever	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Malaise	Symptoms causing no or minimal interference with usual social &	Symptoms causing greater than minimal interference with usual social &	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to

	functional activities	functional activities		perform basic self-care functions
Myalgia	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions

All adverse events related to vaccination, as judged by a medically qualified investigator, occurring during the first 7 days post-vaccination that are observed by the study team/investigator or reported by the participant's parent/guardian, will be recorded on the CRF. The information will be collected both passively and actively.
For active surveillance of a subset of 600 participants, information will be actively collected via phone calls or home visits. Parents of participants not in this subset will be encouraged to go to the 'Adverse Event Monitoring Cell' at the Maniknagar field office which will have a medical doctor and also available on-call 24 hours a day for adverse event monitoring throughout the whole vaccination period + 7 days.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe, 4 = Potentially life-threatening

All Serious Adverse Events (SAEs) observed by the Investigators, members of the study team, or reported by the parent/guardian will be recorded on CRFs.

Throughout the study period

Serious Adverse Events (SAEs), as judged by a medically qualified investigator, observed by the investigator, or members of the study team, or reported by the parent/guardian, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, and action taken. Follow-up information should be provided as necessary.

All SAEs will be recorded in the CRFs, for the entire study duration. Participants with SAEs will be followed by a medically qualified investigator either until resolution or the event is considered stable.

Reporting procedures for serious adverse events

The forms developed by the icddr,b institutional review board (IRB) will be used for reporting all SAEs.

All SAEs occurring within the 6 months post-vaccine administration will be reported to the Data Safety Monitoring Board (DSMB), Directorate General of Drug Administration (DGDA), the PI, and the other study investigators within 24 hours of the site study team becoming aware of the event. A more detailed report form will be completed and sent within the shortest period of the initial report, to all parties mentioned above. Additional and further requested information (follow-up or corrections to the original case) will be detailed in subsequent safety report forms. All SAEs must be reported to the study sponsor within 7 days. Summary reports will be submitted to the icddr,b IRB, and DGDA at the end of the study.

All SAEs will be reported to sponsor at below mentioned email ID:

drugsafety@zyduslife.com, Pavankumar.Daultani@zyduslife.com

Indication for antipyretics and analgesics

Antipyretics will be used in case of fever (axillary temperature >38°C/100.4°F or feels hot to touch) following vaccination. Analgesics will be used in case of pain, or discomfort after getting vaccinated.

Safety monitoring committee

A DSMB will also be formed to oversee the safety component of the study. The DSMB members will not be involved in the study in any way. In addition, a physician with relevant study-related or therapeutic expertise will be identified as an independent safety monitor (ISM). The ISM will not be an investigator for this study. Under any circumstances, the identified ISM would be requested to carry out an independent assessment of the child presented with adverse events. The assessment details will be reported to the PI and DSMB.

Pregnancy follow-up

Follow-up on pregnancy outcomes will be conducted for all married girls identified during routine follow-up as either being pregnant at the time of vaccination or becoming pregnant within three months of vaccination. This follow-up will include monthly visits until the end of the pregnancy, with the final contact occurring after delivery or pregnancy outcome. Starting from the eighth month of gestation, additional weekly follow-ups will be conducted via phone calls. We will record pregnancy-related information considering it as a medically important event.

Immunogenicity study

A subset of 600 participants will be enrolled in the immunogenicity sub-study and will have an additional three face-to-face follow-up visits at the field office at one month (D30/+7 days), at 6 months (D180+/-14 days), and at one year (D365+/-30 days) after vaccination. The first 200 participants from each age stratum (6 months to <2 years, 2 to 4 years, 5 to 15 years) who give their consent will be selected and enrolled for the immunogenicity sub-study.

At these additional visits, the following procedures will be performed:

- Confirmation of continued participation in the study
- Collection of blood (3ml for 6 months to 4 years and 5ml for 5 to 15 years)
- Confirmation of contact details and reiteration of instructions to attend the health care facilities in case of fever for ≥2 days and/or temperature ≥38°C at presentation.

The above information will be recorded in the immunogenicity CRF. Parents/guardians of participants enrolled in the immunogenicity study will be given details of the follow-up appointments.

Surveillance for enteric fever

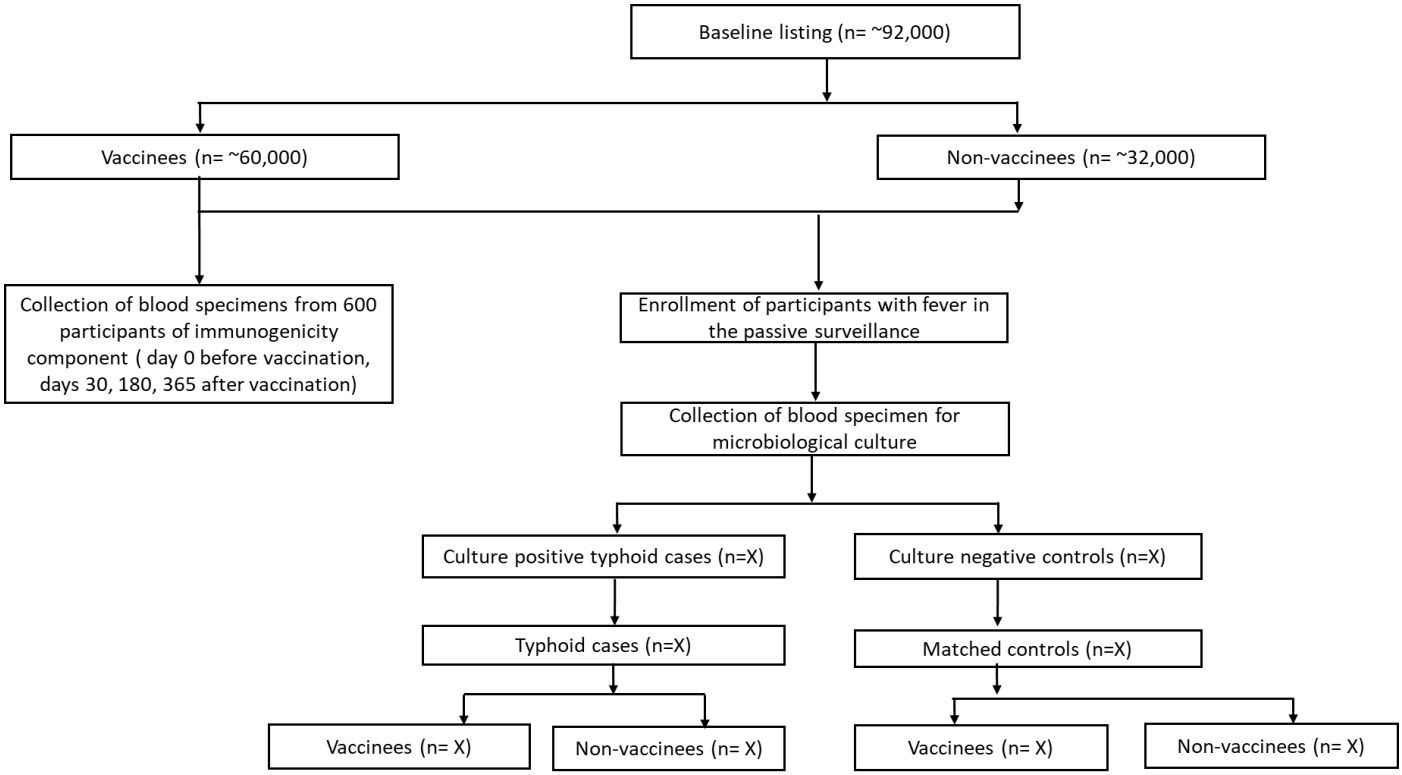
Surveillance for enteric fever will be undertaken at the Maniknagar field office and Mugda Medical College and Hospital for all age-eligible (from 6 months to 15 years) residents recorded in the baseline list. Participants with a subjective history of ≥2 days of fever and/or a temperature of ≥38°C at presentation will be enrolled in the surveillance. A blood sample (3ml) will be collected for microbiological culture. The participants will receive appropriate clinical management. Blood culture-positive patients will be visited at home to confirm their identity, and collect information about their illness and patients will be asked to visit the field office if their condition is not improved. The treatment will be adjusted as necessary, based on laboratory testing.

Study staff will visit households, included in the baseline listing, periodically, to remind them to seek treatment at the Maniknagar field office if their children (both vaccinees and non-vaccinees) develop a fever.

Eligibility criteria for enrollment in the passive surveillance

- Participants information, available in the listing database
- Parent/guardian is willing and competent to provide informed consent. For participants aged 11 to 15 years, informed assent will also be considered
- Participants from the study area who experienced a fever lasting for ≥2 days and/or temperature ≥38^{o}C at presentation [4, 30]
- Participants aged 6 months to 15 years on the first day of the vaccination campaign

Flow diagram



Laboratory procedures

Passive surveillance samples

Microbiological culturing of blood samples collected from enrolled participants will be carried out using BACTEC or BacT/ALERT systems in the icddr,b Clinical Microbiology, Immunology and Molecular Diagnostic Laboratory. The sensitivity and specificity of the blood culture method are 59% and 100% respectively [36]. Organisms from the suspected colonies will be identified by the biochemical tests and final identification will be done by slide agglutination test with *Salmonella-specific* antisera (Denka Sieken, Tokyo, Japan). The pattern of antibiotic susceptibility of *Salmonella* Typhi and *Salmonella* Paratyphi causing enteric fever will be assessed by the disk diffusion method on a Mueller-Hinton agar plate with Oxoid disks containing ampicillin, cotrimoxazole, chloramphenicol, azithromycin, ceftriaxone, ciprofloxacin, nalidixic acid, and cefixime. The procedure will be carried out following GCP guidelines and standard operating procedures. The results of these tests will be recorded in the participant CRF. The isolated strain will be preserved at the Mucosal Immunology and Vaccinology Laboratory for further analysis.

Immunogenicity study samples

Blood samples taken for the immunogenicity sub-study will be transported to the icddr,b laboratory daily. The samples will be processed and stored by trained study staff, following standard operating procedures (SOP). The serum/plasma will be stored and used to measure the anti-Vi-IgG antibody using a commercially available Enzyme-linked Immunosorbent Assay (ELISA) kit (VaccZyme, The Binding Site). This assay will be performed according to the manufacturer's instructions. Laboratory processes will be conducted at the Mucosal Immunology and Vaccinology Laboratory at the icddr,b.

Withdrawal of participants

Participants' parents/guardians can withdraw consent at any point. The investigator may also discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event or disease progression resulting in the inability to continue to comply with study procedures and follow-up

Depending on which aspects participants wish to withdraw from, the withdrawal will result in the cessation of any follow-up calls, visits, or blood tests (as applicable to the subset). No further treatment is required in the study, so no additional action will need to take place. Participants' parents/guardians will have the choice when withdrawing, to withdraw from active study procedures only (follow-up calls and visits) but remain in passive surveillance for the primary outcome, (allowing us to access their hospital records and blood test results), or withdraw from all study contact. Data and blood samples collected prior to the time of participant withdrawal will be kept and analyzed as part of the study data. A participant who withdraws from the study has the option to re-engage at a future date if they choose to do so. All participants who withdraw from the study will be given information on how to re-engage with the study if they so choose. Reasons for withdrawal from the study, if known, will be recorded in the participant's CRF.

Source documentation

Each participant will have a complete source documentation of records including CRF, lab reports, and test results for the entire study period.

Data management

The investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. Octalsoft). Electronic devices and hard copies of CRFs will be used to collect and record data. Data will be collected online and uploaded to a cloud server regularly when electronic devices are brought back to the central field office, and reliable internet is available. Paper CRFs (pCRFs) will also be available as backup, in case of interruption of online data collection, pCRFs will be used for collecting data and data will then be entered into the EDC system when feasible.

Baseline listing data will be collected offline on an electronic device with an internally designed Android application database SQLite and SQL Server. The system will have some logical checks incorporated in the system and it will be checked before saving to the server. Data will be checked periodically using the SQL scripts for detecting errors. Errors will be corrected by the user and transferred to the server again. An audit trail of correction will always be available in the system after correction of data. Data management staff will keep backups from the SQL server database regularly and store the backup on the desktop, laptop, and other removable devices.

All other CRFs will be designed and maintained on EDC system, named Octalsoft, a secure web application for building and managing online surveys and databases. Quality control checks of the system will be performed on a regular basis. Both the EDC system (CRF data) and internally designed Android application will use a relational database via a secure web interface with data checks applied during data entry to ensure data quality.

All participants will be identified by a unique trial-specific number of and/or codes; this will not include any identifiable information. Some personal information will be saved on the Android application designed by icddr,b. Only site research staff and sponsor data managers will have access to view the personal information.

Trial staff will have access to both the internally designed Android application and Octalsoft via a unique username and password. Each trial staff member will have an appropriate level of access to CRFs and collected data, according to their roles and responsibilities within the trial.

All participant data will be stored and maintained on local servers in icddr,b as mentioned above for the duration of the trial. Anonymized data will be shared with the sponsor in India. At the end of the trial, all individually identifiable data will be removed, and fully anonymized data will be retained for further analysis.

Study timeline

Activities	Months																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Agreement																						
Protocol development																						
Regulatory approvals including RRC, ERC, and DGDA																						
Staff recruitment																						
SOP and training materials development																						
Training for vaccination and surveillance																						
Listing of interested children for vaccination in the study area																						
Vaccination																						
AEFI recording																						
SAE recording and reporting																						
Diary card review																						
Patient enrollment in the passive surveillance																						
Patient management																						
Sample collection for imunogenicity and passive surveillance																						
Laboratory analysis																						
Data cleaning																						
Development of statistical analysis plan																						
Data analysis																						
Clinical study report (CSR) development																						
Manuscript writing and submission																						
Dissemination of findings in national and																						

international community																					

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.

A sample size of 515 (103 cases and 412 controls) will be required in this case-control test-negative design study assuming vaccine effectiveness of 80%, vaccine coverage of 65% in the target age-eligible population (6 months to 15 years), 4 controls per case, precision width of ± 10% (20%) and type 1 error rate of 0.05. Considering the incidence rate for typhoid fever of 230 per 100,000 person-years among the non-vaccinated population aged 6 months to 15 years, the target number of vaccinations is ~60,000.

To achieve the target number of vaccinees aged 6 months to 15 years representing ~31% of the total population), the required age-eligible children and population of all age groups will be ~92,000 and ~297,000 respectively.

Data Analysis

Describe 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, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

Focal time

Focal time will be defined as the date of onset of symptoms leading to presentation for care.

Culture-positive case

Eligible febrile patients who will be positive by blood culture for *Salmonella enterica* serotype Typhi and symptoms developed >14 days after vaccination, or, if unvaccinated, >14 days after the midpoint of the vaccination period.

Culture-negative control

Eligible febrile patients who will be negative by blood culture for *Salmonella enterica* serotype Typhi and symptoms developed >14 days after vaccination, or, if unvaccinated, >14 days after the midpoint of the vaccination period.
Given that the sensitivity of the blood culture method is ~59% [36], we will apply the clinical rule, defined in a previous analysis for blood culture-negative typhoid patients, to exclude clinical typhoid patients from the analysis [37]. This exclusion will be helpful for a better evaluation of the magnitude of protection conferred by the vaccine in the proposed study.

Matching of cases and controls

A statistician, blinded to the vaccination status of participants will select blood culture-negative controls (after excluding clinical typhoid patients, vide supra) against each case. Matching of controls for each case will then be done considering the age groups at focal time (6 months to <2 years; 2 to 4 years; and 5 to 15 years) and date of presentation. Controls will be randomly selected on the day of the presentation; if matched controls will not be available on that day, the selection period will be widened for up to a maximum of ±7 days.

Statistical analysis

For the evaluation of vaccine protection, a conditional logistic regression model will be used, considering matched case-control status as the outcome and vaccination status and selected covariates as independent variables, taking non-receipt of vaccine as the referent category for assessment of vaccine protection. Odds ratios for the associations between vaccination and typhoid fever will be estimated by exponentiation of the coefficient for vaccination from the fitted model, and the standard error of the coefficient will be used to calculate 95% confidence intervals for the estimated effect. Protective effectiveness will be calculated as [(1 – odds ratio) X 100%]. The threshold of statistical significance will be considered at P <0.05.

Analysis of immunogenicity and safety data

Geometric Mean Titres (GMT) and seroconversion rate (≥4-fold rise of anti-Vi-IgG antibody titre induced on day 30 compared to day 0 before vaccination) of anti-Vi-IgG antibody titre will be calculated. Analysis of covariance will be used to adjust for imbalances in baseline titres.

In addition, descriptive analyses of safety data will be done for AEFI or SAEs cases.

Shell table for effectiveness of the typhoid conjugate vaccine against culture-confirmed typhoid cases

Overall period	Typhoid Cases		Matched controls		Vaccine effectiveness (VE) (95% CI)			
	Vaccinees n/N (%)	Non-vaccinees n/N (%)	Vaccinees n/N (%)	Non-vaccinees n/N (%)	Crude VE	P value	Adjusted VE	P value
All ages								
6 months - <2 years								
2 - 4 years								
5 - 15 years								
First six months								
All ages								
Second six months								
All ages								

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.

Trial Monitoring

The study will be conducted by the currently approved protocol, ICH GCP, relevant regulations, and standard operating procedures. Data will be evaluated for compliance with the protocol and accuracy of source documents.

Monitoring will be performed by representatives of the icddr,b, and sponsor according to the principles of ICH GCP. The monitoring team will be independent and will not take part in any other study-related procedures.

Monitoring shall be carried out by the sponsor's Clinical Research Associate/Study Monitor or by personnel designated by sponsor. The frequency and methodology of conducting periodic monitoring visit(s) shall be further described in the study specific monitoring plan.

Quality Assurance

A quality assurance audit of this trial will be conducted by the sponsor or sponsor's designee. The quality assurance auditor will have access to all medical records, the investigator's trial related files and correspondence, and the informed consent form documentation that is relevant to this clinical trial.

Serious breaches

A serious breach is defined as "A breach of GCP or the study protocol which is likely to affect to a significant degree

(a) The safety or physical or mental integrity of the participants of the study; or
(b) The scientific value of the study.

If a serious breach is suspected, the DSMB, IRB, and Sponsor must be contacted within one working day.

Protocol deviations

A protocol deviation is any noncompliance with the clinical study protocol, GCP, or site SOP requirements. The noncompliance may be either on the part of the participant, the PI, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

• Compliance with Protocol
• Quality Assurance and Quality Control
• Noncompliance

It is the responsibility of the site to exercise continuous vigilance to identify and report deviations. All deviations must be promptly reported via the appropriate CRF. All deviations from the protocol must be addressed in study participant source documents. A completed copy of reportable protocol deviation forms must be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations must be sent to the icddr,b Ethical Review Committee (ERC) as per its guidelines. The site PI/study staff will be responsible for knowing and adhering to the icddr,b ERC requirements.

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]

Ethical and regulatory considerations

Declaration of Helsinki

The Investigator will ensure that this study will be conducted in accordance with the principles of the Declaration of Helsinki.

Guidelines for Good Clinical Practice (GCP)

The investigators will ensure that this study will be conducted in accordance of Good Clinical Practice and other relevant regulations. All personnel participating in the study will receive online GCP training to ensure their proficiency in these standards.

Approvals

The study protocol, informed consent, assent forms, and participant information sheet will be submitted to the Research Review Committee (RRC) and the Ethical Review Committee (ERC) of icddr,b for their approval.

The Principal Investigator (PI) at icddr,b will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Reporting

The PI will submit the reports to all committees (RRC, ERC, and sponsor) at the end of the study.

Participant confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant/vaccination ID number on all study documents and any electronic database, including the CRF. All documents will be stored securely and only accessible by study staff and authorized personnel. The study will comply with the icddr,b data policy, which requires data to be anonymized as soon as it is practical to do so, and local regulations.

Expenses and Benefits

There will not be any payments or reimbursements made to participants, as an incentive for participant recruitment. It is anticipated that the provision of vaccination will be enough incentive to reach the necessary sample size. Local hospitals, clinics, and vaccination points are being used to deliver all study vaccines, which will not add additional travel or expense to participants and their families. The study will cover the costs of standard care treatment for participants as part of the study, including the cost of tests, antibiotics, and/or other prescribed medications, and in-patient hospital stays and care, if medically necessary.

Other ethical considerations

All efforts will be made to conduct the research in a way that is sensitive to the Bangladeshi culture and social values. Trained study staff will be present at all times during the consent process, and the participant study-related materials (information sheet, consent forms, etc) will be printed in the local language.

The study will be conducted based on the ethical requirements of the icddr,b. Children aged 6 months to 15 years have been selected for the study because of the substantial burden of the disease in both mortality and morbidity, with increased demand for the vaccines.

All samples will be kept for a minimum of 5 years after the end of the study. Storage of these samples may also allow important future research to be done without needing to take new samples from the Bangladeshi healthy population.

Potential participants or their parents/guardians will be notified that they will be able to refuse to have the relevant biological samples stored, without influencing participation in the study or the clinical care of their child. They will also be informed that should they no longer wish for their samples to be retained they may request their destruction.

Use of Animals

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

Not applicable.

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 will be a collaborative study between icddr,b, and Zydus Lifesciences Limited. MTA, MoU, and other agreements are under process for this collaboration. After finalization, these documents will be submitted to the IRB.

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.

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

Study field office

The Maniknagar field office is located in the Dhaka Metropolitan area, approximately 9-10 km from the icddr,b Dhaka Hospital, and has been used for field studies since 2022. Dhaka South City Corporation is densely populated with approximately 3.5 million individuals. This research office has enjoyed a long-standing relationship with this stable community. The Maniknagar field office is composed of two floors, with each floor consisting of 3 rooms; approximately 3000 square feet of research space. This dedicated office building is situated within the same neighborhood as the study population. The facility contains a participant waiting area, an examination room, a staff work and file room, a specimen processing area, and a meeting room. The specimen processing area contains a refrigerator with 24-hour power backup. The building has 24-hour security coverage and internet service. The infrastructure of the facility has made it possible to carry out vaccine and other clinical studies.

Laboratory facilities

The Mucosal Immunology and Vaccinology Laboratory (MIVL) at the icddr,b is a Biosafety Level 2 (BSL2) facility with internal and external quality assurance carried out for tests. All SOPs for studies are updated frequently to meet study requirements. The laboratory is equipped with ten biohazard safety hoods for the processing of biological samples and for maintaining sterile conditions for specimen processing. For the fractionation of samples one bead beater is available, and for the separation of samples temperature-controlled tabletop centrifuges, high-speed centrifuges (Beckman) and ultracentrifuge (Beckman Coulter High Speed Centrifuge) are also available. Three Incubators with carbon-dioxide gas attachment for the study of B-cell and T-cell responses, four incubators and two shaker incubators for bacterial culture and Enzyme Linked Immunosorbent Assay (ELISA) are present. There is a cryostat for sectioning of frozen sections and a microtome for paraffin sections. Five ELISA readers (EON) linked to computers for determining antibody responses in study samples are present. Facilities for Enzyme Linked Immunosorbent Spot (ELISPOT) and Antibody in Lymphocyte Supernatant (ALS) assays are available. The Cellular Technology Limited (CTL) automatic immunospots counters as well as stereomicroscopes for enumeration of Antibody Secreting Cells (ASCs) are also available. There are facilities for carrying out the extraction of lymphocytes from gut biopsies. Two conventional polymerase chain reaction (PCR) machines, two real time PCR (RT-PCR) machines, and one TaqMan PCR machine are available for any preferable gene amplification. Two Luminex machine of xMAP company (Magpix and Intelliflex) and four Fluorescence-Activated Cell Sorting (FACS) machines (two Acuri, Fusion, and Aria III) are present for high throughput analysis. We have liquid nitrogen sample storage facility. In addition, facilities for carrying out intestinal lavages and fecal extracts are used for assessing mucosal antibody responses. There are several refrigerators and freezers maintained at 4°C, -20°C, and -80°C used for storing specimens from vaccine trials and other infectious disease studies.

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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