Phase II, randomized, double-blind, placebo-controlled study of the safety and immunogenicity of the recombinant live attenuated tetravalent dengue virus vaccine admixture TV005 (TetraVax-DV TV005) in healthy adults, adolescents, and children in Dhaka, Bangladesh

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Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
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National Institutes of Health (NIH)

Team Roster

Principal Investigator: Rashidul Haque, MBBS, PhD
Centre for Vaccine Sciences
International Centre for Diarrhoeal Disease Research, Bangladesh
Mohakhali, Dhaka -1212
+880- 0173093859 (phone)
+88 02 9827001-10 ext.3483 (office hours phone)

Co-Principal Investigator: Firdausi Qadri, PhD
Centre for Vaccine Sciences
International Centre for Diarrhoeal Disease Research, Bangladesh
Mohakhali, Dhaka, 1212 Bangladesh
+88 02 9827001-10 ext.2431 (office hours phone)

Co-Principal Investigator: Beth D. Kirkpatrick, MD
Vaccine Testing Center
University of Vermont College of Medicine
Given Building
89 Beaumont Avenue
Burlington, VT 05405
+1 802 656-5822 (phone)

Co-Principal Investigator: Anna Durbin, MD
Center for Immunization Research
Johns Hopkins Bloomberg School of Public Health
Johns Hopkins University
Hampton House, 624 N. Broadway
Baltimore, MD 21205
+1 410 614-4736 (phone)

Co-Investigator: Mohammad Shafiul Alam, PhD
Parasitology Research Group
International Centre for Diarrhoeal Disease Research, Bangladesh
Mohakhali, Dhaka, 1212 Bangladesh
+88 02 9827001-10 ext.3482 (office hours phone)

Co-Investigator: Farhana Khanam, MBBS
Centre for Vaccine Sciences

	International Centre for Diarrhoeal Disease Research, Bangladesh Mohakhali, Dhaka, 1212 Bangladesh +88 02 9827001-10 ext.2413 (office hours phone)
Co-Investigator:	Masud Alam, MBBS Centre for Vaccine Sciences International Centre for Diarrhoeal Disease Research, Bangladesh Mohakhali, Dhaka, 1212 Bangladesh +88 02 9827001-10 ext.3482 (office hours phone)
Co-Investigator:	Benjamin Lee, MD Vaccine Testing Center University of Vermont College of Medicine Given Building Burlington, VT 05405 +1 802 656-7748 (phone)
Co-Investigator:	Marya Carmolli Vaccine Testing Center University of Vermont College of Medicine Given Building Burlington, VT 05405 +1 802 656-9296 (phone)
Co-Investigator:	Mary Claire Walsh Vaccine Testing Center University of Vermont College of Medicine Given Building Burlington, VT 05405 +1 802 656-7764 (phone)
Scientific Investigators:	Stephen S. Whitehead, PhD Laboratory of Infectious Diseases National Institute of Allergy and Infectious Diseases, National Institutes of Health Room 3W10A 6, Building 33 33 North Drive Bethesda, MD 20892, USA Phone: 301-496-7692
Sponsor Medical Monitor:	Marc J. Teitelbaum, MD, MS Clinical Monitoring Research Program (CMRP) Support to Regulatory Compliance and Human Subjects Protection Program / NIAID / NIH Leidos Biomedical Research, Inc. Frederick National Laboratory for Cancer Research

5705 Industry Lane, Room 249
Frederick, Maryland 21704
Phone (301) 228-4707
Mobile (301) 917-4842
Marc.Teitelbaum@nih.gov

Shirley Jankelevich, MD
Clinical Monitoring Research Program (CMRP)
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
Support to: Regulatory Compliance & Human Subjects Protection Program / NIAID / NIH
5705 Industry Lane, Room 248
Frederick, Maryland 21704
Phone 301-846-7322
Fax 301-846-6224
shirley.jankelevich@nih.gov

Clinical Study Site: International Centre for Diarrhoeal Disease Research, Bangladesh
GPO Box 128
Mohakhali, Dhaka, 1212 Bangladesh

Research Laboratory: International Centre for Diarrhoeal Disease Research, Bangladesh
GPO Box 128
Mohakhali, Dhaka, 1212 Bangladesh

Research Laboratory: Vaccine Testing Center
University of Vermont College of Medicine
Given Building
89 Beaumont Avenue
Burlington, VT 05405

Clinical Laboratory: International Centre for Diarrhoeal Disease Research, Bangladesh
GPO Box 128
Mohakhali, Dhaka, 1212 Bangladesh

Institutional Review Board: Ethical Review Committee
(ICDDR,B) International Centre for Diarrhoeal Disease Research, Bangladesh GPO Box 128
Mohakhali, Dhaka, 1212 Bangladesh

Institutional Review Board: Research Review Committee
(ICDDR,B) International Centre for Diarrhoeal Disease Research, Bangladesh GPO Box 128
Mohakhali, Dhaka, 1212 Bangladesh

Institutional Review Board (UVM): Research Protections Office
Institutional Review Board
University of Vermont
213 Waterman Building
85 South Prospect Street
Burlington, VT 05405
802-656-5040 (phone)

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Table 1: List of Abbreviations

Abbreviation	Definition
AE	Adverse event
ALT	alanine transaminase
ANC	Absolute neutrophil count
AST	aspartate aminotransferase
CBC	complete blood count
CIR	Center for Immunization Research
CMI	Cell mediated immunity
CRF	Case report form
CRL	Charles River Laboratories
DENV	Dengue viruses (serotypes DEN1, DEN2, DEN3, and DEN4)
DF	Dengue fever
DGDA	Directorate General of Drug Administration
DHF	Dengue haemorrhagic fever
DSMB	Data Safety Monitoring Board
DSMP	Data safety monitoring plan
DSS	Dengue shock syndrome
EPI	Expanded Program on Immunization
ERC	Ethical Review Committee (icddr,b regulatory body)
FA	Field Assistant
FDA	Food and Drug Administration
FRA	Field Research Assistant
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
ICH	International Conference on Harmonization
IND	Investigational new drug
IRB	Institutional Review Board
JHSPH	Johns Hopkins Bloomberg School of Public Health
LID	Laboratory of Infectious Diseases
MID_{50}	50% Mosquito infectious dose
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NS	Non-structural
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
PBMC	Peripheral blood mononuclear cell
PFU	Plaque-forming units
PI	Principal Investigator
PRNT	Plaque reducing neutralizing antibody titer assay
PT/PTT	prothrombin time/ partial thromboplastin time
RCHSPB	Regulatory Compliance and Human Subjects Protection Branch
ROP	Retro-orbital pain

RRC	Research Review Committee (icddr,b regulatory body)
SAE	Serious adverse event
SCID	Severe combined immunodeficiency
SE	Standard error
SERF	Safety expedited report form
SOP	Standard Operating Procedure
SRCP	Safety Review and Communication Plan
SSP	Study Specific Procedure
SUSAR	Serious and unexpected adverse reaction
TV005	Tetravalent 005
UAE	Unexpected adverse event
UP	Unanticipated problem
UTR	untranslated region
WHO	World Health Organization
Wt	Wild type

Protocol Summary:

Principal Investigator: Dr. Rashidul Haque

Research Protocol Title: Phase II, randomized, double-blind, placebo-controlled study of the safety and immunogenicity of the recombinant live attenuated tetravalent dengue virus vaccine admixture TV005 (TetraVax-DV T005) in healthy adults, adolescents, and children in Dhaka, Bangladesh

Proposed start date: January 2016 **Estimated end date:** February 2020

Background (brief):

a. Burden:

Dengue viruses (DENV) are now the leading arboviral infection globally, with over 2 billion persons at risk of infection with continually expanded regions affected by dengue [1]. The first dengue fever outbreak in Bangladesh occurred in 2000, and the burden of dengue disease in this area has sustained since then [2].

b. Knowledge gap:

A dengue vaccine has not yet been tested on the Indian subcontinent, despite very large populations of persons at risk for infection. In Dhaka, an extremely densely populated city of 12-20 million inhabitants, antibody testing demonstrates that 80% of the population (9.6-16 million persons) has had exposure to dengue.

c. Relevance:

Dengue is an emerging and reemerging arboviral disease of great global public health importance.
Since the outbreak in 2000, dengue fever has had a continued presence throughout Bangladesh. In recent time several live attenuated vaccines for dengue have been developed and tested in different parts of the world. Understanding the safety and immunogenicity of a live attenuated vaccine has never been assessed in Bangladeshi population. Successful completion of this study will give us safety and immunogenicity data of such vaccine in Bangladesh.

Objectives:

Primary Objectives

- Evaluation of the safety of TV005 live-attenuated dengue vaccine in healthy adults, followed by adolescents, and children in a dengue-endemic area of Dhaka (Mirpur), Bangladesh
- Evaluation of vaccine immunogenicity based on neutralizing antibody level to DEN1, DEN2, DEN3, and DEN4 at days 14, 28, 56, and day 180.

Secondary Objectives

- To measure the frequency, quantity, and duration of viremia after vaccination (both vaccine and natural dengue virus) measured at day 7 and 14.
- To determine the number of vaccinees with recoverable vaccine virus or wild type (wt) dengue virus after vaccination.
- To determine the effect of pre-existing dengue antibodies on recovery of vaccine virus and or frequencies of seroconversion in each age group.
- To determine the durability of neutralizing antibody response at 1 year, 2 years, and 3 years after vaccination with TV005.

Methods:

A randomized, step-wise, double-blind, placebo- controlled study design will be employed with 4 age de-escalation cohorts. 192 study participants (48 in each age cohort) will be enroled. Study participants will be screened, and enroled to receive either TV005 vaccine or placebo. There will be 8 follow-up visits to collect information on vaccine safety and immunogenicity. Evaluation of vaccine safety will be determined by the frequency of solicited and unsolicited adverse events. Evaluation of immunogenicity will be measured by Plaque Reducing Neutralizing Antibody Titer (PRNT) assay.

Outcome measures/variables:

The main outcome variables are the following:

- Proportion of study participants with solicited or unsolicited Adverse Events.
- Proportion of study participants who are seropositive or seroconvert following vaccination.
- Proportion of study participants with vaccine virus recovered following vaccination.
- Proportion of study participants with recoverable vaccine virus or wild type virus after vaccination

1 Description of the Research Project

Specific Objectives:

1.1 Primary Objectives

The primary objectives are to determine the safety, immunogenicity, and durability of the TV005 tetravalent live attenuated dengue vaccine in healthy adults followed by assessment in adolescents and children residing in an endemic area in Mirpur, Dhaka Bangladesh.

1 Determine the safety of the TV005 tetravalent live- attenuated dengue vaccine as assessed by the frequency of vaccine-related adverse events (AEs), graded by severity. The study will begin in adults and age de-escalate by cohort based upon the interpretation of safety and immunogenicity data for the adult cohort, provided that stopping criteria are not met following analysis of safety data through Study Day 28 by the Data and Safety Monitoring Boards (DSMBs).

2 Determine the immunogenicity of the TV005 tetravalent live attenuated dengue virus (DENV) vaccine, as assessed by neutralizing antibody titers to DENV-1, DENV-2, DENV-3, and DENV-4 at 28, 56, and 180 days after each vaccination. Monovalent, bivalent, trivalent, and tetravalent seroconversion rates will be determined.

1.2 Secondary Objectives

1 Assess the frequency, quantity, and duration of vaccine viremia following vaccination for both vaccine and wild-type dengue.

2 Determine the number of vaccinees with recoverable DENV-1, DENV-2, DENV-3, and DENV-4 vaccine virus components or wild-type dengue virus during the first 14 days following vaccination. This is defined as recovery of vaccine virus from the blood or serum of a participant and/or by new seropositivity (Plaque Reducing Neutralizing Antibody Titer $(PRNT)_{50} \geq 1:10$) in a previously seronegative study participant or seroconversion to DENV ($\geq$ 4-fold rise in neutralizing antibody titers against DENV-1, DENV-2, DENV-3, or DENV-4) in those volunteers who were seropositive at baseline.

3 Determine the effect of pre-existing DENV antibodies on recovery of vaccine virus from the blood or serum of a study participant and/or seroconversion frequencies to DENV among the different age groups.

4 Determine the durability of neutralizing antibody response at 1 year, 2 years, and 3 years after vaccination with TV005.

1.3 Exploratory Objectives

To evaluate the individual immune response to vaccination examining the following:

1 New serological measures of immunogenicity and vaccine efficacy surround neutralization and enhancement.

2 Cell-mediated immunity including stimulation of T and B cells for measures of adaptive immune response post vaccination

3 Evaluation of innate immune response post vaccination

4 Other immunologic assays may be performed in response to the development of additional assay techniques and current literature and scientific knowledge over the course of the study.

2 Introduction

2.1 Background - Dengue

The World Health Organization (WHO) estimates that dengue fever (DF) is the fastest emerging arboviral infection and is spread by *Aedes* mosquitos. Dengue has far-reaching public health consequences throughout the tropical and sub-tropical regions of the world, primarily in urban and semi-urban areas. Globally, over 2.5 billion people--over 40% of the world's population--are at risk for DF, and more than 75%, or 1.8 billion, live in the Asia- Pacific region (3, 4). WHO estimates that there may be 50-100 million dengue infections worldwide each year (1) and approximately one half million cases annually of the more severe disease, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) (3). An estimated 90% of those individuals with severe dengue infection requiring hospitalization are children less than 5 years of age. Infection with dengue virus (DENV) is the leading cause of hospitalization and death in children in at least 8 tropical Asian countries (4). For these reasons, the WHO has made development of a dengue vaccine a top priority (Resolution WHA 46.31).

Dengue virus was first documented in Bangladesh, known as East Pakistan at the time, in 1964, when the DENV-3 serotype was isolated from a patient with febrile illness. Since this first documented illness, sporadic suspected cases of dengue fever have occurred in Bangladesh between the 1960's and the 1990's (5). In the summer of 2000, the first outbreak of dengue fever and dengue haemorrhagic fever occurred in the major cities of Bangladesh with over 5000 cases of dengue fever reported to the Ministry of Health. While the majority of the cases were dengue fever, approximately 25% of cases were dengue haemorrhagic fever (2). Since that outbreak, dengue fever has had a continued presence throughout Bangladesh. One study has shown the overall seroprevalence of dengue in Dhaka to be approximately 80% (5).

There are 4 serotypes of DENV (DENV-1, DENV-2, DENV-3, and DENV-4), each capable of causing dengue infection ranging from asymptomatic infection or mild and self-limited febrile illness, to a life-threatening disease. Infection with one dengue serotype induces long-lived homotypic immunity to the infecting serotype and short-lived heterotypic immunity to the other serotypes (6). Immunity appears mediated, at least in part, by neutralizing antibodies against the envelope (E) glycoprotein.

Epidemiological studies have demonstrated that most cases of DHF/DSS occur in persons experiencing a second dengue infection with a serotype different from that which caused their first dengue infection (7). For this reason, DHF/DSS occurs predominately in children or adults living in dengue-endemic regions with multiple DENV serotypes circulating simultaneously.

The goal of immunization is to induce a long-lived protective immune response against all 4 DENV serotypes. This can be best and most economically achieved using a live attenuated tetravalent virus vaccine delivered in one or more doses. Development of a live attenuated vaccine is a reasonable goal since it has already been achieved for other related mosquito-borne flaviviruses, including the yellow fever and Japanese encephalitis viruses which are also endemic to tropical regions of the world (3, 8).

In humans, DENV infects predominately monocytes, dendritic cells, and lymphocytes and does not exhibit tropism for any particular organ, except perhaps the liver (9). The majority of primary and secondary infections with DENV are asymptomatic. Following an incubation period of approximately 1 week, self-limiting acute illness, (DF), occurs and is characterized by a febrile period of about 5 days accompanied by systemic symptoms such as headache, malaise, anorexia, arthralgia, and myalgia. Rash (including petechial haemorrhages), lymphadenopathy, leukopenia, thrombocytopenia, and viremia can accompany the fever. Elevations of liver enzymes during DF are common, and the virus is known to infect human hepatocytes (10-12). The virus does not establish persistent infection and is usually eliminated by the end of the second week.

DHF/DSS occurs much less commonly than DF. It develops at the time of defervescence and is characterized by an increased tendency to bleed into the skin or from mucous membranes and by a marked increase in vascular permeability resulting in haemoconcentration and shock. This state of increased vascular permeability is short-lived (a few days) and with proper management is fatal in only about 1% of patients (3). Because previous infection with one DENV serotype can increase the risk for DHF/DSS following infection with a different serotype, it is clear that a dengue vaccine will need to protect against each of the 4 DENV virus serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4.

The DENV genome contains a single open reading frame encoding a polyprotein that is processed by proteases of both viral and cellular origin into 3 structural proteins, namely the capsid (C), membrane, (M) and envelope (E) proteins and at least 7 non-structural (NS) proteins. Each end of the DENV genome consists of an untranslated region (UTR) that is predicted to be highly structured. The E glycoprotein is on the surface of the virion, and immunity is mediated primarily by neutralizing antibodies to this protein. The E protein also uniquely defines each of the 4 DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). The 3′ untranslated region (UTR) is highly conserved between the 4 DENV serotypes.

Studies in mice, Rhesus monkeys, and mosquitoes were designed to evaluate the level of attenuation conferred independently by chimerization and the Δ30 (30 nucleotide deletion in the 3′UTR) mutation (13). This attenuation strategy has been successfully used to design the TV005 tetravalent vaccine proposed to be tested in this clinical trial. The specific objectives of this study are to evaluate the safety, infectivity, and immunogenicity of the TV005 vaccine admixture in a dengue endemic area where volunteers have been variously exposed to DENV. Since the greatest need for a dengue vaccine is among young children living in dengue endemic regions, the planned study is designed as an age de-escalation strategy. The TV005 admixtures will be comprised of four monovalent dengue vaccine candidates representing each of the four DENV serotypes: rDEN1Δ30, rDEN2/4Δ30(ME), rDEN3Δ30/31, and rDEN4Δ30.

2.2 Background - Dengue Vaccines

Numerous studies of wild-type (wt) DENV and live attenuated dengue vaccine candidates have been conducted over the past 60 years. Various live attenuated dengue vaccine candidate viruses have been administered to nearly 1500 study participants in Phase 1 and Phase 2 clinical studies (14-24). The majority of dengue vaccines tested to date have been biologically derived by repeated passage in tissue culture. Although some of these vaccine candidates have appeared promising in early clinical development, none has achieved the ideal balance between reactogenicity and immunogenicity, particularly when included as part of a tetravalent vaccine formulation (22, 23, 25). It has been difficult to achieve adequate antibody responses to all 4 serotypes when the vaccine viruses are administered in the tetravalent formulation.

Common adverse events (AEs) noted in these studies were signs and symptoms of mild DF, notably fever, headache, myalgias, rash, neutropenia, and elevated liver function tests. In the published literature, all subjects who experienced an AE recovered completely and without sequelae. To date, no live attenuated dengue vaccine candidate has induced DHF or DSS. The lack of a suitable licensed tetravalent live attenuated vaccine candidate forms the basis for testing the TV005 candidate vaccine described in this protocol.

2.3 Vaccine Description

The admixture of the candidate vaccine contains 4 live attenuated dengue viruses, each of a different serotype. The description of each component is described below. The monovalent vaccine candidates may be stored at -80°C ± 15°C.

The components of this vaccine candidate have collectively been administered to many volunteers without any significant adverse or permanent effect, however the formulation of the vaccine used in this trial is not a licensed product (24, 27, 35-40). TV005 is currently being evaluated for safety and immunological effects which may result in licensure.

2.3.1 rDEN1Δ30 Vaccine

The vaccine candidate rDEN1Δ30 is a live attenuated virus derived from the DEN1 Western Pacific (WP) wt strain of DENV using recombinant DNA technology. A full-length cDNA clone of the DEN1 WP genome was constructed and a 30-nucleotide deletion was then created in the 3′UTR. Genome-length, capped, RNA transcripts were synthesized, purified, and transfected into qualified Vero cells. Recovered virus was terminally diluted and then amplified by serial passaging in Vero cells. The final amplification of virus was made in serum-free medium, and the titer was determined by plaque titration in Vero cells. From initial transfection through final amplification, only serum-free medium was used for Vero cell culture and propagation of virus. Reagents used during the transfection process and present initially in fluid harvested from the transfection were diluted more than 10^{12}-fold as a result of the biological cloning (terminal dilution) and amplification of the virus. The rDEN1Δ30-1545 seed virus was generated in the Laboratory of Infectious Diseases (LID), National Institute of Allergy and Infectious Diseases (NIAID), and the vaccine was manufactured at Charles River Laboratories (CRL) Biopharmaceutical Services facility in Malvern, PA. The Final Drug Product, rDEN1Δ30-1545 (Lot DEN1#104A) was manufactured in qualified Vero

cells at CRL on 26 August 2004 based on a method developed by LID/NIAID/National Institutes of Health (NIH) and CRL.

2.3.1.1 Final Container rDEN1Δ30

The Final Drug Product was dispensed as 0.6 mL aliquots of approximately $10^{6.8}$ plaque-forming units [PFU]/mL Live Recombinant Dengue Virus Type 1 rDEN1Δ30-1545 Vero Grown Virus Vaccine into 2.0 mL sterile cryovials and stored at -80°C ± 15°C.

2.3.1.2 Composition rDEN1Δ30

The Final Drug Product composition is a concentration of live attenuated rDEN1Δ30-1545 (Lot DEN1#104A) in Leibovitz L-15 Medium containing 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; mono-sodium glutamate, 0.0054 M). The potency of rDEN1Δ30-1545 (Lot DEN1#104A) is $10^{6.8}$ PFU/mL.

2.3.1.3 Investigational Product Label rDEN1Δ30

0804- Vial # Lot DEN1#104A	Live Recombinant Dengue Virus Type 1 rDEN1Δ30-1545 Vero Grown Virus Vaccine
	CAUTION: NEW DRUG LIMITED BY FEDERAL (USA) LAW TO INVESTIGATIONAL USE
	Store at -70°C or below Charles River Laboratories Malvern PA

Figure 1: Label for Final Vial rDEN1Δ30-1545 (Enlarged Sample)

*NOTE: Since manufacture of the Final Drug Product, evaluation of live attenuated dengue virus vaccines (developed at the LID/NIH/ NIAID) in both laboratory and clinical situations indicates that these viruses are stable and potent when stored at a temperature of -80°C ± 15°C. To maintain consistency in Investigational Product storage requirements, all vaccine monovalent candidates will be stored together in a dedicated, locked, and secure freezer at -80°C ± 15°C, and the manufacturer's stability data and confirmation that this storage parameter will be adequate for vaccine storage is documented in the Investigator's Brochure.

2.3.2 rDEN2/4Δ30 (ME) Vaccine

The rDEN2/4Δ30 (ME) virus is a live attenuated chimeric virus derived from rDEN4Δ30. A full-length cDNA copy of rDEN4Δ30 was constructed in which the M and E coding sequences of DEN4 were replaced with those of DEN2 NGC prototype. Genome-length, capped, RNA transcripts were synthesized, purified, and transfected into qualified Vero cells. Recovered virus was terminally diluted and then amplified by serial passaging in Vero cells. The final amplification of virus was made in serum-free medium and the titer was determined by plaque titration in Vero cells. The rDEN2/4Δ30(ME)-1495,7163 seed virus was generated in LID/NIAID and then manufactured at Charles River Laboratories Biopharmaceutical Services facility in

Malvern, PA. The Final Drug Product, rDEN2/4Δ30(ME)-1495,7163 (Lot DEN2/4#106C) was manufactured in qualified Vero cells at CRL on 8 February 2007 based on a method developed by LID/NIAID/NIH and CRL.

2.3.2.1 Final Container rDEN2/4Δ30(ME)

The Final Drug Product was dispensed as 0.6 mL aliquots of approximately $10^{7.8}$ PFU/mL Live Recombinant Dengue Virus Type 2 rDEN2/4Δ30(ME)-1495,7163 Vero Grown Virus Vaccine into 2.0 mL sterile cryovials (Corning #430659) and is stored at -80°C ± 15°C.

2.3.2.2 Composition rDEN2/4Δ30(ME)

The Final Drug Product composition is a concentration of live attenuated rDEN2/4Δ30(ME)-1495,7163 (Lot DEN2/4#106C) in Leibovitz L-15 Medium containing 1X SPG. The potency of rDEN2/4Δ30(ME)-1495,7163 (Lot DEN2/4#106C) is $10^{7.8}$ PFU/mL.

2.3.2.3 Investigational Product Label rDEN2/4Δ30(ME)

Live Recombinant Dengue Virus Type 2 rDEN2/4Δ30(ME)-1495,7163 VERO Grown Virus Vaccine	**Lot DEN 2/4 #106C 0207-vial #**
CAUTION: NEW DRUG LIMITED BY FEDERAL (USA) LAW TO INVESTIGATIONAL USE	
Store at -70±10°C Charles River Laboratories, Malvern, PA	

Figure 2: Label for Final Vial rDEN2/4Δ30(ME)-1495, 7163 (Enlarged Sample)

2.3.3 rDEN3Δ30/31 Vaccine

rDEN3Δ30/31 was constructed by further mutating the DEN3 3′ UTR of the DEN3 Sleman/78 by combining the Δ30 deletion with an additional, 31 nucleotide deletion in the DEN3 3′UTR(23). Since the flavivirus 3′ UTR is non-coding, it is well suited for use as a genetic element to condition attenuation in DENV vaccine candidates. The attenuation phenotype of the rDEN3Δ30/31 virus vaccine is expected to be phenotypically stable since the Δ30 mutation was shown to be stable following administration of the DEN4Δ30 virus vaccine to human study participants (24). Genome-length, capped, RNA transcripts were synthesized from the linearized p3Δ30/31 clone #28 using the AmpliCap SP6 Message Maker Kit (EpiCentre Technologies, Madison, WI; Lot AC650408) and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA).

Virus was recovered in qualified C6/36 cells (Sponsor's Master File, MF #11371) transfected on 6 January 2006 with purified RNA transcripts using DOTAP liposomal transfection reagent (Roche, Indianapolis, IN). An aliquot of the seed virus was submitted to Charles River Laboratories Biopharmaceutical Services (Malvern, PA), for production of a second lot of vaccine. The lot was amplified by 1 passage on qualified

Vero cells in serum free medium, medium containing buffer and essential amino acids and not containing fetal bovine serum, to produce the Final Drug Product, rDEN3Δ30/31Lot DEN3#113B. Final drug product was manufactured on 18 January 2012.

2.3.3.1 Final Container of rDEN3Δ30/31

The Final Drug Product was dispensed as 0.6 mL aliquots of approximately $10^{7.7}$ PFU/mL of Live Recombinant Dengue Virus Type 3 rDEN3Δ30/31 Vero Grown Virus Vaccine in L-15 medium containing 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; mono-sodium glutamate, 0.0054 M) into 2.0 mL sterile cryovials and is stored at -80°C ± 15°C.

2.3.3.2 Composition rDEN3Δ30/31

The Final Drug Product composition is a concentration of live attenuated rDEN3Δ30/31 in Leibovitz L-15 medium containing 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; mono-sodium glutamate, 0.0054 M). The titer of rDEN3Δ30/31 (Lot DEN3#113B) is $10^{7.7}$ PFU/mL.

2.3.3.3 Investigational Product Label rDEN3Δ30/31

Live Recombinant Dengue Virus Type 3 rDEN3Δ30/31 VERO Grown Virus	**Date: 18JAN2012 Vial#: XXXX Lot: DEN3#113B**
CAUTION: NEW DRUG LIMITEDBY FEDERAL (USA) LAW TO INVESTIGATIONAL USE	
Store at -70±10°C Charles River Laboratories, Malvern, PA	

Figure 3: Label for Final Vial rDEN3Δ30/31 (Enlarged Sample)

2.3.4 rDEN4Δ30 Vaccine

The vaccine candidate rDEN4Δ30 is a live attenuated virus derived from the DEN4 Dominica/81 wt strain of DENV using recombinant DNA technology. A full-length cDNA clone of the DEN4 Dominica/81 genome was constructed and a 30-nucleotide deletion was then created in the 3′ UTR. Genome-length, capped, RNA transcripts were synthesized, purified, and transfected into qualified Vero cells. Recovered virus was terminally diluted and then amplified by serial passaging in Vero cells. The final amplification of virus was made in serum-free medium, and the titer was determined by plaque titration in Vero cells. From initial transfection through final amplification, only serum-free medium was used for Vero cell culture and propagation of virus. The rDEN4Δ30-7132,7163,8308 seed virus was generated at LID/NIAID, and vaccine was manufactured at Charles River Laboratories Biopharmaceutical Services facility in Malvern, PA. The Final Drug Product, rDEN4Δ30-7132,7163,8308 (Lot DEN4#109A) was manufactured in qualified Vero cells at CRL on 18 July 2007 based on a method developed by LID/NIAID/NIH and CRL.

2.3.4.1 Final Container rDEN4Δ30

The Final Drug Product was dispensed as 0.6 mL aliquots of approximately $10^{7.2}$ PFU/mL Live Recombinant Dengue Virus Type 4 rDEN4Δ30-7132,7163,8308 Vero Grown Virus Vaccine into 2.0 mL sterile cryovials (Corning #430659) and stored at -80°C ± 15°C.

2.3.4.2 Composition rDEN4Δ30

The Final Drug Product composition is a concentration of live, attenuated rDEN4Δ30-7132,7163,8308 (Lot DEN4#109A) in L-15 medium containing 1X SPG. The potency of rDEN4Δ30-7132,7163,8308 (Lot DEN4#109A) is $10^{7.2}$ PFU/mL.

2.3.4.3 Investigational Product Label rDEN4Δ30

Live Recombinant Dengue Virus Type 4 rDEN4Δ30-7132,7163,8308 VERO Grown Virus Vaccine	**Lot DEN 4 #109A 0707-vial #**
CAUTION: NEW DRUG LIMITED BY FEDERAL (USA) LAW TO INVESTIGATIONAL USE	
Store at -70±10°C Charles River Laboratories, Malvern, PA	

Figure 4: Label for Final Vial rDEN4Δ30-7132, 7163, 8308 (Enlarged Sample)

2.4 Rationale

2.4.1 Animal Studies

Each of the component monovalent vaccine viruses of TV005 has been evaluated in juvenile rhesus monkeys and in the novel rodent model consisting of severe combined immunodeficiency (SCID) mice bearing intraperitoneal tumors of the human liver cell line HuH-7. Replication of these candidate vaccine viruses in the SCID-HuH-7 mouse model was compared with that of their wt parent viruses. The candidate vaccine viruses were attenuated in SCID-HuH-7 mice, with peak decreases in peak titers ranging from 6-fold (rDEN4Δto to 200-fold (rDEN1Δ30) lower than that of their wt parent DENV (13, 26, 28, 29). Juvenile Rhesus monkeys were inoculated subcutaneously with monovalent doses (10^5 PFU) of rDEN1Δ30, rDEN2/4Δ30(ME), rDEN3-3′D4Δ30, rDEN3Δ30/31, rDEN4Δ30, or the wt parent DENV (10, 23, 25, 26). All monkeys inoculated with wt virus became viremic with the mean duration of viremia ranging from 2.8 days (DENV-1) to 5.5 days (DENV-2) (Table 2). Fewer monkeys inoculated with the candidate vaccine viruses were viremic compared with the parent virus for each vaccine candidate except rDEN4Δ30. In addition, the mean number of days of viremia and the mean peak titer were also reduced for each candidate vaccine virus compared with its parent virus (Table 2).

Table 2: Serum antibody responses induced by TV005 when given as a single subcutaneous dose

Vaccine candidate	Dose (log_{10} PFU)	No. of monkeys	No. Viremic	Mean # of days viremic	Mean peak serum titer (log_{10}PFU/ mL)
DENV-1 (wt)	5	4	4	2.8	2.1 ± 0.1
rDEN1Δ30	5	4	2	0.5	0.8 ± 0.1
DENV-2 (wt)	5	4	4	5.5	2.1 ± 0.15
rDEN2/4Δ30(ME)	5	4	1	0.3	0.7 ± 0.02
DENV-3 (wt)	5	4	4	3.5	1.8 ± 0.1
rDEN3Δ30/31	5	4	0	n/a	n/a
DENV-4 (wt)	5	4	4	3	2.7 ± 0.09
rDEN4Δ30	5	6	6	2.5	2.0 ± 0.15

In early experiments, a tetravalent admixture of the live attenuated dengue vaccine candidates was evaluated for safety/toxicity, virus replication, and immunogenicity in Rhesus macaques (30), but contained a DENV-3 component different than that subsequently tested in humans. The admixture included rDEN1Δ30, rDEN2/4Δ30(ME), rDEN3-3′D4Δ30, and rDEN4Δ30 (designated 'TV2'). The animals received 10^5 PFU of each of the component viruses in the admixture in a single subcutaneous injection of 0.5 mL. Monkeys were observed twice daily for mortality and morbidity, as well as for signs of neurologic, haemorrhagic, or dermal disease. No signs consistent with these diseases were observed in any of the animals. Multiple episodes of lymphopenia were seen in all monkeys inoculated with TV2, and in 3 of the 4 monkeys inoculated with placebo. These results were seen throughout the study, including the samples taken on Day -8 and Day 0 prior to inoculation. Mild deviations from the normal reference range were noted on the clinical pathology profiles for most monkeys, but are not likely to be of statistical or biological significance. The clinical profile tested included complete blood count (CBC)and differential, clotting profile (Prothrombin time/Partial thromboplastin time(PT/PTT)), clinical chemistry (Albumin, alanine transaminase (ALT), aspartate aminotransferase (AST), Calcium, Chloride, Cholesterol, Creatinine, Gamma glutamyltransferase (GGT), Globulin, Glucose, Lipase, Magnesium, Osmolality, Phosphorus, Potassium, Sodium, Total Bilirubin, Total Protein, and Triglyceride). Mild transient elevations in ALT and AST not related to the vaccine were seen.

The DENV-4 component of the tetravalent vaccine was the only virus detected in any of the monkeys inoculated with the TV2 admixture. The rDEN4Δ30 vaccine virus was detected at low titer ($10^{1.0}$ to $10^{1.3}$ PFU/mL) in 3 of the 4 monkeys that received TV2, and only on Study Day 2. These findings confirm the attenuation (the absence of dengue vaccine virus detected in serum or dengue vaccine virus detected at a significantly lower level than seen in wild type virus (Table 2)) of each of the component vaccine viruses.

Despite the low or undetectable levels of viremia observed in the animals, the tetravalent vaccine admixture elicited neutralizing antibody titers against the component vaccine viruses. Inoculation of monkeys with TV2 resulted in 100% seroconversion to DENV-1, DENV-2, and DENV-4, and 50% to DENV-3 component. Geometric mean titers for TV2 were ≥ 39, ≥69, ≥ 50, ≥ 70 for the DENV-1, DENV-2, DENV-3, and DENV-4 components respectively.

In summary, the TV2 tetravalent dengue virus vaccine formulation was safe and well-tolerated in Rhesus monkeys and elicited antibody responses against the component viruses.

2.4.2 Clinical Experience with Recombinant Live Attenuated Dengue Vaccine Candidates

Each of the candidate live attenuated DENV vaccines included in TV005 has been evaluated in at least 1 Phase 1 clinical trial as a monovalent vaccine (Table 3). These trials were conducted at the Center for Immunization Research (CIR) at the Johns Hopkins Bloomberg School of Public Health (JHSPH) and at the University of Vermont Vaccine Testing Center. The experience with each of these vaccine candidates is summarized below. The absence of clinically apparent dengue-like illness is likely a result of the high degree of attenuation of the vaccine viruses. Peak viremia titers observed in the studies described below were more than 1,000-fold lower than those observed with symptomatic wildtype DENV infection (31-33).

Table 3: Monovalent components of TetraVax-DV

Vaccine candidate	Lot number	IND number
rDEN1Δ30	DEN1#104A	IND 11677
rDEN2/4Δ30(ME)	DEN2/4#106C	IND 11938
rDEN3Δ30/31	M10377008 DEN3#113B	IND 13886
rDEN4Δ30	DEN4#109A	IND 8463

2.4.2.1 Clinical Experience with LATV DEN1, DEN2, DEN3, and DEN4 Monovalent Vaccines

rDEN1Δ30 was first evaluated at a dose of 10^3 PFU in 2 placebo-controlled Phase 1 studies at the CIR/JHSPH(34). In a single-dose-study, 28 healthy male and non-pregnant female adult study participants, between the ages of 18 and 50, were enroled and were randomly assigned to receive vaccine or placebo. Twenty study participants received vaccine; eight study participants received placebo (vaccine diluent).

In a 2-dose schedule comparison study of rDEN1Δ30 lotDEN1#104A, 2 cohorts of 30 flavivirus-naïve healthy male and non-pregnant female adult study participants between the ages of 18 and 50 were randomly assigned to receive their second dose of vaccine either 120 days or 180 days following their initial dose (35). In each cohort, 25 study participants were randomly assigned to receive vaccine and 5 study participants were randomly assigned to receive placebo. One study participant in this study was replaced after receiving vaccine and a second study participant was replaced after receiving placebo. Thus far, a total of 71 study participants have received rDEN1Δ30 at a dose of 10^3 PFU. The vaccine was found to be safe and immunogenic (Table 4, Table 5, and Table 6**)**. The candidate vaccine was further studied at a dose of 10^1 PFU to determine the 50% human infectious dose (HID_{50}) of this candidate vaccine. Fourteen out of 15

vaccinees (93%) were infected with 10^1 PFU of vaccine, indicating that the HID_{50} is well below 10^1 PFU.

A total of 40 adult flavivirus-naïve study participants have received the rDEN2/4Δ30(ME) candidate DENV-2 vaccine at a dose of 10^3 PFU. rDEN2/4Δ30(ME) was first evaluated at a dose of 10^3 PFU in a Phase 1, placebo-controlled trial conducted at the CIR(36). Twenty-eight healthy male and non-pregnant female adult study participants, between the ages of 18 and 50, were enroled; 20 study participants received vaccine, 8 study participants received placebo (vaccine diluent). The vaccine was found to be safe and immunogenic (Table 4, Table 5 and Table 6). rDEN2/4Δ30(ME) was also evaluated in a 2-dose study at a dose level of 10^3 PFU, with the second dose given at 4 or 6 months post Dose 1. A total of 50 study participants were enroled (25 in each cohort). Twenty study participants received 10^3 PFU of rDEN2/4Δ30(ME) subcutaneously at Day 0 and again at 6 months. Five study participants were enroled as placebo-controls and received vaccine diluent at time 0 and 4 months. Because no subject was infected with the second dose given at 6 months, study participants were not enroled in the cohort evaluating a second dose given at 4 months. The vaccine was found to be safe, well tolerated, and immunogenic (**Tables 4, 5, and 6**).

Eighteen adult flavivirus-naïve study participants were enroled in a trial evaluating the safety and immunogenicity of rDEN2/4Δ30(ME) given as a single subcutaneous dose of 10^1 PFU. Fifteen study participants received vaccine and three received placebo. A dose of 10^1 PFU was given in order to determine the HID_{50} of the candidate vaccine. Fifty-three percent of vaccinees were infected by a dose of 10^1 PFU, indicating that the HID_{50} of this candidate is approximately 10^1 PFU.

rDEN3Δ30/31 has been evaluated at a dose of 10^3 PFU given as a single subcutaneous dose in a Phase 1, placebo-controlled trial conducted at the CIR and at the University of Vermont(37). Seventy male and non-pregnant female adult study participants between the ages of 18 and 50 were enroled. Fifty participants received vaccine and 20 received placebo (vaccine diluent). The vaccine was found to be safe and immunogenic (**Tables 4, 5, and 6**). The candidate vaccine was further studied at a dose of 10^1 PFU to determine the HID_{50} of this candidate vaccine. Eighteen out of 20 vaccinees (90%) were infected with 10^1 PFU of vaccine, indicating that the HID_{50} is well below 10^1 PFU. Because the clinical supply of Lot M10377008 of rDEN3Δ30/31 was exhausted, a second clinical lot of rDEN3Δ30/31, Lot DEN3#113B, was manufactured and has been used in all tetravalent studies initiated since 2012. Lot DEN3#113B will be used for this tetravalent study.

rDEN4Δ30, Lot 4-9 was evaluated in two clinical trials (27, 38). In the first study, 20 healthy male and non-pregnant female adult study participants received 10^5 PFU of the candidate vaccine as a single subcutaneous dose. In the second study, 20 healthy male and non-pregnant female adult study participants received this vaccine at a dose of 10^3 PFU, 10^2 PFU, or 10^1 PFU. Twelve study participants received vaccine diluent as a placebo. The vaccine was found to be safe and immunogenic (**Tables 4, 5, and 6**). The HID_{50} was determined to be less than 10 PFU. Five study participants who received 10^5 PFU of the rDEN4Δ30 candidate vaccine developed a transient elevation in serum ALT

levels. Elevation in serum ALT was abrogated by decreasing the dose of the candidate vaccine (Table 4**).** Thus far, a total of 80 study participants have received rDEN4Δ30.

Because the clinical supply of Lot# 4-9 of rDEN4Δ30 was exhausted, a second clinical lot of rDEN4Δ30, Lot#DEN4#109A, was manufactured. Seventy healthy adult male and non-pregnant female study participants were enroled and received a single subcutaneous 10^3 dose of rDEN4Δ30 (50 study participants) or placebo (20 study participants). The vaccine has been well tolerated by all study participants. The most common vaccine-related AE was asymptomatic, transient maculopapular rash in 20 of 50 (40%) vaccinees (**Table 4**). One vaccinee developed an elevated serum ALT level. Lot#DEN4109A will be used for this tetravalent vaccine study.

2.4.2.2 Summary of Clinical, Virologic, and Serologic Response to Monovalent Vaccines

All of the vaccines described above have been well tolerated and have been found to be both safe and strongly immunogenic when administered as monovalent vaccines. The safety, infectivity, and immunogenicity profiles of the monovalent vaccines were found to be comparable. **(**Table 4, Table 5, and Table 6)(37). Vaccinees did not develop a dengue-like illness at any dose (dengue-like illness is defined in Section 6.3.11.3). Local reactogenicity was minimal in all study participants. The most common AEs observed in all studies were transient neutropenia, rash, and headache (**Table 4**). Fifty-three of 231 vaccinees (23%) developed a transient neutropenia following vaccination. The neutropenia was graded as mild (1,000 – 1500/mm^3) in 48 vaccinees (16%), as moderate (750 – 1499/m^3) in 9 vaccinees (3%) and as severe (< 750/mm^3) in 7 vaccinees (2%). The neutropenia was of short duration (generally 3 – 5 days). All episodes of severe neutropenia were ≤ 4 days. None of the neutropenic study participants developed clinical complications. A total of 92 vaccinees (40%) developed a transient maculopapular rash over the trunk and proximal upper extremities following first vaccination. The rash was characteristically non-pruritic and went unnoticed by the majority of affected study participants. A total of 92 vaccinees (40%) complained of headache; however, this was comparable to the number of headaches reported in placebo recipients (36%). One of the 231 vaccinated study participants developed a fever, though this was determined to be not related to the vaccine. Other AEs experienced in the 28 day follow-up period following vaccination included arthralgia, myalgia, malaise, retro-orbital pain, and injection site reactions. These occurred in fewer than 10% of vaccinated study participants.

Vaccine virus was recovered from the blood of inoculated with each monovalent DENV candidate (**Table 5**). The mean peak titers of the monovalent DENV candidate vaccine viruses were very low, ranging from $10^{0.5}$ PFU/mL (recipients of 10^3 PFU of rDEN3Δ30/31 and rDEN4Δ30) to $10^{1.6}$ PFU/mL (recipients of 10^5 PFU of rDEN4Δ30) (**Table 5**).

Table 4: Clinical response to monovalent DENV vaccine candidates for use in study admixtures

Vaccine candidate	Dose (log_{10}PFU)	No. of participants	% viremic	No. of participants (%) with indicated clinical response				
				Fever	Rash	Headache	Neutropenia[2]	↑ALT
rDEN1Δ30	**3**	**71**	**60**	**1[1] (1.4)**	**22 (31)**	**29 (41)**	**32 (45)**	**1 (1.4)**
rDEN2/4Δ30(ME)	**3**	**40**	**60**	**0 (0)**	**13 (32)**	**12 (30)**	**11 (28)**	**3 (8)[2]**
rDEN3Δ30/31	**3**	**50**	**34**	**0 (0)**	**26 (52)**	**18 (36)**	**2 (4)**	**1 (2)[1]**
rDEN4Δ30 (Lot 4-9)	**3**	**20**	**35**	**0 (0)**	**11 (55)**	**7 (35)**	**5 (25)**	**1 (5)**
rDEN4Δ30 (Lot 109A)	**3**	**50**	**26**	**0 (0)**	**20 (40)**	**26 (52)**	**3 (6)**	**1 (2)**
Placebo[4]	n/a[5]	76	**0**	1 (1)	2 (3)	27 (36)	6 (8)	3 (4)

1. Not related to vaccine.
2. Peak ALT levels in these participants ranged from 1.3 to 1.7 x ULN (upper limit of normal). 2/3 participants with elevated ALT had an ALT above the ULN on Day 0 prior to vaccination despite being normal at screening
3. Participant recorded a fever on 1 occasion. Fever lasted only 2 hours.
4. Includes the placebo recipients enroled in the clinical trial of the monovalent candidates listed in this table.
5. Not applicable.

Table 5: Magnitude, onset, and duration of viremia in participants inoculated with monovalent DENV vaccine candidates for use in tetravalent vaccine admixtures

Vaccine candidate	Dose (log_{10}PFU)	N	(%) with viremia	Mean peak titer ± SE (log_{10} PFU/mL)[1]	Mean day of onset of viremia ± SE	Mean # of days of viremia ± SE
rDEN1Δ30	**3**	**71**	**60**	**1.0 ± 0.08**	**10.0 ± 0.3**	**3.3 ± 0.3**
rDEN2/4Δ30(ME)	**3**	**40**	**60**	**0.5 ± 0.03**	**9.2 ± 0.6**	**3.3 ± 0.6**
rDEN3Δ30/31	**3**	**50**	**34**	**0.6 ± 0.01**	**7.6 ± 0.64**	**3.2 ± 0.5**
rDEN4Δ30 (Lot 4-9)	**5**	**20**	**70**	**1.6 ± 0.1**	**10.5 ± 0.7**	**3.6 ± 0.45**
rDEN4Δ30 (Lot 4-9)	**3**	**20**	**35**	**0.5 ± 0.1**	**9.1 ± 1.2**	**1.6 ± 0.8**
rDEN4Δ30 (Lot 109A)	**3**	**50**	**26**	**0.7 ± 0.1**	**10.8 ± 0.7**	**2.2 ± 0.4**

1. Mean peak titer is calculated only for those participants who were viremic. Lower limit of detection is 0.5 log_{10} PFU/mL.

Table 6: Serum antibody responses induced by each monovalent DENV candidate vaccine given as a single subcutaneous dose

Vaccine candidate	Dose (log_{10})	No. vaccines	%. infected[2]	Geometric mean titer, reciprocal (range)[1]			% seroconverted[3]
				Day 0	Day 28	Day 42	
rDEN1Δ30	**3**	**70**	**94**	**<5**	**170 (<5 - 6309)**	**140 (<5 – 2753)**	**93[4]**
rDEN2/4Δ30(ME)	**3**	**40**	**100**	**<10**	**88 (11– 1043)**	**104 (11 - 1377)**	**100**
rDEN3Δ30/31	**3**	**50**	**82**	**<5**	**84 (14-1715)**	**79 (8-1341)**	**81**
rDEN4Δ30 (Lot 4-9)	**5**	**20**	**100**	**<10**	**567 (72 – 2455)**	**399 (45 – 1230)**	**100**
rDEN4Δ30 (Lot 4-9)	**3**	**20**	**95**	**<10**	**139 (<10 – 2365)**	**129 (15 – 1222)**	**95**
rDEN4Δ30 (Lot 109A)	**3**	**50**	**93**	**<5**	**112 (12 – 892)**	**135 (22 - 748)**	**93**

1. Geometric mean titer calculated only for those participants who seroconverted.
2. Defined as either recovery of vaccine virus from the blood or by seroconversion.
3. Defined as a ≥4-fold rise in serum neutralizing titer compared to Day 0.
4. Vaccine virus was recovered from the blood of 1 subject who did not meet criteria for seroconversion.

Each of the monovalent candidate vaccines induced seroconversion (as defined as a ≥ 4-fold rise in serum neutralizing antibody titer at Study Day 28 or 42 compared with Study Day 0) in a large majority of vaccinees (Table 6). Seroconversion rates ranged from 81% (rDEN3Δ30/31) to 100% (rDEN2/4Δ30(ME), rDEN4Δ30) following a single dose. rDEN1Δ30 induced sterilizing immunity to a second dose of vaccine administered at 4 or 6

months in all vaccinees (data not shown)(32). DEN2/4Δ30 induced sterilizing immunity to a second dose of vaccine administered at 6 months (data not shown)(37).

2.4.2.3 Clinical, Virologic, and Serologic Response to a Tetravalent Admixture TV005

The tetravalent admixture TV005 is a combination of four live attenuated monovalent vaccines, each of a different serotype. The TV005 is comprised of 10^3 of rDEN1Δ30, 10^4 of rDEN2/4Δ30(ME), 10^3 of rDEN3Δ30/31, and 10^3 of rDEN4Δ30 live attenuated monovalent vaccines.

Clinical experience with TV005 (US)

TV005 has been evaluated in healthy flavivirus-naïve adult volunteers in two studies (CIR 268 and CIR 279) at the Center for Immunization Research, Johns Hopkins University and the Vaccine Testing Center, University of Vermont. In CIR 268, 20 volunteers received TV005 and 8 volunteers received a placebo. The protocol was later amended to allow for a second dose of vaccine (or placebo) to be given 6 months later. A limited number of volunteers returned for a second dose. In CIR 279 volunteers received a subcutaneous dose of vaccine (40) or placebo (16) at time 0 and a second dose of the same 6 months later (39).

Virologic response

Virology data from both CIR 268 and CIR 279 is presented together. Following the first dose of TV005, 46/60 (77%) vaccinees had at least one vaccine virus recovered from the blood **(**Table 7**)** Twenty-four vaccinees had only 1 vaccine virus detected in the blood (DEN1Δ30=7; DEN2/4Δ30=1; DEN3Δ30/31=10; DEN4Δ30=6). Seventeen vaccinees had 2 vaccine viruses detected in the blood (DEN2/4Δ30, DEN3Δ30/31=5; DEN3Δ30/31, DEN4Δ30=3; DEN2/4Δ30, DEN4Δ30=4; DEN1Δ30, DEN4Δ30=3; DEN1Δ30, DEN3Δ30/31=2) and 5 vaccinees had 3 vaccine viruses detected in the blood (DEN1Δ30, DEN3Δ30/31, and DEN4Δ30=2; DEN1Δ30, DEN2/4Δ30, DEN3Δ30/31=2; DEN2/4Δ30(ME), DEN3Δ30/31, DEN4Δ30=1). Vaccine virus was recovered from the blood of only 1 vaccine-recipient following the second dose. DENV3Δ30/31 was recovered from the subject only on Study Day 9 following dose 2 (titer = 0.5 $\log_{10}$ PFU/mL). This subject had a $PRNT_{60}$ of 1:12 to DEN3 Slemen/78 on Study Day 180 prior to second dose.

Serologic response

Serologic endpoints differed in the 2 studies (CIR 268 and CIR 279). In CIR 268, seropositivity was defined as a PRNT60 of ≥ 1:10. In CIR 279 seropositivity was defined as a PRNT50 of ≥ 1:10. In addition, in CIR 268 samples were collected only at Study Day 28, 42 and 180 post-vaccination and the peak titer post-each dose was the peak titer that occurred out to Study Day 42. In CIR 279, samples were collected at Study Day 28, 56, 90 and 180 post-vaccination and the peak titer post-each dose was the peak titer that occurred out to Study Day 90.

Following the first vaccination in CIR 268, 80% of vaccinees seroconverted to DENV-1, 60% of vaccinees seroconverted to DENV-2, 80% of vaccinees seroconverted to DENV-3 and 100% to DENV-4 (Table 8). 60% of vaccinees had a tetravalent response, 30% had a trivalent response, 0% had a bivalent response, and 10% had a monovalent response (Table 12).

Following the first vaccination in CIR 279, 92% of vaccinees seroconverted to DENV-1, 97% of vaccinees seroconverted to DENV-2, 97% of vaccinees seroconverted to DENV-3 and 97% to DENV-4 (Table 8). The increase in seropositivity frequency to DENV-2 induced by TV005 in CIR 279 compared to TV003 in CIR 279 was statistically significant (p = 0.006226, Fisher Exact test). Geometric mean peak titers following dose 1 are presented in Table 9. The higher seroconversion frequencies observed in CIR 279 are likely due to the additional day 90 post-vaccination time-point as we noted that antibody titers in some vaccinees continued to rise and some study participants had peak antibody titers at Study Day 90 or sometimes even later. There was no significant boost in antibody titer following a second dose of vaccine (**Table 9**). Titers had declined by Study Day 180 and did not increase appreciably after the second dose. The geometric mean antibody titers recorded post-dose 2 of vaccine were much lower than those observed following dose one. Only one of three study participants who had developed a trivalent antibody response following the first dose of TV005 seroconverted to the missing fourth serotype following the second dose.

2.4.3 Clinical Experience with TV005 in an Endemic Population

TV005 is currently being evaluated in a Phase II safety and immunogenicity clinical trial in volunteers ages 12 months to 50 years in an endemic area in Thailand. Safety data for vaccine related adverse events (AE)s has been completed for the adult cohort only. In this cohort 30 study participants received TV005, and 24 study participants received placebo. These data show that in this population the vaccine is well tolerated. Similar to studies conducted in the United States, mild rash was common following vaccination, occurring in approximately 40% of vaccinated study participants in the adult cohort (48% of all study participants). Fatigue was also common following vaccination, occurring in approximately 33% of vaccinated study participants in the adult cohort. Rash and fatigue were the only two vaccine related AEs that were significantly different between vaccine and placebo recipients (Table 13).

The data from the adolescent cohort are similar to the adult cohort. No safety concerns were noted by the DSMB, and the study has continued to age de-escalate to children ages 5 – 11 years old (data not available).

Conclusions:

TV005 was well tolerated by study participants, and a single dose was highly immunogenic (tetravalent response in 90% of vaccines). Very little boost in antibody titer was observed following a second dose of vaccine administered six months following initial vaccination.

Table 7: Magnitude, onset, and duration of viremia in participants inoculated with TV005, dose 1 (CIR 268 and CIR 279)

Vaccine component	No. participants vaccinated	No. viremic (%)	Maximum titer (log_{10} PFU/mL)	Mean peak titer[1] ± SE	Mean day of onset of viremia (range)	Mean duration in days (range)
DEN1Δ30	60	17 (28)	1.7	0.62 ± 0.1	10.9 (8-15)	2.1 (1-5)
DEN2/4Δ30(ME)		13 (22)	0.5	0.50 ± 0.0	7.5 (6-10)	1.0 (all 1)
DEN3Δ30/31		23 (42)	1.0	0.60 ± 0.0	9.3 (3-14)	2.5 (1-7)
DEN4Δ30		18 (30)	1.5	0.64 ± 0.1	9.3 (6-16)	1.9 (1-6)
	Total Viremic	46 (77)				

Table 8: Serum antibody responses induced by TV005 when given as a single subcutaneous dose

		% of vaccinees seropositive to specific serotype			
Admixture	N	DENV-1	DENV-2	DENV-3	DENV-4
TV005 (268)[1]	20	80	60	90	100
TV005 (279)[2]	39	92	97[3]	97	97

1. Seropositive = 60 percent plaque reduction neutralization titer ($PRNT_{60}$) of ≥1:10
2. Seropositive = 50 percent plaque reduction neutralization titer ($PRNT_{50}$) of ≥1:10
3. Statistically significant (p = 0.006226, Fisher Exact test)

Table 9: Geometric mean antibody titer to each serotype following a single dose of TV005

		Geometric Mean Peak Titer (range)			
Admixture	N	**DENV-1**	**DENV-2**	**DENV-3**	**DENV-4**
TV005 (268)[1]	20	40 (16-91)	44 (10-149)	35 (14-113)	70 (14-338)
TV005 (279)[2]	39	35 (10-190)	91 (12-651)	100 (17-343)	205 (19-2190)

1. Titer calculated using $PRNT_{60}$ (per protocol)
2. Titer calculated using $PRNT_{50}$ (per protocol)

Table 10: TV005 induce a broad neutralizing antibody response to all 4 serotypes

		% with multivalent response (cumulative)				
	N	**Tetravalent**	**Tri-**	**Bi-**	**Mono-**	**None**
TV005 (268)[1]	20	**60**	**30** (90)	**0** (90)	**10** (100)	0
TV005 (279)[2]	39	**90**	**8** (98)	**0** (98)	**2** (100)	0

1. Based on $PRNT_{60}$. Seropositive response is defined as a $PRNT_{60} \geq 1:10$ by Study Day 42.
2. Based on $PRNT_{50}$. Seropositive response is defined as a $PRNT_{50} \geq 1:10$ by Study Day 90.

Table 11: Geometric mean antibody titer to each serotype following a second dose of TV005 (CIR 279)

TV005	**N1**	**DENV-1**	**DENV-2**	**DENV-3**	**DENV-4**
Day 180	33	10 (<5-41)	35 (<5-241)	27 (<5-68)	40 (9-1582)
Peak titer post-dose 2	33	16 (<5-54)	55 (11-297)	36 (10-312)	75 (26-951)

1. Only those participants who received both doses of vaccine were included in this analysis

Table 12: Percent and cumulative seropositivity rates to multiple DENV following the first and second dose of TV005 (CIR 279).

		% with multivalent response (cumulative)				
	N[1]	Tetravalent	Trivalent	Bivalent	Monovalent	None
TV005, dose 1	33	91	9 (100)	0 (100)	0 (100)	0
TV005, dose 2	33	94	6 (100)	0	0	0

1. Only those participants who received both doses of vaccine were included

Table 13: Clinical response to TV005 vaccine candidate in an endemic setting (Thailand)

Vaccine Candidate	No. of participants	No. of participants (%) with indicated clinical response									
		Any AE	ALT ↑	Arthralgia	Rash	Fatigue	Fever	Headache	Myalgia	Neutropenia	Nausea
TV005	30	25 (83)	1 (3)	2 (7)	12 (40)	10 (33)	4 (13)	10 (33)	7 (23)	0 (0)	4 (13)
Placebo	24	15 (63)	0 (0)	1 (4)	2 (8)	0 (0)	0 (0)	6 (25)	3 (13)	1 (4)	2 (8)

2.4.4 Mosquito Transmissibility of Monovalent Vaccine Candidates

For DENV to be transmitted from 1 person to another there is a 10–14 day incubation period within the mosquito. Therefore, for these vaccine viruses to be transmitted to an in-house family member, the subject would have to be viremic with a peak virus titer greater than 10^5 PFU/mL, would have to be bitten by a viable vector mosquito at the peak of viremia, the mosquito would have to live for an additional 10–14 days, and the same mosquito would then have to bite another family member. The peak virus titers of all the live attenuated DENV vaccine viruses tested thus far were at least 1000-fold below the viremia level required for transmission to mosquitoes making risk of transmission minimal.

Both the wt rDEN1 and rDEN1Δ30 demonstrated a low infectivity (50% mosquito infectious dose [MID_{50}] of $>10^3$ PFU/mL)(28). This is consistent with previously reported studies that demonstrated a very low infectivity of DENV-1 for *Aedes aegypti* fed an infectious blood meal. It is unlikely that the vaccine virus would be transmitted from an infected vaccinee to a mosquito since rDEN1Δ30 is poorly infectious for mosquitoes and because of the low level of viremia demonstrated in humans (40).

Transmissibility of rDEN2/4Δ30(ME) to both *Ae. aegypti* and *Toxorhynchites splendens* mosquitoes is reduced compared with both rDEN4 and DEN2-NGC (10). The minimum infectious dose of rDEN2/4Δ30(ME) needed to infect 50% of *Ae. aegypti* mosquitoes (MID_{50}) was ≥10-fold higher than that of either rDEN4 or DEN2-NGC, indicating impaired infectivity for the midgut. rDEN2/4Δ30(ME) was less infectious than either parent virus for *T. splendens*, indicating reduced ability of the chimeric virus to disseminate to the head of the mosquito. It is unlikely that the vaccine virus would be transmitted from an infected vaccinee to a mosquito since rDEN2/4Δ30(ME) is poorly infectious for mosquitoes and because of the low level of viremia demonstrated in humans (36). rDEN2/4Δ30(ME) should be poorly transmitted to mosquitoes, as previously observed for the attenuated rDEN4Δ30 vaccine candidate, which was not transmitted from 10 infected vaccinees to over 300 feeding mosquitoes(27,41).

The parent virus of the rDEN3-3´D4Δ30 and rDEN3Δ30/31 virus vaccines, DEN3-Sleman/78, is very poorly transmitted to mosquitoes: ingestion by *Ae. aegypti* mosquitoes of $10^{4.1}$ PFU of wt DEN3 Sleman/78 infected the midgut of only 4 of 28 (14%) mosquitoes tested and disseminated from the midgut in only 2 of 28 (7%) mosquitoes. The required dose of wt DEN3 Sleman/78 is in excess of 10^5 PFU/mL in blood to allow for transmission to *Ae. aegypti* mosquitoes, the natural vector of DENV(42).

The rDEN4Δ30 virus construct has been extensively studied with respect to its transmissibility to humans by mosquitoes (41). The rDEN4Δ30 was restricted both in its ability to infect the midgut and to cause a disseminated infection in mosquitoes. The ability of rDEN4Δ30 to be transmitted from viremic study participants to *Ae. albopictus* mosquitoes was also evaluated. Mosquitoes fed on study participants on Study Days 7, 8, and 9 post-infection. Vaccine virus was not recovered from any of the 352 mosquitoes that fed on the study participants even though some of the study participants were viremic on each of the days tested(41). The restricted capacity of rDEN4Δ30 to disseminate from the midgut of the mosquito to its head is specified by the Δ30 mutation.

The restricted capacity of rDEN4Δ30 to disseminate from the midgut of the mosquito to its head is specified by the Δ30 mutation. As with the other monovalent attenuated DENV vaccine viruses included in TV005, this mutation is engineered into rDEN3Δ30/31. Ingestion by *Ae. aegypti* mosquitoes of $10^{4.1}$ PFU of wt DEN3 Sleman/78 infected the midgut of only 4 of 28 (14%) mosquitoes tested and disseminated from the midgut in only 2 of 28 (7%) mosquitoes. In addition, the low level of viremia in vaccinated study participants will preclude transmissibility to mosquitoes. rDEN3Δ30/31 should be poorly transmitted to mosquitoes, as previously observed for the attenuated rDEN4Δ30 vaccine candidate, which was not transmitted from 10 infected vaccinees to over 300 feeding mosquitoes(27,41).

Research Design and Methods

3 Study Design

3.1 Overall Study Design

This is a placebo-controlled, double-blind study in normal healthy adult, adolescent, and child volunteers recruited from Mirpur, Dhaka. The purpose of this study is to evaluate the safety, reactogenicity, and immunogenicity of 1 dose of the live attenuated tetravalent dengue vaccine TV005 when given as a subcutaneous injection. The candidate vaccine, TV005, will contain 10^3 PFU of rDEN1Δ30, 10^4 PFU of rDEN2/4Δ30(ME), 10^3 PFU of rDEN3Δ30/31 and 10^3 PFU of rDEN4Δ30. A placebo cohort is included in the study as a control to distinguish vaccine-associated versus non-vaccine-associated AEs. Placebo for this study will be the vaccine diluent, 1X L-15. Placebo is prepared from a specific safety tested lot of 2X L-15 medium (BB-MF #12959) that is obtained from a NIAID contracted repository and mixed 1:1 with sterile water for injection.

After providing written informed consent/assent, participants will undergo eligibility screening, including medical history, physical examination, haematology testing, liver function testing, and hepatitis C screening (for adults and adolescents only), and hepatitis B screening. Pregnancy testing will be performed on female participants as outlined in Section 4.1 #6. Screening will be performed within 30 days prior to Study Day 0. See Section 6 for a detailed description of study visits and procedures.

All clinically significant abnormalities will be reviewed with study participants and they will be referred to an appropriate local care provider for follow-up care. Study participants will be determined to be eligible based on the inclusion and exclusion criteria found in Section 4 of this protocol. For participants who are eligible, the Study Day 0 visit will be scheduled for the participants to receive their vaccination. Pregnancy testing will be repeated on female participants of reproductive age on the day of vaccination, and must be negative prior to vaccination. Participants will be considered enroled in the study only after they are vaccinated (receive either vaccine or placebo). After vaccination, participants will be observed in the clinic for at least 30 minutes in order to assess for any AEs. Study participants will return to the clinic on Study Days 7, and 14 post-vaccination for evaluation by a clinician and to have blood drawn for safety, clinical laboratory studies, and assays to assess for vaccine virus viremia. They will also have a history-directed clinical evaluation performed at each specified visit (see Section 6 for detailed description of study procedures). They will return to the clinic on Study Days 28, 56, 180, 360, and 720 for further evaluation of immune responsiveness. On Study Day 1080 (year 3) participants will return to the clinic for their final visit.

Investigators and or designee will identify suspected dengue and febrile illness cases during the three year duration of the study. The following systems will be utilized:

First line of surveillance
Except for days with scheduled clinic visits, Field Research Assistants (FRAs) or designees will make daily home visits to study participants or parent/legally acceptable representative to collect fever and AE information up to day 14 (Day 0 – Day 14). If study participants can't be reached at the home, FRAs or designees will call the study participants or parent/legal acceptable representative to get information over the phone.

Second line of surveillance
Study participants or parents/legally acceptable or authorized representative (LAR) will be instructed to contact the study Investigator and/or other appropriate study staff or come to the clinic in the event of febrile episodes during entire study period or in case of any untoward medical event.

Third line of surveillance
After Day 14, study staff will contact study participants or their parent/LAR weekly by phone or in person to check for fever and /or AEs through study day 180. After study day 180, study staff will contact study participants monthly, by phone or in person, to remind study participants to contact the clinic if they have a febrile illness. Study staff will also ask study participants if they have been hospitalized for a febrile illness since the last call. If the study participant was hospitalized for a febrile illness, they will be instructed to come to the clinic for suspected Dengue follow-up.

FRAs or designee will assess study participants for solicited local symptoms (see Table 20 and Table 21**)** at the injection site and record this information on surveillance source document on the day of dengue/placebo vaccine administration. FRAs or designee will collect and record temperature through Study Day 14, and AEs through Study Day 28, on the age-appropriate surveillance source document.

3.2 Dosing Strategy

Table 14: Dosing Strategy

Cohort	**Age (years)**	**No. Vaccinee**	**No. Placebo**	**Total**
(01) Adult	18-50	36	12	48
(02) Adolescent	11-<18	36	12	48
(03) Children	5-<11	36	12	48
(04) Young Children	1-<5	36	12	48
	Total:	144	48	192

Each cohort will be enroled in an age de-escalation manner. The first cohort (Cohort 01) of 48 adults will receive one subcutaneous dose of TV005 vaccine or placebo in a 3:1 ratio. Following the Study Day 28, study participants in the adult cohort will have blinded safety data analysed separately evaluating TV005 and placebo. If stopping criteria are not met following analysis of safety data through Study Day 28, enrolment will continue into the next age de-escalation portion of the study based upon the approval of the DSMBs. The second

cohort (Cohort 02) of 48 adolescents will then be enroled into the study. It is estimated that it will take approximately one – two months for DSMB approval to proceed to the next cohort after Study Day 28, including compiling the data report, DSMB review and approval. The sequential enrolment of the final 2 cohorts (Cohort 03 and Cohort 04) will proceed in a similar manner, with DSMB evaluation before each subsequent group. All study participants will be followed for approximately three years after receipt of the vaccine.

3.3 Sample Size and Placebo Ratio

192 study participants (144 vaccinees and 48 placebo recipients) will be enroled in the study. All cohorts will consist of 48 study participants (36 vaccine, 12 placebo). All cohorts will use a 3:1 (TV005: placebo) ratio. The age de-escalation strategy was chosen for the following reasons:

- This study is designed to build upon the previous Phase I and II studies of each monovalent candidate and the tetravalent admixtures, in the United States and Thailand, which used similar cohorts and the similar vaccine to placebo ratios (24, 27, 35, 40).
- Placebo recipients have been included to allow assessment of whether common AEs are vaccine-related.

3.4 Duration of Participation

Study participants will be followed for approximately three years from vaccination. Participants will be screened for eligibility up to 30 days prior to vaccination on Study Day 0.

3.5 Estimated Duration of the Study

The study will span a total of approximately 4 years, from the time of first study vaccination until the last enroled participant completes the last visit. This 4 year study duration includes four cohorts of an approximate 37 month duration (one month of screening and three years of follow-up) and allows for interim safety evaluations by the DSMBs after enrolment of each cohort has been completed and prior to enrolment of the subsequent cohort.

3.6 Treatment Assignment

All study participants will be randomly assigned to receive TV005 vaccine or placebo. Treatment assignment will be done using a random number generator to prepare the sequence in which study participants are assigned to receive TV005 or placebo. Study participants will be randomized in blocks of 4 by the vaccine preparation study staff using a pre-generated randomization code list for all study participants. Each block of 4 will include 3 vaccine recipients for TV005 and 1 placebo recipient. Study participants will be enroled sequentially, and a separate randomization list will be generated for each of the four cohorts.

Clinical staff and study participants will remain blinded to treatment assignment until all participants in that cohort have completed their full three year follow-up period. Study investigators will remain blinded to treatment assignment until study participants have completed follow-up out to their Day 180 visit. Study participants and study staff may only be unblinded after these specified times, or if unblinding is deemed necessary for safety reasons (see Section 3.7).

A master log of treatment assignments in sealed envelopes will be maintained in a record separate from other study records. It will be kept in a locked room with limited access by a designated individual at the International Centre for Diarrhoeal Disease Research,

Bangladesh (icddr,b). A copy of the treatment assignments will also be provided to the NIH DSMB, and ERC DSMB if requested.

3.7 Blinding

This study will be conducted as a double-blind study to avoid biased assessment of AEs. Vaccine or placebo will be prepared and drawn up into syringes by laboratory personnel who are not involved with the clinical assessment of study participants. Syringes are labelled according to Study Specific Procedures with treatment numbers and pre-printed participant numbers. Because vaccine diluent is used as placebo, there will be no difference in the appearance of the syringes, and clinical staff will have no access to the master list of treatment assignments for study participants.

The study participant and clinical staff will not know the treatment group to which study participants are assigned. In addition, other personnel assigned to monitor the study will not know the treatment assignment of the participant.

If the need arises to unblind a specific study participant's treatment assignment for emergency medical management, the Principal Investigator (PI) will contact the assigned designee at icddr,b who is responsible for maintaining the envelopes containing the unblinded information and can obtain the treatment assignment of the study participant in question if needed. Only that specific study participant's assignment will be unblinded. The Sponsor and the DSMBs will be notified within 2 business days. Unblinding will be documented in the participant's study chart. The research monitor and the Ethical Review Committee (ERC) at icddr,b will be notified of the unblinding.

For each age cohort, clinical staff and study participants will remain blinded to treatment assignment until all participants in that cohort have completed their full three year follow-up period. Other study investigators will remain blinded to treatment assignment until all participants in that cohort have completed their Day 180 follow-up visit.

Routine unblinding for Investigators (not clinical staff) will occur when all participants have completed their Day 180 follow-up visit. Once all of the data for each study participant in each cohort up to study day 180 has been entered into the CRFs and uploaded, the clinical designee will notify the site PI that the cohort is ready for unblinding. The site PI will make a formal request for the treatment assignments in writing from the unblinded study staff. The unblinded study staff will then send the treatment assignments to the site PI. Clinical staff and study participants will remain blinded to treatment assignment until each study participant in each cohort has reached their full three year follow-up period.

4 Selection and Enrolment of Participants

4.1 Inclusion Criteria

All of the following criteria must be fulfilled for a participant to qualify for inclusion in this study:

1. Healthy males or females (non-pregnant or lactating) between the ages of 12 months and 50 years at the time of enrolment into the study.

2. Reside in Mirpur Wards 2, 3, or 5 (or other Wards within Mirpur as designated by the PI if needed to reach enrolment targets) at the time of screening.

3. Study participants and/or their parents/guardians/LARs who the Investigator believes can and will comply with the requirements of the protocol (e.g., return for follow-up visits).

4. Good general health as determined by physical examination, laboratory screening, and review of medical history.

5. If the participant is ≥ 18 years of age, an informed consent form signed and dated by the study participant (and by an independent witness if required by local regulations). If the study participant is a minor, an assent form (for participant's age 11-17 years) and informed consent form signed and dated by the participant's properly identified parent(s) or legally acceptable representative. If a participant is < 11 years of age, the participant's parent(s) or legally acceptable representative will consent by signing the consent form for minor participants.

6. If the participant is female, she must not be pregnant or planning to become pregnant up to 28 days post vaccination. Female participants under the age of 18 will be enroled, if they have not yet reached menarche. Females 18 years of age or older, must be properly using a method of pregnancy prevention that is known to be highly effective per the PI. Reliable methods of contraception include: hormonal birth control, condoms with spermicide, diaphragm with spermicide, surgical sterilization, intrauterine device, and abstinence (≥ 6 months since last sexual encounter). For female study participants who are 18 years of age or older pregnancy testing will occur at screening and again before vaccination and they must have a negative pregnancy test prior to vaccination. Additionally, they will receive counselling on the importance of effective contraception during the 30 days prior to vaccination and continuing up to Day 28. Counselling will occur at their screening visit, on all clinic visit days up to Day 28. Female participants 18 years of age and older will be subject to pregnancy testing on the Study Day 28 and 56 visits. If female participants under the age of 18 that have not yet reached menarche prior to enrolment, reach menarche during the study, they will continue to be enroled and followed-up through the entire three year follow-up period.

4.2 Exclusion Criteria

A participant will be excluded from enrolment if any of the following criteria are met:

1. Pregnant or lactating female or female planning to become pregnant within 28 days of receiving an investigational product or planning to discontinue abstinence or contraceptive precautions within 28 days of receiving an investigational product. Menstruating females under the age of 18 will be excluded from being enroled.

2. Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic, renal, autoimmune, hematologic, or endocrine disease or functional defect, as determined by history, physical examination, or screening tests.

3. History of any neurological, psychiatric, or behavioural disorder or seizures, with the exception of a single febrile seizure in childhood.

4. Self-reported or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months, or long-term systemic corticosteroid therapy (prednisone or equivalent, $\geq$0.5 mg/kg/day or 20 mg/day, for more than 2 consecutive weeks within the past 3 months). Inhaled and topical steroids are allowed.

5. Having a height-for-age z-score (HAZ) or weight-for-age z-score (WAZ) of <-3 for children under the age of two. Severe malnutrition as observed by the study physician for all study participants ages 2 years old and older.

6. Hepatitis C virus (HCV) infection by screening and confirmatory assays (screening in adults and adolescents only), or Hepatitis B virus (HBV) infection, by Hepatitis B surface antigen (HBsAg) screening (all participants) or, unwilling to allow HCV (adults and adolescents only) and HBV testing.

7. Screening laboratory values of Grade 1 or above for absolute neutrophil count (ANC), ALT, or platelet count, as defined in this protocol.

8. For children under 5 years of age, screening laboratory values of Grade 1 or above for Haemoglobin.

9. History of allergic reaction likely to be exacerbated by any component of the vaccine; any history of a severe allergic reaction or anaphylaxis.

10. Current alcohol abuse or drug addiction that might interfere with the ability to comply with trial procedures.

11. Any other condition that in the opinion of the Investigator would jeopardize the safety or rights of a participant participating in the trial or would render the participant unable to comply with the protocol.

12. Planned administration of any vaccine from 30 days prior to receipt of the study vaccine and ending 30 days after; with the exceptions of standard infant and child Expanded Program on Immunization (EPI) inactivated vaccines and the inactivated influenza vaccine or the inactivated rabies vaccine (without administration of immunoglobulin) administered to adults or children.

13. Use of any investigational or non-registered drug or vaccine other than the study vaccine within 30 days preceding receipt of study vaccine/placebo or planned use at any time during the study period or history of having received any investigational dengue vaccine at any previous time.

14. Current participation in any clinical or investigational study.

15. Administration of immunoglobulins and/or blood products within 90 days preceding the dose or planned administration at any time during the study period, which might interfere with assessment of the immune response.

16. A planned or anticipated move to a location that will prohibit full participation in the trial for the three year duration.

17. Potential adult volunteers or parents of potential child volunteers, who do not have easy access to a fixed or mobile telephone.

18. Any participant that resides in a household with an individual previously enroled in an older age cohort in this study. Only one participant from each household will be enroled.

19. Any participant identified as a site employee of the Investigator or study clinic, with direct involvement in the proposed study or other studies under the direction of that Investigator or study clinic, as well as any immediate family member (such as husband, wife and their children, adopted or natural) of the clinic employees or the Investigator.

4.3 Elimination Criteria During the Study

The following criteria should be checked at each visit subsequent to the first visit. If any become applicable during the study, it will not require withdrawal of the participant from the study but may determine a participant's evaluability in the per-protocol (PP) analysis. The participant will continue to be evaluated for safety purposes at the regularly scheduled visits. See Section 10.2 for definition of study cohorts to be evaluated.

1. Use of any investigational or non-registered product (drug or vaccine) other than the study vaccine/placebo up to Day 56.

2. Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs up to Day 56. For corticosteroids, this will mean prednisone, or equivalent, ≥0.5 mg/kg/day. Inhaled and topical steroids are allowed.

3. Administration of a licensed vaccine during the period starting from 30 days before dose of vaccine/placebo and ending 30 days after, with the exception of standard infant and childhood EPI "inactivated" vaccinations.

4. Administration of immunoglobulins and/or any blood products up to Day 56.

5. Pregnancy within the 28 days after investigational product administration.

4.4 Contraindications to Vaccination

The following conditions constitute absolute contraindications to administration of the study vaccine; if any of these AEs occur during the study, the study participant may continue study procedures at the discretion of the Investigator. The participant must be followed until resolution of the event, as with any AE.

- Any confirmed or suspected immunosuppressive or immunodeficient condition
- Pregnancy

The following conditions constitute temporary contraindications to administration of the study vaccine:

- Acute disease at the time of vaccination (Acute disease is defined as the presence of a moderate or severe illness with or without fever. Vaccine can be administered to persons with a minor illness such as diarrhoea or mild upper respiratory infection.)

- Fever ≥ 38.0ºC

If any of these temporary contraindications occurs at the time of scheduled vaccination, the study participant may be vaccinated at a later date, within the time window specified in the protocol or excluded from participation at the discretion of the Investigator.

4.5 Participant Withdrawal and Termination Criteria

A study participant will not be considered to have completed the trial if any of the following reasons apply. However, any participant who has received vaccine or placebo will be encouraged to remain in the study for periodic safety evaluations for the duration of the study at the discretion of the Investigator.

1. **Research terminated by Sponsor or Investigator** – applies if the entire study is terminated by the Sponsor or Investigator for any reason.

2. **Withdrawal of consent** – applies to a participant who withdraws consent to participate in the study for any reason.

3. **Noncompliant with protocol** – applies to a participant who does not or is not able to comply with protocol-specific visits or evaluations on a consistent basis such that adequate follow-up is not possible and the participant's safety, and the integrity of the study data, would be compromised by continuing in the trial.

4. **Participant withdrawal** – may occur if the Investigator believes that it is in the best interest of the participant to be withdrawn from the study.

5. **Other –** category used when previous categories do not apply and requires an explanation.

For study participants where the reason for early termination is voluntary withdrawal of consent, the Investigator should attempt to determine the reason for the subject's decision. The study staff will attempt to contact all study participants that withdraw or are terminated to obtain further safety information and to inform them of any significant findings which may affect their safety or health, unless the participant specifies that they do not want to be contacted again. This information will be documented in the source document.

Participants will be allowed to withdraw from the study at any time without prejudice or loss of benefits to which they are entitled. The Investigator or designee will document whether the decision to withdraw from the study was made by the participant or the Investigator and describe the reasons for withdrawal. Information relative to the withdrawal will be documented on the related study documents.

With the exception of expenses resulting from research-related injury, medical costs incurred following a participant's withdrawal from the study will become the responsibility of the participant or the participant's parent/legal guardian.

Any and all data and biologic samples collected from the participant prior to his or her withdrawal may be used by Investigators as indicated in the protocol, unless the participant specifies a wish that the samples are destroyed.

All IRB/ERCs will be notified of any study participant's withdrawal at the time of the continuing review submission.

The Sponsor will be notified of any participant's withdrawal according to the Sponsor's guidelines.

Special Situations:

Pregnancy:
If a female study participant who receives investigational product becomes pregnant up to 28 days after receiving the investigational product, it may pose potential risks or contribute towards an AE for the participant or fetus. If study participant becomes pregnant within 28 days of having received study product, the participant will not be included in the immunogenicity evaluations from her estimated date of conception. She will, however, be encouraged to remain in the study for periodic safety evaluations and will be followed until completion of her pregnancy. If any study participant tests positive for pregnancy within 56 days after receiving the investigational product, the pregnant participant will be followed for the duration of the pregnancy, and the newborn will be assessed for any potential adverse outcomes. The participant will be asked to sign a release of medical information form so that records can be obtained from her clinician or obstetrician regarding the outcome of the pregnancy. All pregnancies occurring within 56 days after receiving investigational product will be reported to the Sponsor, the icddr,b ERC, UVM IRB, the Research Monitor, and to the DSMBs as soon as study staff becomes aware of the pregnancy, and as required by regulations. A Pregnancy Notification/Outcome Form will be completed by the study staff and forwarded to the Sponsor (see Section 7.3 for Adverse Event Reporting details).

Lost to follow-up:
A study participant who is not reachable by telephone or other means of communication and therefore not able to be located is considered lost to follow-up. A participant may be considered lost to follow-up and withdrawn from the study once 3 attempts have been made to contact the participant, followed by non-response to a letter sent to the last known address requesting that the participant contact the clinical site.

Incarceration:
In the event that a study participant becomes incarcerated during the course of the study, every attempt will be made to contact the participant for determination of his/her safety. A participant may be terminated from the study if his/her period of incarceration will make him/her unavailable for scheduled visits.

Immunosuppression:
Study participants will be asked to report any history of immunosuppression at the time of enrolment as part of the exclusion criteria. Investigators will assess participants for immunosuppressive disease by clinical history, physical exam, and medical evaluation. Study participants potentially afflicted with any other self-reported immunosuppressive condition will not be enroled.

5 Vaccine Preparation

5.1 Pre-Vaccine Preparation

Monovalent vaccine virus for this protocol will be stored at a NIAID-contracted repository until requested by the clinical site. Vials of frozen vaccine for administration will be formally requested to be transferred to the clinical site by the PI/designee after local Institutional Review Board (IRB) and Ethical Committee (EC) approvals for the study have been granted and the U.S. Food and Drug Administration (FDA) has been in receipt of the protocol for at least 30 days without issuing a clinical hold. Vaccine may be transferred to the study site prior to IRB approval and FDA review for the purpose of determining the titer of the vaccine only. The icddr,b team will be responsible for obtaining Directorate General of Drug Administration (DGDA) approval for import of the investigational product. The DGDA requires the approved protocol, Research Review Committee (RRC) and ERC approval letters, and an invoice with the drug name, consignee, and consigner to be submitted at the time of application for an import permit for the investigational product.

After transfer to the clinical site, each monovalent vaccine will be stored in a locked and dedicated freezer at -80°C ± 15°C until time of use. After the initial vaccine shipment is received by the clinical site, a pair of vials for each serotype of the vaccine will be thawed. To verify potency, virus titer will be determined by plaque titration of each undiluted monovalent vaccine and after combining into a representative tetravalent admixture held on ice for 4 hours. Additional titration may occur at approximately 6 month intervals to ensure stability of the stored vaccine. The vaccine is supplied as a concentrate that must be diluted to the proper dose prior to administration.

Lab personnel who are not blinded to treatment assignment will be responsible for preparing the vaccine and placebo. Prior to vaccination, the PI/designee will supply a prescription request form including necessary information and the number of doses to be administered to the study staff responsible for vaccine preparation.

Admixture TV005 (10^3 of rDEN1Δ30, 10^4 of rDEN2/4Δ30(ME), 10^3 of rDEN3Δ30/31, and 10^3 of rDEN4Δ30) will be prepared according to the site's Study specific Procedure (SSP). Study staff will prepare the correct dose of vaccine (or placebo) for each study participant in a biosafety hood using aseptic technique. Vaccine will be diluted with 1X L-15 medium, which is prepared from a specific safety tested lot of 2X L-15 medium and sterile water for injection (1:1, v/v). A Type II Master File for L-15 was created and submitted to the FDA (BB-MF #12959) on 27 February 2006. The specific safety testing results for the current lot of 2X L-15 medium are available in the Master File. The diluted vaccine (or placebo) will be drawn up to a volume of 0.5 mL in a 1 mL syringe and labeled according to the SSP. The labeled syringes will be placed in a plastic syringe case and will be transported in an insulated cooler containing wet ice or an electronic insulated cooler to the clinic for administration. Vaccine must be used within 4 hours of being removed from the freezer. Note that the electric coolers do not contain any wet ice, but rather work on airflow and can maintain the desired temperature for approximately 20 hours. In addition, the admixture syringes will be kept in syringe cases within the cooler. These syringe cases are transparent plastic casing which will enclose the syringes.

5.2 Vaccine Storage

Each monovalent vaccine candidate should remain frozen at -80°C ± 15°C until just prior to use on each scheduled vaccination day. Vaccine should never be refrozen for reuse in vaccine preparation. Diluent components will be stored at 2°C to 8°C as per the manufacturer's

recommendation. Vaccine and diluent components should be opened from new containers for each use. No component should be reused for future vaccine or placebo preparation.

5.3 Vaccine Accountability

Laboratory vaccine preparation personnel will maintain an accurate inventory and accountability record of vaccine for this study. Partially used vials of vaccine or placebo components will not be refrozen or reused for future vaccinations.

5.4 Storage Disposition of Used/Unused Supplies

After laboratory personnel have diluted the vaccine and drawn up the syringes for administration, they will remove the label from the vaccine vial and place it in the vaccine preparation form. In this manner, monitoring personnel will be able to verify the accountability of all vaccine vials used for the study. In addition, the number of vaccine vials used will be accounted for in the study specific drug accountability log. The diluent will be accounted for in the general accountability log, with identification of the specific study.

An aliquot of undiluted (if available) and diluted vaccine will be titrated by laboratory personnel after vaccine has been prepared and delivered to the clinical staff. This is done to confirm the potency of vaccine administered to the study participants.

At least 1 aliquot (if available) of diluted vaccine will also be frozen and stored at-80°C ± 15°C at the clinical site for future re-titration if needed. After the syringes have been dispensed and aliquots removed for titration, any remaining vaccine will be destroyed by the laboratory personnel per SSP/SOP. Any unused diluent/placebo from opened vials/bottles will also be destroyed.

6 Study Procedures

The following sections provide a detailed listing of the procedures and studies to be performed in this protocol at designated time points.

6.1 Recruitment and General Screening

Study participants will be recruited from Mirpur, Dhaka Wards, 2, 3, and 5 (or other Wards within Mirpur as designated by the PI if needed to reach enrolment targets). Approximately 100 participants per age group will be approached for recruitment from a household list. If they agree, they will be taken to the clinic for consenting and screening procedures to be performed by a Medical Officer or their designee. Approximately 100 potential participants will be approached in order to recruit 48 study participants in each age cohort. Only one study participant will be recruited per household.

During the screening process, the study participant will read the consent form and will be encouraged to ask questions. The participant may either sign the consent/assent form during the screening visit or return after further consideration. The participant will have a medical history review, pregnancy prevention counselling (if applicable), physical exam, and/or lab specimens drawn during the general screening process after the informed consent/assent is signed.

Approximately 400 adults, adolescents, and children will be screened in order to enrol 192 healthy participants. From the 192 enroled, 144 will receive the tetravalent dengue vaccine, and 48 will receive the placebo. Only participants who have signed the informed consent

and/or assent forms and have met the eligibility and inclusion/exclusion criteria will be enroled in the study.

6.2 Screening Procedures

All participants will undergo the following screening procedures within 30 days prior to vaccination:

1. Explain the study and Informed Consent to the study participant. The consent form will be signed by all adult study participants. An assent form will be signed by all children age 11-17 years of age, in addition to having the consent form signed by the parent(s) or legal guardian(s). A standard adult informed consent will be signed by the parent(s) or legal guardian(s) for participants less than 11 years of age.

2. Ensure that the study participant has signed the Informed Consent/Assent, and receives a copy of the signed Informed Consent and assent forms if applicable.

3. Check eligibility using inclusion and exclusion criteria for study participation.

4. Collect and record demographic data and contact information.

5. Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterilization for female study participants of child-bearing potential. Data will be recorded on appropriate study documents and if the study participant is enroled, the CRF will be uploaded at the time of enrolment (Day0).

6. Perform a complete standard physical examination, including vital signs (height, weight, blood pressure, respiration, and pulse may be recorded depending on age). Data will be recorded in a source document and entered into CRF at the screening visit. If participant is enroled, the CRF will be uploaded at the time of enrolment (Day 0).

7. Obtain urine for rapid point-of-care β-HCG testing in females of child-bearing potential. A positive β-HCG will exclude the participant from the trial.

8. Assign study identification number.

9. Obtain whole blood for the following laboratory screening tests. See Table 16 for blood volumes to be drawn in each age cohort:

    - Complete blood count (CBC) with differential
    - Alanine transaminase (ALT)
    - Prothrombin time/partial thromboplastin time (PT/PTT)
    - Hepatitis B Virus (HBV)
    - Hepatitis C Virus (HCV) for adult and adolescent study participants only

10. Any participant with a positive HBV or HCV test during screening will be referred to an appropriate medical provider who will provide further testing, counselling, and follow up care as needed. These participants would be excluded from enroling in the study.

11. Counsel females of reproductive age or older to avoid becoming pregnant within 28 days of receiving a study vaccination series.

12. Counsel study participants regarding the procedures for capturing any AEs or serious adverse events (SAEs). SAEs will be recorded throughout the entire study period. Participants will be instructed to contact the Investigator or designee immediately should they manifest any signs or symptoms they perceive as serious and for any hospitalizations for any reason (see Section 7.1.2, Serious Adverse Events). Participants will be given an identification card and a set of phone numbers to contact study staff 24 hours a day, 7 days a week.

13. Remind participant of anticipated study visit for dose 1, Study Day 0.

6.3 Detailed Study Procedures

Study procedures to be performed at each visit are listed below. Photographs may be taken of the injection site. In addition, photographs may be taken of other areas of the skin before and/or after vaccination to record the characteristics of any rash that may develop.
The volume of blood to be drawn over the full 156-week of vaccination phase in adults (age $\geq$ 18 years of age) is approximately 257 mL. This amount is well within the Office for Human Research Protections (OHRP) and NIH guidelines (NIH Clinical Center Medical Administrative Policy M 95-9) for blood volumes in clinical research and should not compromise the health of study participants. Please see Table 16 for blood volumes to be drawn from each age cohort. These amounts do not include potential approximate 2 mL blood draws if participants are suspected to have dengue fever or additional amounts as needed to assess for resolution of laboratory AEs. (See Section 6.3.11.3).

On the day of vaccination, more study participants may be invited to the clinic than will be vaccinated. These participants will be alternates and will be vaccinated only if other participants are not available for vaccination or are found to be ineligible on the day vaccination. Up to 48 participants will be assigned to each vaccination day. Up to five alternate participants will be identified for each vaccination day to replace any participants who are unable to attend the assigned first visit date. Those participants who are alternates will be informed that they are alternates when they are invited to the clinic.

Table 15: Study specific procedures

Study Day **Study Activity**	**Screening (D-30 - D-5)**	**Day 0/Dosing**	**Day 7**	**Day 14**	**Day 28**	**Day 56**	**Day 180**	**Day 360**	**Day 720**	**Day 1080**
Conduct household recruiting visits	X									
Education	X	X								
Schedule screening visit	X									
Assign subject ID	X									
Review study procedures	X									
Informed Consent/ Assent (child 11-17)	X	X								
Check Inclusion/Exclusion	X	X								
Check elimination criteria			X	X	X	X	X	X	X	X
Check contra-indications		X								
Medical History	X									
Interim Medical History		X	X	X	X	X	X	X	X	X
Standard Physical Exam	X									
History-directed physical exam		X	X	X	X	X	X	X	X	X
Pre-vaccination temperature		X								
Urine Pregnancy Testing***	X	X			X	X				
Pregnancy prevention education***	X	X	X	X						
Blood Safety Tests: CBC/Diff, ALT	X		X	X						
Blood Safety Tests: PT/PTT	X			X						
HBV, HCV*	X									
Blood for Viremia			X	X**						
Blood for Serology		X		X**	X	X	X	X	X	X
Blood for CMI		X**	X**		X**	X**	X**	X**	X**	X**
Vaccination/ 30 minute observation		X								
Record data from surveillance source document into CRF			X	X	X					

Record concomitant medication		X	X	X	X					
Record Adverse Events		X	X	X	X					
Record Serious Adverse Events		X	X	X	X	X	X	X	X	X
Fever Surveillance/Evaluation of febrile episodes		X	X	X	X	X	X	X	X	X
Scheduling for follow-up visits		X	X	X	X	X	X	X	X	
Reminder for next study visit	X	X	X	X	X	X	X	X	X	
Home visits for fever and AE surveillance on study days 0-14 except for scheduled clinic visits (0, 7, and 14). After day 14 until 6 months, study participants will be contacted by phone or visited at home weekly. After 6 months, study participants will be contacted by phone or in person monthly. Information on AEs will be collected up to day 28.										

***HCV conducted on adults and adolescents only**
****Adult and adolescent age groups only**
*****Pregnancy testing and prevention education if applicable (see Section 4.1#6)**

6.3.1 Immunization Procedure

All cohort study participants will receive vaccine or placebo on Study Day 0. Monovalent vaccine candidates will be kept frozen at -80°C ± 15°C until just before use, whereupon they will be thawed, diluted, combined into a tetravalent admixture, and drawn up for administration (see Section 5 for vaccine preparation). Vaccine or placebo will be kept on wet ice or in a dedicated, temperature-controlled electronic cooler from the time it is diluted until it is delivered to clinical staff for administration. A vaccine volume of 0.5 mL will be delivered by subcutaneous injection in the deltoid region of the upper arm or the thigh in paediatric study participants with a needle of appropriate gauge and length after wiping the injection site with alcohol.

6.3.2 Visit 1, Study Day 0, Administration of Vaccine or Placebo

1. Verify that Informed Consent/Assent was obtained and that the consent/assent form was signed by the study participant and/or parent or guardian and by the study staff.

2. Verify eligibility and that all inclusion and exclusion criteria have been met, and confirm that no contraindications to vaccination are present. If a study participant has any absolute contraindications to vaccination, the participant will be withdrawn from the study. If a participant has a temporary contraindication to vaccination (see Section 4.4), the vaccination may be postponed until resolution of the temporary contraindication.

3. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints and any changes to medical history or exam findings since the screening visit. An examination of the thorax and skin will be performed to evaluate for dengue-like-rash. A pre-vaccination body temperature will be recorded. Any participant found to have a temperature of $\geq$38°C on the day of vaccination will not receive the vaccine on that day, as this is a temporary contraindication to vaccination.

4. Obtain urine for rapid point-of-care β-HCG testing in females of reproductive age (see Section 4.1#6). A positive β-HCG will exclude the participant from the trial per protocol. Ensure the test is negative before proceeding.

5. Perform pregnancy prevention counselling for females of reproductive age.

6. Assign allocated study identification number and ensure study identification number is linked in enrolment log with the vaccine treatment number.

7. Record all concomitant medications in CRF.

8. Obtain whole blood for baseline serology (PRNT). These laboratory studies are drawn as baseline values and will not determine eligibility.

9. Obtain whole blood for baseline Cell Mediated Immunity (CMI) testing for adult and adolescent study participants only. (See Table 16 for CMI blood volume)

10. Administer dose of the vaccine or placebo as described in Section 6.3.1.

11. Observe for at least 30 minutes after vaccination and evaluate for immediate hypersensitivity. Any AEs which occur after vaccination will be recorded by study staff into the CRF.

12. Thermometers will be provided to study participants to monitor for febrile illness during the study period. Surveillance source documents and small rulers will be used by FRAs or designee to record local and general solicited symptoms, including a temperature taken daily for 15 days (Day 0 through 14). The observations will be recorded each day by FRAs or designee and will account for the previous 24 hours. If multiple temperature measurements are taken in one 24 hour period, the maximum temperature will be recorded.
    a. FRAs or designee will record on the surveillance source document daily temperatures, and solicited and unsolicited symptoms (minimum Day 0-14) until the Study Day 14 visit.
    b. After Day 14, FRAs or designee will contact participants weekly by phone or home visit up to day 28, to continue to monitor and record solicited and unsolicited symptoms. To monitor febrile illness, FRAs or designee will also contact participants weekly by phone or home visit up to Study Day 180 and then montly between Day 180 and three year follow-up.

13. FRAs or designee will record on the source document concomitant medication until their next study visit (minimum Day 0-28) after vaccination.

14. Record any AE and SAEs which have occurred from the time of screening. Serious adverse events will be recorded throughout the entire study period. Study participants will be instructed to contact the Investigator or designee immediately should they manifest any signs or symptoms they perceive as serious and for any hospitalizations for any reason (see Section 7.1.2, Serious Adverse Events). They will be given an identification card and a set of phone numbers to contact study staff 24 hours a day, 7 days a week.

15. Study staff will educate the study participants about the need to contact study staff immediately with any acute febrile illness consistent with suspected dengue.

16. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.

17. Study participants will be reminded of subsequent study visits, including the date of the next study visit.

6.3.3 Visit 2, Study Day 7 (+/- 1 day)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3).
2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like-rash.

3. Obtain whole blood for safety labs, to include CBC with differential and ALT according to blood volumes in Table 16**.**

4. Obtain whole blood for viremia evaluation

5. Obtain whole blood for CMI testing for adult and adolescent age cohorts only (**Table 16**).

6. MO to enter new data from the surveillance source document into the CRF.

7. Record any unsolicited or solicited AEs since previous visit.

8. Record concomitant medication since previous visit.

9. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).

10. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.

11. Perform pregnancy prevention counselling for females of reproductive age (see Section 4.1#6).

12. Remind study participant of next scheduled study visit.

6.3.4 Visit 3, Study Day 14 (+/- 1 day)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3).

2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like-rash.

3. Obtain whole blood for safety labs, to include CBC with differential, PT/PTT, and ALT, according to blood volumes in **Table 16** (for participants ≥ 11 years of age only).

4. Obtain whole blood for viremia evaluation in the adult and adolescent cohorts only.

5. Obtain whole blood for serology (PRNT) for adult and adolescent cohorts only.

6. MO to enter new data from the surveillance source document into the CRF.

7. Record any unsolicited or solicited AEs since previous visit.

8. Record concomitant medication since previous visit.

9. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).

10. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.

11. Perform pregnancy prevention counselling for females of reproductive age (see Section 4.1#6).

12. Remind participant of next scheduled study visit.

6.3.5 Visit 4, Study Day 28 (+ 3 days)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3).

2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like-rash.

3. Per clinician discretion, obtain whole blood for safety labs as needed to assess for resolution of any laboratory AEs that were new or ongoing at Study Day 14.

4. Obtain whole blood for serology (PRNT) according to blood volumes in Table 16

5. Obtain whole blood for CMI testing (for adult and adolescent age groups only), and for safety labs as needed to assess for resolution of any laboratory AEs.

6. Obtain urine from females of reproductive age for rapid β-HCG pregnancy testing.

7. MO to enter new data from the surveillance source document into the CRF.

8. Record any unsolicited or solicited AEs since previous visit.

9. Record concomitant medication since previous visit.

10. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).

11. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.

12. Remind participant of next scheduled study visit.

6.3.6 Visit 5, Study Day 56 (+/- 7 days)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3).

2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like-rash.

3. Per clinician discretion, obtain whole blood for safety labs as needed to assess for resolution of any laboratory AEs that were ongoing at study day 28.

4. Obtain whole blood for serology (PRNT) according to blood volumes in Table 16.

5. Obtain whole blood for CMI testing (for adult and adolescent age groups only).

6. Obtain urine from females of reproductive age for rapid β-HCG pregnancy testing.

7. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).

8. Record concomitant medication in the case of new or changed significant or chronic conditions, for medications taken for AEs that were ongoing at Study Day 28, or for those medications taken for an SAE.

9. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.

10. Remind participant of next scheduled study visit.

6.3.7 Visit 6, Study Day 180 (+/- 14 days)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3)

2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like-rash.

3. Per clinician discretion, obtain whole blood for safety labs as needed to assess for resolution of any laboratory AEs that were ongoing at study day 28.

4. Obtain whole blood for serology (PRNT) according to blood volumes in **Table 16**.

5. Obtain whole blood for CMI testing for adult and adolescent age groups only.

6. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived

as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).

7. Record concomitant medication in the case of new or changed significant or chronic conditions, for medications taken for AEs that were ongoing at Study Day 28, or for those medications taken for an SAE.

8. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.

9. Remind participant of next scheduled study visit.

6.3.8 Visit 7, Study Day 360 (+/- 14 days)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3)
2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like rash.
3. Per clinician discretion, obtain whole blood for safety labs as needed to assess for resolution of any laboratory AEs that were ongoing at study day 28.
4. Obtain whole blood for serology (PRNT) according to blood volumes in Table 16.
5. Obtain whole blood for CMI testing for adult and adolescent age groups only.
6. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).
7. Record concomitant medication in the case of new or changed significant or chronic conditions, for medications taken for AEs that wer ongoing at Study Day 28, or for those medications taken for an SAE.
8. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.
9. Remind participant of next scheduled study visit.

6.3.9 Visit 8, Study Day 720 (+/- 14 days)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3)
2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like rash.
3. Per clinician discretion, obtain whole blood for safety labs as needed to assess for resolution of any laboratory AEs that were ongoing at study day 28.
4. Obtain whole blood for serology (PRNT) according to blood volumes in **Table 16**.
5. Obtain whole blood for CMI testing for adult and adolescent age groups only.
6. Record SAEs during the entire study period (participants will be instructed to contact the Investigator immediately should they manifest any signs or symptoms perceived as serious or have any hospitalization for any reason, and/or any febrile illness consistent with suspected dengue).

7. Record concomitant medication in the case of new or changed significant or chronic conditions, for medications taken for AEs that wer ongoing at Study Day 28, or for those medications taken for an SAE.
8. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit.
9. Remind participant of next scheduled study visit.

6.3.10 Visit 9, Study Day 1080 (+/- 14 days)

1. Confirm that study participants are still eligible according to elimination criteria. (See Section 4.3)
2. Obtain vital signs and perform interim medical history and history-directed physical exam, concentrating on any acute complaints. An examination of the thorax and skin will be performed to evaluate for dengue-like rash.
3. Per clinician discretion, obtain whole blood for safety labs as needed to assess for resolution of any laboratory AEs that were ongoing at study day 28.
4. Obtain whole blood for serology (PRNT) according to blood volumes in Table 16.
5. Obtain whole blood for CMI testing for adult and adolescent age groups only.
6. Ensure any SAE during the entire study period were captured and followed to resolution. Active surveillance of serious adverse events will end following this final study visit.
7. Record concomitant medication in the case of new or changed significant or chronic conditions, for medications taken for AEs that wer ongoing at Study Day 28, or for those medications taken for an SAE.
8. Ensure documentation of any febrile illness consistent with suspected dengue since last study visit. Surveillance of suspected dengue will end following this final study visit.
9. Perform study conclusion form and document study conclusion in CRF.

Table 16: Approximate Blood volumes per study visit per cohort

Adult Ages 18-50 **Test**	**Screen**	**Visit 1 Day 0**	**Visit 2 Day 7**	**Visit 3 Day 14**	**Visit 4 Day 28**	**Visit 5 Day 56**	**Visit 6 Day 180**	**Visit 7 Day 360**	**Visit 8 Day 720**	**Visit 9 Day 1080**
CBC/Diff	1 ml		1 ml	1 ml						
ALT/HBV/HCV	4 ml		1 ml	1ml						
PT/PTT	2 ml			2 ml						
Viremia			2 ml	2 ml						
Serology		10 ml		10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
CMI		20 ml	20 ml		20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Total	**7 mL**	**30 mL**	**24 mL**	**16 mL**	**30 mL**	**30 mL**	**30 mL**	**30 mL**	**30 mL**	**30 mL**
Adolescent ages 11-<18 **Test**	**Screen**	**Visit 1 Day 0**	**Visit 2 Day 7**	**Visit 3 Day 14**	**Visit 4 Day 28**	**Visit 5 Day 56**	**Visit 6 Day 180**	**Visit 7 Day 360**	**Visit 8 Day 720**	**Visit 9 Day 1080**
CBC/Diff	1 ml		1 ml	1 ml						
ALT/HBV /HCV	4 ml		1 ml	1ml						
PT/PTT	2 ml			2 ml						
Viremia			2 ml	2 ml						
Serology		5 ml		5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
CMI		8 ml	8 ml		8 ml	8 ml	8 ml	8 ml	8 ml	8 ml
Total	**7 mL**	**13 mL**	**12 mL**	**11 mL**	**13 mL**	**13 mL**	**13 mL**	**13 mL**	**13 mL**	**13 mL**
Ages 5-<11 **Test**	**Screen**	**Visit 1 Day 0**	**Visit 2 Day 7**	**Visit 3 Day 14**	**Visit 4 Day 28**	**Visit 5 Day 56**	**Visit 6 Day 180**	**Visit 7 Day 360**	**Visit 8 Day 720**	**Visit 9 Day 1080**
CBC/Diff	1 ml		1 ml	1 ml						
ALT/HBV	1 ml		1 ml	1ml						
PT/PTT	2 ml			2 ml						
Viremia			2 ml							
Serology		3 ml			3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
Total	**4 mL**	**3 mL**	**4 mL**	**4 mL**	**3 mL**	**3 mL**	**3 mL**	**3 mL**	**3 mL**	**3 mL**
Ages 1-<5 **Test**	**Screen**	**Visit 1 Day 0**	**Visit 2 Day 7**	**Visit 3 Day 14**	**Visit 4 Day 28**	**Visit 5 Day 56**	**Visit 6 Day 180**	**Visit 7 Day 360**	**Visit 8 Day 720**	**Visit 9 Day 1080**
CBC/Diff	1 ml		1 ml	1 ml						
ALT/HBV	1 ml		1 ml	1 ml						
PT/PTT	2 ml			2 ml						
Viremia			2 ml							
Serology		3 ml			3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
Total	**4 mL**	**3 mL**	**4 mL**	**4 mL**	**3 mL**	**3 mL**	**3 mL**	**3 mL**	**3 mL**	**3 mL**

NB: The table is colour coordinated accordingly: Orange = Safety procedures; Green = Study procedures; Blue = Exploratory procedures.

6.3.11 Unscheduled Visits

6.3.11.1 Close-out of Solicited Laboratory Adverse Events

In the event of a grade 1 or higher and unresolved laboratory AE, study participants may be asked to come to the clinic at a time specified by the medical officer or designee to assess resolution of the AE. Additional blood may be drawn at that time as determined to be clinically necessary.

6.3.11.2 Fever Surveillance

First line of surveillance
Field research assistants (FRAs) or designee will make home visits to study participants or parent/legal acceptable representative to collect fever and AE information once per day up to day 14.

Second line of surveillance
Study participants or parents/LAR will be instructed to contact the Study Investigator and/or other appropriate study staff or come to the clinic in the event of febrile episodes during entire study period.

Third line of surveillance
After Day 14, study staff will contact study participants or their parent/legal acceptable representative weekly by phone or in person to check on fever and /or SAEs, or follow-up and resolution of ongoing AEs through Study Day 180. After Study Day 180, study staff will contact study participants monthly by phone or in person to remind them to contact the clinic if they have a febrile illness. Study staff will also ask study participants if they have been hospitalized for a febrile illness since the last call. If the study participant was hospitalized for a febrile illness, they will be instructed to come to the clinic for suspected Dengue follow-up.

6.3.11.3 Definition of Suspected Dengue

Study participants will be instructed to contact the Investigator or study coordinator if they develop fever equivalent to an oral temperature ≥38ºC at any time during the study period following the first vaccination visit.

The Investigator will instruct the participant to monitor fever and any associated symptoms for an additional day.

If 2 days of fever occur, the study participant will be instructed to report to the Investigator for an unscheduled visit to evaluate the participant for suspected dengue as defined below.

The case definition of "suspected dengue" used in this study protocol includes the following criteria:

- The study participant has a fever (equivalent to an oral temperature ≥38ºC) measured at least twice daily or is feverish (feels as if they have a fever but is unable to measure temperature) on two successive days
- No alternative diagnosis by a qualified physician can be made with reasonable certainty

If dengue is suspected, a blood sample (approximately 2 mL of whole blood) will be collected from the study participant and analysed for dengue non-structural 1(NS1) antigen, IgM antibody, and viremia. PCR will also be conducted in order to determine if suspected dengue is vaccine related.

The Investigator will record any symptoms associated with the fever during the suspected dengue evaluation on related study documentation. The participant will be followed by the medical officer or appropriate designee until the fever abates.

If a study participant has a febrile illness determined to not be due to a Dengue virus infection or related to vaccine/placebo administration, further fever work up may be performed at the discretion of the medical officer. The volunteer will be followed until fever abates.

6.3.11.4 Definition of Laboratory Confirmed Dengue

The case definition of laboratory confirmed dengue is as follows. All conditions must apply:

1. The study participant has a fever (equivalent to an oral temperature ≥38ºC) measured at least twice daily or is feverish (feels as if they have a fever but is unable to measure temperature) on two successive days.
2. No alternative diagnosis by a qualified physician can be made with reasonable certainty.
3. DENV is detected by NS1/IgM criterion, PCR, or viremia assay.

Additional testing may be completed at the discretion of the Investigator, if required to confirm a clinical diagnosis.

6.4 Solicited and Unsolicited Adverse Events

FRAs or designee will make home visits or calls to study participants once per day from study Day 0 through study day 14 after vaccination, and weekly thereafter through Study Day 180. After Study Day 180, study staff will contact study participants monthly by phone or in person until the end of the study. They will record temperature and AEs on age-specific surveillance source documents as detailed below. Temperature will be recorded daily from Study Day 0 through Study Day 14. AEs will be collected from the time of informed consent through Study Day 28. Solicited local and general AEs, and a note of whether or not any unsolicited AEs occurred will be recorded on the age-specific surveillance SD. Adverse events will then be entered into the appropriate CRF by MO, or designee, at the next clinic visit. Solicited AEs are described in Table 18, Table 19, and

Table 22. Unsolicited AEs are AEs that are not described in **Tables 18, 19, and 22**. Please see Section 7 for recording and reporting obligations for AEs.

Field staff will instruct study participants how to use thermometers, to take their temperatures if they feel they have elevated temperatures, for dengue surveillance for the duration of the study.

FRAs or designee will measure local reactogenicity at the vaccination site using rulers, and will record on the surveillance source documents.

6.4.1 Solicited Local Injection Site Adverse Events

AEs will be collected from the time of informed consent through Study Day 28. For all study participants, the solicited local symptoms at the injection site listed in **Tables 18 and 19** below will be assessed at the day of dengue/placebo vaccine administration and then every day for 15 days (from Day 0 through Day 14) post-vaccination, and weekly until Study day 180, and monthly between Study day 180 and three year follow-up, by FRAs or designee at home visits or calls using surveillance source documents and rulers.

6.4.2 Solicited General Adverse Events

AEs will be collected from the time of informed consent through Study Day 28. For all study participants, the age-specific solicited general symptoms listed in Table 18 and Table 19 will be assessed at the day of dengue/placebo vaccine administration then every day for 15 days (from Day 0 through Day 14) post-vaccination, and weekly until Study day 180, and monthly between Study day 180 and three year follow-up by FRAs or designee at home visits or calls using surveillance source documents.

For all age groups, oral or axillary temperature will be recorded once daily. If additional temperature measurements are performed at other times of day, the highest temperature will be recorded.
Fever is defined as an oral temperature ≥38°C, axillary temperature ≥38°C, tympanic temperature on oral setting ≥38°C.

See Section 7 for full details of AE monitoring and grading.

6.5 Clinical Laboratory Testing

Using standard techniques, the icddr,b clinical laboratories will perform the following tests:

- Complete blood count plus white blood cell differential.
- PT/PTT
- ALT
- Hepatitis B and C (for adults and adolescents only) screening

Laboratory assays will be performed according to Table 17**.** Urine β-HCG testing will be performed at the clinical trial site using a U.S. FDA-approved pregnancy test kit. Collected scheduled samples for quantitation of vaccine virus titers obtained during the first 14 days after dosing of the candidate vaccine will be stored at-80°C ± 15°C at icddr,b research laboratories, and tested by isolation in Vero cells at the icddr,b research laboratories. Testing of serum samples for suspected dengue will be performed by a rapid NS1 ELISA followed by IgM ELISA at the icddr,b research laboratory. Multiplex PCR will also be run. Serum collected for neutralizing antibody (NAb) plaque reduction neutralization antibody assays

(PRNT assays) will be stored at -20°C or below and assayed at the icddr,b research laboratory. Peripheral Blood Mononuclear Cells (PBMCs) will be isolated and stored in liquid nitrogen at the icddr,b research laboratory and shipped to the University of Vermont for cellular immune studies as detailed below in **Table 17.**

Table 17: Laboratory assays

Marker	Assay Method	Assay Type	Testkit/ Manufacturer	Assay cut-off and unit	Laboratory
CBC/diff, ALT PT/PTT*	Automated assay	Quantitative	Commercial	Refer to table of normal lab values	Local (icddr,b clinical labs)
Vaccine DENV isolation	Vero cell isolation	Qualitative and quantitative	In-house	Positive/negative	icddr,b research labs
NS1 rapid test for suspected Dengue	Automated assay	Qualitative	Commercial	Positive/negative	Icddr,b research labs
NSI/IgM ELISA for suspected Dengue	Automated assay	Qualitative	Commercial	Positive/negative	Icddr,b research labs
Multiplex PCR for suspected Dengue	pcr	Qualitative	In-house	Positive/negative	TBD
Neutralizing Ab to DENV	PRNT	Quantitative	In house (in Vero cells)	The titer giving 50% reduction in viral infection	icddr,b research labs
Anti-HBV, HCV	Automated assay	Qualitative	Commercial	Positive/negative	Local (icddr,b clinical labs)
Human chorionic gonadotropin	Urinalysis	Qualitative	Commercial	As specified by the manufacturer	On-site Mirpur clinic

6.6 Medical History and Concomitant Medications

A complete medical history will be collected during screening. Any changes reported in medical history during each 28-day post vaccination visit will be assessed as possible AEs. After Study Day 28 following vaccination, the medical history will be updated for any new or changed significant or chronic conditions. Study staff will collect current medications as part of the medical history, including over the counter medications and herbal supplements, at the time of enrolment. All changes or updates to medications will be collected through Study Day 28 following vaccination. After Study Day 28, concomitant medications will be collected in the case of new or changed significant or chronic conditions. Medications taken for AEs that occur prior to Study Day 28 and continue past study day 28, and medications taken for SAEs will be recorded throughout the trial.

6.7 Immunology Testing

6.7.1 Antibody Testing

Serum antibody levels to DENV-1, DENV-2, DENV-3, and DENV-4 will be measured by plaque reduction neutralizing antibody assay using standard laboratory protocols. The 50% plaque reduction neutralization titer ($PRNT_{50}$) is defined as the highest dilution of antibody that reduces the number of foci or plaques by 50%, compared to the plaque titer of a mixture of virus with serum from the same participant prior to vaccination.

6.7.2 Other Exploratory Immunological Assays

Additional immunologic assays may be performed on PBMCs to evaluate the individual immune response post-vaccination including:

- New serological measures of immunogenicity and vaccine efficacy surrounding neutralization and enhancement.
- Cell-mediated immunity including stimulation of T and B cells for measures of adaptive immune response post-vaccination.
- Evaluation of innate immune response post-vaccination.
- Other immunologic assays in response to the development of additional assay techniques and current literature and scientific knowledge over the course of the study.
- PBMC phenotyping, to include human leukocyte antigen (HLA) typing of samples, may be performed.

7 Adverse Event Monitoring

7.1 Definitions

7.1.1 Adverse Event

An adverse event (AE) is defined as any untoward or unfavourable medical occurrence in a human subject, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research. All AEs occurring from the time of consent to the day 28 follow-up period will be evaluated for severity, action taken, seriousness, outcome and relationship to the investigational vaccine and to research participation as described in Section 7.2 in this protocol. The relationship to the investigational product and research participation will be determined by the Investigator.

If a diagnosis is clinically evident, the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE. AEs will be collected through the 28-day period following vaccination, to include capture of all solicited local and general AEs recorded on the surveillance source document. Any AEs identified in the 28-day post-vaccination period will be followed until resolution.

AEs are categorized as **solicited AEs and unsolicited AEs**. Solicited AEs include local reactogenicity, systemic reactogenicity, and Laboratory AEs. Solicited AEs are those events which the clinician is specifically evaluating during the 28 day post-vaccination period, as listed in Table 18, Table 19, and

Table 22.

At each contact with the participant, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

• immediately documented in the subject's medical record/source document,

• recorded on the AE CRF and

• reported as outlined to ethical review boards and IND sponsor at continuing review, and DSMB before each meeting outlined in Section11.1.

Table 18: Solicited adverse events: study participants > 5 years of age

Systemic Reactogenicity	Local Reactogenicity
Fever	Injection site pain
Headache	Injection site erythema
Retro-orbital pain	Injection site swelling
Photophobia	Injection site pruritus
Nausea	
Fatigue	
Myalgia	
Arthralgia	
Diffuse rash on trunk	

Table 19: Solicited adverse events: study participants < 5 years of age

Systemic Reactogenicity	Local Reactogenicity
Fever	Injection site pain
Irritability/fussiness	Injection site erythema
Drowsiness	Injection site swelling
Loss of Appetite	Injection site pruritus
Vomiting	
Decrease in activity (fatigue)	
Headache	
Diffuse rash on trunk	

7.1.2 Serious Adverse Event (SAE)

SAEs will be collected from the time of informed consent until study participants three year follow-up period has been completed. An SAE is defined as involving one or more of the following outcomes:

- Death
- Life threatening event, defined as an event that places a subject at immediate risk of death at the time of the event and does not refer to an event that hypothetically might have caused death were it more severe
- Inpatient hospitalization or prolongation of existing hospitalization, defined as at least an overnight stay in the hospital or emergency ward for treatment that would have been inappropriate if administered in the outpatient setting

- Congenital anomaly or birth defect
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Other medically important event*

*Medical and scientific judgment should be exercised in deciding events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed above.

Each AE will be classified by the Investigator/clinically qualified designee as "serious" or "non-serious". An AE needs to meet only one or more of the above criteria to be considered serious.

7.1.3 Unexpected Adverse Events (UAEs)
An adverse event is considered unexpected if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

7.1.4 Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected. SUSARs are reported only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure;
2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug;
3. An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

7.1.5 Unanticipated Problem that is not an Adverse Event (UPnonAE)
An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Adverse Reaction (AR)
An adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of causality.

7.1.6 Pre-existing Conditions, Worsening of Pre-existing Conditions
Stable chronic conditions which are present prior to enrolment and do not worsen are not considered AEs and will be accounted for in the study participant's medical history.

Exacerbation or worsening of pre-existing conditions are defined as AEs and are evaluated using the same criteria described in Section 7.2 in this protocol. Any SAE that occurs prior to enrolment for eligible participants will be recorded using the NIAID SAE report form (the Safety Expedited Report Form or SERF), with the study ID as the identifier.

Protocol Deviation:
Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Serious Protocol Deviation:
A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance:
The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as:

1. Serious: Non-compliance that
    a. Increases risks, or causes harm, to participants
    b. Decreases potential benefits to participants
    c. Compromises the integrity of the NIH-HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

7.2 Assessment of Adverse Events

7.2.1 Identification of Adverse Events

Assessment of safety will include clinical observations and monitoring of haematological, blood chemistry, and immunologic parameters. Safety will be evaluated by monitoring of the study participants for local and systemic adverse reactions during the course of the trial. Participants will be closely monitored for 30 minutes following immunization. Additionally, participants will return to the clinic on Study Days 7, 14, 28, 56, 180, 360, 720, and 1080 following vaccination, at a minimum and may be asked to return more often if warranted. Study staff record temperatures and AEs on the surveillance source document. Any AEs which occur from Day 14 to Day 28 post-vaccination will be captured by questioning of the participant by study staff at the Day 28 visit or by weekly phone calls or home visits. At each visit through Study Day 28 following vaccination, study staff will query the participant about any AEs noted on the surveillance source document and perform a history directed physical exam. A study clinician will be available to study participants by telephone 24 hours a day during the entire three year study evaluation period, and all participants will be provided with a telephone contact card during the initial enrolment visit.

For all enroled study participants, all AEs occurring from the time the informed consent is signed through day 28 will be documented, recorded, and reported. All solicited and

unsolicited AEs will be recorded into source documents during the period after the study participant receives the study vaccine, through and including post-vaccination Study Day 28, and will be entered into the CRF up to the Day 28 visit. In order to ensure maximum participant safety, a study participant may be asked to return to clinic for an unscheduled visit for assessment of resolution of an adverse event at the discretion of the clinician.

All SAEs and SUSARs will be reported following SAE reporting guidelines outlined in Section 7.3.2 of this protocol. SAE severity grading classifications are listed in Table 20**.**

7.2.2 Protocol Specific Adverse Event Definitions

Solicited general adverse events in study participants ≥ 5 years of age

Fever	Oral temperature ≥38°C, axillary temperature ≥38°C, tympanic temperature on oral setting ≥38°C
Diffuse Rash on Trunk (Dengue Vaccine-like Rash):	Macular/ maculo-papular rash, typically found on the trunk and proximal extremities most frequently seen 10-16 days after vaccination.
Headache:	A pain located in the head, over the eyes, at the temples, or at the base of the skull.
Retro-orbital pain (ROP):	Bilateral pain situated behind the orbits of the eye.
Photophobia:	An abnormal sensitivity to or intolerance of light.
Nausea:	Discomfort in the stomach with an urge to vomit.
Fatigue:	Excessive tiredness following minimal exertion.
Myalgia:	Pain in the muscles, found in $\geq$ 2 muscle groups.
Arthralgia:	Pain in a joint, found in $\geq$ 2 joints.

Solicited general adverse events in children < 5 years of age

Fever	Oral temperature ≥38°C, axillary temperature ≥38°C, tympanic temperature on oral setting ≥38°C
Diffuse Rash on Trunk (Dengue Vaccine-like Rash):	Macular/ maculo-papular rash, typically found on the trunk and proximal extremities most frequently seen 10-16 days after vaccination.
Irritability/fussiness:	Excessive irritability, manifested by frequent or inconsolable crying.
Drowsiness:	Fatigue and sleepiness evident more than is normal for the child.
Loss of appetite:	Noticeable decreased desire to eat normally.
Vomiting:	Abnormal frequency of vomiting.
Decreased activity:	Noticeably diminished playing behaviour, lack of interest in normal activities.

Headache (child):	Holding head in hands, sensitivity to loud noise or bright light.

7.2.3 Determination of Severity

The Investigator/designee will assess and grade AE severity using the classifications outlined in Table 18, Table 19, Table 20 and Table 22**.**

Table 20: Severity definitions

Severity	Defined
Grade 0 (Normal)	No symptoms
Grade 1 (Mild)	Event that is easily tolerated, may require 1 dose of medication/treatment
Grade 2 (Moderate)	Event that interferes with daily activity or requires more than 1 dose of medication/treatment
Grade 3 (Severe)	Event that prevents daily activity and requires medical intervention
Grade 4 (Life Threatening)	Event which places the subject at immediate risk of death
Grade 5 (Death)	Event which results in death

Table 21: Assessment of solicited adverse events

Local Reactogenicity	Grade	Severity
Injection Site Pain Injection Site Pruritis	0 1 2 3	Absent Event that is easily tolerated Event that interferes with daily activity Event that prevents daily activity
Injection Site Erythema (redness) Injection Site Swelling *(Record greatest surface diameter in mm)*	0 1 2 3	absent <5 mm 5 mm to 20 mm >20 mm
Systemic Reactogenicity	**Grade**	**Severity**
Fever (oral or equivalent)	0 1 2 3	<38 °C 38° C–38.5°C 38.6°C – 39.1°C ≥39.2°C
Dengue Vaccine Like Rash	0 1 2 3	Normal Rash is present but asymptomatic Rash is symptomatic (pruritus/pain) but does not interfere with function Rash is symptomatic and interferes with function
Headache Retro-orbital pain (ROP) Photophobia Nausea Fatigue Myalgia Arthralgia Irritability/fussiness Drowsiness Loss of appetite Vomiting Decrease in activity (fatigue)	0 1 2 3	Normal Event that is easily tolerated, may require 1 dose of medication/treatment Event that interferes with daily activity or requires more than 1 dose of medication/treatment Event that prevents daily activity and requires medical intervention

Table 22: Assessment of solicited laboratory AEs (43)

Laboratory AEs	Grade	Severity
Haemoglobin (females) (Ages 5 and older)	1 2 3	9.5 - 10.7 gm/dL 8.0 - 9.4 gm/dL ≤7.9 gm/dL
Haemoglobin (males) (Ages 5 and older)	1 2 3	11 – 12.5 gm/dL 9.0 – 10.9 gm/dL ≤8.9 gm/dL
Haemoglobin (Children <5)	1 2 3	10.0 – 11.4 gm/dL 8.5 – 9.9 gm/dL ≤8.4 gm/dL
Neutropenia (reduced ANC)	1 2 3	750 - 999/mm^3 500 – 749/mm^3 <500 mm^3
Leukocytosis (Increased WBCs) (Ages 11 and older)	1 2 3	11,500 - 13,000/mm^3 13,001 - 15,000/mm^3 >15,000 or <1,000/mm^3
Leukocytosis (Increased WBCs) (Ages 4 to <11 years)	1 2 3	15,000 – 16,500/mm^3 16,501 – 18,500/mm^3 >18,500 or <1,000/mm^3
Leukocytosis (Increased WBCs) (Ages 1 year to <4 years)	1 2 3	18,000 – 19,500/mm^3 19,501 – 21,500/mm^3 >21,500 or <1,000/mm^3
Thrombocytopenia (Decreased Platelets)	1 2 3	≥100,000 – 120,000/mm^3 ≥75,000 - 99,999/mm^3 ≤74,999/mm^3
PT	1 2 3	>1.0 - 1.25 x ULN >1.25 - 1.5 x ULN >1.5 x ULN
PTT	1 2 3	>1.0 - 1.66 x ULN >1.66 - 2.33 x ULN >2.33 x ULN
ALT/SGPT	1 2 3	>1.25 - 2.5 x ULN >2.5 - 5.0 x ULN >5.0 x ULN

7.2.4 Relationship of Adverse Events to Investigational Product Vaccine

The Investigator is obligated to assess the relationship between the vaccine/placebo and the occurrence of each AE/SAE. The Investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors as well as the temporal relationship of the event to the vaccine/placebo will be considered and investigated. The Investigator will also consult the

Investigator Brochure in the determination of relatedness between the vaccine and adverse events.

There may be situations when a SAE has occurred and the Investigator has minimal information to include in the initial report to the IRBs, ECs, and sponsor. However, it is very important that the Investigator always makes an assessment of causality for every event prior to transmission of the SAE Report Form, specifically referred to as the Safety Expedited Report Form (SERF). The Investigator may change his/her opinion of causality in light of follow-up information, amending the SERF accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other AEs should be assessed by the Investigator using the following question, "Is there a reasonable possibility that the AE may have been caused by the vaccine/placebo?"

No: The AE is not causally related to administration of the study vaccine(s). There are other, more likely causes and administration of the study vaccine(s) is not suspected to have contributed to the AE.

Yes: There is a reasonable possibility that the vaccine(s) contributed to the AE.

Non-serious and serious AEs will be evaluated as two distinct events. If an event meets the criteria to be determined "serious" (see Section 7.1.2 for definition of serious adverse event), the Investigator will make every attempt to consider ALL contributing factors.

7.2.5 Adverse Event Action Taken

The Investigator/designee will assess the action taken by the study participant or the study staff in relation to the AE using the following classifications:

Action

1 = None
2 = Remedial therapy (≥ 1 dose of medication required)
3 = Discontinued study
4 = Hospitalization
5 = Other

7.2.6 Adverse Event Outcome

The Investigator/designee will assess the outcome of the AE, either at resolution or at the end of the study period, using the following classifications:

Outcome

1 = Resolved
2 = Recovered with sequelae
3= Continuing
4 = Death
5 = Unknown

7.2.7 Adverse Event Seriousness

The Investigator/designee will categorize all AEs as either serious or non-serious, using the criteria defined in Section 7 of this protocol.

Any events defined as serious will also be reported following SAE reporting guidelines outlined in Section 7.3 of this protocol.

7.3 Adverse Event Reporting

7.3.1 Non-Serious Adverse Events

Non-serious AEs will be followed to resolution or until the study ends, and are reported to the Sponsor as requested, to the IRBs/ECs according to IRB/EC policies (to include annual Continuing Review Reports), to the DSMBs as required, and to the U.S. FDA by the sponsor as required for the annual report. AE data will be submitted to the IND Sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

AEs meeting the stopping criteria outlined in Section 7.5 of this protocol will be reported to the Sponsor, the IRBs/ECs, and the DSMBs following the SAE reporting guidelines.

7.3.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to the Clinical Safety Office (CSO) by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported to the CSO within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone 001-301-846-5301
Fax 001-301-846-6224
E-mail: rchspsafety@mail.nih.gov

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF and the SERF.

icddr,b ERC reporting

All SAEs/SUSARs, UAEs, and UPs/UPs involving risk to subject or others are also reported to the icddr,b ERC as per guidelines as below:
The PI is responsible for ensuring timely submission of Serious Adverse Events (SAEs) within 24 hours of knowledge of their occurrence, including measures taken to mitigate/manage the event. In the event any participant experiences multiple SAEs, each event will be reported separately. If detailed information is not available at the time of submission of the initial report, follow-up report(s) will be submitted as soon as information is available. Final outcome of the event should be reported within 72 hours of knowledge of outcome.

UVM IRB
All SAEs/SUSARs, UAEs, and UPs/UPs involving risk to study participants or others will be reported to the UVM IRB as per guidelines below:

- UVM IRB requests that the above events be reported in the annual Continuing Review Report.

Unanticipated Problems (UP) Reporting:
Unanticipated Problems that are also AEs must be reported to the CSO and sent by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the CSO.

Report UPs that are also AEs to the CSO on a SERF or a local IRB UP form.

7.4 Sponsor's Reporting Responsibilities
Serious, unexpected, suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to the U.S. FDA and to all participating Investigators as IND Safety Reports. The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the U.S. FDA as defined in 21 CFR 312.33.

7.5 Stopping Criteria
Halting the study requires immediate discontinuation of study agent administered for all subjects and suspension of enrolment until a decision is made whether or not to continue enrolment and study agent administration. The IRBs/ECs, the IND Sponsor (OCRPRO), CSO, either DSMB, or the FDA (CBER) may stop the study at any time following review of any safety concerns.

The halting rules are outlined below:

• Two or more study participants experience the same or similar SAEs that are unexpected and are possibly, probably, or definitely related to the study agent

OR

• any safety issue that the PI and/or the CSO determines should halt the study

- If a stopping criteria is met, a description of the adverse event(s) or safety issue must be reported by the PI within one business day to the Sponsor, CSO, IRBs/ECs, and DSMBs by fax or email. The IND Sponsor will promptly report to CBER that a stopping criterion has been met.

The IND Sponsor, in collaboration with the PI, IRBs/ECs, and DSMBs, will determine if it is safe to resume the study. The IND Sponsor will notify the Site Investigators of this decision. The conditions for resumption of the study will be defined in this notification. The Site Investigators will notify their local IRBs/ECs of the decision.

7.6 Pausing Criteria

Pausing is the suspension of administration of study agent to a single study participant until a decision is made whether or not to resume administration of the study agent. The pausing criteria for a single study participant include any of the following:

A study participant experiences an SAE that is possibly, probably, or definitely related to a study agent

A study participant experiences a Grade 3 or greater AE that is possible, probably, or definitely related to a study agent. Any safety issue that Site Investigator determines should pause administration of study agent to a single subject.

Any safety issue that the Site Investigator determines should pause administration of a study agent to a single subject.

The IND Sponsor, in collaboration with the PI, may also pause an individual study participant or entire group if a safety concern is identified.

If a pausing requirement is met, a description of the AE(s) or safety issue must be reported to the CSO, the IRBs/ECs, and the DSMBs by the Site Investigator by email within one business day.

The IND Sponsor in collaboration with the PI, will determine if it is safe to resume administration of the investigational product to the study participant/group. The CSO or designee will notify the Site Investigators of this decision. The Site Investigators will notify their local IRB of the decision to resume administration of the study agent prior to resumption.

A subject who does not resume study agent will continue to be followed for safety.

8 Data Collection and Monitoring

8.1 Source Documentation and Data Collection

Complete source documentation (laboratory test reports, hospital or medical records, medical progress notes, etc.) is required for every study participant for the duration of the study. The participant's study record must record his/her participation in the clinical trial and, after unblinding, the randomization treatment received (with doses and frequency) and concomitant medications or other interventions administered, as well as any adverse reactions experienced during the trial.

Study data will be maintained in paper CRFs that are uploaded into DataFax and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Certain CRFs will serve as Source Documents. Data entry into paper CRFs and DataFax will be performed by authorized individuals.

Corrections to the paper source documents must be made by striking through the incorrect entry with a single line (taking care not to obliterate or render the original entry illegible) and

entering the correct information adjacent to the incorrect entry. Corrections must be initialled and dated by the person making the correction whenever possible. Source documentation should support the data collected on the CRFs**,** and must be signed and dated by the person recording and/or reviewing the data.

The Investigator is responsible for the accuracy, completeness and timeliness of the data reported to the Sponsor in the DataFax System. Data reported in the DataFax system should be consistent with source documents. All discrepancies should be explained. Source documentation will be made available for review or audit by the Sponsor or designee, the Sponsor's monitors, and any applicable Federal authorities.

8.2 Study Documentation

Study-related documentation will be completed as required by the IRBs/ECs, the Sponsor, and regulatory authorities. Continuing review documentation will be submitted by the Investigator to the IRBs/ECs by the anniversary date of initial review as specified by each IRB/EC. An annual report will be submitted by the Sponsor to the FDA according to regulations. These reports will provide a brief description of the progress of the investigation as outlined in *21 Code of Federal Regulations* 312.33, and will include any revisions of the protocol if not previously submitted. In addition, annual Continuing Review Reports will be submitted to both the UVM and icddr,b IRBs, per regulations.

The PI and designated icddr,b laboratory personnel will maintain adequate records to account for the disposition of the investigational product, including dates of receipt and quantity, current inventory, and dispensation to study participants. If the study is terminated, suspended, or completed, the designated personnel at icddr,b will destroy all remaining investigational product according to the Sponsor's recommendation.

Investigators will maintain study charts for each volunteer. The study chart will contain the original signed consent form, any clinical and/or laboratory data, and other pertinent paperwork (i.e., source documents). The study chart will be identified by the unique study number assigned to the volunteer. Since the study charts will have documentation with both personal and unique identifiers, the chart is one possible link between study data and a personal identifier.

8.3 Retention of Specimens

All eligible specimens collected as part of this trial will be stored for future research as part of the NIAID and UVM IRB-approved biosample repositories for vaccine research. Participants must provide consent for future use of their samples for research purposes. The informed consent document will clearly include a section in which the participant may consent to allow any future use of retained blood specimens. Study specimens will be stored at icddr,b in accordance with their guidelines for retention of specimens.

If a study participant becomes pregnant, incarcerated, or lost to follow-up, all specimens collected from those study participants will be tested as outlined in the protocol and will be stored for future use, but only if the participant provided consent.

Participants may decide at any time not to allow their samples to be stored for future research. In this case, the Principal Investigator will ensure that all known remaining samples are destroyed and will report the disposition of the samples to the participant and to the IRB.

The decision will not affect the participant's participation in this protocol or in any other protocol sponsored by the NIH.

Samples that are retained by consent of the participant may be used to learn more about dengue or other flavivirus infections and other diseases. Samples will not be sold or used to make commercial products. Investigators within or outside of NIH who wish to study these samples and/or data must first obtain IRB approval. Any clinical information about the sample (with or without patient identifiers) similarly requires prior IRB approval.

All samples stored in the repository will be labelled with the participant's study ID number. This number cannot identify study participants, but is linkable to other research databases (e.g., from questionnaires, clinical assessments, logbooks, etc.) generated only by the primary TV005 study. The repository database will contain only the study subject ID numbers. A master log linking the study subject ID numbers to the names of the study participants will be maintained securely with limited access to authorized research team members. At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol or a repository.

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB.

Access to research samples will be limited using either a locked room or a locked freezer. Samples and data will be stored using codes assigned by the Investigators or their designee. Data will be kept in password-protected computers. Only Investigators or their designee will have access to the samples and data.

8.4 Retention of Records

The PI is responsible for retaining all essential documents listed in the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guideline. Trial-related documents will be maintained by the Investigator in a secure storage facility for a period of 5 years. Records will also be maintained in compliance with IRB and Bangladesh national, state, and federal medical records retention standards. The Sponsor is required to inform the Investigator when documents no longer need to be retained. All trial-related documents will be stored in a way that ensures that confidentiality will be strictly maintained to the extent provided by Bangladesh national, federal, state, and local law.

It is the PI's responsibility to retain copies of source documents until receipt of written notification to the contrary from the Sponsor. All study files, source documents, and trial master files will be maintained and stored in a secure manner in the icddr,b in the office of the Clinical Research Coordinator (or equivalent). No study documents should be destroyed without prior written agreement between OCRPRO/DCR/NIAID, the PI, and the clinical research staff. If the PI wishes to assign the study records to another party and/or move the records to another location, the PI or designee must provide written notification of such intent to OCRPRO/DCR/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written permission must be received by the site prior to destruction or relocation of research records.

8.5 Protocol Compliance
The PI will conduct the trial in exact compliance with the written protocol. The Investigator will not implement any deviation from or change to the protocol without agreement, prior review, and documented approval by the Sponsor and the IRBs/ECs that granted original approval for the study. The DSMBs will be notified of all protocol revisions (other than administrative) and will review any changes to the protocol that involve DSMB oversight or involve changes to the data and safety monitoring plan (DSMP) of the study.

The Investigator may implement a deviation or change in the protocol in order to eliminate an immediate hazard to study participants without prior IRB/EC or Sponsor approval or when the change involves only logistical or administrative aspects of the trial such as a telephone number change. In the event of a medical emergency, the PI shall perform any medical procedures deemed medically appropriate.

As soon as possible, the implemented deviation or change, the reasons for the change, and, if appropriate, the proposed protocol amendment(s) should be submitted to the Sponsor, IRBs/ECs, DSMBs, and to the regulatory authorities.

8.6 Investigator's Brochure
Investigators will receive the current version of the Investigator's Brochure, which comprehensively describes all the available preclinical experience with the experimental vaccine. If relevant new information becomes available during the course of the trial, the Investigators will receive a revised brochure or an amendment to the current version.

8.7 Study Monitoring
Per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored by the Sponsor. Monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the OCRPRO/DCR/NIAID will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff prior to participant enrolment. The plan will outline the frequency of monitoring visits based on such factors as study enrolment, data collection status, and regulatory obligations.

The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare DataFax abstracts with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. Monitors also will inspect the clinical site regulatory files to ensure compliance with regulatory requirements (Office for Human Research Protections-OHRP, FDA) and applicable guidelines (ICH-GCP). During the monitoring visit, the Investigator and/or the designee and other study personnel should be available to discuss the study. The investigator and/or designee will make study documents (e.g., consent forms, DataFax data abstracts and pertinent hospital or clinical records) readily available for inspection by the local IRB, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

The Sponsor will retain original copies of the Form FDA 1572 and copies of other study documents as deemed necessary.

9 Statistical Considerations

9.1 General Design

The goal of this Phase 2 vaccine trial is to determine the safety and immunogenicity of TV005, a live attenuated tetravalent dengue vaccine candidate in healthy human participants in a dengue endemic region. The purpose of this Phase 2 trial is to evaluate the safety and reactogenicity, immune response to the 4 DENV serotypes, and persistence of antibody following subcutaneous administration of 1 dose of TV005 vaccine. With 36 study participants vaccinated in each age cohort, there is 80% power to detect an AE that occurs with a frequency of 1 in 20 participants.

9.2 Study Endpoint Analysis

Primary Objectives

1. To determine the safety of one dose of TV005, as assessed by summarizing the frequency of immediate, systemic, local, and laboratory AEs following vaccination. Adverse events will be displayed in tabular format with line listings of individual clinical and laboratory events classified as immediate, systemic, or local events. Adverse events will be summarized by severity and relationship to vaccine by individuals and by each group.

2. To determine the immunogenicity of 1 dose of TV005, as assessed by determination of the serum plaque reduction neutralization titer 50% ($PRNT_{50}$) toDENV-1, DENV-2, DENV-3, and DENV-4 viruses for each participant at Study Day 0, 14, 28, 56, and 180 post vaccination. Monovalent, bivalent, trivalent, and tetravalent seropositivity and seroconversion frequencies will be determined at these time points.

Secondary Objectives

1. To assess the frequency, quantity, and duration of viremia of each monovalent component of the vaccine after vaccination. The mean peak viremia, mean day of onset of viremia, and mean duration of viremia of each monovalent component within each dose cohort will be calculated.

2. To determine the number of vaccinees infected with the vaccine viruses DENV-1, DENV-2, DENV-3, and DENV-4 or wild-type dengue virus. Infection is defined as recovery of vaccine virus or wild-type dengue virus from the blood or serum of a participant and/or by seropositivity or seroconversion to DENV.
    a. **Seropositivity** will be defined as $PRNT_{50} \geq 1:10$ on or before Day 56 post-vaccination
    b. **Seroconversion** will be defined as ≥ 4-fold rise in DENV-1, DENV-2, DENV-3, or DENV-4 neutralizing antibody titers on or before Day 56 compared with the pre-vaccination antibody titer.
3. To assess the duration of the antibody response in recipients of the tetravalent vaccine. The $PRNT_{50}$ to DENV-1, DENV-2, DENV-3, and DENV-4 will be determined for all specimens collected at Study Day 180 post vaccination.

4. To determine the durability of neutralizing antibody at 1 year, 2 years, and 3 years after vaccination with TV005

Exploratory Objectives

Exploratory objectives may be performed as indicated below and in Section 10.6 (CMI Exploratory Objectives) in addition to additional CMI assays, as available.

1. To evaluate the phenotype of PBMCs at primary infection with the TV005 vaccine
2. To evaluate the cellular immune response to primary infection with the TV005 vaccine
3. To evaluate the innate immune response to primary infection with the TV005 vaccine
4. To evaluate B and T cell memory responses following primary and secondary infections with the TV005 vaccine

10 Statistical methods

10.1 Estimated Sample Size

192 study participants (144 vaccinees and 48 placebo recipients) will be enroled in the study. Each cohort will consist of 48 study participants (36 TV005 vaccine, 12 placebo).

All cohorts will be enroled using 3:1 vaccine to placebo ratio, which was chosen for the following reasons:

1. The study is designed to build upon the previous Phase 1 studies of each monovalent candidate and the tetravalent admixtures, which used similar cohorts and vaccine to placebo ratio.
2. Placebo recipients have been included to assess whether common AEs are vaccine-related.
3. With 36 study participants vaccinated in each age cohort, there is 80% power to detect an AE that occurs with a frequency of 1 in 20 participants.

10.2 Cohort Evaluations

Total vaccinated cohort

The Total Vaccinated Cohort (TVC) will include all vaccinated study participants. . Thus, the TVC for analysis of safety will include all study participants with vaccine administration documented, and the TVC for analysis of immunogenicity will include vaccinated participants for whom data concerning immunogenicity endpoint measures are available, i.e., at least one evaluable post-vaccination NAb PRNT assay result.

Per protocol analysis (PP) of safety

The PP cohort for analysis of safety will include study participants:

1. Who have received the study vaccine/placebo according to their random assignment
2. With sufficient data to perform an analysis of safety (safety follow-up)
3. For whom administration site of study vaccine/placebo is known
4. Who have not received a vaccine not specified or forbidden in the protocol
5. For whom the randomization code has not been broken

Per protocol cohort (PP) for analysis of immunogenicity

The PP cohort for analysis of immunogenicity will include all evaluable study participants (i.e. those meeting all eligibility criteria, complying with the procedures and study visit intervals defined in the protocol, and with no elimination criteria during the study) for whom data concerning immunogenicity endpoint measures are available.

10.3 Safety

The primary safety endpoint is the frequency of vaccine-related AEs, as classified by both severity and seriousness, through active and passive surveillance. Separate assessments of systemic, local, and laboratory reactions will be performed.

The primary analysis for safety will be based on the TVC. If the percentage of enroled participants excluded from the ATP cohort for analysis of safety is more than 5%, a second analysis based on this ATP cohort will be performed to complement the TVC analysis.

10.3.1 Adverse Events

The following parameters will be evaluated for each age cohort and for all cohorts combined:

1. The proportion of enroled study participants with at least one AE (solicited or unsolicited), with at least one general AE (solicited or unsolicited) and with any AE during the solicited follow-up period will be estimated with exact 95% confidence interval (CI) after vaccination and overall.
2. The proportion of study participants reporting each individual solicited local, general, and laboratory AE during the follow-up period will also be estimated with exact 95% CI.
3. The proportion of study participants with at least one report of an unsolicited AE reported up to 29 days (days 0-28) after vaccination will be estimated with exact 95% CI.
4. Similar tabulations will be done for grade 3 AEs and for AEs with relationship to vaccination.
5. Differences in the frequencies of AEs between vaccines and placebos will be determined by Chi squared analysis and likelihood ratios.
6. The proportion of AEs resulting in a medically-attended visit will be tabulated.
7. Serious adverse events and participant withdrawal due to AE(s) will be described in detail.
8. The percentage of study participants in each group who received a concomitant medication to treat study-related AEs will be tabulated with exact 95% CI.
9. For fever, temperatures will be summarized in 0.5^{o}C increments.

10.3.2 Suspected and Confirmed Dengue

The percentage of study participants with suspected or confirmed dengue will be tabulated with exact 95% CI for each age cohort throughout the entire study. Cases will be summarized in terms of clinical and laboratory findings.

10.4 Immunogenicity

Immunogenicity will be measured by serum $PRNT_{50}$ to DENV-1, DENV-2, DENV-3, and DENV-4 viruses for each participant at Study Day 0, 14, 28, 56, 180, 360, 720, and 1080 post vaccination.

Definitions:

- Seropositivity to each serotype will be defined as a $PRNT_{50}$ of $\geq$ 1:10 on or before Study Day 56 (28 for younger cohorts).

- Seroconversion to each serotype will be defined as a ≥ 4-fold in serum neutralizing antibody titer on or before Study Day 56 compared with Study Day 0.

The primary analysis will be based on the PP cohort for analysis of immunogenicity. If more than 5% of enroled study participants are excluded from this PP cohort, second analysis based on the TVC will be performed to complement the PP analysis. At each blood sampling time point, the following parameters will be tabulated. Each of the four age cohorts will be analysed individually.

1. The proportion of study participants who are seropositive per each serotype after vaccination will be estimated with 95% CI.
2. The proportion of study participant who have seroconverted per each serotype after vaccination will be estimated with 95% CI.
3. Differences in seropositivity or seroconversion between study participants with and without pre-existing DENVantibodies will be determined by Chi Square analysis and likelihood ratios.
4. The proportion of study participants with monovalent, bivalent, trivalent, and tetravalent seropositivity and seroconversion will be estimated with 95% CI.
5. An estimate of neutralizing antibody titer for study participants will be determined by geometric mean titer (GMT) for each serotype with 95% CI.
6. The proportion of study participants of each seroptype who are seropositive or seroconvert at day 180 will be estimated with 95% CI to determine the durability of TV005.

10.5 Viremia

The proportion of study participants with vaccine viremia (any serotype and each serotype individually) will be estimated with 95% CI. The mean peak titer will be calculated for each individual serotype. The mean duration of viremia with range will be calculated for each individual serotype. Differences in mean peak titer and duration of viremia between each individual serotype will be measured by Mann-Whitney U analysis.

The proportion of study participants with recoverable DENV-1, DENV-2, DENV-3, and DENV-4 vaccine virus components or wild-type dengue virus during the first 14 days following vaccination will be estimated with 95% CI. This is defined by the recovery of vaccine virus from the blood or serum of a study participant and/ or by new seropositivity ($PRNT_{50} \geq 1:10$) in a previously seronegative study participant or seroconversion ($\geq$ 4-fold rise in neutralizing antibody titers against DENV-1, DENV-2, DENV-3, or DENV-4) in study participants who were seropositive at baseline.

Differences in the recovery of vaccine virus from blood or serum post vaccination between study participants with and without pre-existing DENV antibodies will be determined by Chi Square analysis and likelihood ratio.

10.6 CMI Exploratory Objectives

At study time points Day 0, Day 7, Day 28, Day 56, Day 180, Day 360, Day 720, and Day 1080 the individual immune response to vaccination will be evaluated. Descriptive statistics including mean, standard deviation, range, median 1st, and 3rd quartiles may be tabulated. In addition to descriptive tables, box and whisker plots of the frequencies may be displayed if the data is available.

Because the CMI endpoints are exploratory, all collected specimens may not be analyzed at each time point. If additional CMI assay techniques become available during the course of the study, additional analyses may be performed.

11 Monitoring Plan

11.1 DSMBs: NIAID Intramural and ERC Data and Safety Monitoring Boards

Both the Sponsor (NIAID) and the local ethical committee (ERC) require the formation of a DSMB to review study participant safety. We have outlined the process by which each Board will oversee safety and work together as needed.

The ERC DSMB reflects the disciplines and medical specialties necessary to interpret the data from the clinical trial and may include expertise in clinical aspects of disease/patient population being studied, biostatistics, and clinical trials conduct and methodology. The ERC selects the DSMB chair and determines the terms of membership. The ERC DSMB is constituted to review and evaluate the accumulated study data for participant safety, study conduct, and progress, and when appropriate, efficacy and makes recommendations to the ERC concerning the continuation, modification, or termination of the trial. The ERC DSMB will meet once before the study opens to make recommendations as to whether the study can proceed. The ERC DSMB will also meet after all safety data has been collected up to Day 28 for each cohort. The ERC DSMB will conclude each review with their recommendations to the ERC chair and the Investigator as to whether the study should continue without change, be modified, or terminated.

The NIAID Intramural DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The Board will review the study prior to initiation and once for each age cohort after safety data is obtained for each cohort's initial 28 day participation in the trial. The Board may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all UPs, and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB or IND Sponsor. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

For each DSMB, designated unblinded icddr,b staff will provide the Executive Secretary with blinding codes in a sealed envelope, to be opened in the event that the DSMB requires the information in order to make its recommendations. Prior to each DSMB review, the PI will submit the Sponsor's Medical Monitor's written recommendations and cumulative safety data in a format acceptable, by unblinded cohort if requested. The Boards will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study.

Reports of SAEs will be submitted by the PI to the Boards at the same time they are reported to the Sponsor and IRB. All Unanticipated Problems will be submitted to the DSMBs at the same time they are submitted to the IRB. Safety Reports will be submitted to the DSMBs by the Investigator after their receipt. The PI will notify the DSMBs of any cases of intentional

or unintentional unblinding as soon as possible. The PI will notify the Boards at the time stopping or pausing criteria are met and will obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMBs' recommendations to the IRB of record. DSMBs' Policy Document, which serves as the charter document, will be provided to the University of Vermont IRB at the time of submission of the full protocol packet for review.

The NIAID and ERC DSMB will review safety data simultaneously through enrolment of the youngest cohort (age de-escalation phase of the study). Both DSMBs will share open summaries from their respective meetings. In addition, up to two members from the ERC DSMB may be invited to participate as an ad hoc voting member in the NIAID DSMB. If the local DSMB members are not able to participate, the NIAID DSMB will continue without a local in-country representative and will review the ERC DSMB open summary reports. In the event of differing recommendations between the two DSMBs, the two Boards and/or two authorized individuals will collectively determine an agreeable resolution.

11.2 Sponsor Medical Monitor

Roles and Responsibilities of the Sponsor Medical Monitor

A Medical Monitor (MM) representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The Sponsor MM will be responsible for performing periodic reviews and analyses of aggregate safety data to assure the safety of the participants and compliance with all safety requirements. In this capacity, the MM will: 1) review all AEs and UPs on a regular basis throughout the study, 2) review all SAEs, 3) be available to advise the investigators on study-related medical questions or problems, and 4) evaluate cumulative subject safety data and make recommendations regarding the safe continuation of the study. A Safety Monitoring Plan that has been written for this protocol provides more specific information about the frequency of the data reviews, content to be reviewed, and the format of the meetings between the PI and the Sponsor MM to discuss the results of the analysis of the safety data.

11.3 Safety Review and Communications Plan

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the principal investigator, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

12 Protection of Human Subjects

12.1 Institutional Review Boards

The PI will be responsible for obtaining IRB approvals. Before the start of the study, the appropriate documents (including, but not limited to, the Protocol, Investigator's Brochure, Informed Consent Form, information sheets, and advertisements) will be submitted to the IRBs/ECs, DSMBs, and the Sponsor for approval. A copy of the study approvals (including approval of the informed consent form) is to be maintained in the Investigator study document binder, and a copy will be supplied to the Sponsor. During the study, the Investigator is responsible for providing the IRBs/ECs, and the Sponsor with all documents subject to review (e.g., Protocol Amendments, informed consent form updates,

advertisements, and any written information that may be provided to the participant). Annual reports on the progress of the study will be made to the IRBs/ECs by the Investigator in accordance with IRB guidelines and government regulations.

Administrative changes generally do not require IRB approval but the University of Vermont IRB and local ERC should be notified. The Sponsor must be immediately notified of all administrative changes, including minor and administrative modifications, prior to any action being taken. Any non-administrative changes which may impact study participants' safety, the trial design, or achievement of study objectives will require approval from the Sponsor and the IRBs/ECs. The only exception is a protocol modification that is required immediately to eliminate apparent immediate hazards to a participant. Such a modification may be implemented by the PI and reported to the UVM IRB and local ERC within 48 hours.

The ICF and/or assent forms must be revised to reflect any significant amendment which directly affects volunteers and must also be reviewed and IRB approved with the amendment. Study participants will be consented using the most recently-approved ICF and assent. Participants already enroled in the study will be informed about revisions to the protocol and, depending on the impact of the amendment, may be asked to re-consent. Re-consenting may be accomplished by repeating the informed consent process using the revised ICF, with attention given to the changes, or it may be done using an addendum to the ICF, clearly stating any revisions or new information and summarizing the potential effect upon participation in the study. The new ICF document must be signed and placed into the study record. A signed copy is given to the volunteer.

The PI will be responsible for submitting the Continuing Review Report (CRR) to the icddr,b EC with the assistance of the UVM Study Coordinator. A close-out report should be submitted to the UVM IRB and the icddr,b EC at the completion of the study.

12.2 Informed Consent

In obtaining and documenting informed consent, the Investigator and study staff must comply with the applicable regulatory requirements, GCP guidelines, and ethical principles. The written informed consent form and the assent form (for participants 11-17 years of age) must be approved by the IRBs/ECs prior to its use. The ICF or AF describe the study objectives, risks and potential benefits to participation, and study participant and parent requirements for participation. The ICF will be signed by adult study participants. The AF will be signed by at least 1 parent or acceptable legal representative. Children between the ages of 11 and 17 must mark the AF. Both an ICF signed by the parent/legal guardian and the AF signed by the minor must be signed for a study participant less than 18 years of age to participate in the study. If the parent or legal guardian gives consent for a minor, but the minor does not provide assent, the participant will not be enroled in the study.

Adult participants or the parent or legal guardian of a minor participant may withdraw consent at any time during the course of the trial. A minor study participant may withdraw assent at any time. A copy of the informed consent and assent documents will be offered to the study participant or to the participant's parents or legal guardian. The rights and welfare of the study participants will be protected by emphasizing that the quality of the medical care provided will not be adversely affected if an individual declines participation in the study.

If a child reaches the age of 18 while on study, informed consent will be obtained at that time. Similarly, if a child reaches the age of 11 while on study, assent will be obtained at that time.

Illiterate persons will be asked to mark their thumbprint in lieu of a signature and an impartial witness will also sign.

If a study participant is injured in the process of a study-specific procedure or activity, reasonable and necessary medical care to treat the injury will be provided to the participant at no charge.

12.3 Risks

Risks to the study participants are mainly associated with venipuncture and immunization and are outlined below. Female study participants will be cautioned of the unknown risk of study vaccines to the fetus and will be required to use effective birth control methods through study day 28. Study participants are counselled on pregnancy prevention methods and risks to pregnancy through day 28 of the study.

12.3.1 Venipuncture

The total amount of blood to be drawn throughout the 1080-day duration of the study is approximately 257 mL for cohort 01 (18 – 50), 121 mL for cohort 02 (11 - < 18 years of age), and 33 mL for cohorts 03 and 04 (5 - < 11, and 1 - < 5 years of age respectively). These volumes are well within the Office for Human Research Protections (OHRP) and NIH guidelines (Clinical Center Medical Administrative Policy M 95-9) for blood volumes in clinical research and should not compromise the health of study participants. The total volume of blood to be drawn from paediatric study participants (age $\leq$11 years) will be 3mL/kg or less at any one time, and no more than 46 mL of blood will be drawn over any single 8-week period in the paediatric cohorts.

Risks occasionally associated with venipuncture include bleeding, pain, bruising, or hematoma at the site of venipuncture; light headedness; syncope (rarely); and infection (rarely).

12.3.2 Immunization

Possible local vaccine reactions include pain, swelling, or erythema usually lasting no more than 2 to 3 days, lymphadenopathy, or pruritus at the injection site. Systemic reactions such as macular papular rash and transient benign neutropenia have been observed in some study participants infected with other recombinant dengue vaccine candidates. Other potential systemic reactions that may occur include symptoms of dengue such as fever, headache, eye pain, photophobia, generalized myalgias, arthralgias, elevated ALT, neutropenia, elevated PTT, or decreased platelet count. Immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible, as with any vaccine. Study participants who receive the TV005 vaccine may theoretically be at increased risk for more severe disease (DHF/DSS) if they respond poorly to 1 or more of the components of the vaccine and become infected with a wild type DENV in the future.

Any viral vaccine carries a small risk of Guillian-Barré syndrome (GBS). GBS is a reactive, self-limited, autoimmune disease triggered by a preceding bacterial or viral infection. The clinical manifestations begin gradually, are symmetric, and consist of mild sensory deficits, cranial nerve involvement, and motor weakness that may lead to paralysis. The paralysis will typically spontaneously resolve. This has not been reported following naturally acquired dengue infection or after any investigational dengue vaccination, but remains a small but potential risk.

There is also a theoretical risk associated with breach of confidentiality by participating in this study, although every measure will be taken to assure the confidentiality of participants in this study.

As with any investigational vaccine, there may be risks that are currently unknown. Study participants will be informed of any such risks should further information become available.

> Receiving information concerning positive hepatitis status may cause significant emotional distress and relationship conflicts. Any such study participant shall be referred to an appropriate health care provider for definitive diagnosis and subsequent counseling and treatment. These volunteers will not eligible for participation in the study.

12.4 Benefits

Benefits from participation in this study will include enhanced access to medical care during the screening visit and all subsequent visits. Study participants will receive a full physical exam which may detect medical problems which were previously unknown to the participants, and this may allow earlier diagnosis and treatment of any significant previously unidentified illnesses or health care problems.

Adult and adolescent study participants will receive laboratory screening for hepatitis C infection, and all participants will receive laboratory screening for hepatitis B infection, which could benefit the participant if a previously undiagnosed disease is identified. These individuals will be referred to a medical provider for counselling and treatment. If a study participant has a febrile illness during the course of the study, participation may lead to earlier diagnosis and treatment of dengue fever. Enroled study participants will also receive primary care up to Study Day 180.

12.5 Compensation

Enroled study participants will be compensated with food at all scheduled clinic visits and with 500 Tk (at current exchange rates, approximately $7.00 US dollars) per study visit including the screening visit. The compensation is intended to help offset the cost of travel to the clinic site for study visits and to compensate for loss of income during visits that occur during work hours. Enroled study participants will also receive primary care up to Study Day 180.

12.6 Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, and process and administrative forms will be identified by coded number only to maintain participant confidentiality. Forms, lists, logbooks, appointments books, and any other listings that link subject study ID numbers to other identifying information will be stored in a separate locked file in an area with limited access. A participant's study information will not be released without the written permission of the participant, except as necessary for monitoring by the Sponsor and/or its contractors and the FDA.

Subject identification codes will consist of the study code, TV005, followed by a number designating the age cohort (01 for the adult study participants, 02 for the cohort age 11-<18, 03 for the cohort age 5-<11, and 04 for the cohort age 1-<5), and then by a unique 3 digit identifier, XXX. Three digit subject identifier codes will be assigned in order of enrolment; for example, subject TV005-01-001 will represent the first adult participant enroled into the study to receive an injection.

Data and study files may be accessed by authorized Investigators, UVM and icddr,b study staff, the Sponsor's Clinical Research Associate (CRA) and/ IRB members, the Sponsor (OCRPRO/DCR/NIAID/NIH), the icddr,b ERC personnel, and other regulatory personnel and authorized study personnel as documented on the Study Task Delegation and Signature List, a regulatory document which will be kept on file in the icddr,b Clinical Research Coordinator Section and updated as needed.

12.7 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel during the drawing of blood and during the shipping and handling of all specimens for this study, as currently recommended by regional Occupational Health guidelines and the U.S. Centers for Disease Control and Prevention.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72. Please also refer to individual carrier guidelines (e.g., Federal Express, World Courier) for specific instructions.

Use of Animals

No animals will be used in the execution of this protocol.

13 Study Specific Policies and Procedures

13.1 Collaborations

This study represents collaboration between the OCRPRO/DCR/NIAID/NIH, Johns Hopkins University School of Public Health (JHSPH), University of Vermont Vaccine Testing Center, and International Center for Diarrhoeal Disease Research, Bangladesh (iccdr,b) with roles and responsibilities of each party detailed in the contract agreement. Each organization has specific roles and responsibilities which overlap in certain areas. In summary, icddr,b field clinic, research and clinical laboratories, clinical trial personnel are responsible for preparing for and executing clinical operations; the OCRPRO/DCR/NIAID/NIH through the Regulatory Compliance and Human Subject Protection Program (RCHSPP), Leidos Biomedical Research, Inc., will provide Sponsor support, to include financial, logistic, regulatory, monitoring, and quality assurance sustainment in accordance with regulatory authority guidelines. Financial support and funding distribution and management for the study has been executed in conjunction with JHU.

13.2 Study Sponsor and Funding

The study is sponsored and funded by OCRPRO/DCR/NIAID/NIH through a contract agreement.

Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
OCRPRO/DCR/NIAID/NIH
5601 Fishers Lane
Room 4B11, MSC 9820
Bethesda, MD 20892
Phone: 301-451-5136
Fax: 301-480-1765

Fund transfer between the Sponsor and the icddr,b clinical site will be managed by a contract with Johns Hopkins University School of Public Health.

14 Facilities

The icddr,b has a large multi-disciplinary international and national scientific research staff. Existing field, hospital, laboratory, and office facilities will be used for this study. Scientists at icddr,b have conducted a variety of observational field studies and clinical trials in the Mirpur community where the field site is located.

Existing laboratory facilities of the Immunology and Parasitology Laboratories, Centre for Vaccine Sciences, icddr,b will be used for all clinical aspects of this study, including recruitment, screening visits, administration of the vaccine, follow-up clinic visits, and febrile illness surveillance.

Vaccine storage, accountability, and preparation will be conducted at icddr,b research laboratories with training and assistance from the University of Vermont Vaccine Testing Center.

PRNT assays and viremia assays will be conducted at the icddr,b research laboratories with assistance and training from the University of Vermont Vaccine Testing Center. Exploratory immunology assays will be performed at the University of Vermont Vaccine Testing Center.

Literature Cited

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