Protocol

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

Protocol for: Low N, Bavdekar A, Jeyaseelan L, et al. A randomized, controlled trial of an aerosolized vaccine against measles. N Engl J Med 2015;372:1519-29. DOI: 10.1056/NEJMoa1407417

This supplement contains the following items

1. Original protocol, final protocol, summary of changes

2. Original statistical analysis plan. No changes were made to the statistical analysis plan.

WHO MEASLES AEROSOL PROJECT
Pivotal Trial Clinical Trial Protocol

1 Protocol cover sheet

Study Protocol No.	WHO/MAP/IND/02 Pivotal study to evaluate the immunogenicity and safety of a measles vaccine given by aerosolized inhalation: randomized controlled trial
Version	Version 1: April 2008
Study site	Pune District, Maharashtra, India.
Study Initiation date	
Collaborating institutions	Shirdi Saibaba Hospital (Vadu Hospital), Vadu, Pune, India. King Edward Memorial Hospital and Research Center, Pune, India. Christian Medical College, Vellore, India. National Institute of Virology, Pune, India. Health Protection Agency, London, UK. University of Bern, Bern, Switzerland.
Sponsor	Serum Institute of India Ltd., 212/2 Hadapsar, Pune, 411028, India.
Co-Sponsor	World Health Organization (WHO), Department of Vaccines and Biologicals (IVB), Initiative for Vaccine Research (IVR), 20 Avenue Appia, CH-1211, Geneva 27, Switzerland.
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3 List of abbreviations

ACH	Achieved Level
AE	Adverse Event
ANM	Auxiliary Nurse Midwife
ARC	American Red Cross
BCG	Bacillus Calmette-Guérin vaccine
CDC	Centers for Disease Control and Prevention, USA
CMC	Christian Medical College and Hospital
CRF	Case Report Form
CSD	Common Study Document
DCGI	Drugs Comptroller General, India
DIO	District Immunization Officer
DPT	Diphtheria-Pertussis-Tetanus vaccine
DSMB	Data and Safety Monitoring Board
DSS	Demographic Surveillance System
DT	Diphtheria and Tetanus vaccine
ELA	Expected Level of Achievements
ELISA	Enzyme Linked Immuno-Sorbent Assay
EPI	Extended Programme of Immunization
EPRT	Epidemic Response Team
E-Z	Edmonston-Zagreb
GCP	Good Clinical Practice
GIS	Geographic Information System
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HDSS	Health and Demographic Surveillance System
IB	Investigator's brochure
ICF	Informed Consent Form
IEC	Institutional Ethical Committee
IgG	Immunoglobulin G
IRB	Institutional Review Board
ITT	Intention-to-treat analysis
ICH	Institute of Child Health
INDEPTH	The International Network for Demographic surveillance of Populations and Their Health in developing countries
IVR	Initiative for Vaccine Research, WHO
KEM	King Edward Memorial Hospital and Research Center, Pune, India
MAP	Measles Aerosol Project
MAV	Measles Aerosol Vaccine
MCH	Maternal and Child Health
MEM	Minimal Essential Medium

MO	Medical Officer
MOHFW	Ministry of Health and Family Welfare
MV	Measles Virus
PDG	Project Development Group
PE	Physical Examination
pfu	Plaque forming units
PHC	Primary Health Center
PI	Principle Investigator
PP	Per-protocol analysis
PRNT	Plaque reduction neutralization test
RC	Regional Coordinator
SAE	Serious Adverse Event
SIIL	Serum Institute of India Ltd
SMO	Surveillance Medical Officer
SSH	Shirdi Saibaba Hospital
SSPE	Subacute Sclerosing Panencephalitis
TT	Tetanus Toxoid
UIP	Universal Immunization Program
WFI	Water for Injection
WHO	World Health Organization

4 Background information

4.1 Public health importance of measles

Measles is one of the most common causes of death in children under five-years old globally. In 2000, an estimated 777,000 measles deaths occurred, of which 452,000 were in the African Region of the World Health Organization (WHO). In 2001, WHO and the United Nations Children's Fund published a 5-year strategic plan to reduce measles mortality by half by 2005.[1] In 2003, the World Health Assembly endorsed a resolution urging member countries to reduce the number of deaths attributed to measles by 50% compared with 1999 estimates by the end of 2005.This target was met. Overall, global measles mortality in 2005 was estimated to be 345 000 deaths (uncertainty bounds 247 000 and 458 000 deaths), a 60% decrease from 1999. WHO estimates that in 1999, 58% of all deaths from measles were estimated to occur in the African region and 27% in Southeast Asia. However, by 2005, 50% of all deaths from measles occurred in Southeast Asia and only 37% in Africa.[2] The revised global goal, as stated in the Global Immunization Vision and Strategy 2006–2015 of the World Health Organization and United Nations Children's Fund, is to reduce measles deaths compared to the estimated number in 2000 by 90% by 2010.[3] Important challenges still exist for the achievement of the 2010 goal for reduction of measles mortality. Activities need to be fully implemented in large countries which still have a high measles burden such as India, Pakistan, and Indonesia. Moreover, to achieve this goal, continued progress needs to be made in delivering measles vaccines to the world's children.

4.2 Name and description of the investigational product

4.2.1 Vaccine

The vaccine to be studied in the proposed Pivotal trial in India is the currently licensed measles live, attenuated virus, Edmonston Zagreb strain, manufactured by the Serum Institute of India, Ltd (SIIL) and approved for subcutaneous injection in India and many other countries. It meets the requirements of WHO when tested by the methods outlined in WHO TRS 840 (1994).[4] It is pre-qualified by WHO for sale to UN agencies.

The vaccine is prepared in human diploid cells (MRC5), suspended in minimal essential medium (MEM) and stabilised. The measles vaccine is in the class of prophylactic immunizing agents. The Edmonston Zagreb strain has been shown in Mexican studies to be immunogenic and safe, and capable of maintaining vaccine potency during the nebulisation process.[5]

Clinical trials of a measles vaccine manufactured by a company with experience and expertise in international vaccine regulations, and experience of the procedures for global immunization programmes would be expected to result in an efficient regulatory pathway towards licensure in India and pre-qualification by WHO.

4.2.2 Aerosol device

Nebulisers are classified as medical devices and several are licensed/registered/approved in their country of manufacture. They are currently in use in several countries for the administration by inhalation of therapeutic pharmaceuticals for respiratory diseases.

One nebuliser manufactured in compliance with GMP (the Aerogen Nektar nebulizer) has been selected for use in this trial. This administration device/system will be tested in the study location using the SIIL vaccine.

The Aerogen Nektar nebulizer is a wholly autonomous, battery or mains powered micro-pump nebulizer. The electronic micropump is a technology which produces liquid aerosol in a manner unlike any other technology currently available. As small as 15mm in diameter and wafer-thin, the micro-pump is comprised of a unique dome-shaped aperture plate containing over 1,000 precision-formed tapered holes, surrounded by a vibrational element. When energy is applied, the aperture plate vibrates over 100,000 times per second. This rapid vibration causes each aperture to act as a micropump, drawing liquid through the holes to form consistently sized droplets. The result is a low-velocity aerosol optimized for maximum deep lung deposition.[6]

Performance characteristics for the selected device have been generated for delivery of the measles vaccine by aerosol. This device has been chosen for this proposed study because it has the performance characteristics and vaccine potency retention qualities comparable to the classic Mexican device used in previous trials of several measles vaccines which demonstrated both safety and immunogenicity of measles vaccines by the aerosol route. It was also used during the Phase 1 trial among measles immune healthy subjects 1-35 years old in Pune India, with good safety profile and good immunogenicity results.

4.3 WHO Measles Aerosol Project

The Measles Aerosol Project aims to develop and license at least one method for respiratory delivery of currently licensed measles vaccines by 2009. The assumptions for the project are that the aerosol vaccination devices will use current vaccines and that vaccination will be targeted at children of 12 -59 months for routine vaccination and at individuals of 9 months to 18 years for measles mass campaigns. It is anticipated that clinical testing can be completed by 2009.

Because previous studies of measles aerosol vaccine (MAV) were not carried out with licensure as an aim, WHO is supporting GLP and GCP compliant non-clinical and clinical studies with the aim of achieving licensure. In summary, during the first three years of the project the regulatory pathway has been defined, safety concerns enumerated, preclinical studies have been completed and, devices for clinical trials selected. A pivotal trial is planned in India for the second half of 2008. Licensure of a measles aerosol vaccine will be a critical step towards making reality the promise of aerosol delivery of other vaccines.

WHO/IVR coordinates the Measles Aerosol Project (MAP).The Product Development Group for the Measles Aerosol Project (PDG) is an expert clinical and scientific advisory body to WHO/IVR established to provide independent advice to the IVR regarding the development plan of the measles aerosol vaccine. The Centers for Disease Control and Prevention (CDC), USA and the American Red Cross (ARC) are partners to the MAP. The MAP has received financial support from the Bill and Melinda Gates Foundation.

4.3.1 Summary of findings from non-clinical and clinical studies that are potentially relevant to this trial

4.3.1.1 Non-clinical studies

A standardised laboratory protocol for measles PRNT has been established and validated for use in clinical trials of aerosolized measles vaccines. [7]

A study of measles vaccine degradation by nebulizer devices has been completed. [8] This study assessed vaccine potency retention performance of the three nebulizers used in the Phase 1 trial. The average vaccine potency retained by the Aerogen Nektar nebulizer using the conservative correction for counting bias was 94.7% (95% CI, 85.8-103.7).

GLP animal safety and immunogenicity studies among cynomolgus monkeys (Macaca fascicularis) with the live-attenuated Edmonston-Zagreb measles virus (MV) vaccine have been conducted. Immunogenicity and protective efficacy of aerosol vaccination using devices similar to those previously used in humans were comparable to those in animals vaccinated by injection. No evidence of a safety hazard associated with the route of vaccination was detected.[9]

Toxicity studies have evaluated the safety of pulmonary delivery of Edmonston Zagreb measles vaccine in cynomolgus monkey, a non-human primate which is susceptible to infection with measles.[10] There was no mortality during the study. No effects were seen on body weight evolution, food consumption, ophthalmology, rectal temperature or clinical pathology. Water consumption was increased in measles vaccine treated animals, but urinary volume was not affected. Immunogenicity and viremia following treatment were characterised. A full range of tissues was evaluated for histopathological changes and no treatment-related findings were observed. It was concluded that pulmonary administration of Edmonston Zagreb measles vaccine was well tolerated locally and did not result in any evidence of systemic toxicity.

4.3.1.2 Clinical studies

A systematic review was conducted to examine the immunogenicity and safety of aerosolized measles vaccine (Edmonston-Zagreb or Schwarz strains) 1 month or more after vaccination. In children below 10 months, the studies were heterogeneous. In four comparative studies, seroconversion rates were lower with aerosolized than with subcutaneous vaccine and in two of these the difference was unlikely to be due to chance. In children 10-36 months, the pooled seroconversion rate with aerosolized vaccine was 93.5% (89.4-97.7%) and 97.1% (92.4-100%) with subcutaneous vaccine (odds ratio 0.27, 0.04-1.62). In 5-15-year olds the studies were heterogeneous. In all comparative studies aerosolized vaccine was more immunogenic than subcutaneous. Reported side effects were mild. Aerosolized measles vaccine appears to be equally or more immunogenic than subcutaneous vaccine in children aged 10 months and older. Large randomized trials are needed to establish the efficacy and safety of aerosolized measles vaccine as primary and booster doses.[5]

In 2006, a Phase I trial was initiated in India to assess the safety of measles aerosol vaccine using three different aerosol delivery devices. Preliminary results show good safety and immunogenicity profiles.

Additional details on the preclinical and clinical studies are available in the Investigator's Brochure (IB).

4.3.2 Summary of known and potential risks and benefits to human subjects

The expected risks include those already known to be associated with parenteral measles vaccination. For instance, a mild measles-like illness may follow vaccination. The most common side effects include fever and rash; these occur in 5-15% and 5%, respectively, of persons vaccinated subcutaneously.[11-14] Cough and conjunctivitis may also occur. Thrombocytopenia (platelet count <50,000/mL) occurs in less than one in 30,000 vaccinees approximately 2 weeks post-vaccination and may be more common in persons with a history of immune thrombocytopenia purpura. Only rarely, has this complication been associated with bleeding.[15-19] There is also a risk of encephalitis/ encephalopathy in one of 87,000 to 2,000,000 doses. There has been some controversy regarding whether or not the relationship is causal or coincidental. Nonetheless, cases have clustered in the first 1-2 weeks post-vaccination and may occur up to a month later. In addition, some persons have experienced long-term neurological sequelae.[20-24] As this is a live-attenuated virus vaccine, persons with deficient cell-mediated immunity should not be vaccinated and women who are pregnant should not receive this vaccine secondary to the theoretical risk of fetal infection.

Over the last several years, there has been concern over the possible association between measles vaccination and autism and inflammatory bowel disease. However, carefully conducted studies have failed to confirm these findings and there is no evidence that measles vaccine is associated with or causes these diseases.[25-28]

Reactogenicity associated to the aerosol route has been studied using the Mexican traditional device through a controlled approach comparing frequency of adverse effects among children receiving aerosolized measles vaccines with children receiving subcutaneous vaccination. Most frequent symptoms have been fever, rhinitis, cough, exanthema, conjunctivitis, diarrhea and arthralgias. Among 6-year old children, administration of aerosolized measles vaccine has produced statistically significant lower reactogenicity than subcutaneous route. Among 12-month-old children, no serious temporally associated events were identified in vaccinated children. Only inflammation of the conjunctivae, (injection or reddening, with or without discharge, and/or teary eyes), was more common among the infants given aerosol (34/59, 57%) compared to subcutaneous vaccine (19/55, 35%) ($p = 0.01$). Conjunctival hyperemia ("red eyes" and/or "teary eyes") was reported on at least one day of days 1 to 14, with an incidence of 7% - 14% per day of follow-up among the children in the subcutaneous group, and 12% - 19% per day of follow-up in the aerosol group. None of the children required medical treatment. In summary, the aerosol route of measles vaccine administration has been well tolerated and similar to the subcutaneous route. Episodes of conjunctival hyperemia have been more frequent in the aerosol group in 12-month-old children.

In summary, despite extensive clinical experience with measles aerosol immunization in Mexico and studies in South Africa and Thailand, serious adverse events following

immunization have not been reported. However, some experts have noted the need for more extensive evaluation of the safety of this route of administration.

4.3.2.1 Potential Central Nervous System Effects

Rarely, natural measles infection may be associated with CNS disease, namely encephalitis and sub-acute sclerosing pan encephalitis (SSPE). In contrast, attenuated measles vaccine is not associated with SSPE; on the contrary, measles vaccine is protective against SSPE. There is concern as to whether attenuated vaccine might reach the brain of aerosol vaccinees by retrograde transport via the olfactory nerve fibers. There is also the theoretical risk of exposure of the nasal mucosa to replicating vaccine virus and the possibility of central nervous system side effects due to passage through the cribiform plate of the ethmoid bone. This is an unlikely probability given the extensive public health experience with its use in Mexico. As many as 4 million children have been vaccinated by this method and no serious adverse events, including encephalitis, encephalopathy and death, have been reported. Heretofore, there have not been reports of CNS problems in the large aerosol measles vaccine experience. In addition, during the animal studies described above, no abnormalities in gross pathology or histopathology related to measles infection were detected in any of the organ studied. Furthermore, none of the organ samples tested by a semi quantitative RT-PCR showed measles virus genome including samples of the cribiform plate and an additional sample of the nervus olfactorious with a small piece of the olfactory bulb.

Therefore, there is no available evidence to indicate that the frequency of central nervous system side effects will be greater than that seen among measles non-immune persons receiving measles vaccine parenterally. However, the surveillance of adverse effects includes the search for neurological effects including data indicative of encephalitis or encephalopathy.

4.3.2.2 Potential Environmental Effects

There is the potential risk of transmission of aerosolized measles vaccine virus to health workers and others surrounding the site of vaccination. No contacts of children vaccinated with aerosolized measles vaccine have developed a measles-like illness. However, only one previous study has sought to document person-to-person spread of vaccine virus by serologic assay.[29]Aerosol administration may expose vaccinators to measles vaccine. However, these persons are likely to be measles immune secondary to either previous natural infection or vaccination. Using modern devices such as the one proposed for this trial would reduce this theoretical risk of shedding the vaccine virus from persons vaccinated through the aerosol route.

To minimize risks to health workers during the trial we will explain this possibility before hiring personnel so as to exclude individuals who have not suffered measles or have not been vaccinated within the previous 10 years, or who have a immunosuppressive diseases (e.g. HIV/AIDS, neoplasias, lymphoproliferative disorders, other) or receive immunosuppressive medication. In order to minimize the risk of fetal infection, all female staff of child-bearing age will be required to have a negative urine pregnancy test before hiring and agree to avoid pregnancy during the duration of the study.

4.3.2.3 Special Populations

Persons with respiratory hyperactivity may be at increased risk of bronchospasm following administration of measles vaccine by aerosol. Immunocompromised persons, including those with HIV infection and severe malnutrition, may be at increased risk of adverse effects caused by a live attenuated vaccine administered through the respiratory mucosa. Post licensure it is foreseen that additional clinical trials will specifically evaluate safety of this route of administration among these populations.

4.3.3 Description of and justification of the route of administration, dosage, dosage regimen, and treatment periods

Aerosols delivered to the respiratory mucosa are the natural route of transmission for measles virus, and the most promising non-injectable method of vaccination studied so far and their efficacy is thought to be comparable to injected vaccine.

Measles vaccine is for active immunization against measles. A single dose (containing at least 1,000 pfus) is sufficient to provide prolonged immunity to infection.

In countries where the incidence and mortality from measles is high in the first year of life, the recommended age for immunization against measles is as soon as possible after 9 months of age. Countries where measles is less of a problem may decide on a later date for immunization.

The vaccine is also recommended for use in children and adolescents with no evidence of vaccination or measles infection.

4.3.4 Agreement to ensure that the trial will be conducted in compliance with the protocol, GCP and Indian regulatory requirements (Schedule Y)

The investigator/institution will conduct the trial in compliance with the protocol agreed to by the sponsor and, by the Indian regulatory authority (DCGI) and which will be given approval/ favourable opinion by the Ethics Review Committee. The investigator/institution and the sponsor will sign the protocol, and the Undertaking by the Investigator to confirm this agreement.

4.3.5 Description of the population to be studied

4.3.5.1 Measles and measles vaccination in India

India is a pluralistic, multi-lingual, and multi-ethnic society. It is the world's seventh largest country by geographical area, the second most populous (population 1.12 billion), and the most populous democracy. It borders Pakistan to the west; China, Nepal, and Bhutan to the north-east; and Bangladesh and Burma to the east. India is the world's twelfth largest economy. However, it still suffers from high levels of poverty, illiteracy, malnutrition and environmental degradation.

Measles is endemic in India and is one of the most important causes of childhood morbidity and mortality. Due to the lack of dedicated countrywide surveillance for measles, it is difficult to estimate the true magnitude of the problem. WHO estimates that there was a decline in South-East Asia from an estimated 240 000 (173 000–316 000) measles deaths in 2000 to 178 000 (128 000–234 000) measles deaths in 2006. [30] An important proportion of the remaining disease mortality in this region is believed to occur in India.

Measles vaccination in India is recommended as a single dose, given from nine to 12 months of age.[31] Reported levels of measles vaccine coverage in India vary depending on the source. In 2001 administrative reports showed levels of measles vaccine coverage above 90% while coverage evaluation surveys estimated it at just over 60%. [32]

Vaccine coverage varies within the different states, which leads to a pool of vulnerable target group that is susceptible to the disease. Delhi state has a higher vaccine coverage. Goa, Maharashtra, and Tamil Nadu states reached 84-88 per cent coverage. Six states, Andhra Pradesh, Chhatisgarh, Delhi, Gujarat, Punjab, and Madhya Pradesh achieved coverage of more than 70 per cent and another 5 in the range of 60-70 percent. The majority of 14 states had measles coverage lower than the national average of 55.2 per cent. Moreover studies from the rural, semi urban, slum and community revealed poor vaccine coverage in these areas in India.[33-38]

The District Level and Household Survey evaluated the coverage for measles by district: The report indicates that: 14% of districts achieved coverage below 30% , 24% of districts obtained coverage between 30-50% and 28% of districts coverage achieved above 80%. [39, 40]

WHO estimates that of 26.2 million infants in 2006 who missed receiving their first dose of measles vaccine through routine immunization services by the age of 12 months, 16 million (61%) reside in 5 large countries: India (10.5 million children aged 9–12 months), Nigeria (2.0 million), China (1.2 million), Indonesia (1.2 million) and Ethiopia (1.1 million). [30]

4.3.5.2 Characteristics of Maharashtra State and Pune District.

Maharashtra State is in the west of India on the Deccan Plateau, 150km south east of Mumbai. Pune (population 5 million) is the second largest city in Maharashtra, after Mumbai, and the eighth largest in India. The city is a major industrial centre for the automobile industry and a large computing software centre. The Serum Institute of India Ltd. is also located in Pune. Per capita income in Pune is 50% higher than the national average and income disparity is the lowest in India, according to the Pune Municipal Corporation Environmental Status.

Table 1: Trend from 1991 to 2004 for selected indicators, Maharashtra State

Year	Crude Birth Rate			Crude Death Rate			IMR		
	Total	Rural	Urban	Total	Rural	Urban	Total	Rural	Urban
1991	26.2	28	22.9	8.2	9.3	6.2	60	69	38
1992	25.3	27.4	21.5	7.9	9.1	5.6	59	67	40
1993	25.2	27.1	22.8	7.3	9.3	4.8	50	63	32
1994	25.1	26.9	23	7.5	9.2	5.6	55	34	38
1995	24.5	26	22.4	7.5	8.9	5.4	55	31	34
1996	23.4	24.9	21	7.4	8.7	5.4	48	31	31
1997	23.1	24.4	21	7.3	8.6	5.4	47	32	31
1998	22.5	23.6	20.8	7.7	8.9	5.8	49	31	32
1999	21.1	21.6	20.3	7.5	8.7	5.6	48	58	31
2000	20.9	21.2	20.3	7.5	8.6	5.7	48	57	33
2001	20.6	21	20.1	7.5	8.5	5.9	45	55	27
2002	20.3	20.6	19.8	7.3	8.3	5.6	45	52	34
2003	19.9	20.1	19.4	7.2	8.2	5.6	42	48	32
2004	19.1	19.9	17.9	6.2	6.8	5.4	36	42	27

Source: http://www.maha-arogya.gov.in/achievements/default.htm

4.3.5.3 Immunization and vaccine preventable diseases surveillance practices

The aim of the immunization programme is to give primary immunization at the correct age, the correct dose and appropriate route before the first birthday of child. The Auxiliary Nurse Midwife (ANM) at the sub-center and the trained birth attendants in the villages are the key persons in the delivery of Maternal and Child Health (MCH) Services. Immunization Clinics are utilized for providing regular antenatal services. Immunization services are provided to all the people free of cost through every Health institution. The vaccination schedule as recommended by Govt. of India is followed. Measles vaccination recommendations follow the WHO India schedule, i.e. from 9 to 12 months of age. [31, 33]

The average actual age at measles vaccination is about 10 months (DHS India 1998-99 and personal communication from nursing sister at Shel Pimpelgaon PHC).

Measles vaccination coverage is currently estimated at around 92% from health service data. However, the exclusion of recent immigrants from the denominator but inclusion in the numerator probably results in over-estimates of coverage. The level of immigration is not available but is generally higher in villages near highways.

Health staff enumerate beneficiaries in their respective area. Health Assistants procure vaccine from their institution using the correct cold chain procedure. Vaccination is conducted under the supervision of a Health Assistant. The parents/guardians of those who do not receive vaccine are contacted. Primary vaccinations against tuberculosis (BCG), diphtheria, pertussis, tetanus (DPT), polio and measles, and the first dose of vitamin A are given to children before their first birthday. Booster doses of DPT and polio vaccines are given at 18 to 24 months. Further doses of vitamin A are given at 18, 24, 30 and 36 months. A dose of DT is given to children between 5 to 6 years and a dose of tetanus toxoid is given at 10 years and 16 years. Pregnant women are given 2 doses or a booster dose of TT.

Table 2 summarizes the immunization coverage achieved during 2002-2006.

Table 2: Measles immunization coverage 2002-2007, Maharashtra State

Indicator	2002-2003			2003-2004			2004-2005			2005-2006			2006 2007			2007-2008 (April to Jan 2008)		
	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%
DPT III	2113512	2017046	95	2150681	2110246	95	2055735	2048662	100	2093721	2079367	99	2098904	1586315	76	1974375	1514079	77
OPV III	2113512	2034476	96	2150681	2084555	92	2055735	2049333	100	2093721	2072128	99	2098904	1595884	76	1974375	1476641	75
BCG	2113512	2127471	101	2150681	2173105	94	2055735	2164062	105	2093721	2139148	102	2098904	1664981	79	1974375	1633207	83
Measles	2113512	1978350	94	2150681	1958080	90	2055735	1997355	97	2093721	1984167	95	2098904	1516299	72	1974375	1472809	75
DPT (B)	2106328	1935233	92	2148636	1972534	92	2126485	1947308	92	1703089	1869012	110	2014740	1447327	72	2253480	1462570	65
OPV(B)	2106328	1949264	93	2106328	1974875	92	2126485	1953872	92	1703089	1874653	110	2014740	1442111	72	2253480	1408211	62
DT	2180871	1957099	90	2219884	1900518	86	2329009	2064416	89	2301437	2150579	93	2458596	1724040	70	2467968	1539717	62
TT (10)	2330045	2034823	87	2371691	2067594	87	2329009	2066254	89	2492831	2226557	89	2237233	1776657	72	2467968	1492166	60
TT(16)	2150421	1875999	87	2189255	1822229	83	2126485	1922415	90	2311947	2085794	90	2244801	1585178	71	2253361	1301775	58
TT (M)	2402459	2261068	94	2444708	2143589	87	2355495	2092637	89	2398739	2061763	86	2405384	1723124	72	2254436	1246894	55

Source: http://www.maha-arogya.gov.in/programs/nhp/mchimmunisation/performance.htm

Immunization coverage for Pune district as described in Table 3 (below).

Table 3: Immunization coverage Pune district (1996-2004)

YEAR	BCG	DPT III	POLIO	MEASLES
1996-1997	105	98	97	93
1997-1998	103	98	99	100
1998-1999	104	102	101	99
1999-2000	110	103	103	96
2000-2001	100	102	102	96
2001-2002	104	100	100	99
2002-2003	83	79	79	77
2003-2004	55	51	51	54

Source: http://www.maha-arogya.gov.in/projectandschemes/basic/achievements.htm

Measles is endemic in this State. There is no current active surveillance of measles cases. Reported figures include mainly data from Government hospitals. The state government is currently undertaking a project on integrated communicable disease surveillance.

Table 4, summarizes the number of cases of vaccine preventable diseases reported during the period 2002-2008.

Table 4: Reported cases of vaccine preventable diseases 2002-2008, Maharashtra State.

Surveillance of Vaccine Preventable Diseases														
Years	Diphtheria		Pertussis		N.N.T.		TB (Child)		Measles		Polio		TETANUS (O)	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
2002-03	27	2	8	0	8	2	803	22	1704	3	4	0	70	13
2003-04	27	4	1	0	9	4	536	3	2136	30	3	0	69	14
2004-05	206	11	58	0	14	2	468	1	1790	7	0	0	68	13
2005-06	151	19	6	0	10	3	1062	26	1702	5	5	0	45	5
2006-07	513	1	95	0	67	0	596	6	513	3	1	0	17	4
2007-08 (Up to Jan.08)	497	0	37	0	10	1	142	3	341	6	2	0	26	0

Source: http://www.maha-arogya.gov.in/programs/nhp/mchimmunisation/achievements.htm

Approximately 500 cases are reported from Pune city each year but this is probably a gross underestimation.

4.3.5.4 Trial setting: Vadu Rural area

The proposed study site consists of five contiguous health districts (Vadu, Wagholi, Shel Pimpalgaon, Chakan, and Kendur) comprising over 150 villages, with a total population estimated at about 312,000 in 2007 (Table 5). The site is 30-40km across and the main village in each area is within one hour's drive from central Pune. The health districts lie within three administrative blocks (Shirur, Haveli and Khed) with Vadu spanning two administrative blocks, due to the public/private health care partnership, which is described below, paragraph 4.3.5.4. The population, location and numbers of measles vaccinations delivered in each area are shown in Table 5.

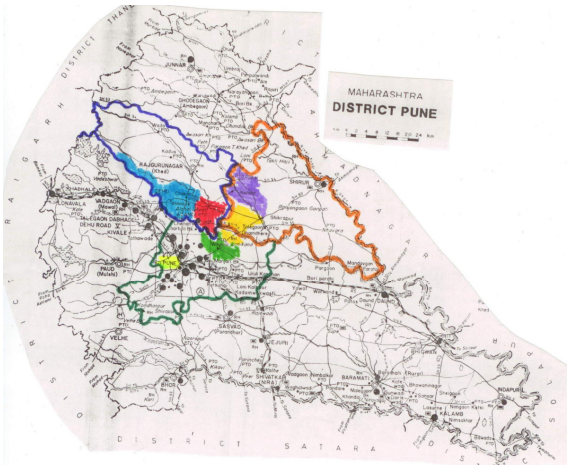


Table 5: Population in study area and number of measles vaccinations performed 2006-7

	Vadu	Wagholi	Shel Pimpalgaon	Chakan	Kendur	Total
Population	74000	49910	63000	99000	26865	312775
Administrative block	Shirur, Haveli	Haveli	Khed	Khed	Shirur	
Distance from Vadu Center (km)	0	15	15	25	10	
Number of EPI clinics	28	12	25	45	14	124
Number of measles vaccine doses given in 2006/07	1322	914	1000	1517	600	5353
Mean number of measles vaccine doses /month	110	76	83	126	50	446
Number of measles vaccine doses in 6 months	661	457	500	759	300	2677

Legend: EPI – WHO Expanded Programme on Immunization

4.3.5.5 Health care facilities in the Study area

Each health district area is covered by a primary health centre (PHC), which usually lies within an administrative block. Each PHC is staffed by a medical officer, nurse, and auxiliary nurse midwife (ANM).

Health care in the Vadu area is provided by the Vadu Rural Health Program, which is under the administration of King Edward Memorial Hospital and Research Centre (KEM), Pune, a privately funded hospital. One of the areas is a Demographic Surveillance Site (DSS) of the INDEPTH Network (an International Network of field sites with continuous Demographic Evaluation of Populations and Their Health in developing countries).

The District Health System is directly responsible for primary health care in the four districts. The PHCs at Kendur, Wagholi, Shel Pimpalgaon, and Chakan provide primary health care services including immunizations in their respective areas served by them. The Chakan and Wagholi PHC area are situated on major highways and are better connected similar to the Vadu area and have multiple private practitioners and small nursing homes capable of providing basic medical care. Chakan also has a rural hospital. The Kendur and Shel

Pimpalgaon PHC areas are remote and have fewer private practitioners and small private nursing homes.

The hospital in Vadu village , Shirdi Saibaba Rural Hospital (SSH), also known as Vadu Rural Hospital, is managed by the KEM Hospital and provides secondary level care to the 22 villages of the Vadu Rural Health Program. The hospital is staffed by a General Medical Officer, Obstetrics and Gynaecology specialist and resident in Obstetrics and Gynecology (all available 24hrs/day, 7days/week) with a surgeon and anesthetist available on call. A pediatrician is also employed. There are also nine registered nurses, a radiology technician and a pharmacist. A sonographer and ophthalmologist visit twice a week. On site facilities include: 7 consulting rooms; 1 male and 1 female ward; 4 patient rooms (private); 2 operating theatres; 1 labor room; 1 vaccine storage room; 1 laboratory (1 room for laboratory work, one for freezer storage); 1 X-ray room and darkroom; 1 sonography room. In addition, there is a separate research wing with a conference room, meeting hall, archive room, computer room, "outreach" room, a study room (used by the Meningococcal Vaccination Project), and a residential area.

4.3.5.6 Current measles immunization practice in the study area

Measles vaccination recommendations follow the WHO India schedule, i.e. from 9 to 12 months of age[31].

4.3.5.7 Identification of children eligible for routine immunization

For routine immunization, health workers compile a list of eligible children every year (in March and April) based on couples eligible for contraceptive and maternal child health services where the woman is aged 15 to 49 years (eligible couples survey, register number R14). Women who become pregnant during the year are listed in the R15 register which details the services received by them during pregnancy and delivery. After delivery, the children are carried forward into the R16 register which details the vaccinations and other services like Vitamin A supplementation received by them till one year of age. A health worker allotted to each geographical area (approximately 8000 people per health worker) is responsible for following up all the eligible couples and children eligible for vaccination in that area on a monthly basis.

4.3.5.8 Procedures during routine immunization clinics

Immunization clinics are held in each village on a permanently set date for every month and clinics are held on that date even if these are holidays and Sundays. For larger villages, clinics are held on the weekly market day to ensure high coverage. The dates of immunization clinics are well known in the local population and reinforced by the local health worker.

All PHCs and SSH are equipped with an ice-lined refrigerator and a vaccine refrigerator for storage of vaccines. Vaccines are supplied by the District Health Office every month. On the immunization clinic day, vaccines are carried by the health worker to the clinic in the village from SSH or the PHC in the morning in a vaccine carrier box. At the end of the clinic, unused vaccine is returned to SSH or PHC where vaccine accountability records are maintained.

At the immunization clinic, the nurse records the child's attendance and vaccines administered in a register. After the clinic these details are transferred to the R16 register, which records the dates of all primary immunizations received by a child. The health workers perform home visits every month in the area assigned to them. A list of children who have

missed a dose is taken from the R16 register and those children are followed up during their routine monthly home visit. Data about the numbers of measles vaccinations delivered each month in the Vadu area are shown in Table 6

Table 6: Numbers of recorded births and measles vaccinations given in immunization clinics in Vadu area, by month

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	All
Measles	96	94	111	98	122	104	105	113	98	87	111	106	1245
(range)	(0-19)	(0-18)	(1-18)	(0-16)	(0-19)	(0-19)	(0-22)	(0-23)	(0-18)	(0-15)	(0-19)	(0-20)	(10-198)
Births	114	94	116	105	97	115	113	95	94	104	100	117	1264
(range)	(0-29)	(0-20)	(1-24)	(0-22)	(0-22)	(0-21)	(0-22)	(0-19)	(1-20)	(0-21)	(0-22)	(2-21)	(16-255)

Legend: Based on data from 20 clinics in Vadu area, collected 2006-7. No data available from two clinics.

4.3.5.9 Research experience of the KEM Hospital and Research Center

The KEM Hospital and Research Center has had a legacy of epidemiological, bio-medical and social science research lasting 20 years. Apart from large community based cohort studies on low birth weight and child survival and fetal origins of adult onset disease, SSH has undertaken community based randomized controlled trials in iron supplementation amongst children. Currently ongoing is a large randomized observer blinded vaccine trial which is assessing the safety and long term immunogenicity of a conjugate meningococcal vaccine against a licensed polysaccharide vaccine.

Vadu has extensive demographic data available from 2003, fieldworkers with experience of collecting health-related data at household level, and experience of designing and conducting large epidemiological studies. The most recent complete census was conducted in July 2007, and updates are conducted every six months. There is currently Geographical Information System (GIS) data available for Vadu (the central village).

5 Trial objectives and purpose

The overall aim of the study is to demonstrate that measles vaccine delivered as an aerosol to the respiratory tract is sufficiently safe and immunogenic to obtain licensure for the nebulizer/vaccine combination device.

5.1 Study Rationale

A large, well-designed and -conducted, randomized controlled trial of the immunogenicity and safety of aerosolized measles vaccine in infants receiving a first dose of measles vaccine is required, in a setting where the vaccine would be used in practice.

An active control vaccine is required because the current subcutaneous vaccine is known to be highly effective and safe and is recommended worldwide. The immunogenicity of aerosolized measles vaccine should be shown to be non-inferior to the subcutaneous vaccine, according to

a pre-determined maximum acceptable difference. Superiority is not a realistic endpoint due to the high levels of serological response produced by subcutaneous vaccine.

5.2 What this trial will add

This trial has been designed to fulfil the regulatory requirements for licensing of a nebulizer/vaccine combination device, if the immunogenicity of aerosolized measles vaccination is shown to be non-inferior to subcutaneous vaccine in the target age group, and has an acceptable safety profile. By conducting the trial in a setting where the new delivery device would be used, existing public health surveillance systems will be strengthened, capacity will be built in the trial area for conducting large scale clinical trials and for the handling and serological processing of large numbers of blood samples.

5.3 Primary objectives

5.3.1 Immunogenicity

To compare the immunogenicity of measles vaccine delivered via a nebulizer/vaccine combination product with a licensed subcutaneous vaccine in infants who are eligible to receive a first dose of measles vaccination but are no older than 12 months.

5.3.2 Safety

To describe the frequency of adverse events following measles aerosol and subcutaneous vaccination.

5.4 Secondary objectives

5.4.1 Immunogenicity

To compare geometric mean antibody titres in infants receiving measles vaccine delivered via a nebulizer/vaccine combination device with subcutaneous measles vaccine;

To compare the immunogenicity of measles vaccine delivered via a nebulizer/vaccine combination device with subcutaneous measles vaccine, according to level of pre-vaccination antibody titre;

To collect blood from subsets of children at 28 days and one year after vaccination. These subsets will be randomly selected from all children enrolled in the Vadu study area because they will be able to be followed up within the infrastructure of an existing demographic and health surveillance system. For these subsets, the secondary objectives are:

- To compare the immunogenicity of measles vaccine delivered via a nebulizer/vaccine combination device with subcutaneous measles vaccine one year after vaccination;
- To describe the evolution of the antibody response to measles vaccine delivered via a nebulizer/vaccine combination device and subcutaneously over time, at days 0, 28, 91, 364 after vaccination;

5.4.2 Safety

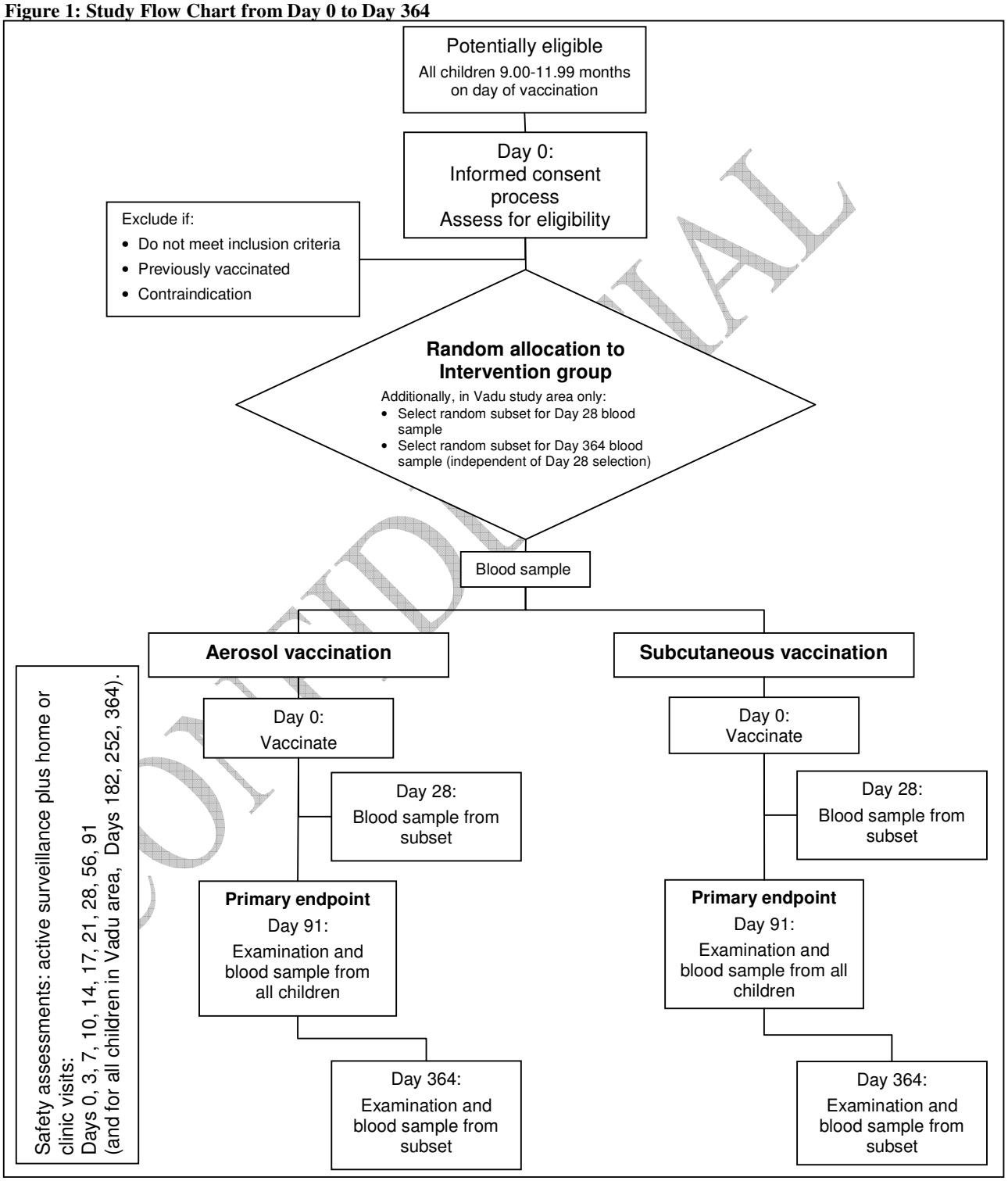
To describe the frequencies of individual adverse events following measles aerosol and subcutaneous vaccination.

6 Trial design

6.1 Type/design of study

Randomized, open-label, active-control, parallel group, non-inferiority trial (Figure 1).

Figure 1: Study Flow Chart from Day 0 to Day 364



6.2 Primary and secondary endpoints

6.2.1 Immunogenicity

6.2.1.1 Primary outcome

Measles seropositivity at day 91 post-vaccination.

6.2.1.2 Secondary outcomes

For all infants:

- Geometric mean titres, pre-vaccination and at day 91;
- Seroconversion (defined as a change from Enzygost OD <0.1 to OD ≥0.1 or PRNT from <120 to ≥120 mIU/mL) measured in paired samples from day 0 and day 91;

For infants from Vadu in 28 and 364 days subsets:

- Geometric mean titres at days 28 and 364 to describe evolution of antibody response at days 0, 28, 91, 364;
- Seropositivity at 364 days in infants with antibody titre ≥ 120 mIU/ml at day 91.

6.2.2 Safety

6.2.2.1 Primary outcome

Adverse events up to and including day 91 post-vaccination;

Adverse events include acute clinical reactogenicity, other adverse events, and serious or unexpected adverse events.

6.2.2.2 Secondary outcome

Frequencies of individual adverse events

6.3 Measures taken to minimize bias

6.3.1 Randomization

See SOP VEL1/1

6.3.1.1 Unit of randomization

Individual child

6.3.1.2 Sequence generation

The allocation sequence will be computer-generated random numbers, in permuted blocks, stratified by area, generated by an independent statistician. The randomization ratio will be 1:1.

6.3.1.3 Allocation concealment

Allocation will be accessed by telephone from the study enrolment site to an operator with a secure web-based system. Allocation will be recorded in the system, along with the unique patient data.

6.3.1.4 Implementation

- A doctor or study nurse at each health centre will obtain the allocation and record it immediately in the Day 0 case report form (CRF).
- The intervention is administered accordingly.

See SOP VEL 2/1 for details of quality assurance, and ensuring that correct allocations have been administered.

6.3.2 Blinding

The trial is open-label and the routes of administration different, so blinding of participants and investigators will not be possible.

6.3.2.1 Blinding of safety assessment

It will not be possible to guarantee the blinding of assessors of safety outcomes. Parents/guardians of participants will know which vaccination route their child received. To minimize bias there will be no record of intervention allocation on CRFs for follow up visits and parents/guardians will be asked identical structured, non-leading questions.

An independent Data Safety and Monitoring Board (DSMB) will have access to unblinded data for the assessment of serious adverse events.

6.3.2.2 Blinding of immunogenicity assessment

Laboratory staff will be blinded to allocation to aerosol or subcutaneous administration (see SOP VEL 3/1 for details).

6.3.2.3 Blinding during laboratory testing

- Blood samples will be labelled with a barcode unique to the patient and blood drawing date, but the patient and sample number (e.g. second sample from this individual) will not be identifiable without the data-linking documents.
- The barcode numbers will be generated at CMC Vellore and the data-linking documents held there. Blood samples and additional identical barcodes will be transported together to the site of serum aliquotting (post serum-separation).
- Each aliquot will be labelled with the same barcode as the original sample. As blood samples will be drawn from both subcutaneous and aerosol vaccine recipients on blood collection days, there will be no predictable pattern (relating to intervention group) in sample receipt at the laboratories for ELISA and PRN testing.
- Test runs will include specimens from both subcutaneous and aerosol arms. As the lab/s conducting the ELISA and PRN tests will have not participated in blood collection and labelling and will have no access to linking data, they will remain blinded.

6.4 Description of the trial combination product (vaccine-aerosol delivery device) and dose

All children will receive monovalent Edmonston-Zagreb (E-Z) strain attenuated measles vaccine manufactured by the Serum Institute of India, Ltd (SIIL).

6.4.1 Dosage form, packaging and labelling of the vaccine

6.4.1.1 Live attenuated measles vaccine

The dose of measles live attenuated lyophilized E-Z vaccine will be not less than 1000 $CCID_{50}$ per dose). The vaccine also contains excipients/stabilisers.

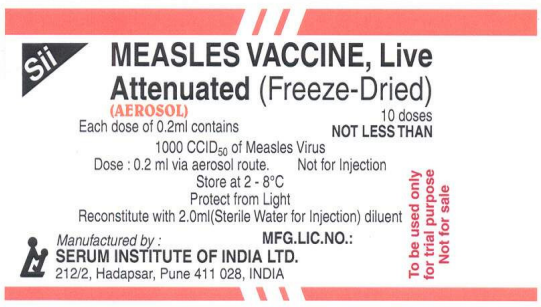
6.4.1.2 Diluent

The diluent for the vaccine is water for injection (WFI). Bulk WFI is prepared by distillation and meets the Indian Pharmacopoeia specifications for WFI.

6.4.1.3 Method of packaging and labelling of the vaccine

Vaccine vials for the aerosol arm will be labelled and packaged to indicate that they are for the clinical trial.

A sample of the label for the aerosol vaccine is shown below:



6.4.2 Vaccine lots

Vaccine lots will be selected as follows:

Item	Batch	Doses	Presentation	Label
Aerosol Vaccine	1	500	50 x 10 dose vials	Clinical label - research only
Aerosol Vaccine	2	500	50 x 10 dose vials	
Aerosol Vaccine	3	500	50 x 10 dose vials	
Aerosol Diluent	A	1500	150 x 2.5 mL amps	Clinical label stating for aerosol vaccine reconstitution only
Subcutaneous Vaccine	1	1500	150 x 10 dose vials	Regular label
Subcutaneous Diluent	B	1500	150 x 5 mL amps	Regular label

All lots will have an expiry date of at least 18 months from date of shipping to the research center. Ten vials of each vaccine batch and 30 ampoules of each diluent batch will be retained by the vaccine manufacturer for retesting.

6.4.2.1 Vaccine delivery device

Aerosolised vaccine will be delivered using the Nektar device (Aerogen/Nektar, San Francisco, See Appendix 1 for full details) to be included.

6.4.2.2 Method of packaging and labelling of the vaccine

A sample of the device over-label is shown below:

Device over-label

For Research Use ONLY
Measles Aerosol Project

6.5 Expected duration of subject participation and description of sequence and duration of trial periods including follow-up.

Duration of subject participation

- Every child will be followed for 91 days after vaccination for ascertainment of the primary immunogenicity and safety outcomes.
- Additionally, all children in the Vadu area will be followed to 364 days after vaccination for ascertainment of adverse events.
- A random subset of 100 of children in each arm from the Vadu area will have blood drawn at day 28.
- A random subset of 100 of children in each arm from the Vadu area will have blood drawn at day 364. The selection of this group will be independent from the selection for the day 28 blood sample and individuals my be selected for blood sampling on both day 28 and 364.

Enrolment period

It is estimated that it will take approximately six months to enrol 2000 participants, based on a 50-80% participation rate. This is assumes that approximately 100 infants will be screened for inclusion in the trial per week.

Duration of follow up periods

The expected duration of the study from initial enrolment to the primary outcome (day 91 visit) is nine to 12 months, based on the enrolment rate above and, the assumption that the number of children available for vaccination is similar to that in previous years.

Duration of the study from initial enrolment to the secondary outcome (day 364 visit) for the subset of children in the Vadu area is 18 to 21 months.

Detailed information on the planned follow-up visits is in Section 8.4.4 including Table 7.

6.6 Stopping rules or discontinuation criteria

The trial will be discontinued according to the stopping rules determined by the DSMB (Appendix 2: Terms of Reference of the Data and Safety Monitoring Board).
The criterion for discontinuation is the occurrence of a single SAE or encephalitis/encephalopathy in one or more subjects which is deemed to be most probably related to the aerosol vaccine.

6.7 Measles vaccine and aerosol delivery device accountability

The investigator will assign a nurse to be responsible for vaccine and aerosol device storage and accountability at the trial site. The investigator/designated person will:

- Store the vaccine and device in the condition that has been specified in writing by the Sponsor in SOP WHO 4/1 and in accordance with the protocol and Indian regulatory requirements.
- Ensure that the vaccine storage temperature is maintained as specified in the protocol. There will be a daily temperature log. (SOP WHO 4/1)
- Maintain records of the vaccine and device delivery, inventory and return. (SOP WHO 4/1)
- Maintain up to date accountability on the vaccine and device accountability log. (SOP WHO 4/1)
- Ensure that the vaccine and device are used only in accordance with the approved protocol.

Written records of receipt and storage of the vaccine and devices, including date received, lot number, quantity received, and dose administered, with the coded identification of the subject, will be recorded. Any known discrepancies in the accountability of the vaccine will be documented. The investigator will not use the vaccine and/or devices in any other manner than that provided for in the protocol.

6.7.1.1 Vaccine Storage, Handling and Transport

The vaccine will be stored, according to the manufacturer's recommendations at 2˚C to 8˚C in a refrigerator in which the temperature is monitored and logged at least once a day. The refrigerator will be secured and located in a limited access area.

6.7.2 Policy and procedure for handling unused investigational product.

(SOP WHO 4/1))

- All unused un-reconstituted measles vaccine will be disposed of according to the manufacturers instructions and WHO[33].
- All reconstituted vaccine not used within 6 hours after reconstitution during an immunization session will be discarded according to WHO recommendations for multi dose open vials.[41] Records will be kept of discarded vaccine.
- All non-reusable components of the nebulizer will be disposed as indicated in SOP NEK 2/1
- The nebulizers used during the trial will be returned to the sponsor at the end of the trial according to sponsor-defined procedures.

6.8 Maintenance of trial randomization codes and procedures for breaking codes

6.8.1 Unblinding of laboratory specimens

Laboratory specimens collected up to day 91 will be unblinded by the designated Sub-Investigator (in charge of data Management and Statistics) at the start of the statistical analysis of the primary outcome and after the database has been locked (see SOP VEL 2/1).

Premature unblinding will be reported immediately to the clinical monitor and the Sponsor and will be documented in the investigator's file. The reason for premature unblinding of the samples should be given.

6.9 Source data

The following documents will be considered source data for this trial:

- Electronic audited copies of the Case Reports Forms and Adverse Events (AE) Investigation Forms
- In addition, the following original documents, data, and records will be considered as source documents:
- hospital records,
- clinical and office charts,
- laboratory notes, memoranda,
- pharmacy dispensing records,
- recorded data from automated instruments,
- copies or transcriptions certified after verification as being accurate copies,
- photographic negatives or files,
- X-rays,
- subject medical files, and
- records kept at the pharmacy, at the laboratories, and at medico-laboratory departments involved in the clinical trial).

7 Selection and withdrawal of subjects

7.1 Inclusion criteria

- Age: from 9.00 to 11.99 months on expected vaccination date;
- Resident in the study area and likely to remain for the duration of the child's involvement in the study;
- Parent/guardian willing for child to be randomized and willing for child to be followed up for at least 91 days.

7.2 Exclusion criteria

- Having received measles, measles-rubella or measles-mumps-rubella vaccine (with confirmation from vaccination card).
- Any contraindication to measles vaccine administration as stated in the WHO measles vaccine position paper: [42]
    - Persons with a history of an anaphylactic reaction to neomycin, gelatine or other components the vaccine should not be vaccinated.
    - Furthermore, measles vaccine is contraindicated in persons who are severely immunocompromised as a result of congenital disease, HIV infection, advanced leukaemia or lymphoma, serious malignant disease, or treatment with high-dose steroids, alkylating agents or antimetabolites, or in persons who are receiving immunosuppressive therapeutic radiation.
    - Administration of immunoglobulins or other antibody-containing blood products may interfere with the immune response to the vaccine. Vaccination should be delayed for 3–11 months after administration of blood or blood products, depending on the dose of measles antibody. Following measles vaccination, administration of such blood products should be avoided for 2 weeks, if possible.
    - Mild, concurrent infections are not considered a contraindication, and there is no evidence that measles vaccination exacerbates tuberculosis. However, vaccination should be avoided if there is high fever or other signs of serious disease.

7.3 Subject withdrawal/discontinuation criteria

- Withdrawal of consent;
- Serious adverse event;
- Any other situation in which the Principal Investigator finds it in the subject's best interest to no longer continue his/her participation in the study.

If this occurs, the subject will be asked to continue with the clinical follow up as per the study protocol but would be excluded from the analysis. Withdrawn subjects will not be replaced.

See section 6.6.

8 Treatment of subjects

8.1 Treatments to be administered

8.2 Aerosolized measles vaccine

A dose of measles vaccine, reconstituted for aerosolisation, containing at least 1,000 plaque forming units and administered by Nektar/Aerogen nebulizer over 20 seconds, as described in SOP NEK 2/1.

The vaccine and nebulizers will be used and maintained according to the manufacturer specifications, as described in SOP NEK 2/1.

8.3 Subcutaneous control

A dose of measles vaccine (0.5ml), reconstituted for subcutaneous administration, containing at least 1,000 plaque forming units and administered as described in SOP WHO 6/1.

The vaccine will be used and stored according to the manufacturer specifications as described in SOP WHO 6/1.

8.3.1 Concomitant medications

Subjects may take all other medications (including rescue medications) as indicated, in line with current Indian MOHFW Guidelines.

Monitoring subject compliance with use of investigational product

Due to the single-dose regime used in this trial, subjects will not be monitored for compliance with dosing schedules as they would be in multiple-dose regimes or those where medications are self/parent/guardian administered. Vaccine administration will occur under direct observation from trial staff for each participant.

8.4 Methods for the trial

8.4.1 Community approval

The trial study area covers more than 150 villages. Preliminary visits will be made to all villages before starting recruitment. Village leaders, influential village residents and other gram panchayat (village level local self government members) will be consulted and asked for verbal agreement for the trial to take place in their village. A written record of meetings will be kept.. Local health providers (family doctors, private clinics, and private doctors) will also be informed of the trial and its purposes.

8.4.2 Enumeration of potential study participants

The method of enumeration will depend on the study area:

- For Vadu, an initial tentative list of all eligible children aged 6 to 10 months will be obtained from the Demographic Surveillance System, Vadu.
- For the other study areas, the study field worker will obtain a list of all eligible children aged 6 to 10 months from local health workers. For full details of this process, please see SOP VAD 3/1. These lists will be in a non-electronic form initially, but in the course of recruitment, details of potential participants will be recorded electronically.

Field workers will visit the parents/guardians of potential participants 2-4 weeks before the infant is 9 months old (Visit 1, see below).

Parents/guardians will be encouraged to consult peers, community leaders and other parents who are study participants. In addition, advertisements about the trial will be displayed in prominent places like gram panchayat offices, temples, and schools in the village.

Parents attending clinics for the vaccination of their child prior to nine months of age (e.g. DPT vaccination at 4 months), will also be given information about the trial.

8.4.3 Vaccination day procedures

The procedures for this visit (Visit 2) are summarized below and described in detail in SOP VAD 4/1.

8.4.4 Follow up schedule

The content of each home or clinic visit shown is described below. The procedures to be followed at each visit are described in SOP VAD 5/1 (home visit and clinic visit).

Table 7: Timetable of home and clinic visits for an individual trial participant

Activity	**Pre-study**	**Main study**											**Long term follow up**			
	ALL INFANTS	**ALL INFANTS**											**SUB-SAMPLE INFANTS†**			
Day	-14	0	3	7	10	14	17	21	28	56	pre-91	91	182	252	pre-364	364
Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Home visit with trial information	x															
Clinic visit for eligibility, randomization and vaccination		X														
Home visit for AE monitoring			x	x	x		x	x	x	x			x	x		
Reminder visit (AE, serology)					x			x			x				x	
Clinic visit for AE examination		x				x			x			x				x
Clinic visit for immunogenicity		X							X¥			X				X
Measles surveillance - case based and outbreaks	X	X											X			
Active surveillance for SAE	X	X											X			

Legend:

AE	Adverse event
SAE	Serious adverse event
X	Procedure undertaken at this time
¥	Only subset – random sample from children in Vadu area
†	Only subset – random sample from children in Vadu area

8.5 Schedules for recruitment and follow up visits, and procedures undertaken at each

8.5.1.1 Visit 1, Identification and recruitment visit (2-3 weeks) before due date for measles vaccination, Figure 2.)

Purpose of visit:

- To inform parents/guardians of potentially eligible children of,
  - Purpose of trial;
  - Potential risks and benefits to participant;
  - Trial methods and requirements from participants (e.g. visits, blood taking, follow up);
  - Process of giving informed consent to participate;
  - Rights of participants, including the right to withdraw without giving a reason at any time;

Procedures:

- **Inform about the trial -** Fieldworkers will give the parents/guardians verbal and written information about the trial and a copy of the consent form in Marathi or Hindi (Document number SD-4, SD-5).
- **Set an appointment for eligibility assessment and vaccination** Fieldworkers will give the parents/guardians an appointment to attend the PHC for Visit 2, if they are willing;
  - Fieldworkers will advise parents/guardians to arrange transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.
- **Provide additional information as required** Fieldworkers will be available in the community to answer and questions arising after this visit.

8.5.1.2 Visit 2, eligibility, randomization and vaccination visit (day 0, minimum of one day after visit 1)

Purpose of visit: to assess the child for eligibility for randomisation and provide measles vaccination, either as part of the trial, or with routine vaccination.

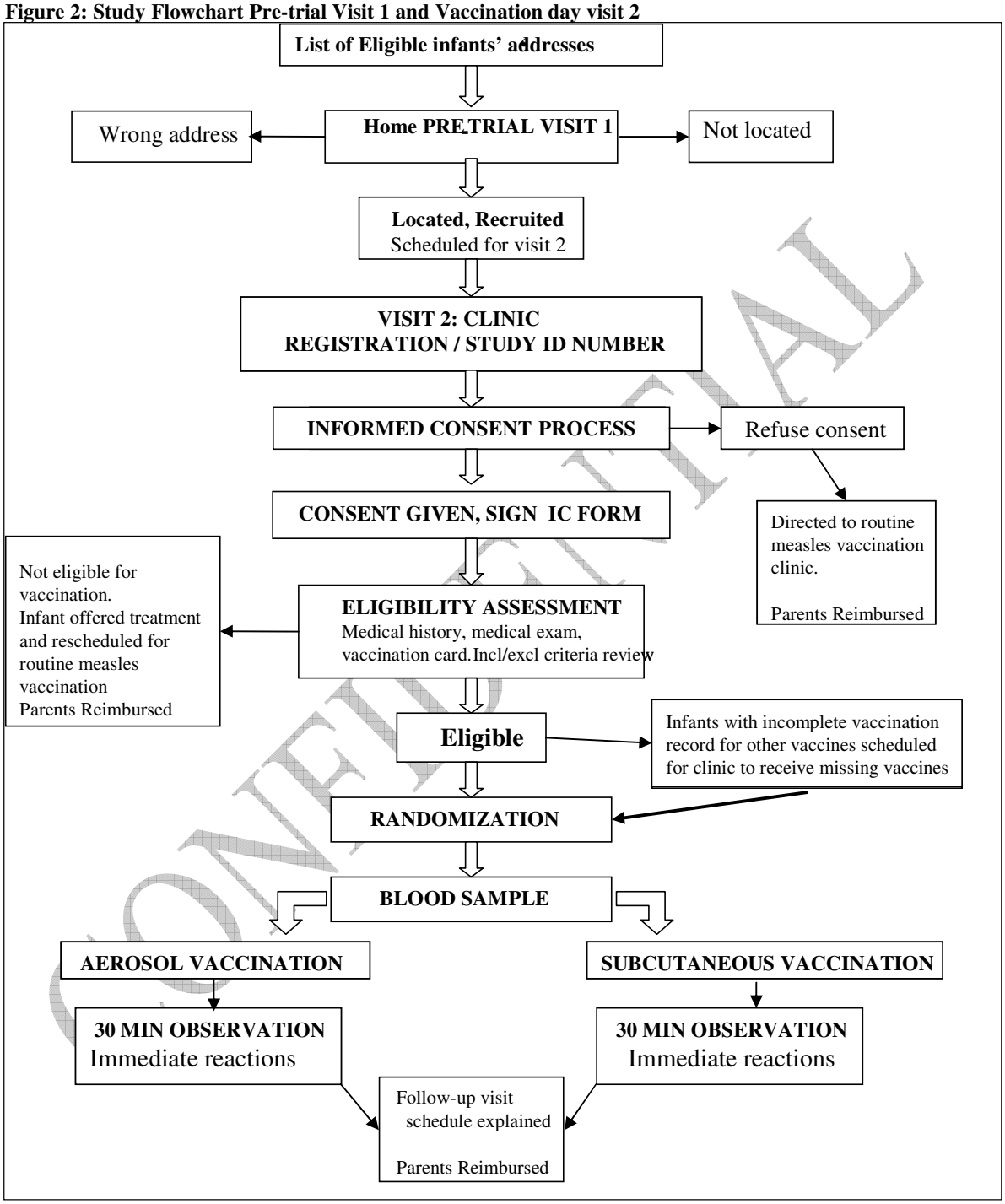
Procedures:

- **Assign a unique ID number –** see SOP VEL1/1)

- **Obtain Informed Consent.** Parents/guardians of eligible children will be informed again of the purpose, risks/benefits and methods of the study and their rights, and will have an opportunity to ask questions. Parents/guardians who are willing for their child to participate will be asked for a signature or thumbprint. The consent form for all children includes consent for medical examination, vaccination, follow up to 91 days and blood sampling at days 0 and 91. In the Vadu area, all parents will also be asked for consent for their child to be followed up to day 364, with the possibility of selection for blood sampling at days 28 and 364. Children may be selected for none, one or both of these 2 additional blood samples. See SOP VAD 4/1 and Consent form, SD -5;
- **Vaccinate children for whom consent is not obtained.** Children without written consent for participation will receive measles vaccine according to Indian MOHFW guidelines. After vaccination, parents/guardians should receive reimbursement as described in the Informed Consent Form (ICF).
- **Assess eligibility.** This is will be conducted according to inclusion/exclusion criteria, Sections 7.1 and 7.2
    - **Take a medical history and vaccination history** - the study doctor will record this information using vaccination cards and verification from clinic records, using a case report form (CRF).
    - **Conduct a medical examination to confirm eligibility** - a physical examination will be undertaken by the Study doctor and recorded in the Case Report Form (See SOP VAD 4/1). Any child found on examination to have a clinically apparent condition that contra-indicates vaccination will be withdrawn from the study and offered referral for treatment.
    - **Vaccination of eligible children with incomplete vaccination schedule** - if the child has an incomplete vaccination history, the parents/guardians will be given an appointment for the missing doses of vaccine to be administered at their local immunization clinic (at a minimum 4 weeks after measles vaccination).
    - **Vaccination of children who are not eligibl**e - all children who are assessed to be ineligible at Visit 2 will be permanently excluded and are ineligible for reassessment for inclusion. These infants will be rescheduled for routine measles vaccination as per Indian guidelines and parents/guardians should receive reimbursement as described in the informed consent form (ICF).
- **Conduct randomization**. If the parents/guardians give consent, the randomization process will occur as described in SOP VAD 4/1. Allocations will be recorded immediately by trial staff;
- **Obtain blood sample**. This will be taken by venepuncture (SOP HPA 1/1);
- **Vaccinate infants.** This will be undertaken according to study allocation. Full details are described in (SOPs NEK 2/1 and SOP WHO 6/1). Vaccination will occur in separate rooms (without direct communication of ventilation systems) for each intervention group. The processes of vaccine storage and transport are described in SOP WHO 4/1.

- **Conduct observation for immediate adverse events.** Children will be observed for 30 minutes after vaccination and observations recorded in the CRF (see below). This is described in detail in SOP WHO 2/1.
- **Provide parents with information on follow up visits.** The study nurse will inform the parents of the subsequent visits and procedures
- **Provide parents with reimbursement of expenses.** The study nurse will ensure that the parents/guardians receive reimbursement as described in the ICF.

Figure 2: Study Flowchart Pre-trial Visit 1 and Vaccination day visit 2



8.5.1.3 Visit 3, AE follow up - home visit (day 3, Figure 3)

Purpose of visit: To collect information about adverse events and concomitant medications (See VAD 5/1).

Procedures:

- **Verify ID code -** Fieldworkers verify the ID code and identification details for the child (see VAD 5/1)
- **Collect information about AE**. Fieldworkers ask parents about adverse events and concomitant medications, using structured questionnaire See SOP WHO 1/1. This will include a question relating to the occurrence of measles symptoms in the family/household.
- **Initial grading of adverse events**. Field workers record this according to predefined criteria (described in Appendix 3).
- **Request advice from the Medical Officer on-call for any AE classified as moderate or severe.** If any of the adverse events identified is classified as moderate or severe the field worker should inform the On-Call Medical Officer immediately and request his/her assistance to confirm the grading and investigation of the AE as described in SOP WHO 1/1
- **Confirm classification of AE.** On-Call Medical Officer assists the field worker to confirm the classification of the AE and complete the CRF.
- **Report and manage AE and SAE –** AE and SAE are reported through the channels described in detail in SOP WHO 1/1 and SOP WHO 3/1. In brief, serious and severe AE are rapidly reported to the Authorised Physician (safety),the PI, the WHO Focal Point, and DSMB, and serious AE are rapidly reported by the PI to the WHO Focal Point and Local ethical committees. At the end of each day, all CRFs with data about adverse events will be checked by a Study Medical Officer to ensure that all adverse events graded as moderate or severe have been followed up, and that fieldworker reports appear consistent. All AE are reported on a regular basis via CRFs to the Data Management Center.
- **Conduct initial causality assessment.** The Authorised Physician (safety) and PI (or their designee) together with the attending Study Medical Officer review the information of regarding any moderate or severe AE and conduct an evaluation of causality. A report is submitted to the DSMB as indicated in the SOP WHO 1/1 and the DSMB TORS Appendix 2.

8.5.1.4 Visit 4, AE follow up - home visit (day 7)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).

8.5.1.5 Visit 5, AE follow up- home visit (day 10)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).
- Fieldworkers give parents/guardians appointment for visit 6 at PHC.
- Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

8.5.1.6 Visit 6, AE follow up -clinic visit (day 14/15)

Purpose of visit: Clinical examination and ascertainment of adverse events.

Procedures:

- **Verify ID Code** – The study nurse verifies the child's identification as described in See SOP VAD 5/1
- **Check for any AE** - Study Medical Officer asks parents/guardians about adverse events using the structured questionnaire and records it in CRF (SOP VAD 5/1).
- **Conduct medical examination** - Study Medical Officer conducts medical examination, including directed neurological examination and records it in the CRF (SOP VAD 5/1).
- **Grade AE** - Study Medical Officer grades adverse events.
- Severe or serious adverse events are reported to Authorized Physician (safety) immediately as described in SOP WHO 3/1.
- For all AE, the Study Medical Officer treats infant or refers for further treatment as appropriate
- All AE (other than SAE) are reported to field workers by the Study Medical Officer for follow-up for AE resolution/outcome
- The Study Medical Officer informs parents of treatment plan
- **Provide parents with information on follow up visits -** the study nurse will inform the parents of the subsequent visits and procedures
- **Provide parents with reimbursement of expenses -** the study nurse will ensure that the parents/guardians receive reimbursement as described in the ICF.

8.5.1.7 Visit 7, AE follow up -home visit (day 17)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).

8.5.1.8 Visit 8, AE follow up -home visit (day 21)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).

- Fieldworkers give parents/guardians of selected children appointment for visit 9 at PHC.
- Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

8.5.1.9 Visit 9, AE follow up -home visit or clinic visit for selected children (day 28-30)

Purpose of visit: To collect information about adverse events and, for selected subset, to draw blood for antibody levels.

Procedures:

- Home visit - AE follow up for all infants: Procedures: As described in visit 3 (above).
- Clinic visit - AE and 28 days blood sample for subset: As described in visit 6 (above plus blood sampling as described in SOP HPA1/1.

8.5.1.10 Visit 10, AE follow up -home visit (day 56 +/- 3 days)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).
- Fieldworkers give parents/guardians children appointment for visit 12 at PHC.

8.5.1.11 Visit 11, Reminder visit- home visit (day before Visit 12)

Purpose of visit: To remind parents/guardians of the clinic appointment.

Procedures:

- Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

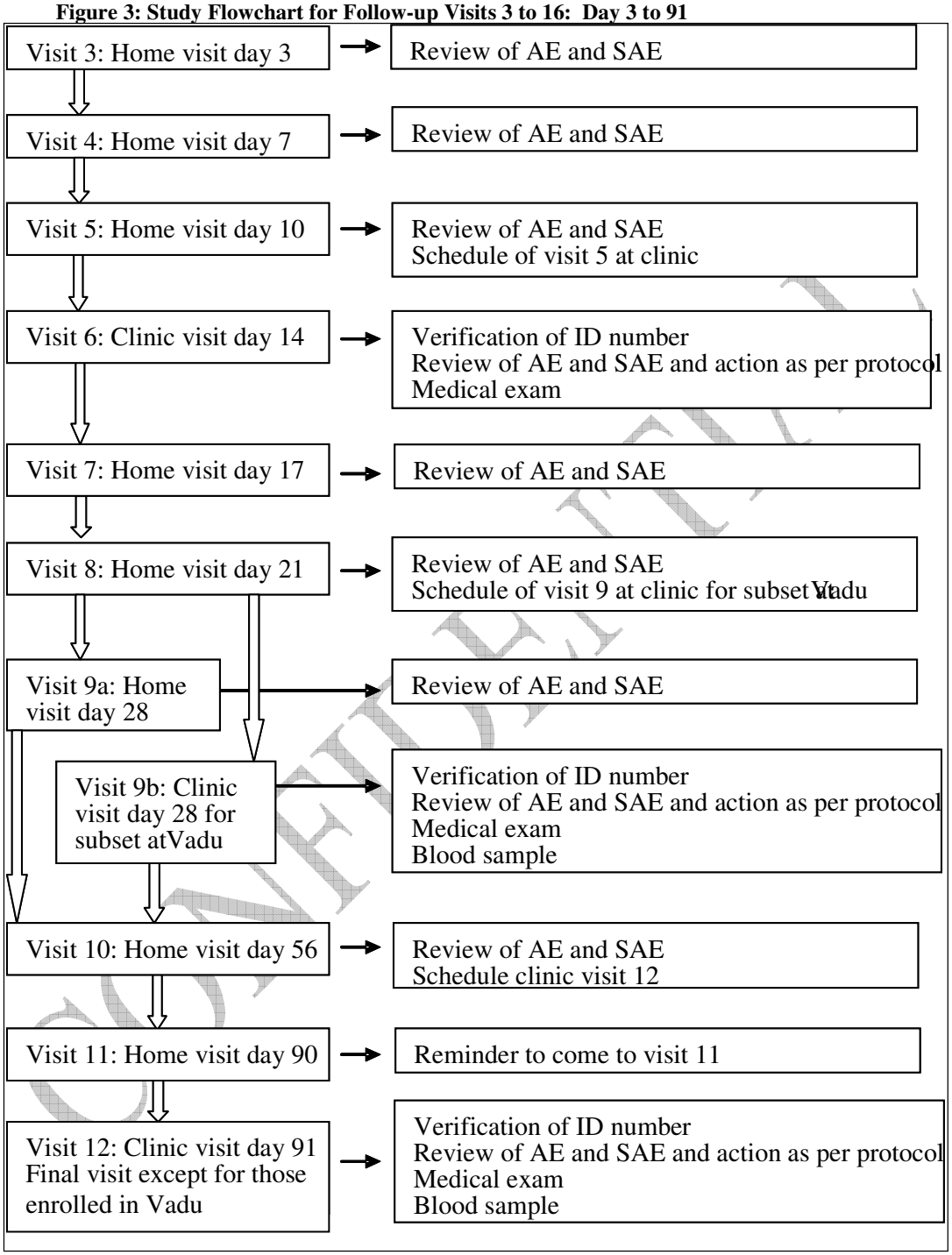
8.5.1.12 Visit 12, AE follow up- clinic visit (day 91 +/- 7 days)

Purpose of visit: Ascertainment of primary trial outcome in all participants.

Procedures:

- As described in visit 6 (above). Structured questionnaire for adverse events, medical examination, including directed neurological examination, recorded in CRF (SOP VAD 5/1). Blood sample from all participants (SOP HPA 1/1)

Figure 3: Study Flowchart for Follow-up Visits 3 to 16: Day 3 to 91



8.5.1.13 Visits 13 and 14, home visits for all children enrolled in Vadu district (day 182 and 252, Figure 4)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).
- Fieldworkers give parents/guardians children appointment for visit 15 at PHC.

8.5.1.14 Visit 15, home visit for subset of children enrolled in Vadu district (pre-Visit 16)

Purpose of visit: To remind parents/guardians of the clinic appointment.

Procedures: Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

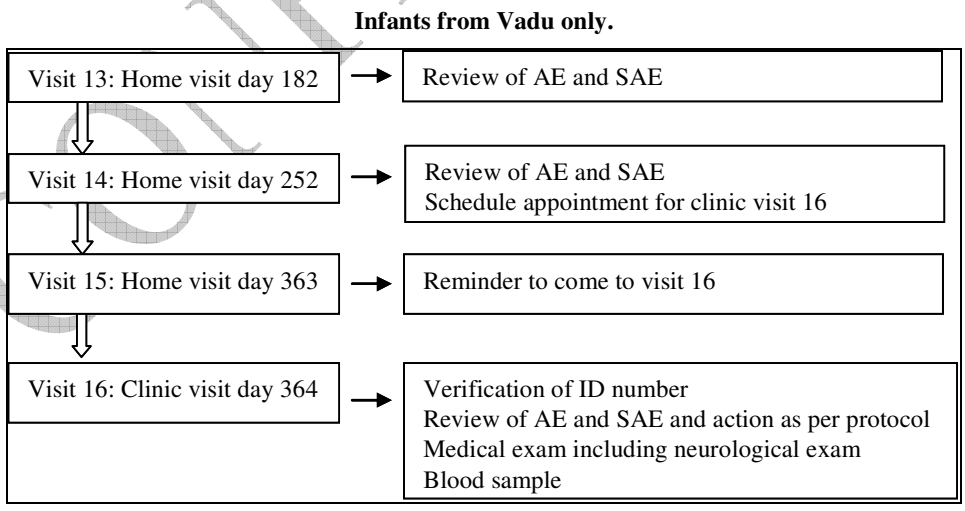
8.5.1.15 Visit 16, clinic visit for all children enrolled in Vadu district (day 364 +/-7 days)

Purpose of visit: Ascertainment of adverse events in all enrolled children in Vadu district and, for selected subset, to draw blood for antibody levels one year after vaccination.

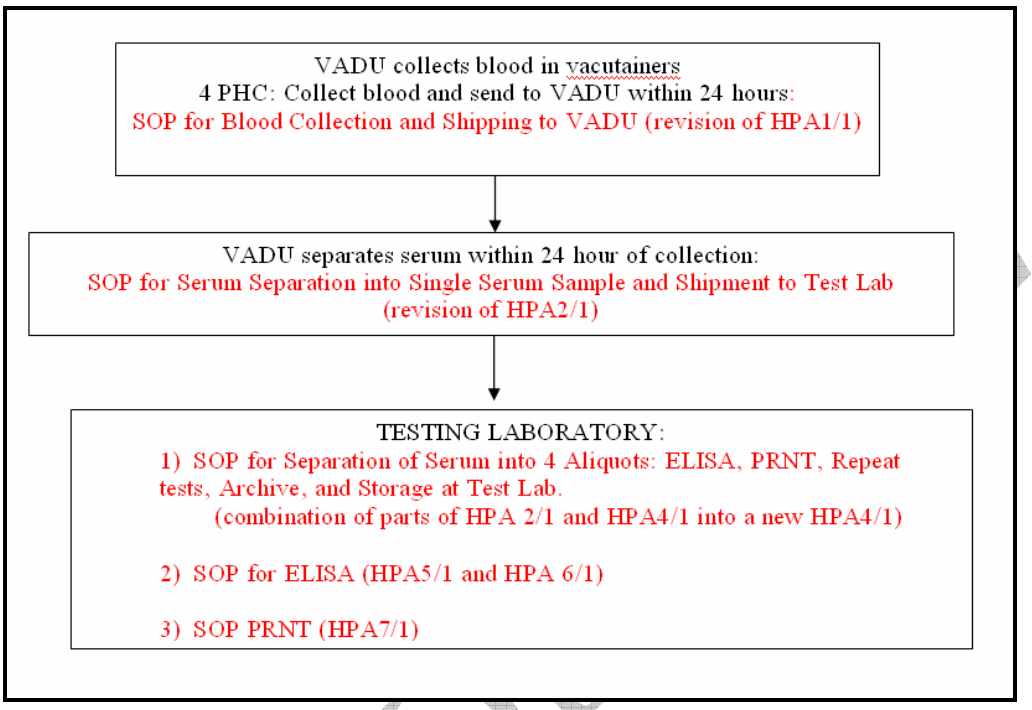
Procedures:

- Clinic visit: As described in visit 6 (above). Structured questionnaire for adverse events, medical examination, including directed neurological examination, venepuncture, recorded in CRF (SOP HPA 1/1).

Figure 4: Study Flowchart for Follow-up Visits 13 to 16: Day 182 to 364.



8.6 Sample collection, storage, tracking, processing, archiving



8.6.1 Collection

- Blood will be taken by venepuncture from participants and collected in gel separation tubes see SOP HPA 1/1

8.6.2 Sample labelling, processing, storage and transport

Each sample will be coded with a unique accession number that permits tracking of the sample through receipt, analysis and reporting. Blood and serum samples (including aliquots of serum) will be labelled as described in section 6.3.2 (blinding) and SOP VEL 3/1.
Each clinical sample will be accompanied by a form to clearly indicate sample coded number and date of collection and what analyses should be performed on it. (SOP HPA 1/1).

- Whole blood samples will be held at 2-8˚C (in ice-chilled, insulated vaccine carriers)
- Whole blood samples will be transported to initial processing site (Laboratory at SSH) on the same day as sample collection.

8.6.2.1 At initial processing site (Lab Vadu Rural Hospital)

- Whole blood samples will be held at 2-8˚C until processing (serum separation) within 24 hours of blood collection. Separation of serum is described in SOP HPA 2/1
- Serum samples will be stored in non-gel tubes.

- Serum samples will be frozen at minus 20˚C.
- Serum samples are sent to testing laboratory once a week.

8.6.2.2 At ELISA/PRNT testing laboratory

- Samples are separated into 4 aliquots (ELISA, PRNT, repeat tests and archiving) SOP HPA 4/1
- Serum samples will be held at minus 20˚C until ELISA or PRNT testing is undertaken

8.6.2.3 Storage of reserve aliquots of serum at testing laboratory in Pune

- Reserve aliquots of serum will be held at minus 20˚C until required or for a minimum of 5 years post-licensure, as required by Indian regulations (See SOP HPA 4/1 for storage after testing).

8.6.2.4 Transportation of samples

- Whole blood samples will be transported from sampling sites to the initial processing site (SSH) by designated trial staff at 2-8˚C (in vaccine carriers).
- Further transportation will be undertaken by a designated laboratory technician from Vadu Rural Hospital and will comply with Indian regulations. Serum samples will be held at minus 20˚C during transportation.

8.6.3 Sample processing

8.6.3.1 Laboratory measurement of immunogenicity

- All serum samples will be tested by Enzygnost Anti-measles-virus/IgG ELISA (Dade-Behring, Marburg, Germany) (SOP HPA 5/1 and SOP HPA 6/1).
- In addition, all specimens with Enzygnost optical density readings <0.1 will be tested by plaque reduction neutralization test (SOP HPA 7/1).

8.6.4 Sample archiving

Unused portions of serum samples will be held at minus 20˚C until a minimum of 5 years post licensure. Samples will be catalogued to allow rapid relocation as required.

8.7 Measurement of safety outcomes

Clinical events following vaccination will be investigated under the categories of: immediate adverse events (IAE); serious adverse events SAE, or other adverse events.

- Data on adverse events will be collected by Medical Officers at clinic visits (Days 0, 14, 28, 91 and, for infants in the Vadu area, day 364), and field workers at home visits (Days 3, 7, 10, 17, 21, 28, 56 and 182, 252 for subset of infants).
- Any adverse event detected during home visits that are graded moderate or severe (see Section 8.7.5, Visit 3 and Visit 6) or are unexpected will be immediately reported to the On-Call Medical Officer, who will examine the child, report the AE and treat, or refer for treatment, as required.

- At the end of each day, all CRFs with data about adverse events will be checked by a Study Medical Officer to ensure that all adverse events that are graded moderate or severe have been followed up, and that reports appear consistent.

8.7.1 Definitions

Acute clinical reactogenicity

Acute reactogenicity includes the expected reactions to vaccination occurring during the first 14 days post vaccination, reported at home visits, or clinic visits.

Adverse event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigation) product, whether or not related to the medicinal (investigation) product.[43] This definition includes all SAE and IAE.

Anaphylaxis

Exaggerated acute allergic reaction, occurring within 2 hours after immunization.[44]
Anaphylaxis is a clinical syndrome characterized by sudden onset AND rapid progression of signs and symptoms AND involving 2 or more organ systems. [45]

Immediate Adverse Events (IAE)

Any adverse event, serious or otherwise, occurring within 30 minutes of vaccine administration. This definition includes SAE occurring in this time period.

Serious adverse event (SAE)

Any adverse event that, at any dose, has one or more of the following attributes:[43] [46]

1. Results in death.
2. Is life-threatening.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
4. Results in persistent or significant disability/incapacity.
5. Results in an important medical event that may not be immediately life-threatening or does not directly result in death or hospitalization, but which may jeopardize the patient.

Unexpected adverse drug reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. that listed in the investigator's brochure). [42, 46] [43, 47]

8.7.2 Adverse event detection, management and reporting

Information about adverse events will be collected through a combination of: structured questionnaires administered at study clinic visits or home visits; active surveillance for events requiring medical treatment or hospitalization, or deaths; and reports from parents/guardians, who will be told by fieldworkers to report any medical conditions immediately on the Study Emergency Number and to seek treatment at SSH or KEM Hospital as appropriate.

The management of patients with adverse events and reporting procedure are described in detail in SOPs WHO 1/1, WHO 2/1 and WHO 3/1. In brief, the investigator/institution will ensure that adequate medical care is provided to a subject for any adverse event. At enrolment, parents/guardians will be given numbers to contact in the case of emergency and informed of other means of seeking medical treatment if they have no immediate access to a telephone. They will also be informed of how to seek medical treatment from either study staff or their local PHC for less urgent conditions. Contact has been made with PHCs regarding their participation in the treatment of study participants who attend PHCs and the reporting of these AE to the research team. SSH is currently the central referral hospital for the study area and in addition oversees PHCs in the Vadu region. Onward referrals from SSH will be to KEM Hospital (which administers SSH). The trial co-investigator at KEM will receive referrals from trial medical officers and ensure that prompt treatment is given. The co-investigator will also ensure mechanisms are in place so that any treatment of a subject in KEM Hospital which does not arrive via the referral method is promptly reported to him. He will monitor clinical care of any trial participant for the duration of their stay at KEM.

The principal investigator is responsible for ensuring that all serious adverse events are reported immediately by telephone or fax to the DSMB and the sponsor, as described in SOP WHO 3/1.

Participants will be reimbursed for expenses incurred in connection with their participation in research. During the period of research if the participant requires treatment for complaints other than those being studied, necessary free ancillary care or appropriate referrals will be provided.[48] The parents/guardians will be informed about the investigations regarding the event.

8.7.2.1 Immediate Adverse Events

See SOP VAD 4/1 (vaccination day)

Pulse rate, respiratory rate, blood pressure and axillary temperature will be measured prior to vaccination and during the 30 minute direct observation period. These will be recorded in the CRF.

Symptoms and signs of immediate reactions, treatments given and outcomes will be recorded and reported, as described in SOP VAD 4/1. Any serious events will be managed and reported as described in SOP WHO 2/1.

8.7.2.2 Additional adverse events

All non-serious adverse events will be assessed using structured questionnaires, either at clinic or home visits. The timing of these visits is described in section 8.5 and SOP VAD 5/1

The choice of specifically solicited adverse events is based on:

- Events observed in the phase 1 MAP trials in India;
- Additional adverse events seen in previous studies of aerosolized measles vaccine;
- Adverse events known to be associated with subcutaneous measles vaccination;
- Adverse events observed in trials of other vaccines administered by the respiratory route.

Detailed list of solicited adverse events and specific grading structure for each event type on a 4 level scale from 0 (none) to 3 (severe) are described in Appendix 3

Atypical measles syndrome

8.7.3 Adverse event reporting procedure

- All adverse events occurring during the trial should be accurately reported in case report form (SOPs WHO 1/1, WHO 2/1, WHO 3/1).
- Adverse events graded as **severe** will be reported by the PI to the WHO focal point within 24h of first knowledge by the Principal Investigator or any trial staff. The DSMB will be informed of these by the WHO focal point within 24 hours.
- The Principal Investigator will report all SAE immediately (within 24h of first knowledge by the principal investigator or any trial staff) to the WHO Focal Point and local ethical committees; even if the adverse event is considered not to be related to the investigation product. Notification will be made by fax and/or by telephone or email communication.
- The Principal Investigator should send promptly, within five working days, the serious adverse event report by fax, email or express mail to the WHO Focal Point.
- Any relevant information concerning the SAE that becomes available after the SAE report has been sent (outcome, precise description of medical history, results of the investigation, copy of hospitalization report, etc.) will be forwarded by the Principle Investigator as soon as possible to the WHO Focal Point.
- For reports of deaths, the investigator should provide WHO Focal Point with any additional requested information, e.g. autopsy reports and terminal medical reports. The anonymity of the subjects shall be respected when forwarding all information
- The WHO Focal Point will comply with the Indian regulatory requirements regarding SAE. The National Regulatory Authorities, WHO ethical committee, DSMB and Clinical Trial Monitor will be notified (e.g. by telephone, facsimile transmission, or in writing) by the WHO focal point as soon as possible (ideally within 24 hours) but no later than 7 calendar days after first knowledge by the WHO focal point that a case qualifies as an SAE, followed by as complete a report as possible within 8 additional calendar days. See SOP WHO 3/1.

8.7.4 Follow-up of Adverse Events and Serious Adverse Events

All AE will be followed up until the participant has reached one of the endpoints as described here.

- An adverse event that is likely to be related to the product and that persists at the end of the trial, or any SAE occurring after termination of the trial and likely to be related to the product, will be followed up by the investigators until the participant has completely recovered, recovered with sequelae, or died.
- All other AE will be followed up until the participant has completely recovered, or they complete the study.

A thorough investigation will be conducted to determine causality. Adverse events will be recorded in detail during the course of the trial, irrespective of the possible causal relationship with the measles vaccine.

8.7.5 Grading of adverse events

Adverse events will be graded using a specific grading structure for each event type on a 4 level scale from 0 (none) to 3 (severe). These are described in Appendix 3

8.7.6 Classification of association of events with vaccination

The investigator will assess whether any adverse event is related to the immunization using the following scale and according to the following definitions (based on Workbook for Investigators, WHO/TDR).

- **Not related:** The event is clearly related to other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy.
- **Unlikely to be related:** The event was most probably produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy, and does not follow a known response pattern to the study vaccine.
- **Possibly related:** The event follows a reasonable temporal sequence from the time of study vaccine administration and/or follows a known pattern to the study vaccine but could have been produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy.
- **Probably related:** The event follows a reasonable temporal sequence from the time of study vaccine administration and/or follows a known response pattern to the study vaccine and could not have been produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy. These events include but are not limited to those that require medical attention and result in withdrawal from follow-up. Any available medical records needed to accurately describe the event will be obtained from the appropriate health care provider.
- **Most probably related:** The event follows a reasonable temporal sequence from the time of vaccine administration; and/or follows a known response pattern to the trial vaccine; and could not have been produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy; and either occurs immediately following trial vaccine administration, or there is positive reaction at the application site.
- **Insufficient data to assess:** There is not enough clinical and/or laboratory information to suggest the relationship between the experience and the trial product.

A report on all **Severe** Adverse Events and all **Serious** adverse events will be submitted to the DSMB within 7 days, following the Terms of Reference of the DSMB for the Measles Aerosol Project (Appendix 2). The DSMB will make the final decision on the classification of serious and severe adverse events.

In addition, the DSMB will receive summary reports of all AE quarterly (see DSMB TORs).

8.8 Measles surveillance in the study area

A surveillance system is to be established (as an entity separate from the trial) in the entire blocks containing the trial areas. It will be established as described in the WHO Best practices for measles surveillance[49] and in the Field Guide -Measles Surveillance & Outbreak

Investigation Guidelines[50] (2005) Government of India, Department of Family Welfare New Delhi.

It is expected that this surveillance system will be operational before the start date of the trial.

The primary purpose of measles surveillance will be to detect, in a timely manner, all areas in the trial whether the measles virus is circulating. Surveillance will be undertaken with support from the Immunization Programme of the State of Maharashtra and with the National Polio Surveillance Project in India. The surveillance system will involve the following:

- **All the health facilities/health providers** in the study area will be trained to implement timely notification of clinically diagnosed measles (probable) cases will be promoted to detect cases and outbreaks. A designated surveillance medical officer (SMO) will conduct monthly active searches in the registry files (outpatient and inpatient records) to ensure all probable measles cases are reported. All measles outbreaks will be serologically confirmed to differentiate them from other fever and rash outbreaks. The surveillance data should be analysed at all levels to determine and improve the immunization strategies.
- In addition, **during the home and clinic visits** the field workers, medical doctors and nurses will solicit information on the presence of cases of rash and fever illness in the household and in the neighbourhood where the infants enrolled in the trial reside. Any probable case will be reported to the designated SMO for it investigation as per the Field Guide -Measles Surveillance & Outbreak Investigation Guidelines.

Every confirmed case of measles (i.e. laboratory or epidemiological confirmation) and any confirmed outbreak in the study area or surrounding areas will be reported within 24 hours to the Principal Investigator by the State of Maharashtra EPI Manager and by the designated SMO. All confirmed measles cases will be managed according to the National guidelines including the administration of Vitamin A.

The designated SMO at each district will collect the surveillance forms (VPD - H002) from all the reporting units, collate them in designated format (VPD - D001 form) and compile the district report. The District Immunization Officer (DIO) will send this routinely every week to the State EPI officer, RC or State SMO by Tuesday of each week. The VPD-D002 form should be used to track completeness and timeliness of reporting from the reporting units. This information should be sent on a quarterly basis by the DIO to the State Programme Officer.

Moreover, the State of Maharashtra will share with the PI investigator monthly updates on the performance of the surveillance systems using the indicators defined in the Field Guide - Measles Surveillance & Outbreak Investigation Guidelines.

The DIO/SMO will analyse the available district/block data on a weekly basis, using and reconciling case information from different surveillance systems to identify outbreaks of measles. As per national guidelines, the presence of measles outbreak should be verified if five or more than five clinically diagnosed cases of measles are identified in a block in a week, or five or more than five confirmed cases of measles occur in an area bordering several blocks in a week, or one or more than one death due to measles occurs in a block in a week.

If possible outbreaks are identified, measles outbreak investigations should be initiated as described in Chapter 5, Measles Outbreak Investigation of the Field Guide -Measles Surveillance & Outbreak Investigation Guidelines. In brief, the DIO and the SMO will oversee that the following actions will take place:

- Identifying the measles outbreaks that need to be investigated and assigning an outbreak number
- Mobilization of Epidemic Response Team (ERT)
- Orientation & planning meeting at the local level
- Conducting measles case search
- Collection and shipment of specimens to the laboratory
- Laboratory confirmation of the outbreak
- Data analysis
- Conversion of data to information for action
- Report writing
- Giving feedback
- Initiating actions including vaccination activities.

8.9 Procedure for monitoring subject compliance

Each subject will be given an appointment card for the next schedule visit to the clinic. If any subjects do not turn up on the scheduled date, the investigator will send the designated field worker to their houses and request them to come to the clinic.

9 Assessment of Immunogenicity

9.1 Primary outcome

Measles seropositivity at day 91 post-vaccination.

Measles seropositive specimens are:

- specimens tested by Enzygnost Anti-measles-virus/IgG (Dade-Behring, Marburg, Germany) enzyme-linked immunosorbent assay with optical density signal **≥ 0.1, or**
- specimens tested by Enzygnost with optical density signal **< 0.1**, that are subsequently tested by plaque reduction neutralization test with a result of ≥ **120 mIU**/ml measles antibody titre.

For further details, please see Sample processing (HPA 1/1, WHO 2/1, WHO 3/1, WHO 4/1), Measurement of outcomes (SOP HPA 5/1, HPA 6/1, HPA 7/1)

9.2 Secondary outcomes

For all infants:

- Geometric mean titres, pre-vaccination and at day 91;
- Seroconversion (defined as a change from Enzygost OD <0.1 to OD ≥0.1 or PRNT from <120 to ≥120 mIU/mL) measured in paired samples from day 0 and day 91;
- As covariates for inclusion in multivariable analyses: age; sex; day 0 antibody titre; study site; crying when vaccine given (aerosol arm only); presence of respiratory tract symptoms at time of vaccination (aerosol arm only). In addition, random subsets of 80 infants each with OD 0.1-0.2 and OD>0.2 at day 91 will be assessed for PRNT antibody concentrations to determine the relationship between PRNT and Enzygost optical densities/concentrations and validate the use of the cut-off of 0.1 for PRNT testing.

For infants in the day 28 blood sample subset or the day 364 blood sample subset:

- Geometric mean titres to describe evolution of antibody response at days 0, 28, 91, 364;
- Seropositivity at 364 days in infants with antibody titre ≥ 120 mIU/ml at day 91.

10 Assessment of Safety

10.1 Primary outcome

Adverse events up to and including day 91 post-vaccination.

10.2 Secondary outcomes

- Frequencies of individual adverse events

For further details of assessment and management of adverse events, please see Section 8.7 and SOPs WHO 1/1, WHO2/1, WHO 3/1.

11 Statistics

11.1 Description of statistical methods

A detailed analysis plan will be finalised prior to locking of the database and commencement of statistical analysis.

11.1.1 Analysis sets

- Primary analysis: per protocol (infants receiving the allocated vaccine and available for follow up at 91 days post-vaccination). For non-inferiority hypothesis, this is the conservative analysis;
- Secondary analysis: intent-to-treat analysis (all infants randomized, analysed in the group to which they were allocated, irrespective of intervention received) of primary and secondary outcomes.

11.1.2 Statistical methods, immunogenicity

- Primary analysis: calculate two-sided 95%CI for the difference in proportion seropositive at day 91.

- Secondary analyses: include calculation of 95% confidence intervals for seropositivity, seroconversion and geometric means as well as multivariable logistic and normal errors regression adjusting for covariates.

11.1.3 Statistical methods, safety

- Primary analysis: proportion (with 95% CI) with any adverse events in aerosol group and in subcutaneous group;
- Secondary analysis: proportions (with 95% CI) of individual adverse events.

11.2 Sample size

11.2.1 Immunogenicity

Total to be enrolled, 2000. Number available for primary analysis (after loss to follow up), 1600. These numbers are based on the following assumptions:

- Significance 2.5%, one-sided for non-inferiority, 5% two-sided for superiority;
- Non-inferiority margin 5%;
- Power for non-inferiority 90% if true seroconversion is 90% in both arms;
- 20% loss to follow up by day 91.
- Calculate two-sided 95%CI for difference in proportion seropositive at day 91. If lower limit of difference greater than -5% then conclude non-inferiority. If lower limit of difference greater than 0% then conclude superiority.

With 100 children sampled at random from those being followed up, based on a standard deviation of post vaccination titres of 0.43, differences of <1.5 fold in geometric mean titres between aerosol and subcutaneous groups between days 28 and 364 would be able to be detected at a 5% level with 80% power. Smaller differences between days 28 and 91, and 91 and 364 would also be detectable.

11.2.2 Safety

To estimate proportions with adverse events in aerosol and subcutaneous groups, the sample sizes above give acceptable precision.

With 1000 infants in each group the precision of estimation of adverse events is:

Observed proportion	95% confidence intervals		
	N=100	N=800	N=1000
0%	0.00 – 3.6%	-	0.00 – 0.37%
1%	0.02 – 5.44%	0.43 – 1.96%	0.48 – 1.83%
2%	0.24 – 7.04%	1.15 – 3.23%	1.26 – 3.07%
5%	1.64 – 11.28%	3.60 – 6.75%	3.73 – 6.54%

If no reactions are observed, the upper 95% confidence interval with a sample size of 1000 is 0.37%, or 1 in 270.

With 100 children sampled at random for follow up to 364 days, the precision for estimating the prevalence of rare events is low. If zero events are observed by day 364 the upper 95%CI will be 3.6% (1 in 28).

11.3 Criteria for the termination of the trial

The trial will be discontinued if a single SAE attributable to the aerosol vaccine is reported. The stopping rules have been determined by the DSMB following explicit written criteria (Appendix 2: Terms of Reference of the Independent Data Safety and Monitoring board).

11.4

11.5 Procedure for accounting for missing, unused and spurious data

11.5.1 Data handling and record keeping

See SOP VEL 2/1 for data management.

11.5.2 Data verification

See SOP VEL 2/1

Source documents including medical records and original laboratory results will be kept in a separate file at the investigator's office. Electronic CRFs will be downloaded with daily frequency at Vadu Rural Hospital dedicated computer and checked for completeness and accuracy. They will be sent to Vellore weekly. The SOP VEL 1/1 describes in detail the process for data collection including the use of audit trails to ensure data security. Printed copies of electronic CRFs will be kept in a room with limited access

11.5.3 Data entry

See SOP VEL 2/1

11.5.4 Data review

See SOP VEL 2/1

11.5.5 Data quality control

See SOP VEL 2/1

Besides range, consistency and missing value checks incorporated in the Study Builder software, further checks will be done using pre-tested syntax in SAS statistical software. If inconsistencies and out of range values are found, the Data Management Centre will produce a Query and Correction sheet for a subject with a query to the corresponding site PI. The PI or designee has to recheck the value and fill the correct value in the sheet with an authorized signature and send back to the Data Management Centre. Based on the filled Query and Correction sheet received from the Site, the data manager will update the Database.

11.5.6 Data sharing and analysis

Data will only be accessed by the sub-investigator in charge of data management and statistical analysis. The DSMB will receive quarterly unblinded reports. See SOP VEL 1/1

Outliers, missing values, and inconsistencies will be identified based on the Validation Check Specification document (in SOP VEL 2/1). If inconsistencies arise then the query will be raised at this level. Based on the query responses, corrections will be made in the original ACCESS database. Having completed all the corrections, the data will be exported to SAS software format. Data analysis will be carried out after all enquiries have been done and the database has been locked. Any data which is still missing after enquiries have been made will be dealt with as described below:

- Missing values
  For the per-protocol (PP) dataset, missing values will be treated as missing at random. For the intention-to-treat (ITT) dataset, the analysis will also be run with imputed values for the missing values, for the best and worst case scenarios. In the best case scenario, we will assume no adverse events occurred and the individuals are seropositive. In the worst case scenario, we will assume an adverse event occurred and the individuals are seronegative.

- Outliers
  For log 10 PRNT values [LPRNT], checks for outliers will be performed prior to the statistical analysis, as part of the data checking. The outliers will also be examined using standardized residuals >3 when looking at log 10 PRNT values [LPRNT].

12 Procedures for reporting any deviations from the original analysis plan

Any change in the planned analysis will be described and documented in the study report including the time (specifically whether prior to or after unblinding occurs) and reason for the change, the procedure used to decide on the change and the nature and content of the data available when the change was made.

12.1 Selection of subjects to be included in the analysis

12.1.1 Analysis sets

Primary analysis: per protocol (infants receiving the allocated vaccine and available for follow up at 91 days post-vaccination). For non-inferiority hypothesis, this is the conservative analysis;

Secondary analysis: intent-to-treat analysis (all infants randomised, analysed in the group to which they were allocated, irrespective of intervention received) of primary and secondary outcomes.

There will be one interim analysis for AE only (see section 12.1.3 below).

12.1.2 Immunogenicity

Primary analysis is to calculate the 2-sided 95%CI for the difference in proportion seropositive at day 91. If lower limit of difference greater than -5% then conclude non-inferiority. If lower limit of difference greater than 0% then conclude superiority.

Secondary analyses will include calculation of 95% confidence intervals for seropositivity, seroconversion and geometric means as well as multivariable logistic and normal errors regression adjusting for covariates.

Secondary analysis of factors associated with seroconversion/response, including:

- pre-vaccination titre
- Age (9m,10m,11m) (also look at interaction with intervention)
- Sex (also look at interaction with intervention)
- PHC
- Crying when vaccine given? (also look at interaction with intervention).
- (Another factor we considered before was acute respiratory infection)
- Explanatory variables for stratification of safety profile

12.1.3 Safety

Primary analysis is proportion (95% CI) with any adverse events in aerosol group with subcutaneous group;

Secondary analysis will describe frequencies of individual adverse events (proportions with 95% CI).

An interim analysis of safety data will be completed. For this analysis, the DSMB will use data from the first 100 children in each arm to complete 91 days of follow up. This will be done as soon as possible after these data are available.

12.2 Contingency plan in the event of confirmed measles cases and outbreaks

Measles cases occurring in trial participants would permit to examine directly the relative vaccine efficacy of aerosol and subcutaneous measles vaccination. Natural boosting of vaccinated individuals may affect the primary outcome of seropositivity at day 91.

If measles cases or outbreaks are detected by the surveillance system we will take the following special steps.

1. Support the implementation of routine Government of India activities for the investigation of cases and control of outbreaks, as appropriate Field Guide -Measles Surveillance & Outbreak Investigation Guidelines (2005) Government of India, Department of Family Welfare New Delhi).

2. **Document all the confirmed measles cases among the children enrolled in the trial or in their households.**

3. **Set up an alternative statistical analysis plan addressing the following elements:**

- Consider those infants confirmed as measles cases (laboratory confirmation) who had day 91 samples taken prior to their date of onset of measles to have unaffected day 91 results.
- Assume that all confirmed measles cases (laboratory confirmation) occurred among sero-negative infants and class them as such in the analysis (and class non-cases according to their ELISA/PRNT sero-status).

4. **If an outbreak is confirmed prior to completion of the recruitment period or the last day 91 follow up, then the recruitment period will be extended after the outbreak** to reach the estimated sample size of 2000 eligible children not confirmed as measles cases.

5. **If an outbreak is confirmed in Vadu DSS after 91 day follow up is completed but prior to the completion of the long term (day 364) follow up, there will be no attempts to recruit additional children** and they will be analysed as described in point 3 above

13 Direct access to Source Data/Documents documents

The investigator will provide written agreement that the investigator /institution(s) will certify that the monitors, the auditors, the Ethics Review Committee members DSMB members and, the regulatory authority representatives (DCGI) will be granted direct access to his original direct access to source data/documents and medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations in India.

14 Quality control and quality assurance procedures

14.1 Protocol changes and protocol deviations

Once the trial has started, the investigator must adhere to the protocol and will ensure that it is strictly followed. The investigator, or person designated by the investigator, will document and explain any deviation from the approved protocol. The investigator will promptly report all important amendments and deviations related to non-compliance with the following protocol provisions: inclusion and exclusion criteria, randomization procedures, blinding procedures, informed consent procedure, assignment of subject identification numbers, dosing and assessment schedules, reporting and procedures for adverse events and, any other protocol deviations. The report will also include description of actions taken to prevent recurrence of the detected deviations.

The investigator will be responsible for reporting any protocol deviations or protocol amendments to the Local Ethics Review Committee. The Sponsor will be responsible for reporting to the National Regulatory Authority and the WHO Ethics Review Committee.

No deviations from, or changes to, the protocol will be initiated without prior written Ethics Review Committee approval/favourable opinion of an appropriate amendment.

An exception to this will be in situations when it is necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial. Under these exceptional circumstances, the investigator should report as soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted to the Ethics Review Committee for review and approval/favourable opinion, and, to the sponsor for agreement.

In the report of the study, protocol deviations will be summarized and grouped into different categories, including:
– those who entered the study even though they did not satisfy the entry criteria.
– those who developed withdrawal criteria during the study but were not withdrawn.
– those who received the wrong vaccine or incorrect dose.
– those who received an excluded concomitant treatment.
- other deviations

Any deviation(s) from the original statistical plan will be described and justified in protocol amendments and/or in the final report, as appropriate.

Using Appendix 4 (Form 1 and Form 2) individual subjects with protocol deviations will be reported and documented.

The sponsor will implement and maintain quality assurance and quality control systems with written SOPs to assure that the trial is conducted and data are generated, recorded, and reported in compliance with the protocol, GCP and the Indian national regulatory requirement(s) requirement for investigational product. This includes the use of independent trial monitors and independent external audits.

14.2 Trial monitoring

Independent clinical trial monitors will be appointed. The monitors will be under the supervision of an experienced Contract Research Organization that will be in charge of ensuring compliance with Good Clinical Practice and Good Laboratory Practice, A trial initiation monitoring visit will be done prior to enrolment of any volunteer at a site and the clinical monitor and investigator will review the protocol, logistics and all trial related procedures. This includes information on the vaccine and aerosol delivery device, procedures for obtaining informed consent, procedures for reporting SAE and procedures for completing the CRFs.

Site monitoring visits will be scheduled by the clinical monitor on a regular basis. During these visits, information recorded in the CRFs will be verified against source documents for accuracy and completion. The clinical monitor will review the informed consent procedures, product accountability and storage, trial documents and trial progress. The clinical monitor will verify that the investigator follows the approved protocol or amendments (if any). S/he will observe trial procedures and will discuss any problems with the investigator.

Monitoring visits will be recorded in the Monitoring Log at the investigator's site, and at the end of the trial a copy of the completed log will be returned to the sponsor.

14.2.1 SAFETY MONITORING

A Data Safety and Monitoring Board (DSMB) will review safety data collected through day 364. See DSMB Terms of Reference

14.3 Consent forms

To allow compliance with GCP principles, the parent/guardian of each infant will be asked for consent regarding direct access to the source documents for monitoring, audit, and inspections. The agreement covering the use of the data or analysis has to be documented in writing, together with the written informed consent for trial participation.

15 Ethical considerations in study design

15.1 Informed consent

See SOP VAD 4/1 and section 8.5, visit 1 and 2 for details
The parents/guardians of potential participants will have the study explained in depth to them and, in addition, will receive a patient information sheet (Study Document 4) and have the opportunity to ask questions or trial staff prior to giving consent. Informed, witnessed, written consent will be recorded on behalf of each participant (by parent/guardian) for trial participation, and other specific aspects of the trial e.g. for day 364 follow up, photography of subjects (Study Document 5).

15.2 Ethical and regulatory approvals

The protocol will be submitted for approval to the Institutional Ethical Committee of KEM Hospital, Pune. In addition, the WHO Ethics Review Committee will review and approve the study.

In addition the protocol will be submitted to the Scientific Advisory Committee of the MOHFW, India. Moreover, the protocol and supporting documentation will be submitted to the Drugs Comptroller General, India in accordance with the Drugs and Cosmetics Act, Schedule Y requirements.

Community leaders will be informed about the social benefits of the study that the data collected in this study will be valuable to the development of a simpler and safer method for measles vaccination and may result in greater numbers of children being protected against measles infection.

15.3 Study data confidentiality

On initiation of the study, the investigator will prepare a file containing documents related to the trial. During the study, the investigator will be responsible for updating the file and

regularly adding trial related documents. The investigator will keep the file in a locked cabinet, in a secure area accessible only to the investigator and authorized study staff.

The investigator file and associated source documents will be retained for at least five years after the licensure of the measles aerosol vaccine. Patient identification codes should be kept for at least 15 years after completion of the trial. **Written approval from all sponsors must be obtained prior to destroying records.**

All study related documents will be kept in locked cabinets at the study site. Filing cabinets with the trial data and the participant information will be locked and accessed only by authorized persons from the sponsor and Regulatory Authorities.

Personal volunteer data will be kept confidential and the privacy of all subjects will be protected in so far as permitted by law. Subject names will appear on the initial documents (CRF) and once enrolled study documents will refer to the subject code (second initial of each name) and assigned study code number Only personnel involved with the study conduct and local and international regulatory agencies may review these records. The investigator will keep in the investigator's files a Subject Identification List and Screening/Enrolment Log (including complete name, age and address).

All proprietary or confidential information communicated to the investigator by or for WHO or communicated to the investigator during the course of and/or as a results of the clinical study is the exclusive property of WHO and/or SIIL, and the investigator will ensure the same shall be kept strictly confidential by him/her or any other person connected with the clinical study and shall not be disclosed, either orally, or in written form, by him/her or such person to any third party without prior written consent of WHO. The investigator shall communicate the results of the clinical study promptly to WHO or its designee.

All rights and interests worldwide in any inventions, know-how, or other intellectual or industrial property rights which arise during the course of and/or as a result of the clinical study which is the subject of the Protocol or which otherwise arise from the information or materials supplied under this Protocol, shall be assigned to, vest in and remain the property of WHO.

15.4 Revaccination of subjects with inadequate immune response

It is ethically responsible to ensure that all children who are shown to have lower than protective antibody titres 91 days post vaccination are revaccinated using standard measles vaccination methods (i.e. subcutaneous measles vaccine as used in this study). To this end, all children found to have PRN titres <120mIU/ml at 91days post vaccination, regardless of vaccination route, will be revaccinated by the subcutaneous route by a team independent to the trial follow up teams. Serological testing will be conducted as soon as is feasible after samples are drawn. Revaccination will occur as soon as possible and a maximum of 6 weeks after the results for each individual is known. Unblinding will only occur to the level of child identification. Study allocation will not be unblinded. Subsequent serological results from children who are revaccinated will not be included in analyses, but they will continue to be monitored for adverse events.

16 Data handling and record keeping

16.1.1 Data archive

Detailed procedures for data archiving at Vadu Rural Hospital and CMC Vellore are described in SOP VEL 2/1

After completion of the study, the Data Management Centre will keep copies of Databases, all CRFs and study related documents in a secure place for at least 5 years in compliance with GCP and regulatory requirement for investigational products.

16.1.2 Record keeping

See SOP VEL 2/1

After completion of the study, the investigators will keep his copies of all CRFs and source documents in a secure place for at least 5 years in compliance with GCP and regulatory requirements for investigational products.

17 Financing and insurance

This study will be supported by World health Organization - Initiative for Vaccine Research. In accordance with GCP guidelines, the Sponsor will provide an insurance policy for the conduct of the trial.

18 Publication policy

This study will be performed within the framework of a larger programme of research, consisting of several individual projects that relate to the development of safe and effective products against measles. In this connection, it has been deemed necessary for all rights to the results of the work to be performed under this project to vest exclusively in WHO. Consequently, General Conditions for publication will be as follows:

1. The Institution shall deliver to WHO at the end of the clinical trial the results of the work performed under this Agreement, unless otherwise agreed.

2. The Institution shall maintain in confidence all results of the work performed under this clinical trial, including information and tangible products of any kind whatsoever, unless authorized by WHO to disclose or publish such results. Notwithstanding the foregoing, the parties hereto recognize the public health benefits that may be achieved through the publication of the results of the project in accordance with normal academic practice.

Therefore, the parties agree that subject to the terms of this general condition 2, the Institution and the Principal Investigator may publish or present the results of this project. In the event of any intended publication or presentation as aforesaid, the Institution and Principal Investigator shall transmit to WHO for its review any material intended to be published or otherwise publicly disclosed, sixty (60) days before it is transmitted to any publisher, editor, referee or

meeting organizer. Within this sixty day review period, WHO may make a written request to the Institution and the Principal Investigator to remove from such material:

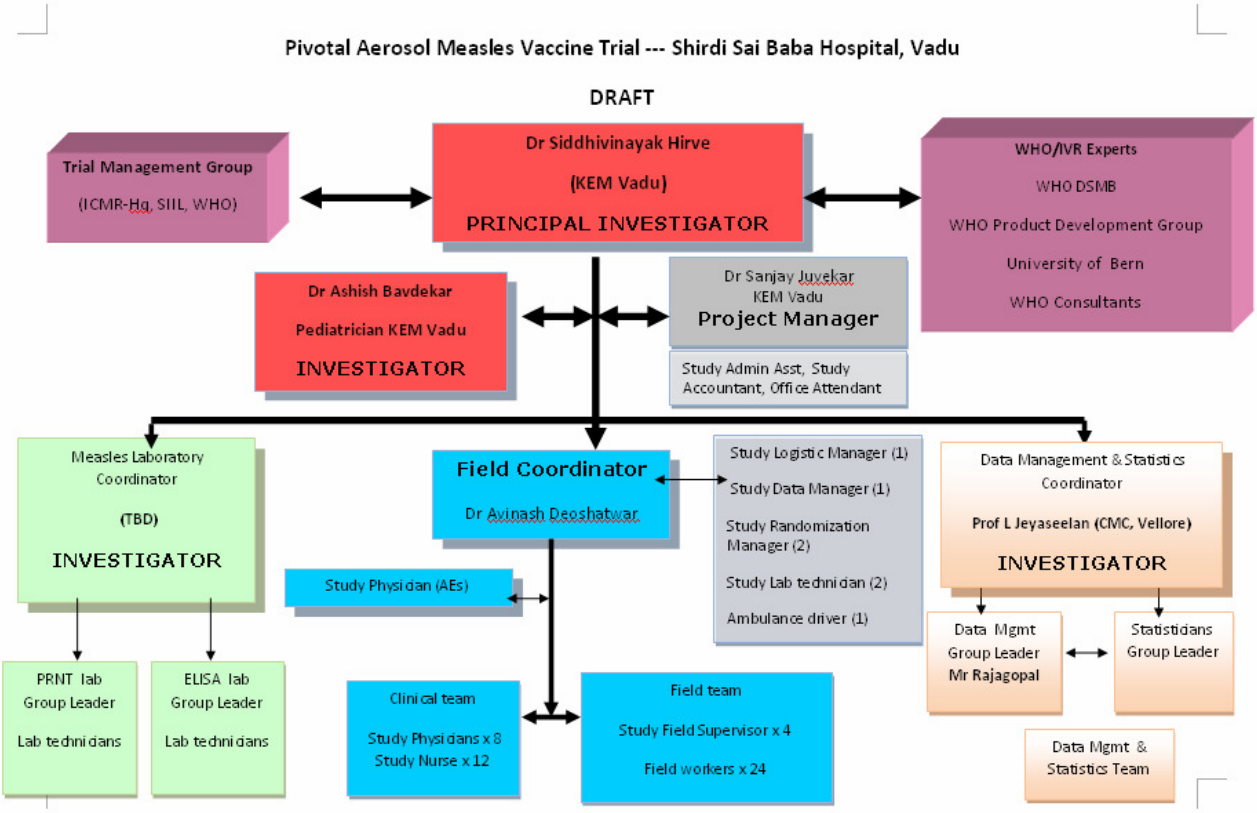
- any proprietary and confidential information disclosed by or on behalf of WHO to the Institution and the Principal Investigator hereunder, as well as
- any results generated hereunder, but only to the extent in order to achieve WHO public sector objectives- WHO reasonably deems it necessary to maintain such results in confidence in order to promote the development of such results into a useful health related product in collaboration with a commercial enterprise.

The Institution and the Principal Investigator agree to comply with any such request. Except to the extent WHO has made a request as aforesaid within the sixty day review period, the Institution and the Principal Investigator shall have the right to proceed with the publication or other public disclosure without further notice to WHO.

3. All rights to the results of the work to be performed under this project, including but not limited to copyright and the right to apply for, hold and exercise patent rights in respect of any invention resulting from the work, are vested exclusively in WHO. The Institution and Principal Investigator shall provide WHO with their full cooperation to permit the effective exercise of the above rights."

19 Organisational structure

Numbers of staff required (study field workers, nurses, doctors)



19.1.1 List of references to literature and data that are relevant to the trial, and that provide background for the trial

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20 Appendices

Appendix 1: Diagram of Vaccine and Nebulizer Components

Appendix 2: Terms of Reference of the Independent Data Safety and Monitoring Board (DSMB).

Appendix 3: Adverse Events, Descriptions and Definitions

Appendix 4: Forms for Protocol Changes

Appendix 5: Study Documents:

SD-1 Investigator's Brochure
SD-2 Study Synopsis
SD-3 Study Timetable
SD-4 Patient/Parent/Guardian Information Sheet
SD-5 Informed Consent Forms for Parent/Legal Guardian
SD-6 Sample CRFs

Appendix 6: Standard Operating Procedures (SOP) for the Study

Note: All SOPs are in draft format and will be finalized during training sessions before the start of the trial.

SOP No.	Title
Vadu Site	
VAD 1/1	Communication with Ethics Committee
VAD 2/1	Training Policy for Study Staff
VAD 3/1	Subject Identification and Recruitment Procedures
VAD 4/1	Informed Consent, Eligibility, Randomization and Vaccination (procedures on day of vaccination)
VAD 5/1	Procedures for Follow-up for Measles Aerosol Clinical Study (day 3 to 365 Post Vaccination)
WHO	
WHO 1/1	Adverse Event Reporting and Management
WHO 2/1	Immediate Adverse Event Reporting and Management
WHO 3/1	Serious Adverse Event Reporting and Management
WHO 4/1	How to Transport, Store and Handle Measles Vaccine, Diluent, Syringes and Safety Boxes
WHO 5/1	Translation back/translation
WHO 6/1	Administration of the Measles Vaccine by the Subcutaneous Route
WHO 7/1	Procedures for Entering Data into CRFs on PDA
Vellore	
VEL 1/1	Statistical Analysis Plan (randomization and allocation concealment)
VEL 2/1	Data management plan (quality assurance and correct allocations administered9
VEL 3/1	Blinding laboratory to allocation
Health Protection Agency	
HPA 1/1	Collection of Blood Specimen (Venous Blood) and Shipping to Vadu Shridi Saibaba Hospital (SSH)
HPA 2/1	Separation of Serum from Blood Samples at Vadu Laboratory and Transport to Testing Laboratory
HPA 3/1	Safe Disposal of Blood Sample Residues and Contaminated Waste
HPA 4/1	Aliquoting of Serum Samples and Storage in Freezers at Testing Laboratory
HPA 5/1	Detection of Anti-Measles Virus IgG by Enzyme Immuno Assay (ELISA) Using Enzygnost Dade Behring Kit and the BEP III ELISA Processor
HPA 6/1	Detection of Anti-Measles Virus IgG by Enzyme Immuno Assay (ELISA) Using Enzygnost Dade Behring: Manual Performance
HPA 7/1	Plaque Reduction Neutralization Test (PRNT) for Measles Antibodies
HPA 8/1	Checking the Calibration of Variable Volume Pipettes
Nektar/Aerogen	
NEK 2/1	Preparation of Vaccine and Nebulizer and Administration by Aerosol Using Aerogen's Clinical Nebulizer to Infants from 9 – 12 Months.

WHO MEASLES AEROSOL PROJECT
Pivotal Trial Clinical Trial Protocol

1 Protocol cover sheet

Study Protocol No.	WHO/MAP/IN/002 Pivotal study to evaluate the immunogenicity and safety of a measles vaccine given by aerosolized inhalation: randomized controlled trial
Version	Version 1: April 2008
Study site	Pune District, Maharashtra, India.
Study Initiation date	
Collaborating institutions	Shirdi Saibaba Hospital (Vadu Hospital), Vadu, Pune, India. King Edward Memorial Hospital and Research Center, Pune, India. Christian Medical College, Vellore, India. National Institute of Virology, Pune, India. Health Protection Agency, London, UK. University of Bern, Bern, Switzerland.
Sponsor	Serum Institute of India Ltd., 212/2 Hadapsar, Pune, 411028, India.
Co-Sponsor	World Health Organization (WHO), Department of Vaccines and Biologicals (IVB), Initiative for Vaccine Research (IVR), 20 Avenue Appia, CH-1211, Geneva 27, Switzerland.
Clinical co-ordinator, WHO Switzerland	Dr Ana Maria Henao Restrepo, Scientist, WHO, Initiative for Vaccine Research, 20 Avenue Appia, CH-1211, Geneva 27, Switzerland. Phone: 00 41 22 791 3402, Fax: 00 41 22 791 4860 E-mail: henaorestrepoa@who.int
Principal investigator (PI):	Dr. Siddhivinayak Hirve, Director, Shirdi Saibaba Hospital, Vadu, Pune, India. to July 2010 Dr. Ashish Bavdekar, Consultant, King Edward Memorial Hospital and Research Centre, Pune, from July 2010

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3 List of abbreviations

ACH	Achieved Level
AE	Adverse Event
ANM	Auxiliary Nurse Midwife
ARC	American Red Cross
BCG	Bacillus Calmette-Guérin vaccine
CDC	Centers for Disease Control and Prevention, USA
CMC	Christian Medical College and Hospital
CRF	Case Report Form
CSD	Common Study Document
DCGI	Drugs Comptroller General, India
DIO	District Immunization Officer
DPT	Diphtheria-Pertussis-Tetanus vaccine
DSMB	Data and Safety Monitoring Board
DSS	Demographic Surveillance System
DT	Diphtheria and Tetanus vaccine
ELA	Expected Level of Achievements
ELISA	Enzyme Linked Immuno-Sorbent Assay
EPI	Extended Programme of Immunization
EPRT	Epidemic Response Team
E-Z	Edmonston-Zagreb
GCP	Good Clinical Practice
GIS	Geographic Information System
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HDSS	Health and Demographic Surveillance System
IB	Investigator's brochure
ICF	Informed Consent Form
IEC	Institutional Ethical Committee
IgG	Immunoglobulin G
IRB	Institutional Review Board
ITT	Intention-to-treat analysis
ICH	Institute of Child Health
INDEPTH	The International Network for Demographic surveillance of Populations and Their Health in developing countries
IVR	Initiative for Vaccine Research, WHO
KEM	King Edward Memorial Hospital and Research Center, Pune, India
MAP	Measles Aerosol Project
MAV	Measles Aerosol Vaccine
MCH	Maternal and Child Health
MEM	Minimal Essential Medium

MO	Medical Officer
MOHFW	Ministry of Health and Family Welfare
MV	Measles Virus
PDG	Project Development Group
PE	Physical Examination
pfu	Plaque forming units
PHC	Primary Health Center
PI	Principal Investigator
PP	Per-protocol analysis
PRNT	Plaque reduction neutralization test
RC	Regional Coordinator
SAE	Serious Adverse Event
SIIL	Serum Institute of India Ltd
SMO	Surveillance Medical Officer
SSH	Shirdi Saibaba Hospital
SSPE	Subacute Sclerosing Panencephalitis
TT	Tetanus Toxoid
UIP	Universal Immunization Program
WFI	Water for Injection
WHO	World Health Organization

4 Background information

4.1 Public health importance of measles

Measles is one of the most common causes of death in children under five-years old globally. In 2000, an estimated 777,000 measles deaths occurred, of which 452,000 were in the African Region of the World Health Organization (WHO). In 2001, WHO and the United Nations Children's Fund published a 5-year strategic plan to reduce measles mortality by half by 2005.[1] In 2003, the World Health Assembly endorsed a resolution urging member countries to reduce the number of deaths attributed to measles by 50% compared with 1999 estimates by the end of 2005.This target was met. Overall, global measles mortality in 2005 was estimated to be 345 000 deaths (uncertainty bounds 247 000 and 458 000 deaths), a 60% decrease from 1999. WHO estimates that in 1999, 58% of all deaths from measles were estimated to occur in the African region and 27% in Southeast Asia. However, by 2005, 50% of all deaths from measles occurred in Southeast Asia and only 37% in Africa.[2] The revised global goal, as stated in the Global Immunization Vision and Strategy 2006–2015 of the World Health Organization and United Nations Children's Fund, is to reduce measles deaths compared to the estimated number in 2000 by 90% by 2010.[3] Important challenges still exist for the achievement of the 2010 goal for reduction of measles mortality. Activities need to be fully implemented in large countries which still have a high measles burden such as India, Pakistan, and Indonesia. Moreover, to achieve this goal, continued progress needs to be made in delivering measles vaccines to the world's children.

4.2 Name and description of the investigational product

4.2.1 Vaccine

The vaccine to be studied in the proposed Pivotal trial in India is the currently licensed measles live, attenuated virus, Edmonston Zagreb strain, manufactured by the Serum Institute of India, Ltd (SIIL) and approved for subcutaneous injection in India and many other countries. It meets the requirements of WHO when tested by the methods outlined in WHO TRS 840 (1994).[4] It is pre-qualified by WHO for sale to UN agencies.

The vaccine is prepared in human diploid cells (MRC5), suspended in minimal essential medium (MEM) and stabilised. The measles vaccine is in the class of prophylactic immunizing agents. The Edmonston Zagreb strain has been shown in Mexican studies to be immunogenic and safe, and capable of maintaining vaccine potency during the nebulisation process.[5]

Clinical trials of a measles vaccine manufactured by a company with experience and expertise in international vaccine regulations, and experience of the procedures for global immunization programmes would be expected to result in an efficient regulatory pathway towards licensure in India and pre-qualification by WHO.

4.2.2 Aerosol device

Nebulisers are classified as medical devices and several are licensed/registered/approved in their country of manufacture. They are currently in use in several countries for the administration by inhalation of therapeutic pharmaceuticals for respiratory diseases.

One nebuliser manufactured in compliance with GMP (the Aerogen Nektar nebulizer) has been selected for use in this trial. This administration device/system will be tested in the study location using the SIIL vaccine.

The Aerogen Nektar nebulizer is a wholly autonomous, battery or mains powered micro-pump nebulizer. The electronic micropump is a technology which produces liquid aerosol in a manner unlike any other technology currently available. As small as 15mm in diameter and wafer-thin, the micro-pump is comprised of a unique dome-shaped aperture plate containing over 1,000 precision-formed tapered holes, surrounded by a vibrational element. When energy is applied, the aperture plate vibrates over 100,000 times per second. This rapid vibration causes each aperture to act as a micropump, drawing liquid through the holes to form consistently sized droplets. The result is a low-velocity aerosol optimized for maximum deep lung deposition.[6]

Performance characteristics for the selected device have been generated for delivery of the measles vaccine by aerosol. This device has been chosen for this proposed study because it has the performance characteristics and vaccine potency retention qualities comparable to the classic Mexican device used in previous trials of several measles vaccines which demonstrated both safety and immunogenicity of measles vaccines by the aerosol route. It was also used during the Phase 1 trial among measles immune healthy subjects 1-35 years old in Pune India, with good safety profile and good immunogenicity results.

4.3 WHO Measles Aerosol Project

The Measles Aerosol Project aims to develop and license at least one method for respiratory delivery of currently licensed measles vaccines by 2009. The assumptions for the project are that the aerosol vaccination devices will use current vaccines and that vaccination will be targeted at children of 12 -59 months for routine vaccination and at individuals of 9 months to 18 years for measles mass campaigns. It is anticipated that clinical testing can be completed by 2009.

Because previous studies of measles aerosol vaccine (MAV) were not carried out with licensure as an aim, WHO is supporting GLP and GCP compliant non-clinical and clinical studies with the aim of achieving licensure. In summary, during the first three years of the project the regulatory pathway has been defined, safety concerns enumerated, preclinical studies have been completed and, devices for clinical trials selected. A pivotal trial is planned in India for the second half of 2008. Licensure of a measles aerosol vaccine will be a critical step towards making reality the promise of aerosol delivery of other vaccines.

WHO/IVR coordinates the Measles Aerosol Project (MAP).The Product Development Group for the Measles Aerosol Project (PDG) is an expert clinical and scientific advisory body to WHO/IVR established to provide independent advice to the IVR regarding the development plan of the measles aerosol vaccine. The Centers for Disease Control and Prevention (CDC), USA and the American Red Cross (ARC) are partners to the MAP. The MAP has received financial support from the Bill and Melinda Gates Foundation.

4.3.1 Summary of findings from non-clinical and clinical studies that are potentially relevant to this trial

4.3.1.1 Non-clinical studies

A standardised laboratory protocol for measles PRNT has been established and validated for use in clinical trials of aerosolized measles vaccines. [7]

A study of measles vaccine degradation by nebulizer devices has been completed. [8] This study assessed vaccine potency retention performance of the three nebulizers used in the Phase 1 trial. The average vaccine potency retained by the Aerogen Nektar nebulizer using the conservative correction for counting bias was 94.7% (95% CI, 85.8-103.7).

GLP animal safety and immunogenicity studies among cynomolgus monkeys (Macaca fascicularis) with the live-attenuated Edmonston-Zagreb measles virus (MV) vaccine have been conducted. Immunogenicity and protective efficacy of aerosol vaccination using devices similar to those previously used in humans were comparable to those in animals vaccinated by injection. No evidence of a safety hazard associated with the route of vaccination was detected.[9]

Toxicity studies have evaluated the safety of pulmonary delivery of Edmonston Zagreb measles vaccine in cynomolgus monkey, a non-human primate which is susceptible to infection with measles.[10] There was no mortality during the study. No effects were seen on body weight evolution, food consumption, ophthalmology, rectal temperature or clinical pathology. Water consumption was increased in measles vaccine treated animals, but urinary volume was not affected. Immunogenicity and viremia following treatment were characterised. A full range of tissues was evaluated for histopathological changes and no treatment-related findings were observed. It was concluded that pulmonary administration of Edmonston Zagreb measles vaccine was well tolerated locally and did not result in any evidence of systemic toxicity.

4.3.1.2 Clinical studies

A systematic review was conducted to examine the immunogenicity and safety of aerosolized measles vaccine (Edmonston-Zagreb or Schwarz strains) 1 month or more after vaccination. In children below 10 months, the studies were heterogeneous. In four comparative studies, seroconversion rates were lower with aerosolized than with subcutaneous vaccine and in two of these the difference was unlikely to be due to chance. In children 10-36 months, the pooled seroconversion rate with aerosolized vaccine was 93.5% (89.4-97.7%) and 97.1% (92.4-100%) with subcutaneous vaccine (odds ratio 0.27, 0.04-1.62). In 5-15-year olds the studies were heterogeneous. In all comparative studies aerosolized vaccine was more immunogenic than subcutaneous. Reported side effects were mild. Aerosolized measles vaccine appears to be equally or more immunogenic than subcutaneous vaccine in children aged 10 months and older. Large randomized trials are needed to establish the efficacy and safety of aerosolized measles vaccine as primary and booster doses.[5]

In 2006, a Phase I trial was initiated in India to assess the safety of measles aerosol vaccine using three different aerosol delivery devices. Preliminary results show good safety and immunogenicity profiles.

Additional details on the preclinical and clinical studies are available in the Investigator's Brochure (IB).

4.3.2 Summary of known and potential risks and benefits to human subjects

The expected risks include those already known to be associated with parenteral measles vaccination. For instance, a mild measles-like illness may follow vaccination. The most common side effects include fever and rash; these occur in 5-15% and 5%, respectively, of persons vaccinated subcutaneously.[11-14] Cough and conjunctivitis may also occur. Thrombocytopenia (platelet count <50,000/mL) occurs in less than one in 30,000 vaccinees approximately 2 weeks post-vaccination and may be more common in persons with a history of immune thrombocytopenia purpura. Only rarely, has this complication been associated with bleeding.[15-19] There is also a risk of encephalitis/ encephalopathy in one of 87,000 to 2,000,000 doses. There has been some controversy regarding whether or not the relationship is causal or coincidental. Nonetheless, cases have clustered in the first 1-2 weeks post-vaccination and may occur up to a month later. In addition, some persons have experienced long-term neurological sequelae.[20-24] As this is a live-attenuated virus vaccine, persons with deficient cell-mediated immunity should not be vaccinated and women who are pregnant should not receive this vaccine secondary to the theoretical risk of fetal infection.

Over the last several years, there has been concern over the possible association between measles vaccination and autism and inflammatory bowel disease. However, carefully conducted studies have failed to confirm these findings and there is no evidence that measles vaccine is associated with or causes these diseases.[25-28]

Reactogenicity associated to the aerosol route has been studied using the Mexican traditional device through a controlled approach comparing frequency of adverse effects among children receiving aerosolized measles vaccines with children receiving subcutaneous vaccination. Most frequent symptoms have been fever, rhinitis, cough, exanthema, conjunctivitis, diarrhea and arthralgias. Among 6-year old children, administration of aerosolized measles vaccine has produced statistically significant lower reactogenicity than subcutaneous route. Among 12-month-old children, no serious temporally associated events were identified in vaccinated children. Only inflammation of the conjunctivae, (injection or reddening, with or without discharge, and/or teary eyes), was more common among the infants given aerosol (34/59, 57%) compared to subcutaneous vaccine (19/55, 35%) ($p = 0.01$). Conjunctival hyperemia ("red eyes" and/or "teary eyes") was reported on at least one day of days 1 to 14, with an incidence of 7% - 14% per day of follow-up among the children in the subcutaneous group, and 12% - 19% per day of follow-up in the aerosol group. None of the children required medical treatment. In summary, the aerosol route of measles vaccine administration has been well tolerated and similar to the subcutaneous route. Episodes of conjunctival hyperemia have been more frequent in the aerosol group in 12-month-old children.

In summary, despite extensive clinical experience with measles aerosol immunization in Mexico and studies in South Africa and Thailand, serious adverse events following

immunization have not been reported. However, some experts have noted the need for more extensive evaluation of the safety of this route of administration.

4.3.2.1 Potential Central Nervous System Effects

Rarely, natural measles infection may be associated with CNS disease, namely encephalitis and sub-acute sclerosing pan encephalitis (SSPE). In contrast, attenuated measles vaccine is not associated with SSPE; on the contrary, measles vaccine is protective against SSPE. There is concern as to whether attenuated vaccine might reach the brain of aerosol vaccinees by retrograde transport via the olfactory nerve fibers. There is also the theoretical risk of exposure of the nasal mucosa to replicating vaccine virus and the possibility of central nervous system side effects due to passage through the cribiform plate of the ethmoid bone. This is an unlikely probability given the extensive public health experience with its use in Mexico. As many as 4 million children have been vaccinated by this method and no serious adverse events, including encephalitis, encephalopathy and death, have been reported. Heretofore, there have not been reports of CNS problems in the large aerosol measles vaccine experience. In addition, during the animal studies described above, no abnormalities in gross pathology or histopathology related to measles infection were detected in any of the organ studied. Furthermore, none of the organ samples tested by a semi quantitative RT-PCR showed measles virus genome including samples of the cribiform plate and an additional sample of the nervus olfactorious with a small piece of the olfactory bulb.

Therefore, there is no available evidence to indicate that the frequency of central nervous system side effects will be greater than that seen among measles non-immune persons receiving measles vaccine parenterally. However, the surveillance of adverse effects includes the search for neurological effects including data indicative of encephalitis or encephalopathy.

4.3.2.2 Potential Environmental Effects

There is the potential risk of transmission of aerosolized measles vaccine virus to health workers and others surrounding the site of vaccination. No contacts of children vaccinated with aerosolized measles vaccine have developed a measles-like illness. However, only one previous study has sought to document person-to-person spread of vaccine virus by serologic assay.[29] Aerosol administration may expose vaccinators to measles vaccine. However, these persons are likely to be measles immune secondary to either previous natural infection or vaccination. Using modern devices such as the one proposed for this trial would reduce this theoretical risk of shedding the vaccine virus from persons vaccinated through the aerosol route.

To minimize risks to health workers during the trial we will explain this possibility before hiring personnel so as to exclude individuals who have not suffered measles or have not been vaccinated within the previous 10 years, or who have a immunosuppressive diseases (e.g. HIV/AIDS, neoplasias, lymphoproliferative disorders, other) or receive immunosuppressive medication. In order to minimize the risk of fetal infection, all female staff of child-bearing age will be required to have a negative urine pregnancy test before hiring and agree to avoid pregnancy during the duration of the study.

4.3.2.3 Special Populations

Persons with respiratory hyperactivity may be at increased risk of bronchospasm following administration of measles vaccine by aerosol. Immunocompromised persons, including those with HIV infection and severe malnutrition, may be at increased risk of adverse effects caused by a live attenuated vaccine administered through the respiratory mucosa. Post licensure it is foreseen that additional clinical trials will specifically evaluate safety of this route of administration among these populations.

4.3.3 Description of and justification of the route of administration, dosage, dosage regimen, and treatment periods

Aerosols delivered to the respiratory mucosa are the natural route of transmission for measles virus, and the most promising non-injectable method of vaccination studied so far and their efficacy is thought to be comparable to injected vaccine.

Measles vaccine is for active immunization against measles. A single dose (containing at least 1,000 CCID50) is sufficient to provide prolonged immunity to infection.

In countries where the incidence and mortality from measles is high in the first year of life, the recommended age for immunization against measles is as soon as possible after 9 months of age. Countries where measles is less of a problem may decide on a later date for immunization.

The vaccine is also recommended for use in children and adolescents with no evidence of vaccination or measles infection.

4.3.4 Agreement to ensure that the trial will be conducted in compliance with the protocol, GCP and Indian regulatory requirements (Schedule Y)

The investigator/institution will conduct the trial in compliance with the protocol agreed to by the sponsor and, by the Indian regulatory authority (DCGI) and which will be given approval/ favourable opinion by the Ethics Review Committee. The investigator/institution and the sponsor will sign the protocol, and the Undertaking by the Investigator to confirm this agreement.

4.3.5 Description of the population to be studied

4.3.5.1 Measles and measles vaccination in India

India is a pluralistic, multi-lingual, and multi-ethnic society. It is the world's seventh largest country by geographical area, the second most populous (population 1.12 billion), and the most populous democracy. It borders Pakistan to the west; China, Nepal, and Bhutan to the north-east; and Bangladesh and Burma to the east. India is the world's twelfth largest economy. However, it still suffers from high levels of poverty, illiteracy, malnutrition and environmental degradation.

Measles is endemic in India and is one of the most important causes of childhood morbidity and mortality. Due to the lack of dedicated countrywide surveillance for measles, it is difficult to estimate the true magnitude of the problem. WHO estimates that there was a decline in South-East Asia from an estimated 240 000 (173 000–316 000) measles deaths in 2000 to 178 000 (128 000–234 000) measles deaths in 2006. [30] An important proportion of the remaining disease mortality in this region is believed to occur in India.

Measles vaccination in India is recommended as a single dose, given from nine to 12 months of age.[31] Reported levels of measles vaccine coverage in India vary depending on the source. In 2001 administrative reports showed levels of measles vaccine coverage above 90% while coverage evaluation surveys estimated it at just over 60%. [32]

Vaccine coverage varies within the different states, which leads to a pool of vulnerable target group that is susceptible to the disease. Delhi state has a higher vaccine coverage. Goa, Maharashtra, and Tamil Nadu states reached 84-88 per cent coverage. Six states, Andhra Pradesh, Chhatisgarh, Delhi, Gujarat, Punjab, and Madhya Pradesh achieved coverage of more than 70 per cent and another 5 in the range of 60-70 percent. The majority of 14 states had measles coverage lower than the national average of 55.2 per cent. Moreover studies from the rural, semi urban, slum and community revealed poor vaccine coverage in these areas in India.[33-38]

The District Level and Household Survey evaluated the coverage for measles by district: The report indicates that: 14% of districts achieved coverage below 30% , 24% of districts obtained coverage between 30-50% and 28% of districts coverage achieved above 80%. [39, 40]

WHO estimates that of 26.2 million infants in 2006 who missed receiving their first dose of measles vaccine through routine immunization services by the age of 12 months, 16 million (61%) reside in 5 large countries: India (10.5 million children aged 9–12 months), Nigeria (2.0 million), China (1.2 million), Indonesia (1.2 million) and Ethiopia (1.1 million). [30]

4.3.5.2 Characteristics of Maharashtra State and Pune District.

Maharashtra State is in the west of India on the Deccan Plateau, 150km south east of Mumbai. Pune (population 5 million) is the second largest city in Maharashtra, after Mumbai, and the eighth largest in India. The city is a major industrial centre for the automobile industry and a large computing software centre. The Serum Institute of India Ltd. is also located in Pune. Per capita income in Pune is 50% higher than the national average and income disparity is the lowest in India, according to the Pune Municipal Corporation Environmental Status.

Table 1: Trend from 1991 to 2004 for selected indicators, Maharashtra State

Year	Crude Birth Rate			Crude Death Rate			IMR		
	Total	Rural	Urban	Total	Rural	Urban	Total	Rural	Urban
1991	26.2	28	22.9	8.2	9.3	6.2	60	69	38
1992	25.3	27.4	21.5	7.9	9.1	5.6	59	67	40
1993	25.2	27.1	22.8	7.3	9.3	4.8	50	63	32
1994	25.1	26.9	23	7.5	9.2	5.6	55	34	38
1995	24.5	26	22.4	7.5	8.9	5.4	55	31	34
1996	23.4	24.9	21	7.4	8.7	5.4	48	31	31
1997	23.1	24.4	21	7.3	8.6	5.4	47	32	31
1998	22.5	23.6	20.8	7.7	8.9	5.8	49	31	32
1999	21.1	21.6	20.3	7.5	8.7	5.6	48	58	31
2000	20.9	21.2	20.3	7.5	8.6	5.7	48	57	33
2001	20.6	21	20.1	7.5	8.5	5.9	45	55	27
2002	20.3	20.6	19.8	7.3	8.3	5.6	45	52	34
2003	19.9	20.1	19.4	7.2	8.2	5.6	42	48	32
2004	19.1	19.9	17.9	6.2	6.8	5.4	36	42	27

Source: http://www.maha-arogya.gov.in/achievements/default.htm

4.3.5.3 Immunization and vaccine preventable diseases surveillance practices

The aim of the immunization programme is to give primary immunization at the correct age, the correct dose and appropriate route before the first birthday of child. The Auxiliary Nurse Midwife (ANM) at the sub-center and the trained birth attendants in the villages are the key persons in the delivery of Maternal and Child Health (MCH) Services. Immunization Clinics are utilized for providing regular antenatal services. Immunization services are provided to all the people free of cost through every Health institution. The vaccination schedule as recommended by Govt. of India is followed. Measles vaccination recommendations follow the WHO India schedule, i.e. from 9 to 12 months of age. [31, 33]

The average actual age at measles vaccination is about 10 months (DHS India 1998-99 and personal communication from nursing sister at Shel Pimpelgaon PHC).

Measles vaccination coverage is currently estimated at around 92% from health service data. However, the exclusion of recent immigrants from the denominator but inclusion in the numerator probably results in over-estimates of coverage. The level of immigration is not available but is generally higher in villages near highways.

Health staff enumerate beneficiaries in their respective area. Health Assistants procure vaccine from their institution using the correct cold chain procedure. Vaccination is conducted under the supervision of a Health Assistant. The parents/guardians of those who do not receive vaccine are contacted. Primary vaccinations against tuberculosis (BCG), diphtheria, pertussis, tetanus (DPT), polio and measles, and the first dose of vitamin A are given to children before their first birthday. Booster doses of DPT and polio vaccines are given at 18 to 24 months. Further doses of vitamin A are given at 18, 24, 30 and 36 months. A dose of DT is given to children between 5 to 6 years and a dose of tetanus toxoid is given at 10 years and 16 years. Pregnant women are given 2 doses or a booster dose of TT.

Table 2 summarizes the immunization coverage achieved during 2002-2006.

Table 2: Measles immunization coverage 2002-2007, Maharashtra State

Indicator	2002-2003			2003-2004			2004-2005			2005-2006			2006 2007			2007-2008 (April to Jan 2008)		
	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%	ELA	ACH.	%
DPT III	2113512	2017046	95	2150681	2110246	95	2055735	2048662	100	2093721	2079367	99	2098904	1586315	76	1974375	1514079	77
OPV III	2113512	2034476	96	2150681	2084555	92	2055735	2049333	100	2093721	2072128	99	2098904	1595884	76	1974375	1476641	75
BCG	2113512	2127471	101	2150681	2173105	94	2055735	2164062	105	2093721	2139148	102	2098904	1664981	79	1974375	1633207	83
Measles	2113512	1978350	94	2150681	1958080	90	2055735	1997355	97	2093721	1984167	95	2098904	1516299	72	1974375	1472809	75
DPT (B)	2106328	1935233	92	2148636	1972534	92	2126485	1947308	92	1703089	1869012	110	2014740	1447327	72	2253480	1462570	65
OPV(B)	2106328	1949264	93	2106328	1974875	92	2126485	1953872	92	1703089	1874653	110	2014740	1442111	72	2253480	1408211	62
DT	2180871	1957099	90	2219884	1900518	86	2329009	2064416	89	2301437	2150579	93	2458596	1724040	70	2467968	1539717	62
TT (10)	2330045	2034823	87	2371691	2067594	87	2329009	2066254	89	2492831	2226557	89	2237233	1776657	72	2467968	1492166	60
TT(16)	2150421	1875999	87	2189255	1822229	83	2126485	1922415	90	2311947	2085794	90	2244801	1585178	71	2253361	1301775	58
TT (M)	2402459	2261068	94	2444708	2143589	87	2355495	2092637	89	2398739	2061763	86	2405384	1723124	72	2254436	1246894	55

Source: http://www.maha-arogya.gov.in/programs/nhp/mchimmunisation/performance.htm

Immunization coverage for Pune district as described in Table 3 (below).

Table 3: Immunization coverage Pune district (1996-2004)

YEAR	BCG	DPT III	POLIO	MEASLES
1996-1997	105	98	97	93
1997-1998	103	98	99	100
1998-1999	104	102	101	99
1999-2000	110	103	103	96
2000-2001	100	102	102	96
2001-2002	104	100	100	99
2002-2003	83	79	79	77
2003-2004	55	51	51	54

Source: http://www.maha-arogya.gov.in/projectandschemes/basic/achievements.htm

Measles is endemic in this State. There is no current active surveillance of measles cases. Reported figures include mainly data from Government hospitals. The state government is currently undertaking a project on integrated communicable disease surveillance.

Table 4, summarizes the number of cases of vaccine preventable diseases reported during the period 2002-2008.

Table 4: Reported cases of vaccine preventable diseases 2002-2008, Maharashtra State.

Surveillance of Vaccine Preventable Diseases														
Years	Diphtheria		Pertussis		N.N.T.		TB (Child)		Measles		Polio		TETANUS (O)	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
2002-03	27	2	8	0	8	2	803	22	1704	3	4	0	70	13
2003-04	27	4	1	0	9	4	536	3	2136	30	3	0	69	14
2004-05	206	11	58	0	14	2	468	1	1790	7	0	0	68	13
2005-06	151	19	6	0	10	3	1062	26	1702	5	5	0	45	5
2006-07	513	1	95	0	67	0	596	6	513	3	1	0	17	4
2007-08 (Up to Jan.08)	497	0	37	0	10	1	142	3	341	6	2	0	26	0

Source: http://www.maha-arogya.gov.in/programs/nhp/mchimmunisation/achievements.htm

Approximately 500 cases are reported from Pune city each year but this is probably a gross underestimation.

4.3.5.4 Trial setting: Vadu Rural area

The proposed study site consists of five contiguous health districts (Vadu, Wagholi, Shel Pimpalgaon, Chakan, and Kendur,) comprising over 150 villages, with a total population estimated at about 312,000 in 2007 (Table 5). The site is 30-40km across and the main village in each area is within one hour's drive from central Pune. The health districts lie within three administrative blocks (Shirur, Haveli and Khed) with Vadu spanning two administrative blocks, due to the public/private health care partnership, which is described below, paragraph 4.3.5.4. The population, location and numbers of measles vaccinations delivered in each area are shown in Table 5.

Note: Three additional health districts have been added to enhance enrolment: Nhavara, Talegaon Dhamdhere and Alandi . Information on these areas is available on request.

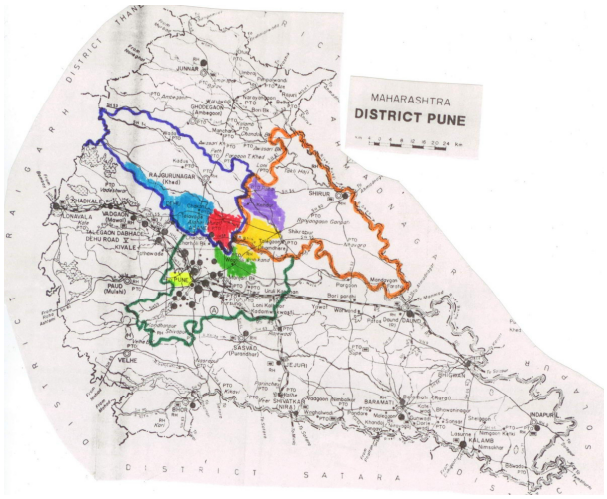


Table 5: Population in study area and number of measles vaccinations performed 2006-7

	Vadu	Wagholi	Shel Pimpalgaon	Chakan	Kendur	Total
Population	74000	49910	63000	99000	26865	312775
Administrative block	Shirur, Haveli	Haveli	Khed	Khed	Shirur	
Distance from Vadu Center (km)	0	15	15	25	10	
Number of EPI clinics	28	12	25	45	14	124
Number of measles vaccine doses given in 2006/07	1322	914	1000	1517	600	5353
Mean number of measles vaccine doses /month	110	76	83	126	50	446
Number of measles vaccine doses in 6 months	661	457	500	759	300	2677

Legend: EPI – WHO Expanded Programme on Immunization

4.3.5.5 Health care facilities in the Study area

Each health district area is covered by a primary health centre (PHC), which usually lies within an administrative block. Each PHC is staffed by a medical officer, nurse, and auxiliary nurse midwife (ANM).

Health care in the Vadu area is provided by the Vadu Rural Health Program, which is under the administration of King Edward Memorial Hospital and Research Centre (KEM), Pune, a privately funded hospital. One of the areas is a Demographic Surveillance Site (DSS) of the INDEPTH Network (an International Network of field sites with continuous Demographic Evaluation of Populations and Their Health in developing countries).

The District Health System is directly responsible for primary health care in the four districts. The PHCs at Kendur, Wagholi, Shel Pimpalgaon, and Chakan provide primary health care services including immunizations in their respective areas served by them. The Chakan and Wagholi PHC area are situated on major highways and are better connected similar to the Vadu area and have multiple private practitioners and small nursing homes capable of providing basic medical care. Chakan also has a rural hospital. The Kendur and Shel

Pimpalgaon PHC areas are remote and have fewer private practitioners and small private nursing homes.

The hospital in Vadu village , Shirdi Saibaba Rural Hospital (SSH), also known as Vadu Rural Hospital, is managed by the KEM Hospital and provides secondary level care to the 22 villages of the Vadu Rural Health Program. The hospital is staffed by a General Medical Officer, Obstetrics and Gynaecology specialist and resident in Obstetrics and Gynecology (all available 24hrs/day, 7days/week) with a surgeon and anesthetist available on call. A pediatrician is also employed. There are also nine registered nurses, a radiology technician and a pharmacist. A sonographer and ophthalmologist visit twice a week. On site facilities include: 7 consulting rooms; 1 male and 1 female ward; 4 patient rooms (private); 2 operating theatres; 1 labor room; 1 vaccine storage room; 1 laboratory (1 room for laboratory work, one for freezer storage); 1 X-ray room and darkroom; 1 sonography room. In addition, there is a separate research wing with a conference room, meeting hall, archive room, computer room, "outreach" room, a study room (used by the Meningococcal Vaccination Project), and a residential area.

4.3.5.6 Current measles immunization practice in the study area

Measles vaccination recommendations follow the WHO India schedule, i.e. from 9 to 12 months of age[31].

4.3.5.7 Identification of children eligible for routine immunization

For routine immunization, health workers compile a list of eligible children every year (in March and April) based on couples eligible for contraceptive and maternal child health services where the woman is aged 15 to 49 years (eligible couples survey, register number R14). Women who become pregnant during the year are listed in the R15 register which details the services received by them during pregnancy and delivery. After delivery, the children are carried forward into the R16 register which details the vaccinations and other services like Vitamin A supplementation received by them till one year of age. A health worker allotted to each geographical area (approximately 8000 people per health worker) is responsible for following up all the eligible couples and children eligible for vaccination in that area on a monthly basis.

4.3.5.8 Procedures during routine immunization clinics

Immunization clinics are held in each village on a permanently set date for every month and clinics are held on that date even if these are holidays and Sundays. For larger villages, clinics are held on the weekly market day to ensure high coverage. The dates of immunization clinics are well known in the local population and reinforced by the local health worker.

All PHCs and SSH are equipped with an ice-lined refrigerator and a vaccine refrigerator for storage of vaccines. Vaccines are supplied by the District Health Office every month. On the immunization clinic day, vaccines are carried by the health worker to the clinic in the village from SSH or the PHC in the morning in a vaccine carrier box. At the end of the clinic, unused vaccine is returned to SSH or PHC where vaccine accountability records are maintained.

At the immunization clinic, the nurse records the child's attendance and vaccines administered in a register. After the clinic these details are transferred to the R16 register, which records the dates of all primary immunizations received by a child. The health workers perform home visits every month in the area assigned to them. A list of children who have

missed a dose is taken from the R16 register and those children are followed up during their routine monthly home visit. Data about the numbers of measles vaccinations delivered each month in the Vadu area are shown in Table 6

Table 6: Numbers of recorded births and measles vaccinations given in immunization clinics in Vadu area, by month

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	All
Measles	96	94	111	98	122	104	105	113	98	87	111	106	1245
(range)	(0-19)	(0-18)	(1-18)	(0-16)	(0-19)	(0-19)	(0-22)	(0-23)	(0-18)	(0-15)	(0-19)	(0-20)	(10-198)
Births	114	94	116	105	97	115	113	95	94	104	100	117	1264
(range)	(0-29)	(0-20)	(1-24)	(0-22)	(0-22)	(0-21)	(0-22)	(0-19)	(1-20)	(0-21)	(0-22)	(2-21)	(16-255)

Legend: Based on data from 20 clinics in Vadu area, collected 2006-7. No data available from two clinics.

4.3.5.9 Research experience of the KEM Hospital and Research Center

The KEM Hospital and Research Center has had a legacy of epidemiological, bio-medical and social science research lasting 20 years. Apart from large community based cohort studies on low birth weight and child survival and fetal origins of adult onset disease, SSH has undertaken community based randomized controlled trials in iron supplementation amongst children. Currently ongoing is a large randomized observer blinded vaccine trial which is assessing the safety and long term immunogenicity of a conjugate meningococcal vaccine against a licensed polysaccharide vaccine.

Vadu has extensive demographic data available from 2003, fieldworkers with experience of collecting health-related data at household level, and experience of designing and conducting large epidemiological studies. The most recent complete census was conducted in July 2007, and updates are conducted every six months. There is currently Geographical Information System (GIS) data available for Vadu (the central village).

5 Trial objectives and purpose

The overall aim of the study is to demonstrate that measles vaccine delivered as an aerosol to the respiratory tract is sufficiently safe and immunogenic to obtain licensure for the nebulizer/vaccine combination device.

5.1 Study Rationale

A large, well-designed and -conducted, randomized controlled trial of the immunogenicity and safety of aerosolized measles vaccine in infants receiving a first dose of measles vaccine is required, in a setting where the vaccine would be used in practice.

An active control vaccine is required because the current subcutaneous vaccine is known to be highly effective and safe and is recommended worldwide. The immunogenicity of aerosolized measles vaccine should be shown to be non-inferior to the subcutaneous vaccine, according to

a pre-determined maximum acceptable difference. Superiority is not a realistic endpoint due to the high levels of serological response produced by subcutaneous vaccine.

5.2 What this trial will add

This trial has been designed to fulfil the regulatory requirements for licensing of a nebulizer/vaccine combination device, if the immunogenicity of aerosolized measles vaccination is shown to be non-inferior to subcutaneous vaccine in the target age group, and has an acceptable safety profile. By conducting the trial in a setting where the new delivery device would be used, existing public health surveillance systems will be strengthened, capacity will be built in the trial area for conducting large scale clinical trials and for the handling and serological processing of large numbers of blood samples.

5.3 Primary objectives

5.3.1 Immunogenicity

To compare the immunogenicity of measles vaccine delivered via a nebulizer/vaccine combination product with a licensed subcutaneous vaccine in infants who are eligible to receive a first dose of measles vaccination but are no older than 12 months.

5.3.2 Safety

To describe the frequency of adverse events following measles aerosol and subcutaneous vaccination.

5.4 Secondary objectives

5.4.1 Immunogenicity

To compare geometric mean antibody titres in infants receiving measles vaccine delivered via a nebulizer/vaccine combination device with subcutaneous measles vaccine;

To compare the immunogenicity of measles vaccine delivered via a nebulizer/vaccine combination device with subcutaneous measles vaccine, according to level of pre-vaccination antibody titre;

To collect blood from subset of children at 28 days and one year after vaccination. These subset will be randomly selected from all children enrolled in the Vadu study area because they will be able to be followed up within the infrastructure of an existing demographic and health surveillance system. For these subsets, the secondary objectives are:

- To compare the immunogenicity of measles vaccine delivered via a nebulizer/vaccine combination device with subcutaneous measles vaccine one year after vaccination;
- To describe the evolution of the antibody response to measles vaccine delivered via a nebulizer/vaccine combination device and subcutaneously over time, at days 0, 28, 91, 364 after vaccination;

5.4.2 Safety

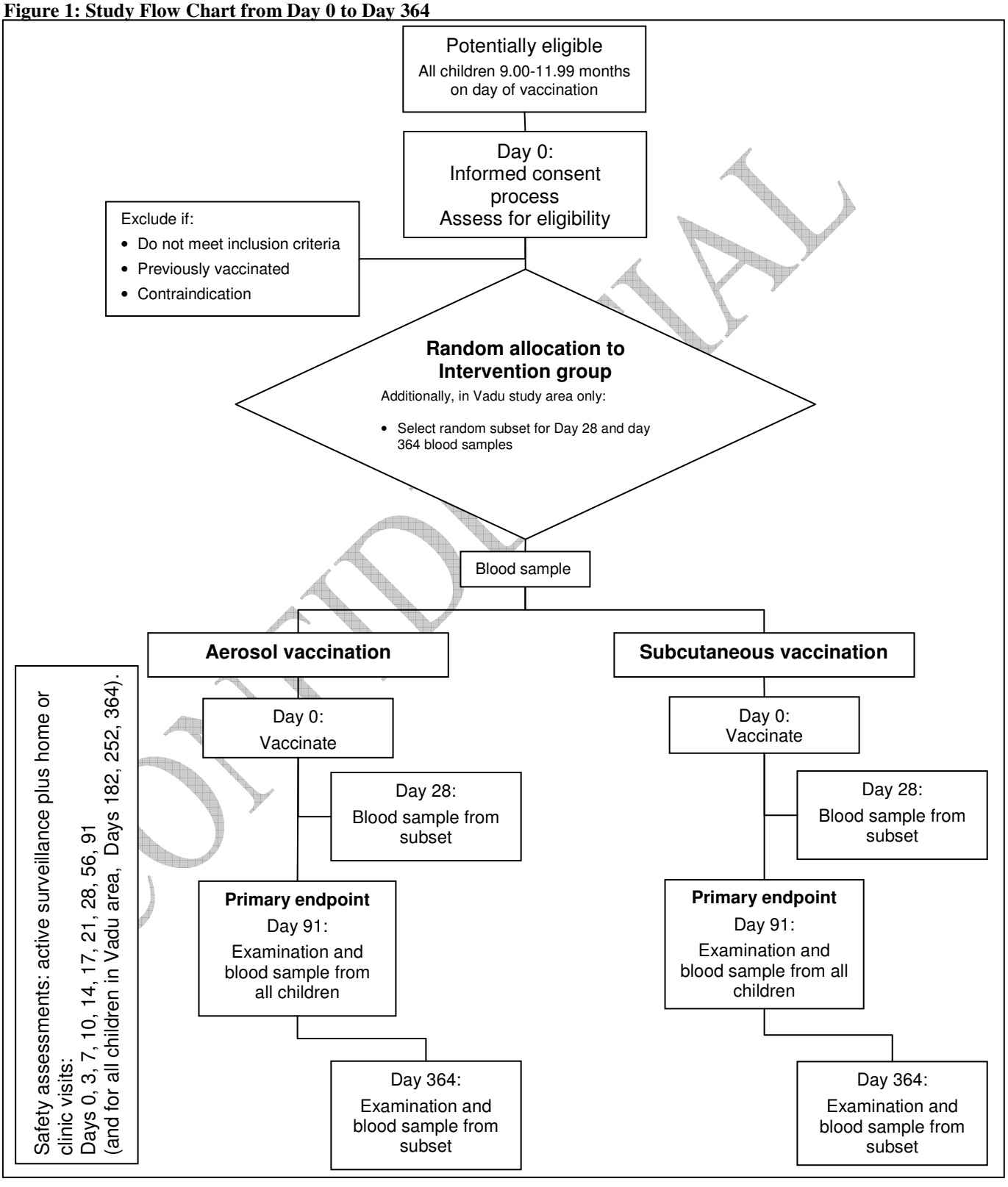
To describe the frequencies of individual adverse events following measles aerosol and subcutaneous vaccination.

6 Trial design

6.1 Type/design of study

Randomized, open-label, active-control, parallel group, non-inferiority trial (Figure 1).

Figure 1: Study Flow Chart from Day 0 to Day 364



6.2 Primary and secondary endpoints

6.2.1 Immunogenicity

6.2.1.1 Primary outcome

Measles seropositivity at day 91 post-vaccination.

6.2.1.2 Secondary outcomes

For all infants:

- Geometric mean titres, pre-vaccination and at day 91;
- Seroconversion (defined as a change from Enzygost OD <0.1 to OD ≥0.1 or PRNT from <120 to ≥120 mIU/mL) measured in paired samples from day 0 and day 91;

For infants from Vadu in 28 and 364 days subset:

- Geometric mean titres at days 28 and 364 to describe evolution of antibody response at days 0, 28, 91, 364;
- Seropositivity at 364 days in infants with antibody titre ≥ 120 mIU/ml at day 91.

6.2.2 Safety

6.2.2.1 Primary outcome

Adverse events up to and including day 91 post-vaccination;

Adverse events include acute clinical reactogenicity, other adverse events, and serious or unexpected adverse events.

6.2.2.2 Secondary outcome

Frequencies of individual adverse events

6.3 Measures taken to minimize bias

6.3.1 Randomization

See SOP VEL 4/1

6.3.1.1 Unit of randomization

Individual child

6.3.1.2 Sequence generation

The allocation sequence will be computer-generated random numbers, in permuted blocks, generated by an independent statistician. The randomization ratio will be 1:1.

6.3.1.3 Allocation concealment

Allocation will be accessed by telephone from the study enrolment site to an operator with a secure web-based system. Allocation will be recorded in the system, along with the unique patient data.

6.3.1.4 Implementation

- A doctor or study nurse at each health centre will obtain the allocation and record it immediately in the Day 0 case report form (CRF).
- The intervention is administered accordingly.

See SOP VEL 2/1 for details of quality assurance, and ensuring that correct allocations have been administered.

6.3.2 Blinding

The trial is open-label and the routes of administration different, so blinding of participants and investigators will not be possible.

6.3.2.1 Blinding of safety assessment

It will not be possible to guarantee the blinding of assessors of safety outcomes. Parents/guardians of participants will know which vaccination route their child received. To minimize bias there will be no record of intervention allocation on CRFs for follow up visits and parents/guardians will be asked identical structured, non-leading questions.

An independent Data Safety and Monitoring Board (DSMB) will have access to unblinded data for the assessment of serious adverse events.

6.3.2.2 Blinding of immunogenicity assessment

Laboratory staff will be blinded to allocation to aerosol or subcutaneous administration (see SOP VEL 3/1 for details).

6.3.2.3 Blinding during laboratory testing

- Blood samples will be labelled with a barcode unique to the patient and blood drawing date, but the patient and sample number (e.g. second sample from this individual) will not be identifiable without the data-linking documents.
- The barcode numbers will be generated at CMC Vellore and the data-linking documents held there. Blood samples and additional identical barcodes will be transported together to the site of serum aliquotting (post serum-separation).
- Each aliquot will be labelled with the same barcode as the original sample. As blood samples will be drawn from both subcutaneous and aerosol vaccine recipients on blood collection days, there will be no predictable pattern (relating to intervention group) in sample receipt at the laboratories for ELISA and PRN testing.
- Test runs will include specimens from both subcutaneous and aerosol arms. As the lab/s conducting the ELISA and PRN tests will have not participated in blood collection and labelling and will have no access to linking data, they will remain blinded.

6.4 Description of the trial combination product (vaccine-aerosol delivery device) and dose

All children will receive monovalent Edmonston-Zagreb (E-Z) strain attenuated measles vaccine manufactured by the Serum Institute of India, Ltd (SIIL).

6.4.1 Dosage form, packaging and labelling of the vaccine

6.4.1.1 Live attenuated measles vaccine

The dose of measles live attenuated lyophilized E-Z vaccine will be not less than 1000 $CCID_{50}$ per dose). The vaccine also contains excipients/stabilisers.

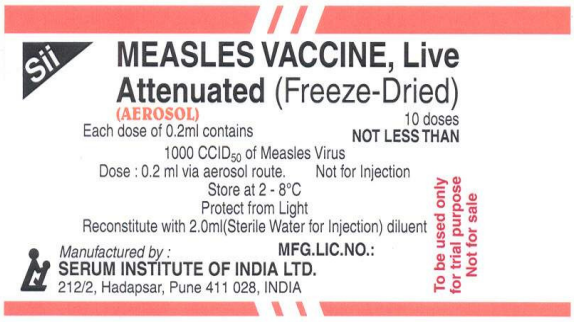
6.4.1.2 Diluent

The diluent for the vaccine is water for injection (WFI). Bulk WFI is prepared by distillation and meets the Indian Pharmacopoeia specifications for WFI.

6.4.1.3 Method of packaging and labelling of the vaccine

Vaccine vials for the aerosol arm will be labelled and packaged to indicate that they are for the clinical trial.

A sample of the label for the aerosol vaccine is shown below:



6.4.2 Vaccine lots

Vaccine lots will be selected as follows:

Item	Batch	Doses	Presentation	Label
Aerosol Vaccine	1	500	50 x 10 dose vials	Clinical label - research only
Aerosol Vaccine	2	500	50 x 10 dose vials	
Aerosol Vaccine	3	500	50 x 10 dose vials	
Aerosol Vaccine	**4**	**1000**	**100 x 10 dose vials**	
Aerosol Diluent	A	1500	150 x 2.5 mL amps	Clinical label stating for aerosol vaccine reconstitution only
Subcutaneous Vaccine	1	1500	150 x 10 dose vials	Regular label
Aerosol Diluent	**B**	**2500**	**250 x 2.5 mL amps**	**Clinical label stating for aerosol vaccine reconstitution only**
Subcutaneous Diluent	B	1500	150 x 5 mL amps	Regular label

All lots will have an expiry date of at least 18 months from date of shipping to the research center. Ten vials of each vaccine batch and 30 ampoules of each diluent batch will be retained by the vaccine manufacturer for retesting.

6.4.2.1 Vaccine delivery device

Aerosolised vaccine will be delivered using the Nektar device (Aerogen/Nektar, San Francisco, See Appendix 1 for full details) to be included.

6.4.2.2 Method of packaging and labelling of the device

A sample of the device over-label is shown below:

Device over-label

For Research Use ONLY
Measles Aerosol Project

6.5 Expected duration of subject participation and description of sequence and duration of trial periods including follow-up.

Duration of subject participation

- Every child will be followed for 91 days after vaccination for ascertainment of the primary immunogenicity and safety outcomes.
- Additionally, all children in the Vadu area will be followed to 364 days after vaccination for ascertainment of adverse events.
- A random subset of 100 of children in each arm from the Vadu area will have blood drawn at day 28 and day 364.
- A random subset of 100 of children in each arm from the Vadu area will have blood drawn at day 364. The selection of this group will be independent from the selection for the day 28 blood sample and individuals my be selected for blood sampling on both day 28 and 364.

Enrolment period

It is estimated that it will take approximately six months to enrol 2000 participants, based on a 50-80% participation rate. This is assumes that approximately 100 infants will be screened for inclusion in the trial per week.

Duration of follow up periods

The expected duration of the study from initial enrolment to the primary outcome (day 91 visit) is nine to 12 months, based on the enrolment rate above and, the assumption that the number of children available for vaccination is similar to that in previous years.

Duration of the study from initial enrolment to the secondary outcome (day 364 visit) for the subset of children in the Vadu area is 18 to 21 months.

Detailed information on the planned follow-up visits is in Section 8.4.4 including Table 7.

6.6 Stopping rules or discontinuation criteria

The trial will be discontinued according to the stopping rules determined by the DSMB (Appendix 2: Terms of Reference of the Data and Safety Monitoring Board).
The criterion for discontinuation is the occurrence of a single SAE or encephalitis/encephalopathy in one or more subjects which is deemed to be most probably related to the aerosol vaccine.

6.7 Measles vaccine and aerosol delivery device accountability

The investigator will assign a nurse to be responsible for vaccine and aerosol device storage and accountability at the trial site. The investigator/designated person will:

- Store the vaccine and device in the condition that has been specified in writing by the Sponsor in SOP WHO 4/1 and in accordance with the protocol and Indian regulatory requirements.
- Ensure that the vaccine storage temperature is maintained as specified in the protocol. There will be a daily temperature log. (SOP WHO 4/1)
- Maintain records of the vaccine and device delivery, inventory and return. (SOP WHO 4/1)
- Maintain up to date accountability on the vaccine and device accountability log. (SOP WHO 4/1)
- Ensure that the vaccine and device are used only in accordance with the approved protocol.

Written records of receipt and storage of the vaccine and devices, including date received, lot number, quantity received, and dose administered, with the coded identification of the subject, will be recorded. Any known discrepancies in the accountability of the vaccine will be documented. The investigator will not use the vaccine and/or devices in any other manner than that provided for in the protocol.

6.7.1.1 Vaccine Storage, Handling and Transport

The vaccine will be stored, according to the manufacturer's recommendations at 2˚C to 8˚C in a refrigerator in which the temperature is monitored and logged at least once a day. The refrigerator will be secured and located in a limited access area.

6.7.2 Policy and procedure for handling unused investigational product.

(SOP WHO 4/1))

- All unused un-reconstituted measles vaccine will be disposed of according to the manufacturers instructions and WHO[33].
- All reconstituted vaccine not used within 6 hours after reconstitution during an immunization session will be discarded according to WHO recommendations for multi dose open vials.[41] Records will be kept of discarded vaccine.
- All non-reusable components of the nebulizer will be disposed of as indicated in SOP NEK 2/1
- The nebulizers used during the trial will be returned to the sponsor at the end of the trial according to sponsor-defined procedures.

6.8 Maintenance of trial randomization codes and procedures for breaking codes

6.8.1 Unblinding of laboratory specimens

Laboratory specimens collected up to day 91 will be unblinded by the designated Sub-Investigator (in charge of data Management and Statistics) at the start of the statistical analysis of the primary outcome and after the database has been locked (see SOP VEL 2/1).

Premature unblinding will be reported immediately to the clinical monitor and the Sponsor and will be documented in the investigator's file. The reason for premature unblinding of the samples should be given.

6.9 Source data

The following documents will be considered source data for this trial:

- Electronic audited copies of the Case Reports Forms and Adverse Events (AE) Investigation Forms
- In addition, the following original documents, data, and records will be considered as source documents:
- hospital records,
- clinical and office charts,
- laboratory notes, memoranda,
- pharmacy dispensing records,
- recorded data from automated instruments,
- copies or transcriptions certified after verification as being accurate copies,
- photographic negatives or files,
- X-rays,
- subject medical files, and
- records kept at the pharmacy, at the laboratories, and at medico-laboratory departments involved in the clinical trial).

7 Selection and withdrawal of subjects

7.1 Inclusion criteria

- Age: from 9.00 to 11.99 months on expected vaccination date;
- Resident in the study area and likely to remain for the duration of the child's involvement in the study;
- Parent/guardian willing for child to be randomized and willing for child to be followed up for at least 91 days (364 days for Vadu subset).

7.2 Exclusion criteria

- Having received measles, measles-rubella or measles-mumps-rubella vaccine (with confirmation from vaccination card).
- Any contraindication to measles vaccine administration as stated in the WHO measles vaccine position paper: [42]
    - Persons with a history of an anaphylactic reaction to neomycin, gelatine or other components the vaccine should not be vaccinated.
    - Furthermore, measles vaccine is contraindicated in persons who are severely immunocompromised as a result of congenital disease, HIV infection, advanced leukaemia or lymphoma, serious malignant disease, or treatment with high-dose steroids, alkylating agents or antimetabolites, or in persons who are receiving immunosuppressive therapeutic radiation.
    - Administration of immunoglobulins or other antibody-containing blood products may interfere with the immune response to the vaccine. Vaccination should be delayed for 3–11 months after administration of blood or blood products, depending on the dose of measles antibody. Following measles vaccination, administration of such blood products should be avoided for 2 weeks, if possible.
    - Mild, concurrent infections are not considered a contraindication, and there is no evidence that measles vaccination exacerbates tuberculosis. However, vaccination should be avoided if there is high fever or other signs of serious disease.

7.3 Subject withdrawal/discontinuation criteria

- Withdrawal of consent;
- Serious adverse event;
- Any other situation in which the Principal Investigator finds it in the subject's best interest to no longer continue his/her participation in the study.

If this occurs, the subject will be asked to continue with the clinical follow up as per the study protocol but would be excluded from the analysis. Withdrawn subjects will not be replaced.

See section 6.6.

8 Treatment of subjects

8.1 Treatments to be administered

8.2 Aerosolized measles vaccine

A dose of measles vaccine, reconstituted for aerosolisation, containing at least 1,000 CCID50 and administered by Nektar/Aerogen nebulizer over 30 seconds, as described in SOP NEK 2/1.

The vaccine and nebulizers will be used and maintained according to the manufacturer specifications, as described in SOP NEK 2/1.

8.3 Subcutaneous control

A dose of measles vaccine (0.5ml), reconstituted for subcutaneous administration, containing at least 1,000 CCID50 and administered as described in SOP WHO 6/1.

The vaccine will be used and stored according to the manufacturer specifications as described in SOP WHO 6/1.

8.3.1 Concomitant medications

Subjects may take all other medications (including rescue medications) as indicated, in line with current Indian MOHFW Guidelines.

Monitoring subject compliance with use of investigational product

Due to the single-dose regime used in this trial, subjects will not be monitored for compliance with dosing schedules as they would be in multiple-dose regimes or those where medications are self/parent/guardian administered. Vaccine administration will occur under direct observation from trial staff for each participant.

8.4 Methods for the trial

8.4.1 Community approval

The trial study area covers more than 150 villages. Preliminary visits will be made to all villages before starting recruitment. Village leaders, influential village residents and other gram panchayat (village level local self government members) will be consulted and asked for verbal agreement for the trial to take place in their village. A written record of meetings will be kept.. Local health providers (family doctors, private clinics, and private doctors) will also be informed of the trial and its purposes.

8.4.2 Enumeration of potential study participants

The method of enumeration will depend on the study area:

- For Vadu, an initial tentative list of all eligible children aged 6 to 10 months will be obtained from the Demographic Surveillance System, Vadu.
- For the other study areas, the study field worker will obtain a list of all eligible children aged 6 to 10 months from local health workers. For full details of this process, please see SOP VAD 3/1. These lists will be in a non-electronic form initially, but in the course of recruitment, details of potential participants will be recorded electronically.

Field workers will visit the parents/guardians of potential participants 2-4 weeks before the infant is 9 months old (Visit 1, see below).

Parents/guardians will be encouraged to consult peers, community leaders and other parents who are study participants. In addition, advertisements about the trial will be displayed in prominent places like gram panchayat offices, temples, and schools in the village.

Parents attending clinics for the vaccination of their child prior to nine months of age (e.g. DPT vaccination at 4 months), will also be given information about the trial.

8.4.3 Vaccination day procedures

The procedures for this visit (Visit 2) are summarized below and described in detail in SOP VAD 4/1.

8.4.4 Follow up schedule

The content of each home or clinic visit shown is described below. The procedures to be followed at each visit are described in SOP VAD 5/1 (home visit and clinic visit).

Table 7: Timetable of home and clinic visits for an individual trial participant

Activity	**Pre-study**	**Main study**											**Long term follow up**			
	ALL INFANTS	**ALL INFANTS**											**SUB-SAMPLE INFANTS†**			
Day	-14	0	3	7	10	14	17	21	28	56	pre-91	91	182	252	pre-364	364
Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Home visit with trial information	**x**															
Clinic visit for eligibility, randomization and vaccination		**X**														
Home visit for AE monitoring			**x**	**x**	**x**		**x**	**x**	**x**	**x**			**x**	**x**		
Reminder visit (AE, serology)					**x**			**x**			**x**				**x**	
Clinic visit for AE examination		**x**				**x**			**X¥**			**x**				**x**
Clinic visit for immunogenicity		**X**							**X¥**			**X**				**X**
Measles surveillance - case based and outbreaks	**X**	**X**											**X**			
Active surveillance for SAE	**X**	**X**											**X**			

Legend:

AE Adverse event
SAE Serious adverse event
X Procedure undertaken at this time
¥ Only subset – random sample from children in Vadu area
† Only subset – random sample from children in Vadu area

8.5 Schedules for recruitment and follow up visits, and procedures undertaken at each

8.5.1.1 Visit 1, Identification and recruitment visit (2-3 weeks) before due date for measles vaccination, Figure 2.)

Purpose of visit:

- To inform parents/guardians of potentially eligible children of,
  - Purpose of trial;
  - Potential risks and benefits to participant;
  - Trial methods and requirements from participants (e.g. visits, blood taking, follow up);
  - Process of giving informed consent to participate;
  - Rights of participants, including the right to withdraw without giving a reason at any time;

Procedures:

- **Inform about the trial -** Fieldworkers will give the parents/guardians verbal and written information about the trial and a copy of the consent form in Marathi or Hindi (Document number SD-4, SD-5).
- **Set an appointment for eligibility assessment and vaccination** Fieldworkers will give the parents/guardians an appointment to attend the PHC for Visit 2, if they are willing;
  - Fieldworkers will advise parents/guardians to arrange transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.
- **Provide additional information as required** Fieldworkers will be available in the community to answer any questions arising after this visit.

8.5.1.2 Visit 2, eligibility, randomization and vaccination visit (day 0, minimum of one day after visit 1)

Purpose of visit: to assess the child for eligibility for randomisation and provide measles vaccination, either as part of the trial, or with routine vaccination.

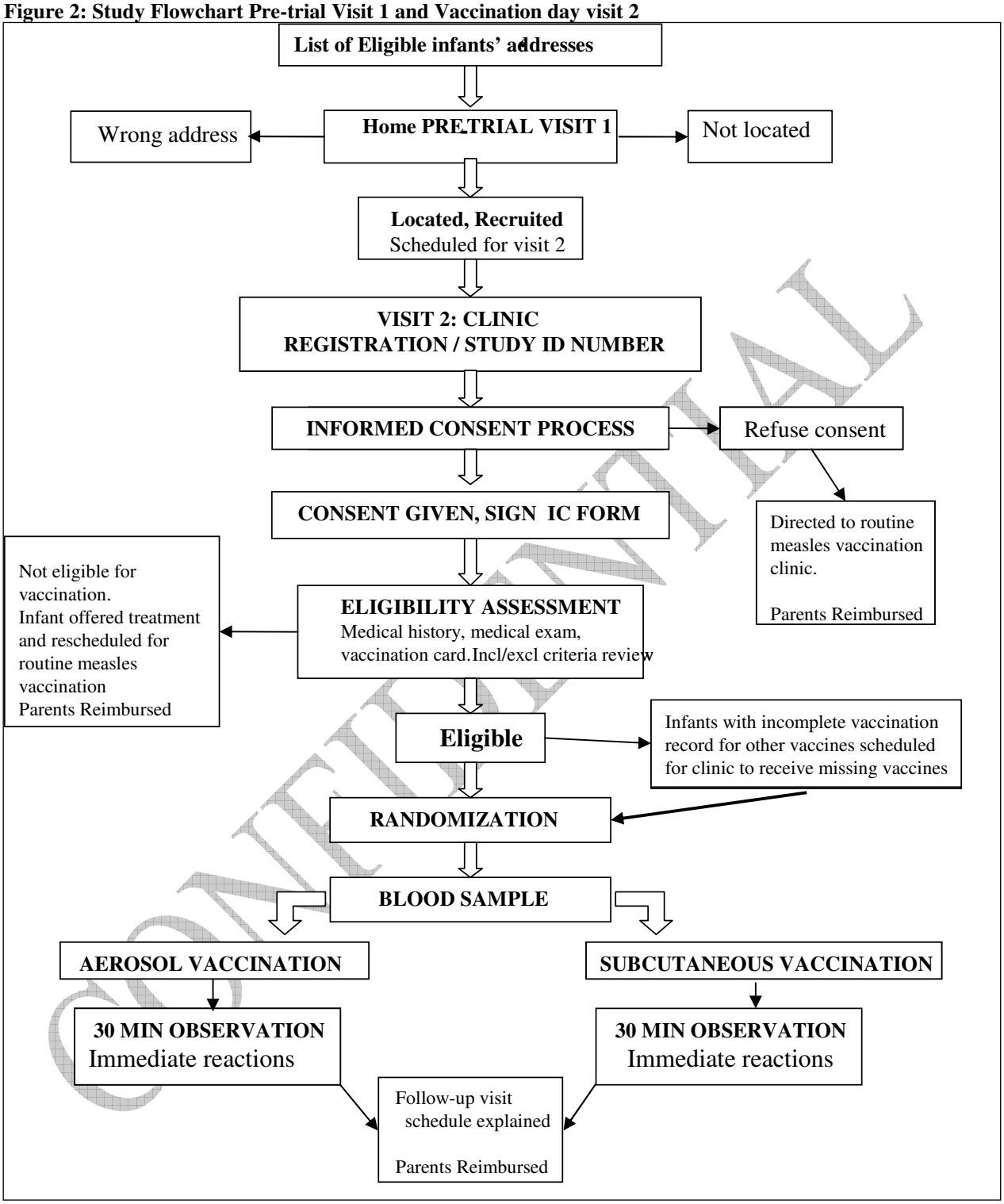
Procedures:

- **Assign a unique ID number –** see SOP VAD 4/1)

- **Obtain Informed Consent.** Parents/guardians of eligible children will be informed again of the purpose, risks/benefits and methods of the study and their rights, and will have an opportunity to ask questions. Parents/guardians who are willing for their child to participate will be asked for a signature or thumbprint. The consent form for all children includes consent for medical examination, vaccination, follow up to 91 days and blood sampling at days 0 and 91. In the Vadu area, all parents will also be asked for consent for their child to be followed up to day 364, with the possibility of selection for blood sampling at days 28 and 364. Children may be selected for none or both of these 2 additional blood samples. See SOP VAD 4/1 and Consent form, SD -5;
- **Vaccinate children for whom consent is not obtained.** Children without written consent for participation will receive measles vaccine according to Indian MOHFW guidelines. After vaccination, parents/guardians should receive reimbursement as described in the Informed Consent Form (ICF).
- **Assess eligibility.** This is will be conducted according to inclusion/exclusion criteria, Sections 7.1 and 7.2
    - **Take a medical history and vaccination history** - the study doctor will record this information using vaccination cards and verification from clinic records, using a case report form (CRF).
    - **Conduct a medical examination to confirm eligibility** - a physical examination will be undertaken by the Study doctor and recorded in the Case Report Form (See SOP VAD 4/1). Any child found on examination to have a clinically apparent condition that contra-indicates vaccination will be withdrawn from the study and offered referral for treatment.
    - **Vaccination of eligible children with incomplete vaccination schedule** - if the child has an incomplete vaccination history, the parents/guardians will be given an appointment for the missing doses of vaccine to be administered at their local immunization clinic (at a minimum 4 weeks after measles vaccination).
    - **Vaccination of children who are not eligibl**e - all children who are assessed to be ineligible at Visit 2 will be permanently excluded and are ineligible for reassessment for inclusion. These infants will be rescheduled for routine measles vaccination as per Indian guidelines and parents/guardians should receive reimbursement as described in the informed consent form (ICF).
- **Conduct randomization**. If the parents/guardians give consent, the randomization process will occur as described in SOP VAD 4/1. Allocations will be recorded immediately by trial staff;
- **Obtain blood sample**. This will be taken by venepuncture (SOP HPA 1/1);
- **Vaccinate infants.** This will be undertaken according to study allocation. Full details are described in (SOPs NEK 2/1 and SOP WHO 6/1). Vaccination will occur in separate rooms (without direct communication of ventilation systems) for each intervention group. The processes of vaccine storage and transport are described in SOP WHO 4/1.
- **Conduct observation for immediate adverse events.** Children will be observed for 30 minutes after vaccination and observations recorded in the CRF (see below). This is described in detail in SOP WHO 2/1.

- **Provide parents with information on follow up visits.** The study nurse will inform the parents of the subsequent visits and procedures
- **Provide parents with reimbursement of expenses.** The study nurse will ensure that the parents/guardians receive reimbursement as described in the ICF.

Figure 2: Study Flowchart Pre-trial Visit 1 and Vaccination day visit 2



8.5.1.3 Visit 3, AE follow up - home visit (day 3, Figure 3)

Purpose of visit: To collect information about adverse events and concomitant medications (See VAD 5/1).

Procedures:

- **Verify ID code -** Fieldworkers verify the ID code and identification details for the child (see VAD 5/1)
- **Collect information about AE**. Fieldworkers ask parents about adverse events and concomitant medications, using structured questionnaire See SOP WHO 1/1. This will include a question relating to the occurrence of measles symptoms in the family/household.
- **Initial grading of adverse events**. Field workers record this according to predefined criteria (described in Appendix 3).
- **Request advice from the Medical Officer on-call for any AE classified as moderate or severe.** If any of the adverse events identified is classified as moderate or severe the field worker should inform the On-Call Medical Officer immediately and request his/her assistance to confirm the grading and investigation of the AE as described in SOP WHO 1/1
- **Confirm classification of AE.** On-Call Medical Officer assists the field worker to confirm the classification of the AE and complete the CRF.
- **Report and manage AE and SAE –** AE and SAE are reported through the channels described in detail in SOP WHO 1/1 and SOP WHO 3/1. In brief, serious and severe AE are rapidly reported to the Authorised Physician (safety),the PI, the WHO Focal Point, and DSMB, and serious AE are rapidly reported by the PI to the WHO Focal Point and Local ethical committees. At the end of each day, all CRFs with data about adverse events will be checked by a Study Medical Officer to ensure that all adverse events graded as moderate or severe have been followed up, and that fieldworker reports appear consistent. All AE are reported on a regular basis via CRFs to the Data Management Center.
- **Conduct initial causality assessment.** The Authorised Physician (safety) and PI (or their designee) together with the attending Study Medical Officer review the information of regarding any moderate or severe AE and conduct an evaluation of causality. A report is submitted to the DSMB as indicated in the SOP WHO 1/1 and the DSMB TORS Appendix 2.

8.5.1.4 Visit 4, AE follow up - home visit (day 7)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).

8.5.1.5 Visit 5, AE follow up- home visit (day 10)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).
- Fieldworkers give parents/guardians appointment for visit 6 at PHC.
- Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

8.5.1.6 Visit 6, AE follow up -clinic visit (day 14/15)

Purpose of visit: Clinical examination and ascertainment of adverse events.

Procedures:

- **Verify ID Code** – The study nurse verifies the child's identification as described in See SOP VAD 5/1
- **Check for any AE** - Study Medical Officer asks parents/guardians about adverse events using the structured questionnaire and records it in CRF (SOP VAD 5/1).
- **Conduct medical examination** - Study Medical Officer conducts medical examination, including directed neurological examination and records it in the CRF (SOP VAD 5/1).
- **Grade AE** - Study Medical Officer grades adverse events.
- Severe or serious adverse events are reported to Authorized Physician (safety) immediately as described in SOP WHO 3/1.
- For all AE, the Study Medical Officer treats infant or refers for further treatment as appropriate
- All AE (other than SAE) are reported to field workers by the Study Medical Officer for follow-up for AE resolution/outcome
- The Study Medical Officer informs parents of treatment plan
- **Provide parents with information on follow up visits -** the study nurse will inform the parents of the subsequent visits and procedures
- **Provide parents with reimbursement of expenses -** the study nurse will ensure that the parents/guardians receive reimbursement as described in the ICF.

8.5.1.7 Visit 7, AE follow up -home visit (day 17)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).

8.5.1.8 Visit 8, AE follow up -home visit (day 21)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).

- Fieldworkers give parents/guardians of selected children appointment for visit 9 at PHC.
- Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

8.5.1.9 Visit 9, AE follow up -home visit or clinic visit for selected children (day 28-30)

Purpose of visit: To collect information about adverse events and, for selected subset, to draw blood for antibody levels.

Procedures:

- Home visit - AE follow up for all infants: Procedures: As described in visit 3 (above).
- Clinic visit - AE and 28 days blood sample for subset: As described in visit 6 (above plus blood sampling as described in SOP HPA1/1.

8.5.1.10 Visit 10, AE follow up -home visit (day 56 +/- 3 days)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).
- Fieldworkers give parents/guardians children appointment for visit 12 at PHC.

8.5.1.11 Visit 11, Reminder visit- home visit (day before Visit 12)

Purpose of visit: To remind parents/guardians of the clinic appointment.

Procedures:

- Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

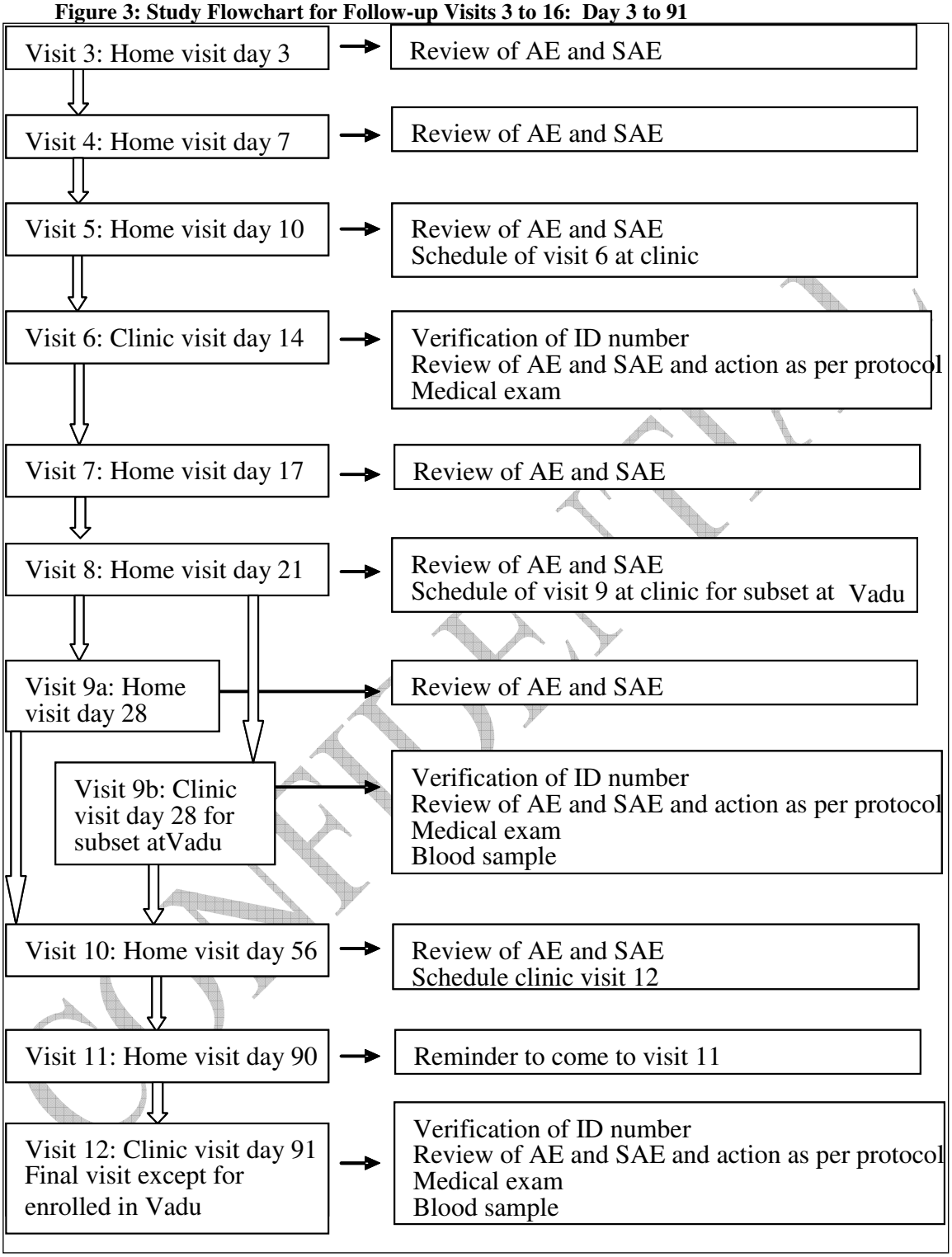
8.5.1.12 Visit 12, AE follow up- clinic visit (day 91 +/- 7 days)

Purpose of visit: Ascertainment of primary trial outcome in all participants.

Procedures:

- As described in visit 6 (above). Structured questionnaire for adverse events, medical examination, including directed neurological examination, recorded in CRF (SOP VAD 5/1). Blood sample from all participants (SOP HPA 1/1)

Figure 3: Study Flowchart for Follow-up Visits 3 to 16: Day 3 to 91



8.5.1.13 Visits 13 and 14, home visits for all children enrolled in Vadu district (day 182 and 252, Figure 4)

Purpose of visit: To collect information about adverse events.

Procedures:

- As described in visit 3 (above).
- Fieldworkers give parents/guardians children appointment for visit 15 at PHC.

8.5.1.14 Visit 15, home visit for subset of children enrolled in Vadu district (pre-Visit 16)

Purpose of visit: To remind parents/guardians of the clinic appointment.

Procedures: Fieldworkers will ensure that parents/guardians have arranged transport to the PHC, and tell them that the costs of travel and time spent on the visit will be reimbursed.

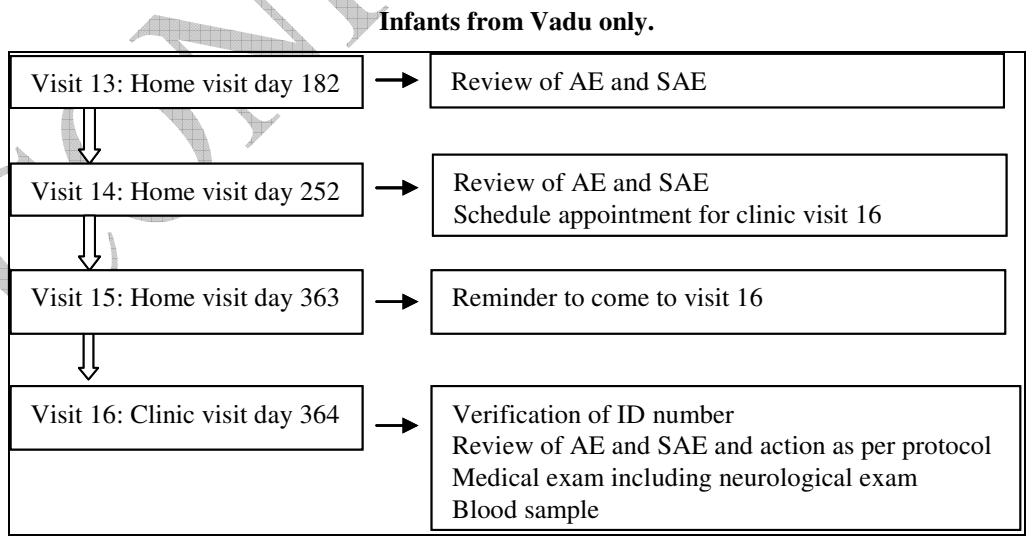
8.5.1.15 Visit 16, clinic visit for all children enrolled in Vadu district (day 364 +/-7 days)

Purpose of visit: Ascertainment of adverse events in all enrolled children in Vadu district and, for selected subset, to draw blood for antibody levels one year after vaccination.

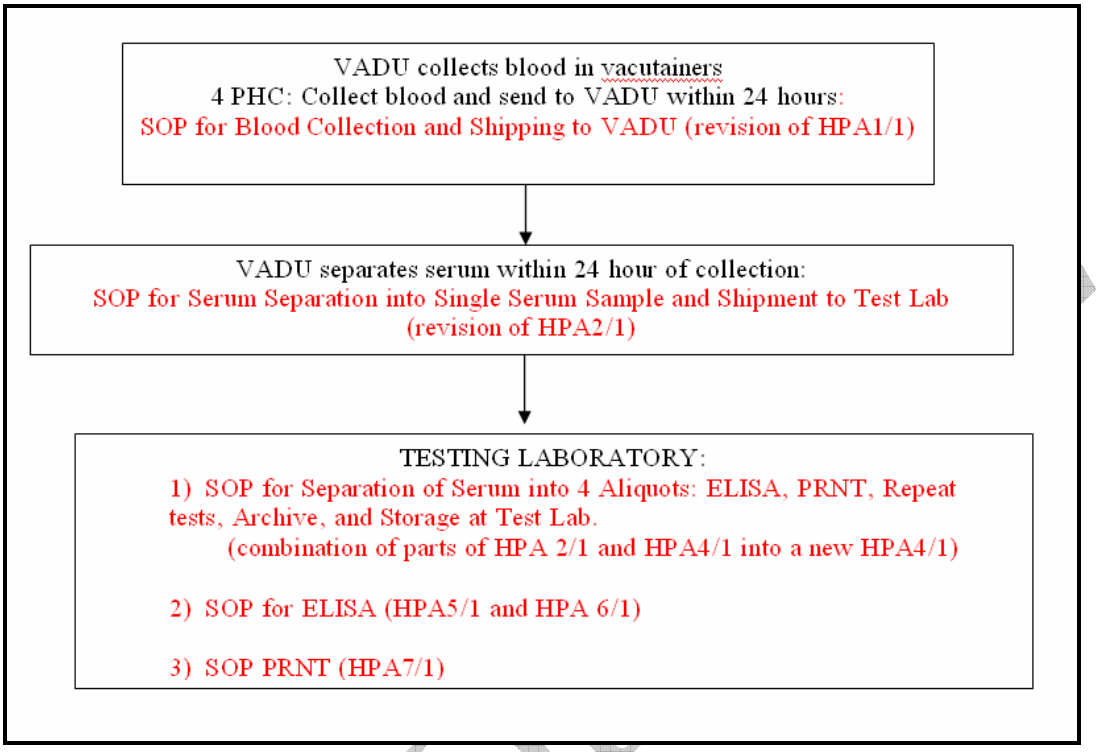
Procedures:

- Clinic visit: As described in visit 6 (above). Structured questionnaire for adverse events, medical examination, including directed neurological examination, venepuncture, recorded in CRF (SOP HPA 1/1).

Figure 4: Study Flowchart for Follow-up Visits 13 to 16: Day 182 to 364.



8.6 Sample collection, storage, tracking, processing, archiving



8.6.1 Collection

- Blood will be taken by venepuncture from participants and collected in gel separation tubes see SOP HPA 1/1

8.6.2 Sample labelling, processing, storage and transport

Each sample will be coded with a unique accession number that permits tracking of the sample through receipt, analysis and reporting. Blood and serum samples (including aliquots of serum) will be labelled as described in section 6.3.2 (blinding) and SOP VEL 3/1.
Each clinical sample will be accompanied by a form to clearly indicate sample coded number and date of collection and what analyses should be performed on it. (SOP HPA 1/1).

- Whole blood samples will be held at 2-8˚C (in ice-chilled, insulated vaccine carriers)
- Whole blood samples will be transported to initial processing site (Laboratory at SSH) on the same day as sample collection.

8.6.2.1 At initial processing site (Lab Vadu Rural Hospital)

- Whole blood samples will be held at 2-8˚C until processing (serum separation) within 24 hours of blood collection. Separation of serum is described in SOP HPA 2/1
- Serum samples will be stored in non-gel tubes.

- Serum samples will be frozen at minus 20˚C.
- Serum samples are sent to testing laboratory once a week.

8.6.2.2 At ELISA/PRNT testing laboratory

- Samples are separated into 4 aliquots (ELISA, PRNT, repeat tests and archiving) SOP HPA 4/1
- Serum samples will be held at minus 20˚C until ELISA or PRNT testing is undertaken

8.6.2.3 Storage of reserve aliquots of serum at testing laboratory in Pune

- Reserve aliquots of serum will be held at minus 20˚C until required or for a minimum of 5 years post-licensure, as required by Indian regulations (See SOP HPA 4/1 for storage after testing).

8.6.2.4 Transportation of samples

- Whole blood samples will be transported from sampling sites to the initial processing site (SSH) by designated trial staff at 2-8˚C (in vaccine carriers).
- Further transportation will be undertaken by a designated laboratory technician from Vadu Rural Hospital and will comply with Indian regulations. Serum samples will be held at minus 20˚C during transportation.

8.6.3 Sample processing

8.6.3.1 Laboratory measurement of immunogenicity

- All serum samples will be tested by Enzygnost Anti-measles-virus/IgG ELISA (Dade-Behring, Marburg, Germany) (SOP HPA 5/1 and SOP HPA 6/1).
- In addition, all specimens with Enzygnost optical density readings <0.1 will be tested by plaque reduction neutralization test (SOP HPA 7/1).

8.6.4 Sample archiving

Unused portions of serum samples will be held at minus 20˚C until a minimum of 5 years post licensure. Samples will be catalogued to allow rapid relocation as required.

8.7 Measurement of safety outcomes

Clinical events following vaccination will be investigated under the categories of: immediate adverse events (IAE); serious adverse events SAE, or other adverse events.

- Data on adverse events will be collected by Medical Officers at clinic visits (Days 0, 14, 28, 91 and, for infants in the Vadu area, day 364), and field workers at home visits (Days 3, 7, 10, 17, 21, 28, 56 and 182, 252 for subset of infants).
- Any adverse event detected during home visits that are graded moderate or severe (see Section 8.7.5, Visit 3 and Visit 6) or are unexpected will be immediately reported to the On-Call Medical Officer, who will examine the child, report the AE and treat, or refer for treatment, as required.

- At the end of each day, all CRFs with data about adverse events will be checked by a Study Medical Officer to ensure that all adverse events that are graded moderate or severe have been followed up, and that reports appear consistent.

8.7.1 Definitions

Acute clinical reactogenicity

Acute reactogenicity includes the expected reactions to vaccination occurring during the first 14 days post vaccination, reported at home visits, or clinic visits.

Adverse event (AE)
Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigation) product, whether or not related to the medicinal (investigation) product.[43] This definition includes all SAE and IAE.

Anaphylaxis
Exaggerated acute allergic reaction, occurring within 2 hours after immunization.[44]
Anaphylaxis is a clinical syndrome characterized by sudden onset AND rapid progression of signs and symptoms AND involving 2 or more organ systems. [45]

Immediate Adverse Events (IAE)
Any adverse event, serious or otherwise, occurring within 30 minutes of vaccine administration. This definition includes SAE occurring in this time period.

Serious adverse event (SAE)
Any adverse event that, at any dose, has one or more of the following attributes:[43 46]

1. Results in death.
2. Is life-threatening.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
4. Results in persistent or significant disability/incapacity.
5. Results in an important medical event that may not be immediately life-threatening or does not directly result in death or hospitalization, but which may jeopardize the patient.

Unexpected adverse drug reaction
An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. that listed in the investigator's brochure). [42, 46 43, 47]

8.7.2 Adverse event detection, management and reporting

Information about adverse events will be collected through a combination of: structured questionnaires administered at study clinic visits or home visits; active surveillance for events requiring medical treatment or hospitalization, or deaths; and reports from parents/guardians, who will be told by fieldworkers to report any medical conditions immediately on the Study Emergency Number and to seek treatment at SSH or KEM Hospital as appropriate.

The management of patients with adverse events and reporting procedure are described in detail in SOPs WHO 1/1, WHO 2/1 and WHO 3/1. In brief, the investigator/institution will ensure that adequate medical care is provided to a subject for any adverse event. At enrolment, parents/guardians will be given numbers to contact in the case of emergency and informed of other means of seeking medical treatment if they have no immediate access to a telephone. They will also be informed of how to seek medical treatment from either study staff or their local PHC for less urgent conditions. Contact has been made with PHCs regarding their participation in the treatment of study participants who attend PHCs and the reporting of these AE to the research team. SSH is currently the central referral hospital for the study area and in addition oversees PHCs in the Vadu region. Onward referrals from SSH will be to KEM Hospital (which administers SSH). The trial co-investigator at KEM will receive referrals from trial medical officers and ensure that prompt treatment is given. The co-investigator will also ensure mechanisms are in place so that any treatment of a subject in KEM Hospital which does not arrive via the referral method is promptly reported to him. He will monitor clinical care of any trial participant for the duration of their stay at KEM.

The principal investigator is responsible for ensuring that all serious adverse events are reported immediately by telephone or fax to the DSMB and the sponsor, as described in SOP WHO 3/1.

Participants will be reimbursed for expenses incurred in connection with their participation in research. During the period of research if the participant requires treatment for complaints other than those being studied, necessary free ancillary care or appropriate referrals will be provided.[48] The parents/guardians will be informed about the investigations regarding the event.

8.7.2.1 Immediate Adverse Events

See SOP VAD 4/1 (vaccination day)

Pulse rate, respiratory rate, blood pressure and axillary temperature will be measured prior to vaccination and during the 30 minute direct observation period. These will be recorded in the CRF.

Symptoms and signs of immediate reactions, treatments given and outcomes will be recorded and reported, as described in SOP VAD 4/1. Any serious events will be managed and reported as described in SOP WHO 2/1.

8.7.2.2 Additional adverse events

All non-serious adverse events will be assessed using structured questionnaires, either at clinic or home visits. The timing of these visits is described in section 8.5 and SOP VAD 5/1

The choice of specifically solicited adverse events is based on:

- Events observed in the phase 1 MAP trials in India;
- Additional adverse events seen in previous studies of aerosolized measles vaccine;
- Adverse events known to be associated with subcutaneous measles vaccination;
- Adverse events observed in trials of other vaccines administered by the respiratory route.

Detailed list of solicited adverse events and specific grading structure for each event type on a 4 level scale from 0 (none) to 3 (severe) are described in Appendix 3.

8.7.3 Adverse event reporting procedure

- All adverse events occurring during the trial should be accurately reported in case report form (SOPs WHO 1/1, WHO 2/1, WHO 3/1).
- Adverse events graded as **severe** will be reported by the PI to the WHO focal point within 24h of first knowledge by the Principal Investigator or any trial staff. The DSMB will be informed of these by the WHO focal point within 24 hours.
- The Principal Investigator will report all SAE immediately (within 24h of first knowledge by the principal investigator or any trial staff) to the WHO Focal Point and local ethical committees; even if the adverse event is considered not to be related to the investigation product. Notification will be made by fax and/or by telephone or email communication.
- The Principal Investigator should send promptly, within five working days, the serious adverse event report by fax, email or express mail to the WHO Focal Point.
- Any relevant information concerning the SAE that becomes available after the SAE report has been sent (outcome, precise description of medical history, results of the investigation, copy of hospitalization report, etc.) will be forwarded by the Principal Investigator as soon as possible to the WHO Focal Point.
- For reports of deaths, the investigator should provide WHO Focal Point with any additional requested information, e.g. autopsy reports and terminal medical reports. The anonymity of the subjects shall be respected when forwarding all information
- The WHO Focal Point will comply with the Indian regulatory requirements regarding SAE. The National Regulatory Authorities, WHO ethical committee, DSMB and Clinical Trial Monitor will be notified (e.g. by telephone, facsimile transmission, or in writing) by the WHO focal point as soon as possible (ideally within 24 hours) but no later than 7 calendar days after first knowledge by the WHO focal point that a case qualifies as an SAE, followed by as complete a report as possible within 8 additional calendar days. See SOP WHO 3/1.

8.7.4 Follow-up of Adverse Events and Serious Adverse Events

All AE will be followed up until the participant has reached one of the endpoints as described here.

- An adverse event that is likely to be related to the product and that persists at the end of the trial, or any SAE occurring after termination of the trial and likely to be related to the product, will be followed up by the investigators until the participant has completely recovered, recovered with sequelae, or died.
- All other AE will be followed up until the participant has completely recovered, or they complete the study.

A thorough investigation will be conducted to determine causality. Adverse events will be recorded in detail during the course of the trial, irrespective of the possible causal relationship with the measles vaccine.

8.7.5 Grading of adverse events

Adverse events will be graded using a specific grading structure for each event type on a 4 level scale from 0 (none) to 3 (severe). These are described in Appendix 3

8.7.6 Classification of association of events with vaccination

The investigator will assess whether any adverse event is related to the immunization using the following scale and according to the following definitions (based on Workbook for Investigators, WHO/TDR).

- **Not related:** The event is clearly related to other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy.
- **Unlikely to be related:** The event was most probably produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy, and does not follow a known response pattern to the study vaccine.
- **Possibly related:** The event follows a reasonable temporal sequence from the time of study vaccine administration and/or follows a known pattern to the study vaccine but could have been produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy.
- **Probably related:** The event follows a reasonable temporal sequence from the time of study vaccine administration and/or follows a known response pattern to the study vaccine and could not have been produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy. These events include but are not limited to those that require medical attention and result in withdrawal from follow-up. Any available medical records needed to accurately describe the event will be obtained from the appropriate health care provider.
- **Most probably related:** The event follows a reasonable temporal sequence from the time of vaccine administration; and/or follows a known response pattern to the trial vaccine; and could not have been produced by other factors such as the patient's clinical state, therapeutic intervention or concomitant therapy; and either occurs immediately following trial vaccine administration, or there is positive reaction at the application site.
- **Insufficient data to assess:** There is not enough clinical and/or laboratory information to suggest the relationship between the experience and the trial product.

A report on all **Severe** Adverse Events and all **Serious** adverse events will be submitted to the DSMB within 7 days, following the Terms of Reference of the DSMB for the Measles Aerosol Project (Appendix 2). The DSMB will make the final decision on the classification of serious and severe adverse events.

In addition, the DSMB will receive summary reports of all AE quarterly (see DSMB TORs).

8.8 Measles surveillance in the study area

A surveillance system is to be established (as an entity separate from the trial) in the entire blocks containing the trial areas. It will be established as described in the WHO Best practices for measles surveillance[49] and in the Field Guide -Measles Surveillance & Outbreak

Investigation Guidelines[50] (2005) Government of India, Department of Family Welfare New Delhi.

It is expected that this surveillance system will be operational before the start date of the trial.

The primary purpose of measles surveillance will be to detect, in a timely manner, all areas in the trial whether the measles virus is circulating. Surveillance will be undertaken with support from the Immunization Programme of the State of Maharashtra and with the National Polio Surveillance Project in India. The surveillance system will involve the following:

- **All the health facilities/health providers** in the study area will be trained to implement timely notification of clinically diagnosed measles (probable) cases will be promoted to detect cases and outbreaks. A designated surveillance medical officer (SMO) will conduct monthly active searches in the registry files (outpatient and inpatient records) to ensure all probable measles cases are reported. All measles outbreaks will be serologically confirmed to differentiate them from other fever and rash outbreaks. The surveillance data should be analysed at all levels to determine and improve the immunization strategies.
- In addition, **during the home and clinic visits** the field workers, medical doctors and nurses will solicit information on the presence of cases of rash and fever illness in the household and in the neighbourhood where the infants enrolled in the trial reside. Any probable case will be reported to the designated SMO for it investigation as per the Field Guide -Measles Surveillance & Outbreak Investigation Guidelines.

Every confirmed case of measles (i.e. laboratory or epidemiological confirmation) and any confirmed outbreak in the study area or surrounding areas will be reported within 24 hours to the Principal Investigator by the State of Maharashtra EPI Manager and by the designated SMO. All confirmed measles cases will be managed according to the National guidelines including the administration of Vitamin A.

The designated SMO at each district will collect the surveillance forms (VPD - H002) from all the reporting units, collate them in designated format (VPD - D001 form) and compile the district report. The District Immunization Officer (DIO) will send this routinely every week to the State EPI officer, RC or State SMO by Tuesday of each week. The VPD-D002 form should be used to track completeness and timeliness of reporting from the reporting units. This information should be sent on a quarterly basis by the DIO to the State Programme Officer.

Moreover, the State of Maharashtra will share with the PI investigator monthly updates on the performance of the surveillance systems using the indicators defined in the Field Guide - Measles Surveillance & Outbreak Investigation Guidelines.

The DIO/SMO will analyse the available district/block data on a weekly basis, using and reconciling case information from different surveillance systems to identify outbreaks of measles. As per national guidelines, the presence of measles outbreak should be verified if five or more than five clinically diagnosed cases of measles are identified in a block in a week, or five or more than five confirmed cases of measles occur in an area bordering several blocks in a week, or one or more than one death due to measles occurs in a block in a week.

If possible outbreaks are identified, measles outbreak investigations should be initiated as described in Chapter 5, Measles Outbreak Investigation of the Field Guide -Measles Surveillance & Outbreak Investigation Guidelines. In brief, the DIO and the SMO will oversee that the following actions will take place:

- Identifying the measles outbreaks that need to be investigated and assigning an outbreak number
- Mobilization of Epidemic Response Team (ERT)
- Orientation & planning meeting at the local level
- Conducting measles case search
- Collection and shipment of specimens to the laboratory
- Laboratory confirmation of the outbreak
- Data analysis
- Conversion of data to information for action
- Report writing
- Giving feedback
- Initiating actions including vaccination activities.

8.9 Procedure for monitoring subject compliance

Each subject will be given an appointment card for the next schedule visit to the clinic. If any subjects do not turn up on the scheduled date, the investigator will send the designated field worker to their houses and request them to come to the clinic.

9 Assessment of Immunogenicity

9.1 Primary outcome

Measles seropositivity at day 91 post-vaccination.

Measles seropositive specimens are:

- specimens tested by Enzygnost Anti-measles-virus/IgG (Dade-Behring, Marburg, Germany) enzyme-linked immunosorbent assay with optical density signal **≥ 0.1, or**
- specimens tested by Enzygnost with optical density signal **< 0.1**, that are subsequently tested by plaque reduction neutralization test with a result of ≥ **120 mIU**/ml measles antibody titre.

For further details, please see Sample processing (HPA 1/1, WHO 2/1, WHO 3/1, WHO 4/1), Measurement of outcomes (SOP HPA 5/1, HPA 6/1, HPA 7/1)

9.2 Secondary outcomes

For all infants:

- Geometric mean titres, pre-vaccination and at day 91;
- Seroconversion (defined as a change from Enzygost OD <0.1 to OD ≥0.1 or PRNT from <120 to ≥120 mIU/mL) measured in paired samples from day 0 and day 91;
- As covariates for inclusion in multivariable analyses: age; sex; day 0 antibody titre; study site; crying when vaccine given (aerosol arm only); presence of respiratory tract symptoms at time of vaccination (aerosol arm only). In addition, random subsets of 80 infants each with OD 0.1-0.2 and OD>0.2 at day 91 will be assessed for PRNT antibody concentrations to determine the relationship between PRNT and Enzygost optical densities/concentrations and validate the use of the cut-off of 0.1 for PRNT testing.

For infants in the day 28 blood sample subset or the day 364 blood sample subset:

- Geometric mean titres to describe evolution of antibody response at days 0, 28, 91, 364;
- Seropositivity at 364 days in infants with antibody titre ≥ 120 mIU/ml at day 91.

10 Assessment of Safety

10.1 Primary outcome

Adverse events up to and including day 91 post-vaccination.

10.2 Secondary outcomes

- Frequencies of individual adverse events

For further details of assessment and management of adverse events, please see Section 8.7 and SOPs WHO 1/1, WHO2/1, WHO 3/1.

11 Statistics

11.1 Description of statistical methods

A detailed analysis plan will be finalised prior to locking of the database and commencement of statistical analysis.

11.1.1 Analysis sets

- Primary analysis: per protocol (infants receiving the allocated vaccine and available for follow up at 91 days post-vaccination). For non-inferiority hypothesis, this is the conservative analysis;
- Secondary analysis: intent-to-treat analysis (all infants randomized, analysed in the group to which they were allocated, irrespective of intervention received) of primary and secondary outcomes.

11.1.2 Statistical methods, immunogenicity

- Primary analysis: calculate two-sided 95%CI for the difference in proportion seropositive at day 91.

- Secondary analyses: include calculation of 95% confidence intervals for seropositivity, seroconversion and geometric means as well as multivariable logistic and normal errors regression adjusting for covariates.

11.1.3 Statistical methods, safety

- Primary analysis: proportion (with 95% CI) with any adverse events in aerosol group and in subcutaneous group;
- Secondary analysis: proportions (with 95% CI) of individual adverse events.

11.2 Sample size

11.2.1 Immunogenicity

Total to be enrolled, 2000. Number available for primary analysis (after loss to follow up), 1600. These numbers are based on the following assumptions:

- Significance 2.5%, one-sided for non-inferiority, 5% two-sided for superiority;
- Non-inferiority margin 5%;
- Power for non-inferiority 90% if true seroconversion is 90% in both arms;
- 20% loss to follow up by day 91.
- Calculate two-sided 95%CI for difference in proportion seropositive at day 91. If lower limit of difference greater than -5% then conclude non-inferiority. If lower limit of difference greater than 0% then conclude superiority.

With 100 children sampled at random from those being followed up, based on a standard deviation of post vaccination titres of 0.43, differences of <1.5 fold in geometric mean titres between aerosol and subcutaneous groups between days 28 and 364 would be able to be detected at a 5% level with 80% power. Smaller differences between days 28 and 91, and 91 and 364 would also be detectable.

11.2.2 Safety

To estimate proportions with adverse events in aerosol and subcutaneous groups, the sample sizes above give acceptable precision.

With 1000 infants in each group the precision of estimation of adverse events is:

Observed proportion	95% confidence intervals		
	N=100	N=800	N=1000
0%	0.00 – 3.6%	-	0.00 – 0.37%
1%	0.02 – 5.44%	0.43 – 1.96%	0.48 – 1.83%
2%	0.24 – 7.04%	1.15 – 3.23%	1.26 – 3.07%
5%	1.64 – 11.28%	3.60 – 6.75%	3.73 – 6.54%

If no reactions are observed, the upper 95% confidence interval with a sample size of 1000 is 0.37%, or 1 in 270.

With 100 children sampled at random for follow up to 364 days, the precision for estimating the prevalence of rare events is low. If zero events are observed by day 364 the upper 95%CI will be 3.6% (1 in 28).

11.3 Criteria for the termination of the trial

The trial will be discontinued if a single SAE attributable to the aerosol vaccine is reported. The stopping rules have been determined by the DSMB following explicit written criteria (Appendix 2: Terms of Reference of the Independent Data Safety and Monitoring board).

11.4

11.5 Procedure for accounting for missing, unused and spurious data

11.5.1 Data handling and record keeping

See SOP VEL 2/1 for data management.

11.5.2 Data verification

See SOP VEL 2/1

Source documents including medical records and original laboratory results will be kept in a separate file at the investigator's office. Electronic CRFs will be downloaded with daily frequency at Vadu Rural Hospital dedicated computer and checked for completeness and accuracy. They will be sent to Vellore weekly. The SOP VEL 1/1 describes in detail the process for data collection including the use of audit trails to ensure data security. Printed copies of electronic CRFs will be kept in a room with limited access

11.5.3 Data entry

See SOP VEL 2/1

11.5.4 Data review

See SOP VEL 2/1

11.5.5 Data quality control

See SOP VEL 2/1

Besides range, consistency and missing value checks incorporated in the Study Builder software, further checks will be done using pre-tested syntax in SAS statistical software. If inconsistencies and out of range values are found, the Data Management Centre will produce a Query and Correction sheet for a subject with a query to the corresponding site PI. The PI or designee has to recheck the value and fill the correct value in the sheet with an authorized signature and send back to the Data Management Centre. Based on the filled Query and Correction sheet received from the Site, the data manager will update the Database.

11.5.6 Data sharing and analysis

Data will only be accessed by the sub-investigator in charge of data management and statistical analysis. The DSMB will receive quarterly unblinded reports. See SOP VEL 1/1

Outliers, missing values, and inconsistencies will be identified based on the Validation Check Specification document (in SOP VEL 2/1). If inconsistencies arise then the query will be raised at this level. Based on the query responses, corrections will be made in the original ACCESS database. Having completed all the corrections, the data will be exported to SAS software format. Data analysis will be carried out after all enquiries have been done and the database has been locked. Any data which is still missing after enquiries have been made will be dealt with as described below:

- Missing values
  For the per-protocol (PP) dataset, missing values will be treated as missing at random. For the intention-to-treat (ITT) dataset, the analysis will also be run with imputed values for the missing values, for the best and worst case scenarios. In the best case scenario, we will assume no adverse events occurred and the individuals are seropositive. In the worst case scenario, we will assume an adverse event occurred and the individuals are seronegative.

- Outliers
  For log 10 PRNT values [LPRNT], checks for outliers will be performed prior to the statistical analysis, as part of the data checking. The outliers will also be examined using standardized residuals >3 when looking at log 10 PRNT values [LPRNT].

12 Procedures for reporting any deviations from the original analysis plan

Any change in the planned analysis will be described and documented in the study report including the time (specifically whether prior to or after unblinding occurs) and reason for the change, the procedure used to decide on the change and the nature and content of the data available when the change was made.

12.1 Selection of subjects to be included in the analysis

12.1.1 Analysis sets

Primary analysis: per protocol (infants receiving the allocated vaccine and available for follow up at 91 days post-vaccination). For non-inferiority hypothesis, this is the conservative analysis;

Secondary analysis: intent-to-treat analysis (all infants randomised, analysed in the group to which they were allocated, irrespective of intervention received) of primary and secondary outcomes.

There will be one interim analysis for AE only (see section 12.1.3 below).

12.1.2 Immunogenicity

Primary analysis is to calculate the 2-sided 95%CI for the difference in proportion seropositive at day 91. If lower limit of difference greater than -5% then conclude non-inferiority. If lower limit of difference greater than 0% then conclude superiority.

Secondary analyses will include calculation of 95% confidence intervals for seropositivity, seroconversion and geometric means as well as multivariable logistic and normal errors regression adjusting for covariates.

Secondary analysis of factors associated with seroconversion/response, including:

- pre-vaccination titre
- Age (9m,10m,11m) (also look at interaction with intervention)
- Sex (also look at interaction with intervention)
- PHC
- Crying when vaccine given? (also look at interaction with intervention).
- (Another factor we considered before was acute respiratory infection)
- Explanatory variables for stratification of safety profile

12.1.3 Safety

Primary analysis is proportion (95% CI) with any adverse events in aerosol group with subcutaneous group;

Secondary analysis will describe frequencies of individual adverse events (proportions with 95% CI).

An interim analysis of safety data will be completed. For this analysis, the DSMB will use data from the first 100 children in each arm to complete 91 days of follow up. This will be done as soon as possible after these data are available.

12.2 Contingency plan in the event of confirmed measles cases and outbreaks

Measles cases occurring in trial participants would permit to examine directly the relative vaccine efficacy of aerosol and subcutaneous measles vaccination. Natural boosting of vaccinated individuals may affect the primary outcome of seropositivity at day 91.

If measles cases or outbreaks are detected by the surveillance system we will take the following special steps.

1. Support the implementation of routine Government of India activities for the investigation of cases and control of outbreaks, as appropriate Field Guide -Measles Surveillance & Outbreak Investigation Guidelines (2005) Government of India, Department of Family Welfare New Delhi).

2. **Document all the confirmed measles cases among the children enrolled in the trial or in their households.**

3. **Set up an alternative statistical analysis plan addressing the following elements:**

- Consider those infants confirmed as measles cases (laboratory confirmation) who had day 91 samples taken prior to their date of onset of measles to have unaffected day 91 results.
- Assume that all confirmed measles cases (laboratory confirmation) occurred among sero-negative infants and class them as such in the analysis (and class non-cases according to their ELISA/PRNT sero-status).

4. **If an outbreak is confirmed prior to completion of the recruitment period or the last day 91 follow up, then the recruitment period will be extended after the outbreak** to reach the estimated sample size of 2000 eligible children not confirmed as measles cases.

5. **If an outbreak is confirmed in Vadu DSS after 91 day follow up is completed but prior to the completion of the long term (day 364) follow up, there will be no attempts to recruit additional children** and they will be analysed as described in point 3 above

13 Direct access to Source Data/Documents documents

The investigator will provide written agreement that the investigator /institution(s) will certify that the monitors, the auditors, the Ethics Review Committee members DSMB members and, the regulatory authority representatives (DCGI) will be granted direct access to his original direct access to source data/documents and medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations in India.

14 Quality control and quality assurance procedures

14.1 Protocol changes and protocol deviations

Once the trial has started, the investigator must adhere to the protocol and will ensure that it is strictly followed. The investigator, or person designated by the investigator, will document and explain any deviation from the approved protocol. The investigator will promptly report all important amendments and deviations related to non-compliance with the following protocol provisions: inclusion and exclusion criteria, randomization procedures, blinding procedures, informed consent procedure, assignment of subject identification numbers, dosing and assessment schedules, reporting and procedures for adverse events and, any other protocol deviations. The report will also include description of actions taken to prevent recurrence of the detected deviations.

The investigator will be responsible for reporting any protocol deviations or protocol amendments to the Local Ethics Review Committee. The Sponsor will be responsible for reporting to the National Regulatory Authority and the WHO Ethics Review Committee.

No deviations from, or changes to, the protocol will be initiated without prior written Ethics Review Committee approval/favourable opinion of an appropriate amendment.

An exception to this will be in situations when it is necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial. Under these exceptional circumstances, the investigator should report as soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted to the Ethics Review Committee for review and approval/favourable opinion, and, to the sponsor for agreement.

In the report of the study, protocol deviations will be summarized and grouped into different categories, including:
– those who entered the study even though they did not satisfy the entry criteria.
– those who developed withdrawal criteria during the study but were not withdrawn.
– those who received the wrong vaccine or incorrect dose.
– those who received an excluded concomitant treatment.
- other deviations

Any deviation(s) from the original statistical plan will be described and justified in protocol amendments and/or in the final report, as appropriate.

Using Appendix 4 (Form 1 and Form 2) individual subjects with protocol deviations will be reported and documented.

The sponsor will implement and maintain quality assurance and quality control systems with written SOPs to assure that the trial is conducted and data are generated, recorded, and reported in compliance with the protocol, GCP and the Indian national regulatory requirement(s) requirement for investigational product. This includes the use of independent trial monitors and independent external audits.

14.2 Trial monitoring

Independent clinical trial monitors will be appointed. The monitors will be under the supervision of an experienced Contract Research Organization that will be in charge of ensuring compliance with Good Clinical Practice and Good Laboratory Practice, A trial initiation monitoring visit will be done prior to enrolment of any volunteer at a site and the clinical monitor and investigator will review the protocol, logistics and all trial related procedures. This includes information on the vaccine and aerosol delivery device, procedures for obtaining informed consent, procedures for reporting SAE and procedures for completing the CRFs.

Site monitoring visits will be scheduled by the clinical monitor on a regular basis. During these visits, information recorded in the CRFs will be verified against source documents for accuracy and completion. The clinical monitor will review the informed consent procedures, product accountability and storage, trial documents and trial progress. The clinical monitor will verify that the investigator follows the approved protocol or amendments (if any). S/he will observe trial procedures and will discuss any problems with the investigator.

Monitoring visits will be recorded in the Monitoring Log at the investigator's site, and at the end of the trial a copy of the completed log will be returned to the sponsor.

14.2.1 SAFETY MONITORING

A Data Safety and Monitoring Board (DSMB) will review safety data collected through day 364. See DSMB Terms of Reference

14.3 Consent forms

To allow compliance with GCP principles, the parent/guardian of each infant will be asked for consent regarding direct access to the source documents for monitoring, audit, and inspections. The agreement covering the use of the data or analysis has to be documented in writing, together with the written informed consent for trial participation.

15 Ethical considerations in study design

15.1 Informed consent

See SOP VAD 4/1 and section 8.5, visit 1 and 2 for details
The parents/guardians of potential participants will have the study explained in depth to them and, in addition, will receive a patient information sheet (Study Document 4) and have the opportunity to ask questions or trial staff prior to giving consent. Informed, witnessed, written consent will be recorded on behalf of each participant (by parent/guardian) for trial participation, and other specific aspects of the trial e.g. for day 364 follow up, photography of subjects (Study Document 5).

15.2 Ethical and regulatory approvals

The protocol will be submitted for approval to the Institutional Ethical Committee of KEM Hospital, Pune. In addition, the WHO Ethics Review Committee will review and approve the study.

In addition the protocol will be submitted to the Scientific Advisory Committee of the MOHFW, India. Moreover, the protocol and supporting documentation will be submitted to the Drugs Comptroller General, India in accordance with the Drugs and Cosmetics Act, Schedule Y requirements.

Community leaders will be informed about the social benefits of the study that the data collected in this study will be valuable to the development of a simpler and safer method for measles vaccination and may result in greater numbers of children being protected against measles infection.

15.3 Study data confidentiality

On initiation of the study, the investigator will prepare a file containing documents related to the trial. During the study, the investigator will be responsible for updating the file and

regularly adding trial related documents. The investigator will keep the file in a locked cabinet, in a secure area accessible only to the investigator and authorized study staff.

The investigator file and associated source documents will be retained for at least five years after the licensure of the measles aerosol vaccine. Patient identification codes should be kept for at least 15 years after completion of the trial. **Written approval from all sponsors must be obtained prior to destroying records.**

All study related documents will be kept in locked cabinets at the study site. Filing cabinets with the trial data and the participant information will be locked and accessed only by authorized persons from the sponsor and Regulatory Authorities.

Personal volunteer data will be kept confidential and the privacy of all subjects will be protected in so far as permitted by law. Subject names will appear on the initial documents (CRF) and once enrolled study documents will refer to the subject code (second initial of each name) and assigned study code number Only personnel involved with the study conduct and local and international regulatory agencies may review these records. The investigator will keep in the investigator's files a Subject Identification List and Screening/Enrolment Log (including complete name, age and address).

All proprietary or confidential information communicated to the investigator by or for WHO or communicated to the investigator during the course of and/or as a results of the clinical study is the exclusive property of WHO and/or SIIL, and the investigator will ensure the same shall be kept strictly confidential by him/her or any other person connected with the clinical study and shall not be disclosed, either orally, or in written form, by him/her or such person to any third party without prior written consent of WHO. The investigator shall communicate the results of the clinical study promptly to WHO or its designee.

All rights and interests worldwide in any inventions, know-how, or other intellectual or industrial property rights which arise during the course of and/or as a result of the clinical study which is the subject of the Protocol or which otherwise arise from the information or materials supplied under this Protocol, shall be assigned to, vest in and remain the property of WHO.

15.4 Revaccination of subjects with inadequate immune response

It is ethically responsible to ensure that all children who are shown to have lower than protective antibody titres 91 days post vaccination are revaccinated using standard measles vaccination methods (i.e. subcutaneous measles vaccine as used in this study). To this end, all children found to have PRN titres <120mIU/ml at 91days post vaccination, regardless of vaccination route, will be revaccinated by the subcutaneous route by a team independent to the trial follow up teams. Serological testing will be conducted as soon as is feasible after samples are drawn. Revaccination will occur as soon as possible and a maximum of 6 weeks after the results for each individual is known. Unblinding will only occur to the level of child identification. Study allocation will not be unblinded. Subsequent serological results from children who are revaccinated will not be included in analyses, but they will continue to be monitored for adverse events.

16 Data handling and record keeping

16.1.1 Data archive

Detailed procedures for data archiving at Vadu Rural Hospital and CMC Vellore are described in SOP VEL 2/1

After completion of the study, the Data Management Centre will keep copies of Databases, all CRFs and study related documents in a secure place for at least 5 years in compliance with GCP and regulatory requirement for investigational products.

16.1.2 Record keeping

See SOP VEL 2/1

After completion of the study, the investigators will keep his copies of all CRFs and source documents in a secure place for at least 5 years in compliance with GCP and regulatory requirements for investigational products.

17 Financing and insurance

This study will be supported by World health Organization - Initiative for Vaccine Research. In accordance with GCP guidelines, the Sponsor will provide an insurance policy for the conduct of the trial.

18 Publication policy

This study will be performed within the framework of a larger programme of research, consisting of several individual projects that relate to the development of safe and effective products against measles. In this connection, it has been deemed necessary for all rights to the results of the work to be performed under this project to vest exclusively in WHO. Consequently, General Conditions for publication will be as follows:

1. The Institution shall deliver to WHO at the end of the clinical trial the results of the work performed under this Agreement, unless otherwise agreed.

2. The Institution shall maintain in confidence all results of the work performed under this clinical trial, including information and tangible products of any kind whatsoever, unless authorized by WHO to disclose or publish such results. Notwithstanding the foregoing, the parties hereto recognize the public health benefits that may be achieved through the publication of the results of the project in accordance with normal academic practice.

Therefore, the parties agree that subject to the terms of this general condition 2, the Institution and the Principal Investigator may publish or present the results of this project. In the event of any intended publication or presentation as aforesaid, the Institution and Principal Investigator shall transmit to WHO for its review any material intended to be published or otherwise publicly disclosed, sixty (60) days before it is transmitted to any publisher, editor, referee or

meeting organizer. Within this sixty day review period, WHO may make a written request to the Institution and the Principal Investigator to remove from such material:

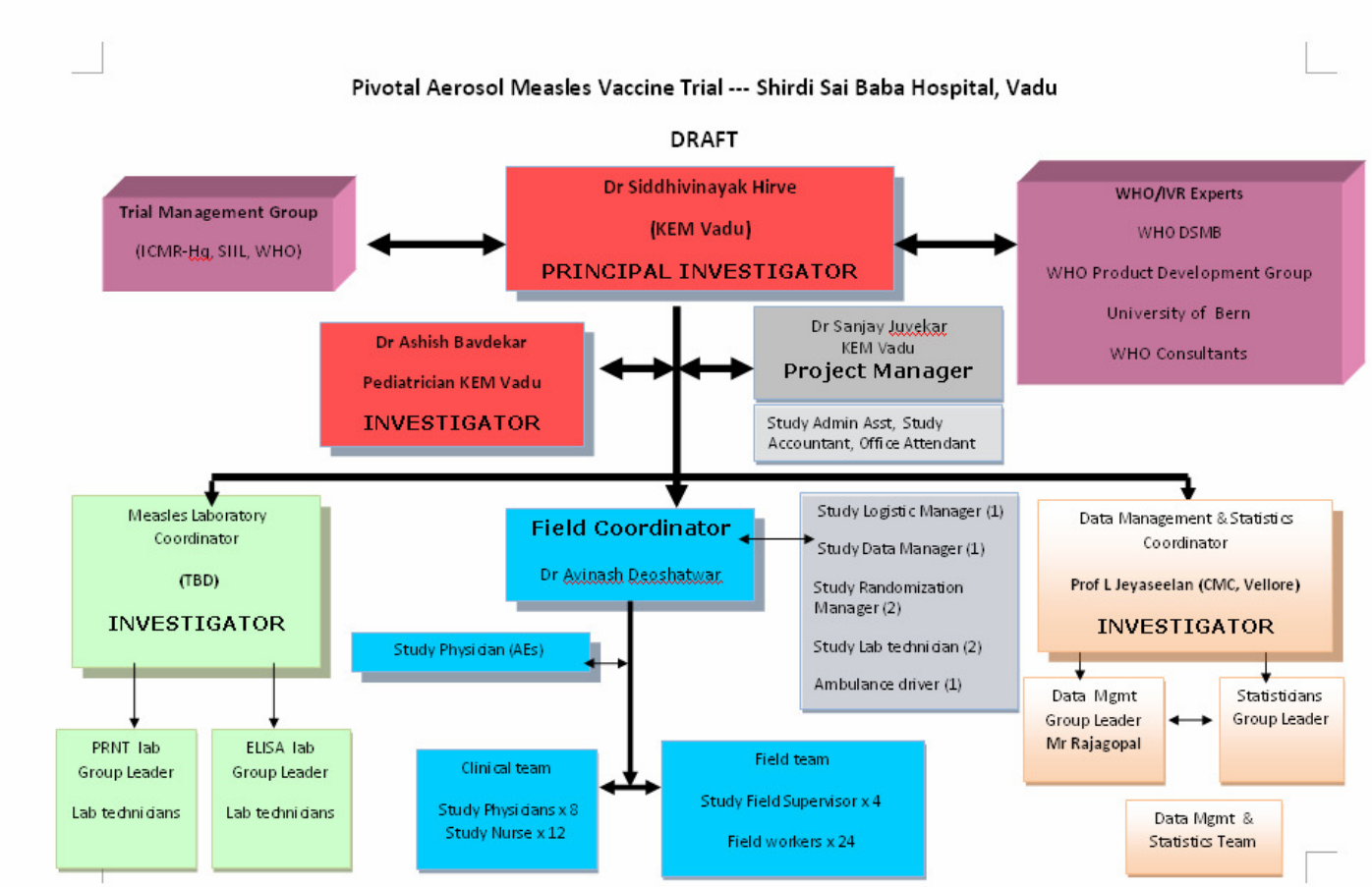
- any proprietary and confidential information disclosed by or on behalf of WHO to the Institution and the Principal Investigator hereunder, as well as
- any results generated hereunder, but only to the extent in order to achieve WHO public sector objectives- WHO reasonably deems it necessary to maintain such results in confidence in order to promote the development of such results into a useful health related product in collaboration with a commercial enterprise.

The Institution and the Principal Investigator agree to comply with any such request. Except to the extent WHO has made a request as aforesaid within the sixty day review period, the Institution and the Principal Investigator shall have the right to proceed with the publication or other public disclosure without further notice to WHO.

3. All rights to the results of the work to be performed under this project, including but not limited to copyright and the right to apply for, hold and exercise patent rights in respect of any invention resulting from the work, are vested exclusively in WHO. The Institution and Principal Investigator shall provide WHO with their full cooperation to permit the effective exercise of the above rights."

19 Organisational structure

Numbers of staff required (study field workers, nurses, doctors)



19.1.1 List of references to literature and data that are relevant to the trial, and that provide background for the trial

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20 Appendices

Appendix 1: Diagram of Vaccine and Nebulizer Components

Appendix 2: Terms of Reference of the Independent Data Safety and Monitoring Board (DSMB). Updated, Nov 26, 2009 to "Data and Safety Monitoring Board Charter"

Appendix 3: Adverse Events, Descriptions and Definitions

Appendix 4: Forms for Protocol Changes

Appendix 5: Study Documents:

SD-1 Investigator's Brochure
SD-2 Study Synopsis
SD-3 Study Timetable
SD-4 Patient/Parent/Guardian Information Sheet
SD-5 Informed Consent Forms for Parent/Legal Guardian
SD-6 Sample CRFs

Appendix 6: Standard Operating Procedures (SOP) for the Study

Note: All SOPs are in draft format and will be finalized during training sessions before the start of the trial.

SOP No.	Title
Vadu Site	
VAD 1/1	Communication with Ethics Committee
VAD 2/1	Training Policy for Study Staff
VAD 3/1	Subject Identification and Recruitment Procedures
VAD 4/1	Informed Consent, Eligibility, Randomization and Vaccination (procedures on day of vaccination)
VAD 5/1	Procedures for Follow-up for Measles Aerosol Clinical Study (day 3 to 365 Post Vaccination)
WHO	
WHO 1/1	Adverse Event Reporting and Management
WHO 2/1	Immediate Adverse Event Reporting and Management
WHO 3/1	Serious Adverse Event Reporting and Management
WHO 4/1	How to Transport, Store and Handle Measles Vaccine, Diluent, Syringes and Safety Boxes
WHO 5/1	Translation back/translation
WHO 6/1	Administration of the Measles Vaccine by the Subcutaneous Route
WHO 7/1	Procedures for Entering Data into CRFs on PDA
Vellore	
VEL 1/1	Statistical Analysis Plan
VEL 2/1	Data management plan (quality assurance and correct allocations administered)
VEL 3/1	Blinding laboratory to allocation
VEL 4/1	Randomization
Health Protection Agency	
HPA 1/1	Collection of Blood Specimen (Venous Blood) and Shipping to Vadu Shridi Saibaba Hospital (SSH)
HPA 2/1	Separation of Serum from Blood Samples at Vadu Laboratory and Transport to Testing Laboratory
HPA 3/1	Safe Disposal of Blood Sample Residues and Contaminated Waste
HPA 4/1	Aliquoting of Serum Samples and Storage in Freezers at Testing Laboratory
HPA 5/1	Detection of Anti-Measles Virus IgG by Enzyme Immuno Assay (ELISA) Using Enzygnost Dade Behring Kit and the BEP III ELISA Processor
HPA 6/1	Detection of Anti-Measles Virus IgG by Enzyme Immuno Assay (ELISA) Using Enzygnost Dade Behring: Manual Performance
HPA 7/1	Plaque Reduction Neutralization Test (PRNT) for Measles Antibodies
HPA 8/1	Checking the Calibration of Variable Volume Pipettes
Nektar/Aerogen	
NEK 2/1	Preparation of Vaccine and Nebulizer and Administration by Aerosol Using Aerogen's Clinical Nebulizer to Infants from 9 – 12 Months.

Please note that several minor corrections have been made to the protocol, mostly typographical errors and a few discrepancies in descriptions different parts of the protocol. These are described below and are highlighted in the protocol.

1) The Protocol number was assigned according to the numbering system decided for the Phase I clinical trials (Sponsor/project/country/number). This was WHO/MAP/IN/002. However, this was typed incorrectly on page 2 of the Protocol as WHO/MAP/IND/02. This has been corrected. This number is now in agreement with all other study documents (CRF, ICF, IND and all SOPs).

2) Correction of the spelling of Principal: from Principle to Principal. Page 6, and page 45.

3) Section 4.3.5.4 page 17. Trial Setting Vadu Rural Area.

   Statement that three additional health centres have been added to the area for recruitment has been added to page 17. The information in the Protocol had not been updated to give details of these areas but the information is available on request.

4) Minor revision to section 5.4.1 Immunogenicity page 21

   To collect blood from a subset~~s~~ of children at 28 days and one year after vaccination. The subset~~s~~ will be randomly selected from all children enrolled in the Vadu study area because they will be able to be followed up within the infrastructure of an existing demographic and health surveillance system.

   Subset is singular.

5) Section 6.3.1 Randomization. See SOP VEL 4/1 page 23

   This procedure was separated from SOP VEL 1/1 “Statistical Analysis” as a separate SOP.

6) Section 6.3.1.2 Sequence generation page 23

   "Stratified by area" has been removed, now reads: "The allocation sequence will be computer-generated random numbers, in permuted blocks, ~~stratified by area~~, generated by an independent statistician."

7) Section 6.4.2.2 title corrected page 26

   Title now “Method of packaging and labelling of the ~~vaccine~~ device.

8) Section 7.1 Inclusion criteria page 28

   Revised point 3 to include subset of infants at Vadu.

Now reads: "Parent/guardian willing for child to be randomized and willing for child to be followed up for at least 91 days (364 days for Vadu subset)."

9) Section 8.2 and 8.3 page 29. Delete "plaque forming units" and replace with "CCID50".

   The potency assay for the measles vaccine is measured in 50% Cell Culture Infectious Doses (CCID50) not in pfu. Change for accuracy of units.

10) Section 8.5.1.2 Visit 2 page 32.

    Assignment of a unique Subject ID number is described in SOP VAD 4/1, not VEL 1/1.

11) Page 45. Delete highlighted phrase "atypical measles syndrome" mistakenly left on this page.

STATISTICAL ANALYSIS PLAN

SOP No. VEL 1/1	**Version-3**
Written by: Dr. L. Jeyaseelan	**Replace Version:** Nil
Edited by: -	**Issue Date:** September 11, 2012
Reviewed by: Dr. Nick Andrews and Dr. Nicola Low	**Effective Date:**
Approved by: Dr. Nick Andrews and Dr. Nicola Low	

Abbreviations

AE : Adverse event
CDMC : Clinical Data Management Centre
CFR : Code of Federal Regulations (USA Food and Drug Administration)
CMC : Christian Medical College (Vellore, India)
CRF : Case Report Form
ELISA : Enzyme-linked immuno-sorbent assay
GCP : Good Clinical Practice
ICH : International Conference on Harmonization
ID : Identification number
MAP : Measles Aerosol Project
MAR : Missing at random
MySQL : My Structure Query Language
PHC : Primary Health Centre
PHP : Personal Home Page
PRNT : Plaque reduction neutralisation test
Promasys : Protocol management system
SAS : Statistical Analyses System
SAE : Serious Adverse Event
SAP : Statistical Analysis Plan
SOP : Standard Operating Procedure
SSH : Shirdi Saibaba Hospital
VCS : Validation Check Specification
WHO : World Health Organization
DSMB : Data Safety Monitoring Board
OD : Optical Density

Table of Contents:

1.0 Study Title:

Pivotal study to evaluate the immunogenicity and safety of a measles vaccine given by aerosolized inhalation: randomized controlled trial.

2.0 Background and Purpose of this Document:

Background:

The pivotal study has been designed to evaluate an aerosolized formulation of measles vaccine as part of the Measles Aerosol Project (MAP). The current subcutaneous measles vaccine is known to be highly effective and safe and is recommended worldwide. The trial has therefore been designed to show that the immunogenicity of aerosolized measles vaccine is not inferior to the subcutaneous vaccine, and has an acceptable safety profile. The pre-determined maximum acceptable difference in the primary outcome between aerosolized and subcutaneous vaccines is 5%.

Purpose:

This document describes principles and procedures for statistical aspects of the randomized controlled trial protocol. The document is referred to as the Statistical Analysis Plan (SAP). The contents of the SAP are in accordance with the guidelines of the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Consolidated Reporting Standards for Trials extension to non-inferiority trials (1).

3.0 Policy and Scope:

The SAP is applicable to procedures for the statistical analysis of data generated during the MAP trial to be undertaken at Vadu, Maharashtra, India. The planned analyses are outlined in the protocol WHO/MAP/IND/002 August 2008, Section 11.

4.0 General Responsibilities:

The Principal Statistician is responsible for carrying out the statistical analysis of the clinical data according to the protocol and this SAP and to ensure that other responsible staff members perform their duties according to this SAP.

5.0 Materials and Equipments:

Hardware:

1. Desktop personal computers with MS Windows Network
2. Laptops with MS Windows Network

Software:

1. Website development : XAMPP 1.6.6a (PHP 5.2.5, MySQL 5.0.51a,Apache), Apache friends group, Germany
2. Statistical Analyses : SAS 9.1, SAS Institute Inc., Cary, NC, USA
3. Audit trail software : Promasys 6.0, PROMASYS BV, Leiden, The Netherlands

6.0 Assessment of Outcomes:

The planned primary and secondary outcomes, and timing of their measurement, are outlined below.

6.1Immunogenicity:

6.1.1 Primary Outcome:

Measles seropositivity at day 91 post vaccination. This is defined as an optical density value (OD) >=0.1 or a plaque reduction neutralisation test (PRNT) result of >=120mIU/ml. Samples with an OD<0.1 at day 91 will be tested by PRNT as well as all samples from the Vadu study site.

6.1.2 Secondary Outcomes:

For all infants:

- Geometric mean ODs, pre-vaccination and at day 91;
- Seroconversion (defined as a change from Enzygnost enzyme-linked immunosorbent assay (ELISA) OD <0.1 to OD >=0.1 (unless PRNT results are available, in which case it is a change from PRNT from <120 to >= 120 mIU/ml) measured in paired samples from day 0 and day 91

For infants from Vadu in 28 and 364 days subsets:

- Geometric mean titres and ODs at days 0,28,91 and 364 and geometric mean fold changes between these time points to describe evolution of antibody response
- Seropositivity at 364 days in infants with antibody titre >=120 mIU/ml at day 91.
- Seropositivity at days 0, 28, 91 and 364 and changes between these time points

6.2 Safety:

6.2.1 Primary Outcome:

Adverse events (AE) up to and including day 91 post vaccination;
AE include acute clinical reactogenicity, other AE, and serious or unexpected AE.

6.2.2 Secondary outcome

Frequencies of individual AE.

7.0 Measures taken to minimize bias:

7.1. Randomization sequence generation:

The randomization sequence will be generated at the Clinical Data Management Centre (CDMC) at Vellore, India using SAS software. The randomization sequence will use 25% of blocks of 4 subjects, 25% of blocks of 6 subjects and 50% blocks of 8 subjects. (Refer SOP Randomization, No: 05 / 02).

In addition, subsets of study participants for additional immunological assessment from the Vadu site will be selected consecutively after the above allocation. Two randomization lists of size 552 with blocks of size 2 and two outcomes (blood draw, no blood draw) have been generated to cover each assessment (day 28 or 364). The same list is used for both study arms. The block randomization will ensure that very close to half are selected for the extra blood draws. There may be some small imbalances between the numbers from each arm selected for the extra examination. (Refer SOP Randomization, No: 05 / 02).

7.2 Allocation concealment:

Treatment allocation will not be able to be determined until the moment of assignment. A website will be created, which can be accessed only by the Randomization Manager at KEM Hospital using a username and password. The website provides access to an application that assigns the treatment allocation. The Medical Officers at the study sites will call the KEM hospital to get the allocation as and when the subject gets recruited into the study. Upon receiving the call the KEM hospital, the Medical Officer at the study site needs to provide the site from which he is calling. Upon receiving the site name, the Operator at the KEM hospital chooses the site on the web page. Then, on the next screen of the web page he/she needs to provide the initials of the child (first name, middle name and the last name), child's date of birth, subject identification number (ID). These are input variables, which will be stored in the database at Vellore. In order to avoid transcribing errors in the entry, the operator needs to retype the subject ID again.

When a unique study ID has been confirmed the person clicks the 'Randomization' button. The next screen gives the allocation as either, 'AEROSOL' or 'SUBCUTANEOUS'. The system records, in a separate database, the details of study site, child's names, Subject ID number, allocation, date and time of randomization. The randomization database will be maintained in Access as backend. This database will be maintained at the CDMC and the backup will be maintained at the backup server which is kept at the Pharmacology Department Museum (old name: Booshnam Moses Computing Center) (Refer SOP Randomization, No: 05 / 02).

7.3 Blinding of Laboratory Staff:

Staff at the study sites and participants' parents will know which treatment has been given. To minimize bias in the assessment of immunogenicity outcomes, laboratory staff will not know the treatment allocation or the order of specimens that they will analyze.

In order to blind laboratory staff to the specimen collection time (day 0, day 28 day 91 or day 364) for the subjects, a single digit random number will be generated and concatenated with the subject ID. This is called the Lab Code. Thus a subject will have a unique Lab Code of 5 digits.

The laboratory will be provided with lists of 22 pairs (day 0 and day 91) of specimen Lab Codes, which will be randomly ordered. Similarly, for the specimens of children who will be followed up at day 28 and day 364, the laboratory will be provided with lists of randomly ordered Lab Codes.

8.0 Sample size:

8.1 Immunogenicity:

The sample size for the primary outcome is determined using the confidence interval (CI) approach, considering where the CI for the treatment effect lies with respect to a pre-specified margin of non-inferiority and the null effect (1).

Total to be enrolled, 2000. This number was based on the following assumptions:

- Significance 2.5%, one sided for non-inferiority, 5% for two sided superiority;
- Non-inferiority margin 5%;
- Power for non-inferiority 90% if true seropositivity is 90% in both arms;
- 20% loss to follow up by day 91, leaving 1600 available for primary analysis.

Sample size for immunogenicity subset:
Those selected for additional testing at day 28 or at day 364 will have ELISA and PRNT test results at these time points as well as at day 0 and 91.

The sample size will be 100 in each arm at each of day 28 and day 364. Based on the estimated variability (Standard deviation) of post subcutaneous vaccination PRNT results of 0.43 on a $\log_{10}$ scale (Nick Andrews, Health Protection Agency London, personal communication) differences of <1.5 fold in geometric mean titres between aerosol and subcutaneous groups at each time point and between each time point would be able to be detected at a 5% significance level with 80% power.

8.2 Safety:

To estimate proportions with AE in Aerosol and Subcutaneous groups, the sample sizes above give acceptable precision.

Table 8.2.1: Range of Precision with Different Levels of Proportions Experiencing Event and Numbers of Participants.

Observed proportions	95% confidence intervals	
	N=800	N=1000
0%	0.00 – 0.46%	0.00-0.37%
1%	0.43-1.96%	0.48-1.83%
2%	1.15-3.23%	1.26-3.07%
5%	3.60-6.75%	3.73-6.54%

The table shows that, if no reactions are observed, the upper 95% confidence interval with a sample size of 1000 is 0.37%, or 1 in 270.

9.0 Data Management and Pre-analysis Statistical Review:

9.1 Data Management:

The data management plan has been developed by the CDMC, Dept., of Biostatistics, Christian Medical College, Vellore. For more details refer to SOP 04/02 Clinical Data Management.

9.1.1 Data Quality Control, Query management and Quality Assurance:

Please refer SOPs Query Generation and Resolution (15/02) and Quality control and Quality assurance (16/02) for details.

9.2 Pre-analysis Statistical Review

This pre-analysis review will be used to make decisions about issues including the exclusion of subjects or data from analysis sets, possible data transformations, and identification of outliers. Important covariates identified in recent research studies might be specified at this stage. The final format of tables will be decided after this review. Decisions taken during the pre-analysis review will be documented.

The steps to be taken in the pre-analysis review are as follows:

1. Each database (e.g. laboratory data, AE data, etc.) will be imported to SAS software format.

2. Basic frequencies and distribution tables and/or plots will be performed for each variable. For example, histograms will be made for continuous variables, such as age, height and weight, etc.

10.0 Description of Patient Flow and Analysis Datasets:

The flow of participants through the trial and follow up will be described in flow charts, as recommended by the CONSORT group (2). The flow diagrams will describe the numbers at each stage and will summarize reasons for exclusion for a 'per-protocol' dataset and an 'intention to treat' dataset (Figures 11.12.7.1 to 11.12.7.3). Tables describing the numbers excluded at each stage will also be produced (Tables 11.12.5.1 to 11.12.5.5B).

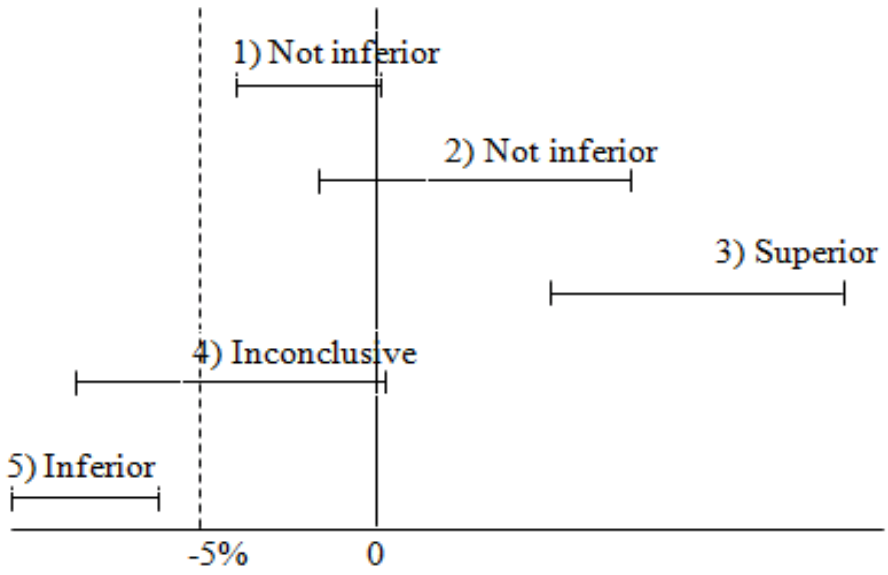
11.0 Statistical Methods:

In this section we first describe the method to be used for the analysis of the primary outcome. We then describe the principles and methods to be used for descriptive and comparative statistical analyses and tables that will be used to report these.

11.1 Analysis of the primary outcome:

The figure shows how the differences in the proportions with the primary outcome and the two sided 95% CI will be interpreted.

Figure 11.1.1:



95% CI for differences in proportions seropositive
(Aerosol - Subcutaneous)

In Figure 11.1.1 non inferiority has been demonstrated for scenarios 1), 2) and 3) since the lower limit of the 95% CI is above -5%. In addition for scenario 3) superiority has been shown because the lower limit does not include 0%. For scenarios 4) and 5) non-inferiority has not been demonstrated and for scenario 5) aerosol is inferior as the upper limit does not include 0%.

Note: Using the Wilson score method, 95% CI will be calculated using SAS software. (Newcombe 1998, Agresti et al 1998)

11.2 Per-Protocol (PP) Analysis Set:

This is the analysis set that will be used for the primary analysis of efficacy. The PP analysis set includes all children with an interpretable result from the blood sample scheduled for 91 days (-7 to +28 days), and without major protocol deviations. Study participants remain in the study up to until the point at which they drop-out or deviate from the protocol (Protocol section 12.0).

Protocol deviations include:

- Study participant incorrectly entered the study, e.g. did not meet age criteria;
- Study participant should have been withdrawn during study but was not;
- Study participant did not receive the vaccine according to the arm to which he / she was randomized;
- Study participant received the wrong dose of vaccine;
- Study participant received a dose beyond the recommended multi-dose vial policy
- Wrong subject ID randomized
- Wrong Subject ID
- Home Visit and Clinic visit on same date
- Visits outside the window period
- Wrong labelling of the CRF
- Wrong labelling of vials
- Informed consent issue

The assessment of long-term immunogenicity in the subset of participants from Vadu, includes children with an, interpretable result from blood sample scheduled for 364 days (-7 to +28 days), and without major protocol deviations.

11.3 Modified Intention to Treat (ITT) Analysis Set:

The 'intention to treat' analysis will be conducted as a secondary analysis. In a non-inferiority trial such as this, the intention to treat analysis is more likely than the per-protocol analysis to give a type I error (falsely claiming non-inferiority) because this kind of analysis tends to minimize differences between the two treatments (1).

The ITT analysis set includes assessments up to 91 days (-7 to +28 days) post-vaccination. All study participants will be included in the group to which they were randomized.

11.4 As Treated Cohort Analysis set:

The primary efficacy analysis for safety includes all children vaccinated, according to the route of vaccination that they received and with at least one safety assessment.

In addition, we will summarize explicitly any severe reactions or serious adverse events (SAE) in children vaccinated by the wrong route.

11.5 Timing of Analyses:

The primary efficacy analysis will be conducted after the last enrolled participant completes the day 91 follow up visit.

Every 3 months the data will be analyzed and a report will be made available for the DSMB. This report will focus on safety.

11.6 Method for Handling Missing Data:

For the primary efficacy analysis there is no plan to impute data values for participants who do not complete follow up visits.

For the PP dataset, missing values will be treated as missing at random. However, preliminary analyses such as age, sex, baseline immunogenicity distribution will be studied between the two groups to examine the validity of this assumption.

For the assessment of immunogenicity, missing values (e.g. no blood taken) are treated as if they are missing at random. The OD values for blood samples taken outside the specified time limits will be treated as missing values.

Note that missing values at 91 days post-vaccination and at other time points will be considered for further analyses. The missing information will be handled by best and worst case scenario methods. Where blood specimens are taken outside the recommended time interval, these results will still be used.

11.7 Significance Level:

This is a non-inferiority trial and the confidence interval approach will be used to determine the trial outcome. For additional analyses, such as those adjusting for covariates, we will not use a fixed ‘statistical significance’ level for reasons described by Sterne and Davey Smith (3). For analyses that require the calculation of p values, we will base our interpretation on examination of the effect size, confidence interval and p value.

11.8 Safety:

We will estimate the proportions (with 95% CI) of AE in each arm according to the definitions of the primary and secondary safety outcomes (Refer section 8.7 in the protocol).

11.9 Covariates to be examined:

Covariates of interest are as follows:

Baseline: Age in months, sex, usual health care provider**,** heart rate, respiratory rate, temperature, height for age, weight for age, any abnormality discovered on systemic examination, pre existing medical conditions (Table 11.12.6.1B)

Others: Observations during vaccination (Table 11.12.6.1D)

The distribution of the baseline covariates by study arm will be described, but not formally tested as randomization will have ensured that any differences are chance differences. For the main comparison of the study arms adjustment for these covariates is not necessary. To investigate the effect of these covariates on study end-points multivariable logistic regression (for binary end points) or normal errors regression (on logged antibody levels) will be performed. Study arm will be included in these analyses and interactions examined as given in section 11.11.

11.10 Sub-group analyses:

No sub group analyses have been planned.

11.11 Interactions with study arm:

Potential interactions will be examined for the following co-variates: sex, age, crying during measles vaccination, and acute respiratory infection.

11.12 Descriptive Analyses:

11.12.1 Distributions of continuous variables and any agreed transformations:

Mean, standard deviation, median, and interquartile range will be generated for continuous variables. The PRNT values will be doubled if the value is >20,000. The PRNT values will be $\log_{10}$ transformed for further analyses such as GMT and 95% CI. Results at the lower limit of the assay will be assigned a value of 10. OD values will also be $\log_{10}$ transformed to calculate geometric means and perform regression. Any OD results of <0.01 will be assigned a value of 0.005 prior to $\log_{10}$ transformation.

11.12.2 Distributions of binary and categorical variables:

Frequency distributions will be shown for binary and categorical variables. The categories will be checked against the Annotated CRF.

11.12.3 Outliers:

For $\log_{10}$ PRNT values, checks for outliers will be performed prior to the statistical analysis as part of the data checking. The outliers will also be examined using standardized residuals >3 when looking at $\log_{10}$ PRNT values.

11.12.4 Flow of Numbers:

The flow of participants through the trial and follow up will be described in flow chart, as recommended by the CONSORT 2010 guidelines and guidance, Reference (3).

Figure 11.12.4.1: Flow of participant numbers in the trial

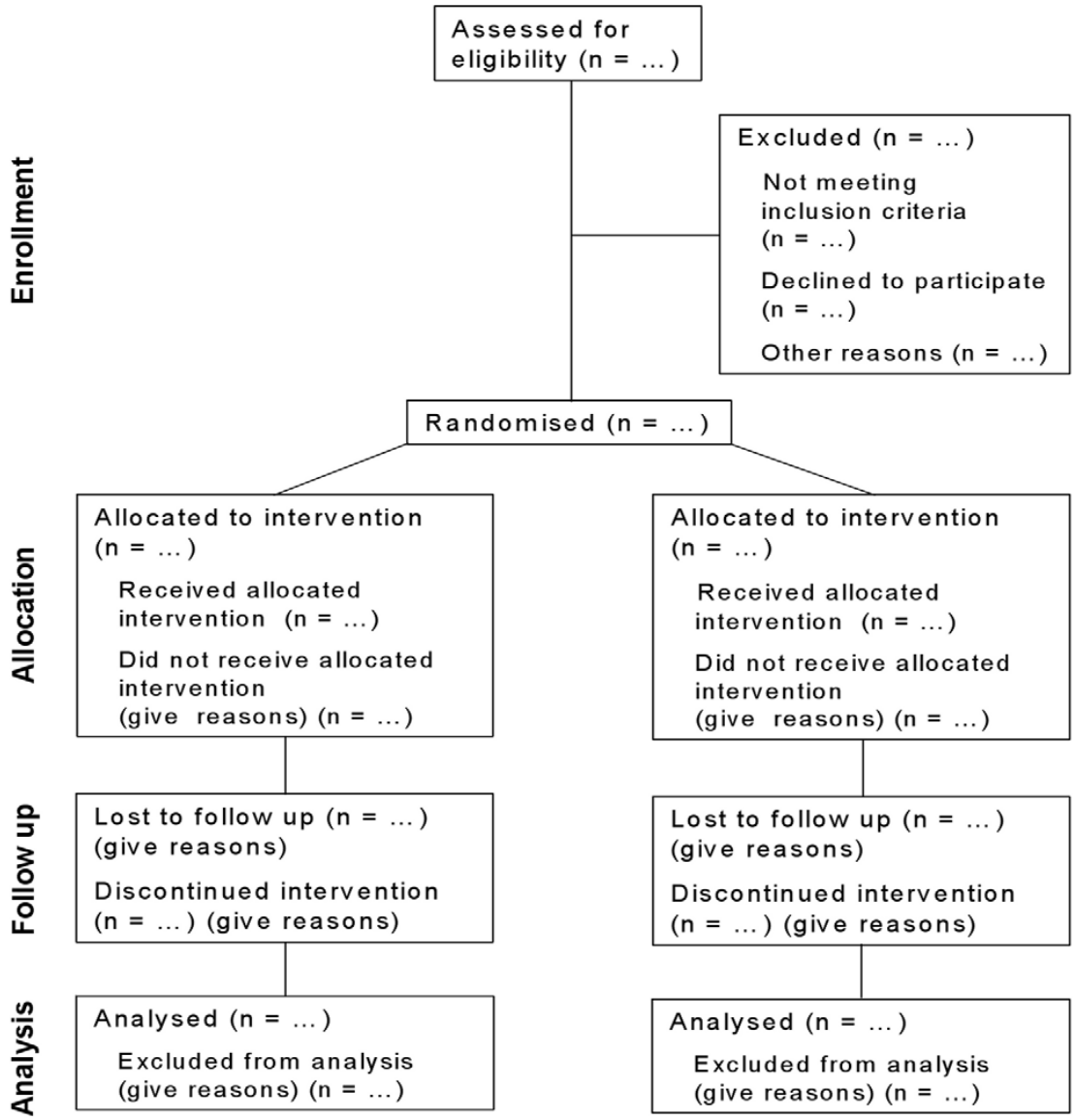


Table 11.12.1: Number of children enrolled and allocated to vaccination by study area, study arm, sex

Study area	**Assess for eligibility**	**Appointment given for Vaccinating**	**Parent willing**	**Accept enrolment**	**Allocated to intervention**	**Aerosol Arm**			**Subcutaneous Arm**		
						Boys	Girls	Total	Boys	Girls	Total
Alandi											
Chakan											
Nhavara											
Pabal											
Shel Pimpalgaon											
Talegaon Dhamdhere											
Vadu											
Wagholi											
TOTAL											

11.12.5 Patient Data Listings:

The data listings of discontinued subjects and the details of completion of enrolled subjects at various time points, the reasons for drop out and the distribution of excluded subjects for efficacy analyses are presented from table 11.12.5.1 to 11.12.5.5B.

Table 11.12.5.1: List of all patients discontinued from the study after enrolment

Subject ID	**Study arm (AER, SC)**	**Days in trial post-vaccination**	**Age**	**Gender**	**Adverse events**	**Major reason discontinued**	**Visit**	**AE / Severity**	**Last visit before discontinuation**

Legend:

AER – aerosol vaccination; SC – subcutaneous vaccination

Table 11.12.5.2: Number of subjects enrolled and followed up at major time points by study area

Primary Health Centre and Time point	Study arm to which participants were randomly allocated	
	Aerosol, n = (% allocated to intervention)	**Subcutaneous, n =** (% allocated to intervention)
Alandi		
Day 0 - Allocated to intervention		
Day 0 - Received allocated intervention		
Day 28		
Day 91		
Chakan		
Day 0 - Allocated to intervention		
Day 0 - Received allocated intervention		
Day 28		
Day 91		
Nhavare		
Day 0 - Allocated to intervention		
Day 0 - Received allocated intervention		
Day 28		
Day 91		
Pabel		
Day 0 - Allocated to intervention		
Day 0 - Received allocated intervention		
Day 28		
Day 91		
Shel Pimpalgaon		
Day 0 - Allocated to intervention		
Day 0 - Received allocated intervention		
Day 28		
Day 91		
Talegaon Dhamdhere		
Day 0 - Allocated to intervention		
Day 0 - Received allocated intervention		
Day 28		
Day 91		
Vadu		
Day0 - Allocated to Intervention		
Day0 - Received allocated Intervention		
Day 28 - clinical assessment		
Day 28 - blood specimen*		
Day 91		
Day 364 - clinical assessment*		
Day 364 - blood specimen*		
Wagholi		
Day 0 - Allocated to Intervention		
Day 0 - Received allocated Intervention		
Day 28		
Day 91		

Legend: *Applies to Vadu subset only

Table 11.12.5.3: Summary of reasons for discontinuation in the clinical trial

Reasons	**Study arm to which the participants were randomly allocated**			
	Aerosol		**Subcutaneous**	
	n	**(%)**	**n**	**(%)**
Screening failure				
Withdrawal of consent				
Refusal				
Loss to follow up				
SAE other than death				
Death				
Others				

Table 11.12.5.4: Summary of protocol deviations that occurred during the clinical trial

Reasons	**Study arm to which participants were randomly allocated**	
	Aerosol	**Subcutaneous**
	n (%)	**n (%)**
Incorrectly provided vaccination		
Aerosol		
Subcutaneous		
Incorrectly entered the study, e.g. inappropriate age		
Should have been withdrawn but was not		
Received the wrong dose of vaccine (e.g. too little or too much)		
Received a dose beyond the recommended multi-dose vial policy		
Other violation of study protocol		
Wrong subject ID randomized		
Subject ID interchange		
Home visit and clinic visit on same date		
Visit outside the window period		
Wrong labelling of the CRF		
Wrong labelling of vials		
Informed consent issue		
Total number of Protocol Deviations		
Number of subjects having protocol deviations		

Table 11.12.5.5: Study participants excluded from the per protocol analysis of Immunogenicity

Subject ID	Allocated intervention	Days in trial post-vaccination	Age	Sex	Reason for exclusion

11.12.6: Distributions of socio-demographic, baseline health and disease status and anthropometric variables

Table 11.12.6.1A: Baseline demographic characteristics of study participants by allocated intervention

Baseline characteristics	Allocated intervention			
	Aerosol		Subcutaneous	
	n	(% of number allocated)	n	(% of number allocated)
Age in months				
9 to 9.99				
10 to 10.99				
11 to 11.99				
Sex				
Male				
Female				
Usual health care provider Government Private Non-governmental organization Other				

Table 11.12.6.1B: Baseline health and disease status of study participants by allocated intervention

Findings	Allocated Intervention			
	Aerosol		Subcutaneous	
	n	(% of number allocated)	n	(% of number allocated)
Pre-existing medical condition				
Significant medical history since birth				
Significant illness in the last three months				
Regular medication for more than one month				
Concomitant medications received during the last four weeks				

Table 11.12.6.1C: Baseline anthropometry and vital signs of study participants by allocated intervention

Findings	**Allocated intervention**			
	Aerosol		**Subcutaneous**	
	mean	**(SD)**	**mean**	**(SD)**
Heart rate				
Respiratory rate				
Temperature				
	n	**(% of number allocated)**	**n**	**(% of number allocated)**
Any abnormality discovered on systemic examination				
Height for age below normal range				
Weight for age below normal range				

Table 11.12.6.1D: Observations during vaccination study participants by Allocated Intervention

Observations	**Allocated Intervention**			
	Aerosol		**Subcutaneous**	
	n	**(% of number allocated)**	**n**	**(% of number allocated)**
Child calm during vaccination				
Child struggling during vaccination				
Child breathing shallow during vaccination				
Child crying during vaccination				
Child holding his/her breath during vaccination				
Child coughing during vaccination				
Child exhibited abnormal behaviour during vaccination				

Figure 11.12.7.1:

Immunogenicity: Flow Chart for Per-Protocol (PP) Analysis

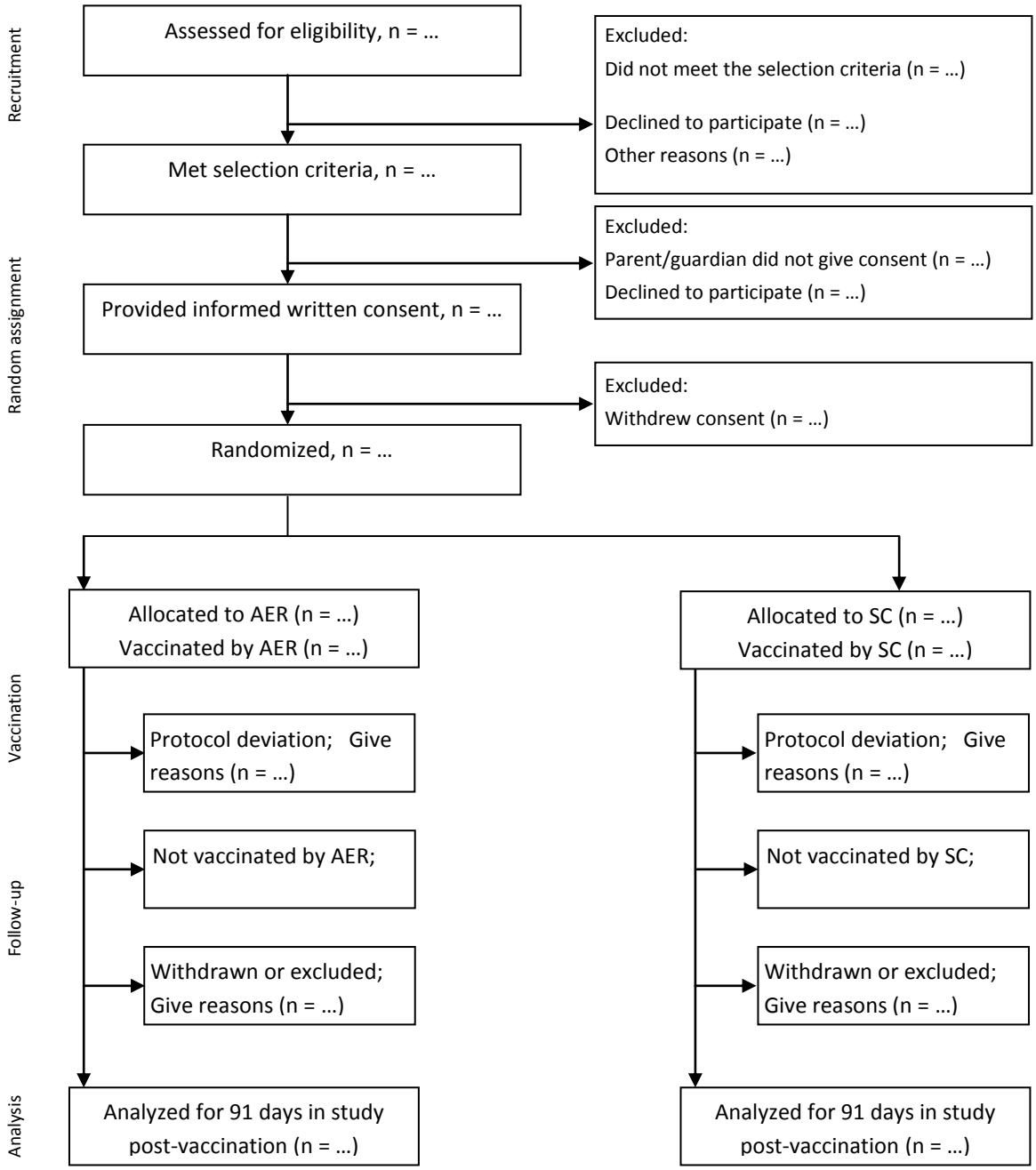


Figure: 11.12.7.2

Immunogenicity: Flow Chart for Intent to Treat (ITT) Analysis

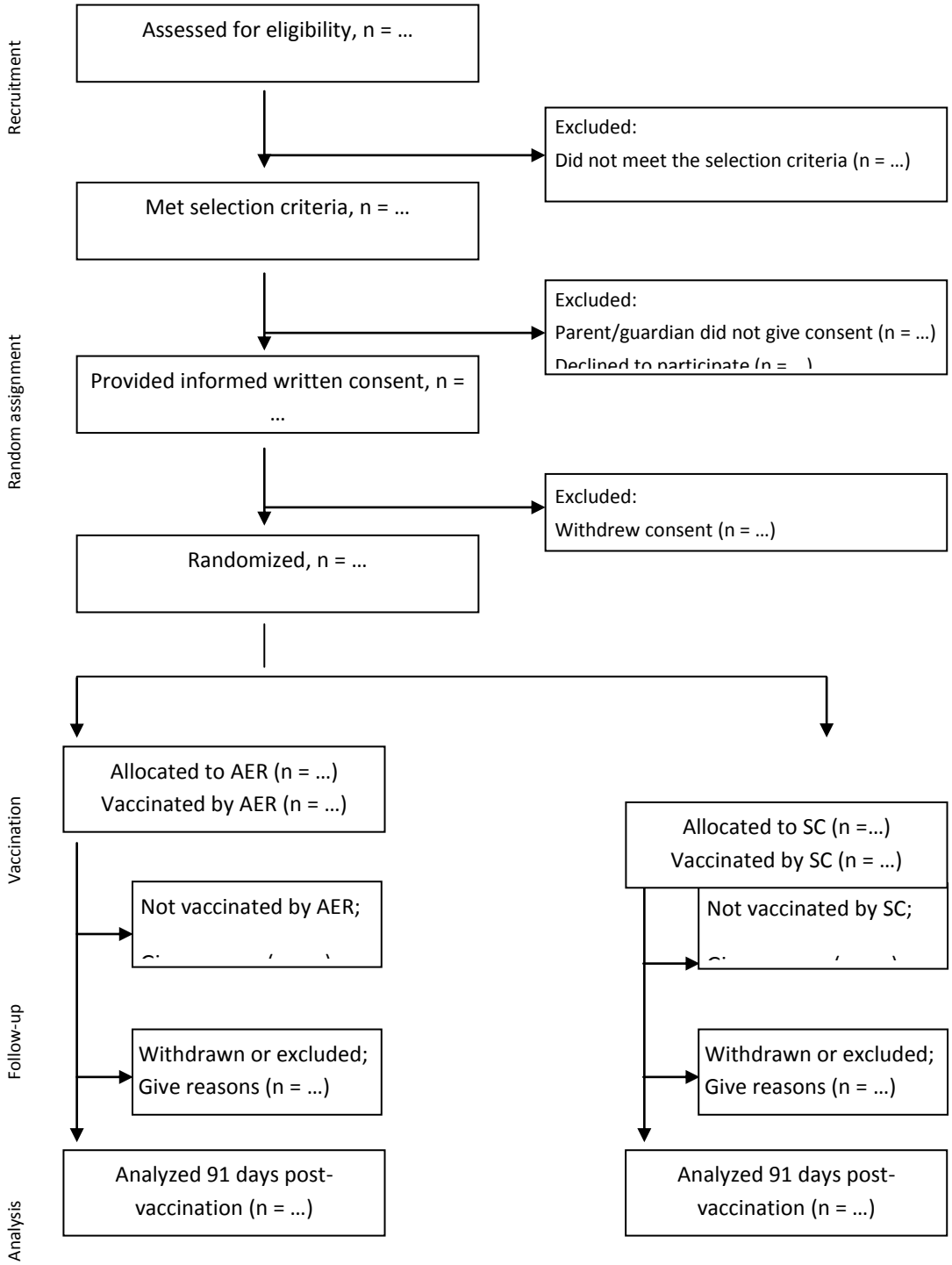
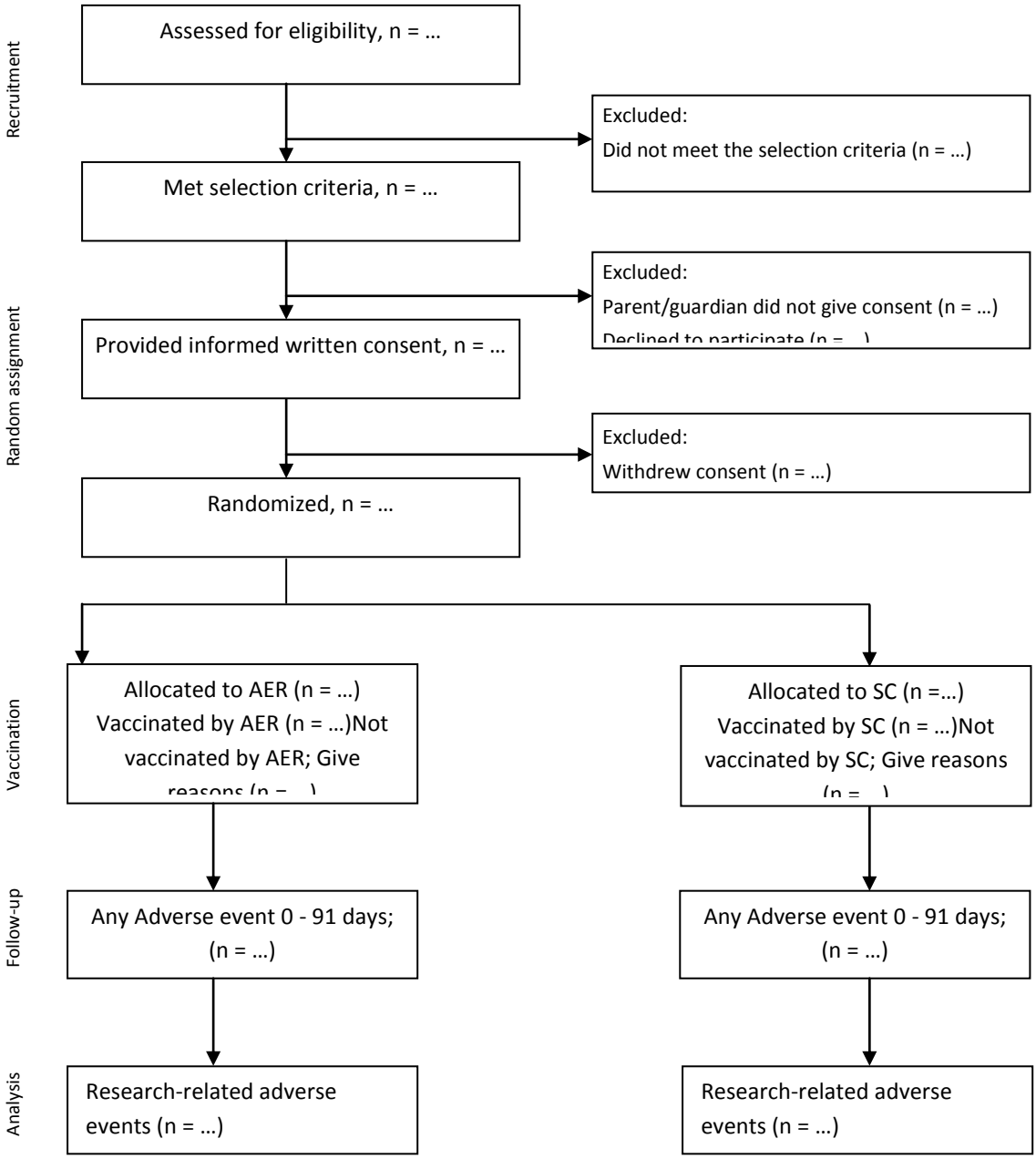


Figure: 11.12.7.3

Safety: Flow Chart of "As Treated" Analysis for Safety



12.0 Efficacy Analyses:

12.1. Efficacy Analyses: Immunogenicity

Table 12.1.1A: Results of ELISA and PRNT, by study group, per-protocol analysis

	Baseline		**Day 91**	
	AER	SC	AER	SC
	n=__	n=__	n=__	n=__
ELISA result (optical density)				
Median				
Interquartile range				
Min – Max				
Geometric mean titre				
95% CI	n=__	n=__	n=__	n=__
PRNT result (mIU/ml)				
Median				
Interquartile range				
Min – Max				
Geometric mean titre				
95% CI				

Legend:

AER – aerosol; SC - subcutaneous

For PRNT titre, includes subjects whose OD values are < 0.1 and all from Vadu site

Table 12.1.1B: Results of ELISA and PRNT, by study group, ITT analysis

	Baseline		**Day 91**	
	AER	SC	AER	SC
	n=__	n=__	n=__	n=__
ELISA result (optical density)				
Median				
Interquartile range				
Min – Max				
Geometric mean titre				
95% CI	n=__	n=__	n=__	n=__
PRNT result (mIU/ml)				
Median				
Interquartile range				
Min – Max				
Geometric mean titre				
95% CI				

Note: For PRN titre, the subjects whose OD values are < 0.1 and from Vadu site

12.1.2: Day 91 ELISA and PRNT results

Table 12.1.2A: Aerosol Group

	PRNT <120	**PRNT >=120**	**PRNT not done**	**Total**
Day 91 ELISA <0.1				
Day 91 ELISA >=0.1				
Day 91 ELISA Not done				
Total				

Legend:
Boxes highlighted in yellow represent results classified as seropositive at day 91

Table 12.1.2B: Subcutaneous Group

	PRNT <120	**PRNT >=120**	**PRNT not done**	**Total**
Day 91 ELISA <0.1				
Day 91 ELISA >=0.1				
Day 91 ELISA Not done				
Total				

Legend:
Boxes highlighted in yellow represent results classified as seropositive at day 91

Table 12.1.3: Measles seropositive at day 91 post-vaccination (all infants) and difference in positivity between study arms

	AER n/N	**AER%, 95% CI**	**SC n/N**	**SC%, 95% CI**	**Difference (AER-SC), 95% CI***
Seropositive at day 91					

Legend:
AER – aerosol; SC – subcutaneous;
* This difference is used to interpret non-inferiority, estimated by Wilson score method (5) (6).

Table 12.1.4A: Comparison of serological outcomes, by study group and time of sample collection: **Per Protocol cohort**

Time point	**AER**		**SC**		**Difference (AER-SC) 95% CI**
	n	**%**	**n**	**%**	
Day 0, seropositive					
Day 91, seropositive					
Day 91, seroconversion					

Legend:
AER – aerosol; SC - subcutaneous

Table 12.1.4B: Comparison of serological outcomes, by study group and time of sample collection: **ITT cohort**

Time point	AER		SC		Difference (AER-SC) 95% CI
	n	%	n	%	
Day 0, seropositive					
Day 91, seropositive					
Day 91, seroconversion					

Legend:
AER – aerosol; SC - subcutaneous

Table 12.1.5A: Comparison of serological outcomes, by study group and time of sample collection for Vadu subset: **Per Protocol cohort**

Time point	AER		SC		Difference (AER-SC) 95% CI
	n	%	n	%	
Day 0, seropositive					
Day 28, seropositive					
Day 28, seroconversion					

Legend:
AER – aerosol; SC - subcutaneous

Table 12.1.5B: Comparison of serological outcomes, by study group and time of sample collection for Vadu subset: **ITT cohort**

Time point	AER		SC		Difference (AER-SC) 95% CI
	n	%	n	%	
Day 0, seropositive					
Day 28, seropositive					
Day 28, seroconversion					

Legend:
AER – aerosol; SC - subcutaneous

Table 12.1.6A: Comparison of serological outcomes, by study group and time of sample collection for Vadu subset: **Per Protocol cohort**

Time point	AER		SC		Difference (AER-SC) 95% CI
	n	%	n	%	
Day 0, seropositive					
Day 364, seropositive					
Day 364, seroconversion					

Legend:
AER – aerosol; SC - subcutaneous

Table 12.1.6B: Comparison of serological outcomes, by study group and time of sample collection for Vadu subset: **ITT cohort**

Time point	**AER**		**SC**		**Difference (AER-SC) 95% CI**
	n	**%**	**n**	**%**	
Day 0, seropositive					
Day 364, seropositive					
Day 364, seroconversion					

Legend:
AER – aerosol; SC - subcutaneous

Table 12.1.7: Day 0 and 91 ELISA and PRNT results with Sero-Conversion. (highlighted in yellow)

			Day 91							
Baseline (Day 0)			**ELISA <0.1**			**ELISA >=0.1**			**ELISA Not done**	
		PRNT	**<120**	**>=120**	**Not done**	**<120**	**>=120**	**Not done**	**<120**	**>=120**
	ELISA <0.1	<120								
		>=120								
		Not done								
	ELISA >=0.1	<120								
		>=120								
		Not done								
	ELISA Not done	<120								
		>=120								
		Not done								

Note: Highlighted in grey are the ones, which are "Not Applicable". That is, which are seropositive or unknown at baseline

Table 12.1.8: Geometric mean titres, pre-vaccination and at day 91 for all infants

GMTs				
	AER	**SC**	**Ratio AER/SC**	**95% CI for ratio**
Day 0 OD (all)				
Day 91 OD (all)				
OD (OD >0.1)				
PRNT (OD<0.1)				

12.2 Secondary End Point (For infants from Vadu in 28 and 364 days subset): ITT Cohort

12.2.1Analysis: Geometric mean titres and ODs at days 0, 28, 91 and 364 to describe evolution of antibody response;

Table 12.2.1.1A: Descriptive statistics of infants age 9 to 11.99 months at vaccination and change in ELISA titer for subset of subjects at Vadu followed to day 364

	Baseline		**Day 28**		**Day 91**		**Day 364**	
	AER	SC	AER	SC	AER	SC	AER	SC
	n=__	n=__	n=__	n=__	n=__	n=__	n=__	n=__
ELISA Titer: (Δ OD) Median Min – Max GMT 95% CI								

Table 12.2.1.1B: Descriptive statistics of infants age 9 to 11.99 months at vaccination and change in PRN titer for subset of subjects at Vadu followed to day 364

	Baseline		**Day 28**		**Day 91**		**Day 364**	
	AER	SC	AER	SC	AER	SC	AER	SC
	n=__	n=__	n=__	n=__	n=__	n=__	n=__	n=__
PRN Titer: (mIU/ml) Median Min – Max GMT 95% CI								

Table 12.2.1.2: Geometric mean titres, pre-vaccination and at days 28, 91, 364 for infants in the Vadu subset

GMTs						
	AER		SC		Ratio AER/SC	95% CI
	n	GMTs	n	GMTs		
28 days subset						
Day 0 OD						
Day 28 OD						
Day 91 OD						
Day0 PRNT						
Day 28 PRNT						
Day 91 PRNT						
364 days subset						
Day 0 OD						
Day 91 OD						
Day 364 OD						
Day 0 PRNT						
Day 91 PRNT						
Day 364 PRNT						

12.2.1.3: Seropositivity at 364 days in infants with PRNT ≥ 120 mIU/ml at day 91

12.2.1.3A: Aerosol group

	Day 364			
Day 91	**PRNT**	**<120**	**>=120**	**Total**
	<120			
	>=120			
	Total			

12.2.1.3B: Subcutnaeous group

	Day 364			
Day 91	**PRNT**	**<120**	**>=120**	**Total**
	<120			
	>=120			
	Total			

12.2.1.4: Seropositivity at days 0, 28, 91 and 364 and changes between these time points

Table 12.2.1.4.1: Measles seropositivity at days 0, 28, 91 and 364:

	n		**% (95% CI) for difference**	
	AER	**SC**	**AER**	**SC**
Seropositivity at day 0				
Seropositivity at day 28				
Seropositivity at day 91				
Seropositivity at day 364				

12.3 Risk factor Analyses:

As covariates for inclusion in multivariable analyses: age; weight for age; sex; day 0 antibody titre; study site; crying when vaccine given (aerosol arm only); presence of respiratory tract symptoms at time of vaccination (aerosol arm only).

Table 12.3.1: Risk Factors for Seronegative of Aerosol Arm children

Risk Factors/Covariates	**Seropositivity***		
	OR	**95%CI**	**P**
Age (months):			
9	1.0		
10			
11			
11.99			
Sex (Male)			
Usual health care provider			
Government	1.0		
Private			
Non-governmental organization			
others			
Weight for Age			
Normal	1.0		
Abnormal			
Height for Age			
Normal	1.0		
Abnormal			
Day 0 Antibody Titre			
Sites:			
Vadu	1.0		
Pabal			
Talegoan Dhamdhere			
Nhavare			
Chakan			
Shel Pimpalgaon			
Wagholi			
Alandi			
Crying when vaccine given (yes):			
Respiratory Track Symptoms at the time of vaccination (Yes)			
Any abnormality discovered on systematic examination(Yes)			
Pre existing medical condition(Yes)			

*Modelled for seronegatives.

13.0 Safety Analyses:

13.1: Reactogenic Events:

Table 13.1.1A (1): Frequency of adverse events reported during the first 30 minutes after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of AEs	% of AE	95% CI for percentage of AE		Number of AEs	% of AE	95% CI for percentage of AE	
			Lower CI	Upper CI			Lower CI	Upper CI

Table 13.1.1A (2): Number of subjects experiencing at least one adverse event during the first 30 minutes after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population		Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population	
			Lower CI	Upper CI			Lower CI	Upper CI

[#] One subject may have more than one AE. So, 'Number of persons having AE' counts expected to overlap

** Denominator: Total number of subjects, all sites

Table 13.1.1B (1): Frequency of adverse events reported from 31 minutes 14 days after vaccination and number of subjects experiencing at least one adverse event

Adverse Events	Aerosol				Subcutaneous			
	Number of AEs	% of AE	95% CI for percentage of AE		Number of AEs	% of AE	95% CI for percentage of AE	
			Lower CI	Upper CI			Lower CI	Upper CI

Table 13.1.1B (2): Number of subjects experiencing at least one adverse event from 31 minutes to 14 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population		Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population	
			Lower CI	Upper CI			Lower CI	Upper CI

[#] One subject may have more than one AE. So, 'Number of persons having AE' counts expected to overlap

** Denominator: Total number of subjects, all sites

Table 13.1.1C (1): Frequency of adverse events reported from 15 days to 28 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of AEs	% of AE	95% CI for percentage of AE		Number of AEs	% of AE	95% CI for percentage of AE	
			Lower CI	Upper CI			Lower CI	Upper CI

Table 13.1.1C (2): Number of subjects experiencing at least one adverse event from 15 days to 28 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population		Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population	
			Lower CI	Upper CI			Lower CI	Upper CI

[#] One subject may have more than one AE. So, 'Number of persons having AE' counts expected to overlap

** Denominator: Total number of subjects, all sites

Table 13.1.1D (1): Frequency of adverse events reported from 29 days to 56 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of AEs	% of AE	95% CI for percentage of AE		Number of AEs	% of AE	95% CI for percentage of AE	
			Lower CI	Upper CI			Lower CI	Upper CI

Table 13.1.1D (2): Number of subjects experiencing at least one adverse event from 29 days to 56 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population		Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population	
			Lower CI	Upper CI			Lower CI	Upper CI

[#] One subject may have more than one AE. So, 'Number of persons having AE' counts expected to overlap

** Denominator: Total number of subjects, all sites

Table 13.1.1E (1): Frequency of adverse events reported from 57 days to 91 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of AEs	% of AE	95% CI for percentage of AE		Number of AEs	% of AE	95% CI for percentage of AE	
			Lower CI	Upper CI			Lower CI	Upper CI

Table 13.1.1E (2): Number of subjects experiencing at least one adverse event from 57 days to 91 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population		Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population	
			Lower CI	Upper CI			Lower CI	Upper CI

[#] One subject may have more than one AE. So, 'Number of persons having AE' counts expected to overlap

** Denominator: Total number of subjects, all sites

Table 13.1.1F (1): Frequency of adverse events reported from 92 days to 364 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of AEs	% of AE	95% CI for percentage of AE		Number of AEs	% of AE	95% CI for percentage of AE	
			Lower CI	Upper CI			Lower CI	Upper CI

Table 13.1.1F (2): Number of subjects experiencing at least one adverse event from 92 days to 364 days after vaccination

Adverse Events	Aerosol				Subcutaneous			
	Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population		Number of persons having AE[#]	Proportion in population ** (in %)	95% CI for Population	
			Lower CI	Upper CI			Lower CI	Upper CI

[#] One subject may have more than one AE. So, 'Number of persons having AE' counts expected to overlap

** Denominator: Total number of subjects, all sites

13.2: Severity of reactions

The Distribution of Severity of Adverse Events:

Table 13.2.1A: Number of AEs reported according to severity in the Aerosol group

Time Points	Severity of AE			Total	SAE n (% of events)
	Mild n (% of events)	Moderate n (% of events)	Severe n (% of events)		
0-30mins					
31 mins to day 14					
Day15-28					
Day29-56					
Day57-91					
Day 92-364*					
Total					

*Applies to Vadu subset only

Table 13.2.1B: Number of AEs reported according to severity in the Subcutaneous group

Time Points	Severity of AE			Total	SAE n (% of events)
	Mild n (% of events)	Moderate n (% of events)	Severe n (% of events)		
0-30mins					
31 mins to day 14					
Day15-28					
Day29-56					
Day57-91					
Day 92-364*					
Total					

*Applies to Vadu subset only

13.3: Causality

Table 13.3.1A: Number of moderate and severe Adverse Events and relationship to vaccination, Aerosol group

Time Points	**Relationship to Vaccination**						**Total**
	Not Related **n (% of events)**	Unlikely to be Related **n (% of events)**	Possible **n (% of events)**	Probable **n (% of events)**	Most Probably **n (% of events)**	Insufficient data to assess **n (% of events)**	
0-30mins							
31 mins to day 14							
Day15-28							
Day29-56							
Day57-91							
Day 92-364*							
Total							

*Applies to Vadu subset only

Table 13.3.1B: Number of moderate and severe Adverse Events and relationship to vaccination, Subcutaneous Group

Time Points	**Relationship to Vaccination**						**Total**
	Not Related **n (% of events)**	Unlikely to be Related **n (% of events)**	Possible **n (% of events)**	Probable **n (% of events)**	Most Probably **n (% of events)**	Insufficient data to assess **n (% of events)**	
0-30mins							
31 mins to day 14							
Day15-28							
Day29-56							
Day57-91							
Day 92-364*							
Total							

*Applies to Vadu subset only

13.4: Listing of AEs by subject

Table 13.4.1: AE listing by Subject

subject ID(Sex)	Age (Day)	Vaccinated Date	AE	Date of AE onset	Days to start AE	Date of AE stops	Duration of AE existence (in days)	Concomitant Medications given for the Visit	AE treatment outcome

14.0 Interim Analyses:

An interim analysis of safety data will be completed for this analysis. This includes only safety assessment events reported within 91 days after vaccination, and no laboratory results will be included. The DSMB will use data from the first 100 children in each arm. This will be done as soon as possible after these data are available.

Table 14.0.1: Frequency of all AEs by study group and time since vaccination

Name	**Time since vaccination**																	**Total**	
	Visit 2 0 – 30 mins		**Visit 3 31 mins – 3 days**		**Visit 4 4 – 7 days**		**Visit 5 8 – 10 days**		**Visit 6 11 – 14 days**		**Visit 7 15 – 16 days**		**Visit 8 17 – 21 days**		**Visit 9 22 – 28 days**		**..**		
	AER	**SC**	**AER**	**SC**	**AER**	**SC**	**AER**	**SC**	**AER**	**SC**	**AER**	**SC**	**AER**	**SC**	**AER**	**SC**		**AER**	**SC**
Adverse Events Mild, Moderate &Severe* ** Anorexia																			
Conjunctivitis																			
Coryza																			
Cough																			
Crying																			
Diarrhoea																			
Difficulty																			
Fever																			
Irritability																			
Local reaction																			
Malaise																			
Rash																			
Seizure																			
Shivering																			
Vomit																			
Wheeze																			
Others																			
Total																			

Table 14.0.2: Frequency of AE that have been deemed probably related and most probably related* by study group and time since vaccination

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Adverse Events Mild, Moderate & Severe* ** Anorexia																			
Conjunctivitis																			
Coryza																			
Cough																			
Crying																			
Diarrhoea																			
Difficulty																			
Fever																			
Irritability																			
Local reaction																			
Malaise																			
Rash																			
Seizure																			
Shivering																			
Vomit																			
Wheeze																			
Others																			
Total																			

Table 14.0.3: Frequency of AE that have been deemed probably related and most probably related* by study group, severity and time since vaccination

Part1: Mild and Moderate counts together

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Adverse Events Mild or Moderate Anorexia																			
Conjunctivitis																			
Coryza																			
Cough																			
Crying																			
Diarrhoea																			
Difficulty																			
Fever																			
Irritability																			
Local reaction																			
Malaise																			
Rash																			
Seizure																			
Shivering																			
Vomit																			
Wheeze																			
Others																			
Total																			

Part 2: Severe counts

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Adverse Events Severe* ** Anorexia																			
Conjunctivitis																			
Coryza																			
Cough																			
Crying																			
Diarrhoea																			
Difficulty																			
Fever																			
Irritability																			
Local reaction																			
Malaise																			
Rash																			
Seizure																			
Shivering																			
Vomit																			
Wheeze																			
Others																			
Total																			

Part 3: Serious Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Serious Adverse Events** Result in death																			
Is life threatening																			
Results in persistent or significant disability or incapacity																			
Requires hospitalization or continuation of existing hospitalization																			
Important medical event																			
Total																			

***** *1. Not related 2.unlikely to be related 3.Possible 4. Probable 5. Most probably 6. Insufficient data to assess*

****** *1. Results in death 2. Is life threatening 3. Results in persistent or significant disability or incapacity 4. Requires hospitalization or continuation of existing hospitalization 5. Important medical event (may not result in death but may jeopardise subject)*

******* *As defined in the protocol list of solicited adverse events*

Table 14.0.4: Characteristics of duration of adverse event separately for severity, causality and by administered intervention

Adverse Event	Mild																Moderate																Severe											
	Possible				Probable				Most Probably				Not Related				Possible				Probable				Most Probably				Not Related				Possible				Probable				Most Probably				Not Related			
	AER		SC		AER		SC		AER		SC		AER		SC		AER		SC		AER		SC		AER		SC		AER		SC		AER		SC		AER		SC		AER		SC	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	N	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Anorexia																																												
Conjunctivitis																																												
Coryza																																												
Cough																																												
Crying																																												
Diarrhoea																																												
Difficulty																																												
Fever																																												
Irritability																																												
Local																																												
Malaise																																												
Rash																																												
Seizure																																												
Shivering																																												
Vomit																																												
Wheeze																																												

15.0 Trial Termination:

The trial will be terminated according to the stopping rules determined by the DSMB. The criterion for termination is the occurrence of the single serious adverse event or encephalitis/encephalopathy in one or more subjects which is deemed to be most probably related to the Aerosol vaccine.

16.0 Reference List

(1) UNDP/World bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). Workbook for investigators. 2002; p 119.

(2) Piaggio G, Elbourne DR, Altman DG, Pocock SJ, Evans SJ. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. JAMA 2006 Mar 8;295(10):1152-60.

(3) Moher D, Schulz KF, Altman D. CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomised Trials. PLOS Medicine 2010 Mar; Vol 7; Iss 3.

(4) Sterne JAC, Smith GD, Cox DR. Sifting the evidence{---}what's wrong with significance tests? Another comment on the role of statistical methods. BMJ 2001 Jan 27;322(7280):226-31.

(5) Newcombe RG. Interval estimation for the difference between independence proportions: Comparison of eleven methods. Statistics in Medicine 1998;17: 873-890

(6) Alan Agresti and Brent A. Coull. Approximation is better than “Exact” for Internal Estimation of Binomial Proportions. American Statistical Association, 1998; 52:119-126

Appendix 1:

Data Safety Monitoring Board (DSMB) Reports:

1.1 Immediate reports:
Refers to reports of any for Fatal or Life-Threatening Suspected Unexpected Serious Adverse Reactions (SUSARs). These reports should include basic information (e.g. date of vaccination and vaccine lot) and data from the CRF Serious Adverse Events case report form. As indicated in the SOPs WHO 1-3 reporting should take place immediately – within 24 hours- of first knowledge by CMC .

1.2 Regular reports (every 3 months):
These reports contain basic information regarding progress with recruitment (Table 1.2.1). A histogram chart was proposed to illustrate the information on the proposed table (Figure1.2.1). An aggregated information on Fatal or Life-Threatening Suspected Unexpected Serious Adverse Reactions (SUSARs) to vaccination is given (Table 1.2.2A and Table 1.2.2B). Information will be shown by study arm (Aerosol or SQ), type of adverse event and time of onset after vaccination. The laboratory results will be provided only if requested by the DSMB.

Table 1.2.1: Number of children enrolled and vaccinated by study area, study arm, sex

Study area	Assess for eligibility	Allocated to Intervention	Received allocated Intervention	Aerosol Arm			Subcutaneous Arm		
				Boys	Girls	Total	Boys	Girls	Total
1.									
2.									
3.									
4.									
5.									
6.									
7.									
8.									
TOTAL									

Remarks: _____

The median age of children at randomisation was_____ (interquartile range____ to ____ days) (range __ to____ days).

Figure 1.2.1: Histogram for Table 1.2.1 data

Table 1.2.2A: Frequency of Fatal or Life-Threatening Suspected Unexpected Serious Adverse Reactions (SUSARs) by study group and time since vaccination

Part 1: Serious Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Serious Adverse Events** Result in death																			
Is life threatening																			
Results in persistent or significant disability or incapacity																			
Requires hospitalization or continuation of existing hospitalization																			
Important medical event																			
Total																			

Part 2: Unexpected Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Unexpected Adverse Events**																			

Part 3: Severe Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Severe Adverse Events* ** Anorexia																			
Conjunctivitis																			
Coryza																			
Cough																			
Crying																			
Diarrhoea																			
Difficulty breathing																			
Fever																			
Irritability																			
Local																			
Malaise																			
Rash																			
Seizure																			
Shivering																			
Vomit																			
Wheeze																			
Others																			
Total																			

Table 1.2.2B: Frequency of Fatal or Life-Threatening Suspected Unexpected Serious Adverse Reactions (SUSARs) that have been deemed *probably related* and *most probably related* to vaccination* by study group and time since vaccination
Part 1: Serious Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Serious Adverse Events** Result in death																			
Is life threatening																			
Results in persistent or significant disability or incapacity																			
Requires hospitalization or continuation of existing hospitalization																			
Important medical event																			
Total																			

Part 2: Unexpected Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Unexpected Adverse Events**																			

Part 3: Severe Adverse Events

Name	Time since vaccination																	Total	
	Visit 2 0 – 30 mins		Visit 3 31 mins – 3 days		Visit 4 4 – 7 days		Visit 5 8 – 10 days		Visit 6 11 – 14 days		Visit 7 15 – 16 days		Visit 8 17 – 21 days		Visit 9 22 – 28 days		..		
	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC	AER	SC		AER	SC
Severe Adverse Events* ** Anorexia																			
Conjunctivitis																			
Coryza																			
Cough																			
Crying																			
Diarrhoea																			
Difficulty																			
Fever																			
Irritability																			
Local reaction																			
Malaise																			
Rash																			
Seizure																			
Shivering																			
Vomit																			
Wheeze																			
Others																			
Total																			

***** *1. Not related 2.unlikely to be related 3.Possible 4. Probable 5. Most probably 6. Insufficient data to assess*

****** *1. Results in death 2. Is life threatening 3. Results in persistent or significant disability or incapacity 4. Requires hospitalization or continuation of existing hospitalization 5. Important medical event (may not result in death but may jeopardise subject)*

******* *As defined in the protocol list of solicited adverse events*

17. Additional Analyses

17.1. Sero-positivity at Day91: By Vaccinated Sequential Number

17.1.1. Difference in Sero-positivity between study arms by Vaccination Sequential Number

Table17.1.1.1: Measles seropositive at day 91 post-vaccination (all infants) and difference in positivity between study arms by Vaccination Sequential Number

Seropositive at Day91, by VacSeqNo	AER n/N	AER% 95% CI	SC n/N	SC% 95% CI	Difference (AER-SC) 95% CI
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Legend: AER-aersol; SC-subcutaneous;
*This difference is used to interpret non-inferiority,estimated by Wilson score method.

17.2. Sero-positivity at Day91: By Study Arm

17.2.1. Difference in Sero-positivity between study arms by Study Arm

Table17.2.1.1: Measles seropositive at day 91 post-vaccination (all infants) and difference in positivity between study arms by Study Arm

Seropositive at Day91 by Study arm	**AER n/N**	**AER% 95% CI**	**SC n/N**	**SC% 95% CI**	**Difference (AER-SC) 95% CI**
Vadu					
Pabal					
Talegaon					
Wagholi					
Nhavara					
Shel Pimpalgaon					
Chakan					
Alandi					

Legend: AER-aersol; SC-subcutaneous;
*This difference is used to interpret non-inferiority,estimated by Wilson score method.

17.3. Comparison of baseline characteristics

17.3.1. Per – Protocol dataset

Table 17.3.1.1: Comparison of baseline characteristics: **Per-Protocol dataset**

		Included subjects		Excluded Subjects* (with Reasons)	
		n	%	n	%
Age in months	9.0-9.9				
	10.0-10.9				
	11.0-11.9				
Sex	Male				
	Female				
Health care	Govt.				
	Private				
	Non-gov				
	Other				
Pre-existing medical condition	Yes				
	No				
Significant illness in last 3 months	Yes				
	No				
PHC	Alandi				
	Chakan				
	Nhavara				
	Pabal				
	Shel Pimpelgaon				
	Talegaon Dhevdhere				
	Vadu				
	Wagholi				
Seropositive at baseline	Yes				
	No				

17.3.2. Intention To Treat dataset

Table 17.3.2.1: Comparison of baseline characteristics: **Intention To Treat dataset**

		Included subjects		Excluded Subjects* (with Reasons)	
		n	%	n	%
Age in months	9.0-9.9				
	10.0-10.9				
	11.0-11.9				
Sex	Male				
	Female				
Health care	Govt.				
	Private				
	Non-gov				
	Other				
Pre-existing medical condition	Yes				
	No				
Significant illness in last 3 months	Yes				
	No				
PHC	Alandi				
	Chakan				
	Nhavara				
	Pabal				
	Shel Pimpelgaon				
	Talegaon Dhevdhere				
	Vadu				
	Wagholi				
Seropositive at baseline	Yes				
	No				

*such as ‘loss-to-follow ups’, ‘protocol deviation’ and other exclusion reasons will have separate columns