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Glossary of terms

ACIP	Advisory Committee on Immunization Practices
AD	Auto-disable
AEFI	Adverse events following immunization
CDC	Centers for Disease Control and Prevention
CRF	Case Report Form
CRS	Congenital rubella syndrome
DTP3	Third dose of diphtheria–tetanus–pertussis vaccine
ELISA	Enzyme-linked immunosorbent assay
EPI	Expanded Program on Immunization
GIVS	Global Immunization Vision and Strategy
GVAP	Global Vaccine Action Plan
icddr,b	International Center for Diarrhoeal Disease Research, Bangladesh
IgM	Immunoglobulin M
IMCI	Integrated management of childhood illness
ITAG	SEAR Immunization Technical Advisory Group
JRF	Joint Reporting Form
MCV0	Zero dose of measles containing vaccine
MCV1	First dose of measles containing vaccine
MCV2	Second dose of measles containing vaccine
MDG	Millennium development goal
MMA	Maternal measles antibodies
MMR	Measles, mumps, rubella vaccine
MR	Measles and rubella vaccine
MRI	Measles and Rubella Inititative
NML	National measles-rubella laboratory
NRA	National Regulatory Authority
ORI	Outbreak Respond Immunization
PID	Participant Identification Number
PT	Proficiency testing
RC	Regional Committee
RCV	Rubella containing vaccine
RED	Reach Every District
RRLs	Regional Reference Laboratories
RT-PCR	Reverse transcription polymerase chain reaction
RVC	Regional Verification Commission (for Measles Elimination)
SAGE	Strategic Advisory Group of Experts on Immunization
SEAR	South-East Asia Region
SIAs	Supplementary Immunization Activities
SOP	Study operating procedure
WHO	World Health Organization

Project Summary

Principal Investigator: Dr. K. Zaman

Research Protocol Title: Assessing immunogenicity of Measles-Rubella vaccine at 6 and 9 months of age

Proposed start date: As soon as possible **Estimated end date:** 1 year from starting

Background (brief):

Measles:

Prior to the development and widespread use of measles vaccines, measles was estimated to cause between five and eight million deaths annually. Measles continues to cause high morbidity and mortality among children worldwide. It is one of the leading causes of vaccine preventable childhood death with annual estimated measles deaths in 2014 reaching 114,900 (1). Measles is an acute viral respiratory illness. It is characterized by fever and malaise, cough, coryza, and, conjunctivitis followed by maculopapular rash. Children under 5 years of age, who live in overcrowded conditions, and who are malnourished (have vitamin A deficiency), and children with immunological disorders are at higher risk for developing complications such as pneumonia, diarrhoea, encephalitis, and blindness or death. In some low income countries, case-fatality rates among young children may reach 5–10%.(2, 3). Measles virus is highly infectious with reproductive rate of 12-18(4).

Rubella:

Rubella is mild, self-limited illness caused by a rubella virus that usually occurs during childhood. Children develop few or no symptoms, however adults may have low-grade fever, headache, malaise, mild coryza and conjunctivitis. Rubella is transmitted through direct or droplet contact from nasopharyngeal secretions. Rubella infection during early pregnancy may result in serious consequences, including miscarriages, stillbirths, and a constellation of severe birth defects in infants known as congenital rubella syndrome (CRS). The risk of congenital infection and defects is highest during the first 12 weeks of gestation and decreases after the 12th week of gestation, and defects are rare after the 20th week of gestation.(5, 6)

Infants acquire passive immunity to measles and rubella by transplacental transfer of maternal antibodies. (7, 8) These measles and rubella antibodies offer some protection for infants during first several months of life and wane over time, making infants susceptible to measles and rubella infection. The duration of protection offered by maternal antibodies is correlated with the concentration of antibodies transferred transplacentally. Women who had prior natural infection typically have higher titers of antibodies compared with women with vaccine-induced antibodies.

Infants born to mothers with naturally acquired immunity typically have higher titers of passively acquired antibodies. Vaccine-derived maternal antibodies transferred to the infant decline to an undetectable level sooner than naturally-derived maternal antibodies.

This trial will be conducted in collaboration with icddr,b in Matlab, Bangladesh, a site where several vaccine trials have been conducted in the recent past. Bangladesh introduced MR into routine immunization schedule in 2012 and conducted a nationwide MR SIA (Supplementary Immunization Activities) targeting 52 million children aged 9 months – 15 years of age in 2014. A second measles only vaccine dose is now administered to children at 15-18 months of age.

Knowledge gap:

Recent measles outbreaks in many countries show a bimodal age distribution with a high proportion of cases <9 months of age [before the age of routine first dose of measles and rubella (MR) vaccine], as well as increasing numbers of cases among adolescents and young adults. This may be explained in part by the fact that many infants are born to mothers who have been vaccinated against measles. Infants born to mothers with vaccine induced immunity against measles and rubella have lower concentrations of maternal antibodies at birth and lose protective levels of antibodies 2-4 months sooner compared with infants born to mothers with naturally induced immunity. Recent studies show that infants in both groups lose protective levels of measles and rubella antibodies by age of 4 – 6 months that is before the recommended age of 9 months for the first dose of measles containing vaccine (MCV) (9-11).

During large measles outbreaks, WHO recommends using an MCV starting at 6 months to prevent measles among young infants. This dose of MCV should be considered only as MCV 0 dose and parents are advised to bring their infants to receive a routine MCV first dose at 9 months. Most studies of immunogenicity of measles containing vaccine in infants <9 months were conducted using a measles single antigen vaccine. There is a limited data on immunogenicity of MR vaccine administered at < 9 months of age and its impact on immunogenicity of MR vaccine routinely administered at 9 months. As more countries are introducing MR vaccine in their routine immunization program (at 9 months) we propose a study that will assess the immunogenicity of MR vaccine at 6 months (MCV 0 dose) and its impact on immunogenicity of MR vaccine administered at 9 months (MCV1 dose).

Relevance:

The primary purpose of the study is to assess the immunogenicity of MR vaccine when delivered at 6 months. In addition, the study will establish the equality of MR vaccine seroconversion administered at 9 months following administration of an earlier MR vaccine dose at 6 months of age compared to MR vaccine dose administered at 9 months without previous MR vaccination. This study will also provide additional data on safety and tolerance of MR vaccine given at 6 months, and impact of maternal antibodies on immunogenicity of MR vaccine at 6 months.

Hypothesis:

Our study hypothesis is that the proportion of children seroconverting after administration of MR vaccine dose at 9 months, and who had received an MR vaccine dose at 6 months of age, is not different from that obtained after administration of MR vaccine dose at 9 months in infants who had no previous dose of MR vaccine.

Primary objectives:

1. To assess immunogenicity of MR vaccine at 6 months of age
2. To assess immunogenicity of MR vaccine at 9 months of age among children without prior measles and rubella vaccination, compared with MR vaccine immunogenicity among those who had a prior MR vaccination at 6 months of age

Secondary objectives:

1. To assess the frequency of adverse reactions following administration of MR vaccine at 6 months
2. To compare the immunogenicity of the MR vaccine first dose administered at 6 months vs at 9 months.
3. To assess the proportion of mothers with undetectable, detectable and protective levels of measles and rubella antibodies
4. To determine the extent of variation in measles antibodies in women of child bearing age in a population with a long standing measles vaccination program
5. To determine the extent of variation in rubella antibodies in women of child bearing age in a population where rubella vaccine have been recently introduced
6. To determine if variation in antibody levels in infants at 6 months is predominately explained by variation in starting antibody levels in the mother in this population
7. To estimate the half-life of decay of measles and rubella antibodies in infants

Methods:

This study will be an open-label, randomized, 2-arm clinical trial in healthy infants in Bangladesh. 620 children will be enrolled and randomized at 6 months of age to one of two study arms. The study design and participant evaluations are illustrated in Table.

Table: Schematic representation of study arms

Arm		Age of participant				
		6 months	9 months	11 months	Sample size	Enrollment target (sample size + 10% attrition)
A	MR 6 & 9 months	MR •	MR •	•	279	310
B	MR 9 months	•	MR •	•	279	310
	Total (infants)				558	**620**
	Mothers	•			558	**620**

• Indicates blood collection
MR- Measles -rubella vaccination

Outcome measures/variables:

The primary end-point assessment will be based on **measles antibody titers (mIU/ml) (measured by the multiplex bead assay (MBA))** and rubella titers (IU/mL) (measured by ELISA) on sera collected 8 weeks after completion of vaccination series (at 11 months of age). Immune response is defined at 8 weeks after completion of vaccination series, as seropositivity post-vaccination if pre-vaccination antibody was undetectable (i.e. seroconversion), or a 4-fold or greater increase in antibody titers among pre-vaccination seropositives after adjusting for decline in maternal antibodies. Seroprotection is defined as antibody level of ≥ 120 mIU/mL for measles and >10IU/mL for rubella. We will calculate the proportion of children attaining post-vaccination protective levels of antibodies.

Hypothesis to be tested

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

Our study hypothesis is that the proportion of children seroconverting after MR vaccine dose at 9 months following administration of an earlier MR vaccine dose at 6 months of age (Arm A) is not different compared with the immunogenicity obtained when MR vaccine dose is administered at 9 months to infants who have had no previous dose of MR vaccine (Arm B). A secondary hypothesis is the incidence and spectrum of adverse reactions following immunization will be similar between doses given at 6 and 9 months (Arm A), and at 9 months.

The following questions are to be answered by the study:

1. What is immunogenicity of MR vaccine at 6 months of age?
2. What is the impact of the 6-month MR dose on immunogenicity of the 9-month MR dose?
3. What are the frequency and severity of adverse reactions following MR vaccination at 6 months and 9 months?
4. What is correlation between the measles and rubella antibody levels in mothers and level of maternal antibodies in infants

Specific Objectives

Primary objectives

1. To assess immunogenicity of MR vaccine at 6 months of age
2. To assess immunogenicity of MR vaccine at 9 months of age among children without prior measles and rubella vaccination, compared with MR vaccine immunogenicity among those who had a prior MR vaccination at 6 months of age

Secondary objectives:

1. To assess the frequency of adverse reactions following administration of MR vaccine at 6 months
2. To compare the immunogenicity of the MR vaccine first dose administered at 6 months vs at 9 months.
3. To assess the proportion of mothers with undetectable, detectable and protective levels of measles and rubella antibodies

4. To determine the extent of variation in measles antibodies in a convenience sample of women of child bearing age in a population with a long standing measles vaccination program
5. To determine the extent of variation in rubella antibodies in a convenience sample of women of child bearing age in a population where rubella vaccine have been recently introduced
6. To determine if variation in antibody levels in infants at 6 months is predominately explained by variation in starting antibody levels in the mother in this population
7. To estimate the half-life of decay of measles and rubella antibodies in infants

Background

Measles

Prior to the development and widespread use of measles vaccines, measles was estimated to cause between five and eight million deaths annually. In the pre-vaccine period, >90% of individuals were infected by 10 years of age. Wide scale introduction of safe and effective measles vaccines has greatly reduced the burden of this disease. For example, the recent global measles update shows that during 2000–2014, the number of annually reported measles cases worldwide decreased 69%, from 853,479 to 267,482, and measles incidence decreased 73%, from 146 to 40 cases per million population. Although the number of annually reported measles cases worldwide decreased due to measles vaccination programs, measles continues to cause high morbidity and mortality among children. It is one of the leading causes of vaccine preventable childhood death with annual estimated measles deaths in 2014 reaching 114,900 (1).

Measles is an acute viral respiratory illness. Measles is caused by single-stranded, enveloped RNA virus with one serotype. Measles virus is highly infectious (reproductive rate of 12-18) and occurs only in humans; measles virus is transmitted by aerosolized respiratory droplets and by direct contact (4). It is characterized by fever and malaise, cough, coryza, and conjunctivitis followed by maculopapular rash. The rash spreads from the head to the trunk to the lower extremities. The incubation period ranges from 7 to 21 days. An infected person is contagious during the time from 4 days to 4 days after the rash appears. The severity of measles depends on a number of host and environmental factors. Infants have more severe and longer duration of measles illness, with high mortality.(7, 12) Children under 5 years of age who live in overcrowded conditions or who are malnourished (have vitamin A deficiency), and children with immunological disorders are at higher risk for developing complications such as pneumonia, diarrhoea, encephalitis, and blindness or death. In some developing countries, case-fatality rates among young children may reach 5–10%.(2, 3)

Rubella

Rubella is an enveloped single-stranded RNA virus. Humans are the sole host of the virus. Rubella is a mild, self-limited illness that usually occurs during childhood. The rubella rash occurs in 50-80% of infected persons. Children develop few or no symptoms; however, adults may have a prodrome (1-5 days) of low-grade fever, headache, malaise, mild coryza and conjunctivitis. Postauricular, occipital and posterior cervical lymphadenopathy is characteristic, and typically precedes a maculopapular, erythematous and often pruritic rash. Rubella is transmitted through direct or droplet contact from nasopharyngeal secretions. The incubation period of rubella is 12-23 days. Persons with rubella are most infectious when rash is erupting, but they can shed virus from 7 days before to 7 days after rash onset. Up to 70% of adult women with rubella may develop arthralgia or arthritis. Complications of rubella include thrombocytopenic purpura and encephalitis. Postinfectious encephalitis occurs in approximately 1/6000 rubella cases; however, in the Pacific Islands, Hong Kong and Tunisia, there has been a documented increase in frequency to 1:500.(13, 14) During 2000-2014, the number of rubella cases worldwide declined by 95% from 670,894 (in 102 countries) to 33,068 (in 161 countries) (15).

Congenital rubella syndrome

Rubella infection during early pregnancy may result in serious consequences, including miscarriages, stillbirths, and a constellation of severe birth defects in infants known as congenital rubella syndrome (CRS). The risk of congenital infection and defects is highest during the first 12 weeks of gestation and decreases after the 12th week of gestation; and defects are rare after the 20th week of gestation. The most common congenital manifestations are sensorineural hearing impairment, ophthamologic (i.e., cataracts, pigmentary retinopathy), and cardiac (i.e., patent ductus arteriosus, peripheral pulmonic stenosis) (5, 6, 14)

Rubella /CRS control and elimination

High rubella vaccine coverage has significantly reduced rubella and CRS in both developed and developing countries. In 2014, a total of 33,068 rubella cases were reported to WHO from 102 countries, a 95% decrease from the 670,894 rubella cases reported in 2000 from 161 countries. In the Americas, the last endemic rubella and CRS cases were reported in 2009, and the region was declared free of endemic rubella virus transmission in April 2015.(15)

This suggests that elimination of rubella and CRS are feasible. Recently, the Pan American Health Organization announced that the Americas Region has become the first region to stop transmission of endemic rubella virus and declared elimination of rubella and CRS from the region. This landmark achievement culminates a 15-year region-wide effort to widely implement MMR vaccination in the

Western Hemisphere(16). Two other regions (European Region and Western Pacific Region) have established rubella elimination goals. South-East Asia region was the last region to adopt measles elimination and rubella/CRS control goals by 2020. Eight of the 11 countries in the SEAR have already introduced combined MR vaccines into their routine immunization schedules (e.g. Bangladesh in 2014 (17); India and Indonesia are the two largest countries that plan to introduce MR vaccine in the region in the nearest future). Given that rubella is not as highly infectious as measles and because the effectiveness of 1 dose of a rubella containing vaccine is ≥95% even given at 9 months of age, only 1 dose of rubella vaccine is required to achieve rubella elimination if high coverage is achieved.(13)

The World Health Organization (WHO) recommends that countries take the opportunity offered by accelerated measles control and elimination activities to introduce rubella containing vaccines.(13)

Measles and rubella vaccines

Measles vaccines

In 1963, the live attenuated measles containing vaccine (MCV) (Edmonston B strain) was licensed in the US. Two more attenuated live MCVs were derived from the Edmonston strain in 1965 (Schwartz strain) and in 1968 (Moraten strain)(4). In the US, the Moraten strain is currently used. There are other Edmonston-derived strains used internationally, including the Schwartz or the Edmonston-Zagreb strain (4). In addition, other attenuated MCVs derived from local wild-type measles virus strains are used internationally: the Leningrad-16 strain (Russian Federation), the Shanghai-191 strain (People's Republic of China),and CAM-70 and AIK-C strains (Japan)(4). The vaccine effectiveness of all strains is ~ 85% when given at 9-11 months of age, and 90-95% when given at ≥12 months of age.(18)

The recommended age for measles immunization in developing countries is 9 months, particularly in countries with ongoing transmission and in which the risk of measles mortality among infants remains high. In countries with low rates of measles transmission (mostly developed countries), the vaccine is recommended at 12 months of age because of the higher seroconversion rates achieved at this age. (4) The MCV1 effectiveness depends on the age at vaccination, presence of passively acquired maternal measles antibodies, maturity of the immune system, HIV infection, other immunosuppressive conditions, and in some circumstances, concurrent acute infections.(19).

Rubella vaccines

Since 1970s, rubella vaccine has been available and proven to be safe and highly effective. Studies show that one dose of rubella-containing vaccine is ≥95% effective when administered at 9 months of age and confers long-term (likely lifelong) protection against rubella (13, 20).

The RA27/3 strain is the most widely used throughout the world because of its consistent immunogenicity, induction of resistance to reinfection, and low rate of adverse events.(14)

In most countries, rubella vaccine is combined with measles and/or mumps vaccines (MR or MMR).

Measles-rubella vaccine

Measles-rubella vaccines have been commercially available since the 1980s. Serum Institute of India is one of the main suppliers of MR vaccine in the market. A recently published study from Bangladesh showed that when MR vaccine is administered at 9 months, 74% and 100% of the vaccine recipients had seroprotective levels of measles and rubella virus antibodies respectively.(21)

Measles and rubella/CRS elimination goals

Under the Global Vaccine Action Plan, measles and rubella are targeted for elimination in five WHO Regions by 2020. For measles elimination, the target year for the European and Eastern Mediterranean Regions is 2015, and for the African Region and South-East Asian Regions is 2020. For rubella elimination, the target year for the region of the Americas and European Region are 2010 and 2015, respectively. The Western Pacific Region has not set a targeted date for rubella elimination.

Passive immunity

Infants acquire passive immunity to measles and rubella by transplacental transfer of maternal antibodies. (7, 8) These antibodies offer some protection for infants prior to receipt of MR vaccine. Mothers who have had natural infection in the past typically have higher titers of antibodies compared with women who have vaccine-induced antibodies and because of their lower titer, vaccine-derived maternal antibodies transferred to the infant decline to an undetectable level sooner than naturally-derived maternal antibodies. This has been documented by researchers who tested paired mother and infant serum samples for measles and rubella antibodies [1, 2] (some studies included blood samples drawn at week 36 of pregnancy and cord blood sample) [3, 4]. They reported that infants of vaccinated women have significantly lower antibody concentrations than did infants of naturally immune women, and infants of vaccinated women lose protective levels of antibodies sooner (i.e., by 6-8 months) than infants of naturally immune women [4].

A study in Matlab, Bangladesh(22) reported that in 1993, 67% of infants by age 5 months and 95% of infants by age 8 months had measles antibody titers below the threshold for protection leaving them susceptible to natural infection. As a result, a rising trend of measles cases below the eligible age of first routine measles vaccination, typically 9 or 12 months of age, has been observed. From 2004-2006, in Bangladesh an increase in the proportion of measles cases among infants <9 months of age was noted in

6 selected outbreaks. In these outbreaks 54% of cases were 6-8 months of age. From 2002-2009, 10% of confirmed measles cases in WHO African Region occurred among infants <9 months of age(23). In China, measles cases have been noted to have a bimodal distribution with majority of the cases either in adults up to age 30 in infants younger than 8 months, the routine age of first dose of measles vaccine. To improve the accuracy of interpretation of study results it would be prudent to test mothers of enrolled infants for measles and rubella antibodies. This will also enable us to determine the proportion of mothers with undetectable, detectable and protective levels of antibodies, and to assess the relationship between the level of antibodies in mothers and their infants' maternal antibodies.

Breastmilk does not give infants antibodies that protect against measles and rubella. Antibodies that are transferred through breastmilk are mostly IgA antibodies which mostly protect against gastrointestinal infections.

Response to early vaccination

Response to early measles vaccination, prior to 9 months of age, is affected by prematurity of the infant's immune system as well as neutralization of vaccine virus by circulating maternal antibodies. A number of studies have evaluated immunogenicity of early vaccination with single antigen measles vaccine. Studies conducted in the years after licensure of measles vaccine reported 60%-70% seroconversion with measles vaccine in infants <9 months of age. Additionally, some studies reported a suboptimal immune response to later vaccination in children who had been previously vaccinated at <1 year of age. Recently, in a study in children in California, USA, Gans et al reported no difference in cell-mediated immune response in children vaccinated at 6, 9 or 12 months (24). Gans et al. also reported that seroconversion in 6 month old infants was 77% among those who did not have maternal antibodies compared with 96% in those 12 months of age (24). Overall, while measures of humoral immunity (seroconversion and geometric mean titers) were lower in 6 month old infants compared with those 12 months of age, cell-mediated immunity responses in these two groups of infants did not differ. Significantly, early measles vaccination at 6 months of age was not associated with altered immune response to second dose of measles vaccine. A subsequent study of 55 infants by Gans et al confirmed the prior findings and reported an overall seroconversion of 86% in 6 month old infants. The only trial that evaluated the immunogenicity of early rubella vaccination was conducted in Brazil in 1990-91 and reported >90% seroconversion by 6 months of age in 24 infants (25).

Seroconversion and immune response

Seroconversion is defined as a change from an antibody seronegative to seropositive status after exposure to an antigen. Immune response definition includes seroconversion or a 4-fold or greater

increase in antibody titers among pre-vaccination seropositives, at 8 weeks after completion of vaccination series, after adjusting for decline in maternal antibodies.

Typically, this is demonstrated by a rise in IgG titers. Following measles vaccination, antibodies first appear between 12 (when hemagglutination-inhibition (HI) and neutralization (Nt) tests used) and 15 days and peak 21-28 days after vaccination (4).

Study Rationale

In countries with low levels of circulating measles virus, and lower risk for measles infection, MCV1 is recommended at 12 months of age. In countries with high risk for measles, MCV1 is recommended at 9 months. In the past decade, many countries have been experiencing measles outbreaks with a high proportion of cases among infants < 9 months of age, below the recommended age of routine MCV1.

Recent publications suggest that this may be due to the fact that the majority of infants now are born to mothers with vaccine induced immunity to measles, and who lose maternal antibodies much earlier, by age of 4-6 months.(10, 11, 26) For example, in a measles outbreak in Malawi in 2010, 14% (17,858) of the estimated 134,000 cases occurred in children 0-8 months. In 2013, in a measles outbreak in Sri Lanka approximately 34% of measles cases were in children 6-12 months of age. Furthermore, in 2013 Jordan experienced a large measles outbreak with high proportion of young infants affected (6-9 months). WHO measles outbreak response guidelines recommend vaccinating children as young as 6 months during outbreaks. As an example, in response to a measles outbreak, Sri Lanka and Jordan conducted ORI that included infants 6 months of age despite the limited evidence on immunogenicity of MR vaccine at that age.

Some researchers suggest that routine MCV should be given before age of 9 months based on published data showing that infants born to mothers with vaccine induced measles immunity are born with lower concentration of maternal measles antibodies (MMA) and lose protection against measles infection at an earlier age. (10, 26) Measles vaccine immunogenicity depends on several factors, including presence of maternal measles antibodies (i.e., passively acquired measles antibodies may neutralize vaccine virus before a complete immune response develops resulting in primary vaccine failure.), maturity of immune system of the vaccine recipient, and strain of the measles vaccine used. So any decisions to alter the age of MCV1 dose should balance the potential risk of primary vaccine failure against the risk of measles infection and measles related complications, including death. Immunogenicity of measles vaccine given at 6 months is well studied; however, data on immunogenicity of combined MR vaccine administered at 6 months and its impact on MR vaccine effectiveness given at 9 months, is limited.

Because the number of infants born to mothers with vaccine induced immunity has been steadily increasing and most countries will be using MR vaccine routinely in EPI, it is timely to conduct a study to assess immunogenicity of MR vaccine given at 6 months and its impact on a subsequent 9 month MR dose. The first dose of MCV given at 6 months is frequently referred to as MCV0, indicating that two subsequent doses are needed to attain population seroprotection levels necessary to stop endemic transmission of the measles virus.

To improve the accuracy of interpretation of study results it would be prudent to test mothers of enrolled infants for measles and rubella antibodies. Without the knowledge of maternal antibody status it would be difficult to interpret results of the infants with low or undetectable levels of maternal antibodies. Testing maternal serum samples would help us to understand whether low or undetectable baselines are due to decay or absence of maternal antibodies. This will also enable us to determine the proportion of mothers with undetectable, detectable and protective levels of antibodies, and to assess the relationship between the level of antibodies in mothers and their infants' maternal antibodies.

Research Design and Methods

Setting: Study area

This study will be conducted in a single site in rural Bangladesh at Matlab. Matlab lies in the delta of the river Ganges, about 55 km south east of Dhaka. The area has high population density of more than 700 per km^2 and most people live by subsistence farming and travel by both waterways and road. Matlab is a major rural field site for icddr,b, where for the past 50 years continuous health and demographic information was collected on >200,000 population. (27) icddr,b, formerly Cholera Research Laboratory, has implemented a health research programme since 1963. In addition, icddr,b has conducted a Maternal, Child Health & Family Planning services program since 1978.[1] A central treatment facility, staffed by physicians and paramedics, provides free therapy for >25,000 diarrhea patients a year. A Health and Demographic Surveillance System (HDSS) has been maintained in Matlab since 1966. The villages of the HDSS area are divided into an icddr,b "intervention area" where the icddr,b provides maternal, child health and family planning services,(28) and a "government comparison

[1] "Health and Demographic Surveillance System - Matlab: Volume Forty Seven Registration of Health and Demographic Events 2013 [SR126, April 2015]" available from http://www.icddrb.org/publications/cat_view/52-publications/10049-hdss-annual-reports

area" where the government health system provides these services. The demographic information collection is conducted in both intervention and government areas.

From 2007 to 2010, icddr,b, the Ministry of Health (MOH) in collaboration with other international organizations conducted several randomized vaccine effectiveness studies, including the most recently published study entitled "Non-interference of rotavirus vaccine with measles-rubella vaccine at 9 months and improvements in anti-rotavirus immunity: a randomized trial."(21)

Study design

This study will be an open-label, randomized, 2-arm clinical trial. 620 children will be enrolled and randomized at 6 months of age to one of two study arms. The primary objectives of this study are to assess the immunogenicity of MR vaccine at 6 months, and assess equality of MR vaccine seroconversion administered at 9 months following administration of an earlier MR vaccine dose at 6 months of age compared with MR vaccine dose administered at 9 months only without previous MR vaccination. The study will enrol generally healthy 6 month old infants living in Matlab and who have never received an MR vaccine dose and have no history of measles or rubella. Participants will be followed to 11 months of age.

Infants in this study will be randomly assigned to one of two arms. Infants in Study Arm A will receive MR vaccine at 6 months of age (at enrolment) and at 9 months. Infants in Study arm B will receive MR vaccine only at 9 months.

Blood specimens will be collected from all infants at 6, 9 and 11 months of age. The 6 month sample is a pre-vaccination sample and mainly will be used to determine maternal antibody levels. For arm A, the sample collected at 9 months before the second MR dose will be used to assess antibody levels after the first MR dose at 6 months of age. For study Arm B, the 9 month sample will be used to assess measles and rubella antibody decay rate. The sample collected at 11 months will be used to assess immune response to either a two dose or a one dose schedule (arms A and B, respectively). The study design and participant evaluations are illustrated in the Table.

Table: Schematic representation of study arms

Arm		Age of participant				
		6 months	9 months	11 months	Sample size	Enrollment target (sample size + 10% attrition)
A	MR 6 & 9 months	MR •	MR •	•	279	310
B	MR 9 months	•	MR •	•	279	310
	Total (infants)				558	**620**
	Mothers	•			558	**620**

• Indicates blood collection
MR- Measles -rubella vaccination

Blood specimens will be collected from mothers of all enrolled infants at time of enrolment.

Study enrolment and withdrawal

A sample of 620 infants at age 6 months (180 days, +/- 7 days) of age (310 per each study arm) will be enrolled from population living in Matlab area. Inclusion and exclusion criteria are provided below.

Inclusion criteria: Infants

- Healthy infants at 6 months (180 days, +/- 7 days) of age (calendars will be provided to staff)
- A parent or guardian that consents for participation in the full length of the study
- A parent or guardian that is able to understand and comply with planned study procedures

Inclusion criteria: Mothers

- Mothers of infants that meet inclusion criteria.
- ≥18 years of age
- Mothers who consent to participate in the full length of the study

Exclusion criteria: Infants

- Family that is unable to participate in the full length of the study
- A diagnosis or suspicion of immunodeficiency disorder either in the infant or mother
- A diagnosis or suspicion of bleeding disorder that would contraindicate parenteral administration of MR vaccine or collection of blood by venipuncture
- Acute infection or illness at the time of enrolment (6 months) that would require infant's admission to a hospital

- Receipt of any measles or rubella containing vaccine prior to enrolment (i.e., before age 6 months) outside of study based upon documentation or parental recall
- Known history of laboratory confirmed measles or rubella infection
- A diagnosis of rubella infection in mother during pregnancy
- A diagnosis of congenital rubella syndrome in infant
- Known allergy/sensitivity or reaction to measles-rubella containing vaccine or contents of measles-rubella containing vaccine
- Persons with a history of an anaphylactic reaction to any components of the vaccine
- Infants from premature births (<37 weeks of gestation)

Exclusion criteria: Mothers

- Refuses to give blood samples. (Note: If the mother agrees for her child to participate in the study, but refuses to give a blood samples herself or blood samples cannot be obtained, the child will still be enrolled.)
- A diagnosis or suspicion of immunodeficiency disorder
- A diagnosis or suspicion of bleeding disorder that would contraindicate collection of blood by venipuncture

Temporary exclusion

- Acute febrile illness (≥38°C) at the time of enrolment
- Family will be requested to bring back the child 1-2 days later or when child feels better.

Discontinuation criteria

Enrolled participants may be excused from the study for the following reasons:

- Withdrawal of consent for participation for any reason
- Request by parents of participant to terminate study procedures on enrolled participant
- Receipt of any measles or rubella containing vaccine (M, R, MR or MMR) outside of study based on documentation or parental recall after enrolment (6 months) or second visit at 9 months
- Identification of immunodeficiency disorder, bleeding disorder or another medical condition for which continued participation, in the opinion of the investigator, would pose a risk to the participant to continue in the study
- Receipt of immunosuppressive medications
- Allergic reaction to a prior dose of measles or rubella containing vaccine or its contents necessitating withdrawal
- Premature termination of the study

- The participant has moved to another area outside of Matlab and is not available for follow up visits
- In case of a withdrawal from study, the local investigator should talk to a parents and document reasons why they are withdrawing, and document any adverse events or conditions that necessitate withdrawal of a participant from the study.

Termination of study

There seem to be no anticipated reasons that may result in suspension or termination of the study. Nevertheless, there are possible causes that may warrant termination of study:

- Unexpected high number of severe adverse reactions related to use of MR vaccine or any other safety concern then any ethical committee overseeing the study, Ministry of Health or CDC can terminate the study for any safety reasons.
- A large measles or rubella outbreak in the community
- An outbreak response immunization activity with MR vaccine targeting infants aged 6 months and older in the area in response to a large measles or rubella outbreak

Measles or rubella diagnosis; measles or rubella outbreak in the community

If during the course of the study, an infant is diagnosed with a laboratory confirmed measles or rubella infection – it will not be a reason for discontinuation. If an infant is diagnosed with measles or rubella study staff will make sure that the child is non-infectious (at least 4 days after rash onset) then parents will be asked to bring the child to study site for a follow up visit

Concomitant medications and vaccines

- There will be no restrictions on the use of medications or treatments for concomitant diseases. If child is prescribed an immunosuppressive medication then the child will be excluded from the study.
- Parents will be encouraged to get their child vaccinated for other routine childhood vaccines; upon completion of the study, all parents will be reminded to take their child to their assigned vaccination centre to receive a second dose of routine measles vaccine at 15 months of age

Adherence to group assignments

Study ID and group assignment will be recorded in the participant's Case Reporting Form (CRF). A log book will be maintained by the study team. Participants will be issued a study ID card that will contain the following key information: participant's name, study ID, and assigned group. A study team member will verify the information recorded on the study ID and will select appropriate procedures required during each visit.

If a participant misses the scheduled vaccine or is incorrectly provided a dose then a study supervisor will be immediately informed to conduct necessary review and decide actions to be taken to resolve the current issue and prevent such issues in future.

Description of MR vaccine

Only MR vaccine will be used during the study. MR vaccine will be selected from the list of WHO prequalified vaccines and those that are commercially available during the study (the list is available here: http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/). The major manufacturer of the live MR vaccine is Serum Institute of India Pvt. Ltd. Measles-rubella vaccine will be provided on site in its commercial presentation. A multi-dose vial with its lyophilised active component needs to be reconstituted with excipient diluent supplied before use. Sterile, disposable and auto-disable syringes will be provided separately.

Product storage and stability

Measles and Rubella Virus Vaccine Live USP™ must be stored in vaccine refrigerators at Matlab Health and Research Centre (MHRC) at +2°C to +8°C (36°F to 46°F) and the diluent must not be frozen. Vaccine vials should be protected from light.

MHRC has appropriate back-up power generators and the vaccine room is staffed by study vaccine accountability personnel.

MR vaccine will be transferred daily to each fixed site clinic according to study procedures using cold storage boxes. Storage temperature will be monitored daily and documented on an appropriate form. In case of freezing or accidental disruption of the cold chain, the vaccine should not be administered and the investigator or the responsible person should contact study investigator to receive further instructions.

MR vaccine

- A new vial will be opened and reconstituted for each participant per visit.
- The vial will be discarded after each participant.

Dosage, Preparation and Administration of Study Intervention

MR vaccine will be reconstituted with provided diluent and administered per WHO EPI immunization guidelines. MR vaccine will be given in a single dose of 0.5 ml. If reconstituted vaccine is not used within the first two hours then it should be discarded. One vial will be used per participant per visit. All vaccines will be provided either by study physicians or by study nurses and documented by study physicians.

Study procedures

Study arm assignment procedures

This is an open-label randomized trial that will be conducted at the icddr,b site in Matlab. After the enrolment parents would know what study arm they are matched with and whether their infant is going to receive one or two doses of MR vaccine during the trial.

Randomization procedures

Participants will be randomized to two arms using a block randomization scheme.

A computer-generated block randomization scheme will be used with random block sizes and 1:1 allocation ratio. The sealed envelopes containing assignment numbers and instructions will be delivered to the recruitment sites and will be opened only at time of enrollment.

Each new enrolled participant will be assigned the next ID number on the list. The study staff enrolling participants will not have *a priori* knowledge of the randomization scheme. As this is an open-label trial, the arm assignment of participants will be known to study staff and participant's parents after randomization.

Field workers will inform parents of infants under 6 months (180 days, +/- 7 days) about the study and briefly explain the study procedures. If interested to participate, parents will be asked to visit the study clinic when their infant turns 6 months (180 days, +/- 7 days) of age, to obtain comprehensive information on the study and if the parents agree to complete enrolment procedures.

Enrolment visit – 6 months of age

(interview, infant's and mother's blood draw, vaccine administration to infants in Study Arm A)

During this visit, the following procedures will be conducted:

1. The study physician will confirm that the infant is eligible to participate. If the infant is eligible, all study procedures will be explained to the parents. If parents agree to participate then they will read or a staff member will read the consent form for them and a parent will be asked to sign/give thumb impression in the consent form.
2. A study doctor will obtain a medical history and conduct a medical examination to assess whether the infant is eligible to take part in the study.
3. Infants who are not eligible per inclusion and exclusion criteria will be excused from study participation and it will be documented in the case reporting form.

4. Participants who meet temporary exclusion criteria at enrollment will be excused for the day and will be requested to return several days later, but as long as their age is 180 days, +/- 7 days. A note will be made in the case reporting form.
5. The eligible infant will be given a study ID and assigned to a study arm (arm A or B) according to a pre-determined randomization scheme. Further details of the randomization scheme are described in the 'Data Analysis' section.
6. Parents will be interviewed and socio-demographic and clinical information will be collected as per standardized study questionnaire.
7. One blood specimen (minimum of 1 ml) will be collected by venipuncture from all enrolled infants following the enrollment. During visits in which the child receives vaccine, blood samples will be drawn before vaccine administration.
8. One blood specimen (1 ml) will be collected by venipuncture from mothers of all enrolled infants following the enrollment.
9. Infants randomized to the study arm A will be administered the first dose of MR vaccine. Vaccine will be administered only if an adequate blood specimen has been collected from the participant.
10. The participant will be observed for 30 minutes for any adverse reactions from administration of the vaccine or the blood collection.
11. A research assistant will schedule the second visit. All participants will be requested to return 3 months later after enrolment, i.e., at 9 months of age.

<u>Follow up home visit (2 days after MR vaccination). Safety monitoring.</u>

- Children in study arm A, who received a 6 month dose of MR vaccine, will be visited by a field research assistant at their homes 2 days after MR vaccination. This visit is to identify if any participants experienced any adverse events following immunization.
- The research assistant will inquire about and record any potential adverse events after the 6 month dose of MR. All finding will be captured in the AEFI form.

<u>Study site visit at 9 months of age. Blood draw and MR vaccination.</u>

During the 9 month visit, the following procedures will be conducted:

1. The study physician will complete the study questionnaire and examine the participant
2. 1 ml of blood will be drawn from each infant (both study arms A and B).
3. Study vaccine will be administered to ALL infants (both study arm A and B). The participant will be observed for 30 minutes to monitor for any adverse reactions
4. Schedule the next visit at 11 months of age

MR vaccination at 9 months is now routinely given in Bangladesh. Therefore, there will be no follow-up household safety monitoring visit after the 9 month MR dose during the study.

Temporary exclusion

- Participants who meet temporary exclusion criteria for vaccination will be excused for the day and will be requested to return several days later, but as long as their age is 9 months, +/- 7 days. A note will be made in the study log book.

Study site visit at 11 months of age. Blood draw and Study exit.

This visit will be the third and last planned study visit for participants. During this visit, the following procedures will be conducted:

1. The study physician will complete the follow up form and examine the participant
2. 1 ml of blood will be drawn from each infant (from both study arms A and B).
3. At study exit, parents will be reminded to bring their child to the fixed site clinic or another EPI facility to receive their second dose of measles vaccine according to the new EPI schedule (at age 15 months).

Laboratory evaluations

Clinical Laboratory Evaluations

No clinical laboratory evaluations will be offered in this study. Laboratory assays described in the protocol are for research purposes only and laboratory results will not to be used for clinical diagnosis.

Special Assays or Procedures

Blood samples will be obtained at 6 months from all infants (at enrolment) and mothers and tested for measles and rubella antibodies. The baseline levels will identify infants who have transplacentally transferred maternal antibodies been exposed to natural measles infection before the enrolment to the study. The mothers' antibody levels will be determined and they will be classified as "susceptible" or "protected" (seroprotection definitions will be the same: titer of ≥ 120 mIU/mL for measles and rubella >10IU/mL).

For participants in the study group A, the immune response to the 6 month MR dose will be measured at 9 months (blood drawn during the second visit) to evaluate both the proportion seroconverting (from negative to protected), 4-fold or greater increase in antibody titers among pre-vaccination seropositives, after adjusting for decline in maternal antibodies, as well as the magnitude of the immune response (expressed as geometric mean titers of the antibody response). The rate of decay of maternal measles and rubella antibodies will be calculated in infants in arm B based on the difference of antibody levels measured at 6 months (baseline) and 9 months. We will incorporate the model of maternal measles and rubella decay for infants in arm A to measure post-vaccine antibody levels and impact of the maternal

antibody to immune response in infants who had measles or rubella antibodies at enrolment. In the recently published study from Bangladesh, baseline measles virus serum neutralizing antibody seroprotection and anti–rubella virus IgG seroprotection at 9 months of age was low (0.42-1.25% and 0-0.42%, respectively) (21). If during this study, we determine that most children have no detectable levels of maternal measles or rubella antibodies at 6 months it would be impossible to calculate the decay. In this case, we will refer to antibody decay rates reported elsewhere (29, 30).

THE FOLLOWING CHANGES ARE MADE TO THE PROTOCOL:

Since the inception of the project and completion of the field data collection a newer assay, multiplex bead assay (MBA) for measles and rubella has been developed. It has been validated by the CDC measles and rubella laboratories.

Multiplex Bead Assay (MBA) for Serum Antibody Detection of Measles and Rubella

Multiplex bead assay detects antibodies against viral proteins in the serum of individuals who have been exposed to measles or rubella either by vaccination or natural infection. The MBA works by reading the fluorescent intensity of antigen-specific antibodies in serum binding to antigen coated beads and converting to antibody concentration using a standard curve. This is a novel IgG test for measles and rubella used at research labs. It is not commercially available and requires a significant amount of preparatory work to run the test and is not yet used to diagnose or confirm recent infection (IgM). Developing this capacity is not required for routine measles and rubella surveillance which the Bangladesh National Lab performs. Moreover, icddr,b has no prior experience in running MBA tests.

Measles and Rubella MBA testing will be conducted by the Measles, Mumps, Rubella, and Herpesvirus Laboratory at CDC, a WHO reference laboratory for measles virus. These tests will be will be performed as described by Smits et al.(31) The analytical cut-off value in the measles MBA assay is 153 mIU/ml. The concentration of antibody ≥153 mIU/ml will be considered to be protective for measles. The analytical cut-off value in the rubella MBA assay is 9.36 IU/ml. The concentration of antibody ≥ 9.36 IU/ml will be considered to be protective for rubella. There may be a formal comparison of rubella IgG concentrations between MBA and the standard EIA results.

Following testing of the samples by MBA, 5%-10% of the samples will also be tested by PRN for quality control testing.

Additional information regarding this assay is included in a detailed protocol maintained at the study laboratory.

Serum Measles IgG Antibody Detected by ELISA

Measles IgG testing will also be conducted by the Measles, Mumps, Rubella, and Herpesvirus Laboratory at CDC.

A commercial enzyme-linked immunosorbent assay (ELISA) will be used for the qualitative detection of IgG antibodies to Measles virus in human serum (Zeus Scientific Inc). Antigen specific antibody in the serum specimen binds to immobilized antigen and is complexed with an antibody linked to an enzyme. The color intensity of the solution depends upon the antibody concentration in the original test sample.

Calibrators included in each run are used to calculate a cutoff OD value for positive samples as determined by the manufacturer. A manufacturer provided correction factor for each lot of kits is printed on the kit box. To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator. Index standard ratios (ISR) are calculated for each specimen by dividing its OD value by the cutoff OD. An ISR<0.90 indicates no significant amount of IgG antibodies to Measles detected (negative). An ISR>1.10 indicates that IgG antibodies specific to Measles were detected (positive). Specimens with ISR in the equivocal range (0.91 - 1.09) will be retested in duplicate giving three test results; the final result is the one that appears in 2 of 3 results.

The Zeus Scientific ELISA has a sensitivity of 96.06% and specificity of 70.5%. This assay has been shown to have 86% agreement with the plaque reduction neutralization assay, the gold standard assay used to determine immune protection to measles at the protective titer of 120 mIU/ml. *(Latner et.al., 2020, in press).*

Additional information regarding the specifics of this assay is included in a detailed protocol maintained at the study laboratory.

Serum Measles Neutralizing Antibody Detected by PRNT

This test will be run as quality control testing for MBA, using 5-10% of samples. The plaque reduction neutralization test (PRNT) is considered a 'gold standard' laboratory test for measles vaccination immunogenicity studies as it is a functional antibody assay, measuring serum neutralizing antibodies (SNA).(21, 32) This testing requires a cell culture line and it will be standardized with the laboratory in CDC. The PRNT is not normally used in measles surveillance and developing this capacity is not required for routine measles operation which Bangladesh has in the National Lab. Moreover, icddr,b has no prior experience in doing measles PRNT.

Measles PRNT will be conducted by the Measles, Mumps, Rubella, and Herpesvirus Laboratory at CDC, a WHO reference laboratory for measles virus. PRNT will be performed as described by Cohen et al.(32) Assays will be standardized using WHO 2nd International Standard for measles antibody containing 5000 mIU/mL, which enables the 50% neutralizing antibody end-point dose (titer, ND50) of test samples to be transformed to antibody concentrations in terms of mIU/mL. The analytical cut-off value in this assay is ND50 < 1/8; this is the lowest dilution at which sera are tested. The interpretive cut-off value is ≥ 120mIU/mL; this is the concentration of antibody considered to be protective. The term seroconversion will be used to refer to subjects whose SNA antibody levels changed from less than the interpretive cut-off value of 120 mIU/mL to equal or greater than the interpretive cut-off value of 120 mIU/mL.

Additional information regarding this assay is included in a detailed protocol maintained at the study laboratory.

Serum Rubella IgG Antibody Detected by ELISA

Rubella testing will also be conducted by the Measles, Mumps, Rubella, and Herpesvirus Laboratory at CDC, a WHO reference laboratory for rubella virus using a commercially available ELISA kit. For this test serial dilutions will be prepared at the CDC laboratory for the testing. The Zeus ELISA Rubella IgG test kit will be used to test for IgG class antibodies to Rubella virus in samples. In this assay, purified virus antigen is adsorbed onto a solid phase and the subject's serum is added. Any virus-specific antibody binds to the antigen, and virus-specific IgG is detected using anti-human IgG. The binding of virus-specific IgG is measured using a chromogen substrate. The colour intensity of the solution depends upon the antibody concentration in the original test sample. With this assay, a positive Index Standard Ratio (or optical density ratio) ≥1.10 (when converted to IU/mL, corresponds to ≥10 IU/mL) is positive for IgG antibody to rubella virus, and is considered protective for rubella.(33)

Additional information regarding this assay is included in a detailed protocol maintained at the study laboratory.

Specimen collection, handling and testing

Following universal precautions, 1 ml of blood will be collected from participants by venepuncture into a vacutainer at enrolment and at scheduled time-points during follow-up before administration of vaccine.

If informed consent is obtained from parents, the serum specimens will be stored for up to 5 years after the end of the study for future evaluation related to the vaccines effectiveness.

Processing of Sera

Immediately after collection at the fixed site clinic, the blood specimen tube will be inverted 5 times, labelled with the unique study participant number and the collection date, and stood upright to clot for at least 30 minutes at room temperature, before transfer via cool box to icddr,b for processing. At the Matlab laboratory, specimens will be centrifuged at 3000 rpm for 5-10 minutes before aliquoting into 2 vials of at least 200µl each. All aliquots will be stored at -20°C.

Aliquoting of Sera

The same day as specimen collection and processing, specimen aliquoting will be performed at icddr,b laboratory. This procedure should be completed participant-by-participant to avoid mixing blood tubes according to the following steps:

- After centrifugation, the technician in charge of aliquoting specimens should carry out the operation by taking the blood collection tubes one by one from the centrifuge.
- The operator will only place in a rack the number of cryovials (2 vials per subject) necessary for the aliquoting of one participant's specific blood specimen and will affix the completed labels onto the vials, checking the study participant number and collection date.
- Each serum specimen will be divided into 2 aliquots as follows:
- First aliquot: at least 200µl for measles and rubella immunogenicity assays.
- Second aliquot: remaining serum for back up stored at icddr,b.
- The study participant number, date of collection, number of aliquots obtained, and the date and time of division will be specified on a Serologic Specimen Log form. On this form, comments may be made on the quality of specimens (e.g., hemolyzed, contaminated, etc.).
- Tubes with clotted blood will be discarded following standard biohazard waste disposal protocol.
- The next participant blood collection tube should be taken out of the centrifuge, and the procedure repeated for this subject's specimen.
- Immediately following aliquoting, serum specimens to be shipped to CDC should be frozen at -20°C. Back up specimens will be stored at -80°C at icddr,b.

International Specimen Shipment

Frozen sera will remain frozen during routine shipments to the laboratory at icddr,b and to the CDC measles/rubella laboratory in Atlanta. Shipment of serum will be made according to internationally recognized standards for transport of specimens.

Long-term specimen storage

The parents will be requested to permit storage of blood specimens for no more than 5 years for potential future use in studies. All specimens will be destroyed after 5 years from the end date of the study (the date of last study visit of the last participant to be enrolled). Specimens will be destroyed as per standard procedures of the laboratory for destruction of biohazardous waste.

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

Sample size

The study will be powered to address the primary objectives. Sample size calculations are based on equality comparisons with 10% attrition.

Assuming 50% MR seroconversion to a dose at 6 months of age, a sample size of 271 per arm will provide a precision of +/-5% with a 0.90 probability of obtaining an estimate within the desired range. Adjusting for 10% attrition, the enrolment target is 302. Assuming a seroconversion for measles of 75% following a single dose given at 9 months of age, a seroconversion of 65% for a 9 month dose preceded by a 6 month dose, and power of 80%, a sample size of at least 279 per arm is required. Adjusting for 10% attrition, this sample size is inflated to 310. The overall study samples size will be 620 infants. 620 mothers of infants will also be enrolled. (Table)

Time to endpoint (and the corresponding objective)

Primary endpoint

- Time from randomization at 6 months to collection of blood at 9 months of age (To assess immunogenicity of MR vaccine at 6 months of age)
- Time from randomization to collection of blood at 11 months of age (To assess immunogenicity of MR vaccine at 9 months of age among children without prior measles and rubella vaccination, compared with MR vaccine immunogenicity among those who had a prior MR vaccination at 6 months of age)

Secondary endpoint

- Time from randomization 11 months (To assess the frequency of adverse reactions following administration of MR vaccine at 6 months)
- Time from randomization to 11 months of age (To compare the immunogenicity of the MR vaccine first dose administered at 6 months vs at 9 months)

- Collection of blood from mothers at time of enrolment to assess measles and rubella titers, as only one blood sample will be collected. (To assess the proportion of mothers with undetectable, detectable and protective levels of measles and rubella antibodies)
- Time from randomization to 9 and 11 months (To assess the correlation between measles and rubella antibody levels in women and levels of maternal antibodies in infants)
- Time from randomization to 9 months, Arm B (To estimate the half-life of decay of maternal measles and rubella antibodies in infants)

The primary end-point assessment is based on measles titers by MBA (mIU/ml) and rubella titers (IU/mL) by ELISA on sera collected 12 weeks after the first dose (time between 6 and 9 months) and 8 weeks after completion of vaccination (time between 9 and 11 months). Immune response is defined as a 4-fold increase in antibody titers 12 weeks after vaccination (post-6-month visit) compared with 6-month pre-vaccine titers accounting for the expected decline in maternal antibody titers or a change from antibody-negative to positive (i.e. seroconversion). However, quantitative data (i.e., determining titers) will allow seroconversion defined by other -fold increases to also be assessed.

We will calculate the rate of decay for those infants that have maternal antibodies detected at enrolment (at 6 months) in arm B (anti-measles or anti-rubella IgG levels at 6 months vs 9 months). We will incorporate the model of maternal decay for infants in arm A to measure post-vaccine antibody levels and impact of the maternal antibody to immune response. However, if we determine that most children have no detectable levels of maternal measles or rubella antibodies at 6 months it would be impossible to calculate the decay rate (in Arm B). In this scenario, we will refer to antibody decay rates reported elsewhere (29, 30).

Outcome measures/variables:

The primary end-point assessment is based on measles titers by MBA (mIU/ml) and rubella titers (IU/mL) by ELISA on sera collected 12 weeks after MR 6-month dose of vaccination (i.e., at 9 months) for study Arm A.

Immune response is defined as a 4-fold increase in antibody titers 12 weeks after vaccination (post-6-month visit) compared with 6-month pre-vaccine titers accounting for the expected decline in maternal antibody titers or a change from antibody-negative to positive (i.e. seroconversion). In addition, quantitative data on antibody titers will allow us to assess the magnitude of seroresponse show as –N-fold increases of pre- and post-vaccine titers. Seroprotection is defined as titer of ≥ 120 mIU/mL for measles and rubella >10IU/mL

Secondary outcomes:

Several secondary outcomes will be assessed:

1) Secondary outcome is based on measles titers by MBA (mIU/ml) and rubella titers (IU/mL) by ELISA on sera collected 8 weeks after MR 9-month dose of vaccination (i.e., at 11 months) – post two dose series for participants in Arm A and 1 dose for arm B.
2) The safety profile of MR vaccine will be assessed by incidence of specific symptoms/problems. They will be categorized into one of the following three categories:
    a) Immediate reactions (with emphasis on allergic reactions), occurring in the first 30 minutes post vaccination observed by study staff at the fixed site clinic.
    b) Adverse events (AE) following 6-month MR dose that may occur during the 48 hours after 6-month MR vaccine administration. AEs will be graded and subcategorized as those deemed related to vaccination or not by the investigator.
    c) Serious adverse events (SAEs) occurring from vaccination during the course of the study, identified or observed by study staff and/or reported by parent at any time during the study period. SAEs will be subcategorized as those deemed related to vaccination or not by the investigator and reviewed by the local Data and Safety Monitoring Board (DSMB) and icddr,b ERC committee.

Data Analysis

This is an open-label trail clinical trial that compares immunogenicity of MR vaccine schedules. The study will recruit 620 participants in 2 arms. Participants will be enrolled at 6 months of age and followed at 9 months and 11 months of age for a total of 5 months of follow-up.

General analytical issues

The data will be analysed by study investigators that include epidemiologists and statisticians. Statistical packages that would be used for the analysis include R, SAS and STATA.

The immunogenicity of each MR vaccination schedule will be determined by measuring antibody levels:

- Blood samples will be obtained before each MR dose given (at 6 and 9 months) and at 11 months (i.e. 2 months after the last MR dose given at 9 months).
- Immune response is defined as a 4-fold increase in antibody titers 12 weeks after vaccination (post-6-month visit) compared with 6-month pre-vaccine titers accounting for the expected decline in maternal antibody titers or a change from antibody-negative to positive (i.e. seroconversion).
- The antibody titers (if present and detectable) at 6 months of age will be the starting point for the expected decline in maternal antibody.

- Laboratory quantitative data (i.e., titers) will allow seroconversion defined by other -fold increases to also be assessed. Seroprotection is defined as titer of ≥ 120 mIU/mL for measles and rubella >10 IU/mL.

We will assume that antibodies detected at 6 months are maternally-derived as no measles outbreaks have been detected in the area for the last few years; the number of reported measles cases has steadily declined since 2012 in Bangladesh (34) and Bangladesh conducted a nationwide MR campaign in 2013.

If the measles and rubella antibody titers are present and detectable at 6 months of age (at time of enrolment), these titers will be considered as the baseline IgG level for the expected decline in maternal antibody.

The rate of decay of maternal measles and rubella antibodies will be calculated for infants in study arm B based on change of measles and rubella antibody titers between 6 months and 9 months of age. This decay rate of measles and rubella antibodies will be incorporated in an analytical model when measuring post-6-month MR vaccine dose antibody response in infants in Study Arm A, and we will assess the impact of the maternal measles and rubella antibodies to immunogenicity of a 9-month MR vaccine dose. Seroprotection for measles is defined as concentration of ≥ 120 mIU/mL and rubella >10IU/mL.

Possible limitations:

Taking into account that baseline measles or rubella antibodies detected at 6 months will be assumed as maternal antibodies, this may possibly lead to overestimation of the MR seroconversion rate. In some infants, measles or rubella antibodies may have developed in response to natural infection. It is impossible to determine with laboratory methods whether baseline measles or rubella antibodies are maternal or developed post-infection.

A major limitation of many studies in which mother-infant paired serum samples were tested, was ascertainment of mothers' past measles or rubella vaccination or measles and rubella natural infection status. Some authors used age (i.e., year of birth, born in pre- measles or rubella vaccination era) as a proxy for determining the past vaccination or disease status. One recent study minimized recall bias as the researchers ascertained mothers' status (vaccine induced or post-infection antibodies) by reviewing their medical charts. [4]

Primary analytical approach

Intention-to-treat (ITT) analysis will be conducted on all enrolled participants who are randomized and receive two measles-rubella vaccines (at 6 and 9 months, study arm A) or one measles-rubella vaccine dose (at 9 months, study arm B).

Children who are seropositive (≥1:120 mIU/mL) for measles before vaccine administration (at 6 months of age, study arm A; at 9 months, study arm B) will be included in the ITT analyses and will be included in the safety analysis. Similarly, children who are seropositive (≥10 IU/mL) for rubella before vaccine administration (9 months of age) will also be included in the ITT analyses.

To evaluate primary and secondary objectives, the analysis will be restricted to participants who

- Have not withdrawn consent for receipt of MR vaccine and follow-up evaluations since enrolment
- Have adequate blood specimen for serological analysis from the enrolment visit, and
- Have adequate blood specimen for serological analysis from study site visits at 6, 9 and 11 months of age (For presence of maternal measles and rubella antibodies – 6 months, seroconversions: 9 months of age and 11 months)

Secondary analytical approach

In addition, we will also perform a per protocol analysis.

To evaluate primary objective, per protocol analysis will be restricted to participants who

- Have not withdrawn consent for receipt of vaccines and follow-up evaluations since enrolment
- Have adequate blood specimen for serological analysis from each visit within 3 days of 6, 9 and 11 months of age, and
- Have received all scheduled vaccines within 3 days of scheduled visit date

Using the above described primary analytical approach we will

- Compare the proportion of participants demonstrating immunogenicity in response to measles and rubella vaccines across study arms and use chi-square test to test for statistical significance across study arms.
- Compare the proportion of participants demonstrating seroconversion at 9 months in both study arms. We will use chi-square test to test for statistical significance
- Compare the change in median titers of measles and rubella antibodies by study arm
- Compare immunogenicity of MR vaccine administered at 6 months (Arm A) vs 9 months (Arm B). Analyse the relationship of infants' immunogenicity to MR vaccine and mothers' level of antibodies
- Analyse the relationship between the levels of infants' maternal measles and rubella antibodies and mothers' antibodies
- Analyse results of adverse events by study arm and compare by age and arm of the study.

Data Safety Monitoring Plan (DSMP)

Records to be kept

Participant data will be collected using case report forms (CRFs). Personal identifiers will not be recorded on any CRFs, biological specimens or electronic records. Participants will be identified by a participant identification number (PID), which will be provided after enrolment and randomization. The PID will be used to link information recorded in CRFs and results from laboratory analysis of biological specimens.

Data entry, cleaning and storage

Records of participants will be kept in a locked cabinet with access restricted to study staff and investigators. All computer records will be kept in a locked room. The computers will be password protected. Only the study staff and investigators will be authorized to access the digital records.

Data from the CRFs will be entered into an electronic database. Checks will be performed to identify and correct data entry errors. After the completion of analysis and publication of study results, the study forms and database will be archived. The forms will be stored in a locked cabinet with access restricted to investigators. The database will be password protected.

Site monitoring plan

Prior to start of the study, Standard Operating Procedures (SOPs) will be developed by investigators in consultation with study staff. Study staff will be trained in study procedures and human subjects' management. After start of the study monitoring of study site will be conducted twice.

During these monitoring visits the following study documents and procedures would be reviewed:

1. Participant records, including consent forms
2. CRFs and other supporting data
3. Adverse event forms
4. Data Safety and Monitoring Board (DSMB) reports
5. Laboratory specimen records
6. Vaccine and biological materials storage and records
7. Any additional medical records
8. Measures to ensure protection of study participants
9. Measures to ensure compliance with study protocol, and accuracy and completeness of records

10. Any regulatory files associated with the study will also be inspected to ensure all regulatory and reporting requirements are being followed

Site monitoring visits will be conducted by CDC staff that have not interacted with study participants. Site monitoring reports will be shared with site PI.

Safety monitoring

Live attenuated measles vaccines currently in use have an excellent track record for safety and efficacy. Measles vaccine was introduced in Bangladesh in 1979 and since then has been used widely in the country. Recently, Bangladesh conducted a nationwide MR SIA targeting 52 million children aged 9 months – 15 years of age in 2014, and introduced MR into the routine immunization schedule.

Few children may experience some adverse effects from MR vaccine. Most adverse effects are mild and transient (local reaction at injection site, fever, and rash) with no long term sequelae. One serious but very rare adverse effect of measles vaccine is anaphylaxis following vaccination.

Fever of 39.4° C or higher (≥ 103° F) occurs in approximately 5% to 15% of vaccine recipients between the 7th and 12th day after vaccination and lasts approximately 1 to 2 days. (4) Rash occurs in approximately 5% of recipients, beginning 7 to 10 days after vaccination and lasting for 1 to 3 days. Determining the etiology of a rash illness after measles vaccination can be difficult because other infections, such as human herpesvirus type 6 and human parvovirus type 19, can often occur early in life and can be confused with a vaccine reaction. (4)

The rate of anaphylaxis is approximately 1 to 3.5 per million doses administered. Children with immediate hypersensitivity to eggs can be safely immunized with measles vaccines, and no special precautions are necessary.(4)

All participants will be observed for 30 minutes post vaccination to monitor for any immediate adverse reactions to the vaccine. Properly skilled medical personnel will be immediately available in case of an emergency in the event of an unexpected adverse reaction. The study physician will record at the end of this period the presence of any potential reaction to the vaccine. At the end of each study visit, the physician or study coordinator will provide the parents with a phone number for a study nurse to call if they have any questions or experience any reactions to the vaccine.

A study staff will visit the house of the participant to inquire and record any potential adverse event in 48 hours after the first dose of MR administered at 6 months (study arm A).

Should a serious illness occur while enrolled in the study (requiring a physician's visit or hospitalization), parents will be instructed to seek medical care immediately. Medical care for expected

minor illnesses that will develop during the follow up period in study participants such as rash, fever or respiratory infections as well as any adverse outcomes of the vaccination judged to be possibly, probably or definitely related (detailed below) to study vaccines or vaccination will be provided free of charge.

Recording, monitoring and reporting of adverse events

During each study visit the study staff will question the parents of the participant about possible adverse events (AE) since the previous study visit and record this in the CRF. Reported AE will be recorded in the AE form. The AE form will include a description of the event, time of onset, assessment of severity, relationship to study product, and time of resolution/stabilization of the event.

Reports of AE will be periodically discussed by the team of investigators. The study team will review the potential relationship and classify the relationship into:

1. Unable to judge
2. Not related
3. Probably not related
4. Possibly related
5. Probably related
6. Definitely related

Interpretation of vaccine-relationship to AE will be based on the type of event, the relationship of the event to the time of vaccine administration, the known biology of the vaccine and the investigators' medical judgment.

An AE will be considered a serious adverse event if it meets any of the following criteria:

1. Death during the study period
2. Hospitalization
3. Anaphylaxis associated with vaccine administration

All serious adverse events will be notified by the principal investigator to the regulatory agencies, ethical review committees and Data Safety Monitoring Board (DSMB) within 24 hours. However, the investigational team will report clusters of AE (at least three within one week) to the DSMB periodically for further evaluation.

Data safety monitoring board (DSMB)

The study will be monitored by a DSMB constituted by icddr,b with input by Ethical Review Committee (ERC). The DSMB will include representation outside of icddr,b. The DSMB is expected to convene once prior to the start of the study after ethical and regulatory approval of study protocol. The DSMB will convene meetings at the time of starting the study, near the middle of the study and at the study completion. The DSMB will be responsible for establishing study stopping rules. Immunogenicity data will not be available for DSMB meetings as all blood specimens will be analysed after the completion of study field activities.

Ethical Assurance for Protection of Human rights

Justification for involving a vulnerable population

Infants will be enrolled at 6 months of age. Enrolment at this early age is required as the study is assessing the immunogenicity of MR vaccine that is recommended at this age during outbreak response activities in response to outbreaks with infants affected at age < 9 months (age for measles routine vaccination). In addition, some researchers have suggested that the first measles vaccination should be given at a time point earlier than 9 months of age, based on the reports that infants born to mothers with vaccine induced immunity against measles may have lower concentrations of maternal antibodies and may lose protection by maternal antibodies at an earlier age.(26, 35, 36) All infants will be administered MR vaccine at age 9 months and some will receive an earlier dose at 6 months. Routine MR second dose will be given at 15 months as per current national immunization schedule endorsed by MoHFW in Bangladesh.

Potential benefits and compensation to participants

Participants will receive all recommended vaccines as per the vaccination schedule of Government of Bangladesh. EPI vaccines will be supplied by the government of Bangladesh. If a child has missed a routine vaccine dose (Pentavalent or polio) that she or he needed to get before the age of 6 months, parents will be encouraged to get their child vaccinated for other routine childhood vaccines.

Some infants in the study will be randomly selected (study arm A) to receive MR vaccine at 6 months, 3 months earlier than the current age (9 months) for MCV1. This will provide early protection against measles and rubella to those infants who seroconvert. All infants will also receive MR vaccine at 9 months as study participants. MR dose scheduled to be administered at 9 months as part of routine immunization will be cancelled. Children then will continue on to receive measles vaccine at 15 months per routine immunization schedule.

During study visits, participants will be examined by study physicians. They will assess infants' growth and development. In addition, participants will be offered advice on diagnosis and treatment of any other illness they experience during the study period. Treatment related cost will be covered by the study.

Potential risks to participants

The potential risks and discomfort to participants stem from blood collections and adverse reactions to MR vaccine.

Immunogenicity of the MR vaccine can only be assessed from testing the blood specimens. Multiple blood draws would be necessary as immunogenicity is measured by determining the change in antibody titers. Moreover, enrolled infants may have maternal antibodies against measles and rubella acquired transplacentally. The first blood draw at 6 months (for infants in both study arms A and B) will establish the baseline maternal antibody titers. The titers are assumed to decay at a fixed rate. Subsequent blood draws at 9 and 11 months will aid in assessing immunogenicity of MR vaccine as per the primary and secondary objectives. Collecting blood samples from infants in Arm B would help us determine what proportion of infants are additionally protected at 9 months when compared to infants in Arm A. It would also help us determine the rate of background exposure to measles and rubella circulation between 6 and 9 months (only 50% of rubella cases are symptomatic).

The minimal amount of blood necessary for testing is 1 ml. The most common effect of blood draws is pain at the site of collection. In order to minimize the discomfort and risks associated with blood draw, blood from infants will be drawn only by qualified staff experienced in collection of blood in infants. Sterile equipment and technique and alcohol swabs will be used to reduce the risk of infection from blood draw. Pressure will be applied at the site of blood draw to minimize bleeding from the site of blood draw.

MR vaccine (dose is 0.5ml) per WHO EPI immunization guidelines will be administered following all safcty norms. Thc most common sidc-cffcct of MR vaccinc injcction is pain and rcdncss at thc sitc of administration. The site will be used for monitoring of local reactions.

MR vaccine may have some traces of neomycin. Rarely participants may react to these substances though these reactions. One serious but extremely rare adverse effect to measles vaccine is anaphylaxis. The risk is extremely low - 1 in 3.5 million doses administered(4). Overall, adverse events, with the exception of anaphylactic reactions, are less likely to occur after receipt of a second dose of measles containing vaccine.

It is important to recognize the early symptoms of anaphylaxis and treat such cases immediately. Therefore all infants who receive study vaccine dose will be observed for 30 minutes by medical officer and if any signs of anaphylactic reaction are noticed the medical officer will initiate treatment on the site. Treatment will be free of charge. This child will not receive future MR vaccine doses. Special notes will be made in his medical card. Although we do not expect any additional side effects the 6 months infants will be followed for 48 hours after receipt of the first MR study dose because they receive MR vaccination before the age of routine vaccination in Bangladesh. Most common adverse events in infants at 6 months after administration of measles containing vaccine are fever, mild rash, redness and swelling of injection site. Most adverse effects are mild and self-limited.

Forty eight hours after vaccine administration at 6 month a study staff will visit respondents at their home to monitor and document any adverse effects following immunization.

All costs will be provided by the study for infants who experience severe adverse events in this study.

Participant Privacy

Personal identifiers, including birth date and sex will be collected and recorded on study case report forms (CRFs) and each participant will be given a study identification card with Current Identification Number (CID)/Registration Identification Number (RID) number. Other information collected by study staff and recorded on a linking document containing the study participant identifier will include the name of the participant, name of the parents, CID number, location/address of residence, and other identifiers important for ensuring follow-up throughout the study period. The CID number on the card and the linking document provides the link between the participant and his/her study information. Although this linking document will be stored separately from the study CRFs containing study data, a potential risk for "loss of confidentiality" does exists.

To avoid this risk, participants will be assigned a unique study participant number that will be used to identify the participant and link, using the linking document, an individual to his/her study data and/or biological specimens. This linking document will be destroyed once data are finalized. Whenever feasible, use of identifiers, such as name and addresses, will be avoided and the unique study participant numbers will be used instead. Paper-based records (i.e., CRFs) will be kept in a separate, secure location with controlled access and will only be accessible to personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Individual participants will not be identified in any study related reports, and all study results will be reported in aggregate only.

Biological specimens (serum) will be identified by study participant number, and specimen collection date. Following collection, the specimens will be processed according to specimen handling/processing guidelines and stored at Matlab Health Research Centre. Serum specimens will be shipped to the United States Centers for Disease Control and Prevention (CDC). The links between study data and the study specimens will not be destroyed, but no personal-identifying information associated with the study will be stored on site with the specimens.

Institutional Review Board (IRB) and ethical review

The protocol with letters of consent and other associated appendices will be reviewed and approved by the Ethical Review Committee (ERC) and Research Review Committee (RRC) at the icddr,b before any participants are enrolled. The ERC and RRC at the icddr,b will be responsible for oversight of the study.

All participants will be below the legal age of consent as participants will be enrolled at 6 months of age. Parents or legal guardians of potential participants will be informed that their participation in this study is strictly voluntary and that they are free to withdraw from the study at any time. A signed consent form will be obtained from the parent or legal guardian of the participant. Irrespective of literacy status of parents the consent form will be explained in the presence of a witness not directly related to the study. The consent form will describe the purpose of the study, the procedures followed, confidentiality of recorded information, and the risks and benefits of participation. Consent forms will be translated from English to Bangla, the local language. A copy of the consent form will be given to the parent or legal guardian of the participant and this would be noted in the participant record. Any subsequent modifications to the protocol will be submitted to the ERC and RRC at the icddr,b for additional approval.

Participant confidentiality procedures

All CRFs, laboratory specimens and other reports including reports of adverse events will be identified by PID to maintain participant confidentiality. No identifiers will be recorded on CRFs, other study reports and laboratory specimens. All records will be stored in a locked cabinet. All electronic records will be password protected. Access to paper and electronic records will be restricted to study staff and investigators.

The manufacturers of vaccines used in the study may use study data and results for their vaccine product regulatory files and may also present this data and study results to regulatory agencies. No identifiers will be shared with vaccine manufacturers or regulatory agencies. All participant data will be identified by PID.

Use of animals
Not applicable.

Dissemination and use of findings
icddr,b and CDC investigators will produce a detailed report of the study findings as well as an executive summary. These findings will be shared with all stakeholders including the government of Bangladesh EPI program, through a dissemination meeting. In addition, a manuscript of the findings will be submitted to peer reviewed journals for publication.

Collaborative Arrangements
This study includes two partners with the following roles in the study:

1. International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b)
    - Collaborate in the development of the study protocol and submission through ethical and scientific committees in Bangladesh.
    - Select study sites and study staff and develop a budget for study implementation
    - Develop standard operating procedures, study forms and training material. Conduct training and supervise study staff.
    - Carry out implementation of the study procedures and coordinate logistics for sample collection, processing and storage; as well as study vaccine storage and administration.
    - Record and provide treatment for potential adverse events developed during the study.
    - Enter data collected in questionnaires into a database for future analysis.
    - Collaborate with CDC investigators in the analysis, interpretation and writing of the study results for study reports and manuscripts to be published in peer review journals
2. Centers for Disease Control and Prevention, Atlanta, GA, USA
    - Design of the study and provision of funding for its implementation
    - Submission of the protocol for CDC ethical clearance
    - Provide support in the training of study staff
    - Monitor study implementation through visits to the study sites in coordination with the icddr,b
    - Test serum specimens for the determination of antibodies against measles and rubella (laboratory at the Division of Viral Diseases) and enter laboratory data into an electronic database
    - Lead data analysis, interpretation of the results and writing detailed reports and manuscripts for publication in peer reviewed journals in collaboration with icddr,b investigators

Facilities Available

Field site

The study will be carried out in rural area at Matlab, Bangladesh. CDC and icddr,b (The International Centre for Diarrhoeal Disease Research, Bangladesh) have previously collaborated to conduct several clinical trials including polio, measles and rubella and rotavirus trials at this site.

Clinical facilities

icddr,b has large multi-disciplinary international and national scientific research staff. Existing field, hospital, laboratory and office facilities will be used for this study. icddr,b scientists have conducted a variety of vaccine studies including rotavirus, polio and measles-rubella.

Laboratory facilities at icddr,b

Existing laboratory facilities in icddr,b will be used to store and process specimens prior to shipment to CDC, Atlanta.

Laboratory facilities in CDC

The Measles, Mumps, Rubella, and Herpesvirus Laboratory at CDC, a WHO reference laboratory for measles and rubella laboratory of the Division of Viral Diseases at the US Centers for Disease Control and Prevention, Atlanta has the necessary staff, infrastructure and equipment to determine levels of antibodies against measles and rubella in the sera of study subjects using neutralization testing. This testing on blood requires specific training, equipment and cell culture capability that is not currently available at the laboratory of the icddr,b or at any other laboratory in Bangladesh.

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