Reactogenicity and Protectivity Following Measles- Rubella (MR) Routine Immunization in Indonesian Infants and Children

Phase IV

PMS-MR-0417

CLINICAL TRIAL PROTOCOL

Sponsor **PT BIO FARMA (PERSERO)**
Jl. Pasteur No. 28 Bandung – 40161 Indonesia

August 2017

Reactogenicity and Protectivity Following Measles-Rubella (MR) Routine Immunization in Indonesian Infants and Children

PMS-MR-0417
CLINICAL TRIAL PHASE IV
(POST MARKETING SURVEILLANCE)

Sponsor	PT. BIO FARMA (PERSERO) Jl. Pasteur No. 28 Bandung – 40161 INDONESIA
Investigational Product	Measles-Rubella **(**MR) Vaccine
Manufacturing Sites	Serum Institute of India PVT.LTD. 212/2, Hadapsar, Pune 411028, India
Principal Investigator	Dr. dr. Dominicus Husada, SpA(K)
Sub-Investigators	dr. Dwiyanti Puspitasari, SpA(K) dr. Leny Kartina, SpA(K)
Medical Advisor	Prof. Dr.dr. Ismoedijanto, SpA(K) Prof. dr. Parwati S. Basuki, SpA(K)
Biometry	Dr. dr. Windhu Purnomo, MS
Monitors	Dr**.** Novilia Sjafri Bachtiar, dr., M.Kes. Rini Mulia Sari, dr. Julianita Fahmi, dr. Asep Irham Fattahul Qur'an, dr
Biological Laboratory	Rini Mulia Sari, dr. Yani Sukriyani Lestari
Version Number	1.0
Date	August 2017

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SYNOPSIS

Sponsor	PT. Bio Farma (Persero)
Trial Title	Reactogenicity and Protectivity Following Measles-Rubella (MR) Routine Immunization in Indonesian Infants and Children.
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	Rini Mulia Sari, dr.
	Julianita Fahmi, dr.
	Asep Irham Fattahul Qur'an, dr
Biological Laboratory	Rini Mulia Sari, dr.
	Yani Sukriyani
	Lestari
Trial Centre	Department of Child Health Dr. Soetomo Hospital/School of Medicine, Airlangga University, Surabaya, East Java.
Trial Design	Cohort Study (Prospective non-intervention study)
Trial Phase	2
Protocol Number	PMS-MR-0417
Study Initiation Date	September 2017
Study Period	September 2017 – April 2018
Planned Sample Size	• A total 600 subjects; 9-12 months and 18-47 months • Pre and post immunization sera obtained from 200 infants and children.
Primary Objective	• To assess serious immediate systemic events within the first 30 minutes after Measles-Rubella (MR) vaccine in

	infants and children.
Secondary Objectives	• To assess local reaction after Measles-Rubella (MR) vaccine in infants and children. • To assess systemic event after Measles-Rubella (MR) vaccine in infants and children. • To evaluate the protectivity against Measles and Rubella after 1 dose of MR vaccine in in infants and children. • To describe antibody response to Measles and Rubella after 1 dose of MR vaccine in infants and chidren.
Inclusion Criteria	1. Healthy infants (9-12 months) or children (18-47 months) 2. Will receive MR routine immunization. 3. Parents have been informed properly regarding the study and signed the informed consent form. 4. Parents will commit themselves to comply with the instructions of the investigator and the schedule of the trial.
Exclusion Criteria	MR vaccine given simultaneously with other vaccination, except Pentabio (DTP/HB/Hib)
Investigational Product	Measles and Rubella Vaccine, live attenuated (Serum Institute of India) Form : Freeze dried Dose : 1 dose 0.5 ml contains not less than 1000 CCID50 of measles virus and 1000 CCID50 of rubella virus. **Batch Number:** 012M6104, 012M6105, 012N6107A, 012N6107B, 012N6139, 012N6121B, 012N6123, 012N6124, 012M6145, 012M6146, 012M6147, 012N6121A, 012N6140A, 012M6149, 012N6151
Vaccination Schedule	V1 (Day 0) : one dose of immunization
Serology Schedule	V1 (Day 0) : before immunization

(Sub Study)	V2 (Day 0 + 28D) : 28 days after immunization
Evaluation Criteria	**Primary Evaluation Criteria:** • Number and percentage of subjects with at least one serious immediate systemic events within 30 minutes after vaccination. **Secondary Evaluation Criteria** **<u>Safety</u>** • Number and percentage of subjects with at least one local reaction and systemic event occurring within 72 h after vaccination. • Number and percentage of subjects with at least one local reaction and systemic event occurring between day 4-14 after vaccination. • Number and percentage of subjects with at least one local reaction and systemic event occurring between 15 days to 28 days following vaccination. • Any serious adverse event occurring from inclusion until 28 days after immunization **<u>Immunogenicity</u>** • Percentage of subjects with anti measles titer ≥8(1/dil) and anti rubella titer ≥11 IU/ml, 28 days after one dose of MR vaccine in Infants & Children. • Serological response to MR vaccine in infants and children : GMT, percentage of infants with increasing antibody titer ≥ 4 times and/or percentage of infants with transition of seronegative to seropositive.

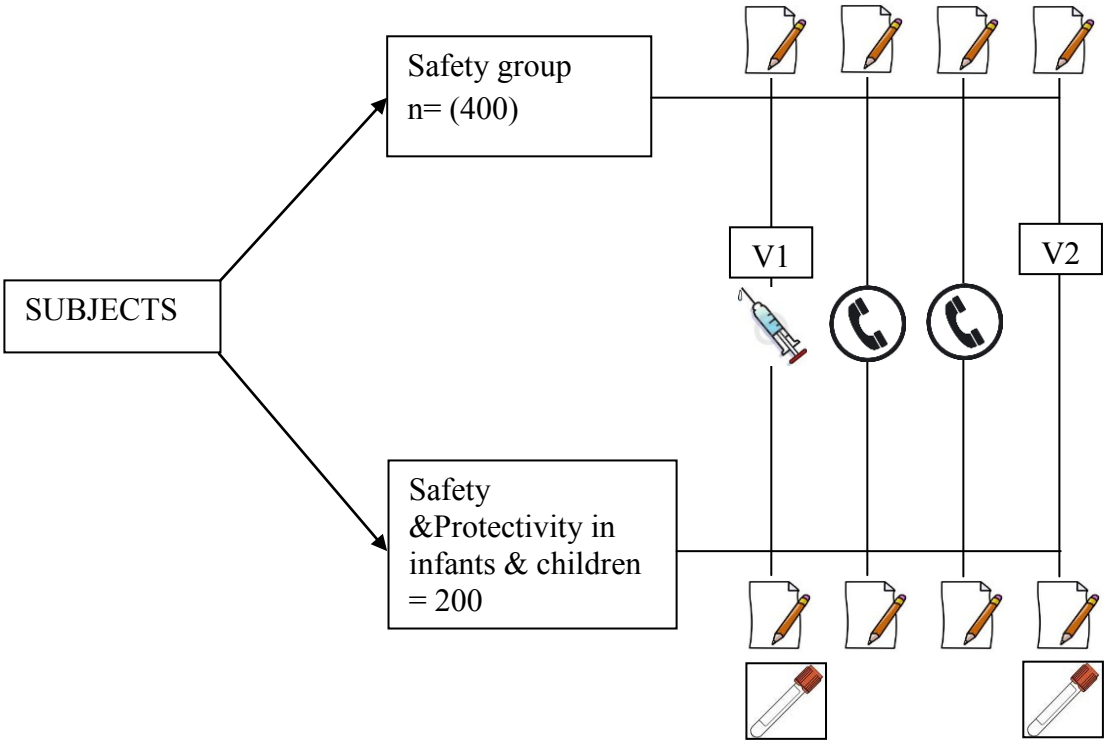
FLOW CHART

Schedule

Visit Number	V1	V2
Visit Intervals (Days)	Day 0	V1+28D
Age of infants/Children	9-12 months (infants) 18-47 months (children)	
Time Windows (Days)		-4/+7D

Informed consent signed	X	
Inclusion & Exclusion Criteria	X	
Past Medical History	X	
Physical Examination	X	X
Blood Sampling (4 ml) Sub Study A and B	X	X
Vaccination Dose	X VAX1	-
Immediate surveillance (30 min)	X	
Assessment of Local Reactions & Systemic Events	X	X
Diary Cards Provided	DC1	-
Diary Cards Collected		DC1
Prior & Concomitant Therapies	X	X
Termination Record/Final	X	X
Serious Adverse Events	→	

FLOWCHART



V1 = Day 0

V2 = V1 + 28 days

V1 + 7D = Safety follow up by phone

V1 + 14D = Safety follow up by phone

 = serology = 4mL

 = MR immunization

 = Immunization reactions recording

 = Safety follow up by phone

ABBREVIATIONS AND SYMBOLS USED

CCID	Cell Culture Infective Dose
CRF	Case Report Form
CRS	Congenital Rubella Syndrome
D	Day (D1 = Day 1)
DC	Diary Card
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
IEC	Institutional Ethics Committee
IRAEF	Initial Report for Adverse Event Form
IRB	Institutional Review Board
M	Month
MR	Measles-Rubella
MMR	Mumps-Measles-Rubella
NIHRD	National Institute of Health Research and Development
RCV	Rubella Containing Vaccine
SAE	Serious Adverse Event
V	Visit (V1 = Visit 1)
VAX	Vaccination
WHO	World Health Organization

GLOSSARY

1. **Blinding**; a procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s). In case of measurement blinding, the technician being unaware of the measure sample(s) before or after immunization.
2. **Case report form (CRF)**; A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.
3. **Clinical Trial/Study;** Any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamics effects of an investigational product(s), and/or identify any adverse reactions to an investigational product(s), and/or to study absorption, distribution, metabolism, and excretion of an investigational product(s) with the objects of ascertaining its safety and/or efficacy. The terms clinical trial and clinical study synonymous.
4. **Impartial witness;** a person, who is independent of the trial, who cannot be unfairly influenced by people involved with the trial, who attend the informed consent process if the subject or the subject's legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the subject.
5. **Immunogenicity;** capability of inducing an immune response.
6. **Informed consent;** a process by which a subject voluntary confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent in documented by means of a written, signed, and dated informed consent form.
7. **Investigator;** a person responsiblefor the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator. See also subinvestigator.
8. **Monitoring;** the act of overseeing the progress of a clinical trial and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, SOPs (Standard Operating procedures), GCP (Good Clinical Practice), and the applicable regulatory requirement(s).

9. **Sponsor;** an individual, company, institution, or organization which takes responsibility for the initiation, management, and/or financing of a clinical trial.
10. **Subinvestigator;** any individual member of the clinical trial team designated and supervised by the investigator at a trial site to perform critical trial-related procedures and/or to make important trial-related decisions (e.g., associates, residents, research fellows). See also investigator.

1. BACKGROUND AND RATIONALE

1.1 Introduction

In 2013, Ministry of Health Indonesia issued the regulations on immunization implementation which aims to decrease morbidity, disability and mortality because of the diseases that can be prevented by immunization. One of the specific purposes is achieving measles elimination in 2018 and rubella control in 2020.[1]

There were several diseases that become world's concern and global commitments which were followed by countries such as polio eradication, measles elimination-rubella control, and maternal neonatal tetanus elimination.[1]

World Health Organization (WHO) released The Global Measles and Rubella Strategic Plan 2012-2020 in 2012. The vision was to achieve and maintain a world without measles, rubella and congenital rubella syndrome (CRS). By the end of 2015, the goals were to reduce global measles mortality by at least 95% compared with 2000 estimates and to achieve regional measles and rubella/CRS elimination goals. Thus, by the end of 2020, it is expected to achieve measles and rubella elimination in at least five WHO regions.[2]

There were some milestones that were needed to fulfil by the end of 2015, such as reduce annual measles incidence to less than five cases per million and maintain that level, achieve at least 90% coverage with the first routine dose of measles-containing vaccine (or measles- rubella-containing vaccine as appropriate) nationally, and exceed 80% vaccination coverage in every district or equivalent administrative, achieve at least 95% coverage with M, MR or MMR during supplementary immunization activities (SIAs) in every district, establish a rubella/CRS elimination goal in at least three additional WHO regions, and establish a target date for the global eradication of measles.[2]

[1]Peraturan Menteri Kesehatan Nomor 42 Tahun 2013 tentang Penyelenggaraan Imunisasi. 2013.
[2]WHO. Global Measles and Rubella Strategic Plan 2012-2020. 2012.

1.2 Epidemiology

Measles is an acute viral infectious disease. References to measles can be found from as early as the 7th century. The disease was described by the Persian physician Rhazes in the 10th century as "more dreaded than smallpox". Before a vaccine was available, infection with measles virus was nearly universal during childhood, and more than 90% of persons were immune by age 15 years. Measles is still a common and fatal disease in developing countries. The World Health Organization estimates there were 164.000 deaths globally from measles in 2008.[3]

Measles virus infection is one of the most important infectious diseases of humans and has caused millions of deaths since its emergence as a zoonotic infection thousands years ago. In the Region of the Americas, intensive immunization and surveillance efforts have, since November 2002, stopped endemic transmission of measles virus, in part based upon the successful Pan American Health Organization strategy of nationwide measles vaccine campaigns and high routine measles vaccine coverage.[2]

Measles occurs throughout the world. However, interruption of indigenous transmission of measles has been achieved in the United States and other parts of the Western Hemisphere.

Measles is a human disease. There is no known animal reservoir, and an asymptomatic carrier state has not been documented. Measles transmission is primarily person to person via large respiratory droplets. Airborne transmission via aerosolized droplet nuclei has been documented in closed areas (e.g., office examination room) for up to 2 hours after a person with measles occupied the area.
Measles is highly communicable, with greater than 90% secondary attack rates among susceptible persons. Measles may be transmitted from 4 days before to 4 days after rash onset. Maximum communicability occurs from onset of prodrome through the first 3-4 days of rash.[3]

According to Indonesian Health Profile 2013, incidence rate for measles was 6.53 per 100.000 and the immunization coverage was 97.85%. In 2012, the incidence rate was 4.64 per 100.000 and the immunization coverage was 99.3%. There were 128

[3]Centers for Disease Control and Prevention.Epidemiology and Prevention of Vaccine-Preventable Diseases. Atkinson W, Wolfe S, Hamborsky J, eds. 12th ed., second printing. Washington DC: Public Health Foundation, 2012.

outbreaks with a total 1677 cases in 2013. Based on laboratory confirmation, 24 outbreaks were rubella's.[4]

Rubella occurs worldwide. Rubella is a human disease. There is no known animal reservoir. Although infants with CRS may shed rubella virus for an extended period, a true carrier state has not been described. Rubella is spread from person to person via airborne transmission or droplets shed from the respiratory secretions of infected persons. There is no evidence of insect transmission. Rubella may be transmitted by person with subclinical or asymptomatic cases (up to 50% of all rubella virus infections). Rubella is only moderated contagious. The disease is most contagious when the first rash appears, but virus may be shed from 7 days before to 5-7 days or more after rash onset. Infants with CRS shed large quantities of virus from body secretions for up to 1 year and can therefore transmit rubella to persons caring for them who are susceptible to the disease.[3]

The most important and serious consequence of rubella is congenital rubella infection. When primary rubella infection occurs in a pregnant woman, the virus can infect the placenta and fetus during the period of viraemia. The risk of congenital infection is related to the gestational age at the time of maternal infection. The outcome of a primary rubella infection during pregnancy includes: spontaneous abortion; stillbirth/fetal death; infant born with CRS; infant born with congenital rubella infection without congenital defects and birth of a normal infant.[5]

The most common defects of CRS are hearing impairment (unilateral or bilateral sensorineural), eye defects (e.g. cataracts, congenital glaucoma, or pigmentary retinopathy), and cardiac defects (e.g. patent ductus arteriosus, or peripheral pulmonic stenosis). Other clinical manifestations may include microcephaly, developmental delay, purpura, meningoencephalitis, hepatosplenomegaly, low birth weight and radiolucent bone disease. Children with CRS may develop late-onset manifestations including endocrine abnormalities (e.g. diabetes mellitus, thyroid dysfunction), visual abnormalities (e.g. glaucoma, keratic precipitates), and neurological (e.g. progressive panencephalitis), in addition to developmental manifestations which include autism.[5, 6]

[4]Indonesia. Kementrian Kesehatan RI. Profil Kesehatan Indonesia Tahun 2013. Jakarta: Kementerian Kesehatan RI. 2014.
[5] WHO. The Immunological Basis for Immunization Series: Module 11: Rubella, Geneva. 2008.
[6] Cooper LZ, Alford CA. Rubella. In: Remington J et al., eds. Infectious diseases of the fetus and the newborn infant, 6th ed. Philadelphia, Elsevier, WB Saunders, 2006:894-926.

1.3 Prevention and Control of Infection among Humans

Measles- and rubella-containing vaccines are among the most cost-effective public health tools available.[2] At least one dose of rubella-containing vaccine, as combination MMR (or MMRV) vaccine, is routinely recommended for all children 12 months of age or older. The high response rate to a single dose of rubella vaccine (≥95%) and the long-term persistence of protection in vaccinees do not support a routine requirement for second dose of rubella vaccine. However, based on the indications for a second dose of measles-containing and mumps-containing vaccines, a second dose of MR or of MMR is now offered in most countries. A rubella-containing vaccines (RCV) is usually administered at age 12–15 months, but it can also be administered to children aged 9–11 months and to older children, adolescents and adults.In most countries, rubella vaccine is given as MR or MMR, and the age of administration follows the schedule for measles – that is, the first dose is usually given at 9 months or 12–15 months and a second dose at 15–18 months or 4–6 years.[7]

1.4 Rationale

Measles returns when we let down our guard. Rubella also remains a threat to pregnant women and their foetuses in particular, with more than 100.000 children born each year with congenital rubella syndrome (CRS), which includes heart defects, blindness and deafness.[2]

To achieve and maintain a world without measles, rubella and congenital rubella syndrome (CRS), there are 5 components of the strategy needed to fulfil, such as achieve and maintain high levels of population immunity by providing high vaccination coverage with two doses of measles- and rubella-containing vaccines; monitor disease using effective surveillance and evaluate programmatic efforts to ensure progress; develop and maintain outbreak preparedness, respond rapidly to outbreaks and manage cases; communicate and engage to build public confidence and demand for immunization; and perform the research and development needed to support cost-effective operations and improve vaccination and diagnostic tools.[2]

[7] WHO, Weekly epidemiological record, Rubella vaccines: WHO position paper, Geneva 2011, 29(86); 301-316

All countries that have not yet introduced rubella vaccine, and are providing 2 doses of measles vaccine using routine immunization or SIAs, or both, should consider including RCVs (Rubella Contains Vaccine) in their immunization programme. Two doses of measles vaccine through a combination of routine immunization and supplementary immunization activities (SIAs) as part of accelerated efforts to reduce measles mortality or regional elimination efforts. In countries with ongoing transmission in which the risk of measles mortality among infants remains high, MCV1 should be administered at age 9 months. At the community level, SIAs rapidly increase population immunity and thereby interrupt measles transmission.[7]
Indonesia will conduct catch up campaign program for MR in August and September 2017. Since October 2017 the immunization routine will be changed from Measles to Measles-Rubella (MR) vaccine.

1.5 Previous MR Vaccine (SII) Study

Study 01 was carried out by Indra Bhargava et al in 1360 prepubertal and adolescent girls to evaluate reactogenicity of the vaccine. Out of them, 30 girls were evaluated for immunogenicity profile. A 100% seropositivity was observed for measles and rubella in all the subjects post-vaccination. Local adverse events reported were pain and swelling (3.46 %), redness and tenderness (1.74 %), nodule (0.29 %). Systemic adverse events were mild fever (5.29 %), high fever (0.15 %), cold and coryza (2.87 %), red eye (1.10 %), rash (0.59 %), cervical lymphadenopathy (0.22 %), arthralgia (0.37 %), arthritis (0.22 %). The study result shows the vaccine is highly immunogenic. All the events resolved without any sequelae. No serious adverse event was reported.[8]

In Study 02, Dr Dutta et al assessed immunogenicity in 84 Indian children of 4-12 year of age. Post vaccination seropositivity was 96.43% for measles and 91.67% for rubella. GMTs of antibodies of measles rose from pre-vaccination values of 19.861 U/ml to 80.352 U/ml. Similarly, GMT for rubella rose from 12.794 IU/ml to 64.565 IU/ml after the vaccination. Adverse events reported were pain (13 %) and induration (16.66 %) at injection site and fever (4.76 %). All three reactions were transient and resolved without any sequelae. No serious adverse event was reported. Difference

[8] Bhargava I. A study of immunogenicity and reactogenicity of rubella containing vaccine produced by Serum Institute of India ltd. Pune India in prepubertal and adolescent girls. Indore 1999.

between pre immunization and post-immunization IgG levels for measles and rubella was statistically significant. The vaccine was found highly safe.[9]

In 2000, Albania conducted a mass immunization campaign with MR vaccine for children of 1-14 years of age. A total of 867,000 doses of MR vaccine of SIIL were administered for an estimated coverage of 99 %. From the routine surveillance for the cases of measles and rubella, it was observed that the mass immunisation campaign had a positive impact on interruption of measles and rubella. The number of measles cases dropped from 2386 in 1997 to 18 cases in 2001. In cases of Rubella, the same was reduced from 721 cases in 1998 to 10 cases in 2001. Obviously the campaign had a dramatic impact on the epidemiology of these two diseases and demonstrated the effectiveness of the vaccine. Total 231 cases of campaign-associated adverse events following immunization (AEFI) were reported, including 15 hospitalizations. Adverse events like fever (9), arthralgia (1.15), allergic reaction (11.5) and syncope (237) were reported per million doses of MR vaccine. 2 cases encephalitis/ encephalopathy, and 1 case each of aseptic meningitis, seizures, Guillain-Barré syndrome and anaphylaxis were reported. Most probably, cases of encephalitis, Guillain-Barré syndrome and aseptic meningitis were unrelated to the MR vaccine.[10]

In 2001, South Korea implemented a national immunization campaign with SIIL MR Vaccine. The campaign covered a total of 4853, 233 individuals of 8-16 years of age. As an effect, South Korea announced in late 2006 that interruption of indigenous measles transmission has been achieved. This makes South Korea the first country in the World Health Organization's (WHO) Western Pacific Region to declare measles elimination. The Ministry of Health and Welfare received total 1199 adverse event reports. Most reported reactions were minor e.g. fever, headache & rash. Only 2 cases of serious adverse events were reported that included one case of anaphylaxis and one of acute disseminated encephalomyelitis. Three episodes of mass hysterical illness

[9] Dutta A. K., et al. A study of immunogenicity and reactogenicity of MR vaccine produced by Serum Institute of India ltd. New Delhi; India. 2000-01.
[10] Bino S, Kakarriqi E, Xibinaku M, Ion-Nedelcu N, Bukli M, Emiroglu N, Uzicanin A. Measles-rubella mass immunization campaign in Albania, November 2000. J Infect Dis. 2003 May 15;187 Suppl 1:S223-9

occurred among clusters of school children, which were diagnosed as hyperventilation syndrome due to anxiety and obviously unrelated to MR vaccine.[11]

In a mass immunization campaign in Iran in December 2003, more than 33 million doses of MR vaccine of SIIL vaccine were administered to the population of 5 to 25 years of age. Simultaneously, a study was conducted in 1940 individuals vaccinated during the campaign across the country to evaluate immunity against rubella vaccine using a cluster sampling design. Blood samples were collected in these individuals before and 1 month after the MR vaccination. 61.9 % of vaccinees were immune against rubella before vaccination, and 38.1% were susceptible. At the end of 1 month, 98% of the susceptible group acquired immunity against rubella after vaccination. Study concluded that the mass vaccination in December 2003 provided appropriate immune coverage among vaccinees. Safety evaluation was not performed in this study.[12] Another study was undertaken on 810 pregnant women who were vaccinated inadvertently with MR vaccines in mass immunisation campaign or became pregnant within 30 days. These women were grouped to susceptible and immune against rubella before vaccination by the status of IgG avidity response to rubella vaccine. The susceptible women were then followed up to delivery time and their neonates were followed up to one year. In no case, signs of congenital rubella syndrome were found. The study concluded that the MR vaccination during the pregnancy does not cause any harmful effect on pregnant women and their children.[13]

In mass campaign in Kyrgyz Republic around 1.8 million persons of 7-25 years of age were vaccinated with MR vaccine of SIIL in 2001. The vaccination coverage among the targeted age group was 98.7 %. Total 130 events were reported throughout the campaign. Of these, Only 12 cases met the WHO definition of adverse events. Most of vaccine induced adverse events were mild and of short duration. Rates of reported adverse event were syncope (4.9), fever (1.5), hyperpyrexia (0.5), mild rash (1.5), anaphylactic shock (0.05), encephalomyelitis (0.05), and anaphylactoid reaction

[11] Pless RP, Bentsi-Enchill AD, Duclos P. Monitoring vaccine safety during measles mass immunization campaigns: clinical and programmatic issues. J Infect Dis. 2003 May 15;187 Suppl 1:S291-8.
[12] Hamkar R, Jalilvand S, Mokhtari-Azad T, Jelyani KN, Nategh R. Evaluation of immunity against rubella in Iranian after mass campaign for measles-rubella vaccination on December 2003. Am J Infect Control. 2006 Nov;34(9):588-92.
[13] Hamkar R, Jalilvand S, Abdolbaghi MH, Esteghamati AR, Hagh-Goo A, Jelyani KN, et al. Inadvertent rubella vaccination of pregnant women: evaluation of possible transplacental infection with rubella vaccine. Vaccine. 2006 Apr 24;24 (17):3558-63.

(0.05) per 100,000 doses of MR vaccine administered. The National expert group investigating the association of adverse event with vaccination found that encephalomyelitis was not associated with vaccination.[14]

In nationwide immunisation campaign to eradicate the rubella and measles, a total of 1635,445 persons of 15- 45 years of age were vaccinated successfully. Total 981 adverse events were reported possibly related to vaccination. Rates of reported adverse events were rash (12.9), lymphadenopathy (6.8), fever (6.18), headache (3.12) and arthritis or arthralgia (3.12) per 100,000 doses of MR vaccine administered. No serious adverse event was reported.[15]

A cross-sectional study was performed in pregnant women of 15 to 29 years of age, who were inadvertently vaccinated with MR vaccine during the mass immunisation campaign or who became pregnant within 30 days thereafter. Of 2 292 women, 288 were susceptible, 316 immune, 1 576 indeterminate, 8 ineligible, and 104 lost to follow-up. Of a total of 1 580 newborns followed up, 9 (0.6%) tested positive for rubella-specific IgM in the first serum sample. Of these 9 children, 5 were born to susceptible women. In one child the wild rubella virus was identified through genotypical characterization, and the child's clinical condition was compatible with CRS. The 4 IgM positive newborns of susceptible mothers had no clinical abnormality at 1-year follow-up.[16]

[14] Study Report, 2002. Evaluation of the 2001 National Immunization campaign against Measles and Rubella conducted by Ministry of Health, Kyrgyz Republic.
[15] Centers for Disease Control and Prevention (CDC). Nationwide campaign for vaccination of adults against rubella and measles—Costa Rica, 2001.MMWR Morb Mortal Wkly Rep. 2001 Nov 9;50(44):976-9.
[16] Da Silva e Sa GR, Camacho LA, Siqueira MM, Stavola MS, Ferreira DA. Seroepidemiological profile of pregnant women after inadvertent rubella vaccination in the state of Rio de Janeiro, Brazil, 2001-2002. Rev Panam Salud Publica. 2006 Jun;19(6):371-8.

2. OBJECTIVES

2.1 Primary Objective

- To assess serious immediate systemic events within the first 30 minutes after Measles-Rubella (MR) vaccine in infants and children

2.2 Secondary Objectives

- To assess local reaction after Measles-Rubella (MR) vaccine in infants and children.
- To assess systemic event after Measles-Rubella (MR) vaccine in infants and children.
- To evaluate the protectivity against Measles and Rubella after 1 dose of MR vaccine in in infants and children.
- To describe antibody response to Measles and Rubella after 1 dose of MR vaccine in infants and chidren.

3. TRIAL DESIGN AND METHODOLOGY

3.1 Trial Design

This was a Post Marketing Surveillance study. Each subject who received MR vaccines according to National Immunization Programme were recorded. A total 600 subjects; 9-12 months and 18-47 months, who received MR vaccine involved in this trial.

There was **sub-study** to evaluate the protectivity and safety after Measles /Rubella Immunization. Beside the safety assessment, pre and post immunization sera were obtained from 200 infants (aged 9-12 months) and children (aged 18-47 months).

Since it was catch up campaign program for MR in children below 15 years of age, it would be difficult to recruit a children aged 18-47 months who have not been immunized yet. Therefore, it was not necessary to obtain equal number of subject for infants and children.

3.1.1 Treatment Allocation Procedures

Sub Study: After checking inclusion and exclusion criteria and collecting a signed informed consent from the parents, or the witness, the investigator allocated an inclusion number **from 001 to 200.**

Safety study: After checking inclusion and exclusion criteria and collecting a signed informed consent from the parents, or the witness, the investigator allocated an inclusion number **from 201 to 600**.

3.2 Trial Population

3.1.2 Inclusion Criteria

- Healthy infants (9-12 months) or children (18-47 months).
- Received MR routine immunization
- Parents had been informed properly regarding the study and signed the informed consent form.
- Parents commited themselves to comply with the instructions of the investigator and the schedule of the trial.

3.2.2 Exclusion Criteria for Infants:

- MR vaccine given simultaneously with other vaccination, except Pentabio (DTP/HB/Hib)

3.3 Ethical Considerations/Protocol Review

3.3.1 Protocol Review

Before the inclusion of the first subject in the centre, the protocol had been signed by the investigators and sponsor's representatives and approved by the Quality Assurance Division of Bio Farma, then by the Institutional Ethics Committee and Indonesian Regulatory Authorities.

3.3.2 Ethical Considerations

The investigator were responsible for obtaining approval of the protocol from the Institutional Ethics Committee before start of the trial, as well as approval of all amandments in compliance with local law. Copies of these approvals were forwarded by the investigator to Bio Farma with the composition (names and qualification of the members) of the Institutional Ethics Committee.

3.3.2.1 Informed Consent

The parents's subject gave his/her written informed consent before the inclusion, after had been informed of the nature of the trial, the potential risks and his/her obligations (Appendix 2). Informed consent signed by an impartial witness (hierarchically independent of the investigator and not specified on the list of trial contributors) if the subject's legally acceptable representative is not able to read and sign the form.

By signing the consent form, the witness will attest that the information in the consent form and any other written information were accurately explained to the parents, or to his/her legally acceptable representative, and apparently understood.

3.3.2.2 Subject Benefits/Potential Risks

The subjects enrolled in this trial would potentially gain benefit from vaccination with a Measles/Rubella vaccine. As to the risks, refer to the package insert of Measles/Rubella vaccine.

3.4 Trial Calender/Timelines

Recruitment	: September 2017- January 2018
End of Follow-up for 28 after last injection	: February 2018
Serology testing	: January-February 2018
Data Management	: October 2017- February 2018
Statistical analysis	: March 2018
Clinical Report	: April 2018

3.5 Trial Centres

This trial will be implemented in one center, Department of Child Health Dr. Soetomo Hospital/School of Medicine, Airlangga University, Surabaya, East Java.

The location of recruitment will be conducted at nine puskesmas in three districts in East Java:

- Kabupaten Kediri (Puskesmas Gurah, Puskesmas Adan-Adan and Puskesmas Ngasem).
- Kabupaten Gresik (Puskesmas Manyar, Puskesmas Cerme, and Puskesmas Balongpanggang).
- Kabupaten Lamongan (Puskesmas Sugio, Puskresmas Karenggeneng, Puskesmas Babat).

3.6 Vaccination and Serology Schedule

- Immunization Schedule

  Any immunization of MR vaccine in infants (9-12 months) or children (18-47 months

- Serology Schedule (Sub Study)

  Visit 1 (Day 0) : - vaccination (1st dose)
  - 1st blood sample (V1)

  Visit 2 (V2= V1+28 days –4/+7 days) : - 2nd blood sample.

3.7. Case Report Form and Data Collection

All information will be recorded by the investigator or a designated person in the Case Report Forms prepared by Bio Farma. The form should be filled out using with black-ink, in capital letter and signed by the investigator.

Explanations must be given for all missing information. All incorrect data should be striked-through, then signed and dated by the investigator (except date corrections). "White-out" correction fluid should not be used.

3.8 Subject Diaries and Interim Histories

Parent's subjects will keep an observation card (diary) to assess and record information for local/systemic reactions for 28 days following immunization, with special attention within the first three days. Safety data from the diary card could be controlled by a visit of the nurse (or field visitor) to the subject's house or communication by phone The local reaction assessment will involve assessment of the vaccination site. The systemic event assessment will involve daily axillary temperature readings (parents will be supplied with a thermometer and instruction how to use it) and recording any systemic complaints such as irritability on a diary card. Any medical office visit, emergency room visit or hospitalization for any reason will be recorded throughout the trial period. Moreover, any serious adverse event occurring throughout the trial period should be reported immediately and will also be recorded in the CRF.

3.9. Procedure for Obtaining, Handling of Serum Samples (Sub Study)

3.9.1 Obtaining Serum Samples

Four ml of blood was collected in vacutainer tubes at visits one (V1) before vaccination and V2 (V1 + 28 (-4/+7)) days. The person in charge of blood drawing verified the subject's identity and should check that the initials on the laboratory request are those of the subject just before taking the blood sample. Then, he/she wrote the subject's initials on all labels of the corresponding band. He/she affixed one label onto the Vacutainer tube immediately prior to blood sample drawing. It is absolutely necessary to obtain a sterile blood sample.

After clotting at room temperature from 30 minutes to 2 hours, blood samples were centrifuged at 3000 rpm for 15 minutes and the sera was separated aseptically into 4 aliquots (0.5 ml each) within 24 hours after the sampling. Sera were rapidly stored in a freezer at –20°C to-80°C.

Each blood sample was labeled with sticker, which indicates the blood sampling stage (V1 and V2), the trial code, the inclusion number and the subject's initials. The labels were mention neither the group nor the vaccine injected to allow the blinding of serology.

3.9.2 Handling Serum Samples

3.9.2.1 Storage Conditions

Each sample properly labelled should be frozen at –20°C to –80°C. Temperature should be monitored and documented on the appropriate form during the entire trial.

3.9.2.2 Method and Timing of Measurement

Antibody titers are measured at visit V1 (Day 0) and at visit V2 (V1+28 days –4/+7 days). These titers will be evaluated by the following assay techniques:

Antigen	Methods	Units
Measles	Neutralization test	1/dil
Rubella	ELISA	IU/mL

Immunogenicity measurements:

Antibody against measles will be performed by neutralization test with the concentration in 1/dilution while antibody against rubella will be performed by ELISA, with the concentration in IU/ml. The tests will be performed from the preimmunization (V1) and post immunization (V2) after the blinding procedure.

All tests (neutralization & ELISA) will be conducted in Immunology Laboratory of Clinical Trial Department of Bio Farma. These tests already validated by Clinical Trial Department and approved by Quality Assurance Division. This lab has been certified for ISO 14001:2004, ISO 9001:2008 and OHSAS 18001:2007.

Anti-measles will be tested using neutralization assay. Briefly, plasma samples in serial dilutions were incubated with 100 TCD50 of measles virus for 1 hour at 36 ± 1 °C in an atmosphere of 5% CO_2 and plated onto confluent Vero in 96-well plates.

After incubation, citophatic effect (CPE) were counted. The measles serum standard and internal controls will be tested in parallel with the samples. Titers ≥ 8 will be considered protective.

Anti-rubella will be tested using commercial kit Anti-Rubella virus ELISA IgG Euroimmun. The Quality control will be evaluated for each running test.

3.9.3 Blinding Procedure

The serology testing should be started only after the samples have been blinded. The blinding code and list will be prepared by the statistician. The blinding procedure should be witnessed by the investigator and team. The samples were stored in the cryobox according to the new code. The key of the blinding code will be kept by the statistician and the investigator. The blinding code will be opened after the result of serology testing has been received by the investigator.

3.10 Clinical Supplies

Protocols, CRFs, and diaries will be provided by Bio Farma.

4. PRODUCTS

4.1 Investigational Product Characteristics: Measles/Rubella vaccine (Serum Institute of India)

4.1.1 Product Description

Measles/Rubella vaccine is prepared from the live, attenuated strains of Edmonston-Zagreb measles virus and Wistar RA 27/3 rubella virus. Both measles and rubella viruses are propagated on human diploid cells (HDC). The vaccine is lyophilized and is provided with diluent. The product has the appearance of a yellowish-white dry cake.

4.1.2 Composition

Form : Freeze dried

Dose : 1 dose 0.5 ml contains not less than 1000 CCID50 of measles virus and 1000 CCID50 of rubella virus.

Batch Number : 012M6104, 012M6105, 012N6107A, 012N6107B, 012N6139, 012N6121B, 012N6123, 012N6124, 012M6145, 012M6146, 012M6147, 012N6121A, 012N6140A, 012M6149, 012N6151

4.1.3 Preparation

The vaccine should be reconstituted only with the entire diluent supplied (Sterile water for injections) using a sterile syringe and needle. With gentle shaking the dried cake is easily dissolved. After reconstitution the vaccine should be used immediately. One vial will be used for one subject only.

4.2 Precautions for use

The vaccine must be stored at +2°C to +8°C and should not be frozen.

The skin at the sites of injection will be cleaned and disinfected prior to injection. The vaccinator should use a separate sterile syringe for each individual trial part. The vaccines should be injected deep subcutaneously.

4.3. Labeling and Packaging

Commercial label will be used.

4.4. Storage and Shipment Conditions

4.4.1. Shipment Conditions

All of vaccines uses in this study were distributed according to routine immunization program.

4.5.2 Storage Conditions

Vaccines shall be stored at a temperature ranging from +2°C to +8°C (in a refrigerator). Temperature should be monitored and documented on the appropriate form (see operating guidelines) during the entire trial.

In case of deep freezing or accidental disruption of the cold chain, vaccines should never be administered and the investigator or the responsible person should contact the Monitor to receive further instructions.

5. TRIAL ADMINISTRATION

5.1 Personnel Involved in the Trial

Principal Investigator	Dr. dr. Dominicus Husada, SpA(K)
Medical Advisor	Prof. Dr. dr., Ismoedijanto, SpA(K);
	Prof. dr. Parwati S. Basuki, SpA(K)
Sub Investigator	dr. Dwiyanti Puspitasari, SpA(K)
Biometry	Dr. dr. Windhu Purnomo, MS
Biological Laboratory	Rini Mulia Sari, dr.
	Yani Sukriyani
	Lestari
Monitor	Dr**.** Novilia Sjafri Bachtiar, dr., MKes.
	Rini Mulia Sari, dr.
	Julianita Fahmi, dr.
	Asep Irham Fattahul Qur'an, dr

5.2. Visit Procedures

⇒ Visit 1. (D0) 1st vaccination & 1st blood sample

a. Provide parents's subjects with relevant information concerning the trial.
b. Obtain informed consent, dated and signed by the mother/ father or the legally acceptable representative.
c. Check eligibility criteria.
d. Allocate an inclusion number, chronologically to the enrolment of the child.
e. Perform a physical examination.
f. Take a pre-vaccination blood sample (V1, 4 ml) for sub study
g. Inject the one dose of vaccine.
h. Put the initial name and the inclusion number of the subject on the used vial.
i. Record the code of vaccine in the CRF and the list of participant.
j. Keep the subject under observation for 30 minutes and evaluate the immediate local reactions and systemic events.
k. Complete CRF.
l. Provide the parent's subjects with 1st diary card (DC1).

m. Inform the parent's/subjects that they should contact the investigator or the study nurse in case of any Serious Adverse Event.
n. Instruct the parents/subjects to return for visit 2.

Safety data within 7 days (V1 +7 days) and 14 days after immunization (V1+14 days) will be followed up by phone.

Investigator or designated person will record the information in the CRF.

⇒ Visit 2. 2nd blood sample (V1+28 days –4/+7 days)

a. Perform a physical examination.
b. Check the possible Serious Adverse Events, which occurred since the last visit.
c. Report the safety data from DC1 to CRF.
d. Check the concomitant therapies and record in the CRF.
e. Take a post-vaccination blood sample 4 ml (for sub study)
f. Finalize the CRF.
g. Completion the study.

5.3 Lost to Follow-up Procedures

In the case of subjects who fail to appear for a follow-up examination, extensive effort should be undertaken to locate or recall them or at least to determine their health status. These efforts should be documented in the subject's CRF and source documents.

5.4 Termination Classification

Definitions:

- Discontinuation by the investigator: an observation is considered a discontinuation by the investigator when the latter decides to terminate the subject's participation for medical reasons (for subject's safety), for personal reason (it is his opinion that the subject cannot continue to participate), etc.
- Drop-out: a subject included in the trial is said to have dropped out after deciding, on his own volition, to terminate his participation in the trial. Subjects may decide to withdraw their subject from the trial at any time. The investigator should make sure,

however, that withdrawal was not due to an adverse event. The reason for withdrawal should be noted in the space provided for this purpose in the CRF.

- Lost to follow-up: the subject could not be found in spite of the investigator's researches.
- Death.

5.5 Monitoring, Auditing, and Archiving

5.5 1 Routine Monitoring

5.5.1.1 Set-up Visit

A set-up visit will be performed before the inclusion of the first subject. The monitor will verify and document that the material to be used during the trial has been received and that the investigational team has been properly informed about the trial, regulatory requirements, and the SOPs established by Bio Farma.

5.5.1.2 Follow-up Visit

The Monitor will carry out regular follow-up visits.

The investigator commits himself to be available for these visits and to allow the monitoring staff direct access to subject medical file and CRFs. The Monitors are committed to professional secrecy.

During the visits, the Monitors:

- will carry out a quality control of the trial progress: respect to protocol and operating guidelines, data collection, signature of consent forms, sample and product management, cold chain monitoring, completion of document, and appearance of SAE,
- will check the CRFs and other documents,
- will assess the inclusions in order to evaluate the number of complete or ongoing observations.

Monitors will discuss any problem with the investigator and define, after conservation, the actions to be taken.

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5.5.1.3 Close-out Visit

A close-out visit will be performed at the end of the trial. Its goals are to make sure that:

- the center has all the documents necessary for archiving,
- all samples have been shipped,
- all unused material has been recovered,
- all products have been returned to the sponsor.

5.5.2 Audits and Inspections

If necessary, a quality assurance audit could be carried out by Bio Farma Quality Assurance Department or by independent auditors to make sure that the trial has been conducted according to the protocol and the applicable regulations.

An inspection may be conducted by Indonesian Regulatory Authorities.

The investigator shall allow direct access to trial documents.

5.5.3 Archiving

The investigator must keep all trial documents provided by Bio Farma for **at least 5 years** after the completion or discontinuation, whatever the center (private, hospital, and institution).

The investigator will inform Bio Farma of any address change.

6. ADVERSE EVENT REPORTING

6.1 Definitions

⇒Adverse event (AE):

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

⇒Serious adverse event (SAE):

A serious adverse event (experience) is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening. The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have cause death, if it were more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.
- Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

⇒Adverse drug reaction (ADR):

All noxious and unintended responses to medical product related to any dose should be considered adverse drug reactions. The phrase "responses to a medicinal product"

means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

⇒Unexpected adverse drug reaction:

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational medicinal product).

6.2 Expected Reactions

Anticipated reactions to the trial vaccines are those expected forMeasles/Rubella Vaccine and should be mild and transient. Reactions that have been reported may be found in the package insert of Measles/Rubella vaccine.

6.3 Safety Data Collection and Management Procedures

The safety data will be collected up to 6 month after the injection. During this period, each patient will be provided with a diary card to record the appearance, the duration and the intensity (coded 1,2 or 3) of any local reaction and any systemic event expected or not. The intensity of local reactions will be assessed using a plastic bangle presented in Appendix 4.

For any subject, a local reaction is defined as the occurrence of one or several reaction(s) (expected or unexpected) at the injection site within 28 days following vaccination. Local reactions are:

- **Local pain**
- **Redness**
- **Induration**
- **Swelling**
- **Other local reactions**

For any subject, a systemic event is defined as the occurrence of one or several symptom(s) (solicited and unsolicited) following vaccination. Systemic events are:

- **Fever (axillary temperature ≥ 38°C)**[17]
- **Rash**
- **Other systemic events**

Subjects will measure the intensity of local reactions in using a plastic bangle (See appendix 4)[7]. The intensity will be coded in the Diary and CRF as follows:

	1=Mild	**2=Moderate**	**3=Severe**
Local pain for infants (9-12 months)	Reacts when site is touched	Cries when site is touched	Cries when limb is moved
Local pain for children (18-47 months)	Mild pain to touch	Pain with movements	Significant paint at rest
Redness/swelling/ Induration Other local reactions	Reaction completely included in the smaller circle	Largest diameter of the reaction included between the two circles	Reaction beyond the largest circle
Fever	38.0 – 38.4°C	38.5 – 38.9°C	≥39.0°C
Rash *	Localized, minimum symptom	Generalized, (mild symptoms: pruritus, tenderness), no sign of superinfection	Generalized, (severe symptoms: pruritus, tenderness), potential for superinfection
Other systemic events*	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity, requires medical intervention

*An urine sample should be taken if a rash occurs 4-12 days after vaccination, to be performed the isolation of measles and or Rubella virus.

**Other systemic events should refer to published case definition for example Brighton Collaboration Definition.

[17] FDA, Guidance for Industry, Toxicity Grading Scale for Healthy adult and adolescent volunteers enrilled in Preventive Vaccine Trials, April 2005.

Collection and follow-up of adverse events

Adverse events will be collected as indicated in the CRF.

Adverse events likely to be related to the product, whether serious or not, which persist at the end of the trial will be followed up by the investigator until their complete disappearance. The investigator will inform the Medical Responsible or the Monitor of the date of final disappearance of the adverse event and will document it on a correction sheet.

Moreover, any serious adverse event likely to be related to the product and occurring after trial termination should be reported by the investigator to Bio Farma according to the procedure described below.

6.4 Reporting of Serious Adverse Events

Every serious adverse event occurring throughout the trial should be reported to the Ethic Committee, Regional Vaccine Safety Advisory Committee and to the sponsor, by the investigator as soon as he/she is alerted of it i.e. within 24 hours, even if the investigator considers that the adverse event is not related to the treatment.

Notification should be made:

- by phone/email; then the investigator should immediately send the completed alert form to the Ethic Committee, Regional Vaccine Safety Advisory Committee and to the sponsor.

The copy of alert form should be sent to Bio Farma. The investigator should then fill in the SAE reporting form as soon as possible, i.e. within five working days or seven calendar days. This form should be signed by the investigator and sent by fax or express mail to the sponsor and Ethics Committee. Bio Farma as a sponsor should send the reporting form for SAEs-related product (Adverse Drug Reaction) to the NRA within 15 calendar days since the cases was found.

6.5 Causality Assessment

Causality is the relationship between two events (the cause and the effect), where the second event is a consequence of the first. A direct cause is a factor in absence of which the effect would not occur (necessary cause). Sometimes there are multiple

factors that may precipitate the effect (event) or may function as co-factors so that the effect (event) occurs).

Causality assessment is the systematic review of data about AEFI case; it aims to determine the likelihood of a casual association between the event and the vaccines(s) received. For individual cases, one tries to apply the evidence available on the basis of the history and time frame of the event to arrive at a casual likelihood.

Case selection of cases for causality assessment should focus on:

- Serious AEFI
- The occurrence of events above the expected rate or of unusual severity;
- Signals generated as a result of individual or clustered cases as these could signify a potential for large public health impact.

WHO Classification[18]:

I. Case with adequate information for causality conclusion

A. Consistent casual association to immunization: A case with adequate information for causality conclusion

A1. Vaccine product-related: An AEFI that is caused or precipitated by a vaccine due to one or more of the inherent properties of the vaccine product

A2. Vaccine quality defect-related reactions: An AEFI that is caused or precipitated by a vaccine due to one or more quality defects of the vaccine product, including the administration device, as provided by the manufacturer.

A3. Immunization error-related reaction: An AEFI that is caused by inappropriate vaccine handling, prescribing or administration and that thus, by its nature is preventable.

A4. Immunization anxiety-related reaction: An AEFI arising from anxiety about the immunization.

[18] WHO. Causality Assessment of an Adverse Event Following Immunization. Geneva: WHO; 2013.

B. Indeterminate

B1. Temporal relationship is consistent but there is insufficient definitive evidence that vaccine caused the event (it may be a new vaccine-linked event). This a potential signal and needs to be considered for further investigation.

B2. Reviewing factors result in conflicting trends of consistency and inconsistency with casual association to immunization (i.e. it may be vaccine-associated as well as coincidental and it is not possible clearly to favour one or the other.

C. Inconsistent causal association to immunization (coincidental): This could be due to underlying or emerging condition(s) or conditions caused by exposure to something other than vaccine.

Case without adequate information for causality conclusion

This case is categorized as "unclassifiable" and requires additional information for further review of causality. The available information on unclassifiable cases should be placed in a repository or an electronic database which should be periodically reviewed to see if additional information is availavle for classification and to perform analyses for identifying signals.

The decision to modify or discontinue the trial, or to break individual or all study codes may be made after mutual agreement between the sponsor and the investigator(s).

Regulatory Requirements

In order to comply with current regulations on serious adverse event reporting to Health Authorities and to allow Bio Farma to carry out a precise analysis of the safety of the developed products, the investigator pledges to document accurately the event, to respect notification deadlines, to provide Bio Farma with all necessary information and if requested by the sponsor, to give access to source documents.

Bio Farma pledges to inform Health Authorities as soon as it is informed of any serious adverse event likely to be related to the product. The sponsor also pledges to inform the Authorities of any trial discontinuation and specify the reason for discontinuation.

Blood Sampling

A blood sample to be taken as soon as possible might be requested in case of serious adverse event if it can help in analyzing the SAE. Four milliliters of blood will be taken in a dry tube.

Urine Sampling

An urine sample to be taken if a rash occurs 4-12 days after vaccination, to performed the isolation of measles and or Rubella virus. An urine sample will be taken in a sterile urine container at day 3-5 after rash occured.

Determination of SAE

Determination of SAE and its causal relationship with the product (vaccine) will be held by Regional Advisory Committee on Adverse Event. This special committee member consists of experts from multidisciplinary field, i.e. medicine (pediatrics, immunology, neurology, vaccine experts, forensic etc.), public health, biostatistics, health law, etc.

7. EVALUATION CRITERIA

7.1 Primary Evaluation Criteria

7.1.1 Definitions of the Criteria

Number and percentage of subjects with at least one serious immediate systemic events within 30 minutes after vaccination.

7.1.2 Parameters to be Measured

Serious immediate systemic events occurring within 30 minutes after immunization will be assessed with 95% CI.

7.2 Secondary Evaluation Criteria

7.2.1 Definition of the Criteria

Safety

- Number and percentage of subjects with at least one local reaction and systemic event occurring within 72 h after vaccination.
- Number and percentage of subjects with at least one local reaction and systemic event occurring between day 4-14 after vaccination.
- Number and percentage of subjects with at least one local reaction and systemic event occurring between 15 days to 28 days following vaccination.
- Any serious adverse event occurring from inclusion until 28 days after immunization

Immunogenicity

- Percentage of subjects with anti measles titer ≥8(1/dil) and anti rubella titer ≥11 IU/ml, 28 days after one dose of MR vaccine in Infants & Children.
- Serological response to MR vaccine in infants and children : GMT, percentage of infants with increasing antibody titer ≥ 4 times and/or percentage of infants with transition of seronegative to seropositive.

7.2.2 Parameters to be Measured

- Local reactions and systemic events occurring within 72 hours, between day 4- 14 and 15 to 28 days following injection will be assessed with 95% CI.
- Any Serious Adverse Events occurring over the study period will be described
- The GMT, seroprotection and seroconversion rates before and after immunization will be described per antigen with 95% confidence interval (CI).

7.2.3 Method and Timing of Measurement

Immunogenicity assessment:

Antibody titers will be measured at visit V1 (Day 0) and at visit V2 (V1+28 days – 4/+7 days).The anti-measles will be tested by neutralization method and anti-rubella will be tested by ELISA. The titer ≥ 8 IU/mL for Measles and ≥ 11 IU/mL for Rubella are considered to be protected. This test will be conducted in Clinical Trial Department of Bio Farma, had been validated and approved by Quality Assurance Division.

Safety assessment:

Parents will record the presence of any local or systemic symptom within 24h, 72h, 14 days and 28 days following injection in the diary card. They will answer all questions in the diary for solicited and unsolicited events during this period.

Safety data within 7 days and 14 days after immunization will be followed up by phone. The investigator will assess the intensity (code 1, 2 or 3), duration, and relation of each adverse event to the trial vaccines.

Local and systemic reactions, expected or not, occurring after 24h, within 72h, 14 days and 28 days after injection will be evaluated by interviewing the subjects during the post surveillance visits: V1, and V2. Particularly, the axillary temperature was measured for 14 days after vaccination, in the evening and/or at time of febrile peak, and the highest temperature was recorded in the diary card, expressed as Celsius degrees, using a thermometer. The trial team record the information in the CRF.

8. BIOMETRY

8.1 Statistical Methods and Data Analysis

8.1.1 Determination of Sample Size

Sample size was calculated using formula for phase IV study which the incidence rate of AEFI already known.

$$\text{Formula : } N = \frac{\left[z_{1-\alpha}\sqrt{\lambda_0} + z_{1-\beta}\sqrt{(\lambda_0 + \delta)}\right]^2}{\delta^2}$$

λ_0 = Incidence rate

δ = Incidence rate anticipated

$z_{1-\alpha}$ =significance level α coefficient

$z_{1-\beta}$ =power 1-β coefficient

The calculation was based on the incidence rate of fever (0.02%) following MR immunization.

Table With the known incidence rate of the adverse reaction (WHO) with 95%CI and power 80%, the minimal sample size:

Adverse reaction	**Incidence rate (λ_0)**	**Incidence rate anticipated (δ)**	**Sample size**	**+ 20% DO Sample size**
Measles				
Injection Site reactions	0.17	0.17	58.29	69.95
Fever	0.05	0.05	198.19	237.83
Rash	0.05		198.19	237.83
Mumps				
Injection Site Reactions	0.17	0.17	58.29	69.95
Fever	0.02	0.02	495.48	594.57
Acute Arthralgia	0.25	0.25	39.64	47.57
Acute Arthritis	0.1	0.1	99.10	118.91

From the above table, the highest sample size was obtained from the incidence rate of fever (496 doses), if predictably 20% drop out, it required 600 doses.

Sample size formula for protectivity evaluation

Hypothesis tests for a population proportion (Two sides)

$$n = \frac{\{Z_{1-\alpha/2}\sqrt{Po\,(1-Po)} + Z_{1-\beta}\sqrt{Pa(1-Pa)}\}^2}{(Pa - Po)^2}$$

$\alpha = 5$

$1 - \beta = 90$

Po= 0.92

Pa = 0.9

n = 77

Po 92% were taken from a study conducted by Serum Institute of India in 2000, which found that the percentage seropositivity post vaccination MR was 91,67% for Rubella component.

Approximately needs 77 subjects to be involved in this sub study. If 25% subjects were added, approximately 100 subjects should be involved; 200 subjects from infants and children. Since there will be a catch up campaign program for MR in children below 15 years of age, it will be difficult to recruit a children aged 18-47 months who have not been immunized yet. Therefore it is not necessery to obtain equal number of subject for infants and children.

8.1.2 Data Sets to be Analysed

7.1.2.1 Definition of the Population

"Full Analysis Set" (Intention To Treat, ITT): Every subject included in the study will be analysed in this population, except if he/she did not receive any injection of one of the study vaccines. This analysis to determine the safety profile.

"Per Protocol Subjects" (PP): Following non-compliant subjects will be excluded from this population:

- Subjects included without meeting at least one inclusion criterion
- Subjects included despite meeting at least one non-inclusion criterion
- Subjects found non-compliant with the immunization or blood sampling schedule.
- Subjects vaccinated at least once with the wrong vaccine (non-compliance with the randomization schedule).
- Subjects excluded from the ITT analysis.

7.1.2.2 Populations Used in the Analysis

Safety Analysis:

All included and vaccinated subjects will be analysed.

Immunogenicity Analysis:

The immunogenicity analysis will be conducted on the Per Protocol population and also will be conducted on the All Randomized population, each subject being analyzed according to the group attributed by the randomization process.

8.1.3 Statistical Methodology

Safety analysis

At each follow-up visit, the following parameters will be computed:

- Number and percentage of subjects with at least one local reaction (within the first 30 minutes, 72-hours, 4-14 days and 15-28 days post vaccination), with the frequencies of each type of local reactions.

- Number and percentage of subjects with at least one systemic event(within the first 30 minutes, 72-hours, 4-14 days and 15-28 days post vaccination), with the frequencies of each type of event.
- Number and percentage of subjects with at least one Serious Adverse Event, with the frequencies of each type of event within 28 days after each

Immunogenicity analysis

- ♦ Seroprotection and seroconversion rates at last visit (V2): the following parameters was presented: crude rates with their 95% confidence intervals (computed using the exact binomial probability)
- ♦ Geometric means of titers (GMTs) with their 95% CI will be presented at V1 and V2

9. ADVERSE EVENTS COMPENSATION, STIPENDS AND CONFIDENTIALITY

9.1 Confidentiality

9.1.1 Confidentiality of Data

Prior to initiation of the trial, the investigator will sign a fully executed confidentiality agreement with Bio Farma.

9.1.2 Confidentiality of Subject Records

Confidentiality of patient records will be ensured by identifying documents using inclusion number and subject initials (first three letters of the subject's name).

9.2 Adverse Events Compensation and Insurance

Since this is a Post Marketing Surveillance study, for any adverse events that occured during the study will be handled according to local goverment regulation.

Bio Farma shall not be liable for all claims from third parties caused by or resulting from malpractice and/or negligence or willful misconduct of investigators and its staff.

If there is any doubt raised by cause and effect relation between adverse reactions and their participation on this clinical study, judgment of Regional Vaccine safety Advisory Committee or NRA shall be needed.

10. PUBLICATION POLICY

The final report will be prepared by a publication committee which includes the investigators and representatives of Bio Farma. It will be signed by the coordinating (or principal) investigator.

11. BIBLIOGRAPHICAL REFERENCES

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12. APPENDICES

The following is a non-comprehensive list of possible appendices:

Appendix 1: Personnel involved in the trial

Appendix 2: Information Sheet and Informed Consent Form

Appendix 3: Sample SAE Reporting Form

Appendix 4: Plastic Bangle Model

Appendix 5: Signature/Investigator's Agreement to the Protocol

APPENDIX 1: Personnel Involved in the Trial

Medical Advisor	Prof. Dr. dr. Ismoedijanto, SpA(K)
	Prof. dr. Parwati S. Basuki, SpA(K)
Principal Investigator	Dr. dr. Dominicus Husada, SpA(K)
Sub-Investigators	dr. Dwiyanti Puspitasari, SpA(K)
	dr. Leny Kartina, SpA(K)
Biometry	Dr. dr. Windhu Purnomo, MS
Monitor	Dr. Novilia Sjafri Bachtiar, dr., M.Kes.
	Rini Mulia Sari, dr
	Julianita Fahmi, dr
	Asep Irham Fattahul Qur'an, dr
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Biological Laboratory	Rini Mulia Sari, dr.
	Yani Sukriyani
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	PT. Bio Farma (Persero)
	Jl. Pasteur No. 28, Bandung – Indonesia
	Telp.: +62-22-2033755 Fax.: +62-22-2041306

APPENDIX 2: Signatures

Signatures Investigator's Agreement to Protocol of

Reactogenicity and Protectivity Following Measles- Rubella (MR) Routine Immunization in Indonesian Infants and Children

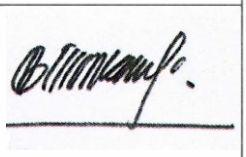
I have read and agree to conduct this trial according to the procedures outlined in this protocol and in accordance with local regulations and Good Clinical Practices.

Function	Name	Date	Signature
Principal Investigator	Dr. Dominicus Husada, dr., DTM&H., MCTM(TP), SpA(K)		
Sub-investigator	Dwiyanti Puspitasari, dr., DTM&H., MCTM(TP), SpA(K)		
Sub-investigator	Leny Kartina, dr., SpA(K)		

Signatures Investigator's Agreement to

Reactogenicity and Protectivity Following Measles- Rubella (MR) Routine Immunization in Indonesian Infants and Children

I have read and agree to conduct this trial according to the procedures outlined in this protocol and in accordance with local regulations and Good Clinical Practices.

Function	Name	Date	Signature
Medical Advisor	Prof. Dr. dr. Ismoedijanto, SpA(K)		
Monitor	Dr. Novilia Sjafri Bachtiar, dr., M.Kes.	15-8-2017	
Monitor	Rini Mulia Sari, dr.	15-8-2017