Magnitude of Measles and Rubella Viruses: Evidence from Selected Health Facilities of Ethiopia

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A research Thesis submitted to Addis Ababa University, College of Health Sciences, School of Biomedicine and Laboratory Sciences, for partial fulfillment of the requirements for a Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology track)

Addis Ababa, Ethiopia

June, 2026

Addis Ababa University

School of Graduate Studies

This is to certify that the Thesis prepared by Rahel Fekade, entitled: Magnitude of *Measles* and *Rubella* Viruses among Children from selected health facilities in Ethiopia, and submitted in partial fulfillment of the requirements for the degree of Master of Laboratory Science in Diagnostic and Public Health Microbiology track. Complies with the regulations of the University and meets the accepted standard with respect to originality and quality.

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Acknowledgments

All glory to God for his guidance and support in every aspect of my work. I would like to express my sincere gratitude and appreciation to my Advisors, Dr. Kassu Desta (College of Health Sciences, School of Biomedicine and Laboratory Sciences) and Mr. Biniyam Dagne for their supervision, constructive suggestions and for sharing their valuable expertise during title selection, valuable suggestions and comments on this Thesis work.

I want to express my sincere thanks to the Ethiopian Public Health Institute for the financial support for this project, for providing excellent mentorship and for giving me project-related training during Thesis development.

My great thanks also go to Addis Ababa University for financial support and for providing an MSc opportunity, and to do this Thesis as part of partial fulfillment for an MSC degree.

I would also like to express my deepest gratitude to Addis Ababa University, Department of Medical Laboratory Sciences and all my instructors at Addis Ababa University.

Last but not least, I want to thank all my family for their support and encouragement throughout my work.

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Abbreviations and Acronyms

AAU	Addis Ababa University
CDC	Centers for Disease Control
CFR	Case fatality ratio
CI	Confidence Interval
CRS	congenital Rubella syndrome
COVID-19	Corona Virus-2019
ELISA	Enzyme-Linked Immunosorbent Assay
EPHI	Ethiopian Public Health Institute
EPI	Extended Program for Immunization
EQA	External Quality Assurance
HCW	Health Care Workers
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Inter quartile range
MRCV	Measles and Rubella Conjugated Vaccine
MV	Measles virus
PI	Principal Investigator
PT	Proficiency testing
QC	Quality Control
RV	Rubella Virus
RCV	Rubella Conjugated Vaccine
RNA	Ribose nucleic acid
SPSS	Statistical Package for the Social Sciences
SSPE	subacute sclerosing panencephalitis
VPD	Vaccine-Preventable Disease
WHO	World Health Organization

Abstract

Background*: Measles and Rubella* remain the most common vaccine-preventable infections, particularly in children. An estimated 11 million cases and 95,000 fatalities of measles were reported worldwide in 2024. Among the top ten high-burden nations, Ethiopia is ranked seventh. Measles and rubella are two of the most common infections in Ethiopia that have a significant impact on children. With an annual incidence of 50 cases per 105 people, measles and rubella ranked third among the most prevalent illnesses that significantly contribute to morbidity and mortality in children under five, behind pneumonia and diarrhea.

Objective: to determine the epidemiology and associated factors of *Measles* and *Rubella* among children attending surveillance health facilities in Ethiopia.

Methods: A multi-center cross-sectional study was employed between October 2024 and May 2025 among measles and rubella-suspected children from selected health facilities in Ethiopia. Socio-demographic data, clinical data, and blood samples were collected from each participant. Data for the study were captured at different stations; patient enrollment took place in the health facility and samples were collected and submitted to the National Vaccine-Preventable Diseases (NVPD) Reference Laboratory. IgM ELISA-based detection of *Measles* and *Rubella* was performed following standard protocols. Epi-Info Version 7.2 was used to enter the data, and SPSS 20.0 was used for analysis. Measures of descriptive statistics were calculated to describe the research variables. The statistical relationship between the variables was evaluated using a logistic regression model.

Results: A total of 651 measles-suspected participants enrolled**.** The median age of enrolled participants was 5 years, and 623(95.7%) had Maculopapular rash. Of them, 140 (41.4 %), 170 (50.3%), and 28 (4.3%) had laboratory-confirmed *Measles, Rubella*, and *Measles-Rubella* dual infection, respectively. The positivity rate reaches a maximum level between 6 and 15 days after the onset of symptoms. Overall age-specific prevalence ranges between 20% and 60%. A higher frequency of 60% was seen at the age of one year. The analysis showed that people who were living in rural areas (AOR: 1.46, 95%CI=1.05-2.02), contact with symptomatic cases (AOR: 1.33, 95%CI =1.17-1.84), children missing vaccinations (AOR: 3.16, 95%CI =2.04-5.72), and lack of trust in immunization programs (AOR: 1.39, 95%CI =1.09-1.96) were independently associated factors. In contrast, previous history of infection (AOR: 0.57, 95%CI =0.42-0.79) and

attending postnatal care visits (AOR: 0.63, 95%CI =0.45-0.89) showed a protective association for *Measles* and *Rubella* infection.

Conclusion: This study reported a high prevalence of *Measles* and *Rubella* infection in Ethiopian children. The current study recommends adequately managing cases, strengthening the vaccination campaign, and reinforcing the routine EPI program, identifying hot spot areas, and assessing the risk of a large outbreak in a sustained manner.

Keywords: Epidemiology, Associated factors, Measles, Rubella, Children, Ethiopia

1. Introduction

1.1 Background

Rubella and Measles are both viral infections that present similarly, are highly contagious, and are serious airborne diseases that can result in severe complications and even death. The measles virus (MV) belongs to the Paramyxoviridae family and is classified under the morbillivirus genus. MV possesses a 16 kb negative-sense RNA genome that is non-segmented and encapsulated within a lipid envelope. The complete measles genome comprises 15,894 nucleotides and encodes six structural proteins along with two non-structural proteins. These structural proteins are linked to the viral RNA and the envelope [1].

The Rubella Virus (RV) is responsible for causing Rubella, also known as German measles, an infectious disease linked to an RNA-enveloped virus from the Togaviridae family and the Rubivirus genus. This virus possesses a single-stranded positive-sense RNA genome comprising 9,762 nucleotides, which encodes two nonstructural proteins and three structural proteins (one nucleocapsid and two envelope proteins). RV affects both children and adults, typically presenting as a febrile illness. Additionally, RV is a significant contributor to birth defects and fetal mortality when contracted by pregnant women. As a teratogen, RV is a leading cause of congenital Rubella syndrome (CRS), a congenital defect [2].

Measles and Rubella are spread via the airborne route and can last for up to 2 hours in the airspace where an infected person breathes, coughs, or sneezes. Measles is highly communicable, and if one person has it, 90% of the people close to that person who are not immune will also become infected. The Infected people can spread measles to others from 4 days before through 4 days after the rash appears [3]. The signs and symptoms include: Fever, rash, and the “three C’s”: cough, coryza (redness and swelling of the nasal mucosa) and conjunctivitis (red, watery eyes) can also have: koplik spots (scattered blue-white tiny spots on a bright red background) may appear inside the mouth, malaise, diarrhea, Anorexia, and lymphadenopathy are common in Measles complications [4]. Children younger than 5 years of age and adults older than 20 years of age are more likely to suffer from Complications from measles, which can include acute otitis media, which affects approximately 1 in 10 children with the disease and may lead to permanent hearing impairment. Pneumonia, affecting about 1 in 20 children with measles, is the leading

cause of death from measles in young children. Another complication is subacute sclerosing panencephalitis (SSPE), a rare but deadly condition that can develop 7-10 years after a natural measles infection. SSPE involves progressive brain swelling with no known cure, and most individuals diagnosed with it die within 1-3 years. However, some experience a more rapid disease progression, resulting in death within three months of diagnosis[4,5]. The likelihood of developing SSPE may be greater in those who contract measles before reaching the age of two. The incidence of SSPE declined by at least 90% in countries that have practiced widespread measles vaccination [5].

Rubella is contracted when a person inhales the virus released into the air by an infected person through breathing, sneezing, or coughing. Initially, the virus targets cells in the upper respiratory tract and subsequently disseminates through the bloodstream, replicating within the lymphoid tissues of the nasopharynx [6]. This process results in the infection of various organ systems. After the virus is transmitted through the respiratory route and replicates in the nasopharynx and nearby lymph nodes, viremia develops 5–7 days post-exposure [6]. This allows the virus to spread throughout the body and cross the placenta, causing fetal harm by destroying cells. Conjunctivitis, arthralgia, and arthritis are the most frequent side effects of mitotic arrest, an acute illness marked by fever and rash that is spread by respiratory droplets and direct contact. Rarely, more serious side effects, such as encephalopathy and hemorrhage, occur in pregnant women: miscarriage, stillbirth, birth defects, and placental infection that results in transplacental infection of the fetus. Some of the complications of rubella result from host immune responses, including arthritis, thrombocytopenic purpura, and encephalitis [6-7]. Research findings suggested that rubella infection in pregnancy was also associated with autism. Autism can result from a variety of harmful factors, including genetic, environmental, prenatal, and pharmacological exposures. The exact origins of autism are mostly unknown. While congenital rubella syndrome (CRS) is the most widely known result of rubella exposure, rubella has also been investigated as a viral cause of autism [8].

Measles and rubella infections do not presently have a specific therapy. The WHO advises vitamin A supplementation be given to all children with acute measles at vaccination contacts after age 6 months, which many children receive in addition to the MRCV vaccine [8]. It has been demonstrated that giving vitamin A to children with measles reduces both the severity of

the disease and the case-fatality rate. Basic immunological processes depend on vitamin A, encompassing dendritic cell, T cell, and NK cell development, maturation, migration, and activation. On the day of the measles diagnosis, one dose (50,000 I.U. for infants under six months, 100,000 I.U. for infants between six and eleven months, and 200,000 I.U [9] for children over twelve months) should be given, followed by another dose the next day. Fluids such as ORS, antipyretics, nutritional therapy, and giving women folic acid supplements are frequently recommended for simple instances [9].

The childhood immunization schedule recommends two doses of the Measles conjugated vaccine (MCV) and the Measles-Rubella conjugated vaccine (MRCV), spaced at least four weeks apart, for every kid. Every newborn should have proof of immunity, such as verification of at least one vaccination. The vaccination should be administered twice to some adults and adolescents [10]. On or after the first birthday, the first dose of MCV or MRCV should be administered. Any vaccination administered before 12 months of age should not be included in the series. Two doses of the MCV or MRCV vaccine should be given to children who received the vaccination before the age of 12 months. The first dose should be given when the kid is at least 12 months old [10].

Those who did not respond to the first dose of MCV or MRCV are advised to receive a second dose to develop immunity. Before a child enters kindergarten or first grade, the second dose of the MCV or MRCV vaccine should normally be administered at age 4–6. For children who have not yet had two doses of MCV or MRCV, the preadolescent health visit at age 11–12 can be used as a catch-up opportunity to confirm immunization status and deliver the vaccine. One month (i.e., at least 28 days) following the first dose, the second dose of MCV or MRCV may be given. When they start school, children who have already received two doses of the MRCV vaccine spaced at least four weeks apart, with the first dose given no earlier than their first birthday, do not require a second dose. Children who do not have proof of sufficient vaccination against measles, rubella, and mumps or other valid proof of immunity to these illnesses [10].

1.2 Statement of the problem

Measles and rubella remain major causes of morbidity among vaccine-preventable diseases, particularly among children. Despite the availability of safe and effective vaccines, these diseases continue to pose significant public health challenges worldwide [11]. In 2024, an estimated 11 million measles cases and approximately 95,000 measles-related deaths were reported globally, with the majority occurring among unvaccinated or under-vaccinated children younger than five years of age. Ongoing transmission is largely driven by suboptimal immunization coverage and disparities in vaccine uptake across different geographic regions. Consequently, measles continues to circulate in many parts of the world, and outbreaks occur even in settings with relatively high routine immunization coverage. According to the latest reports from the World Health Organization (WHO), millions of people remain at risk of measles infection globally [12]. The number of measles cases reported in 2024 was among the highest recorded over the past 25 years, with major outbreaks documented across several regions of the world. In 2023, approximately 83% of children worldwide received their first dose of the measles-containing vaccine by their first birthday through routine immunization programs. During the period from 2022 to 2023, a total of 196,171 rubella cases were reported to WHO through case-based surveillance systems. However, the true burden of rubella remains inadequately documented in many countries [11]. It is estimated that more than 100,000 infants were born with congenital rubella syndrome (CRS) globally in 2023. Furthermore, the COVID-19 pandemic substantially disrupted immunization services and disease surveillance systems worldwide. The resulting decline in vaccination coverage and surveillance activities left millions of children vulnerable to vaccine-preventable diseases, including measles and rubella, increasing the risk of outbreaks and disease resurgence [12].

According to the WHO 2024 report, ten countries account for approximately 75% of the global burden of measles and rubella. Among these, five are located in Africa: Ethiopia, Sudan, Somalia, Nigeria, and the Democratic Republic of Congo[13]. In sub-Saharan Africa, measles continues to be a major cause of morbidity and mortality. In 2023, the African region reported 125,957 measles cases. WHO reports further indicate that the majority of measles- and rubella-related deaths occurred in sub-Saharan Africa. The COVID-19 pandemic significantly disrupted immunization programs and surveillance activities, resulting in an estimated 16.6 million

children missing scheduled supplementary measles vaccinations between January 2020 and April 2021. These disruptions created substantial immunity gaps and weakened disease surveillance systems across the continent [14]. During this period, eight African countries experienced major measles outbreaks affecting tens of thousands of individuals. In 2023, the Democratic Republic of Congo experienced one of the world's largest measles epidemics, with reported deaths exceeding 6,000. Despite ongoing control efforts, measles remains endemic in many African countries and continues to pose a serious public health threat [12-14]. Similarly, rubella remains endemic throughout much of the African region, with a considerable proportion of cases occurring among children under five years of age and women of reproductive age. However, the true burden of rubella remains inadequately documented because of limited surveillance and underreporting in many African countries [14].

Ethiopia ranks seventh among the ten countries with the highest burden of measles globally. In the country, measles and rubella remain important causes of childhood illness and are among the nationally notifiable diseases. Among vaccine-preventable diseases (VPDs), measles is one of the leading contributors to morbidity and mortality among children under five years of age, ranking third after pneumonia and diarrhea [14]. Despite ongoing control efforts, measles continues to be a significant public health concern in Ethiopia, with an estimated incidence of 50 cases per million population per year, far exceeding the national measles elimination target of fewer than one case per million population. The disease remains endemic, with cases reported annually, and an estimated case fatality rate ranging from 3% to 6%. In 2023, Ethiopia reported 16,814 laboratory-confirmed measles cases and 182 associated deaths, corresponding to a case fatality ratio (CFR) of 1.1%. In 2022, active measles outbreaks were documented in 44 woredas across eight regions, including Afar, Amhara, Harari, Oromia, Southern Nations, Nationalities and Peoples' Region (SNNPR), South West Ethiopia Peoples' Region, Tigray, and Somali. Although Ethiopia does not maintain a separate surveillance system for rubella, rubella cases are identified through the integrated measles surveillance program [15,16]. Suspected measles cases that test negative for measles are subsequently tested for rubella infection. Through this surveillance system, approximately 18 rubella outbreaks are detected and reported annually [16].

Measles and rubella are predominantly childhood diseases and continue to pose significant health risks to children worldwide. In densely populated settings, measles most frequently affects children aged 3–4 years, whereas in less crowded urban and rural areas, the highest incidence is

typically observed among children aged 5–10 years. Although a safe, effective, and affordable measles vaccine has been available for more than four decades, measles remains one of the leading causes of death among vaccine-preventable diseases, particularly in developing countries. Deaths associated with measles and rubella are often the result of disease-related complications rather than the infections themselves. Common complications include pneumonia and diarrhea, which occur due to the immunosuppressive effects of the infections. In addition to mortality, these diseases can result in severe and lifelong disabilities, including neurological impairment, blindness, and hearing loss. Furthermore, measles is recognized as one of the most highly contagious infectious diseases, contributing to its rapid transmission and frequent outbreak occurrence, especially in populations with inadequate immunity [14-16].

One of the factors contributing to low vaccine uptake in Ethiopia is the limited availability of reliable information on the burden of vaccine-preventable diseases. To address this gap, the WHO African Region supports member countries in generating data on rubella incidence through the integrated measles case-based surveillance system. Under this surveillance framework, epidemiological information and blood specimens are collected from individuals presenting with suspected measles infection. The specimens are initially tested for measles-specific immunoglobulin M (IgM) antibodies using standard enzyme-linked immunosorbent assay (ELISA) methods. Samples that test negative for measles IgM antibodies are subsequently analyzed for rubella-specific IgM antibodies using standard ELISA procedures. This integrated approach enhances the detection and monitoring of rubella cases while strengthening surveillance activities for both diseases [11-16].

The true incidence of measles and rubella is often underestimated by surveillance systems due to several factors, including the nonspecific nature of some clinical manifestations, challenges in clinical diagnosis, inadequate surveillance infrastructure, and limited laboratory capacity for case confirmation. Consequently, many cases remain undetected or unreported, resulting in an incomplete understanding of the actual disease burden. To address these gaps, it is essential to ensure that all suspected outbreaks are comprehensively investigated and documented, expand sentinel surveillance systems in countries such as Ethiopia, and strengthen mechanisms that enhance the sensitivity of case detection.

Rubella infection, in particular, is frequently underreported because many cases are either not recognized by healthcare providers or remain unnoticed by infected individuals due to the mild or asymptomatic nature of the disease. As a result, rubella outbreaks may occur and spread without clinical recognition. Furthermore, adverse outcomes associated with rubella infection, including congenital rubella syndrome, are often inadequately documented, even in developed countries. Therefore, periodic epidemiological studies and active surveillance efforts are necessary to improve disease detection, accurately characterize transmission patterns, and generate reliable evidence for effective prevention and control strategies.

To our knowledge, there is limited data on the current Epidemiology of diseases circulating in the central, eastern and southeastern parts of Ethiopia. Thus, it is important to identify predisposing factors for causing *Measles* and *Rubella* in this specific geographic region of Ethiopia, which could contribute to the strengthening of the disease control program of the country.

1.3 Significance of the study

Effective *Measles* and *Rubella* control programs require the initiation of appropriate vaccination as well as an understanding of the epidemiology of the disease. Moreover, monitoring the outbreak of the disease is very important and information based on active surveillance is used as an indicator for control and prevention of the disease. Understanding the magnitude and associated factors is important to detect if the disease is influenced by the host, environmental and evolutionary background of the virus.

Studies of this nature are essential for determining the incidence of measles and rubella, understanding their transmission dynamics, monitoring their distribution, and facilitating the early detection of outbreaks. Furthermore, the evidence generated from such studies contributes to a better understanding of disease epidemiology and provides valuable information for strengthening prevention and control strategies. The findings can also support the revision and development of health policies and inform evidence-based recommendations for public health authorities. Ultimately, these data serve as an important resource for guiding interventions aimed at reducing the burden of measles and rubella and advancing disease control and elimination efforts.

The information generated during such a study was publicized, which is important to protect against transmission, and it is useful to detect vaccination quality. Eastern and central Ethiopia are expected to have high *Measles* and *Rubella* cases, like the other parts of the country, because of higher population flow and overcrowding. However, recent data are scarce on the detection of *Measles* and *Rubella*, identification of the disease and identification of the most dominant cases.

This study provides important evidence for identifying cases that frequently remain unreported because they are either not recognized by healthcare professionals or go unnoticed by affected individuals. As a result, outbreaks may occur and spread without timely clinical detection. Furthermore, adverse outcomes associated with these infections are often inadequately documented, even in countries with well-established healthcare systems. Therefore, periodic studies incorporating active disease surveillance are essential to improve case detection, accurately characterize disease patterns, and generate reliable data to support effective public health interventions and disease control strategies.

2. Literature Review

Substantial progress has been achieved globally in reducing the burden of vaccine-preventable diseases (VPDs). Nevertheless, the resurgence of measles and rubella outbreaks in recent years has emerged as a significant challenge to global disease control and elimination efforts. Understanding the epidemiology and incidence of these diseases is essential for assessing their global distribution and transmission patterns. However, existing evidence is largely derived from localized studies, limiting a comprehensive understanding of the worldwide burden and temporal trends of measles and rubella. Furthermore, there remains a scarcity of studies evaluating the global disease burden and long-term epidemiological trends of measles.

A systematic review and meta-analysis conducted to estimate national-level measles case-fatality ratios (CFRs) in low- and middle-income countries reported an overall CFR of 2.6% among individuals aged 0–34 years in community-based settings. The study found that CFRs were consistently higher in hospital-based settings than in community-based settings, reflecting the greater severity of cases presenting to healthcare facilities. The highest case-fatality ratios were observed among children younger than one year of age, with mortality risk declining progressively with increasing age. Across all geographical regions, the sub-Saharan African region had the highest estimated CFRs in both community- and hospital-based settings in 2019, highlighting the substantial burden of measles-related mortality in the region [17].

A systematic review and meta-analysis conducted in European Union/European Economic Area (EU/EEA) countries and the United Kingdom assessed the prevalence of measles susceptibility among healthcare workers (HCWs) and examined associated demographic characteristics. The study reported that 13.3% of HCWs remained susceptible to measles. Comparisons between female and male HCWs showed a relative risk (RR) of 0.92, while age-group comparisons yielded an RR of 2.7. The findings highlighted a greater susceptibility among individuals born during the post-vaccination era and emphasized the importance of mandatory vaccination policies for healthcare workers. The authors recommended enhanced efforts to identify unvaccinated HCWs and promote vaccination uptake to reduce the risk of disease transmission [18].

A retrospective cross-sectional study conducted in Uganda investigated measles-rubella positivity and associated risk factors following the 2019 measles-rubella vaccination campaign.

The study demonstrated a significant reduction in both suspected cases and laboratory positivity rates, indicating the effectiveness of the campaign in reducing disease burden. However, children younger than nine months and unvaccinated individuals remained at a higher risk of infection. Furthermore, receipt of at least one dose of the measles-rubella vaccine was associated with a lower likelihood of testing positive, with increasing vaccine doses providing greater protection against infection [19].

An expert review examining progress toward measles elimination and its implications for rubella control reported that recent measles resurgence has been accompanied by a shift in disease occurrence toward older children, adults who were missed by earlier vaccination programs, and infants too young to be vaccinated. Although the elimination of measles and rubella is biologically achievable, successful implementation will require stronger political commitment, innovative vaccination strategies, enhanced surveillance systems, sustainable funding, and improved population immunity across all age groups [20].

A study assessing the burden, epidemiological patterns, and surveillance gaps of rubella in Nigeria found that the highest incidence occurred during the first four months of the year, with most cases affecting individuals younger than 15 years of age in both rural and urban settings. The study highlighted that rubella and congenital rubella syndrome (CRS) remain neglected public health problems due to the absence of dedicated surveillance systems, national incidence estimates, and routine vaccination policies. Because rubella surveillance is integrated within the measles surveillance system and only measles-negative samples are tested for rubella, substantial underreporting may occur. The authors emphasized the need to strengthen surveillance systems and introduce routine rubella vaccination programs in Nigeria [21].

A twelve-year laboratory-based surveillance study conducted in Senegal revealed that children younger than five years and those aged 5–15 years were the most vulnerable groups for measles and rubella infection, respectively. The study underscored the importance of maintaining high-quality surveillance and immunization coverage to achieve disease elimination goals. It also highlighted that rubella incidence may be underestimated due to the lack of a rubella-specific case definition and the practice of testing only measles-negative cases. Despite relatively low

incidence rates, both diseases remain endemic in Senegal, posing challenges to regional elimination efforts [22].

A sentinel surveillance study of congenital rubella syndrome in South Africa identified an increase in laboratory-confirmed CRS cases from four cases in 2012 to 37 cases in 2015 in the absence of a public rubella vaccination program. Most mothers of affected infants were between 14 and 30 years of age, indicating an immunity gap among women of reproductive age. The study emphasized the importance of introducing rubella-containing vaccines (RCVs) and maintaining continuous CRS surveillance to monitor vaccine impact and support rubella control initiatives [23].

An ecological study using global rubella surveillance data reported 139,486 rubella cases worldwide, of which 11% occurred among vaccine-eligible populations. Global incidence declined substantially from 13.9 cases per million population in 2007 to 1.7 cases per million in 2018. However, incidence remained higher among vaccine-ineligible populations, particularly among children younger than 15 years and women of reproductive age. The study concluded that rubella vaccination has significantly reduced disease burden and recommended the introduction of rubella-containing vaccines in countries that have not yet adopted them to prevent CRS-related morbidity and mortality [24].

In Ethiopia, a retrospective analysis of measles surveillance data from Bale Zone between 2013 and 2019 identified 4,241 reported measles cases, with children younger than four years being the most affected group. The highest prevalence was reported in 2019, and the case-fatality rate was estimated at 3.07 per 1,000 population. The findings indicated that poor immunization coverage contributed substantially to the continued burden of measles in the area, with Ginir district reporting the highest number of cases [25].

A similar retrospective study conducted in Addis Ababa reported 4,203 suspected measles cases, of which 27.5% were laboratory confirmed and 12.2% were clinically compatible. The study found that measles reporting rates were highest among younger children. Although some patients had received at least one dose of measles vaccine, a considerable proportion remained unvaccinated or had unknown vaccination status, highlighting ongoing gaps in immunization coverage [26].

An 18-year retrospective study in Eritrea examined confirmed measles cases, surveillance performance, and associated risk factors. Although the country reported relatively low measles incidence, substantial year-to-year variability was observed. The study noted the potential for underestimation due to underreporting and incomplete laboratory testing. The median age of measles cases was seven years, suggesting that vaccination campaigns targeting only younger children may fail to reach susceptible older age groups. Factors associated with laboratory-confirmed measles included age, geographical location, healthcare facility level, vaccination status, and year of disease onset. Persistent inequalities in vaccine coverage and surveillance systems were identified as barriers to elimination efforts [27].

A study investigating a rubella outbreak among schoolchildren in Addis Ababa reported a higher prevalence of rubella infection among females (12.7%) than males (9.3%). The study also found a higher proportion of infections among children younger than five years of age compared with older children. The findings suggested the occurrence of outbreaks among rubella-susceptible populations, likely due to the absence of rubella vaccination and the presence of susceptible women of childbearing age, emphasizing the need for enhanced rubella control measures in Ethiopia [28].

3. General objectives

The general objective of the study was to determine the magnitude and associated factors of *Measles* and *Rubella* among children attending selected surveillance facilities in Ethiopia

3.1 Specific objectives

The Specific objectives of this study were:

1. To determine the magnitude of *Measles* and *Rubella* based on various factors such as age, immunization status and other host factors in the era of increased vaccination coverage from selected health facilities in Ethiopia between 2024 and 2025.
2. To assess the proportion of mixed infection and associated factors among *Measles* and *Rubella*-infected patients in Ethiopia from 2024 to 2025.
3. To describe associated factors based on the clinical and demographic profile of children presenting with *Measles* and *Rubella* infection in Ethiopia from 2024 to 2025.

4. Materials and Methods

4.1 Study Setting

This study was a cross-sectional health institution-based study conducted in selected health facilities in Ethiopia. The study was conducted between October 2024 and May 2025 in Ethiopia. The estimated population size of Ethiopia was more than 135 million at the time of the study, and health facilities with high patient loads were selected.

4.2 Study design

A cross-sectional study design was used to conduct the study.

4.3 Study period

The study was conducted between October 2024 and May 2025.

4.4 Study Area

The study was conducted in twelve surveillance facilities in different regions of Ethiopia, including Shashamene Referral Hospital, Dodola Hospital, ALERT Specialized Hospital, Lalibela Health Center, Finote Selam General Hospital, Bir Sheleko Hospital, Maykadra Health Center, Assayita Hospital, Dire Dawa Hospital, Warder Hospital, Bambasi Hospital, and Gambella General Hospital. The facilities were selected based on their high annual patient reports.

4.5.1 Source Population

The source population was all children under 15 who had visited the selected hospitals and health centers for medical care during the study period,

4.5.2 Study population

The study population for this study was *Measles* and *Rubella* symptomatic children at selected hospitals and participants during the study period.

4.6 Inclusion and Exclusion criteria

4.6.1 Participant Inclusion Criteria

- Patients who are willing to give informed consent/assent.
- All suspected *Measles* and *Rubella* cases meet the case definition of febrile rash with at least one of the symptoms: cough, coryza or conjunctivitis.

- All symptomatic children with less than 15 years of age.

4.6.2 Participant Exclusion Criteria

- Individuals who had received any vaccination within the two weeks preceding symptom onset were excluded from the study, as post-vaccination adverse events may produce clinical manifestations similar to those of the diseases under investigation, potentially affecting the accuracy of case identification and diagnosis.
- Patients with an unacceptable sample quality.

4.7 Variables of the Study

4.7.1 Dependent variables:

- Burden of Measles and Rubella
- Proportion of mixed infection

4.7.2 Independent variables:

- Demographic variables
- Age at the beginning of signs and symptoms
- Sex
- Region (Address they came from)
- Educational status
- Vaccination status
- Presence of co-morbidity
- Clinical Factors

4.8 Sampling and Sample Size Determination

4.8.1 Sample size calculation

For objectives I and **II**: the sample size was determined by a single population proportion formula assuming a prevalence of 39.4 %, marginal error of 5% at 95% confidence interval (CI) (Mekonen G et al, 2016) [29].

$$n = \frac{(Z/2)^2 \times p(1-p)}{d^2}$$

Z = the standard normal deviation at 95% confidence level =1.96

p = 39.4%, the proportion of prevalence

d = the desired precision (marginal error), 5%

$$= \frac{(1.96)^2 \times 0.394(1-0.394)}{(0.05)^2} = 367 + (10\% * 367) = 404$$

For objectives III

Table 1: Sample size calculation for factors associated with rubella and measles infection

	Factors	Outcome Variable	Prevalence (%)	Initial sample Size	Non-response rate	Final sample size	Reference
1	Measles (Age)	Age group 5-15 years	51%	384	38	422	Mengistu B et al., 2022 [30]
2	Rubella (Age)	<10 years	76.5 %	276	28	304	Gemechu D et al., 2022 [31]

The sample size of the third objective was 422.

Finally, to increase precision, a larger sample size was taken; therefore, a total of **422** participants were included in this study.

4.8.2 Sampling Method

The health facilities were selected purposely because of their service at the start of the study period, and then the profiles of all *Measles* and *Rubella* cases in the hospitals and health centers were assessed. Then, non-probability purposive sampling was utilized to identify participants meeting the criteria of the study's sampling. If they met the inclusion criteria, and they were requested to participate in the study.

4.9 Measurement and Data Collection

4.9.1 Data Collection Procedure

Data were collected by trained health professionals using structured questionnaires and data extraction forms at selected health facilities. Eligible participants were identified based on the inclusion criteria and informed about the objectives and procedures of the study. Participants were assured that all information would be kept confidential and that their decision to participate or decline would not affect the healthcare services they received. Written informed consent was obtained before enrollment.

For participants who consented to participate, a pretested questionnaire and data extraction tool were used to collect socio-demographic and clinical information. Data collection continued consecutively until the required sample size was achieved. Information gathered included age, educational level, occupation, gestational age, and vaccination status. Additional data on socio-demographic characteristics, healthcare access, nutritional status, and other potential risk factors for measles and rubella infection were obtained through interviews with mothers or caregivers of children. Vaccination status was verified through review of immunization cards whenever available and supplemented by caregiver recall of vaccination history.

The tool was pretested and modified accordingly. The pretest was on 5% of the sample size. The patients were recruited consecutively till the sample size allocated for that was reached. Health professionals collected data by face-to-face interviews using structured questionnaires. Blood samples were taken under aseptic techniques. A 3- 5 mL blood sample was collected from study participants based on the type of clinical symptoms into test tubes, and serum was separated and transported to the laboratory immediately. The specimens were transported with a triple packaging system under +2 to +8 °C to the VPDL, EPHI, for laboratory testing. The sample was transported as soon as possible on the day of collection; however, in case of delay in transportation, it was stored in a refrigerator and stored at a temperature of –20 degrees centigrade till analysis. The author and professionals who worked with the author analyzed all serum samples in the VPD laboratory.

4.9.2 Laboratory Methods

4.9.2.1 Sample collection

A venous blood sample was collected using established techniques as follows: Prepare all blood collection supplies (sample rack, sample request/ blood collection form, sample storage log) and necessary labels. Label each sample tube with the sample ID (use the same ID number for the same participant for all the samples collected at the same time point). Explain the procedure to the participant and put on clean gloves. Assemble the needle or butterfly needle into the Vacutainer holder, being sure that it is firmly seated in the threads. Loosely place the Vacutainer tube into a holder, but do not puncture the top.

Apply a tourniquet above the venous area to be punctured, preferably at the mid-arm position. Clean the area where the vein is visible using a swab with a disinfectant in a circular motion. Repeat with a second wipe and allow the area to dry. Use a Vacutainer and needle to puncture the vein. Loosen the tourniquet immediately after blood flow is established.

Allow blood in the red top or serum separator tube to clot, then centrifuge. After clotting and centrifugation, an aliquot of serum was transferred to individual cryovials. Keep all the tubes in an upright position in the rack. Remove the needle from the vein and apply pressure with a swab on the punctured area to stop bleeding., tell the subject to continue applying pressure at least for one minute or until there is no more bleeding from the punctured site check the specimens are labelled correctly and fill in the specimen collection form completely and accurately, document the time and date of blood sample collection on the specimen collection form. Place sample tubes upright on a sample rack for transportation and storage in liquid nitrogen. Remove and discard used gloves in the waste bin for contaminated materials [33].

To extract the serum, whole blood should be allowed to coagulate before being centrifuged for ten minutes at 1000 × g. The blood should be refrigerated until the clot completely retracts from the serum (no more than 24 hours) if a centrifuge is not available. To prevent removing red blood cells, the serum should be carefully extracted using a fine-bore pipette. It should then be aseptically transferred to a sterile container labeled with the patient's name or identity, the date of collection, and the kind of specimen. [33].

4.9.2.2 Storage and transportation of serum specimens from the study sites to EPHI-VPD

Serum samples were maintained at a temperature of 4–8°C until shipment and for no longer than seven days. For storage periods exceeding seven days, specimens were frozen at −20°C and transported to the testing laboratory using ice packs to preserve sample integrity. Repeated freeze–thaw cycles were avoided because they can adversely affect the stability of IgM antibodies and compromise test results.

All serum specimens were transported to the laboratory as promptly as possible within 2 days, without delaying shipment to await additional sample collection. Each specimen was placed in a uniquely labeled, sealed vial and packaged in a leak-proof plastic bag or pouch containing absorbent material, such as cotton wool, to absorb any accidental leakage. The packaged specimens were then placed in insulated transport containers with sufficient ice packs to maintain the required temperature during transit. Corresponding data collection forms were enclosed separately in sealed envelopes and securely attached to the shipment package. The specimens were positioned centrally within the container and surrounded by additional ice packs to ensure optimal temperature control. Before dispatch, transportation arrangements and shipping schedules were coordinated, and the responsible postal or courier service was notified of the shipment details to facilitate timely and secure delivery.

4.9.2.3. Enzyme-Linked Immunosorbent Assay (ELISA) for Measles

Laboratory confirmation of measles virus (MV) infection was based on the detection of either the virus itself or measles-specific IgM antibodies. Virological diagnosis can be achieved through virus isolation, direct detection of viral antigens, or identification of viral RNA. Serological diagnosis was performed by detecting measles-specific IgM antibodies in a single serum specimen collected during the symptomatic phase of illness. In this study, measles IgM antibodies were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Germany) following the manufacturer's instructions [34–36].

The assay employs an antibody-capture ELISA principle, in which patient IgM antibodies are captured by anti-human IgM antibodies coated onto the microplate wells. After washing to remove unbound serum proteins and other immunoglobulins, measles antigen is added and binds

to any measles-specific IgM antibodies present in the sample. Following a subsequent washing step, the bound antigen is detected using an enzyme-conjugated anti-measles monoclonal antibody. Addition of a chromogenic substrate results in a color reaction, the intensity of which indicates the presence or absence of measles-specific IgM antibodies in the specimen [34–36].

The Euroimmun Measles IgM ELISA kit contains anti-human IgM-coated microtiter plates, positive and negative control sera, recombinant measles nucleoprotein antigen and control antigen, enzyme-conjugated anti-nucleoprotein antibodies, as well as the required substrates, buffers, and diluents. Test results are interpreted using the ratio of the sample optical density (OD) to the calibrator OD rather than raw OD values. A ratio of less than 0.8 is considered negative, values between 0.8 and 1.1 are regarded as borderline or equivocal, and values equal to or greater than 1.1 are interpreted as positive for measles-specific IgM antibodies [34–36].

4.9.2.4 Enzyme-Linked Immune Sorbent Assay (ELISA) for Rubella

Detection of rubella-specific immunoglobulin M (IgM) antibodies in serum is the standard method for the rapid laboratory diagnosis of rubella infection. In this study, rubella IgM testing was performed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Germany) in accordance with the manufacturer's instructions [37–39].

The assay is based on an indirect ELISA principle in which rubella antigens are immobilized on the surface of microplate wells. Diluted serum specimens, along with positive and negative controls, are added to the wells, allowing rubella-specific antibodies present in the samples to bind to the coated antigens. Following incubation, the wells are washed to remove unbound proteins and other serum components. Subsequently, horseradish peroxidase (HRP)-conjugated anti-human IgM antibodies are added to detect bound rubella-specific IgM antibodies. After a further washing step to remove excess conjugate, a chromogenic substrate is added, resulting in the development of a colored reaction product. The intensity of the color produced is directly proportional to the concentration of rubella-specific IgM antibodies present in the sample. The reaction is terminated using a stop solution, and the absorbance is measured at 450 nm using an ELISA microplate reader after blanking against the substrate control [37].

Test validity and result interpretation were performed according to the manufacturer's recommendations. Results were determined based on the ratio of the sample optical density (OD)

to the calibrator OD rather than on raw OD values alone. Samples with a ratio below 0.8 were considered negative, ratios between 0.8 and 1.1 were classified as borderline or equivocal, and ratios equal to or greater than 1.1 were interpreted as positive for rubella-specific IgM antibodies [37–39].

4.10 Quality assurance

To maintain the quality and integrity of the data, the principal investigator (PI) and assigned supervisors closely supervised all study activities throughout the data collection period. Routine monitoring was conducted to ensure data completeness, consistency, accuracy, and adherence to ethical standards. Quantitative data were entered into Epi Info version 7 using a coding structure developed specifically for the study. After data entry, all records were verified against the original data collection instruments to confirm their accuracy and reliability. Data cleaning and validation procedures were systematically implemented at every stage of data management.

The PI oversaw and evaluated the entire data collection process on an ongoing basis. Completed questionnaires were checked daily for completeness and internal consistency before being entered into the database. To reduce the likelihood of data entry errors, information was independently entered twice into Microsoft Excel, and the two datasets were compared for discrepancies. Any differences identified were reviewed and corrected using the source documents.

Laboratory data quality was ensured through comprehensive internal and external quality assurance measures. The laboratory routinely participated in quality assessments, quarterly quality control (QC) testing, and annual external quality assurance (EQA) proficiency testing (PT) programs. Furthermore, the laboratory maintained annual accreditation through the WHO Global Measles and Rubella Laboratory Network, demonstrating compliance with internationally recognized standards. Throughout the study period, the laboratory retained its accredited status and consistently achieved an accuracy rate of 95% or higher in both QC and PT evaluations. Internal and external control materials were included in every assay run, and laboratory findings were released only when all quality control requirements were fulfilled, confirming the validity of the test results.

4.11 Data Analysis and Interpretation

Before data entry, each questionnaire was edited and coded and a data entry template was prepared. Records were cross-checked with hard copies after completing cleaning. Collected data were checked for completeness and consistency before coding and entry. The data were cleaned and entered into Epi Info version 7 and subsequently exported to SPSS version 20 for further statistical analysis. For the descriptive component of the study, line-list data were analyzed using Microsoft Excel 2010, and the results were presented through tables, charts, and graphs. Measles and Rubella cases were described according to person, place, and time characteristics. Descriptive statistical methods were employed to summarize the data and generate frequencies, percentages, tables, and graphical presentations.

Bivariate analysis was used to compare the distribution of variables. Multivariate analysis was used to describe the association and to control for confounders and a 95% CI was used to determine the significance of the associations. To assess the association between infection status and potential risk factors, logistic regression analysis was performed using demographic characteristics, previous disease history, and vaccination status as independent variables. Variables with a statistically significant association were identified based on a p-value of less than 0.05. Candidate variables were subsequently included in a multivariable logistic regression model to determine independent predictors of infection while controlling for potential confounding factors. The adjusted analysis was used to identify factors significantly associated with infection after accounting for the effects of other covariates.

4.12 Operational Definition

Clinical measles is defined as fever and a maculopapular rash accompanied by at least one of the following three symptoms: cough, coryza, and conjunctivitis.

Immunization: The process of introducing foreign antigens to the child's body to produce antibodies that help to fight against infectious diseases.

Cases of measles are defined as clinical measles in a person with laboratory-confirmed measles virus infection and/or an epidemiological link to a laboratory-confirmed case.

Suspected: is defined as a case with signs and symptoms consistent with the clinical criteria of measles**.**

Laboratory confirmed: This is defined as a suspected case that meets the laboratory criteria for measles case confirmation.

Epidemiologically linked: is defined as a suspected case that has not been adequately tested by the laboratory and which was in contact with a laboratory-confirmed measles case 7–18 days before the onset of rash.

Endemic is defined as a case resulting from endemic transmission of the virus (i.e., virus present in the territory for ≥12 months) as confirmed by laboratory testing or epidemiological linkage.

Imported is defined as cases with Virological or epidemiological evidence, or both, of exposure outside the region or country during the 7–18 days (for measles) or 12-23 days (for rubella) before rash onset.

Unknown origin: is defined as cases where the origin of infection cannot be determined.

4.13 Ethical Considerations

Ethical clearance for the study was secured from the Institutional Review Board of Addis Ababa University, Department of Medical Laboratory Sciences (Protocol number: DRERC/762/24/MLS). Following this, letters of permission were written to the respective facilities of the Hospital and Health Centers. Permission was obtained from the Ethiopian Public Health Institute, participating facilities, and health offices before starting data collection. The inclusion and exclusion criteria set during the protocol design were strictly respected throughout the study. Patient-based data were collected from the volunteer participants using only codes, and no names were used in connection with the results. All participants were informed about the purpose and expected outcome of the study, and informed assent and consent were obtained. All the information collected through the study was kept confidential, and analysis was carried out without revealing the identity of individuals to ensure privacy.

4.14 Dissemination and Utilization of Results

The findings of this study were presented to the School of Medical Laboratory Science, and the results were disseminated to the concerned body in the study area. The findings were also disseminated to different organizations (governmental and non-governmental) that have contributed to improving and preventing the spread of Measles and Rubella. Findings were presented in different seminars and workshops to disseminate, and they may also be submitted to peer-reviewed journals for possible publication.

5. Results

5.1 Socio-demographic characteristics of the study population

During the study period, 677 *Measles* and *Rubella* suspected participants were recruited, of which 26 participants were excluded from the study; 12 participants were excluded due to unlabeled, mislabeled, and mismatched data, 9 participants were excluded due to delayed transport of the sample, and 5 participants were excluded due to gross hemolysis or lipemic serum samples, leaving 651 participants for further analysis (**Figure 1**).

A total of 651 children less than 15 years of age were included in the final analysis, with a median age of 5 (IQR 3-8) years. Of these, 335 (51.3%) were female, and 420 (64.5%) were urban dwellers. In addition, 360 (55.2%) had a recorded history of previous infection, 365 (60.5%) had a history of contact with symptomatic cases, 379 (59.2%) were vaccinated with MCV (MR vaccine), and 171 (31.3%) participants had received a single dose of vitamin A supplement within the past six months (**Table 1**). Children's primary guardian: 196 (30.1%) of them had no formal education, 284(43.6%) were farmers, 250(38.4%) were unmarried, 296(45.5%) had no history of postnatal care visits, and 447 (68.7%) had distrust of the immunization program (**Table 1**).

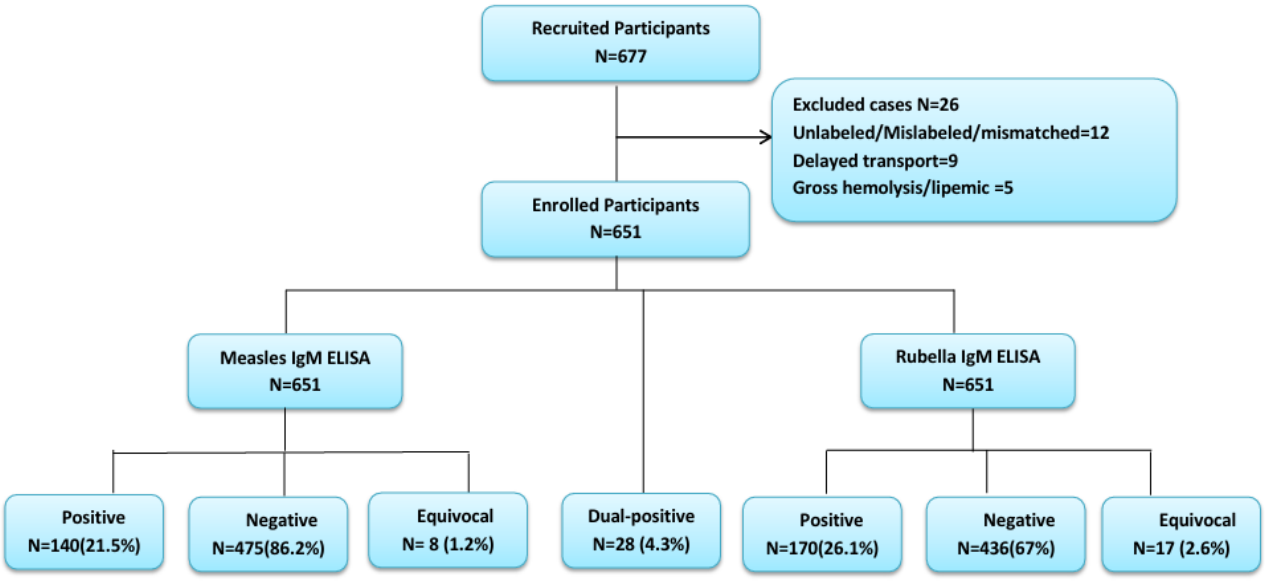


Figure 1: *Measles* and *Rubella* cases identified among children <15 years attending selected health facilities in Ethiopia

Table 1: Socio-demographic and clinical characteristics of participants

Characteristics		**Frequency (%) n= 651**
Gender	Female	335 (51.3)
	Male	316 (48.7)
Age, Years	Median (IQR)	5(3–8)
	<1	44 (6.7)
	1–4	234 (35.9)
	5-8	262 (40.2)
	9-12	82 (12.6)
	13-15	29 (4.4)
Residence	Urban	420(64.5)
	Rural	231(35.5)
Educational status of the primary guardian	No formal education	208 (31.9)
	Primary (1-8)	316 (48.5)
	Secondary (9-12)	88 (13.5)
	Higher education	39(6)
Occupation of primary guardian	House wife	196(30.1)
	Farmer	284(43.6)
	Government employee	42(6.4)
	Private employee	129(19.8)
Marital status of the primary guardian	Unmarried	250(38.4)
	Married	349(53.6)
	Divorced/Widowed	52(8)
History of previous infection	Yes	360(55.2)
	No	291(44.7)
History of contact with symptomatic cases	Yes	395(60.7)
	No	256(38.3)

Vaccination	Yes	379(59.2)
	No	120(18.4)
	Unknown	152(23.5)
History of vitamin A supplementation	Yes	173(31.3)
	No	285(51.7)
	Unknown	193(35)
Had a postnatal care visit	Yes	355(54.5)
	No	296(45.5)
Distrust immunization programs	Yes	204(31.3)
	No	447(68.7)

5.2 Clinical manifestations and confirmed cases by days following symptom onset

The included participants had one or more clinical signs and symptoms; maculopapular rash was noted in 623(95.7%) of participants, followed by cough in 471 (72.5%), and fever in 446 (68.3%). Koplik spots were found in 227 (34.9%) of participants, while only 167(25.6%) of participants had lymphadenopathy. The remaining symptoms, coryza, conjunctivitis, and arthralgia, were noted with the lowest frequency (**Figure 2**).

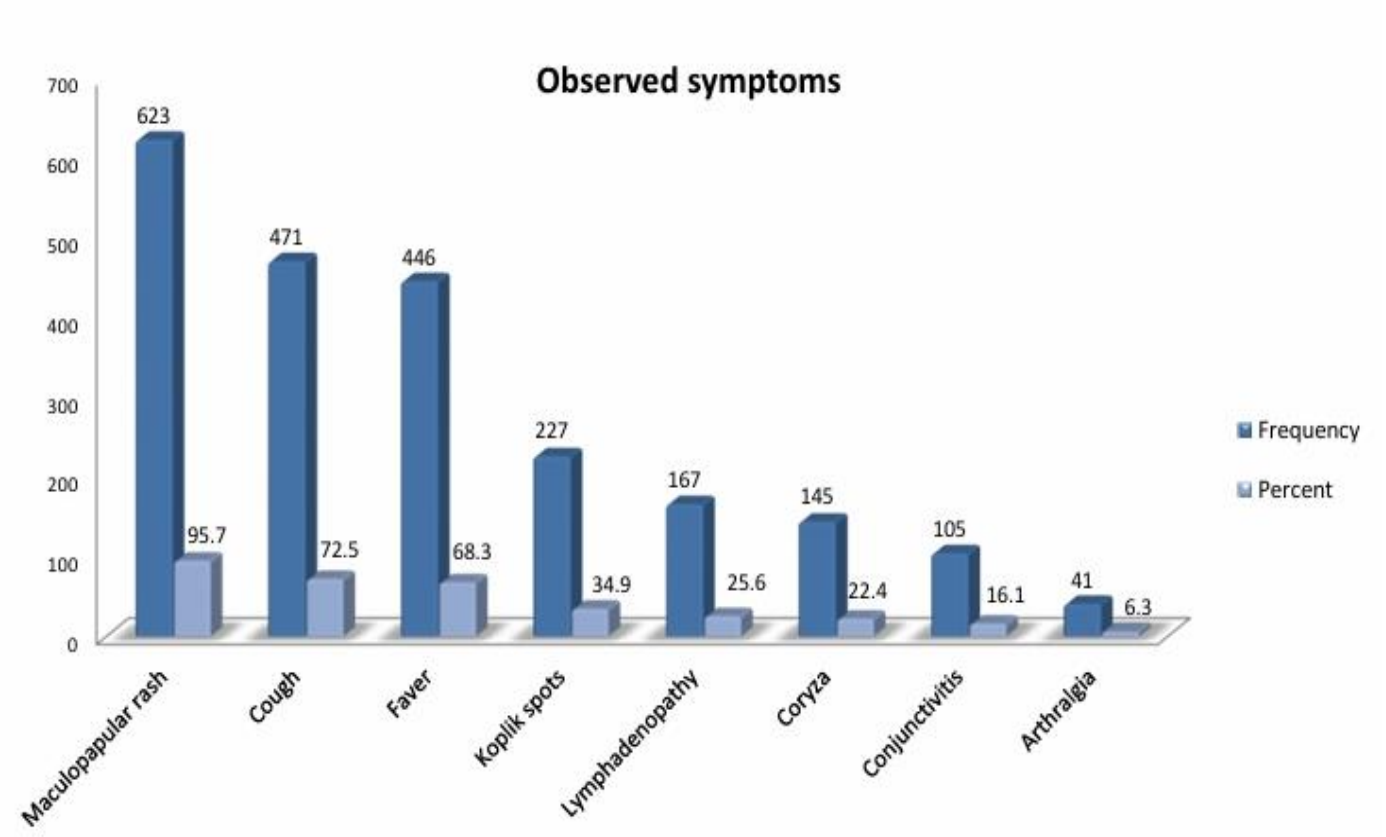


Figure 2: Proportions of individual symptoms among *Measles and Rubella*-suspected children <15 years attending selected health facilities in Ethiopia.

Measles and *Rubella* positivity rate correlated with the peak IgM concentration and negativity with the IgM concentration decline. Among suspected participants, 318 (94%) had confirmed *measles*, and *Rubella* cases were detected with in fourth to nineteen days after symptom onset. The proportion of IgM ELISA-confirmed cases increased from 5 (1.5%) on the third day to 31 (9%) on the fifth day after symptom onset. The positivity rate increased to a maximum level approximately at 6–15 days after the onset of symptoms and then began to decrease until reaching its minimum of 4(1.1%) at 21–25 days (**Figure 3**).

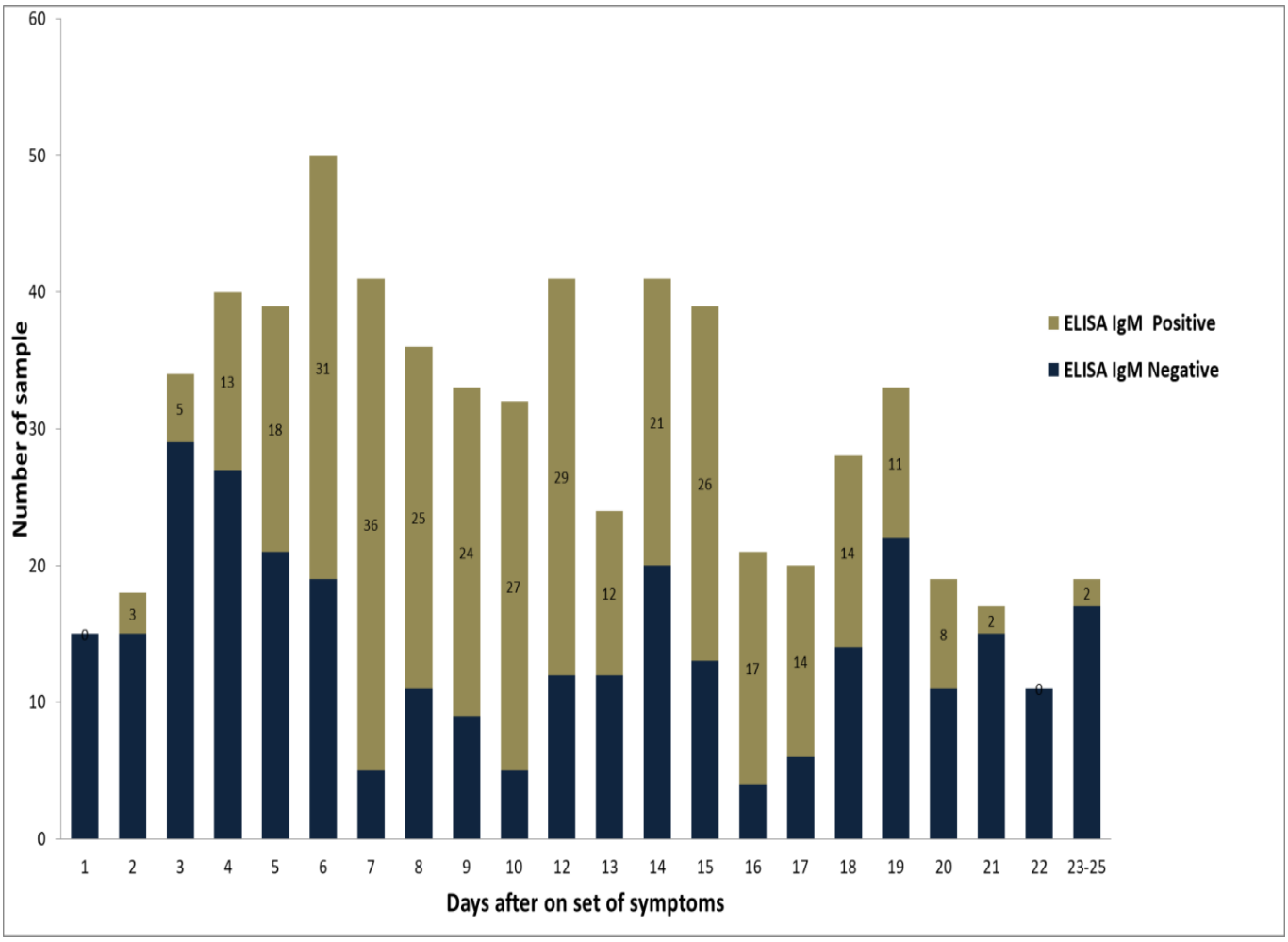


Figure 3: Proportion of IgM ELISA confirmed cases with days after onset of symptoms attending selected health facilities in Ethiopia

5.3 Prevalence of Measles and Rubella

Overall, 338 (61.3%) cases were detected among the 651 MR-suspected participants. Of the total positive cases, 140 (41.4 %) of the study participants were positive for anti-Measles IgM only, 170 (50.3%) positive for *Rubella* IgM only, while the remaining 28 (4.3%) were found to be positive for both *Measles* and *Rubella*. The higher proportion of *Measles* cases, 50 (14.8%), was identified in the 1-4 years age group, 71 (21%) among females, and 83 (24.5%) among urban dwellers. Based on clinical history, the proportions of identified *Measles* cases among previously confirmed cases and those who had contact with a known MR patient were 60 (17.7%) and 82 (24.3%), respectively. The higher proportion of *Rubella* cases, 76 (22.5%), was identified in the 5-8 years age group compared to other groups. Ninety-three (27.5%) were female, and 109 (32.2%) were urban residents. Based on clinical history, the proportions of identified *Rubella* cases among previously confirmed cases and those who had contact with a known MR patient were 95 (28.1%) and 116 (34.3%), respectively (**Figure 1& Table 2**).

Table2: Distribution of confirmed *Measles* and *Rubella* cases by selected demographic characteristics of children from selected facilities of Ethiopia.

Variable	Confirmed Measles cases n (%)	Confirmed Rubella cases n (%)	Co-infected cases n (%)
Gender	N=338		
Male	69 (20.4)	77 (22.8)	16 (4.7)
Female	71 (21)	93 (27.5)	12 (3.5)
Age, Years	N=338		
<1	16 (4.7)	5 (1.5)	6 (1.8)
1–4	50 (14.8)	60 (17.7)	8 (2.4)
5-8	42 (12.4)	76 (22.5)	10 (2.9)
9-12	22 (6.5)	24 (7.1)	3 (0.9)
13-15	10 (2.9)	5 (1.5)	1 (0.3)
Residence	N=338		
Rural	57 (16.9)	61 (18)	16 (4.7)
Urban	83 (24.5)	109 (32.2)	12 (3.5)
Previous infection	N=338		
Yes	60 (17.7)	95 (28.1)	11 (3.2)
No	80 (23.7)	75 (22.2)	17 (5)
Contact cases	N=337		
Yes	82 (24.3)	116 (34.3)	20 (5.9)
No	57 (16.9)	54 (16)	8 (2.4)

5.4 Proportion of mixed infections

A total of 28 (4.3%) participants were coinfected with both *Rubella* and *Measles*. The higher MR prevalence was found in males (16, 4.7%), those in the 5–8 years age group (10,2.9%), those living in rural areas (16,4.7%), those without a history of MR infection (17, 5%), and those who had contact with a known MR patient (20,5.9%) **(Table 2**).

5.5 Age-specific prevalence of Rubella and Measles infections

Overall age-specific prevalence ranges between 20% and 60%. A higher frequency of 60% was seen at the age of one year, followed by 55% at fourteen years and 54% at five years of age. Sero-positivity reduced by less than 20% or by two-thirds at the ages of 11 and 13 years (**Figure 4**).

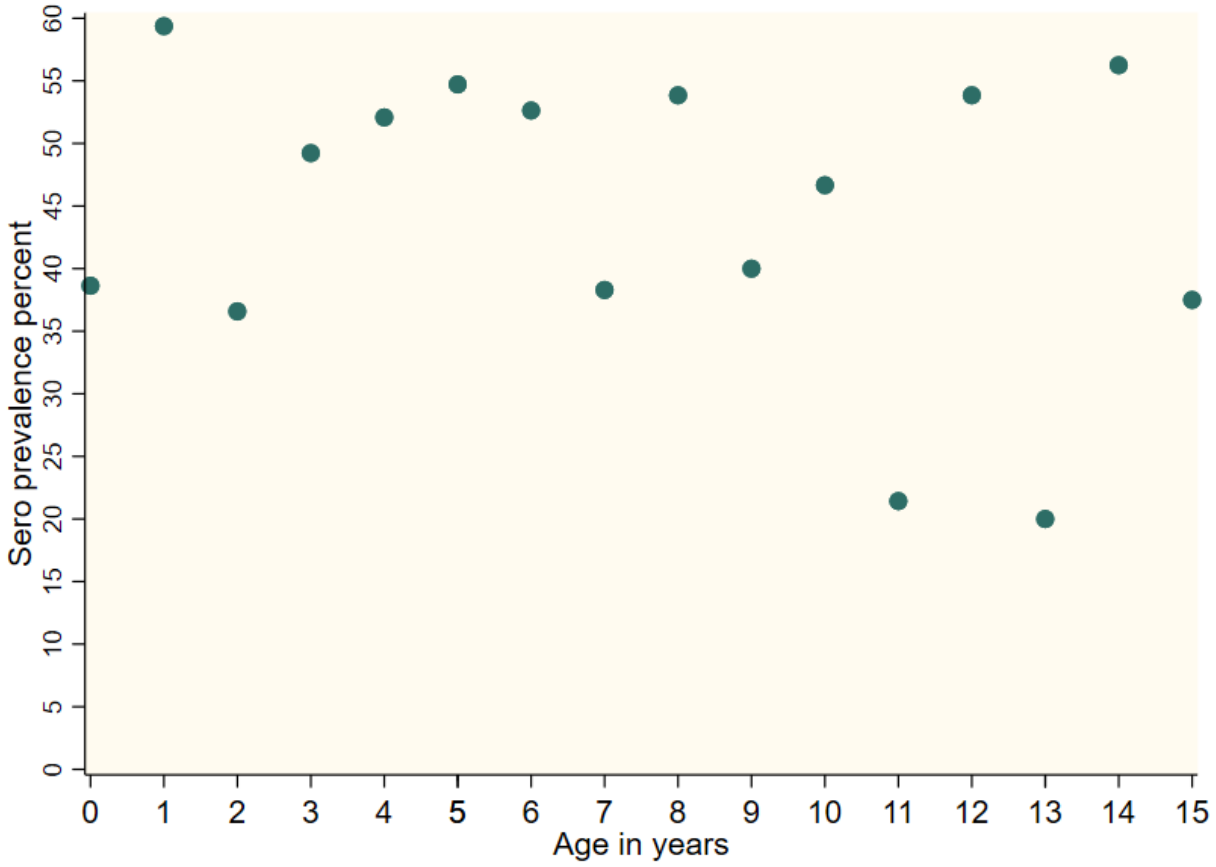


Figure 4: Age-specific prevalence of *Measles* and *Rubella* among children attending selected health facilities in Ethiopia.

5.6 Factors associated with Rubella and Measles infections

To identify independently associated factors, a bi-variable logistic regression model was used for all variables that met the assumptions. Variables having a P-value < 0.05 in the bi-variable analysis were then subjected to a multivariable logistic regression model. The adjusted model revealed a statistically significant association between testing positive and a number of factors, such as living in a rural area, having a previous infection, having contact with symptomatic individuals, missing vaccinations, not attending postnatal care visits, and lack of trust in immunization programs.

The findings show that the category of children living in rural areas had 1.46 (95%CI =1.05-2.02) times the odds of positivity compared to children living in urban areas. In addition, contact from symptomatic cases, children missing vaccinations, and lack of trust in immunization programs had 1.33 (95%CI =1.17-1.84), 3.16 (95%CI =2.04-5.72), and 1.39 (95%CI =1.09-1.96) times the odds of developing *Measles* and *Rubella* diseases compared to their counterparts, respectively. In contrast, mothers attending postnatal care visits and a previous history of infection had 0.63 (95%CI =0.45-0.89) and 0.57 (95%CI =0.42-0.79) times the odds of protective association for *Measles* and *Rubella* infection (**Table3 and Table4**).

Table 3: Univariate analyses of factors associated with *Measles* and *Rubella* incidence

Characteristics	**Positive n (%)**	**Negative n (%)**	**COR (95% CI)**	**P-Value**
Sex	N= 338	N=313		
Male	162 (47.9)	173 (55.3)	1	0.061
Female	176 (52.1)	140 (44.7)	1.34 (0.99–1.83)	
Age, Years	N=338	N=313		
<1	27 (7.9)	17 (5.4)	1	
1–4	118 (34.9)	116 (37.1)	0.64 (0.33-1.24)	0.185
5-8	128 (37.9)	134 (42.8)	0.60 (0.31-1.16)	0.127
9-12	49 (14.5)	33 (10.5)	0.93 (0.44-1.98)	0.86
13-15	16 (4.7)	13 (4.1)	0.77 (0.30-2.01)	0.599
Residence	N=338	N=313		
Urban	204 (60.4)	216 (69)	1	
Rural	134 (39.6)	97 (31)	1.46 (1.06-2.02)	0.022
Previous infection	N=338	N=313		
Yes	166 (49.1)	194 (62)	0.6 (0.44 – 0.82)	
No	172 (50.9)	119 (38)	1	0.001
History of contact with symptomatic cases	N-=337	N=313		
Yes	218 (64.7)	177 (56.5)	1.41(1.02 – 1.92)	
No	119 (35.3)	136 (43.5)	1	0.037
Vaccination status	N=266	N=233		
Vaccinated	171 (64.3)	208 (89.3)	1	
Not Vaccinated	95 (35.7)	25 (10.7)	4.62(2.85-7.51)	0.002
History of vitamin A supplementation	N=239	N=219		
Yes	70 (29.3)	103 (47)	0.47(0.32 – 0.70)	<0.001
No	169 (70.7)	116 (53)	1	
Had a postnatal care visit	N=337	N=314		

Yes	174 (51.6)	181 (57.6	0.94 (0.43–1.27)	0.001
No	163 (48.4)	133 (42.4)	1	
Distrust in immunization programs	N=338	N=313		
Yes	119 (35.2)	85 (27.2)	1.46(1.03 – 2.06)	0.032
No	219 (64.8)	228 (72.8)	1	

Table 4: Multivariate analysis showing factors associated with *Measles* and *Rubella* Incidence

Associated Factor	Adjusted Odds Ratio	95%CI		P-value
		Lower	Upper	
Residence	1.46	1.05	2.02	0.023
Previous history of Measles or Rubella infection	0.57	0.42	0.79	0.001
History of contact with symptomatic cases	1.33	1.17	1.84	0.04
Vaccination status	3.16	2.04	5.72	0.001
Vitamin A supplementation	0.92	0.81	1.04	0.183
Postnatal care visit	0.63	0.45	0.89	0.009
Distrust in immunization programs	1.39	1.09	1.96	0.048

6. Discussion

The purpose of a successful *Measles-Rubella* control program is to prevent the virus from spreading. To achieve this, a coordinated public health response, immunization, and a robust surveillance system with improved laboratory-based confirmation of suspected cases are becoming increasingly important. *Measles* or *Rubella*-specific immunoglobulin M (IgM) ELISA serology is the current accepted technique for the rapid laboratory identification of *Measles* and *Rubella* viruses. This study aimed to determine the epidemiology and laboratory-based surveillance of *Measles* and *Rubella* infection in children under the age of 15 years. In the present study, 338 (51.9%) of *Measles* and *Rubella* cases were detected; of these, 41.4% were *Measles* cases, and 50.3% were *Rubella* cases. These findings are lower than a meta-analysis study, which identified a pooled prevalence of *Measles*-confirmed cases in Ethiopia of 67.6% among suspected cases [40] and 60% in Nigeria [41]. However, the finding is higher than outbreak investigations conducted in the Somali region, which reported 33% for measles, and in South Sudan, which reported 34% for measles and 33% for rubella [42,43]. The possible reasons for these findings could be suboptimal vaccination coverage, delays to routine immunization services, internal conflicts, internal displacement, facility closures, and vaccine shortages, which may be the likely reason for Ethiopia's high *Measles* and *Rubella* prevalence.

The findings of this study indicate a higher prevalence of *Rubella* compared to *Measles* among suspected cases. These findings are consistent with earlier evidence from Uganda, where *Rubella* IgM positivity (23%) exceeded that of *Measles* (12.5%), and another comparable finding from the Central African Republic showed that 29.7% of cases were laboratory-confirmed as *Measles*, and an additional 35.9% were positive for *Rubella* from patients with *Measles* IgM-negative and equivocal samples [44,45]. The likely reason for these findings could be that the surveillance system is solely developed for *Measles* and is generally examined for *Rubella* when the patients are negative for *Measles*, the clinical manifestations are generally subclinical, or symptoms are mild and often go unnoticed, contributing to increased transmission, and the *Rubella* conjugated vaccine was introduced later.

The study demonstrated that a substantial proportion of *Measles* and *Rubella* coinfection was detected among suspected symptomatic cases. The observed coinfection rate was 4.3%, indicating simultaneous circulation of both viruses in the population. The coinfection proportions

have been reported in Asia, with rates ranging from 1% to 5% in studies conducted in China and India [46, 47]. Indicating that such findings reflect a broader regional pattern in settings where *Measles and Rubella* remain endemic or occasionally epidemic. The detection of dual positivity may represent true coinfection. However, it may also be influenced by antibody cross-reactivity [48]. The indirect ELISA-based IgM detection used in this study has improved diagnostic accuracy by using disease-specific antigens and reducing the possibility of non-specific binding through several washing processes. The concordant findings from studies using both ELISA and nucleic acid–based assays support the validity of true coinfection [46]. From a public health perspective, even a 4.3% coinfection rate is important because it highlights ongoing viral transmission and possible outbreak activity. This underscores the need to strengthen routine immunization with the combined *Measles–Rubella* vaccine and improve surveillance sensitivity.

Our findings showed that 59.2% of children had been vaccinated; unvaccinated children had a threefold higher risk of being positive than vaccinated children did. In Ethiopia, despite the availability of a combined *Rubella* and *Measles* vaccination program, which is carried out in three ways: first, for available children aged 9 months for the first dose and 15 months for the second dose, secondly, through periodic campaigns for children under the age of 14 years, and thirdly, through case-based surveillance for pregnant women during CRS monitoring [49]. In Ethiopia, studies have reported low vaccine coverage, with first-dose (MCV1) and second-dose (MCV2) coverage estimated at 61% and 53%, respectively [49]. The proportion is also lower in conflict-affected areas, internally displaced groups, difficult-to-reach rural populations, pastoralist populations, developing regions, and refugee camps [49, 50]. Therefore, expanding access to vaccination for underserved populations, strengthening existing policy, improving immunization programs, and achieving greater than 95% coverage in high-burden countries remains critical for achieving national and global measles control targets.

The results of our study showed that the age-specific distribution was higher in children aged less than one year. Similar findings from Vietnam show that in a measles outbreak, 50% of the infections occurred in babies younger than 9 months [51]. Several studies also reveal that most newborns have protective antibodies at birth, which begin to decline after 2 months and may fall below the protective threshold by 4 to 6 months [52, 53]. Furthermore, beyond six months, most

infants lack protective antibodies, and protective antibody titers fall below protective thresholds, making the newborn susceptible to infection.

In this study, we found that participants who had a previous history of infection were significantly less likely to develop the disease compared with those with no previous infection. Previous studies have shown that naturally acquired antibodies can survive longer after infection than vaccine-induced antibodies [54]. This link could be attributed to the establishment of immunity upon subsequent natural infection with the same pathogen; memory cells generate a more potent and quicker secondary immune response. However, in LMIC settings, malnutrition and immunological deficiencies may limit the efficacy of this protection.

According to our findings, individuals who had a history of contact with symptomatic cases were 1.33 times more likely to get *Measles* and *Rubella* infection than those who had no history of contact during that time. The results of this study are consistent with various studies conducted in Ethiopia, Indonesia, and Kenya [55–57]. Studies have shown that people who had previously come into contact with *Measles* cases in their village, home, or school were more likely to contract the disease than people who did not. The risk of a susceptible contact contracting *Rubella* and *Measles* was 50% and 90%, respectively [56, 57].

This study showed that children whose mothers were not attending postnatal care visits had greater odds of being infected with *Measles* and *Rubella*. This outcome is consistent with a study conducted in Ethiopia, where mothers who did not attend postnatal care had 3.4 times higher odds of being infected with *Measles* and *Rubella* compared with those who attended postnatal care in 2024, which demonstrates that maternal awareness of vaccination practices and their utilization of health services are important factors influencing the completion of vaccinations [58]. In Ethiopia, despite improvements in maternal and child care (MCH) services, deliveries in medical facilities only reached 64% in 2025, while postnatal care (PNC) visits only reached 36% in the same year [59]. This demonstrates significant gaps in MCH services and the ongoing work needed to improve these. We emphasize the value of ANC and PNC visits, which provide possibilities for routine immunization programs and health education.

Another risk factor identified by our analysis was a lack of trust regarding immunization initiatives. According to studies, digital claims, false information, religious concerns, and fear of adverse effects are among the potential causes [60]. The results reported highlight the attitudes and beliefs of mothers or primary guardians toward immunizations. In order to create an atmosphere that is conducive to immunization, public health interventions and raising awareness about the significance of vaccination should be based on education programs and community mobilization.

7. Strengths and Limitations of the Study

Limitation

The study has several limitations. First, we are unable to see possible follow-up clinical features and the outcome of the patients infected with *Measles* and *Rubella*, due to a lack of adequate resources. Second, not all patients had complete immunization records. Third, mothers were not included in the study. Fourth, the participants were collected from selected facilities; the results may not be applied to the entire country.

Strength

The results of this study have been strengthened by the large and representative sample size, the availability of clinical data, the reporting of the coinfection rate, which is usually underreported, and the quality of the laboratory data (all tests were carried out at a laboratory recognized as a center of excellence by WHO).

8. Conclusion

This study reported a high prevalence of *Measles* and *Rubella* in Ethiopian children under the age of fifteen years. For both infections, the prevalence was 4.3% of coinfection cases. This study found a greater prevalence of *Rubella* than *Measles* because our surveillance system is solely developed for *Measles* and is generally examined for *Rubella* when it is negative for Measles. Age-specific distribution was higher in children aged less than one year. Among suspected participants, confirmed *Measles* and *Rubella* cases were detected six to fifteen days after symptom onset.

Missing vaccines and contact with measles cases were among the primary factors of the incident. Potential contributing factors to this disease include failing to attend postnatal care visits, having a previous infection, and lacking trust in immunization programs.

9. Recommendation

- The increase in prevalence calls for enhancing surveillance and laboratory diagnostic capacities.
- The incidence of rubella infection is underestimated globally due to insufficient surveillance, reporting systems, and the subclinical nature of the disease; thus, we recommend an effective disease-specific surveillance system.
- A vaccine-preventable disease requiring herd immunity reached by having a coverage of greater than 95% at all levels; therefore, an urgent vaccination campaign with health education interventions is highly recommended.
- Adequately managing cases, initiating a selective vaccination campaign for vulnerable groups, and reinforcing the routine EPI program.
- Identify hot spot areas and assess the risk of a large outbreak.

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11. Annex

Annex I: Questionnaire for Data Collection: Magnitude of *Measles* and *Rubella* among Children

Q.NO	Questions	Responses	Skips Pattern
1	Full name of the interviewer	_____	
2	The phone number of the interviewer		
3	Date of interview (EC)	_____/_____/_____ DD/MM/YYYY	
4	Name of Diagnostic Center:	_____	
5	Patient Reg. No. (Medical Record No)		
6	Unique Identification Number of the patient:	☐☐☐☐ (Facility Code + Patient Sequential No.)	
7	Date of Diagnosis (EC)	_____/_____/_____ DD/MM/YYYY	
8	Age	☐☐ Years	
10	Sex	Male……..…1 Female ……...…….2	
9. Current Patient	A.13.1Region		

Address (a *place where the patient has lived for at least 6 months*)	A.13.2 Zone/Sub-city		
	A.13.3 Woreda		
	A.13.4 Kebele		
	Patient contact		
10	Residence	Rural……..…1 Urban ……...…….2	

11	For how long has your child been sick with current symptoms (in days)	☐☐ days
12	Did the child have the same symptoms before the current illness?	Yes…….1 No ..……..…2 Unknown….……....3
13	Any Chronic illness in the child?	1) Yes 2) No. If Yes, specify _____
14	Has your child had any of these symptoms within the past week?	**Yes** **No** Malaise …..…….1 2 Skin rash…….…….1 2 Fever.…….……..….1 2 Joint Pain…….…….…....1 2 Other Specify…….…….....1 2

		_____
15	Have you ever heard of rubella infection?	Yes……..….1 No ..……2
16	Have you ever heard of a measles infection?	Yes……..….1 No ..……2
17	Have you vaccinated your child against Rubella and Measles?	Yes……..….1 No..…….2
18	If yes to question 18	Where …….. When ……
19	Immunization Status:	1) Immunized with a single dose of vaccine 2) Immunized with two doses of the vaccine 3) Not Immunized

20	Have you ever vaccinated your children against rubella?	Yes …… No …….
21	If yes to question 21	□ Where …….. □ When ……
22	Childhood residence	□ Rural □ Urban
23	Clinical sign and symptom	1)Fever 2) Cough 3) Coryza 4) Conjunctivitis 5) Day of onset of rash 1) Day 1 2) Day 2 3) Day 3 4) other Specify_____ 6) Diarrhoea 7) Seizures 8) Others (specify)_____

Q.NO	Questions	Responses	Skips Pattern
1	Full name of the interviewer	_____	
2	The phone number of the interviewer		
3	Date of interview (EC)	_____/_____/_____ DD/MM/YYYY	
4	Name of Diagnostic Center:	_____	
5	Patient Reg. No. (Medical Record No)		
6	Unique Identification Number of the patient:	☐☐☐☐ (Facility Code + Patient Sequential No.)	
7	Date of Diagnosis (EC)	_____/_____/_____ DD/MM/YYYY	
8	Gestational age of pregnancy Age (Weeks)	☐☐ weeks	
9. Current Patient Address (a *place where the patient has lived for at least 6 months*)	A.13.1Region		
	A.13.2 Zone/Sub-city		
	A.13.3 Woreda		
	A.13.4 Kebele		
	Patient contact		

10	Residence	Rural……1 Urban ……2	
11	Marital status	1. Unmarried 2. Married 3. Divorced 4. Widowed	

12	Educational Status of the Client	Primary ……...1 Secondary ……..…2 others …….…….....3
13	For how long have you been sick with your current symptoms (in days)	☐☐ Weeks
14	Did you have the same symptoms before your current illness?	Yes…….1 No ..……..…2 Unknown….……....3
15	Have you ever had a	☐ Miscarriage Yes (how many)……. No ☐ Stillbirth Yes (how many)…… No ☐ Preterm delivery Yes (how many) …...No ☐ Abnormal/malformed child? Yes (how many) …….No
16	Have you had any of these symptoms within 3 months of getting pregnant or in this pregnancy?	**Yes** **No** Malaise …..…….1 2 Skin rash…….…….1 2 Fever.…….…….…1 2 Joint Pain…….…….....1 2 Other Specify…….…….....1 2 _____ _____

17	Have you ever heard of rubella infection?	Yes……..….1 No ..……2 Unknown……....3
18	Have you ever been vaccinated against rubella?	Yes……..….1 No..…….2
19	If yes to question 18	Where …….. When ……
20	Have you ever vaccinated your children against rubella?	Yes …… No …….
21	Have you ever vaccinated your children against rubella?	Yes …… No …….
22	If yes to question 21	□ Where …….. □ When ……
23	Childhood residence	□ Rural □ Urban
24	Current residence	□ Rural □ Urban

Annex III. Consent form for Parents/Guardians of children, English version

Title of the study: Magnitude and **laboratory-based surveillance of *Measles* and *Rubella* virus infection in Ethiopia**

Name of the investigator: Rahel Fekade

Name of the Participant:_____

Hospital number:_____

Good morning/afternoon/evening.

I am the site focal person for a study conducted by the Ethiopian Public Health Institute. This study will be conducted at selected health facilities in the country. The study aims to improve the national *Measles* and *Rubella* prevention and control in Ethiopia.

Study Procedure：This study will be conducted by taking your blood specimen. If you are willing to participate in the study, we will test your blood in the laboratory. You will receive treatment based on the findings.

Risks and benefits of participating in the study

An estimated 20-25-minute interview will be conducted for you. There is no risk you face due to the interview. The results of this study will be used to improve MV& RV diagnosis and treatment in the country.

Confidentiality

The information you give me and your laboratory analysis results will be kept confidential. Personal identifying information will not be shared outside of the study.

The right to refuse or stop participation at any time

Participation in this study is solely based on your willingness. If you are not willing to participate in this study, you can withdraw at any time.

I have read and understood this consent form and the information provided to me regarding participation in the study. I have been informed about the nature of the study. I am aware that if I have any questions during this study, I should contact the investigator.

By signing this consent form:

Name and signature/thumb impression of the participant /parents/guardian

Name _____ Signature_____ Date_____

Annex VI.Consent form for Parents/Guardians of children Afan-Oromo version

Mata duree qorannichaa: **hordoffii laabraatoorii bu'uura godhachuun infekshinii vaayirasii mezili fi ruubeelaa Itoophiyaa keessatti**

Maqaa qorataa: Rahel Fekade

Maqaa Hirmaataa:_____ .

Lakkoofsa hospitaalaa:_____

Nagaa ganama/waaree/galgala.

Ani qorannoo dhaabbata fayyaa hawaasaa Itoophiyaatiin gaggeeffameef site focal person dha, qorannoon kun dhaabbilee fayyaa biyyattii filataman irratti kan gaggeeffamu ta'a. Qorannichi Ityoophiyaa keessatti ittisaa fi to'annoo mizili fi ruubeelaa biyyoolessaa fooyyessuuf kan kaayyeffate ta'uu ibsameera.

Adeemsa Qo'annoo：qorannoon kun saamuda dhiiga keessanii fudhachuun kan gaggeeffamu ta'a. Qorannicha irratti hirmaachuuf fedhii yoo qabaattan, argannoo irratti hundaa'uun laaboraatoorii yaala argattan keessatti dhiiga keessan ni qoranna.

Balaa fi faayidaa qorannicha irratti hirmaachuu

Tilmaamni daqiiqaa 20-25 gaaffii fi deebii isiniif ni gaggeeffama. Sababa interview kanaan balaan isin mudatu hin jiru. Bu'aan qorannoo kanaa biyyattii keessatti qorannoo fi yaala mizili fi ruubeelaa fooyyessuuf kan oolu ta'a.

Iccitii eeguu

Odeeffannoon isin naaf kennitan fi bu'aan xiinxala laabraatoorii keessanii iccitii ta'ee ni eegama. Odeeffannoon dhuunfaa adda baasu qorannichaan alatti hin qoodamu.

Mirga yeroo kamiyyuu hirmaannaa diduu ykn dhaabuu

Qo'annoo kana irratti hirmaachuun fedhii kee qofa irratti hundaa'a. Yoo qorannoo kana irratti hirmaachuuf fedhii hin qabne yeroo barbaaddetti ofirraa baasuu dandeessa.

Unka hayyamaa kanaa fi odeeffannoo hirmaannaa qorannichaa ilaalchisee naaf kenname dubbisee hubadheera. Waa'ee maalummaa qorannichaa naaf ibsameera. Yeroo qorannoo kanaa gaaffii yoo qabaadhe qorataa qunnamuu akkan qabu nan beeka.

Unka hayyamaa kana mallatteessuudhaan:

Maqaa fi mallattoo / qubbee harkaa hirmaataa /warra/guddistuu

Maqaa _____

Mallattoo_____

Guyyaa_____ .

Annex V. Consent form for Parents/Guardians of children, Amharic version

የጥናቱ ርዕስ፡ **በኢትዮጵያ የኩፍኝ ቫይረስ ኢንፌክሽን እና በቤተ ሙከራ ላይ የተመሰረተ ክትትል**

የመርማሪው ስም፡- ራሄል ፈቃደ

የተሳታፊው ስም፡_____

የሆስፒታል ቁጥር፡_____

ሰላም ኖት

በኢትዮጵያ የህብረተሰብ ጤና ኢንስቲትዩት ለሚካሄደው ጥናት ሳይት ፎካል ሰው ነኝ ይህ ጥናት የሚካሄደው በሀገሪቱ በሚገኙ የጤና ተቋማት ነው። ጥናቱ በኢትዮጵያ ያለውን ብሔራዊ ኩፍኝ እና ኩፍኝ መከላከልና መቆጣጠርን ለማሻሻል ያለመ ነው።

የጥናት ሂደት ይህ ጥናት የሚካሄደው የደምህን ናሙና በመውሰድ ነው። በጥናቱ ለመሳተፍ ፍቃደኛ ከሆናችሁ ደምዎን በማግኘት ላይ በመመስረት ህክምና በሚያገኙበት ላቦራቶሪ ውስጥ እንፈትሻለን።

በጥናቱ ውስጥ የመሳተፍ አደጋዎች እና ጥቅሞች

ከ20-25 ደቂቃ የሚገመት ቃለ መጠይቅ ይደረግልሃል። በቃለ መጠይቁ ምክንያት የሚያጋጥሙዎት ምንም አይነት ስጋት የለም። የዚህ ጥናት ውጤት የ ምርመራ እና ህክምናን በሀገሪቱ ለማሻሻል ጥቅም ላይ ይውላል።

ሚስጥራዊነት

ለእኔ የምትሰጠኝ መረጃ እና የላብራቶሪ ትንታኔህ ውጤት በሚስጥር ይጠበቃል። የግል መለያ መረጃ ከጥናቱ ውጭ አይጋራም።

በማንኛውም ጊዜ ተሳትፎን አለመቀበል ወይም ማቆም መብቶች

በዚህ ጥናት ውስጥ መሳተፍ በእርስዎ ፍላጎት ላይ ብቻ የተመሰረተ ነው። በዚህ ጥናት ለመሳተፍ ፈቃደኛ ካልሆኑ በማንኛውም ጊዜ ማቋረጥ ይችላሉ።

ይህንን የስምምነት ቅጽ እና በጥናቱ ውስጥ ተሳትፎን በተመለከተ የተሰጠኝን መረጃ አንብቤ ተረድቻለሁ። ስለ ጥናቱ ተፈጥሮ ተብራርቻለሁ. በዚህ ጥናት ወቅት ማንኛውም አይነት ጥያቄ ካለኝ መርማሪውን ማነጋገር እንዳለብኝ አውቃለሁ።

የተሳታፊው/ወላጆች/አሳዳጊው ስም እና ፊርማ/አውራ ጣት ፊርማ

ስም _____

ፊርማ_____

ቀን_____

Annex VI: Standard Operating Procedures Measles ELISA detection:

Principle: Enzyme-Immuno Assay for the detection of human IgM antibodies to measles virus in serum and plasma.

The RF absorbent binds to the IgG present in the test sample. Any rheumatoid factor in

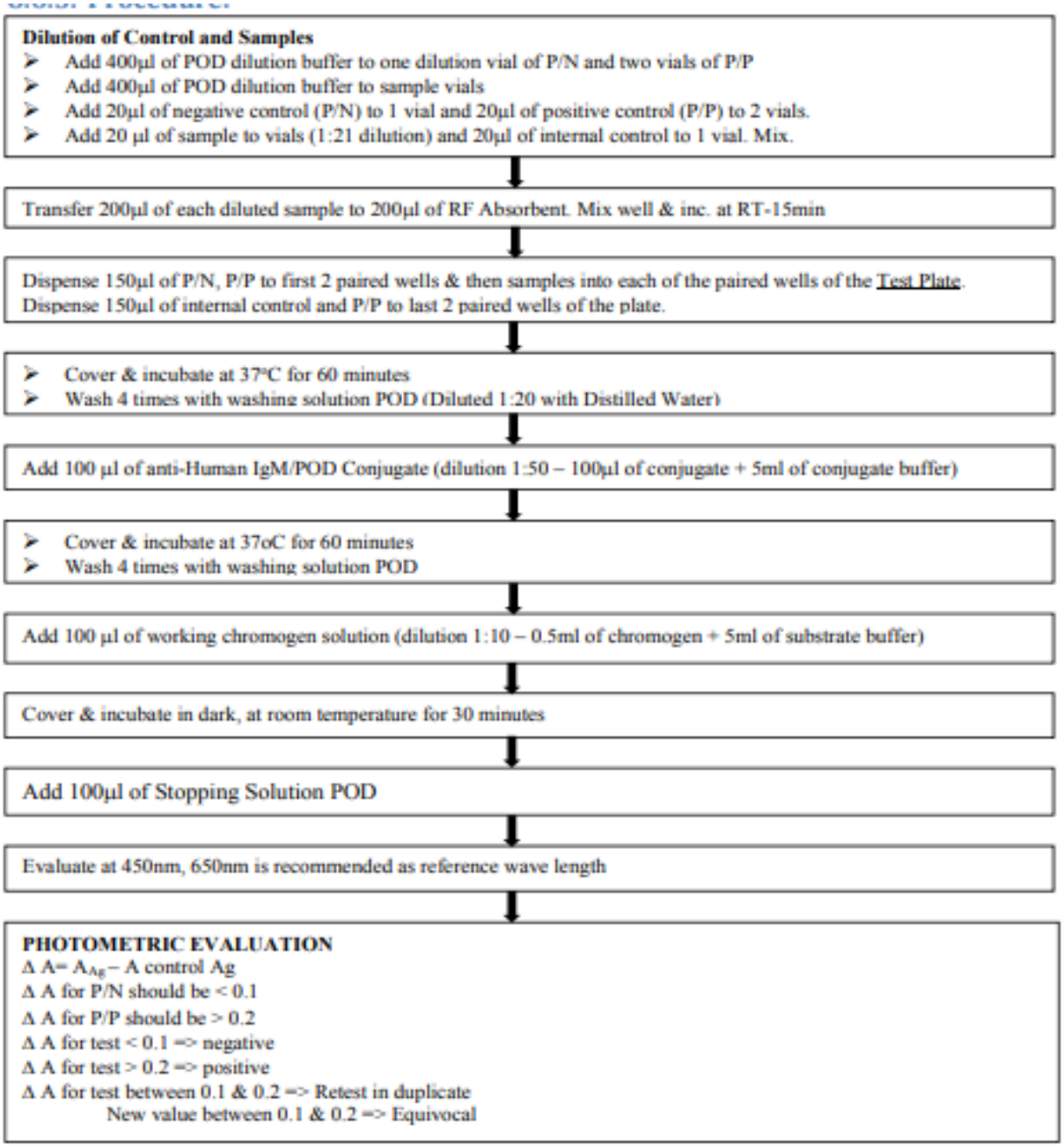
the sample binds to the resulting immune complexes and is thus eliminated. The RF absorbent

precipitates up to 15mg IgG/ml, and this enhances the sensitivity of the IgM test. IgM in the test sample, which is specific for the virus, binds to the virus antigen on the plastic surface of the test plate. The anti-human IgM/POD conjugate binds to this complex. The enzyme component of the conjugate catalyzes the working chromogen solution, producing a blue color. This reaction is terminated by the addition of the stopping solution POD, and the yellow colour is formed, which is read at 450nm. IgM directed against the cellular antigen is measured in the same way in the well, with a control antigen. The difference between the color intensities in the well coated with antigen and in the well coated with control antigen is the measure of the concentration and immunochemical reactivity of the virus antibodies detected in the sample.

Interpretation of results:

- A NEGATIVE result means that virus-specific IgM cannot be detected. The patient either is NOT acutely infected with the measles virus or, if infected or vaccinated, is still unable to produce IgM specific for the virus. If exposure to the virus is suspected despite a negative finding, a second sample should be collected no less than 7 days later and should be tested together with the first sample. If a sample is
- POSITIVE indicates that virus-specific IgM has been detected. This is proof of a recent infection. In over half the measles patients, anti-measles IgM is detectable on the first day of the rash itself and can be detected for about 3 months.
- If a result is EQUIVOCAL after a re-test, this is indicative of virus infection. Here, too, a second sample must be collected no less than 7 days later and tested together with the first sample.

Procedure


Dilution of Control and Samples
Add 400µl of POD dilution buffer to one dilution vial of P/N and two vials of P/P
Add 400µl of POD dilution buffer to sample vials
Add 20µl of negative control (P/N) to 1 vial and 20µl of positive control (P/P) to 2 vials.
Add 20 µl of sample to vials (1:21 dilution) and 20µl of internal control to 1 vial. Mix.
Transfer 200µl of each diluted sample to 200µl of RF Absorbent. Mix well & inc. at RT-15min
Dispense 150µl of P/N, P/P to first 2 paired wells & then samples into each of the paired wells of the Test Plate.
Dispense 150µl of internal control and P/P to last 2 paired wells of the plate.
Cover & incubate at 37°C for 60 minutes
Wash 4 times with washing solution POD (Diluted 1:20 with Distilled Water)
Add 100 µl of anti-Human IgM/POD Conjugate (dilution 1:50 – 100µl of conjugate + 5ml of conjugate buffer)
Cover & incubate at 37oC for 60 minutes
Wash 4 times with washing solution POD
Add 100 µl of working chromogen solution (dilution 1:10 – 0.5ml of chromogen + 5ml of substrate buffer)
Cover & incubate in dark, at room temperature for 30 minutes
Add 100µl of Stopping Solution POD
Evaluate at 450nm, 650nm is recommended as reference wave length
PHOTOMETRIC EVALUATION
Δ A= A Ag – A control Ag
Δ A for P/N should be < 0.1
Δ A for P/P should be > 0.2
Δ A for test < 0.1 => negative
Δ A for test > 0.2 => positive
Δ A for test between 0.1 & 0.2 => Retest in duplicate
New value between 0.1 & 0.2 => Equivocal

Annex VII: Standard Operating Procedures ELISA Worksheet

POD BUFFER
Volume added to control vials – _____
Volume added to sample vials – _____

RF ABSORBENT
Volume added to sample vials – _____

CONTROL / SAMPLES
Volume of P/P – _____
Volume of P/N – _____
Volume of samples – _____

ELISA SET UP
Volume added - _____ Time - _____

INCUBATION
Period -_____ From _____to _____ Temperature - _____

WASHING
Time - _____ No. of cycles -_____

CONJUGATE
Dilution - _____Volume added - _____ Time - _____

INCUBATION
Period -_____ From _____to _____ Temperature - _____

WASHING
Time - _____ No. of cycles -_____

SUBSTRATE
Dilution - _____Volume added - _____ Time - _____

INCUBATION
Period -_____ From _____to _____ Temperature - _____

STOPPAGE
Volume added - _____ Time - _____

READING
Wavelength - _____ Time - _____

Annex VIII: Standard Operating Procedures Rubella ELISA detection

Rubella IgM ELISA

1. Purpose

This document establishes the procedures for performing a rubella IgM ELISA using the Euroimmun Rubella IgM ELISA commercial test kit. This assay is used for the detection of rubella-specific IgM antibodies in patient serum. Detection of specific IgM antibodies in a serum sample collected within the first few days after rash onset can provide presumptive evidence of a current or recent rubella virus infection. The optimum time-point for collection of serum is five days after the onset of symptoms (fever and rash) when >90% of cases will be IgM positive. On the day of rash onset, only about 50% of cases are IgM-positive. Therefore, if the serum collected less than five days after onset is negative, a second sample would be necessary to confirm/rule out rubella. When a patient with suspected rubella has been recently vaccinated (<30 days before blood draw), IgM cannot distinguish rubella disease from the response to vaccination. Determination of the rubella genotype is necessary when rubella symptoms occur following exposure to wild-type virus, and rubella-containing vaccine has been provided as post-exposure prophylaxis.

2. Background and Test Principle

Rubella virus is a human pathogen belonging to the Togavirus family of RNA viruses. The virus is transmitted by respiratory secretions and causes a mild disease characterized by rash and fever. Rubella infection during pregnancy may lead to severe damage to the fetus, a condition known as congenital rubella syndrome. To detect rubella IgM antibodies in a serum sample, microtiter wells are coated with rubella antigen. The sample is added to the well, and any antibodies specific to the antigen present will bind to the bound antigen. After the removal of unbound material, rubella-reactive IgM antibodies in the sample can be detected using a commercial anti-human IgM antibody that is conjugated with an enzyme. After washing to remove unbound conjugate, a chromogen/substrate is added. This reacts with the conjugate to produce a color change. The color intensity can be quantified photometrically and is proportional to the level of IgM antibody bound.

3. Related Documents

- Euroimmun IgM Results Worksheet QC
- Euroimmun Job Aid

4. Equipment

- Absorbance Microplate Reader
- Vortex
- Automated Microplate Washer (optional)
- Freezer -20ºC
- Refrigerator, 4ºC

5. Reagents and Media

- In-house positive serum control
- In-house negative serum control
- Euroimmun Rubella IgM Kit (Catalog # EI 2590-9601-M for 96 tests)
    - Microtiter test strips (STRIPS)
    - Positive Control serum (ready to use, POS CONTROL)
    - Negative Control serum (ready to use, NEG CONTROL)
    - Calibrator (ready to use, CAL)
    - Enzyme conjugate (ready to use, CONJUGATE)
    - Washing solution (WASH BUFFER 10X)
    - Sample buffer (ready to use, SAMPLE BUFFER)
    - Stopping solution (ready to use, STOP SOLUTION)
    - Chromogen/Substrate (ready to use, SUBSTRATE)
    - Quality control certificate

6. Supplies, Other Materials

- PPE (lab coat, gloves, goggles)
- Test sera
- Distilled or deionized water

- Microcentrifuge tubes, titer tubes, or deep well plates
- Squeeze bottle (if not using automated washer)
- Micropipetters 1-20µl, 20-200µl, and 200-1000µl
- Multichannel pipetter 50-300µl or 20-200 µl (optional)
- Pipette tips
- Serological pipettes 5ml, 10ml, 25ml
- Pipette aide
- Thermometer for room temperature
- Paper towels
- Reagent reservoirs
- Container to discard waste
- 10% bleach solution
- Tape

7. Safety Precautions

- Follow universal precautions while working with blood and/or blood products.
- Wash your hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause erroneous results.
- Never pipette by mouth or allow reagents or patient samples to come into contact with skin.
- Handle all blood and serum as if capable of transmitting disease.

8. Sample Information / Processing

- Store serum between 2-8ºC if testing will take place within 1 week. If specimens are to be kept for longer periods, store at -20ºC or colder.
- Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibodies.
- Samples that are improperly stored may yield erroneous results.
- Contaminated samples should not be tested due to the risk of incorrect results.
- If paired sera are to be collected, acute samples should be collected as soon as possible after the onset of symptoms. The second sample should be collected 14 to 21 days

after the acute specimen was collected. Both samples must be run on the same plate.

9. Quality Control

- Use only distilled or deionized water and clean glassware.
- Prepare working dilutions of the assay reagents identically for each run.
- Strictly observe incubation times and temperatures.
- Match patient identifiers with serum dilution tubes and the location of sera on test plates with calculated optical density (OD) values.
- A record must be kept of each assay as the test is performed. Note the reagents, lot numbers, and expiration dates. Record how dilutions are made and the timing of each step.
- Record and monitor control values for in-house positive and negative sera controls to review assay performance over time (optional).
- Do not use kits after the expiration date marked on the kit has been reached.

A. Controls

- Include a calibrator, positive control and negative control on each run.
- Run in-house positive and negative sera controls on each run in addition to the controls that are included in the kit. Store in-house sera control at - 20ºC. Plot the results of in-house controls over time to track trends.

B. Accepted Values

- The OD of the calibrator must be in the range given on the quality control certificate.
- The positive control ratio must fall within the range given on the quality control certificate.
- The negative control ratio must fall within the range given on the quality control certificate.
- If the controls are run in duplicate, the mean of the two readings should be used to determine the calibrator value and the ratios of the positive and negative controls.

C. Repeat the assay if quality control requirements are not met. If the assay fails QC again on the retest, discuss the next course of action with a supervisor.

10. Test Procedure (refer to kit insert)

- Bring all reagents to room temperature (18-25ºC). Use a calibrated thermometer to verify that the room temperature is within the specified range.
- Prepare the plate layout on the Euroimmun IgM results worksheet and Job Aid. Calculate the number of wells needed for the assay, including calibrator, positive, negative, and in-house controls (IHC).
- Using the worksheet, determine the amount of working stock Wash buffer needed and the amount of concentrated Wash and distilled water needed to make a 1:10 dilution.
- Vortex controls and samples before use.
- Dispense 1000 µl of sample buffer into micro/titer tubes or deep well plates, one for each sample to be tested, including in-house controls (IHC).
- Samples must be diluted before use. The three kit controls are ready for use—DO NOT DILUTE.
- Dilute test samples 1:101 with the sample buffer, being sure to switch tips between each sample. (ie, add 10 µl of sample to 1000 µl of sample buffer) Mix well by using a vortex or repeatedly pipetting up and down at least 10 times. Do not aerosolize.
- Incubate the diluted test samples for 10 minutes at room temperature (18-25ºC) before adding to the wells.
- Dilute the 10X washing buffer concentrate with distilled water following the calculations on the Euroimmun worksheet. (e.g, if running 4 strips, add 20 ml washing buffer to 180 ml distilled water) *Buffer can be stored for up to 28 days at 2-8°C. If using a refrigerated buffer, bring it to room temperature before proceeding.*
- Open the bag containing the micro-test strips and remove any unneeded strips from the frame. Tape the remaining test strips into the frame to prevent them from ejecting during the wash process. Replace the extra strips and the desiccant in the bag and reseal carefully. Return to the refrigerator.
- Add 100 µl of diluted samples and controls into the appropriate wells, changing tips for

each sample, as follows:

- **Well A1 calibrator**
- **Well, B1 positive control**
- **Well, C1 negative control**
- **Well D1 in-house positive control serum (IHC)**
- **Well E1 in-house negative control serum (IHC)**
- **Well F1 patient (test) serum**
- **Well, G1 patient (test) serum**
- **Well, H1 patient (test) serum**

- Incubate the test plate for 30 minutes at room temperature (18-25ºC)
- After the incubation is complete, wash all wells with diluted washing solution as follows:
  - Aspirate or shake out the incubation solution; blot on a paper towel
  - Fill each well with 300 µl washing solution (450 µl if using an automated washer)
  - Leave the wash buffer in the wells for 30-60 seconds
  - Aspirate or shake out the washing solution
  - Repeat the washing procedure 2 more times (total 3)
  - Blot any remaining liquid on a paper towel
  - Complete removal of the last wash is critical for accurate performance of the test
- Add 100 µl of conjugate to each well (including kit control wells) and incubate for 30 minutes at room temperature (18-25ºC).
- Wash three times as described above.
- Add 100 µl of Chromogen/substrate solution to each well, including the kit controls, and incubate for 15 minutes at room temperature (18-25ºC). **Protect the plate** and **the reagent from light.**
- Add 100 µl stop solution to each well.
- If the plate reader cannot shake, gently shake the microplate to ensure a homogeneous distribution of the solution by tapping on the side of the frame a few times, being sure not to spill or aerosolize the liquid.

- Read optical density within 30 minutes of adding the stop solution. Read at 450 nm, reference wavelength between 620 nm and 650 nm (e.g., 630 nm).

11. Manual Calculations

- The Euroimmun manual validation worksheet can be used to manually calculate results.
- Fill out the upper portion of the worksheet with pertinent information.
- Enter the raw OD values in column B.
- Determine the Ratio of all kits and in-house controls by dividing the raw OD by the raw OD of the calibrator.
- Determine the validity of the assay by answering the questions listed. If this section indicates all data pass, the assay is valid, and results can be reported.
- Determine all sample Ratios.
- Based on the ratio value determined, a qualitative result can be determined and entered into the Interpretation column as either positive, borderline, or negative, as described in the table in section 12.

The ratio value for the positive and negative controls and each specimen is determined by dividing the specimen OD value by the cutoff calibrator OD.

For example:

  - OD obtained for the calibrator = 0.325
  - OD obtained for patient serum = 0.60
  - Ratio value = 0.60/0.325 = 1.846

12. Calculations

- The Euroimmun IgM results worksheet can be used to prepare the plate layout as well as calculate results.
- Fill out the upper portion of the worksheet with pertinent information.
- Using the Quality Control Certificate from the kit, fill in the range value for the calibrator, positive control valid range, and negative control valid range.
- At the bottom of the page is a template to prepare the plate layout.
- Using this information, determine the total number of wells being used on the plate. Enter this data into the indicated well for this information in either the cell if using a plate

washer or the cell if manually washing.

- The information contained in the gray box will automatically calculate the amount of Wash buffer to process the number of samples on the plate.
- Once raw OD values have been obtained, enter them into the indicated bottom section for raw OD values.
- Determine the validity of the assay by verifying the information contained in the red box under Quality Control. If this section indicates all data pass, the assay is valid, and results can be reported.
- The Ratio column automatically calculates the ratio based on the OD value and calibrator value as described below.
- Based on the ratio value determined, a qualitative result appears in the Result column as either positive (Pos), borderline (B), or negative (Neg), as described in the table in section 12.

13. Interpretation

Ratio	Result	Interpretation
<0.80	Negative	No significant detectable level of rubella IgM antibody
≥0.8 to <1.1	Borderline	The sample should be retested.
≥1.10	Positive	A significant level of detectable rubella IgM antibody. Indicative of current or recent infection.

In cases of repeated borderline test results, an additional patient sample should be taken 7 days later and retested in parallel with the first patient sample.

14. In-House Controls (IHC)

- Each run of the ELISA test must include both a positive and a negative IHC serum.
- If the negative IHC serum is positive, the run needs to be repeated.
- The positive IHC serum needs to be tracked using a Levey-Jennings chart. Each positive IHC OD value is plotted to give a visual indication of the stability of the method over time. The chart determines the number of standard deviations (SD) the actual result is from the mean. Ideally, the IHC should not exceed 3 SD.

Declaration

I, the undersigned, declare that this Thesis is my original work, has not been presented for an MSc degree in this or any other university and that all sources of materials used for the Thesis have been duly acknowledged.

By: Rahel Fekade (BSc)

Signature:_____

Date of submission:_____

This Thesis has been submitted with our approval as advisors.

Advisor: Kassu Desta (PhD, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Biniyam Dagne (PhD fellow, Researcher)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.