ORAL FLUID AS ALTERNATIVE SPECIMEN TO SERUM IN THE DETECTION OF MEASLES AND RUBELLA VIRUS-SPECIFIC IGM ANTIBODY BY ENZYME IMMUNOASSAY IN KENYA

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Oral Fluid as Alternative Specimen to Serum in the Detection of Measles and Rubella Virus-Specific IGM Antibody by Enzyme Immunoassay in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

The research is dedicated to my loving parents Mr. and Mrs Elijah and Rachel Bet, my daughter Valerie, my son Edric, my sister Rodha, and brothers Enock and Erick.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

CVR	Centre for Virus Research.
DBS	Dried blood spot
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
IgM	Immunoglobulin M
KEMRI	Kenya Medical Research Institute
MOH	Ministry of Health
OD	Optical density
rpm	Revolution per minute
RT	Room temperature
RT-PCR	Reverse Transcription Polymerase Chain Reaction
POD	PolyPOD
WHO	World Health Organization
EDTA	Ethylenediamine tetraacetic acid
RF	Refractive Index

ABSTRACT

Measles is a severe, vaccine-preventable disease that causes extensive morbidity and mortality in large parts of the world. Despite the widespread use of measles vaccine, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella, 278 358 reported cases of measles and an estimated 164 000 deaths from measles occurred worldwide in 2008. Vaccine coverage is highly variable between World Health Organization global regions. Measles has been eliminated in the Americas but continues to be endemic in the African and South-East Asia regions, where vaccine coverage is less than 80%. These regions account for approximately 94% of all global measles deaths. In Kenya measles and rubella are still prevalent due to poor vaccination coverage in the neighboring war tone countries such as Somalia and Southern Sudan. Diagnosis of measles/rubella in a laboratory set up is usually done by serological examination of particular IgM from blood sample removed from the veins through puncture. The usual sample collection method is invasive hence not acceptable. There is need to develop and utilize a non-invasive sample collection technique such as saliva or oral fluid for diagnosis. This study was aimed at assessing the feasibility of oral fluid as an alternative method for the detection of measles/rubella-virus specific IgM in routine surveillance of measles and rubella. The study utilized a prospective laboratory based cross sectional design using matched serum/oral fluids which were collected during an emerging outbreak across Kenya of illness characterized by rashes. With a calculated sample size of 180 participants, only 176 participants enrolled and completed the study. Pairs of 176 samples were investigated by enzyme immunosorbent assay for IgM precise antibodies. The statistical analysis used was kappa (k) statistics to measure inter-observer variations. The occurrence of rubella was 26.7% and 23.3% when detected using serum and oral fluid methods, respectively. When tested against the gold standard (serum), specificity and sensitivity of rubella IgM in oral fluid was 93% and 86%, respectively. The prevalence of measles among the vaccinated participants was between 4.2% in Nairobi west, Nakuru North and Wajir East being the least and Nairobi North 45.8% being the highest. Nairobi north district recorded the highest percentage (male 33.3% and 66.7% female) because the region is occupied by refugees from Somalia and Southern Sudan. Rubella virus prevalence among the vaccinated participants was high compared to negative and indeterminate results combined. Nairobi North recorded the highest prevalence (64.3%) of rubella for the two genders while Wajir had the lowest prevalence (3.6%) among the vaccinated participants in all the Sub-Counties in this study. There was some degree of agreement between the two methods since the Kappa value was 0.80. On the other hand, prevalence of measles from oral fluid and serum was 39.8% and 31.8%, respectively. When tested against the gold standard, specificity and sensitivity of measles IgM in oral fluid was 97% and 96%, respectively. Kappa statistic value was 0.26 suggesting fair agreement between the two methods. The study concludes that the use of oral fluid specimen is the best alternative for measles/rubella diagnosis since it is simple to collect, non-invasive and more acceptable than serum. This alternative method can be applied in varied clinical set up and is more applicable to disease surveillance programs. The merit of oral fluids as a specimen for diagnosis is easy and safe to collect. It is also easily shipped to the laboratory. The results and

findings of this study strongly suggest the use of saliva in conducting disease surveillance and epidemiological surveys and studies in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Measles is a severe, vaccine-preventable disease that causes extensive morbidity and mortality in large parts of the world. Despite the widespread use of measles vaccine, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella (MMR), 278 358 reported cases of measles and an estimated 164 000 deaths from measles occurred worldwide in 2008 (Dabbagh *et al.,* 2009). Vaccine coverage is highly variable between World Health Organization (WHO) global regions. Measles has been eliminated in the Americas but continues to be endemic in the African and South-East Asia regions, where vaccine coverage is less than 80%. These regions account for approximately 94% of all global measles deaths (Choi *et al.,* 2008). Outbreaks continue to occur in other global regions, primarily as a result of measles virus importation into areas where vaccine coverage has fallen to a suboptimal level and a susceptible cohort has accumulated (Vyse *et al.,* 2006; Choi *et al.,* 2008). Most regions have elimination goals and elimination strategies based on the maintenance of high vaccination coverage, for which political commitment is required. A key component of elimination plans is surveillance to monitor impact (Choi *et al.,* 2008).

In Kenya, the Expanded Program on Immunization (EPI) was started in 1980 with the first dose of measles-containing vaccine (MCV) or MCV1 given at 9 months of age. Accelerated control for measles was started in 2002 when the first catch-up supplemental immunization activity (SIA) that targeted children aged 9 months to 14 years was conducted followed by periodic SIAs and the establishment of a case-based surveillance for measles with laboratory confirmation. In addition, the Reach Every District approach has been implemented in the country since 2003 to improve RI coverage. Measles is diagnosed using clinical manifestation according to world health organization case definition (WHO, 2009). Alternatively, it can be diagnosed in the laboratory by isolation of RNA measles virus from the specimen obtained from respiratory tract and positive confirmation of measles IgM antibodies from the

serum samples (Helfand, 2008).

Saliva can be collected for testing specific measles IgA from individual participants where blood collection is problematic. Contacts with measles participants are epidemiological pointer hence strong evidence during diagnosis since measles is spread through contact with body fluids of infected persons. Contacts with infected individuals with body fluids such as saliva, semen, vaginal discharge or mucus may causes infection (Perry & Halsey, 2004). Complications with measles is moderately regular, it range from relatively mild and less serious diarrhoea, to pneumonia, Otitis media and acute encephalitis (and rarely subacute sclerosing panencephalitis); corneal ulceration leading to corneal scarring. Complications are usually more severe in adults who catch the virus (Simons *et al.,* 2012). Rubella is a disease caused by a virus which can be passed from the mother to the unborn foetus hence teratogenically acquired by the unborn baby. When the mother gets infected with rubella virus during first trimester of pregnancy, then the virus will teratogenically infect the foetus causing congenital disorders (Mawson & Croft, 2019). The virus always infects young adults, children and adolescent alike. Mostly, almost 50% of rubella infections are asymptomatic hence there is need for laboratory confirmation. The infection can be subclinical meaning asymptomatic or the infections could be mild in children and adults (Sanders, 2010; White *et al.,* 2012). The rubella virus infection can lead to congenital defects and miscarriage (Bouthry *et al.,* 2014). The following are symptoms of rubella virus infection; lymph nodes inflammation, rash referred to as maculopapular rash as well as mild catarrhal symptoms. The inflammation of lymph nodes also called lyphadenopathy will begin five to seven days before appearance of the rash and up to two days afterwards (Nomoto *et al.,* 2020). The rubella disease has an incubation period of fourteen to eighteen days. It affects the respiratory tract by infecting the nasopharynx and multiplies. It also enters the surrounding lymph nodes multiplies resulting to viraemic phase which starts within four to five days after infection and spreads to the whole body (Murray, 1998).

In Kenya measles and rubella are still prevalent due to poor vaccination coverage in the neighbouring war tone countries such as Somalia and Southern Sudan. The

people from this countries are running to Kenya as refugees hence the emerging outbreaks of measles and rubella in some parts of the country. The most common differential diagnosis for measles is rubella. About 50.0% of measles suspected cases turn out to be rubella in Kenya (Robertson *et al.*, 2003). During measles surveillance, the world health organization recommends that rubella should be included. Diagnosis of measles/rubella in a laboratory set up is usually done by serological examination of particular IgM from blood sample removed from the veins through puncture. The usual sample collection method is invasive hence not acceptable. These tests are performed in the laboratory hence crucial for confirmation of measles/rubella outbreaks and sporadic cases. The use of saliva as a specimen is non-invasive and may be preferred by participants. Therefore, there is need to develop and utilize a non-invasive sample collection technique such as saliva or oral fluid for diagnosis.

1.2 The Problem Statement

The most common differential diagnosis for measles is rubella. In Kenya a study by carried out by Robertson *et al.*, (2003) found that roughly thirty to fifty percent of all the suspected cases of measles after laboratory diagnosis turn out to be rubella. The WHO has recommended a laboratory test for rubella virus in case of measles outbreaks and surveillance system. To aide in diagnosis, WHO has further established both regional and global laboratory networks for measles and rubella testing and surveillance. Several laboratory techniques have been used in the study and detection of rubella infection. These include serological assays which is often done using blood serum or plasma. Serum has been the sample of choice in the measles/rubella laboratory testing. However, several challenges have been experienced especially in the developing countries. Taking of venous blood samples in most settings is not always practical hence limiting its use especially outside the clinical settings. Due to miss-diagnosis, WHO recommends skilled personnel to draw blood from the participants using an invasive procedure (WHO, 2009). The collection of samples especially from children has always been challenging and tormenting procedure. The collected blood needs to be processed into serum after which it is preserved and transported using a cold chain to a laboratory where the diagnosis is done. It is also a fact that handling of serum especially during massive

outbreaks is associated with higher risk of other infections like HIV, HBV compared to other body fluids (Robertson *et al.,* 2003). To overcome these challenges in developing countries especially in Kenya, there is need for research to find new, reliable and safer diagnostic methods, which would be used in resource-limited settings and for mass investigations of outbreaks.

1.3 Justification

Measles and rubella infections are endemic in Kenya (WHO, 2009) especially in remote areas such as North-eastern Counties like Garisa, Mandera and Wajir. The most common laboratory assays for diagnosis of measles/rubella acute infection have been IgM detection in serum. In the current study, oral fluids were utilized as alternative specimen to either serum or plasma. Oral fluid is also easy to collect and is also non-invasive (Parry *et al.,* 2000) that makes it acceptable to the participants. The current method is being introduced in Kenya due to challenges of collecting serum and the need for clod chains during sample transportation to the laboratory. Kenya is a vast country with skewed infrastructural development. Measles outbreaks normally take place in remote areas of Kenya especially towards north eastern where laboratory network is not well connected. Collecting blood samples from such places and transporting them to Nairobi is a challenge hence the need to utilize alternative specimen such as oral fluid which can be transported with ease. This study assesses the feasibility of oral fluid in the diagnosing of rubella and measles infection. The WHO has noticed a continuous need to develop sensitive and specific test for both diagnostic and sero-epidemiological surveys of measles/rubella using safer and easily collected specimens other than serum. The study findings has filled the gap on the role played by oral fluid as an alternative method for measles rubella diagnosis other than plasma or serum. The study findings will inform policy and also may aid in the establishment of new policies to guide in measles rubella diagnosis in Kenya. The government should prioritise the right towards easy and safe methods of diagnosis of infectious diseases especially measles and rubella in Kenya.

1.4 Research Questions

1. What are the demographic and clinical characteristics of the study

participants?

2. Is it possible to investigate/detect measles and/or rubella-virus specific IgM in simultaneously collected paired serum and oral fluid specimens from a participants?
3. What is the sensitivity and specificity of using oral fluid to detect measles and/or rubella-virus specific IgM relative to the serum gold standard?

1.5 Objectives

1.5.1 General Objective

To assess the feasibility of using oral fluid as an alternative specimen to serum in the detection of measles and rubella-virus specific IgM using Enzyme Immunoassays in Kenya.

1.5.2 Specific Objectives

1. To determine the demographic and clinical characteristics of the study participants
2. To investigate measles and/or rubella-virus specific IgM in paired serum and oral fluid specimens obtained from each study participant
3. To determine the sensitivity and specificity of using oral fluid to detect measles and/or rubella-virus specific IgM relative to the serum gold standard

1.6 Hypotheses

1.6.1 Null hypothesis

Oral fluid cannot be used as alternative specimen to serum for detection of measles/rubella virus infection using measles/rubella IgM enzyme immunoassay in Kenya.

1.6.2 Null hypothesis per specific objectives

1. Demographic and clinical characteristics of the study participants in this study cannot be determined
2. Measles and/or rubella-virus specific IgM in paired serum and oral fluid specimens cannot be investigated/detected
3. Sensitivity and specificity cannot be determined using oral fluid to detect measles and/or rubella-virus specific IgM relative to the serum gold standard

CHAPTER TWO

LITERATURE REVIEW

2.1 Measles and rubella virus diagnosis

Studies have found that roughly thirty to fifty percent of all the suspected cases of measles in Kenya after laboratory diagnosis turn out to be rubella (Richardson, 2001). The WHO has recommended a laboratory test for rubella virus in case of measles outbreaks and surveillance system (WHO, 2009).

2.1.1 Measles Virus

Measles, is a communicable disease also known as rubeola or morbilli, is a disease caused by a virus called paramyxovirus from the genus Morbillivirus which affects the respiratory system. The illness is characterised by an incubation period of 6 to 19 days (median 13 days) (Richardson, 2001), a prodromal period of 2 to 4 days with upper respiratory tract symptoms, conjunctivitis, Koplik's spots on mucosal membranes, and high fever, followed by a widespread maculopapular rash that persists, with fever, for 5 to 6 days (Furuse, 2010). A history of fever of up to three days or more with at least one the following signs/symptoms, coryza, cough or conjunctivitis and an observed Koplik's spots is a sure clinical diagnosis of measles.

The measles virus enters the body via conjunctiva or the upper respiratory tract before spreading to the surrounding lymph nodes. When they are at the lymph nodes, destruction begins leading to an intense leucopoenia (Wolfson 2007). A process called primary viraemia follows which ensures that the viruses are distributed throughout a system called R-E and the entire respiratory tract. Followed by secondary viraemia in which the pathogen (virus) spreads further to the bladder, viscera, kidney and the skin. Due to the delayed hypersensitivity reactions, the rash and Koplik’s spots appears. The viral markers referred to as antigen are not present in the lesion itself (CDC 2000).

Measles virus is an enveloped, non-segmented negative-sense, single-stranded RNA virus belonging to the *paramyxoviridae* family in the *Mononegavirales* order. The

pleomorphic particles diameter, range from 120 to 250 nanometres. This is enclosed with proteins that are utilized and involved during replication and transcription of the virus. These are nucleocapsid protein N which is associated with enzymatic active phosphoprotein P and the L protein also called large protein. The non-structural proteins V and C arose from the P gene. The bilayered lipid envelope is partly of cellular origin with the matrix protein M inside and bears a fringe of spike-like projections containing the haemagglutination (H) and the haemolytic and cell fusion (F) activities (Figure 2.1 by (Murray, 1998). Virion infectivity disappears when the envelope is distorted impulsively and when lipid solvents is used in virus treatment.

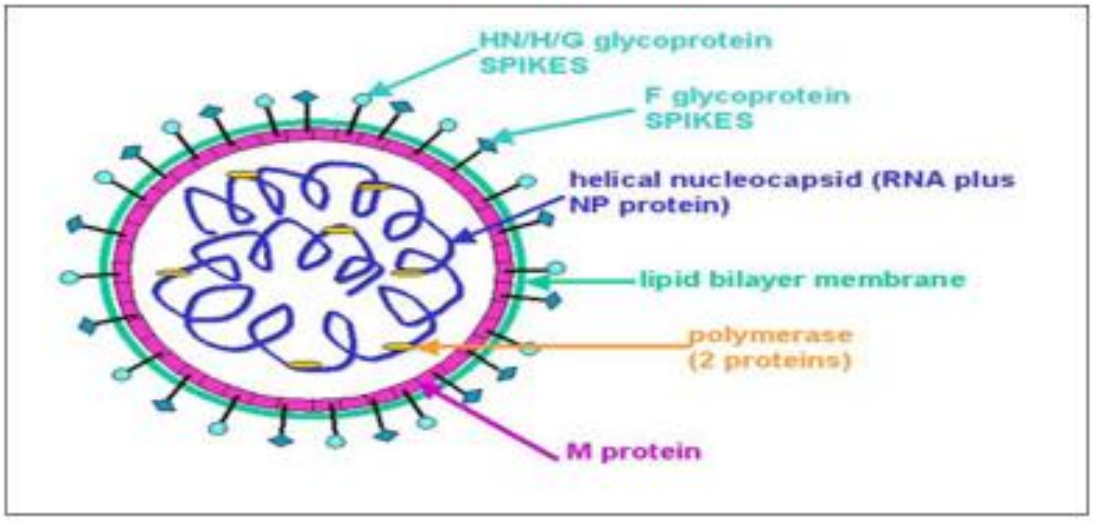

Figure 2.1: Schematic diagram of the measles virion

2.1.2 Incidence and prevalence of measles

Risk factors of measles include; Children with immunodeficiency due to HIV or AIDS, leukemia, alkylating agents, or corticosteroid therapy, lack of vaccination, poor nutritional status/malnutrition (WHO, 2009). Other risk factors include; traveling internationally, having a vitamin A deficiency among others. Worldwide, there are an estimated 21 million cases of measles each year (Wolfson 2007), but the incidence ranges from 0 to 10/100,000 people in countries with widespread immunization programmes such as the USA, UK, Mexico, India, China, Brazil, and Australia (WHO, 2009). In the USA, before licensing of effective immunizations, more than 90% of people were infected by the age of 15 years. After licensing in

1963, incidence fell by about 98% (CDC 2000). The mean annual incidence in Finland was 366/100,000 in 1970 (Ventola, 2016), but steadily decreased since the launch of vaccination in 1982, with three cases in 1995 and no cases in 1996 (WHO, 2009). Similarly, the annual incidence declined to zero in Chile, the English-speaking Caribbean, and Cuba during the 1990s, when immunization programmes were introduced (WHO, 2000).

The WHO Africa Regional Office (AFRO) has established measles elimination indicators, including: 1) measles incidence <1 case per million population at national level; 2) ≥95% MCV1 coverage in RI at national and at sub-national levels; 3) 95% coverage in measles SIAs and outbreak response immunization activities (ORI); 4) ≥80% of districts (sub-national levels) investigating one or more suspected measles cases within a year; and 5) reported non-measles febrile rash illness rate of ≥2 per 100,000 population at national level (Masresha *et al.*, 2018).

In Kenya, the Expanded Program on Immunization (EPI) was started in 1980 with the first dose of measles-containing vaccine (MCV) or MCV1 given at 9 months of age. Accelerated control for measles was started in 2002 when the first catch-up supplemental immunization activity (SIA) that targeted children aged 9 months to 14 years was conducted followed by periodic SIAs and the establishment of a case-based surveillance for measles with laboratory confirmation. In addition, the Reach Every District approach has been implemented in the country since 2003 to improve RI coverage. With the promulgation of the new constitution in 2010, Kenya adopted a decentralized government and replaced the eight provinces and existing districts with 47 semi-autonomous counties. In 2013, health services were fully decentralized to the counties to run the devolved functions. Measles case-based surveillance therefore shifted from being district-reported to county-reported. A second dose of MCV (MCV2) was introduced in 2013 and is recommended for children aged 18 months (Ngina *et al.*, 2018). Kenya developed a national measles elimination strategic plan 2012-2020 as a road map for eliminating measles transmission (Masresha *et al.*, 2018).

2.1.3 Diagnosis of Measles Virus

In areas with a low incidence of measles, the diagnosis of measles by clinical presentation is often complicated because of the sporadic nature of the illness as well as the widespread occurrence of other rash-causing illnesses. In addition, many measles cases in previously vaccinated or immunosuppressed individuals according to WHO case definition are excluded because they do not meet the criteria. Therefore, confirmation of measles virus infection must be made using laboratory-based methods as a confirmation (Ratnam *et al.,* 2000). Measles-specific immunoglobulin M (IgM) serology is the standard test for the rapid laboratory diagnosis of measles, and IgM testing is now almost purely performed with commercial enzyme immunoassay (EIA) kits (Bolotin *et al.,* 2017). Antibody detection is the most versatile and a common method utilized in diagnosis of measles. In acute, uncomplicated measles, a significant rise in antibodies specific measles IgG between acute and convalescent phase serum specimens is generally considered diagnostic. A positive test result for specific IgG antibodies in a one serum specimen indicates past infection with measles virus or measles vaccination, but does not ensure protection from infection or re-infection. Detection of antibodies specific IgM in a serum specimen collected within the first few days of rash onset can provide a good presumptive diagnosis of current or recent measles virus infection. Therefore, the IgM assay is the test of choice for rapid diagnosis of measles cases. The enzyme immunoassay (EIA) is a method commonly utilized in detecting measles-specific IgM and IgG antibodies. Both capture and indirect formats for IgM detection are available commercially and most perform well (Husada *et al.,* 2020). However, in countries where disease prevalence is low, intensified surveillance typically implemented during and after an importation will result in some false positive IgM results since no assay is 100% specific.

Use of serum in specific IgM for measles using Enzyme Immunoassays (EIAs) are the most laboratory assays recommended in diagnosing acute stage of measles hence enough for programs leading to measles control. During characterization of measles virus using molecular techniques, serum samples are not enough or not ideal. Laboratory test of measles can be improved only when the collection of samples is simplified. Currently EIAs the golden diagnostic tool for measles since no other field nor has laboratory method been developed. A non-invasive method for specimen

collection is ideal which does not require cold chains nor does it need technically sophisticated equipment (Coughlin *et al.,* 2017). Filter paper collection of saliva and oral fluid swabs specimen are two methods that are ideal for both diagnostic methods and for molecular characterization of measles virus. These two methods are better as compared to collection of blood serum. They are easy to collect, safe since it is non-invasive and does not require refrigeration during transportation to the laboratory for diagnosis (Badawi & Ryoo 2016).

2.2 Rubella Virus

Rubella is a virus belonging to *Togaviridae* family in the genus *Rubivirus* (Murray, 2006). It is roughly spherical with a diameter of 60-70nm. It has an icosahedral neucleocapsid containing a single stranded, positive sense RNA genome. It has a complex lipid envelope (Figure 2.2 by Murray, 1998). The virus contains three structural proteins. The two in the envelope are E1 and E2 and one in the core called C protein that surrounds the RNA. The envelope protein is glycoprotein that exists as heterodimer that project from the virus to form 6- to 8-nm spikes. E1 appears to be the dominant surface molecule and is associated with neutralizing and haemagglutinating epitopes (Battisti *et al.,* 2012). Only one serotype of the virus is known, but phylogenetic tree analysis of the coding region of E1 indicates the existence of at least 7 distinct genotypes represented in 2 clades. Rubella virus causes congenital disorders to babies who are produced by women who had the disease during their pregnancy (Bouthry *et al.,* 2014).

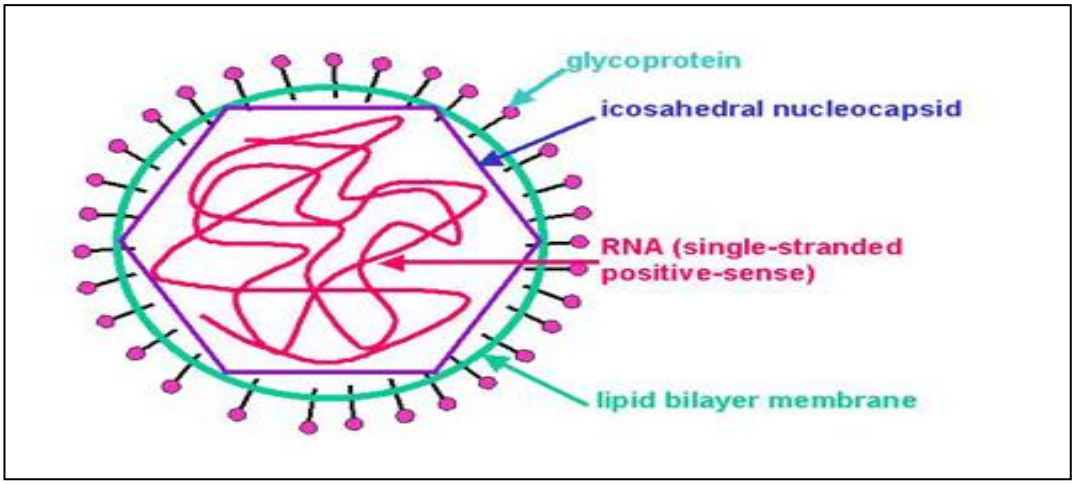

Figure 2.2: Schematic diagram of the rubella virion

2.2.1 Incidence and prevalence of Rubella Virus

In the pre-immunization era in the UK, rubella was rare under the age of 5 years, with the peak incidence being between 5 and 10 years of age (WHO, 2014). Serological surveys around the world found that by late adolescence/early adulthood, 80% of women had been infected, (Sharma *et al.,* 2011). Many industrialized countries have a rubella vaccination policy that makes their populations have relatively low rubella susceptibility rates compared to those in the developing world where such policies may not exist. A study by Manakongtreecheep & Davis, (2017) on Rubella susceptibility showed that, in Kenya specifically Nairobi, about 7.3% of Kenyan and 15% of Asian origin but reside in Kenya in the Aga Khan Hospital were found to be susceptible to rubella, compared to 3.6% in comparison to samples collected in Kenyatta National Hospital. The ethnic difference in Rubella antibody tire among the Asians and Africans in Kenya could have been generated by chance due to the small size of their study sample. While Sero-prevalence in Uasin Gishu district stands at 80% and increases from 59% among ages 4–6 years to 94% in ages 14–20 years (Kombich *et al.,* 2009).

2.2.2 Pathogenesis of Rubella Virus

The following are risk factors to contracting rubella virus; contact with infected participants, failure to get vaccinated, overcrowding, poor immune response to the vaccine and being medical personnel (WHO, 2009). Rubella virus is acquired via the respiratory route. The Rubella virus interacts with specific host cell surface receptors and enters the cell via receptor-mediated endocytosis. From this point, the virus uncoats its envelope and releases its genome into the cell. After a latency period of up to ten hours, viral RNA synthesis can be detected within the host cell. The viral RNA induces the translation of the viral non-structural proteins (Boulant *et al.,* 2015). These non-structural proteins facilitate the modifications that must be made to rubella virus proteins before new virus particles can be synthesized. Once rubella virus has assembled its RNA-containing nucelocapsid core, it begins to bud from the cell membrane. Maturation of the rubella virus is marked when it eventually buds

from the host cell membrane, encapsulated by an envelope composed of host cell lipids and the viral proteins. The viruses first infect the nasopharynx, multiply in the respiratory tract lining and in the surrounding lymph nodes and then enter into the blood stream. In the blood streams the viruses multiplies further leading to viraemia between five to seven days after which it spreads to the rest of the body up to the skin. The rubella viruses can be recovered from the nasopharynx before and after infection. These viruses can be isolated from up to 2 weeks after the appearance of the rash or 1 week before rash appearance (Namoto *et al.,* 2020). Incubation period for rubella averages 14-18 days but can range from 12-23 days. A short prodromal phase occurs before the rash appears in the adolescents and adults but not in children. In children a rash is usually the first manifestation. This prodrome has the following signs: low-grade fever, headache, malaise, anorexia, mild conjunctivitis, coryza, sore throat, lymphadenopathy involving the suboccipital, post-atricular and cervical lymph nodes (Boulant *et al.,* 2015.

2.2.3 Diagnosis of Rubella Virus

Rubella is often the next option with the diagnosis of suspected measles cases that turn out to be negative. The WHO has recommended the laboratory test for rubella virus in case of measles outbreaks and surveillance system. It has also established regional and global laboratory networks for measles and rubella testing. Several laboratory techniques have been used in the study and detection of rubella infection. Generally, about 30% to 50% cases of suspected measles infections are confirmed to be cases of rubella (Robertson *et al.,* 2003). Varied methods in the laboratory have been used in detecting rubella virus in participants specimens including: reverse transcription (RT)-PCR in which RNA from rubella virus is extracted from a participants sample by Southern blot hybridization (hybridization using RT-PCR plus hybridization), RT-PCR from rubella virus RNA isolated from an infected tissue which is culture hence tissue culture (culture plus RT-PCR), immunofluorescent assay (IFA) of a low-background to identify virus proteins in tissue culture cells infected with viruses, and a replicon-based technique to identify contagious virus (Zhu *et al.,* 2007). The World Health Organization considers blood serum samples for virus detection as the ‘gold standard’ for laboratory confirmatory cases.

Diagnosis of many viral diseases has always relied on the antibody detection in serum or plasma, (WHO 2007). A study done in Peru showed that rubella virus infection is typically diagnosed by the identification of rubella virus-specific immunoglobulin M (IgM) antibodies in serum, but approximately 50% of serum samples from rubella cases collected on the day of rash onset are negative for rubella virus-specific IgM (Helfand *et al*., 2007).

Rubella case detection commonly done with the identification of rubella- IgM specific antibodies in the serum from cases that are suspected of rubella virus illness. When the child is infected with rubella virus after birth, specific IgM are not detected up to several days after rash appearance. However, the rubella virus is normally present and detectable within the nasopharynx or the throat from a few days up to about five days after the onset of the rash, making rubella virus detection a possible and reliable option for diagnosis at this stage (Mercader *et al.,* 2012). On the other hand measles IgM serology allows the testing of single sera specimen and is a sure diagnostic when the result is positive. The IgM serum antibody level peaks within 2 days after start of the rush (WHO 2007).

2.3 Dried Blood Spots in Measles/ Rubella Virus Detection

The dried blood spot has widely been used in epidemiological studies as an alternative to serum. This form of dried blood spot has proved to be effective in IgM detection and solved the problem of cold chain transportation. This technique has been applied to measles diagnosis and can also be applicable with rubella (Hefand *et al.,* 2001). Recent studies have suggested that filter paper dried blood spots (DBS) are suitable for laboratory detection of measles-specific IgM (De Swart *et al.,* 2001). They compared the detection of specific IgM and IgG antibodies of rubella virus in DBS to that of blood serum samples collected by healthcare workers in a hospital set up where participants were diagnosed with rubella virus infection. The foetus of mothers with rubella virus infection during the first trimesters of their pregnancy has high percentages of congenital rubella syndrome (Riddell *et al*., 2003).

To reduce the risk in formation of congenital rubella syndrome, a haemagglutination inhibition (HI) test has been done routinely to identify the anti-RV antibodies in serum of pregnant mothers. The method is ideal and very useful for infection screening though it is not useful for detecting the occurrence of viral infection. The methods used for determining recent viral infection is Enzyme-linked immunosorbent assay (ELISA). It detects anti-RV IgM in serum hence useful in detecting recent infections. Anti-RV specific IgM have been shown to appear in serum from the second or third week after infection and to disappear by the end of the first or second month (De Swart *et al.,* 2001). Some studies have also described the use of dried blood samples with enzyme immunoassay method (EIA) in the investigation of the rash illness and assessment of children following vaccination for measles, mumps and rubella (Tipples *et al.,* 2004).

2.4 The Use of oral fluid in measles and rubella Virus Detection

The use of oral fluid has been successfully used in measles, rubella, and mumps laboratory based surveillance in UK (WHO 2007). The use of saliva in other studies has shown higher sensitivity for nucleic acid detection. Testing of oral fluids as an alternative to serum has many advantages for surveillance of measles/rubella virus (Maple, 2015). This led in 1994 to its implementation as a laboratory confirmatory

test for rubella cases (Charlton & Severini, 2016).

Oral fluid is also easy to collect, non-invasive and is more acceptable to the population. Some studies on detection of rubella-specific antibodies from saliva specimens have been previously described. The use of antibody-capture radio immunoassay, showed that virus IgM specific was detected in all (100%) saliva samples of participants suspected of rubella infection collected between one and five weeks following the onset of the illness (Charlton & Severini, (2016) . In a community-based study of notified cases of rubella in England and Wales they revealed that the sensitivity of saliva rubella IgM testing was 81%. However, the sensitivity rose to 90% when results from specimens collected outside the recommended period (1-6 weeks after onset). Specimens that took more than 1 week to reach the laboratory were excluded in the study (Maple, 2015). Antibody test for saliva has been developed for RV **(**Charlton & Severini, 2016).

The study that was carried out to compare test assays showed that ELISA test with saliva was more sensitive than the results of the immunoassay and that the results correlated better with the serum IgG result than the results of radio immunoassay. It had an overall sensitivity of 82% and a rank correlation of 0.68, whereas the sensitivity and rank correlation for the radioimmunoassay were 74% and 0.45, respectively (Sheppard *et al.*, 2001). The ability to detect IgM in sera and saliva was compared with the ability to detect rubella virus RNA in saliva by reverse transcription-PCR (RT-PCR) by using paired samples taken the first four days after the appearance of the rash from suspected rubella cases during an outbreak in Perú (Leland & Relich, 2016).

Sera were tested for IgM by both indirect and capture enzyme immunoassays (EIAs), and saliva was tested for IgM by a capture EIA. Tests for IgM in serum were more sensitive for the confirmation of rubella than the test for IgM in saliva during the 4 days after rash onset (Emily *et al.*, 2009). The use of oral fluid as a method of antibody detection has widely been used for its advantage and studies have been done on hepatitis A and B, measles, mumps, and human parvovirus B19 and Dengue fever (Mercader *et al.*, 2006). As a non-invasive alternative, oral fluid is a body fluid

that contains antibodies of diagnostic significance, and the body content of salivary crevicular fluid reflects that of plasma. Therefore, it is possible to detect antibodies to a variety of viral antigen in oral fluid especially by use of sensitive antibody-capture assay (Soares *et al.,* 2015). Ohuma *et al.,* (2009) in a study in Kenya evaluated the effectiveness of a measles vaccine campaign in rural Kenya, based on oral-fluid surveys and mixture-modelling analysis. Specimens were collected from 886 children aged 9 months to 14 years pre-campaign and from a comparison sample of 598 children aged 6 months post-campaign. Their results confirm the effectiveness of the campaign in reducing susceptibility to measles and demonstrate the potential of oral-fluid studies to monitor the impact of measles vaccination campaigns in Kenya rural setup.

2.5 Sensitivity and specificity

For clinically diagnosed measles confirmation, the utilization of serum specific IgM EIAs are the most recent current laboratory test recommended. There are two methods which works well, these are both indirect and capture EIA formats (Ratnam et al., 2000), most of these tests have reported reasonably high sensitivity (83%–89%; especially higher after rash onset which is the first week) and specificity (95%–100%), when serum samples were collected three to twenty-eight days after the rash onset (Biellik et al., 2002). The main advantage of EIAs method is that it is usually performed with a single specimen of serum, usually rapid, simple and easy to perform by a well-trained technician. It also requires very small volumes of specimen about 2mL of serum. It is generally used in diagnosis of acute measles infection from rash onset to four weeks afterwards. The test requires advanced equipment and can be done within four hours, they are robust in respect to signal to noise differences and reproducible as well. Therefore, the test cannot be done at bedside since it requires sophisticated equipment such as EIA reader. Majority of the kits are commercially prepared, are of good quality and relatively have a long shelf life. The sensitivity and specificity of the assays are very good; the specificity appears to be sufficiently high that small outbreaks of febrile rash illnesses due to other causes are not misdiagnosed as measles. The sensitivity of the test is normally lower at the first few days of rash appearance, though the limitation has not been

significant epidemiologically for measles control programs (Hersh 2002; Biellik 2002). An assay of slightly reduced sensitivity in the first week after rash onset (e.g., 70%–80%) is probably sufficient for case based surveillance, although some isolated cases might be misclassified as not measles. However, measles outbreaks should still be detected, because the WHO recommends that five to ten samples be tested to obtain laboratory confirmation of outbreaks of suspected measles (WHO, 2001; Murray 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The current study was nested within a prospective cross sectional study design which was based in the laboratory. However, the current study was a retrospective study, utilizing samples from a surveillance survey. The study was done from June to December 2010 a period of six months. Emerging outbreaks of measles and rubella predetermined the collection of samples. Routinely, blood was collected for confirmation of measles and rubella. Additional study tools were supplied to areas experiencing outbreaks and sensitisation meetings were held to update teams from MOH on the study purpose and data collection tools.

3.2 Study sites

Samples were collected from the following districts currently referred to as Sub-Counties; Nairobi West, Nairobi North, Nakuru North, Fafi, Garissa, Wajir East, Wajir South, Wajir West Kaloleni, Kamukunji, Lagdera, Tana River, Taveta, Turkana North, and Kakuma (Sudanese living in Kenya). These were areas with outbreaks of rash-like infections in some part of the country were used to select the study site. Sample collection tools were dispatched and specimens were collected from participants who consented. They were then transported to KEMRI measles laboratory for testing and processing. The current study was nested in a WHO program which was investigating measles outbreaks in Kenya. The programme was confined to the study sites stated above since there was measles/rubella outbreaks in the counties. The study areas are shown in the Map of Kenya Figure 3.1.

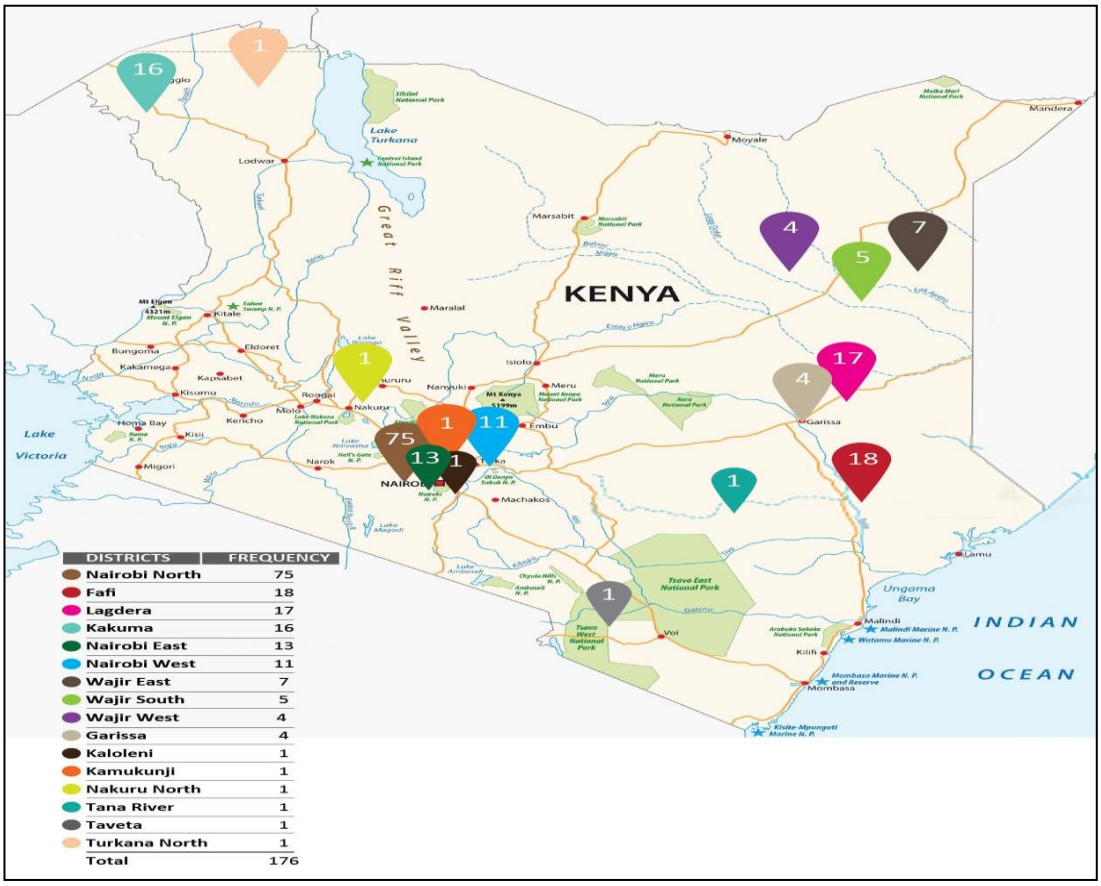

Figure 3.1: Map of Kenya showing the study areas

3.3 Study population

A standard WHO case definition for measles/rubella infection based on the clinical presentation (maculopapular rash, fever, cough, coryza and conjunctivitis) was used to select the population of interest. The vaccination status, age and sex of the study population was considered in this study.

3.3.1 Inclusion criteria

All the participants identified by the clinician to be presenting with symptoms suggestive of measles or rubella infection as per the standard WHO case definition.

3.3.2 Exclusion criteria

- Participants who do not fit the WHO case definition for measles/rubella

infection.

- Participants who meet the WHO case definition for measles/rubella infection who do not consent to participate in the study.

3.4 Sample size determination

According to the Kenyan Ministry of Health (MoH) surveillance system of 2008-2009, the rubella sero-prevalence was 13.5% (MoH, 2015) across the country especially in the endemic regions selected as study sites in the current study.

Hence the sample size determination will be determined as earlier described by Cochran, (1963):

$$N = \frac{Z^2 p(1-p)}{d^2}$$

Where N = minimum sample size requirement.

Z = 1.96 (standard error)

p= the prevalence of Rubella virus in Kenya. (13.5%)

d = 0.05 (the inverse of 95% confidence limit sometimes referred to as degree of freedom

N=1.96^2 0.135(1-0.135)/ 0.05^2

= 179.44

≈ 180 samples

For each Sub-County, purposive sampling was done using simple random sampling methods.

3.5 Sampling methods/criteria

The study utilized purposive sampling method to choose the study sites that had been selected by the WHO program due to measles/rubella outbreaks. Purposive sampling (also known as judgment, selective or subjective sampling) is a sampling technique in which researcher relies on his or her own judgment when choosing the study site or population to participate in the study. The study population were selected through simple random sampling methods. Those who meet case division were assigned random numbers and those choosing odd numbers were selected for the study until the sample size was reached.

3.6 Specimen collection and processing

Informed consent of the participants was sought with the assistance of a health officer. Demographic and clinical information of the participants was obtained and included age, sex, date of the rash onset/appearance (using the structured questionnaire; Appendix 2) before specimen collection. The specimen collected were blood and oral fluid samples from each consenting study participant simultaneously on the rash onset or within 28 days after the onset. The participants were picked consecutively until the sample size was achieved per Sub-County.

3.6.1 Blood collection for serum harvesting

Three millilitres of venous blood were collected in a red-topped (no EDTA) vacutainer tube containing a clot activator using the appropriate phlebotomy technique described by WHO, (2007). The blood samples after collection were allowed to clot fully for 15 to 30 minutes before harvesting of serum by centrifugation at 3000 rpm for 10 minutes so as to separate the serum and the whole blood. Serum was stored at 4^{o}C until analysis for Measles virus IgM by ELISA using a commercial enzyme immunoassay.

3.6.2 Oral fluid

Oral fluid was collected using an ORACOL test kit (Malvern Medical Developments, Worcester, UK). The device has a sterile swab, with an absorbent material that was placed into the participant's mouth between the lower cheek and

gum and left in until adequately moistened. The pad was removed and inserted in the bottom of a vial containing preservative. Consistency in labelling for the two samples from a participant was ensured. The same label also appeared in the questionnaire. Serum and oral fluid was shipped in a cool box to KEMRI for laboratory processing. The oral fluid in the swabs was extracted as soon as it reached the laboratory by adding 1ml of the virus transport medium to the tube containing the oral fluid swab. The swab was then agitated by vortexing to ensure foaming of the transport medium. The swab was removed from the tube by twisting motion; centrifugation at 2000 rpm for 5 minutes to ensure that much liquid was recovered from the swab. Extracted fluid was ready for measles/rubella virus IgM capture test using a commercial enzyme immunoassay kit

3.7 Laboratory Test Procedures

Rubella- and measles-specific IgM status (+ve/ –ve) in oral fluid and serum was determined as follows:

3.7.1 Diagnosis of Measles Virus IgM in Serum by ELISA

The Enzygnost® Anti-Measles-Virus/IgM immunoassay (Siemens Healthcare Diagnostics Products, Marburg, Germany) was utilized for analysis of serum samples in order to detect Measles-Virus IgM. Sterile distilled water was used as negative controls while a known sample which is positive for measles virus stocked from a previous surveillance was used as a positive control. In the IgM-capture EIA, IgM antibody in the participant's serum is bound to anti-human IgM antibody adsorbed onto a solid phase. This step is non virus specific. The plate is then washed, removing other immunoglobulins and serum proteins. Viral antigen is then added and allowed to bind to any virus-specific IgM present. After washing, bound antigen is detected using anti-virus monoclonal antibody conjugated with an enzyme, following which a detector system with chromogen substrate reveals the presence or absence of virus-specific IgM in the test sample. In some formats, the antigen-monoclonal antibody-enzyme complex is premade, eliminating one binding and washing cycle.

3.7.2 Diagnosis of Rubella Virus IgM in Serum by ELISA

The Enzygnost® Anti-Rubella-Virus/IgM immunoassay (Siemens Healthcare Diagnostics Products, Marburg, Germany) was utilized in analyzing the serum samples in order to detect Rubella-Virus IgM. Enzyme Immunoassay is used for quantitative and qualitative determination and detection of specific IgM antibodies for rubella virus in plasma and serum for human beings. The enzyme Immunoassay is processed using Elisa processor. The test was specifically developed for testing single specimen, not for pooled samples. This product is for in-vitro diagnosis. The RF absorbent binds to the IgG present in the test sample. Any rheumatoid factor in the sample bind to the resulting immune complexes and are thus eliminated. The RF absorbent precipitates up to 15mg IgM/ml (value refers to undiluted sample) and thus also removes the virus specific IgG. This effect enhances the sensitivity of the IgM test. The IgM antibodies in the tested sample which are specific for rubella virus bind to the antigen in the well of the test plate. The anti-human IgM/POD (POD – Poly-POD solution) conjugated binds to the specific antibodies. The enzyme component of the conjugate catalyses the working chromogen solution, producing a blue colour. This reaction is terminated by the addition of stopping solution POD with the colour changing to yellow. IgM directed against cellular antigens is measured in the same way in the well coated with control antigen. The difference between the colour intensities in the well coated with antigen and in the well coated with control antigen is a measure of the concentration and immunochemical reactivity of the virus antibodies detected in the sample. Micro titration plate is coated with inactivated rubella virus antigen. The wells in the left row of each strip are coated with antigen derived from baby hamster kidney cell culture (BHK21) infected with rubella virus, and the wells on the right row are coated with non-infected cells (control antigen).

3.7.3 Diagnosis of Measles Virus IgM in Oral fluid by ELISA

The Microimmune Measles IgM Capture Enzyme Immunoassay kit (Microimmune Ltd, Middlesex, UK) was utilized for detecting Measles IgM in oral fluid. The undiluted, extracted oral fluid was added to anti-human IgM coated microtitre wells. IgM in the specimen bound to the wells and after washing, recombinant measles

nucleoprotein (rMeNP) antigen was added. After washing the wells, a monoclonal antibody to the rMeNP conjugated to horseradish peroxidase was added. After washing tetramethylbenzadine (TMB) substrate was added to reveal the presence of specific IgM. The presence of measles specific IgM resulted in a colour change in the TMB from colourless to blue and then yellow on terminating the enzyme reaction. The colour change and intensity was monitored using a spectrophotometric plate reader set at 450nm with a correction filter between 620 and 650 nm. The presence of measles specific IgM was indicated by optical density values above the cut-off. Sterile distilled water were used as negative controls while confirmed positive specimen stocked in the laboratory from a previous study were used for positive controls.

3.7.4 Diagnosis of Rubella Virus IgM in Oral fluid by ELISA

The Microimmune specific Rubella IgM Capture Enzyme Immunoassay kit (Microimmune Ltd, Middlesex, UK) was utilized in detecting Rubella Specific IgM in oral fluid. The undiluted, extracted oral fluid was added to anti-human IgM coated microtitre wells in duplicate. IgM in the specimen bound to the wells and after washing; recombinant rubella antigen (rRA) was added to one of the duplicate sample wells and diluent to the other well. Rubella specific IgM in the sample, if present, bound the rRA. After washing the wells, a monoclonal antibody to the rRA conjugated to horseradish peroxidase was added. After washing tetramethylbenzadine (TMB) substrate was added to reveal the presence of specific IgM. The presence of rubella specific IgM resulted in a colour change in the TMB from colourless to blue which then to yellow on stopping the reaction with 0.5M HCl. The colour change and intensity was monitored using a spectrophotometric plate reader set at 450nm with a correction filter between 620 and 650 nm. The presence of measles specific IgM was indicated by optical density values above the cut-off in the antigen well compared to the control well.

3.8 Specificity and sensitivity of oral fluid diagnosis

The entire tests were calculated in relation to serum results. The tests were relative specificity and sensitivity, negative predictive value (NPV) and positive predictive value (PPV) of oral-fluid. The results of the serum assay were used as the gold standards for reference. Concordance between the oral fluid-based and corresponding serum-based assay results was evaluated by considering all sample pairs in the study (Appendix 5).

		Serum Positive	**Serum Negative**
Tests	**Oral fluid Positive**	A	B
	Oral fluid Negative	C	D

A: True positive: the participants have the disease and the test is positive.

B: False positive: the participants does not have the disease but the test is positive.

C: True negative: the participants does not have the disease and the test is negative

D: False negative: the participants have the disease but the test is negative.

3.9 Data Management - analysis and presentation

The data was entered into Epi-info statistical software for analysis. All the information was stored in CDs and flash disk to back up the work before data was analysed. Hard copy data was kept in cabinets under lock and key. The performance of measles, rubella EIA on oral fluid was assessed by calculating the proportion of individuals with measles or rubella IgM and among those without the measles or rubella IgM in the oral fluid. The proportion of the individuals with anti-

measles/rubella IgM antibody was assessed with the accuracy of antibody-capture EIA in oral fluid samples noted. For the comparison of the measles/rubella specific IgM antibodies in serum and oral fluid and samples by antibody capture EIA where Elisa reader took the optical density readings from the readings from each plate. The cut-off and validity of each plate are obtained from the recommendation from the kit protocol. Qualitative comparison was done using a two-by-two table with positive and negative results. Descriptive results were presented in the form of graphs, charts and tables to show the association. This was used for comparison purpose of the two samples, serum being the goal standard.

Kappa (k) statistic was utilized in order to compute the inter-observer variability. Kappa is a measure of this difference, standardized to lie on a -1 to 1 scale, where if k = 1 that would indicate perfect agreement, if k = 0 that is exactly what would be expected by chance, and if k = negative values that would indicate agreement less than chance, i.e. potential systematic disagreement between the observers (the serum and oral fluid optical density results).

3.10 Ethical Considerations

Ethical clearance was sought from KEMRI'S Scientific Steering Committee number 1839 and Ethical Review Committee and also from Jomo Kenyatta University of Agriculture and Technology. The study was carried out according to KEMRI guidelines and regulations on human specimen use and care and the globally established ethics for laboratory use and standard operating procedures as found in the WHO guidelines. Potential participants were who presented with a rush like illness were identified by a clinical officer. The study set up and purpose was explained to study participants after which informed consent was sought. Informed consent was obtained from children over 12 years of age. Parent/ guardian provide assent for children who have not attained the age of 12 years and those who were not able to consent. The participant experienced little pain when blood was being withdrawn but this did not pose any health risk since the equipment (needle and swabs) used to get this specimen were sterile. Participant's name was not used to maintain confidentiality. All hard copy data were stored in designated lockers and

access was limited to the principal investigator. Passwords only known to the principal investigator were used to safeguard electronic data.

3.11 Study Limitations

Limitations of this study were; poor responds from the participants, refusals to sign the consent form and failure to return the questionnaires. Missed identification of the rash or the participants ignores the rash and fails to report to the hospital were also limitations to the study.

3.12 Benefit of the Study

Findings were presented to the World Health Organization (WHO), Kenya Expanded Program on Immunization (KEPI) and Ministry of Health. The findings provided useful information to improve efficiency of the disease surveillance system in Kenya. The useful information from this study is that oral fluid can be used as an alternative to serum in measles/rubella diagnosis and surveillance. Quality and reliability of laboratory diagnosis was enhanced.

CHAPTER FOUR

RESULTS

4.1 Response rate

A total of 180 participants were recruited in this study. However, data from 4 of them was incomplete. A response rate of 97.8% (176 participants) was obtained which was considered acceptable.

4.2 Demographic and clinical characteristics of the respondents

4.2.1 Gender distribution of respondents/participant

The anticipated sample size for this study was 180 (100%) participants but only 176 participants enrolled and completed the study. There were 98 (55.7%) males and 78 (44.3%) females as shown in Figure 4.1

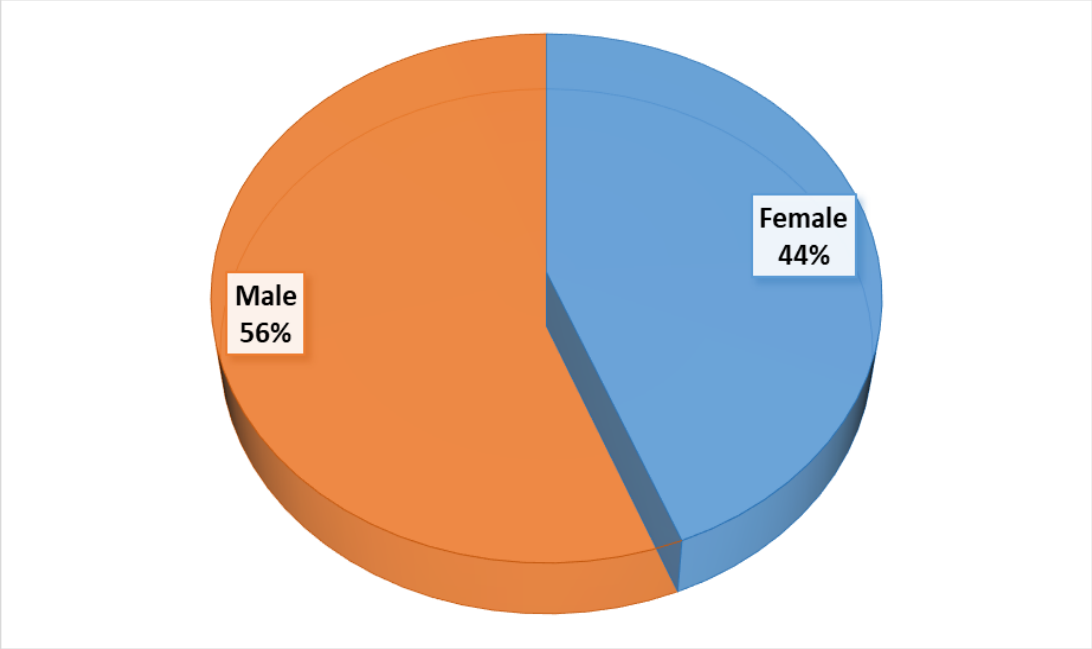

Figure 4.1: Sex/gender of participants

4.2.2 Age distribution of the participants

In this study, 107(60.8%) participants were aged between 0-5, 38(21.6%) were aged

between 6-10, 12(6.8%) were aged between 11-15, 8(4.5%) were aged between 16-20, 5(2.8%) were aged between 21-25, 1(0.6%) were aged between 31-35, 3(1.7%) were aged between 36-40 while 2(1.1%) were aged between 41-45 years old (Table 4.1).

Table 4.1: Categories of participants by age

Age category (Years)	Frequency (n)	Percent (%)
0-5	107	60.8
6-10	38	21.6
11-15	12	6.8
16-20	8	4.5
21-25	5	2.8
31-35	1	0.6
36-40	3	1.7
41-45	2	1.1
Total	176	100.0

Table 4.2 shows comparison of participants' age and sexes. The results showed that, 62(63.3%) male participants were aged between 0-5, 20(20.4%) were aged 6-10, 7(7.1%) were aged 11-15, 4(4.1%) were aged 16-20, 2(2%) were aged 21-25, while 1(1%) was aged between 31-35, 36-40 and 41-45 years old, respectively. Chi-square test revealed that there was no significant statistical relationship between the participants' age categories and their gender. The mean Kappa value of 0.507 means that there was agreement that there was no significant statistical relationship between age categories and the gender in relation to measles and rubella infection although there were more males compared to female participants in this study.

Table 4.2: analysis of gender verse age categorization of the participants

Age in years	Male (n) (%)	Female (n) (%)	Total (n) (%)	p-value	Kappa
0-5	62(63.3)	45(57.7)	107(60.8)	0.932	0.507
6-10	20(20.4)	18(23.1)	38(21.6)		
11-15	7(7.1)	5(6.4)	12(6.8)		
16-20	4(4.1)	4(5.1)	8(4.5)		
21-25	2(2)	3(3.8)	5(2.8)		
31-35	1(1)	0(0)	1(0.6)		
36-40	1(1)	2(2.6)	3(1.7)		
41-45	1(1)	1(1.3)	2(1.1)		
Total	98(100)	78(100)	176(100)		

4.2.2.1 Association between participant's age and measles IgM

Table 4.3 present results of associations between participant's age and measles IgM on serum and oral fluid samples. Analysis of the study results, revealed that, 56(31.8%) samples of the participants tested positive, 118(67%) tested negative while the 2(1.1%) of them had indeterminate when their serum was tested for measles IgM across all the age categories. Chi-square test that yielded a p-value of 0.000 hence revealing that there was significant statistical relationship between participants' age categories and measles IgM test results on serum. Further analysis yielded a Kappa mean statistic value of 0.131 showing that there was agreement that there was significant statistical relationship between the participants' age categories and measles serum IgM test results in serum.

On oral fluid samples, 70(39.8%) tested positive, 98(55.7%) tested negative while 8(4.5%) were indeterminate for measles IgM across all the age categories. Chi-square test that yielded a p-value of 0.691 revealing that there was no significant statistical relationship between participants' age categories and measles IgM on oral fluid. It was further confirmed by the Kappa mean statistic value of 0.324 showing that there was an agreement that there was no significant statistical relationship between the participants' age categories and IgM on measles oral fluid samples.

Table 4.3: Analysis of participants' age verse measles IgM on serum and oral fluid

Samples	Age (years)	Test results				Statistics	
		Positive (n) (%)	Negative (n) (%)	Indeterminate (n) (%)	Total (n) (%)	p-value	Kappa
Measles serum IgM	0-5	28(26.2)	78(72.9)	1(0.9)	107(100)	0.000	0.131
	6-10	14(36.8)	24(63.2)	0(0)	38(100)		
	11-15	6(50)	6(50)	0(0)	12(100)		
	16-20	4(50)	4(50)	0(0)	8(100)		
	21-25	1(20)	4(80)	0(0)	5(100)		
	31-35	1(100)	0(0)	0(0)	1(100)		
	36-40	2(66.7)	1(33.3)	0(0)	3(100)		
	41-45	0(0)	1(50)	1(50)	2(100)		
	Total	56(31.8)	118(67)	2(1.1)	176(100)		
Measles oral fluid IgM	0-5	39(36.4)	64(59.8)	4(3.7)	107(100)	0.691	0.324
	6-10	16(42.1)	20(52.6)	2(5.3)	38(100)		
	11-15	7(58.3)	5(41.7)	0(0)	12(100)		
	16-20	4(50)	3(37.5)	1(12.5)	8(100)		
	21-25	2(40)	2(40)	1(20)	5(100)		
	31-35	1(100)	0(0)	0(0)	1(100)		
	36-40	1(33.3)	2(66.7)	0(0)	3(100)		
	41-45	0(0)	2(100)	0(0)	2(100)		
	Total	70(39.8)	98(55.7)	8(4.5)	176(100)		

Table 4.4 Presents analysis results for measles IgM on serum and oral fluid samples. The study showed that, 51(72.9%) samples tested positive for measles IgM on oral

fluid also tested positive when on serum, 18(25.7%) tested negative on serum and 1(1.4%) was indeterminate on serum.

Likewise, 4(4.1%) samples of oral fluid that tested negative for measles IgM tested positive on serum. Other sample [93(94.9%)] tested negative for measles IgM on serum and 1(1%) was indeterminate on serum. One [1(12.5%)] sample that was indeterminate on oral fluid tested positive on serum IgM while 7(87.5%) tested negative for measles IgM on serum. The Chi-square test results that yielded a p-value of 0.000 showing that there was significance statistical relationship between participants the test results of measles IgM both on serum and oral fluid among the participants age categorization. Further analysis yielded a Kappa mean statistic value of 0.000 showing that the agreement that there was statistical relationship between the participants' age categories and Measles IgM test on oral fluid and serum.

Table 4.4: Analysis of measles IgM test results on serum verse oral fluids

Test	**Test results**				**Statistics**	
	Measles oral fluid				**p-value**	**Kappa**
Measles serum	**Positive (n) (%)**	**Negative (n) (%)**	**Indeterminate (n) (%)**	**Total (n) (%)**		
Positive	51(72.9)	4(4.1)	1(12.5)	56(31.8)	0.000	0.000
Negative	18(25.7)	93(94.9)	7(87.5)	118(67)		
Indeterminate	1(1.4)	1(1)	0(0)	2(1.1)		
Total	**70(100)**	**98(100)**	**8(100)**	**176(100)**		

4.2.2.2 Association between participant's age and rubella IgM

Table 4.5 presents results on analysis of participants' age verse measles IgM test results on serum and oral fluid. The study revealed that, 47(26.7%) samples tested positive, 113(64.2%) tested negative while 16(9.1%) were indeterminate for rubella IgM in serum across all the age categories. Chi-square test yielded a p-value of 0.204 revealing that there was no significant statistical association between participants' age categories and rubella IgM on serum.

Kappa mean statistic value was 0.412 hence revealing that there was no significant statistical relationship between the participants' age categories and Rubella IgM test results on serum. There were 40(22.7%) samples which tested positive, 117(66.5%) tested negative while 19(10.8%) were indeterminate for rubella IgM on oral fluid across all the age categories in this study. Chi-square test yielded a p-value of 0.321 showing that there was no significant association between participants' age categories and rubella IgM on oral fluid. The Kappa mean was 0.150 hence showing that there was no significant statistical relationship between the participants' age categories and Rubella IgM on oral fluid.

Table 4.5: Analysis of participants' age verse measles IgM on serum and oral fluid

Samples	**Age (years)**	**Test results**				**Statistics**	
Test		**Positive (n) (%)**	**Negative (n) (%)**	**Indeterminate (n) (%)**	**Total (n) (%)**	**p-value**	**Kappa**
Rubella serum IgM	0-5	28(26.2)	71(66.4)	8(7.5)	107(100)	0.204	0.412
	6-10	12(31.6)	22(57.9)	4(10.5)	38(100)		
	11-15	3(25)	9(75)	0(0)	12(100)		
	16-20	1(12.5)	6(75)	1(12.5)	8(100)		
	21-25	2(40)	2(40)	1(20)	5(100)		
	31-35	0(0)	1(100)	0(0)	1(100)		
	36-40	0(0)	1(33.3)	2(66.7)	3(100)		
	41-45	1(50)	1(50)	0(0)	2(100)		
	Total	47(26.7)	113(64.2)	16(9.1)	176(100)		
Rubella oral fluid IgM	0-5	23(21.5)	72(67.3)	12(11.2)	107(100)	0.321	0.150
	6-10	13(34.2)	21(55.3)	4(10.5)	38(100)		
	11-15	1(8.3)	11(91.7)	0(0)	12(100)		
	16-20	1(12.5)	7(87.5)	0(0)	8(100)		
	21-25	2(40)	2(40)	1(20)	5(100)		
	31-35	0(0)	1(100)	0(0)	1(100)		
	36-40	0(0)	2(66.7)	1(33.3)	3(100)		
	41-45	0(0)	1(50)	1(50)	2(100)		
	Total	40(22.7)	117(66.5)	19(10.8)	176(100)		

Table 4.6 is a cross tabulation of rubella test results on oral fluid verse serum. The results showed that, 35(87.5%) samples from the participants tested positive for rubella IgM on oral fluid and also tested positive for rubella on serum. About 4(10%) samples tested negative for rubella IgM on serum while 1(2.5%) were indeterminate for rubella on serum.

Some [7(6%)] samples tested positive for rubella IgM on serum, 101(86.3%) tested negative for rubella IgM on serum while 9(7.7%) were indeterminate. The Chi-square test results showed that the p-value was 0.000 hence there was significance statistical association between the test results for rubella IgM on both serum and oral fluid. Kappa mean statistic value was 0.000 showing that there an agreement that there was statistical relationship between the participants' age categories and rubella IgM test results on oral fluids and serum.

Table 4.6: Cross-tabulation of rubella test results on oral fluid verse serum

Test	**Test results**				**Statistics**	
	Rubella oral fluid IgM				**p-**	
Rubella IgM	**Positive (n) (%)**	**Negative (n) (%)**	**Indeterminate (n) (%)**	**Total (n) (%)**	**valu e**	**Kap pa**
Positive	35(87.5)	7(6)	5(26.3)	47(26.7)	0.000	0.00
Negative	4(10)	101(86.3)	8(42.1)	113(64.2)		0
Indetermi nate	1(2.5)	9(7.7)	6(31.6)	16(9.1)		
Total	40(100)	117(100)	19(100)	176(100)		

4.2.3 Distribution of respondents per Sub-County

The study was carried out in 15 Sub-Counties within Kenya. Refugees from South Sudan living in Kenya were also captured. The highest samples of 75(42.6%) was reported from Nairobi North, followed by Fafi (10.2%), Lagdera (9.7%), Kakuma (9.1%), Nairobi East (7.4%) and Nairobi West (6.2%). All the other Sub-Counties had below 5% each (Table 4.7).

Table 4.7: Participants distribution per Sub-County

Sub-County	Frequency	Percentage (%)
Nairobi North	75	42.6%
Fafi	18	10.2%
Lagdera	17	9.7%
Kakuma	16	9.1%
Nairobi East	13	7.4%
Nairobi West	11	6.2%
Wajir East	7	4.0%
Wajir South	5	2.8%
Wajir West	4	2.3%
Garissa	4	2.3%
Kaloleni	1	0.6%
Kamukunji	1	0.6%
Nakuru North	1	0.6%
Tana River	1	0.6%
Taveta	1	0.6%
Turkana North	1	0.6%
Total	176	100.0

4.2.4 Vaccination status of the respondents prior to the study

The study population was divided into those who were: vaccinated and non-vaccinated (Table 4.8). Among the participants 49.0% of the male and 46.2% of the females had not been vaccinated. Those who had been vaccinated were 51.0% of males and 53.8% of the female participants. In total 92(52.3%) participants had been vaccinated while 84(47.7%) had not been vaccinated prior to this study. This reveals that less than half (47.3%) of the participants had not been immunized.

Table 4.8: Vaccination status of the participants

Vaccination status	Gender		Total
	Male	Female	

Non-vaccinated	49.0%	46.2%	84(47.7%)
Vaccinated	51.0%	53.8%	92(52.3%)
Total	**100.0%**	**100.0%**	**176(100.0%)**

4.2.5 Rubella and measles prevalence among the vaccinated, non-vaccinated

Among the participants, the prevalence of measles was 13.6% in serum and 17.6% in oral fluid among the vaccinated. Measles prevalence among the non-vaccinated participants was 18.8% in serum and 87.5% in oral fluid. Rubella prevalence was 15.9% in serum and 78.6% in oral fluid among the vaccinated participants. Rubella prevalence among the non-vaccinated was 10.2% in serum and 68.4% in oral fluid. Some of the samples were indeterminate (2.8% measles in serum and 4.5% rubella in serum) for the two disease when tested on the serum. All the samples were sensitive on oral fluid for both measles and rubella (Table 4.9).

Table 4.9: Rubella and measles prevalence among the vaccinated, non-vaccinated

IgM per sample	Vaccinated (%)	Non-vaccinated (%)
Measles IgM in serum	13.6	18.8
Indeterminate Measles IgM in serum	00.0	2.8
Measles IgM in oral fluid	17.6	87.5
Rubella IgM in serum	15.9	10.2
Indeterminate Rubella IgM in serum	00.00	4.5
Rubella IgM in oral fluid	78.6	68.4

4.3 Measles and rubella specific IgM detection among the participants

4.3.1 Measles specific IgM detection among the participants

4.3.1.1 Prevalence of measles among the genders of the participants

The prevalence of measle IGM on serum among the gender of the participants were 32(32.7%) males and 24(30.8%) females. Among the participants 66(67.3%) males

and 54(69.2%) were found to be negative and 1% males and 1.3% females were indeterminate. On the other hand, the prevalence of IGM for measles on oral fluid samples among the participants genders were; 40(40.8%) males and 30(38.5%) females were positive. Those who test negative were; 54(55.1%) males and 44(56.4%) females tested negative while 4(4.1%) males and 4(5.1%) females were indeterminate (Table 4.10).

Table 4.10: Prevalence of measles among the gender of participants

Measles		Male	Female	Total
IGM Serum	Positive	33(32.7%)	24(30.8%0	57(31.8%)
	Negative	64(66.3%0	53(67.9%)	117(67.0%)
	Indeterminate	1(1.0%)	1(1.3%)	2(1.1%)
	Total	100.0%	100.0%	176(100.0%)
IGM Oral fluid	Positive	40(40.8%)	30(38.5%)	70(39.8%)
	Negative	54(55.1%)	44(56.4%)	98(55.7%)
	Indeterminate	4(4.1%)	4(5.1%)	8(4.5%)
	Total	100.0%	100.0%	176(100.0%)

4.3.1.2 Prevalence of measles in different regions among the vaccinated participants

The prevalence of measles in the different district investigated were variable. Fafi and Lagdera Sub-County recorded a prevalence of 13.3%, respectively among vaccinated male participants. There were no vaccinated female participants in the two Sub-Counties. Nairobi north district recorded the highest percentage (male 33.3% and 66.7% female) totalling to 45.8% for both genders. Garissa, Nairobi East, Nairobi West and Nakuru North recorded an average of 6.7% among the male participants while Garissa and Nairobi North had an average prevalence of 11.1% among the female participants (Table 4.11).

Table 4.11: Prevalence of Measles serum IgM among the Vaccinated

Measles IgM	Sub-County	Male	Female	Total

Positive	Fafi	13.3%		8.3%
	Garissa	6.7%	11.1%	8.3%
	Lagdera	13.3%		8.3%
	Nairobi East	6.7%	11.1%	8.3%
	Nairobi North	33.3%	66.7%	45.8%
	Nairobi West	6.7%		4.2%
	Nakuru North	6.7%		4.2%
	Wajir East		11.1%	4.2%
	Wajir West	13.3%		8.3%
	Total	100.0%	100.0%	100.0%
Negative	Fafi	8.6%	9.1%	8.8%
	Kaloleni		3.0%	1.5%
	Lagdera	11.4%	6.1%	8.8%
	Nairobi East	11.4%	12.1%	11.8%
	Nairobi North	54.3%	57.6%	55.9%
	Nairobi West	8.6%	9.1%	8.8%
	Tana River	2.9%		1.5%
	Wajir East		3.0%	1.5%
	Wajir West	2.9%		1.5%
	Total	100.0%	100.0%	100.0%

4.3.1.3 Prevalence of Measles among non-vaccinated participants in different regions

The prevalence of Measles serum 1gM among the non-vaccinated participants were relatively higher compared to the vaccinated individuals. Fafi, Lagdera and Nairobi East recorded an average of 10.5% among non-vaccinated male participants with Lagdera and Nairobi North recording an average prevalence of 15.4% among the female participants. Nairobi North District recorded the highest prevalence (21.1%) among the male participants while Fafi recorded a prevalence of 23.1% among the female participants. Sudanese living in Kenya were all non-vaccinated with a prevalence of 15.8% among the males and 30.8% among the female participants (Table 4.12).

Table 4.12: Prevalence of Measles among the non-vaccinated from different regions

Measles IgM	Sub-County	Sex		Total
		Male	Female	
Positive	Fafi	10.5%	23.1%	15.6%
	Garissa	5.3%		3.1%
	Lagdera	10.5%	15.4%	12.5%
	Nairobi East	10.5%		6.3%
	Nairobi North	21.1%	15.4%	18.8%
	Nairobi West	5.3%		3.1%
	Sudanese living in Kenya	15.8%	30.8%	21.9%
	Turkana North	5.3%		3.1%
	Wajir East		15.4%	6.3%
	Wajir South	10.5%		6.3%
	Wajir West	5.3%		3.1%
	Total	100.0%	100.0%	100.0%
Negative	Fafi	7.1%	13.6%	10.0%
	Garissa		4.5%	2.0%
	Kamukunji	3.6%		2.0%
	Lagdera	10.7%	9.1%	10.0%
	Nairobi East	3.6%		2.0%
	Nairobi North	35.7%	40.9%	38.0%
	Nairobi West	3.6%	9.1%	6.0%
	Sudanese living in Kenya	17.9%	18.2%	18.0%
	Taveta	3.6%		2.0%
	Wajir East	7.1%	4.5%	6.0%
	Wajir South	7.1%		4.0%
	Total	100.0%	100.0%	100.0%
Indeterminate	Nairobi North	100.0%		50.0%
	Wajir South		100.0%	50.0%
	Total	100.0%	100.0%	100.0%

4.3.1.4 Analysis of measles IgM in serum

The Pearson Chi-Square gives the statistical significance levels for measles specific IgM when serum was used for diagnosis when the test is positive 0.395, when negative 0.992 and when indeterminate 0.157. All these values are >0.05. This shows that there is significant association between measles test in serum and oral fluid. It also shows that there was significant association between test results for vaccinated

and non-vaccinated participants with p-value of 0.0375 as shown in Table 4.13.

Table 4.13: Chi square test for Measles IgM in Serum

Vaccination	Measles_Serum_IgM		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Not done	Positive	Pearson Chi-Square	1.548[a]	2	.461	.00	.00
		Likelihood Ratio	1.890	2	.389		
		Linear-by-Linear Association	.001	1	.980		
		N of Valid Cases	32				
	Negative	Pearson Chi-Square	.903[b]	2	.637		
		Likelihood Ratio	.909	2	.635		
		Linear-by-Linear Association	.786	1	.375		
		N of Valid Cases	50				
	Indeterminate	Pearson Chi-Square	2.000[c]	1	.157		
		Continuity Correction[d]	.000	1	1.000		
		Likelihood Ratio	2.773	1	.096		
		Fisher's Exact Test				1.000	.500
		Linear-by-Linear Association	1.000	1	.317		
		N of Valid Cases	2				

Table 4.13: continued

Vaccination	Measles_Serum_IgM		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Done	Positive	Pearson Chi-Square	1.739[e]	1	.187		
		Continuity Correction[d]	.070	1	.792		
		Likelihood Ratio	2.035	1	.154		
		Fisher's Exact Test				.0375	.0375
		Linear-by-Linear Association	1.667	1	.197		
		N of Valid Cases	24				
	Negative	Pearson Chi-Square	3.252[f]	2	.197		
		Likelihood Ratio	3.389	2	.184		
		Linear-by-Linear Association	2.176	1	.140		
		N of Valid Cases	68				

4.3.2 Prevalence of specific rubella virus IgM among the participants

4.3.2.1 Prevalence of rubella among the genders of the participants

The prevalence of Rubella IGM in serum revealled that; 23(23.5%) males and 24(30.8%) females were positive, 69(70.4%) males and 44(56.4%) females were negative while 6(6.1%) males and 10(12.8%) females were indeterminate. The prevalence of rubella in oral fluid among the participants when compared in terms of gender revelled that; 24(24.5%) males and 17(21.8%) females were positive. Those who were negative for rubella virus IgM were; 67(68.4%) males and 50(64.1%) females while 7(7.1%) males and 11(14.1%) females were indeterminate (Table 4.14).

Table 4.14: Prevalence of Rubella among the sexes of the participants

Rubella		Sexes		Total
		Male	Female	
IGM serum	Positive	23(23.5%)	24(30.8%)	47(26.7%)
	Negative	69(70.4%)	44(56.4%)	113(64.2%)
	Indeterminate	6(6.1%)	10(12.8%)	16(9.1%)
	Total	100.0%	100.0%	176(100.0%)
IGM Oral Fluid	Positive	24(24.5%)	17(21.8%)	41(23.3%)
	Negative	67(68.4%)	50(64.1%)	117(66.5%)
	Indeterminate	7(7.1%)	11(14.1%)	18(10.2%)
	Total	100.0%	100.0%	176(100.0%)

4.3.2.2 Prevalence of rubella in different regions among vaccinated participants

Among the participants 20% of vaccinated females from Fafi sub-county tested positive for Rubella virus. Lagdera and Nairobi East sub-counties recorded a prevalence of 11.1% each among the male participants with the lowest prevalence of 5.6% from vaccinated male participants from Nairobi wets and Wajir west. Nairobi North recorded the highest prevalence of 66.7% among the males and 60.0% among the females. The region recorded recorded the highest prevalence was Nairobi North (64.3% in total) while Wajir recorded the least prevalence of 3.6% among the vaccinated participants. The results for the negative test and indeterminate among vaccinated male and female were few compared to the positive test as shown in Table 4.15.

Table 4.15: Rubella prevalence in different regions among vaccinated participants

Rubella IgM	Sub- County	Sex		Total
		Male	Female	
Positive	Fafi		20.0%	7.1%
	Lagdera	11.1%		7.1%
	Nairobi East	11.1%	10.0%	10.7%
	Nairobi North	66.7%	60.0%	64.3%
	Nairobi West	5.6%	10.0%	7.1%
	Wajir West	5.6%		3.6%
	Total	100.0%	100.0%	100.0%
Negative	Fafi	17.2%	3.4%	10.3%
	Kaloleni		3.4%	1.7%
	Lagdera	13.8%	6.9%	10.3%
	Nairobi East	10.3%	13.8%	12.1%
	Nairobi North	34.5%	58.6%	46.6%
	Nairobi West	10.3%	6.9%	8.6%
	Nakuru North	3.4%		1.7%
	Tana River	3.4%		1.7%
	Wajir East		6.9%	3.4%
	Wajir West	6.9%		3.4%
	Total	100.0%	100.0%	100.0%
Indeterminate	Garissa	33.3%	33.3%	33.3%
	Nairobi North	66.7%	66.7%	66.7%
	Total	100.0%	100.0%	100.0%

4.3.2.3 Prevalence of rubella in different regions among non-vaccinated participants

The prevalence of rubella virus among the non-vaccinated participants was 11.1% from Fafi, Nairobi West, Taveta, Wajir east and Wajir south and Sudanese living in Kenya, respectively. Nairobi North recorded the highest prevalence among the non-vaccinated male (33.3%) and females (40.0%). Participants positive for rubella virus among the non-vaccinated cohort were few compared to those who tested negative and indeterminate (Table 4.16).

Table 4.16: Rubella prevalence in different regions among non-vaccinated participants

Rubella IgM	Sub- County	Sex		Total
		Male	Female	
Positive	Fafi	11.1%	10.0%	10.5%
	Garissa		10.0%	5.3%
	Lagdera		20.0%	10.5%
	Nairobi North	33.3%	40.0%	36.8%
	Nairobi West	11.1%	10.0%	10.5%
	Sudanese living in Kenya	11.1%		5.3%
	Taveta	11.1%		5.3%
	Wajir East	11.1%	10.0%	10.5%
	Wajir South	11.1%		5.3%
	Total	100.0%	100.0%	100.0%
Negative	Fafi	9.1%	18.2%	12.7%
	Garissa	3.0%		1.8%
	Kamukunji	3.0%		1.8%
	Lagdera	12.1%	4.5%	9.1%
	Nairobi East	6.1%		3.6%
	Nairobi North	33.3%	31.8%	32.7%
	Nairobi West	3.0%	4.5%	3.6%
	Sudanese living in Kenya	15.2%	31.8%	21.8%
	Turkana North	3.0%		1.8%
	Wajir East	3.0%	4.5%	3.6%
	Wajir South	9.1%	4.5%	7.3%
	Total	100.0%	100.0%	100.0%
Indeterminate	Fafi		25.0%	10.0%
	Lagdera	16.7%	25.0%	20.0%
	Nairobi East	16.7%		10.0%
	Nairobi North	16.7%		10.0%
	Sudanese living in Kenya	33.3%	25.0%	30.0%
	Wajir East		25.0%	10.0%
	Wajir West	16.7%		10.0%
	Total	100.0%	100.0%	100.0%

4.3.2.4 Chi square test for Rubella IgM in serum

Analysis of results revealed that there was significant association between the rubella IgM in serum test results for vaccinated and non-vaccinated participants in these study. The p-values were 0.00 (non-vaccinated) and 0.05(vaccinated), since they are

less than 0.05, then they are significant at 95% confidence interval. The chi square rest are presented in table 4.17

Table 4.17: Chi square test for Rubella IgM in serum among the participants

Non-Vaccinated	Rubella serum IgM		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
	Positive	Pearson Chi-Square	2.647[a]	2	.266		0.00
		Likelihood Ratio	3.419	2	.181		
		Linear-by-Linear Association	2.151	1	.142		
		N of Valid Cases	19				
	Negative	Pearson Chi-Square	1.615[b]	2	.446		
		Likelihood Ratio	1.950	2	.377		
		Linear-by-Linear Association	.625	1	.429		
		N of Valid Cases	55				
	Indeterminate	Pearson Chi-Square	.972[c]	2	.615		
		Likelihood Ratio	1.323	2	.516		
		Linear-by-Linear Association	.042	1	.838		
		N of Valid Cases	10				

Table 4.17: continued

Vaccinated	Rubella serum IgM		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
e	Positive	Pearson Chi-Square	.745[d]	3	.863		
		Likelihood Ratio	1.065	3	.785		
		Linear-by-Linear Association	.083	1	.773		
		N of Valid Cases	28				
	Negative	Pearson Chi-Square	2.352[e]	2	.308		
		Likelihood Ratio	3.131	2	.209		
		Linear-by-Linear Association	1.775	1	.183		
		N of Valid Cases	58				
	Indeterminate	Pearson Chi-Square	.667[f]	1	.414		
		Continuity Correction [g]	.000	1	1.000		
		Likelihood Ratio	.680	1	.410		
		Fisher's Exact Test				1.000	0.0500
		Linear-by-Linear Association	.556	1	.456		
		N of Valid Cases	6				

4.3.3 Comparison of measles and rubella oral fluid and serum

The prevalence of measles in serum was 31.8% while that in oral fluid was 39.8%. The prevalence of rubella on the other hand, was 26.7% in serum and 23.3% in oral fluid. The number of indeterminate samples was 1.1% for measles serum IgM and 9.1 % for rubella serum IgM. An indeterminate result means that the test kit shows a weak positive reaction, perhaps because of an unknown antibody cross-reaction, because of cross-contamination with another person's sample or because of another technical error. The results are as shown in Table 4.18.

Table 4.18: Oral fluid and serum prevalence comparison

Out come	Measles		Rubella	
	Serum	Oral fluid	Serum	Oral fluid
Positive	56 (31.8)	70 (39.8)	47(26.7)	41(23.3)
Negative	118 (67.0)	98 (55.7)	113 (64.2)	117 (66.5)
Indeterminate	2 (1.1)	8 (4.7)	16 (9.1)	18 (10.2)
Total	176	176	176	176

Key: Figures in brackets are percentages

4.4 Sensitivity and specificity

4.4.1 Sensitivity and specificity for measles

4.4.1.1 Measles test

Calculations based on results obtained from Table 4.4 for measles tests are:

Expected agreement

$$p_e = [(n_1/n) * (m_1/n)] + [(n_0/n) * (m_0/n)]$$

$$= [(69/183) * (69/183)] + [(114/183) * (114/183)]$$

$$= 0.53$$

Therefore, the observed agreement is simply the percentage of all tests for which both serum and oral fluid evaluations agree, which is the sum of a + d divided by the total n yields,

$$P_0 = (a + d)/n$$

$$= (66 + 111)/183$$

$$= 0.64$$

Therefore,

$$\text{Kappa, } K = \frac{P_0 - P_e}{1 - pe} = = \frac{0.64 - 0.53}{1 - 0.53} = 0.26$$

From the Kappa, K value = 0.26 it can be concluded that there was a fair agreement that Measles Oral Fluid cannot be used as an alternative Specimens for Measles Serum.

The gold starndard test for measles is normally serum which was used to determine sensitivity and specifity of using oral fluid to detect measles. Table 4.19 gives these results.

Table 4.19: Sensitivity and specificity for measles

Test	**Outcome**	**Positive**	**Negative**
Measles Tests in serum	Positive	66 (true positive)	3 (false positive)
	Negative	3 (false negative)	104 (true negative)
Total		**69**	**107**

The results showed that 62.7% tested negative while 37.3% tested positive. For the oral fluid 61% of the participants tested negative while 39% tested positive. Sensitivity showed that 96% of those who tested positive with measles serum tested positive again when they were later tested with measles oral fluid. Specificity showed that 97% of those who tested negative when they were tested with measles serum again tested negative when they were later tested with measles oral fluid.

$$\text{Positive Predictive } Value = \{a/(a + b)\}\, x100$$

$$= \{66/(66 + 3)\}\, x100$$

$$= 96\%$$

Of the participants who tested positive, 96% of them had measles infections against 4% of them who were not infected at the time of testing.

$$\text{Negative Predictive } Value = \{d/(c + d)\}\, x100$$

$$= \{111/(3 + 111)\}\, x100$$

$$= 97\%$$

Of the participants who tested negative, 97% of them did not have measles infections against 3% of them who had infections (Table 4.20).

Table 4.20: Sensitivity and specificity for Measles virus

Measles test	**Serum**	**Oral fluid**
Sample size	176	176
Sensitivity (%)	96	96
95% CI	0.05	0.05
Specificity (%)	97	97
NPV	97	97
PPV	96	96

*NPV- Negative predictive values, PPV- Positive predictive values, CI – Confidence Interval

4.4.2 Sensitivity and specificty for rubella

4.4.2.1 Rubella test

Calculations based on results obtained from Table 4.6 for rubella tests are:

$$p_e = [(n_1/n) * (m_1/n)] + [(n_0/n) * (m_0/n)]$$

$$= [(64/195) * (64/195)] + [(131/195) * (131/195)]$$

$$= 0.56$$

Therefore,

The observed agreement is simply the percentage of all tests for which the two test

evaluations agree, which is the sum of a + d divided by the total n in the Table below.

$$P_0 = (a + d)/n$$

$$= (55 + 122)/195$$

$$= 0.91$$

Therefore,

$$\text{Kappa, } K = \frac{P_0 - P_e}{1 - pe} = = \frac{0.91 - 0.56}{1 - 0.56} = 0.80$$

From the Kappa K value of 0.80, it can be deduced that there was Substantial agreement that Rubella Oral Fluid cannot be used as an alternative Specimens for Rubella Serum.

Serum test for rubella showed that 68.9% of the participants tested negative while 31.1% tested positive. Oral fluid on the other hand revealed that 63.8% tested negative while 36.2% tested positive (Table 4.21).

Table 4.21: Sensitivity and specificty for rubella (n=176)

Test	Outcome	Positive	Negative
Rubella Tests in serum	Positive	55 (true positive)	9 (false positive)
	Negative	9 (false negative)	103 (true negative)
Total		64	112

Sensitivity reveals that 86% of the participants who tested positive Rubella Serum again tested positive with Rubella Oral Fluid. Specificity revealed that 93% of the participant who tested negative with Rubella Serum again tested negative with Rubella Oral Fluid.

$$\text{Positive Predictive } Value = \{a/(a + b)\}\, x\, 100$$

$$= \{55/(55 + 9)\}\, x100$$

$$= 86\%$$

Of the participants who tested positive, 86% of them had Rubella infections against 14% of them who did not have Rubella infections.

$$Negative\ Predictive\ Value\ = \{d/\ (c\ +\ d)\}\ x100$$

$$= \{122/\ (9 + 122)\}\ x100$$

$$= 93\%$$

Of the participants who tested negative, 93% of them did not have Rubella infections against 7% of them who had Rubella infections (Table 4.22).

Table 4.22: Sensitivity and specificity for rubella virus test in oral fluid

Test	**Outcome**	**Positive**	**Negative**
Rubella Tests in oral fluid	Positive	66 (true positive)	3 (false positive)
	Negative	3 (false negative)	104 (true negative)
Total		**69**	**107**

CHAPTER FIVE

DISCUSSION

5.1 Participants characteristics

The data was collected from Fafi, Garissa, Kaloleni, Kamkunji, Lagdera, Turkana North, Nairobi East, Nairobi West, Nairobi South, Tana River, Nakuru North, Sudanese living in Kenya, Taveta, Wajir East, Wajir West and Wajir South. The majority of the participants (42.6%) were from Nairobi North and Kaloleni while Kamkunji, Nakuru North, Tana River, Taveta and Turkana North comprised less than 1% each with other regions falling in between this range.

The study revealed that there were more male (55.7%) than female (44.3%) participants in this study. These shows that males were vulnerable to measles/rubella disease compared to the female. When compared in terms of the specimen used for diagnosis, the study showed that, the prevalence of measle IGM on serum were 32.7% among the males and 30.8% among the females. On the other hand, the prevalence of IGM for measles on oral fluid was 40.8% among the males and 38.5% among the females. The prevalence of Rubella IGM in serum among the males was 23.5% and 30.8% among the females. The prevalence of rubella in oral fluid was 24.5% among the males and 21.8% among the females. These results implies that males are more vulnerable to both measles and rubella viruses. The oral fluid and serum gives comparable results when measles/rubella IgM were tested. In this study, Chi-square test revealed that there was no significant statistical relationship between the participants' age categories and their gender. A Kappa value of 0.507 showed that there was no significant association between different age groups and the gender in relation to measles and rubella infection although there were more males compared to female participants in this study.

The results of this study has shown that most (60.8%) of the participants were aged 0-5 years. People of young age are the most at risk of contracting measles disease due to their naïve immune system. Also young aged are known to have a low immune system since it is still developing. Therefore, their immune system is

compromised due to the young age whether vaccinated or non-vaccinated as evidenced by the results of this study. The prevalence of measles among the age groups was inversely proportional to the age of the participants. These means that the prevalence reduced with age from 60.8% at age 0-5 years to 1.1% at age 41-54 years in this study.

Majority (67.0%) of the participants tested negative for measles IgM in serum samples. There was p-value of 0.000 on Chi-square test. Therefore, there was significant associations between participants' age categories and measles IgM test results on serum. There was a Kappa mean statistic of 0.131 hence there was significant relationship between the participants' age categories and measles serum IgM test results in serum. The results of this study implies that age is a contributing factor to measles acquisition. On the other hand, the test results for measles IgM on oral fluid samples was 39.8% positivity, 55.7% negativity across all the age categories. The p-value was 0.691 according to Chi-square test, thus there was significant difference between participants' age categories and measles IgM on oral fluid. A Kappa mean statistic value of 0.324 confirmed that there was no significant association between the participants' age categories and IgM on measles oral fluid samples. The results means that oral fluid can be used as an alternative to serum although serum still remains the preferred sample for measles diagnosis.

According to the results of this study, rubella prevalence was not proportional to the age of the participants. These mean that anybody can acquire rubella virus regardless of the age. It was further confirmed by Chi-square test with a p-value of 0.204 showing that there was significant difference between participants' age categories and rubella IgM on serum and oral fluid (p=0.321). Kappa mean statistic value of 0.412 for rubella IgM on serum and 0.150 on oral fluid hence revealing that there was no significant statistical relationship between the participants' age categories and Rubella IgM.

Comparison of serum and oral fluid as diagnostic sample for rubella, the Chi-square test showed that there was significance statistical association (p=0.000) between the test results from the two types of samples. Further analysis by Kappa mean statistics

(0.000) confirmed that there was indeed statistical relationship between the participants' age categories and rubella IgM test results on oral fluids and serum. Hence in this case, oral fluid can replace serum in rubella IgM diagnosis.

The study investigated the prevalence of measles in different Districts of Kenya currently Sub-Counties. The prevalence of measles among the vaccinated participants was between 4.2% in Nairobi west, Nakuru North and Wajir East being the least and Nairobi North 45.8% being the highest. The measles affected more males than females in all the Districts/Sub Counties investigated in this study. The results implies that measles vaccine is not 100% protective against the virus. Nairobi north district recorded the highest percentage (male 33.3% and 66.7% female) because the region is occupied by refugees from Somalia and Southern Sudan. Vaccination might not have been effective among these populations because of conflicts in their countries. Vaccine viability are affected by different factors such as storage temperature, dose and time. Hence these population could have received expired vaccines or they did not complete the doses due to political instabilities in their countries.

The prevalence of Measles serum IgM among the non-vaccinated participants were relatively higher compared to the vaccinated individuals. Nairobi North District and a group denoted Sudanese living in Kenya had were among the non-vaccinated with a high prevalence of measles among the participants. In this study, the Sudanese living in Kenya (refugees) were all non-vaccinated with a total prevalence of 21.9% and 15.8% among the males and 30.8% among the female. The prevalence of measles among the non-vaccinated individuals was relatively high compared to vaccinated individuals in the different study sites in this study. These shows that although measles vaccine is not 100% protective against the virus, it has some degree of protection. The study also revealed that a person living in Nairobi North is most likely to suffer from measles disease compared to the other studied regions. It is also true to say that due to non-vaccination practise among the Sudanese living in Kenya, they are highly vulnerable to measles virus infection.

Rubella virus prevalence among the vaccinated participants was high compared to

negative and indeterminate results combines. An indeterminate result means that the lab cannot tell for sure if the results is either positive or negative. If an indeterminate result is not reproduced in other tests, it is almost certainly simply a false positive reaction. However, if more than one type of test gives an indeterminate result, it will be necessary to retest with several types of test. In this study, Nairobi North recorded the highest prevalence of 64.3% for the two genders while Wajir had the lowest prevalence (3.6%) among the vaccinated participants in all the Sub-Counties in this study. Nairobi North Sub-County is inhabited by Somalis hence the high prevalence since vaccination is not widely practised in their country due to political instability. The prevalence of rubella virus among the non-vaccinated participants in this study showed that, Nairobi North Sub-County was leading with 38.6%. Vaccination against different pathogens should be protective but, in this study, however, this study has showed that the prevalence of Rubella among the vaccinated participants was high as compared to the non-vaccinated participants.

With regards to immunization, the study found out that a total number of 92 participants had been vaccinated while 85 of them had not been vaccinated prior to this study. This implies that there was poor immunization coverage in the study areas. Some of the study areas had populations which originate from conflict endemic countries such as Somali and Southern Sudan where immunization is a challenge due to political instability. In Kenya and the world at large, children are immunized against measles, mumps and rubella at 40 weeks of birth according to world health organization standards WHO (2005). Immunization coverage is very poor in area where there are inadequate health facilities, coupled with political instability.

In most countries, based on the previous diagnosis, measles/rubella is diagnosed based on clinical criteria. However, with low immunization rates, the numbers of mild or asymptomatic infections increased and medical personnel have less capacities and expertise in diagnosing measles/rubella. In the current study the prevalence of measles and rubella using serum was 31.8% and 26.7%, respectively while the prevalence using oral fluid was 39.8% and 23.3%, for measles and rubella respectively. Miss diagnosis can lead to inappropriate vaccination campaigns and

wasted resources or missed opportunities to prevent transmission. Therefore, laboratory confirmation has become important to measles control programs in the world according to studies of Helfand *et al.,* (2007). Generally, the current laboratory methods that exist depend mostly on the detection of significant rises in measles or rubella antibody or the detection of Measles/rubella antibodies in blood. Therefore, saliva detection of measles specific IgM antibody is not practical for widespread use in children (WHO, 2009). However, the results of the current study have proved that saliva can be used for diagnosis since there was significant association between the prevalence of measles and rubella when tested using serum and oral fluid samples among the participants. Blood collection requires specially trained staff and sterile equipment, which restricts its widespread use. Therefore, the use of saliva/oral fluid which is a non-invasive method for confirming measles infection could have an important role in surveillance in communities with limited measles/rubella transmissions (Helfand *et al.,* 2008) as confirmed by the results of the current study.

5.2 Measles and/or rubella-virus specific IgM in serum and oral fluid specimens

Laboratory diagnosis of acute measles/rubella is usually achieved by serology assays for measles/rubella specific IgM antibody. For comparison of measles/rubella specific IgM antibody in oral fluid and serum, 176 paired blood and fluids samples from the oral cavity were collected 1-28 days after the onset of rash. Hence in agreement with a study by Talat *et al.,* (2003) who took blood and saliva of suspected cases of measles/rubella 1-14 days after rash onset. The prevalence of specific IgM for Measles antibodies in serum was noted to be high in Nairobi North District amongst the vaccinated group which was 45.8%. The highest amongst the non-vaccinated in serum was 21.9% amongst the Sudanese living in Kenya. For the oral fluid the total prevalence amongst the non-vaccinated was 87.5% while the vaccinated group recorded a prevalence of 17%. Measles specific IgM was detected in 31.8% for serum and 39.8% in oral fluid. The number of measles seropositive was high when tested using oral fluid as compared to serum. This study indicates that oral fluid is more feasible in detecting measles IgM than the serum. In other studies, done by Oliveira et al., (1998) which compared IgM antibody concentration in serum and saliva according to the days after rash onset found out that the first 5 days

following onset of rash, infection was verified by salivary test in 88.7% measles cases. However, between 6 and 14 days after rash onset, virus-specific IgM was detected in 100% of saliva specimens, demonstrating the high sensitivity of salivary IgM antibody detection as compared to serum. In this study the specimen was collected 1 to 28 days after the appearance of the rash and the results compares well with studies done by Helfand *et al.,* (1996) who found out that the optimal time for collection of samples was 1 to 4 weeks after onset of rash hence in agreement with the findings of the current study. The IgM capture tests for measles specific antibodies are often positive on the 4^{th} day of the rash onset. However, in the first 72 hours after rash onset, up to 30% of tests for IgM antibody may give false negative results. Some samples gave inconclusive results also referred to as indeterminate when serum (1.1%) was tested 4.5% on oral fluid for measles IgM. The indeterminate could be as results of poor specimen transportation and storage, some participants reported the case 28 days after rash appearance or onset causing the IgM not to be captured by the test kit. The discordant pairs of oral fluid and serum specimen, this study may have resulted from samples being collected too early in the course of the IgM antibody response. In addition to optimizing time for specimen collection, ingestion of water may affect oral fluid results as well and this resulted in high percentage of indeterminate when the oral fluid was used for diagnosis. Talat *et al.,* (2003) in his study found that one of the cases who had a positive result for serum sample, had drank water 2 minutes before collection of saliva specimen which could explain the presence of indeterminate results in the current study although the questionnaire did not investigate water uptake before sample collection. It is possible that her saliva was diluted with water hence could either give an indeterminate or a negative result depending on the degree of dilution. Rubella and parvovirus B19 seem to be responsible for a few misdiagnosed cases of measles in the United Kingdom and other infectious causes of measles-like illness need to be sought (Robertson *et al.,* 2003; CDC 2002), which could also true for this study, although this study did not test whether the participants had been infected with both Rubella and parvovirus B19.

The serum and oral fluid were also tested for specific IgM antibodies for rubella virus; the study captured vaccinated and non-vaccinated participants. The non-

vaccinated and vaccinated prevalence of specific Rubella IgM in serum was high in Nairobi North District, 36.8% and 64.3%, respectively. The prevalence of specific Rubella IgM in serum was low in the Sudanese living in Kenya. The number of the participant's vaccinated amongst this group was zero. The total prevalence of specific Rubella IgM in serum and oral fluid was 26.7% and 23.3%, respectively. About 3.4% of those who were seropositive turned out to be negative when the oral fluid was tested. This result compares well with studies done by Oliveira *et al.,* (2008) found out that of all seropositive for rubella 84% of their saliva were rubella IgM positive. The number of those who were indeterminate when tested using serum were 9.1% while oral fluid were 10.2%. The indeterminate were rerun twice so as to confirm their validity. In this case the indeterminate results were because of the delay in reporting time by the participants hence leading to antibody conversion from IgM to IgG. Direct detection also enables diagnosis of cases of IgG-positive participants as re infections after natural immunity or secondary vaccine failures which develop with no IgM production. According to a study done by Castillo *et al.,* (2002) a rate of 63.6% of the sera were positive by PCR, in contrast to another report that showed amplification in only 24% of cases. Again, early sampling could account for this difference (CDC, 2005).

A total number of 92 (52.3%) of the participant had been vaccinated while 84 (47.7%) had not been vaccinated prior to the study. Some of the participants who had been vaccinated against the two disease turned out to be positive because the vaccine administered was either not effective or due to poor immunization response. The Sudanese living in Kenya had not been vaccinated at all this is because of poor vaccination coverage and surveillance in their country. Kalou (2014) in their study found out that virus-specific IgM antibody was present in 91% of saliva samples collected 2 weeks after measles vaccination therefore their results are in agreement with the findings of this study.

In the current study, both measles and Rubella Specific IgM in serum and oral fluid were tested and compared. More than half of suspected measles case turned out to be rubella cases. Hence in agreement with a study by Robertson *et al.,* (2003) who found that approximately 30 to 50% of suspected measles cases turn out to be rubella

cases. Timing of specimen collection is an important factor in determining whether viral specific IgM will be detected, however this factor was not put into consideration in this study because all the specimen was collected within the range specified by WHO. The mildness of the majority of measles/rubella cases makes participants reluctant to seek medical assistance.

Asymptomatic rubella cases denoting outbreaks in developing countries may occur even in the communities which are being monitored. Alternative specimen for rubella diagnosis is oral fluid which comes handy since it has several advantages over serum or plasma specimen. It is more acceptable to majority of participants and applicable to children, (Masresha *et al.*, 2018, 1996) reuse of disposable equipment is avoided and occupational risk from needle stick injuries is eliminated (Tarigan *et al.*, 2015). Detection of IgM anti-body in saliva samples for virus diagnosis has mostly been performed by oral mucosal transudate (Kalou 2014; Kingsbury. 2007). Kalou (2014) indicated that using whole saliva as a diagnostic medium had shown good sensitivity and specificity although the current findings indicted otherwise. Talat *et al.*, (2003) in a similar study demonstrated that saliva from the oral cavity of participants contains specific IgM antibodies in the right concentrations which were high enough to be used for diagnosis.

Irrespective of the advantages offered by oral IgM assays, this study identified that many cases of rubella and measles would go undetected until later stages when applying oral assays. This is because there is less concentration of ribonucleic acid paramyxovirus in saliva than in serum. This implies that oral assays should be used together with other specimen when diagnosing measles and rubella but should only be used as an alternative specimen for detecting measles/rubella specific IgM. Although the results of this study support the use of oral fluid specimens for diagnosing measles/rubella, there is need to optimize sampling time to detect IgM antibody after rash onset. The discordant pairs of oral fluid and serum samples data in the study may have resulted from samples being collected too early or too late in the course of the IgM antibody response. In this study the specimen was collected from the participants almost 4 to 28 days from the time the rashes appeared on their skins. On average the entire population sample took 4.98 days to test for the diseases,

a figure that falls in between the 95% confidence interval with the lower bound of 4.49 days and 5.48 days. The 4 days was the median number of days an individual took between the day of rashes appearance and the day of specimen collection. The findings of this study are in agreement with the work done by Helfand *et al.,* (2007) which showed that serum of persons suspected for Rubella Specific IgM increased from 76% in the first three days after rash onset to 88% from day 4 to 28 after rash onset. The large number of undetected cases using saliva could be because the study was conducted or samples were collected soon after the appearance of rash as participants sought medical assistance not later than five days after the onset of rash.

There was significant association between the test results of measles IgM in serum and oral ($p<0.005$) fluid in this study. These support the fact that oral fluid ca be used as an alternative to serum in measles surveillance and diagnosis. The study also showed that the prevalence of measles among the vaccinated and no-vaccinated had significant associations ($p=0.0375$). Chi square test showed that there was significant associations between the rubella IgM in serum test results for the participants who had been vaccinated ($p=0.05$) and those who had not been vaccinated ($p=0.00$). These imply that measles and rubella vaccination is not 100% protective against the virus. The study population in this study were vulnerable to measles/rubella disease since majority originated from regions where vaccination coverage is very poor.

5.3 Sensitivity and specificity for measles/rubella-virus specific IgM oral fluid

Sensitivity and specificity for measles IgM when tested on both serum and oral fluid were 96% and 97%, respectively. Rubella sensitivity and specificity for the two tests were 86% and 93%, respectively. In this study both sensitivity and specificity of serum and oral fluid were in agreement. In a study carried out in Ethiopia by Nokes *et al.,* (2001) they revealed that the sensitivity and specificity generally were 97% and 87% for measles and 79% and 90% for rubella. There was agreement between oral-fluid and serum results for 96% of observations for measles and 81% for rubella.

In each instance the difference between the agreement expected by random processes and that observed was statistically significant. In the current study the two test were not statistically significant ($t<0.05$). Talat et al., (2003) also found similar results for both saliva and serum measles/rubella specific IgM ($p<0.05$). The negative and positive predictive values depend on the prevalence of the disease in the population and therefore are useful in providing clinical guidance to the participants and clinicians. In this study specificity and sensitivity were the same as the negative and positive predictive values. These implies that the test samples are reliable and can be used interchangeably.

The finding of the current study has revealed that fluids from the oral cavity can be used as an alternative sample to serum during diagnosis of recent measles/rubella illness. The oral fluid specimen is accepted widely, therefore should be utilized in facilitating investigation of measles/rubella outbreak. It should also aid in disease control both in national and regional public health laboratories globally. According to Holm-Hansen *et al.,* (2010) the use of alternative body fluids has other advantages: reluctance to submit to venepuncture is circumvented, reuse of disposable equipment is avoided, and the occupational risk from needle stick injuries is eliminated. Culture and religious beliefs of some traditions do not accept blood sample collection from the veins and sometimes there are vein problems after venepuncture hence additionally increases the difficulty in getting samples for testing (Holm-Hansen 2010).

This study hypothesis was to determine the possibility of specific IgM for rubella and measles in oral fluid assays can be used as an alternative to Rubella and Measles Serum IgM assays in the first four days after the rash onset. Kappa was used to check the association or relationship of the two set of test that is oral fluid verse serum in detecting measles/rubella antibodies. The calculation is based on the difference between how much agreements were actually present ("observed" agreement) compared to how much agreement would be expected to be present by chance alone ("expected" agreement) is determined.

Kappa, K value = 0.26 was obtained between oral fluid and serum test for measles.

This shows that there was a fair agreement that Measles Oral Fluid can be used as an alternative Specimens for Measles Serum. Fair agreement means the specimen oral fluid can be used as an alternative specimen to serum for diagnosis of measles virus. For Rubella, the Kappa K value = 0.80 was obtained showing that there was good agreement that Rubella Oral Fluid can be used as an alternative Specimens for Rubella Serum. It was found out that Rubella had a higher level of agreement in the detection of IgM than in measles. From these results, it can therefore be concluded that oral fluid can be used as an alternative to Rubella and Measles serum IgM assays. Similar conclusions were reported by Piacentini *et al.,* (1993) who obtained kappa coefficient of 1.0 indicating an excellent agreement of serum and saliva results which suggested that oral fluid can be used as a diagnostic tool for recent HBV infection. Further, Amado *et al.,* (2006) obtained kappa coefficient of 0.78, in total anti-HAV antibody assay, which indicated that the agreement between serum and oral fluid was good.

The existing national surveillance system for measles/rubella, which relies on clinically diagnosed cases, lacks the precision required for effective disease control. Oral fluid is a valid alternative to serum for IgM detection, and salivary diagnosis could play a major role in achieving measles elimination as evidenced by the result of this study. This study therefore highlights the need of incorporating a simple assay such as enzyme linked immunosorbent assay for salivary measles/rubella IgM for public health laboratories nationwide. Timing of specimen collection is an important factor in determining whether viral specific IgM will be detected, however this factor was not put into consideration in this study because all the specimen was collected within the range specified by WHO.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

On average the entire population sample took 4.98 days after the onset of the rash to test for the diseases. Among the participants 52.3% had received vaccination while 39.8% had not been vaccinated and 7.9% did not know their vaccination status prior before the commencement of the study. Slightly more males 98 (55.7%) than females 78 (44.3%) participated and they were of different age groups. Therefore, male gender according to the results of this study is a risk factor to measles/rubella virus. Vaccination is not 100% protective against measles/rubella virus since some participants had been vaccinated yet their specimen was positive for the virus.

1. The result presented in this study indicates that fluids from the oral cavity referred to as oral fluid specimen can be a good substitute for serum in measles and rubella surveillance in Kenyan setting. In this study it is true to say that the participants who were seropositive for rubella were seronegative for measles and vice versa.
2. Sensitivity and specificity for measles IgM when tested on both serum and oral fluid were 96% and 97%, respectively. Rubella sensitivity and specificity for the two tests were 86% and 93%, respectively. Therefore, the hypothesis that - Oral fluid cannot be used as alternative specimens to serum for detection of measles/rubella virus infection using measles/rubella 1gM enzyme immunoassay is rejected in favour of the alternative hypothesis.
3. The chi square test revealed that all the p-values were less than 0.05 hence the study reject the null hypothesis in favour of the alternative that state that Oral fluid can be used as alternative specimen to serum for detection of measles/rubella virus infection using measles/rubella IgM enzyme immunoassay.

6.2 Recommendations

1. The findings provided is useful information to improve efficiency of the disease surveillance system in Kenya. The useful information from this study is that oral fluid can be used as an alternative to serum in measles/rubella diagnosis and surveillance.
2. There is need to optimize sampling time from the time of clinical presentation for serum and oral fluid to detect measles/rubella specific IgM antibody after rash onset which was not done in this study. This can assist reduce the number of indeterminate samples.
3. A study to monitor temperature for oral fluid over long periods of transportation and also to determine possibility of not using cold chain for this specimen is recommended to be undertaken.

There is need to optimize sampling time from the time of clinical presentation for serum and oral fluid to detect measles/rubella specific IgM antibody after rash onset which was not done in this study. This can assist reduce the number of indeterminate samples.

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APPENDICES

Appendix 1: Consent form

Introduction

Hallo. I am Rose Chelangat from Jomo Kenyatta University of Agriculture and Technology. This information form seeks informed consent for your participation in a study that seeks to determine dried blood spot and oral fluid as an alternative specimen to serum in the detection of rubella virus-specific IgM by enzyme immunoassay test.

Project title: ORAL FLUID AS ALTERNATIVE SPECIMENS TO SERUM IN THE DETECTION OF MEASLES/RUBELLA VIRUS-SPECIFIC IgM BY ENZYME IMMUNOASSAY A program in Kenya

Purpose

This study is being done to:

1. To determine the demographic and clinical characteristics of the study participants
2. To detect measles and/or rubella-virus specific IgM in simultaneously collected paired serum and oral fluid specimens obtained from each study participant
3. To determine the sensitivity and specificity of using oral fluid to detect measles and/or rubella-virus specific IgM relative to the serum gold standard

Procedure

If you agree to participate or your child to participate for the ones under 18 years in this study, you will receive an identification number. A trained interviewer will ask questions pertaining to the study for two minutes. Trained laboratory personnel will collect 2mililiters of blood and a swab of saliva until the pad is socked. The procedure will be like the way you brush your teeth but this will be done on your gums and cheek. This is after consent/assent has been obtained.

Benefits of participation

There is no direct benefit to the Participant but the findings will provide useful information to improve efficiency of the disease diagnosis system in Kenya that will be a non-painful procedure as to the case of blood collection.

Risks/ Discomforts

You will experience little pain when blood is being withdrawn but this does not pose any health risk since the equipment (needle and swabs) used to get this specimen is sterile.

Alternatives to participation

The alternative to participate is not to participate without penalty.

Confidentiality

Any information you provide during the study will be kept strictly confidential. All hard copy data will be stored in designated lockers and access will be limited to the principle investigator. Using passwords only known to the principle investigator will

safeguard electronic data. At the ministry of health data records concerned with the measles and rubella surveillance the test results will not be disclosed to any person without authorization from the ministry of health. Your name will not appear on any study document and instead, you will be given an identification number.

Voluntaries

Your participation in this study is completely voluntary. You are free to choose whether or not to participate in this study. You are also free to withdraw from the study at any time you wish to without penalty.

Who to contact

You are encouraged to ask any questions to clarify any issues at any time during your participation in the study. If you need more information on the study, contact Rose Chelangat through 0722 27 93 92, the principle investigator of the study and if you need more information on the rights to participation contact KEMRI/ Ethical Review Committee through 0722 20 59 01, 0733 40 00 03, or 2722541 extensions 3307

Declaration

I have read and understood the study information. I have been given the opportunity to ask questions about the study. I understand that my taking part is voluntary; I can withdraw from the study at any time and I will not be asked any questions about why I no longer want to take part. I understand my personal details will be kept private. I

hereby consent to participate in the study as has been explained and as I have understood.

Participants' signature:

.....

Witness thumb print (if unable to write)

……

……

Date:

.....

Researcher's name:

.....

Researcher's signature:

.....

Date:

.....

SWAHILI TRANSLATION

IAMBATISHO II: Ridhaa

Kuanzishwa

Hallo. Mimi ni Rose Chelangat kutoka Jomo Kenyatta Chuo Kikuu cha Kilimo na Teknolojia. Fomu hii inataka habari ridhaa kwa ajili ya ushiriki wako katika utafiti inataka kuamua kama damu doa na mate kama kielelezo badala ya sehemu ya maji ya damu katika kutambua IgM katika maradhi ya rubela virusi maalum na mtihani enzyme immunoassay.

Kichwa cha utafiti: ORAL FLUID AS ALTERNATIVE SPECIMENS TO SERUM IN THE DETECTION OF MEASLES/RUBELLA VIRUS-SPECIFIC IgM BY ENZYME IMMUNOASSAY

Kusudi

Utafiti huu hufanyika:

• Ili kujua matumizi ya mate, na damu kavu katika utambuzi wa rubela ambayo kutatua tatizo la maumivu ya kuwa ni uzoefu wakati wa kupata damu kwa mtihani huo

• Ili kulinganisha utendaji wa mate na damu doa iliyokaushwa.

• Kwa kuamua maambukizi ya rubela kutumia mate, matangazo ya kavu damu na majimaji ya damu.

Utaratibu

Kama wakubaliana na kushiriki au mtoto wako kushiriki kati ya wale wa chini ya miaka 18 katika utafiti huu, mtahini atakuuliza maswali yanayohusu utafiti kwa dakika mbili. Mtu kutaka maabara atakusanya, milimita mbili ya damu na usufi ya mate mpaka kwenye pedi. Utaratibu utakuwa kama njia ya kuzugua meno yako lakini hii itafanyika kwenye ufizi wako na shavu. Hii ni baada ya ridhaa ya kutiwa saini kupatikana.

Faida ya kushiriki.

Hakuna faida ya moja kwa moja na Mshiriki lakini matokeo utatoa taarifa muhimu za kuongeza ufanisi wa mfumo wa ugonjwa wa utambuzi katika Kenya ambayo itakuwa ni utaratibu yasiyo ya chungu kama kesi ya ukusanyaji damu.

Hatari

Uzoefu utakuwa maumivu kidogo wakati wa kutoa damu lakini hii haina hatari yoyote ya afya tangu vifaa (sindano na swabs) za kutumika ili kupata damu na mate ni hasaa.

Njia mbadala ya ushiriki

Hatua hiyo ya kushiriki ni kutoshiriki na hakuna adhabu yoyote.

Usiri

Taarifa yoyote, kutoa wakati wa utafiti zitatunzwa madhubuti ya siri. Takwimu zote nakala ngumu itahifadhiwa katika makabati mteule na upatikanaji utakuwa mdogo na uchunguzi wa kanuni. Anayetaka kutumia tu anajulikana kwa uchunguzi wa kanuni ya mapenzi kulinda takwimu za elektroniki. Katika wizara ya kumbukumbu za takwimu za afya na wasiwasi na surua na rubella ufuatiliaji matokeo ya mtihani haitakuwa wazi kwa mtu yeyote bila ya ruhusa kutoka kwa wizara ya afya. jina lako halita onekana kwenye hati yoyote ya utafiti na badala yake, utakuwa ukipewa namba kitambulisho.

Hiari kushiriki.

ushiriki wako katika utafiti huu ni hiari kabisa. Wewe una uhuru kuchagua kama wataka kushiriki katika utafiti huu. pia una uhuru kuchagua kama wataka kutoshiriki waweza kuondoka kutoka utafiti wakati wowote bila adhabu.

Utakao wasiliana nao

Wewe ni huru kuuliza maswali na kufafanua masuala yoyote wakati wowote katika ushiriki wako katika utafiti. Kama unahitaji habari zaidi juu ya utafiti huo, wasiliana na Rose Chelangat kupitia 0722 27 93 92, mpelelezi kanuni ya utafiti na kama unahitaji habari zaidi juu ya haki wasiliana na ushiriki KEMRI / kimaadili Kamati ya Uchunguzi kupitia 0722 20 59 01, 0733 40 00 03, au 2722,541 ugani 3307.

Tamko

Nimesoma na kuelewa taarifa ya masomo. Nimepewa nafasi ya kuuliza maswali juu ya utafiti. Mimi nimeelewa kwamba sehemu yangu ya kuchukua ni hiari, ninaweza kuondoka kutoka utafiti wakati wowote bila kuulizwa maswali yoyote. Nimeelewa maelezo yangu binafsi kuwa agizo binafsi na ni siri.

Washiriki signature: ....

Shahidi thumb magazeti (kama hawawezi kuandika) ... ...

Tarehe: ....

Mtafiti jina la: ....

Mtafiti's signature: ....

Tarehe: ....

Appendix II: Questionnaire

EPID NUMBER

Province-------- District --------- Year of onset ----------

Case number. ----------

PARTICIPANTS DETAILS sex ----- ------- age in years----------

Male female

VACCINATION STATUS FOR MMR (RUBELLA) ----- ------

Yes no

LAST DATE OF VACCINATION _/ _/ _

DATE OF ONSET OF MACULOPAPULAR RASH _/ _/ _

DATE OF SAMPLE COLLECTION _/ _/ _

TYPE OF SPECIMEN COLLECTED; Blood ---------- OF----------, DBS----------.

LABORATORY DETAILS

DATE OF SAMPLE RECEPTION IN LAB _/ _/ _

TYPE OF SPECIMEN RECEIVED; Blood ----------, OF----------, DBS---

---

RESULTS

IgM DETECTION IN:

OF ods ----------. DBS ods ----------. SERUM ods ----------.

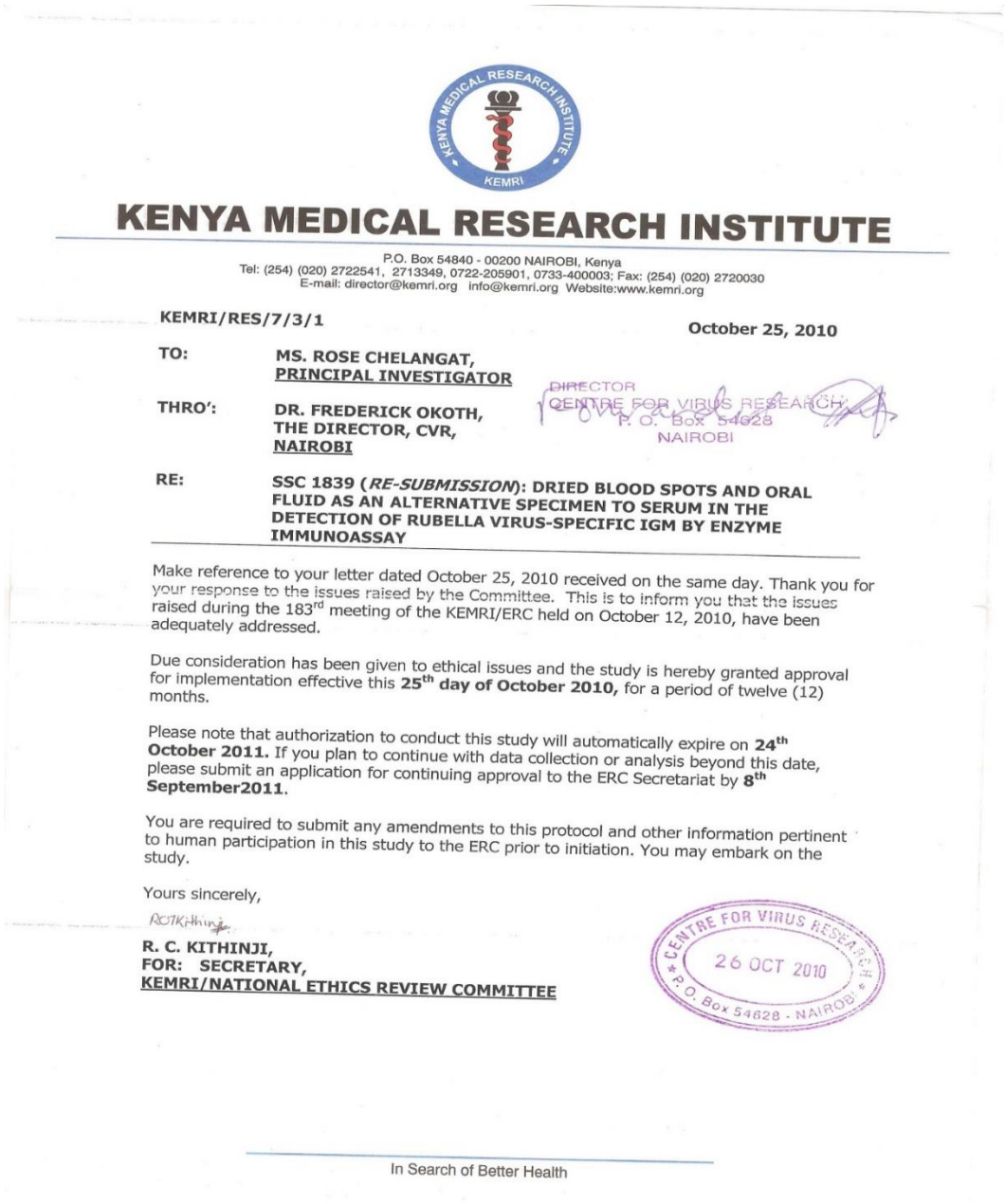
LAB TECH NAME: ----------

LAB TECH SIGNATURE: ----------

Preparation of transport media

Transport media	100mls volume
10% foetal calf serum	10mls
0.2% tween 20 (sigma)	200µl
Phosphate buffered saline	90mls
0.5% Gentamicin (50 mg/ml stock)	500µl
0.2% Fungizone (250 µg/ml stock)	200µl
Red food dye	50µl

Appendix III: Kenya Medical Research Institute Ethical clearance/approval



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1

October 25, 2010

TO: MS. ROSE CHELANGAT,
PRINCIPAL INVESTIGATOR

THRO': DR. FREDERICK OKOTH,
THE DIRECTOR, CVR,
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

RE: SSC 1839 (*RE-SUBMISSION*): DRIED BLOOD SPOTS AND ORAL FLUID AS AN ALTERNATIVE SPECIMEN TO SERUM IN THE DETECTION OF RUBELLA VIRUS-SPECIFIC IGM BY ENZYME IMMUNOASSAY

Make reference to your letter dated October 25, 2010 received on the same day. Thank you for your response to the issues raised by the Committee. This is to inform you that the issues raised during the 183rd meeting of the KEMRI/ERC held on October 12, 2010, have been adequately addressed.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective this **25th day of October 2010,** for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on **24th October 2011.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **8th September2011**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the ERC prior to initiation. You may embark on the study.

Yours sincerely,

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

CENTRE FOR VIRUS RESEARCH
26 OCT 2010
P.O. Box 54628 - NAIROBI

In Search of Better Health

Appendix IV: Kenya Medical Research Institute scientific clearance letter

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
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E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/6772

11th August, 2010

Rose Chelangat

Thro' Director, CVR
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

REF: SSC No.1839 (Revised) – Dried blood spots and oral fluid as alternative specimens to serum in the detection of rubella virus – specific IgM by enzymes immunoassay

I am pleased to inform you that the above-mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its **170th meeting** held on **3rd August, 2010** and has since been approved for implementation by the SSC.

Kindly submit 4 copies of the revised protocol to the Secretary SSC as soon as possible.

The SSC however, advises that work on this project can only start when ERC approval is received.

for

Sammy Njenga, PhD
SECRETARY, SSC

CENTRE FOR VIRUS RESEARCH
12 AUG 2010
P. O. Box 54628 - NAIROBI

Appendix V: Sensitivity and specificity

The sensitivity is the ability of the test to correctly identify those participants with the disease.

Sensitivity = True positives/ True positives +False positives

The specificity is the ability of the test to correctly identify those participants without the disease.

The positive predictive value is a proportion that is used to answer the question: 'How likely is it that this participant has the disease given that the test result is positive?'

Positive predictive value = True positives/True positives + False positives

The negative predictive value answers the question: 'How likely is it that these participants does not have the disease given that the test result is negative?'

Negative predictive value = True negatives /True negatives + False negatives

Appendix VI: Publication

Advances in Life Science and Technology
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Vol.50, 2016

Assessing feasibility of using Oral Fluid assay as Alternative method in the Detection of Rubella Virus-Specific IgM Antibodies in routine disease surveillance Programme in Kenya

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1. Jomo Kenyatta University of Agriculture and Technology
2. University of Kabianga, Department of Biological Sciences
3. Kenya Medical Research Institute (Centre for Virus Research)

Abstract

Background: The WHO recommends the inclusion of rubella testing in the measles surveillance system. Laboratory diagnosis of measles and rubella virus infection is achieved by serological assay for specific IgM from a sample of blood drawn by vein puncture. This conventional method of sample collection is invasive and less acceptable.

Aim: To assess feasibility of using oral fluid as an alternative method in the detection of rubella-virus specific IgM in routine surveillance of rubella

Settings and Design: A prospective laboratory-based cross-sectional study using matched oral fluid and serum collected from emerging outbreaks of rash-like illnesses across Kenya.

Methods and Material: Matching samples of 176 patients were investigated for IgM specific antibodies using enzyme linked immunosorbent assays.

Statistical analysis used: The kappa (k) statistic was used to measure inter-observer variations.

Results: The prevalence of rubella using serum and oral methods was 26.7% and 23.3% respectively. Sensitivity and specificity for rubella IgM in oral fluid when tested against the gold standard was 86% and 93% respectively. Kappa statistic value was 0.80 suggesting substantial agreement between the two methods.

Conclusion: The study showed that oral fluid method is a promising simple alternative, non-invasive and more acceptable specimen of choice for rubella diagnosis. The alternative method will be more applicable to disease surveillance programmes where clinical settings are varied. The advantage of this method of sample collection is ease and safety with minimum requirement for shipment to laboratory. These findings will support the entire disease surveillance system in Kenya and also can have extended use in conducting epidemiological studies.

Key words: Oral fluid, serum, diagnosis, surveillance, prevalence, diseases, measles and rubella

1. Introduction

Since inception of measles surveillance in Kenya, the assay method of choice has been using blood for IgM detection using a commercially manufactured ELISA kit. As a standard procedure, rubella testing is also performed using the same sample. This is because rubella infection presents with almost similar clinical manifestations as measles.

The WHO recommended rubella testing as a differential diagnosis of measles in routine measles surveillance system of member countries. In support of the initiative, it established a global measles and rubella laboratory network [23]. Data collected over the years showed that rubella is endemic in Kenya and 30% to 50% of suspected measles cases are laboratory confirmed as being caused by rubella virus [23]. This has led to introduction of Measles and Rubella (MR) Vaccine into the National Immunization schedule.

Rubella virus is a teratogenic virus that causes congenital disorders to the children who are born to mothers who had the infection during the first trimester of their pregnancy [4]. Rubella virus affects children, adolescents and the young adults. Around 50% of the rubella infections are subclinical and can only be confirmed through laboratory testing. It may be asymptomatic or mild infection in adults and children [3]. This infection during pregnancy especially during the first trimester can result in miscarriage, stillbirth to congenital defect if the foetus is not miscarried [22]. The main symptoms include inflammation of the lymph nodes and a maculopapular rash that may be preceded by mild catarrhal symptoms. Lyphadenopathy occurs from 5-7 days before the onset of the rash and up to 2 days after [5]. Rubella virus infection occurs via the respiratory route. It infects the nasopharynx and multiplies in the lining of the respiratory tract and in local lymph nodes before getting to viraemic phase that begins within 4-5 days after the infection and spreads to the rest of the body. Rubella virus has an incubation period of 14-18 days [14].

In patients where phlebotomy is not possible, saliva can be collected for salivary rubella-specific IgA testing. Positive contact with other patients known to have rubella adds strong epidemiological evidence to the diagnosis. The contact with any infected person in any way, including semen through sex, saliva, or mucus, can cause infection [19].

In this study oral fluid was used as an alternative method for detection of rubella IgM. This method was explored because of the challenges posed by collection, maintenance and safety of blood sample. In addition published literature has shown that transportation of oral fluid does not require low temperatures during shipment when compared to blood [17].

2. Materials and methods

Ethical consideration

Ethical clearance was sought from Ethical Review Committee and also from Jomo Kenyatta University of Agriculture and Technology. Authority was also sought from the Ministry of Health since the samples were collected as part of routine surveillance of measles and rubella. The research was conducted in accordance with KEMRI guidelines on human sample use and care and the internationally accepted principles for laboratory procedures using standard operating procedures [26].

Study site and design

The study site was determined by outbreaks of rash-like illness in the country. On notification of emerging rash-like outbreaks, samples were collected using an established disease surveillance system. The samples were collected from different districts in Kenya and transported to the Kenya Medical Research Institute (KEMRI) laboratory for processing. The districts were; Fafi, Garissa, Kaloleni, Kamukunji, Lagdera, Nairobi North, Nairobi West, Nakuru North, Tana River, Taveta, Turkana North, Wajir East, Wajir South, Wajir West and Kakuma (Sudanese living in Kenya).This was a prospective laboratory-based cross-sectional study design over a period of six months from June- December 2010.

Study population

Samples were collected using the WHO standard [25] case definition for based on the clinical presentation under routine surveillance system.

Sample size

A hundred and eighty (180) matched samples were collected based on the Ministry of Health (MoH) surveillance system 2008-2009 requirement for laboratory confirmation of suspected measles outbreaks.

Specimens collection

Samples of oral fluid and blood were collected from each study participant within 28 days of rash onset. Venous blood (3mm) was collected in a vacutainer tube technique described by WHO [25]. Whole blood specimen was allowed to fully clot for 15-30 minutes at room temperature before harvesting of serum. Serum was transported using cool boxes with frozen ice packs and stored at 4°C waiting in the laboratory until analysis. The period of storage within the laboratory was less than 3 days.

Oral fluid was collected using an ORACOL test kit (Malvern Medical Developments, Worcester, UK). The device has a sterile swab, with an absorbent material that was placed into the patient's mouth between the lower cheek and gum and left in until adequately moistened. The pad was removed and inserted in the bottom of a vial containing preservative. Consistency in labelling for the two samples from a patient was ensured. The same identification number was entered in the case-based request form. The oral fluid in the swabs was extracted as soon as it arrived in the laboratory by adding 1ml of the virus transport medium to the tube containing the oral fluid swab. The swab was then agitated by vortexing to ensure foaming of the transport medium. The swab was removed from the tube by a twisting motion; centrifugation at 2000 rpm for 5 minutes to ensure that much liquid was recovered from the swab. Extracted fluid was stored at 4°C until analysis was done.

Diagnosis of Rubella Virus IgM using Serum method

The Enzygnost®Anti-Rubella-Virus/IgM immunoassay (Siemens Healthcare Diagnostics Products, Marburg, Germany) was used to analyse the serum specimens to detect Rubella-Virus IgM. Enzyme Immunoassay is for both qualitative and quantitative determination of IgM antibodies to rubella virus in human serum and plasma. The test was developed for testing individual samples, not for pooled samples. The method is ELISA-based and uses a commercially available kit therefore manufacturer instructions were used. Results were interpreted qualitatively using optical densities (OD). The reading of optical density was done at 450/650 nm using spectrophotometric plate reader.

Diagnosis of Rubella Virus IgM using Oral fluid method.

The Microimmune Rubella IgM capture Enzyme Immunoassay kit (Microimmune Ltd, Middlesex, UK) was used to detect Rubella IgM in oral fluid. Samples and test reagents were brought to room temperature (18-24 °C) prior to testing. Manufacturer instructions were followed in the testing using the kit. The reading of optical density was done at 450/650 nm using spectrophotometric plate reader.

Oral fluid diagnostic test interpretation Data analysis and presentation

In each test, the relative sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of oral-fluid results were calculated relative to serum being the gold standard. Concordance between the oral fluid-based and corresponding serum-based assay results was evaluated by considering all sample pairs in the study.

Descriptive findings were presented in form of tables, graphs and charts to show the comparison of the results. The kappa (k) statistic was used to measure inter-observer variability.

Results

Rubella seropositivity using serum and oral fluid were 26.7% and 23.3% respectively (Table 1) Samples took an average of three days to arrive in the laboratory.

Table 1: Comparison of oral fluid and serum prevalence

Out come	*Rubella*	
	Serum	*Oral fluid*
Positive	*47(26.7)*	*41(23.3)*
Negative	*113 (64.2)*	*117 (66.5)*

- ***Rubella seropositivity using serum and oral fluid were 26.7% and 23.3% respectively.***
- ***Samples took an average of three days to arrive in the laboratory***

Sensitivity and specificty for rubella

Serum test for rubella showed that 64.2% of the participants tested negative while 26.7% tested positive. Oral fluid on the other hand revealed that 63.8% tested negative while 36.2% tested positive.

In the rubella test, concordance was confirmed in 176 of samples resulting in sensitivity rate of 86%.and specificity rate of 93%

Table 2: Sensitivity and specificity for Rubella virus

Rubella test	*Sample size*	*Sensitivity (%)*	*95% CI*	*Specificty (%)*	*95% CI*	*NPV*	*PPV*
Serum	*176*						
Oral fluid	*176*	*86*	*0.05*	*93*	*0.05*	*93*	*86*

- ***Serum test for rubella showed that 64.2% of the participants tested negative while 26.7% tested positive.***
- ***Oral fluid on the other hand revealed that 63.8% tested negative while 36.2% tested positive.***
- ***In the rubella test, concordance was confirmed in 176 of samples resulting in sensitivity rate of 86%.and specificity rate of 93%***

Rubella test

The calculation based on the difference between how much agreements were actually present ("observed" agreement) compared to how much agreement would be expected to be present by chance alone ("expected" agreement). The statistical analysis was necessary to determine whether the oral fluid assays were effective in detecting the ribonucleic acid paramyxovirus in the same manner as invasive serum assays. Since the hypothesis of this study was to examine whether Rubella oral fluid IgM assays can be used as an alternative to Rubella serum IgM assays, the study checked the relationship between the two tests using this tool, the calculation based on the difference between how much agreements were actually present ("observed" agreement) compared to how much agreement would be expected to be present by chance alone ("expected" agreement) is determined. Calculations based on results for rubella tests are 0.56

Therefore, observed agreement is simply the percentage of all tests for which the two test evaluations agree, 0.91 Kappa, K is 0.80.

Discussion

The findings in this study confirmed that an oral fluid sample can be used as an alternative to blood in the surveillance of rubella in Kenya. This proposed and tested method will be useful especially post introduction of measles and rubella (MR) vaccination in Kenya (ref). As a follow of the introduction of MR vaccine in routine immunization, active surveillance of rubella cases in assessing the impact of the intervention and also serosurveys will benefit greatly from these results. The samples used were collected from geographically diverse regions in Kenya confirming versatility of the new method. Failure to perform laboratory confirmation of suspected outbreaks can lead to missed opportunities to prevent transmission. Laboratory confirmation has

become important to both measles and rubella control programs world over [7]. Existing laboratory methods rely largely on the detection of significant rises in rubella antibody titer or the detection of rubella IgM antibody. Collection of blood sample is often considered invasive requiring specially trained persons which can limit usability. A non-invasive method of sample collection can have an important role especially in areas with limited resources[12].

The findings in this study demonstrated high level of concordance between serum and oral fluid IgM results which was demonstrated by the high specificity and sensitivity rates and an acceptable agreement using Kappa statistic. Other investigators have also confirmed versatility of oral fluid for rubella IgM detection. The results of Talat *et al.,* yr?[24] showed indeed that whole saliva contains IgM antibody at concentrations high enough to be diagnostically useful. Its applicability has been tested widely as demonstrated by Author? Where antibody-capture radio immunoassay, showed that virus specific IgM was detected in 100% of rubella saliva samples collected between 1 and 5 weeks after onset of disease [21]. Also in another community-based study in England and Wales found out that the sensitivity of saliva rubella IgM testing was 81% when compared to blood. Another study done in Ethiopia by Nokes *et al.,*[15] showed that the overall sensitivity and specificity were 79% and 90% for rubella in a study done in Ethiopia.. The, timing of salivary specimen collection is another important factor in determining whether viral specific IgM will be detected. Although this study did not focus on duration after rash onset for sample collection several studies have confirmed that rubella IgM can be detected well shortly after rash onset but within 14 days of rash onset [7]

The mildness of the majority of rubella cases makes parents and medical practitioners reluctant to take blood for diagnosis. Moreover, in developing countries rubella outbreaks can occur with no clinical recognition, even in a community in which health is being monitored. The use of non-invasive specimens for diagnosis offers several advantages over blood such as: acceptability to patients, applicability to children and reuse of disposable equipment can be ruled out. In addition occupational risk from needle stick injures is eliminated. The present work and other studies indicate that oral fluid is a viable alternative to serum for monitoring the impact of vaccination programmes and disease surveillances in the future.

Data has shown that approximately 30 to 50% of suspected measles cases are often confirmed as being caused by rubella virus (, [23]. Diagnosis of rubella using oral fluid samples instead of serum samples offers many advantages that can support disease surveillance programmes in any country [12]. Among the many advantages are ease of specimen collection, non-invasiveness and also comparative acceptability in many communities. .

The results demonstrated that oral fluid specimens are a convenient alternative to serum for diagnosis of recent rubella infection. The widespread acceptability of oral fluid collection should facilitate the investigation of rubella outbreaks and have an important role in controlling the disease in regional and national public health laboratories worldwide. Additionally, refusal in collecting blood samples due to cultural or religious traditions and vein puncture related problems may increase the difficulty in obtaining specimens for testing [8].
Oral fluid is a valid alternative to serum for IgM detection of rubella antibodies that could play a major role in surveillance of rubella and also for use in rubella serosurveys. This study therefore confirms the potential of incorporating enzyme linked immunosorbent assay using oral fluid as a sample of choice in detection of rubella IgM. For routine surveillance and epidemiological surveys nationwide and also for other regions with similar settings. Moreover, the turnaround time for results in this method does not deviate from that of the gold standard yet it offers additional advantages comparatively.

5. Conclusion

The study showed that oral fluid method is a promising simple alternative, non-invasive and more acceptable specimen of choice for rubella diagnosis. The alternative method will be more applicable to disease surveillance programmes where clinical settings are varied. The advantage of this method of sample collection is ease and safety with minimum requirement for shipment to laboratory. These findings will support the entire disease surveillance system in Kenya and also can have extended use in conducting epidemiological studies.

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