SEROPREVALENCE AND MOLECULAR DETECTION OF RUBELLA VIRUS AMONG WOMEN OF REPRODUCTIVE AGE IN KADUNA STATE, NIGERIA

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BY

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AUGUST, 2017

DECLARATION

I hereby declare that the work presented in this dissertation entitled **"Seroprevalence and Molecular Detection of Rubella Virus among Women of Reproductive Age in Kaduna State, Nigeria"** has been carried out by me in the Department of Microbiology, Ahmadu Bello University Zaria, Kaduna State, Nigeria, under the supervision of Dr. A.B. Suleiman and Prof. S.E. Yakubu. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Deborah Akut _____ _____

Signature Date

CERTIFICATION

This project dissertation entitled "Seroprevalence and Molecular Detection of Rubella Virus among Women of Reproductive Age in Kaduna State, Nigeria" by Deborah Akut meets the regulations governing the award of Masters Degree in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this research work to God Almighty and to my parents Mr. and Mrs Akut Abui for their support, encouragement and Prayers.

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ABSTRACT

Rubella (German measles) is a vaccine preventable disease caused by Rubella virus. The public health importance of rubella relates to the teratogenic effects of the virus when acquired in the early months of pregnancy. The purpose of the study was to determine the Seroprevalence and detect the presence of Rubella virus among women of reproductive age in Kaduna State, Nigeria. A total of 360 women between the ages of 15 and 45 years attending the selected health facilities were enrolled for the study and blood and throat swab specimens obtained from these women were analysed using ELISA and Nested RT-PCR techniques. The prevalence of Rubella virus IgG and IgM antibodies obtained among the 360 women examined was 343(95.3%) for Rubella IgG antibodies and 93(25.8%) for Rubella IgM antibodies. Women between the age group of 40 and 45 had the highest prevalence of 100% while women between the age group of 25 and 29 years had the lowest prevalence of 92.9% for Rubella IgG antibody. Women between the age group of 15-19 years had the highest prevalence of 31.8% while those between the ages of 20 and 24 years had the lowest of 19.6% for rubella IgM. Some of the socio-demographic and risk factors associated with rubella in this study were Senatorial district of the women, history of vaccination, joint pains ($P<0.05$). A positivity rate of 13% was obtained by polymerase chain reaction. Efforts should be made to make routine screening part of antenatal care.

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ABBREVIATIONS AND SYMBOLS

µl: microlitre

bp: base pair

CDC: Centres for Disease Control

cDNA: Complementary Deoxyribonucleic Acid

C-protein: Capsid protein

CRS: Congenital Rubella Syndrome.

DMSO: Dimethyl sulfoxide

E1: envelope glycoprotein 1

E2: envelope glycoprotein 2

EDTA: ethylene diamine Tetra-acetic acid

ELISA: Enzyme Linked Immuno-Sorbent Assay

Ig: Immunoglobulin

IgA: Alpha (α) Immunoglobulin

IgG: Gamma (ɣ) Immunoglobulin

IgM: Mu (µ) Immunoglobulin

IU/ml: International Unit per millilitre

kDa: KiloDalton

LGA: Local Government Area

ml: Millilitre

MMR: Measles Mumps and Rubella

M-MuLV: Molony Murine Leukaemia virus

NIP: National Immunisation Programme

nm: nanometre

NPC: National Population Commission

NSP: non-structural proteins

nt: nucleotide

O. D: Optical Density

ORF: Open Reading Frame

PAGE: polyacrylamide gel electrophoresis

PBS: Phosphate Buffered Saline

PCR: Polymerase Chain Reaction

pmol: Pico mol

RCV: Rubella containing vaccines

RNA: Ribonucleic acid

RT-nPCR: Reverse Transcription Nested Polymerase Chain Reaction

SDS: sodium dodecyl sulphate

TBE: tris borate ether

TMB: 3,3′.5,5′- Tetramethylbenzidine

TORCH: Toxoplasmosis, Rubella, Cytomegalo virus, Herpes Simplex and Others (Syphilis, Varicella zoster, Parvovirus B19)

UTR: untranslated region

UV: Ultraviolet

VTM: Viral Transport Medium

WHO: World Health Organisation

CSTE: Council of State and Territorial Epidemiologists

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Rubella is a mild viral illness commonly known as German measles though its etiologic agent differs from Measles (Nayeri and Thung, 2013; NICE, 2013). It is also called 'three day measles' because of the short duration of the rash (Bubuk *et al.,* 2002; Elias and Ruselle, 2014). Rubella rash starts on the face and neck and spreads centrifugally to the trunk and extremities within 24 hours (Heymann, 2008; Pickering *et al.,* 2012; Elias and Ruselle, 2014). The rash begins to fade on the face on the second day and disappears throughout the body by the end of the third day (Gabbe *et al.,* 2002; Heymann, 2008; Elias and Ruselle, 2014). The disease is contagious and can affect people of all ages and sex (Hobman *et al.,* 2007). The name Rubella was coined from Latin and it means "little red". Rubella was initially considered to be a variant of measles or scarlet fever and thus called "third exanthematous disease of childhood". It was first described as a separate disease in the German medical literature by German physicians, de Bergan and Orlow, hence the common name "German measles" (Lee and Bowden, 2000; Atkinson, 2011).

The etiologic agent of the disease is Rubella virus (Deka, *et al.,* 2006; Murry, 2006). It is an enveloped, non-segmented, positive-stranded, Ribonucleic acid (RNA) virus which replicates in the cytoplasm (Frey, 1994; Hobman *et al.,* 2007). It is the only member of the Rubi virus genus belonging to the family; toga viridae (Lee and Bowden, 2000; Chantler *et al.,* 2001; Heymann, 2008).

The rubella virus genome consists of less than 10,000 nucleotides and encodes five proteins including three structural proteins: E1 glycoprotein, E2 glycoprotein and capsid protein and two non-structural proteins: P150 and P90. The E1 glycoprotein is a structural

glycoprotein with neutralising epitopes (WHO, 2000; Chantler *et al.,* 2001; Plotkin and Reef, 2004). According to a report on the meeting organised by World Health Organisation (WHO) in 2004 to discuss the standardization of nomenclature for describing the genetic characteristics of wild-type rubella virus, the E1 gene sequence between genome positions 8731 and 9469 was recommended as a target site for routine genotyping analysis for molecular epidemiology (WHO, 2005).

Maternal infections caused by viruses like *Toxoplasma gondii*, Rubella virus, Cyto megalo virus and Herpes simplex virus, transmissible *in utero* at various stages of gestation can lead to unfavourable pregnancy outcome (Surpam *et al.,* 2006). Primary infections caused by the above mentioned infectious agents are the major causes of abortions, still births and congenital defects among foetuses of infected mothers; among these agents, Rubella virus is most consistent in its harmful effects on the foetus (Surpam *et al.*, 2006).

Unlike most Toga viruses, Rubella virus is not transmitted by arthropods (Lambert *et al.*, 2015), Humans are the main reservoirs of the virus (De Santis *et al.,* 2006; Ilkow *et al.,* 2010). The usual portal of entry of rubella virus is the respiratory epithelium of the nasopharynx (Elias and Ruselle, 2014). The virus is transmitted via the aerosolized particles from the respiratory tract secretions of infected individuals (Elias and Ruselle, 2014). The virus attaches to and invades the respiratory epithelium where it replicates in the nasopharynx and regional lymph nodes and then spreads to other parts of the body through the blood (Elias and Ruselle, 2014). The teratogenic properties of the virus makes it possible for vertical transmission from an infected mother to her foetus via the placenta thereby infecting the foetus and interfering with foetal cell development (Edlich *et al.,* 2005; Rasmussen, 2010). Rubella virus is included in the TORCH Complex (Toxoplasmosis, Rubella, Cytomegalo virus, Herpes Simplex virus and others which includes Syphilis, Varicella Zooster, Parvo B19 virus) of perinatal infections because of its

teratogenicity. Babies with congenital rubella syndrome (CRS) may shed the virus for more than a year in pharyngeal secretions and urine (Best, 2007; Atkinson, 2011). These children become significant sources of infection to other infants and most importantly to pregnant female contacts (CDC, 2004; Vijaylakshmi *et al.,* 2004). Humans are the only natural host and known reservoir of the virus (De Santis *et al*., 2006; Heymann, 2008; Ilkow *et al*., 2010).

Richardson *et al.* (2001) reported that the disease has an average incubation period of 14 days but ranges from 2-3 weeks during which the patient is contagious typically for about one week before he/she develops a rash and for about one week thereafter (Agbede *et al.,* 2011). Increased susceptibility to infection might be inherited as there is some indication that Human Leukocyte Antigen (HLA)-A1 or factors surrounding A1 on extended haplotypes are involved in virus infection or non-resolution of the disease (Honeyman *et al.,* 1975; Forrest *et al.,* 2002).

Rubella virus is the mildest of common viral exanthems (Brooks *et al.,* 2004) and common symptoms associated with the disease include low-grade fever, swollen lymph nodes and a short-lived mobilliform rash (Brooks *et al.,* 2007). These symptoms are often confused with similar rash associated illnesses caused by other viral and non-viral pathogens or even some drugs, making differential diagnosis unreliable (Hobman *et al.,* 2007). Most patients with rubella develop mild symptoms and recover without any complications or sequelae because the infection is usually benign and self-limiting in children or adults. On the contrary, when the infection occurs among young women who are susceptible to the virus during early pregnancy, especially within the first trimester it may cause foetal death or congenital rubella syndrome (CRS) (Creasy *et al.,* 2004; Agbede *et al.,* 2011). Congenital rubella syndrome is characterised by multiple malformations such as deafness, eye abnormalities, congenital cardiac disease and neurological abnormalities

and children who are born asymptomatic may develop these abnormalities later in life (WHO, 2000; Bantavala and Brown, 2004).

Rubella invokes a serological response that is detectable at the onset of its characteristic rash and this response continues to evolve over the next few weeks (Hobman *et al.,* 2007). Viral-specific IgM antibodies are first detected 10 days after infection and it peaks at about 4 weeks after infection (Hobman *et al.,* 2007). This may persist for over 7 months after an acute infection. Anti-rubella virus antibodies are present in all immunoglobulin classes, including IgG, IgA, IgD, and IgE three weeks after infection (Hobman *et al.,* 2007).

Serologic testing for rubella specific IgM antibody is the most commonly used method for diagnosis of rubella. Diagnosis can also be made by demonstration of sero-conversion of rubella-specific IgG antibody titers and by detection of virus either through virus culture or Polymerase Chain Reaction (Reef *et al.,* 2006).

There is no specific treatment for Rubella but the disease can be prevented by vaccination with rubella vaccine, a single dose of the vaccine is more than 95% effective (Lambert *et al.,* 2015). Often, it is given in combination with Measles and Mumps vaccine known as the Measles, Mumps and Rubella (MMR) Vaccine (Atkinson, 2011).

Widespread outbreaks usually occurred every 6-9 years in the United States and 3-5 years in Europe, mostly affecting children in the 5-9 years age group before the vaccine to *Rubella* was introduced in 1969 (Reef *et al*., 2002) and ever since the vaccines were introduced, occurrences of the disease have become rare in such countries with high prevalence (Reef *et al*., 2002). Outbreaks however, still arise, usually in developing countries where the vaccine is not available (Reef, 2006).

1.2 Statement of the Problem

Rubella is an infection that is common in many areas of the world (De Santis *et al.*, 2006; Huong, 2014). Though, the burden of CRS is not well documented in all countries, WHO estimated that more than 100,000 cases of CRS occur every year in developing countries (WHO, 2000; Vijaylakshmi *et al.,* 2004). The incidence of CRS in developing countries ranged from 0.4 to 4.3 per 1000 live births as reported by Robertson *et al.* (2003). According to a report by World Health Organization (2012) cases of Rubella have increased in Nigeria from 450 in 2010 to 3,691 in 2011.

Rubella is a mild illness in adults and children and about 25-50% of cases are asymptomatic (Brooks *et al.,* 2004a; Dontigny *et al.,* 2008). As at 2002, Forty-three percent (43%) of countries all over the world do not have a national immunization programme for rubella (Bantavala and Brown, 2004). Despite the fact that safe and effective vaccine for the prevention of rubella has been available for more than 40 years, there has been no global recommendation for inclusion of the vaccine in the routine immunization programme of most developing countries, including Nigeria.

Foetal infection rates are near 80% in the first trimester, dropping to 25% in the late second trimester and increasing again in the third trimester to 35% at 27–30 weeks' gestation and nearly 100% beyond 36 weeks' gestation when maternal infection/exposure occurs in the first trimester (Gabbe *et al.,* 2002). The risk of congenital defects has been reported to be 90% when maternal infection occurs before 11 weeks of gestation, 33% at 11–12 weeks, 11% at 13–14 weeks, 24% at 15–16 weeks, and 0% after 16 weeks (Gabbe *et al.,* 2002).

Studies carried out in the past on rubella virus seroprevalence in Nigeria, has revealed rubella IgG seroprevalence in pregnant women to be 68.5% for a study carried by Bamgboye *et al.* (2004) in Ibadan; 53.0% in Benin (Onakewhor and Chiwuzie, 2011);

54.1% in Maiduguri (Bukbuk *et al.,* 2002); 97.9% in Zaria (Mohammed *et al.,* 2010) and 93.1% in Zaria (Olajide *et al.,* 2015).

1.3 Justification of the Study

Rubella in a susceptible pregnant woman within the first trimester of pregnancy can cause congenital rubella syndrome (CRS), with potentially devastating consequences for the developing foetus (Banatvala and Brown, 2004; Lanzieri *et al.,* 2004). Therefore, the susceptibility of women in the reproductive age group to rubella virus, especially before gestation, is closely related to the potential risk for congenital infection and its sequelae (Gershon, 2000; CDC, 2002). Children who are infected with the virus before births are at risk of growth problems, intellectual disability, defects of the heart and eyes, deafness, liver, spleen and bone marrow problems (Scott and Baron, 2015).

According to Olajide *et al.* (2015), women in Nigeria within the reproductive age group are not routinely screened for rubella as such there is the need to carry out studies on Rubella so as to generate epidemiological data on rubella, create awareness and sensitize healthcare administrators and providers.

In spite of the high mortality rate in Nigeria, women and children are not routinely vaccinated against Rubella since it is not among the diseases recommended for vaccination in the national programme on immunization. Efforts to realize significant political commitment and investment in rubella control and elimination in Nigeria has not yielded significant result (Adewumi *et al.,* 2015). Most people who are vaccinated for measles within the country receive monovalent measles vaccine rather than rubella-containing vaccines (RCVs) like trivalent measles-mumps-rubella (MMR) vaccine advertised on the platform of National Immunization Programme (NIP) (Adewumi *et al.,* 2015).

It is insufficient to diagnose rubella and congenital rubella syndrome from clinical information alone because some infected individuals may be asymptomatic or have mild symptoms and also, certain viruses such as measles, human parvovirus B19 and enterovirus which causes symptoms similar to rubella can affect such diagnosis (Bantavala and Brown, 2004; Tipple *et al.*, 2004; WHO, 2005). In order to avoid misdiagnoses of the disease, there is the need to test for Rubella-specific antibodies in women of reproductive age attending various health care facilities since clinical immunity is usually parallel to serum Rubella antibody titres (Ender, 1985; CDC, 2002).

Cases of CRS can also be misclassified since some patients with CRS may have a single symptom or defect or may have positive result for Immunoglobulin class M (IgM) enzyme immune assays without clinical symptoms (CDC, 2001; Chantler *et al.,* 2001; Zheng *et al.,* 2003) as such Molecular techniques such as Polymerase chain reaction can be employed to confirm the presence of the virus (Reef *et al.,* 2006).

Despite the availability of safe and effective vaccines and the successful control of rubella virus and congenital rubella virus in developed countries, substantial control has not been institutionalised in developing countries including Nigeria (CDC, 2010; Adewumi *et al.,* 2015). This study therefore is meant to assess immunity to rubella, identify subjects with primary infection and consequently make appropriate recommendations aimed at facilitating control.

1.4 Aim of the Study

The aim of this research was to determine the seroprevalence and molecularly detect the presence of Rubella virus in women of reproductive age in Kaduna State, Nigeria.

1.5 Objectives of the Study

The objectives of this study therefore, were to:

1. determine the presence of IgG and IgM antibodies to rubella virus in the study population using Enzyme-linked immunosorbent assay (ELISA).
2. confirm the presence of the virus using Reverse transcription nested polymerase chain reaction (RT-nPCR).
3. determine the socio-demographic factors associated with rubella virus infection in the studied population using a structured questionnaire.

1.6 Research Hypothesis

Null Hypothesis (H_O): There is no significant association between Rubella virus and women of reproductive age in Kaduna State, Nigeria.

Alternate Hypothesis (H_1): there is a significant association between Rubella virus and women of reproductive age in Kaduna State, Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Rubella and Congenital Rubella

In 1740, Friedrich Hoffmann, a German physician, gave the first clinical description of "German measles" ("Rötheln" in German). In the year 1814 George de Maton described rubella as distinct from other disease such as Measles and Scarlet fever in the German Medical Literature hence the common name German measles (Lee and Bowden, 2000). The virus was referred to as "rubella" (meaning "little red") in connection with an outbreak at a school in India in 1841 (CDC, 2013). Henry Veale, a Scottish clinician serving as an English Royal Artillery surgeon, suggested renaming the virus "Rubella" to replace "Rötheln" in 1866 (Lee and Bowden, 2000).

Rubella was accepted as a distinct disease by the International congress of medicine in 1881 (Best *et al.*, 2005; Banatvala and Peckham, 2007). In 1914 the aetiology of the virus was postulated by Hess based on his work with Monkeys. In 1938, S. Tasaka and Y. Hiro, Japanese scientists, confirmed the aetiology of the virus by successfully transmitting rubella from sick children to healthy children but did not find the causative agent of the disease (CDC, 2013). In 1941, the teratogenic effect of Rubella was recognised by Norman McAlister Gregg an Ophthalmologist in Australia who had noticed an unusual number of infants with cataract following rubella epidemic in 1940. Subsequently, rubella infection during early gestation was associated with the devastating array of congenital defects comprising the "Congenital Rubella Syndrome" (Best *et al.*, 2005; Banatvala and Peckham, 2007). In 1960, Thomas Weller, M.D., a Harvard School of Public Health researcher, isolated rubella virus from his 10-year-old son who was infected with the virus.

In 1962, Rubella virus isolated in cell culture by Parkman and Weller and Neutralization test was developed (Best *et al.,* 2005; Banatvala and Peckham, 2007). 1965-1967, attenuated vaccine strains were developed and rubella virus was first visualised by electron microscopy in 1967 (Best *et al.,* 2005; Banatvala and Peckham, 2007).

Three rubella vaccines were licensed in the United States in 1969: HPV-77:DE-5 Meruvax (duck embryo), HPV-77:DK-12 Rubelogen (dog kidney), and GMK-3:RK53 Cendevax (rabbit kidney) strains. HPV-77:DK-12 was later removed from the market because there was a higher rate of joint complaints following vaccination with this strain. In 1971, a combination of measles, mumps, and rubella (MMR) vaccine was licensed for use and in 1979, the RA 27/3 (human diploid fibroblast) strain (Meruvax-II, Merck) was licensed and all other strains were discontinued. Rubella virus genome was sequenced in 1986 (Best *et al.,* 2005; Banatvala and Peckham, 2007).

World Health Organisation recommended immunisation policies for the elimination of congenital rubella syndrome in the year 2000 and a combination of Measles, Mumps, Rubella and Varicella (MMRV) vaccine was licensed for use in 2005 (CDC, 2013). In 2006, 123 countries of the world included rubella vaccination in their national immunization programmes (Best *et al.,* 2005; Banatvala and Peckham, 2007).

2.2 Rubella Virus Classification

Rubella Virus is classified as the only member of the genus *Rubivirus* within the family *Togaviridae* (Murphy *et al.,* 1995). The name "togavirus" is derived from the Latin word "toga," meaning cloak or shroud, referring to the envelope of the virus (Murphy *et al.,* 1995). The family Toga viridae also contains a second genus; Alphavirus which contains several viruses of clinical and economic significance for humans, mammals and birds (Jose *et al.,* 2009).

Alpha viruses and Rubella Virus share a similar gene order and expression strategy since they belong to the same family (Dominguez *et al.,* 1990). They differ from each other in that the virions of alpha viruses are icosahedral, their nucleocapsids are assembled in the cytoplasm, and their virions bud from the plasma membrane (Straus and Straus, 1994). Rubella Virus virions on the other hand are pleomorphic, their nucleocapsids are assembled on Golgi membranes and then budding of the virus into this organelle (Frey, 1994). Rubella virus possesses a single antigenic type that does not cross-react with other members of the toga virus group (CDC, 2015). It is most closely related to group A arbo viruses, such as Eastern and Western equine encephalitis viruses (CDC, 2015). Rubella virus has a half life of 1 hour at 37 °C outside of the host (Walther and Ewald, 2004).

The virus is relatively temperature labile but is more heat stable than measles virus; it is inactivated after 30 minutes at 56°C, 4 minutes at 70°C, and 2 minutes at 100°C. It degrades rapidly with conventional freezing at -20°C but stable at -60°C and below and when freeze-dried with stabilizers (Prince and Prince, 2001). It can be repeatedly frozen and thawed without loss of titre when stabilised with protein. Rubella virus can be inactivated by; Lipid solvents, weak acids, weak alkalis and Ultraviolet (UV) light (Chantler *et al.,* 2001). It is also susceptible to a wide range of disinfectants and is inactivated by 1% sodium hypochlorite, 70% ethanol and formaldehyde (Prince and Prince, 2001). The Virions are not stable at a pH less than 6.8 or greater than 8.0 (Chantler *et al.,* 2001).

2.3 Rubella Virus Structure and Genome

Rubella virus (Figure 2.1) is roughly spherical in shape with a diameter of 60-70 nm (nanometres). It is composed of an icosahedral nucleocapsid containing a single-stranded, positive sense RNA genome (Best and Banatvala, 2007). The nucleocapsid is 40nm in diameter and is surrounded by a complex lipid envelope upon which the envelope

glycoproteins are arranged. The virus contains three structural proteins, two of the structural proteins namely; Envelope glycoprotein 1 (E1) and Envelope glycoprotein 2 (E2) are believed to be responsible for attachment to host cells. It is also believed that an acidic condition (pH $\leq$ 6.0) induces conformational changes in the glycoprotein making attachment of the viral envelope to host cells more likely. The third structural protein which is also called Capsid or C protein is located in the core surrounding the viral nucleic acid (Frey, 1994).

The envelope glycoproteins, E1 and E2, exist as heterodimers that project from the virus to form 6-nm to 8-nm surface spikes (Frey, 1994). The envelope glycoprotein E1 appears to be the dominant surface molecule and is associated with neutralizing and haemagglutinating epitopes (Chaye *et al.,* 1992). The Rubella Virus Capsid in comparism with Alpha viruses has been suggested to have a T = 3 icosahedral symmetry, whereas Alpha viruses have a T = 4 symmetry (Frey, 1994; Strauss and Strauss, 1994; Chantler *et al.,* 2001).

Small variations exist between various genomes of Rubella Viruses in terms of the length of their genomes but most genomes are 9762 nucleotides (nts) in length (Zhou *et al.,* 2007). They have similar genomic structure when compared to other Toga viruses. The nitrogenous base composition of the rubella virus genome includes; Guanine (G) 30.8%, Cytosine (C) 38.7%, Adenine (A) 14.9% and Uracil (U) 15.4% (Zhou *et al.,* 2007). It is one of the RNA viruses with the highest Guanine and Cytosine (G+C) contents sequenced so far with about 69.5% of the genome consisting of Guanine and Cytosine nucleotides (Dominguez *et al.,* 1990; Frey, 1994). The viral genome contains a methyl^{7}guanosine cap at the 5′ terminus and a polyadenylate tail at the 3′ terminus (Frey, 1994). The cap serves as a recognition site for Ribosome and is required for efficient translation (Zuckerman *et al.,* 2009).

The genome also contains two non overlapping open reading frames (ORFs): the 5′ proximal ORF and the 3′ ORF (Best and Banatvala, 2007). The 5′ proximal ORF which contains 6,345 nts is translated from 40S genomic RNA. It codes for non structural proteins (NSPs) p150 and P90, including a RNA-dependent RNA polymerase that is essential for viral genome replication (Mathew *et al.,* 2009).

The 3′ proximal Open Reading Frame on the other hand contains 3,185 nts, it is translated from the 24S sub genomic RNA and it codes for the viral structural proteins (the capsid protein and the two envelope glycoprotein) (Frey, 1994). Rubella virus also contains three untranslated regions (UTRs) in its genome which includes; a 40-nt sequence at the 5′ end (5′ UTR), an approximately 118-nt sequence in the intragenic region (IR) between the two ORFs and a 59-nt sequence at the 3′ end (3′ UTR) (Frey, 1994). The genome also includes 5' and 3' *cis*-acting elements (CAEs) containing multiple stem-loops, that are important for viral replication, transcription of the Sub-Genomic RNA, and RNA packaging (Pogue *et al.,* 1993; Chen and Frey, 1999).

2.4 Rubella Virus Proteins

The two long open reading frames (ORF) contained in the genomic RNA of rubella virus codes for non-structural proteins and structural proteins using the 5′-proximal ORF and 3′-proximal ORF respectively (Frey, 1994; Mathew *et al.,* 2009).

2.4.1 Structural proteins

The following structural proteins namely Capsid protein, Envelope glycoprotein 1 and Envelope glycoprotein 2 are coded for by the subgenomic RNA of rubella virus (Frey, 1994).

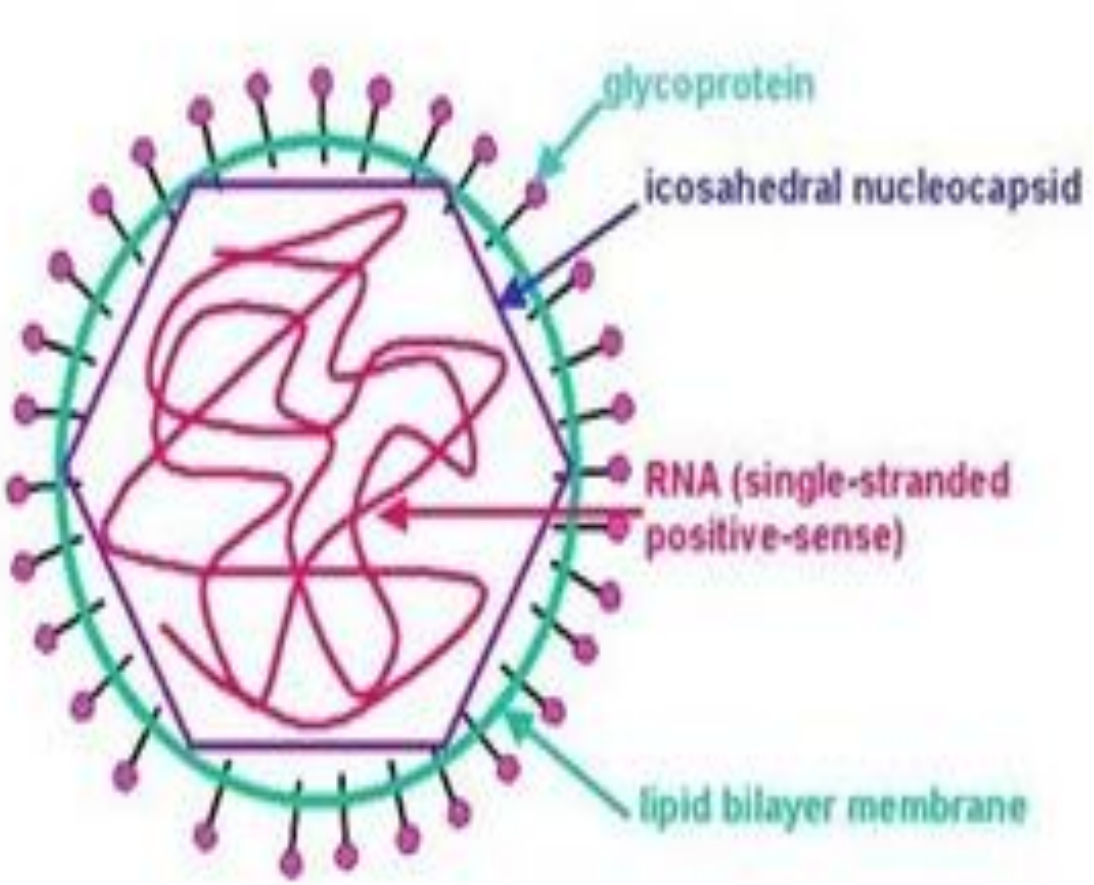


Figure 2.1 Structure of rubella virus and genome

Source: Hunt, 2011

2.4.1.1 Capsid protein

The Capsid protein or C-protein is also known alternatively as Coat Protein. It is a non-glycosylated, phosphorylated, disulfide-linked homodimer with a reported molecular mass of 33 to 38 Kilodalton (kDa) (Vaheri and Hovi, 1972; Oker-Blom, 1983; Bowden and Westaway, 1989; Marr *et al.,* 1991). The capsid protein contains clusters of Proline and Arginine residues, which have been postulated to be involved in binding to the Rubella Virus genomic RNA to form the viral nucleocapsids (Bowden and Westaway, 1989; Frey, 1994). As reported by Lui *et al.* (1996) a 28-amino-acid domain containing a large number of basic residues appears to be directly involved in binding to the RNA genome. However, the interaction of the capsid protein with the viral RNA may not be solely dependent on the density of these basic residues because other basic regions within the protein were found to bind poorly. On the Rubella Virus genome, a 29-nucleotide (nt) stretch (nt 347 to 376) interacts with the capsid protein, although it is not clear whether this is sufficient for packaging of the genome (Liu *et al.,* 1996).

The capsid protein interacts with the genomic RNA and assembles into icosahedric core particles. The resulting nucleocapsid eventually associates with the cytoplasmic domain of E2 at the cell membrane, leading to budding and formation of mature virions. Phosphorylation negatively regulates RNA-binding activity, possibly by delaying virion assembly during the viral replication phase. Capsid protein dimerizes and becomes disulfide-linked in the virion, modulates genomic RNA replication and modulates subgenomic RNA synthesis by interacting with human. It Induces perinuclear clustering of mitochondria and the formation of electron-dense inter-mitochondrial plaques, both of which are hallmarks of rubella virus infected cells. Apart from forming the nucleocapsid, interaction of the capsid protein with the E1 and E2 glycoproteins is required for assembly and budding of the virion (Qiu *et al*., 1994).

The capsid protein also has other non-structural functions in the infected host cell. The capsid protein has been reported to regulate the viral RNA transcription and replication (Chen *et al*., 2004). The capsid protein causes dramatic changes in the morphology and distribution of mitochondria in infected cells (Beatch *et al*., 2005). It also inhibits mitochondrial import in infected cells (Ilkow *et al*., 2010). It has also been shown to bind to proteins such as mitochondrial matrix protein (p32), prostate apoptosis response (Par-4) protein and Bax, which are involved in apoptotic pathways in host cells, thereby influencing apoptosis mechanisms in infected cells (Beatch and Hobman, 2000; Beatch *et al*., 2005; Ilkow *et al*., 2011). This function may form the basis for the observed delay in lytic release of virus during rubella infection as compared to other RNA viruses (Ilkow *et al*., 2011).

2.4.1.2 Envelope glycoprotein 1 (E1)

Envelope glycoprotein 1, alternatively known as Spike glycoprotein 1 is a class II viral fusion protein. Fusion activity by E1 glycoprotein is inactive as long as E1 is bound to E2 in mature virion. After virus attachment to target cell and clathrin-mediated endocytosis, acidification of the endosome would induce dissociation of E1/E2 heterodimer and concomitant trimerization of the E1 subunits. This E1 homotrimer is fusion active, and promotes release of viral nucleocapsid in the cytoplasm after endosome and viral membrane fusion. E1 cytoplasmic tail modulates virus release, and the tyrosines residues are critical for this function (Battista *et al.*, 2012).

The Envelope glycoprotein 1 is 481 amino acids long and is more exposed on the virion surface than Envelope glycoprotein 2. The E1 protein is also the major antigenic determinant on the virus. It is responsible for receptor recognition (Cong *et al*., 2011) and membrane fusion of the virus with the endosomal membranes at low-pH (Yang *et al*.,

1998). The E1 glycoprotein binds to the recently identified receptor, myelin oligodendrocyte glycoprotein (MOG) (Cong *et al*., 2011).

2.4.1.3 Envelope glycoprotein (E2)

Envelope glycoprotein 2 (E2), alternatively known as Spike glycoprotein E2 is responsible for viral attachment to target host cell; it is able to achieve this function by binding to the cell receptor. Its transport to the plasma membrane depends on interaction with E1 protein; the interaction between E1 and E2 protein is critical for the transport of the E2 protein to the plasma membrane (Battista *et al.*, 2012).

The E2 glycoprotein is 282 amino acid residues in length. It is essential for proper folding and maturation of the E1 glycoprotein (Garbutt *et al*., 1999; DuBois *et al.*, 2013). Envelope glycoprotein (E2) is not easily accessible to glycosidases (Ho-Terry and Cohen, 1980) and monoclonal antibodies (Pettersson *et al*., 1985), which implies that E2 is probably buried in the membrane and/or concealed by the E1 on the surface.

2.4.2 Non structural proteins

The synthesis and processing of rubella virus proteins takes place through high-molecular weight polyprotein precursors. The genomic RNA is approximately 10 kb long and mimics cellular mRNA by having a 5′-cap and a 3′-polyA tail (Frey, 1994). The 5′ ORF of the genomic RNA is translated from this RNA, producing a large non-structural polyprotein replicase precursor called P200. This precursor is capable of synthesizing a genome-length negative-sense RNA using the infecting positive-sense genomic RNA as a template (Liang and Gillam, 2001). P200 is subsequently cleaved into two non-structural replicase proteins, P150 and P90. The process is catalyzed by a protease embedded within the polyprotein. P150 contains methyl/guanylyl-transferase and Adenosine Diphosphate-ribose phosphatase domains as well as the protease, while P90 contains a helicase domain and the RNA dependent-RNA polymerase (Liang and Gillam, 2001). Following cleavage,

using the genome length negative-sense RNA as a template, two positive-sense RNAs are synthesized: the genomic RNA and a sub genomic RNA (Liang and Gillam, 2001).

The non-structural proteins of rubella virus are translated as a polyprotein precursor from the viral genomic RNA. The polyprotein is later cleaved into two products, p90 and p150, through the proteolytic activity of p150's carboxy terminal region. These two proteins together form the viral replicase (Hobman *et al.*, 2007).

2.5 Congenital Rubella Syndrome

Congenital rubella syndrome (CRS) is an illness resulting from infection with rubella virus during pregnancy; usually during the early months in pregnancy. It results in serious consequences such as miscarriages, foetal death or stillbirths and a constellation of severe birth defects in infants (Reef *et al.*, 2000). The risk of congenital infection and defects is highest during the first three months of gestation and decreases after the 3rd month of gestation with defects rare after the 5th month of gestation (Peckham, 1972; Miller *et al.*, 1982; South and Sever, 1985).

Congenital Rubella Syndrome has many manifestations and virtually all organs of the body are affected. It basically, affects three core organs namely, the optic lens, the cochlea and the heart (Forrest *et al.*, 2002). In addition, it affects other organs like the lungs, liver, spleen, brain, kidney, bone marrow, bones and endocrine organs leading to encephalitis, mental retardation, pneumonia, hepatitis, thrombocytopenia, metaphyseal defects, diabetes mellitus and thyroiditis (Vardas, 2011). The most prevalent pathologies are cataracts, cochlea atrophy and patent ductus arteriosus, other pathology that occur frequently in these three core organs includes glaucoma, central auditory imperceptions and peripheral pulmonic stenosis. Some infected infants may appear normal at birth, and defects like auricular sensorineural deafness, may be detected later (Vardas, 2011).

Maternal immunity, either after vaccination or naturally derived, is generally

protective against intrauterine rubella infection (Aboudy *et al.,* 1997 and Bullens *et al.,* 2000). However, there have been cases of Congenital Rubella Syndrome after maternal re-infection (Aboudy *et al.,* 1997). Therefore, CRS should always be considered in a foetus or neonate with a clinical picture suggestive of congenital infection (Bullens *et al.,* 2000). It should be noted that no case of CRS has been reported when maternal re-infection occurred after 12 weeks of pregnancy (Robinson *et al.,* 1994).

2.6 Rubella Virus Life Cycle and Coding Strategy

The virus enters the host cell through receptor mediated endocytosis via its receptor; the Myelin Oligodendrocyte glycoprotein (Cong *et al.,* 2011). As early endosomes mature into late endosomes, acidification triggers the fusion of the rubella virus envelope to the endosomal membrane and the uncoating of the capsid protein (Magliano *et al.,* 1998; Wu *et al.,* 2009) and the release of the genomic RNA into the Cytosol of the Cytoplasm. This genomic RNA serves as template for the synthesis of the non-structural proteins and a 40S negative sense RNA strand (Lee and Bowden, 2000). The positive, single-stranded RNA at first only acts as a template for the translation of the non-structural proteins, which are synthesized as a large polyprotein P200 and are then cleaved into single proteins P150 and P90 by the viral protease domain of P150 (Marr *et al.,* 1994).

RNA replication occurs in replication complexes (located in the cytoplasm) created from modified lysosomes which are morphologically termed Cytopathic vacuoles (Magliano *et al.,* 1998). The processing of P200 into P150/P90 regulates the switch from the synthesis of negative strand to positive strand (Liang and Gillam, 2001).

The 40S negative polarity RNA functions as a template for the genomic RNA and a 24S sub-genomic RNA encoding the structural proteins (Marr *et al.,* 1994; Lee and Bowden, 2000). The non structural proteins and replicating RNA remains in close association with the endosome and stimulate the formation of spherule-like membrane

invaginations intruding into the lumen of the endosome (Magliano *et al.,* 1998). The nascent spherules remain attached to the endosomal membrane via a neck structure and provide a protected environment for viral RNA replication and for the synthesis of non-structural proteins (Marr *et al.,* 1994). The 24S sub-genomic RNA is transported to the rough endoplasmic reticulum and translated polyprotein precursor is processed by a cellular signalase leading to the formation of C, E1 and E2 structural proteins (Tzeng and Frey, 2005). The E1 and E2 glycoproteins then become embedded into the Endoplasmic Reticulum and the Capsid protein likewise remains membrane associated (Tzeng and Frey, 2005). After being transported through the Golgi apparatus, the capsid protein reaches the modified endosome and assembles with the newly synthesized genomic RNA expelled from the invaginations. As infections proceeds, the rubella virus- modified endosome attracts several intracellular organelles, such as rough endoplasmic reticulum, Golgi apparatus and Mitochondria together constituting the replication complex also termed Rubella factory (Novoa *et al.,* 2005; Fontana *et al.,* 2010). The nucleocapsid of the RV then matures via budding through the intracellular membrane, including the Golgi complex, Endoplasmic Reticulum and Cytoplasmic vacuoles or occasionally at the plasma membrane (Fontana *et al.,* 2010; Risco *et al.,* 2003).

2.7 Pathogenesis and Clinical Syndrome

The infection is caused by Rubella virus in early childhood or adult life. it is usually mild, self limiting and often asymptomatic. The infection is contracted via the respiratory route. Rubella virus replicates in the nasopharynx and lung first, then spreads to the lymph nodes and appears in the blood. The resulting viremia transfers the virus to various tissues and the skin clinically apparent postnatal rubella is characterised by maculopapular rash, lyphadenopathy and fever (Best and Banatvala, 2007). The rash is the most prominent feature that exists in most of the cases. It begins with the face and spreads

to other part of the body. Infection in adults can be more severe and can occasionally be complicated by more significant symptoms such as arthropathy, thrombocytopenia and post infectious encephalopathy (Frey, 1994; Escuedero *et al.,* 2000).

In seronegative or immune deficient pregnant women, the virus can cross the placenta and infect the foetus during maternal viremia. Rubella virus is capable of spreading widely throughout the developing foetus and almost any organ can be infected. A chronic and generally non-cytolytic infection is then established. The embryo is at risk until the 20^{th} week of pregnancy (Lee and Bowden, 2000; De Santis *et al.,* 2006). By disturbing the ontogeny of fetal organs congenital Rubella Virus infection may lead to serious developmental abnormalities (Lee and Bowden, 2000; De Santis *et al.,* 2006). Common clinical manifestations of CRS include; congenital heart defect (most frequently patent ductus arteriosus, pulmonary artery or valvular stenosis), cataract, glaucoma or retinopathy, hearing loss, psychomotor retardation, Skin rash at birth, low birth weight and hepatoslenomegaly (Lee and Bowden, 2000). Although the teratogenic effects of this virus is not yet fully understood, numerous noteworthy studies suggest that the clues lies in the unusual features and unique cellular effects of Rubella virus replication (Lee and Bowden, 2000; Duszak, 2009).

2.8 Laboratory Diagnosis of Rubella

The diagnosis of Rubella is usually conducted using the following method: serological method, polymerase chain reaction, viral culture and molecular typing.

2.8.1 Serological Methods

Several techniques are available for serologic testing, including the following: Enzyme-linked immunosorbent assay (ELISA), Immunofluorescent assay (IFA), Latex agglutination (LA) test, Haemagglutination inhibition (HI) test, Complement fixation (CF)

test, Passive hemagglutination antibody (PHA) test, Hemolysis-in-gel test. Among all the serologic tests available, ELISA is the most widely used because it is relatively inexpensive, technically easy to perform, rapid, and very sensitive (Elias and Ruselle, 2014).

2.8.1.1 Enzyme linked immunosorbent assay (ELISA)

Enzyme linked immunosorbent assay is the most frequently used method for Rubella antibody screening and diagnosis because it is sensitive and adaptable and can be readily automated (WHO, 2007). From a public health standpoint, attempting to confirm a rubella infection in a pregnant woman or a newborn infant is important; a pregnant woman exposed to rubella should be tested immediately for a rubella-specific antibody.

The presence of rubella-specific IgG is evidence that the patient is immune. An infant with congenital rubella syndrome shows the IgG antibody from the mother, which disappears in a few months, and an elevated IgM antibody level because of antibody production by the infant. The presence of the IgM antibody usually indicates recent infection because IgM does not cross the placenta from the mother as does IgG. After 1 year, confirming the diagnosis of congenital rubella syndrome in an infant with serology alone is very difficult.

Asymptomatic rubella infection can be diagnosed by a positive rubella-specific IgM antibody test or a significant rise in IgG antibody level between acute- and convalescent-phase tests. The acute-phase IgG serum specimen should be collected as soon as possible after exposure, whereas the convalescent-phase IgG specimen should be collected $\geq$7-14 days (preferably 2-3 weeks) later (Centers for Disease Control and Prevention and Council of State and Territorial Epidemiologists 1997).

2.8.1.2 Rubella IgG/IgM ELISA test principle

Purified rubella antigen is coated on the surface of micro wells. Diluted patient serum is added to wells, and the rubella IgG/IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB (3, 3′.5,5′- Tetramethylbenzidine) Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of IgG/IgM specific antibody in the sample. The results are read by a micro well reader compared in a parallel manner with calibrator and controls.

2.8.2 Reverse transcription polymerase chain reaction

It is a highly sensitive and specific diagnostic tool that can be used to detect the presence of rubella virus after growth in tissue culture or directly in clinical specimens (WHO, 2011). Some of the preferred clinical specimens for detecting rubella RNA in both CRS cases and suspected acute cases are Pharyngeal samples; including throat swabs, nasal swabs, or nasal aspirates (Edlich *et al.,* 2005). Urine samples may also contain rubella virus. Collection of both pharyngeal and urine samples, when feasible, can increase the likelihood of detecting the virus. In Reverse Transcription Polymerase Chain Reaction, the RNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR.

2.8.2.1 Principle for nested RT-PCR

Nested Reverse Transcription polymerase chain reaction is a type of RT-PCR that involves two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product. The target

DNA undergoes the first run of polymerase chain reaction with the first set of primers. The selection of alternative and similar primer binding sites gives a selection of products, only one containing the intended sequence.

The product from the first reaction undergoes a second run with the second set of primers. It is very unlikely that any of the unwanted PCR products contain binding sites for both the new primers, ensuring the product from the second PCR has little contamination from unwanted products of primer dimmers, hairpins, and alternative primer target sequences.

2.8.3 Molecular typing

Molecular typing can help determine the origin of the virus, which virus strains are circulating and whether these strains have become endemic. It provides important epidemiologic information to track the epidemiology of the disease (Icenogle *et al.*, 2006). Specimens submitted for rubella virus isolation or detection by RT-PCR can also be used for molecular typing. However, the appropriate specimens include throat swabs and cerebrospinal fluid. Specimens for molecular typing should be obtained as soon as possible after diagnosis (CDC and CSTE, 1999).

2.8.4 Viral culture

Rubella grows in a wide range of cell lines. It induces a Cytopathic effect (CPE) only in continuous cell lines such as RK13 (derived from rabbit kidney epithelia cells) and Vero cells (derived from African green monkey kidney) (Elias and Ruselle, 2014). Immunofluourescense assay is often used to identify the presence of the virus in culture. Rubella virus can be isolated from nasopharyngeal, blood, throat, urine, or cerebrospinal fluid specimens from rubella and CRS cases but best results come from throat swabs (WHO, 2007). Specimens needed for viral culture should be obtained as soon as possible

(within 4 days) after rash onset. Clinical specimens for virus isolation should be obtained from all patients (or from some patients in each outbreak) at the time of the initial investigation since viral isolation is a valuable epidemiologic tool (CDC, 1999). Viral isolation is labour intensive, time consuming, costly and is not routinely used for diagnosis (WHO, 2011; Elias and Ruselle, 2014).

2.9 Treatment for Rubella

There is no specific treatment for rubella (Heymann, 2008). Patients with congenital rubella need supportive care not only in the neonatal period but also throughout life for permanent impairments like deafness and heart defects (Chantler, *et al.,* 2001; Atreya *et al.,* 2004; Horstmann *et al.,* 1976). There have been no reports of successful treatment with antiviral drugs (Horstmann *et al.,* 1976).

2.10 Rubella Virus Prevention

Rubella infection may be acquired from an infected asymptomatic person or from a patient during the incubation period for which infected persons may begin to shed the virus and, therefore, are contagious before the onset of symptoms (Agbede *et al.,* 2011; Elias and Ruselle, 2014). As a result, the most effective preventive strategy for rubella infection is the administration of rubella vaccine (Jazbec *et al.,* 2004; Elias and Ruselle, 2014).

2.10.1 Rubella vaccine

The only licensed rubella vaccine in the United States since 1979 is the 27/3 strain, which is grown in human diploid cell cultures. It is available in three (3) forms: as a combined vaccine with measles, mumps, and rubella (MMR) (Ilkow *et al.,* 2010), which is most widely used; a combined vaccine with measles, mumps, rubella, and varicella (MMRV), licensed by the United States Food and Drug Administration on September 6,

2005; and in monovalent rubella vaccine, which is less frequently used. All three (3) forms of vaccine are administered by subcutaneous injection at a standard dose of 0.5 mL.

After administration of a single dose of rubella vaccine, protective serum antibody develops in at least 95% of recipients older than 1 year (Rafiei *et al.,* 2013). Studies have shown that a single dose confers long-term immunity, probably lifelong immunity, against clinical and asymptomatic infection in more than 90% of immunized persons (Rafiei *et al.,* 2013).

2.10.1.1 Measles, Mumps and Rubella (MMR) Vaccine

Children are recommended to receive 2 doses of Measles, Mumps Rubella vaccine. The first dose of MMR is received at age 12-15 months. The second dose of MMR is received at age 4-6 years. Children who have not received the second dose by the time they enter school should receive it as soon as possible but no later than age 11-12 years (Institute for Clinical Systems Improvement, 2008).

Persons who have not received at least 1 dose of the vaccine or who have no serologic evidence of immunity to rubella are susceptible to rubella and should be immunized with MMR vaccine. MMR vaccine is especially recommended for all adults at risk of rubella infection like college students, military recruits, and health care personnel.

All post pubertal females without documentation of immunity should be vaccinated unless they are known to be pregnant (Elias and Ruselle, 2014). Birth before 1957 or clinical diagnosis of rubella is not acceptable evidence of infection for women who could become pregnant because it is only presumptive evidence, not proof of immunity. Women who receive the vaccine should be counselled not to become pregnant within 28 days of vaccine administration (Elias and Ruselle, 2014).

2.10.1.2 Precautions and contraindications of MMR vaccine

Enhanced replication of vaccine viruses may occur in persons who have immune deficiency diseases and in other persons with immunosuppression. Severe immunosuppression may be caused by many disease conditions, such as congenital immunodeficiency, HIV infection, hematologic or generalized malignancy and by therapy with immunosuppressive agents like large doses of corticosteroids (Elias and Ruselle, 2014). For some of these conditions, all affected persons are severely immunocompromised. For other conditions, such as HIV infection, the degree to which the immune system is compromised depends on the severity of the condition, which, in turn, depends on the disease or treatment stage. Ultimately, the patient's physician must assume the responsibility for determining whether the patient is severely immunocompromised on the basis of clinical or laboratory assessment (Elias and Ruselle, 2014).

It has been shown that combined MMRV vaccine is associated with an increased risk of febrile seizure occurring 5-12 days following vaccination at a rate of 1 in 2300-2600 in children aged 12-23 months compared with separate MMR vaccine and varicella vaccine administered simultaneously (Klein *et al.*, 2010; Hviid, 2011). As a result, the CDC Advisory Committee on Immunization Practices (ACIP) recommends that separate MMR and varicella vaccines be used for the first dose, although providers or parents may opt to use the combined MMRV for the first dose after counselling regarding this risk (Marin *et al.*, 2010). MMRV is preferred for the second dose (at any age) or the first dose if given at age 48 months or older (O'Leary *et al.*, 2012; Huang, 2013).

It is estimated that there is a slightly higher risk of febrile seizures in children aged 12-23 months vaccinated with the MMRV when compared with separate MMR and varicella vaccine administration. The period of risk for febrile seizures is from 5-12 days

after receipt of the vaccine. However, there is no increased risk of febrile seizures among patients aged 4-6 years receiving the MMRV. Thus, the American Academy of Paediatrics recommends that either MMR and varicella vaccines separately or the MMRV be used for the first dose of measles, mumps, rubella, and varicella vaccines administered at age 12-47 months. For the first dose of measles, mumps, rubella, and varicella vaccines administered at ages 48 months and older, and for dose 2 at any age (15 month to 12 years), use of MMRV is preferred (American Academy of Paediatrics, 2011). Data from post licensure studies do not suggest that children aged 4-6 years who received the second dose of MMRV vaccine had an increased risk for febrile seizures after vaccination compared with children the same age who received MMR vaccine and Varicella vaccine administered as separate injections at the same visit (Marin *et al.*, 2010).

Measles Mumps and Rubella vaccine should be administered to all asymptomatic persons infected with HIV who do not have evidence of severe immunosuppression (CD4 >15%) (Angel *et al.*, 1996). It also should be considered for all symptomatic persons infected with HIV who do not have evidence of severe immunosuppression because persons infected with HIV are at increased risk of severe complications if infected with the natural strain of rubella virus (BHIVA, 2006).

The benefit of immunizing non-severely immunosuppressed patients infected with HIV with MMR vaccine outweighs any vaccine adverse effects. Studies have shown that the immunologic response to live-antigen and killed-antigen vaccines may decrease as HIV progresses and that vaccination early in the course of HIV infection may be more likely to induce an immune response (Elias and Ruselle, 2014). Therefore, immunizing infants infected with HIV without severe immunosuppression with MMR vaccine as soon as possible after age 1 year is important. Consideration should be given to administration of

the second dose of MMR vaccine as soon as 28 days after the first dose rather than waiting until the child is ready to enter kindergarten or first grade (BHIVA, 2006).

Measles Mumps and Rubella vaccine should not be given to pregnant women because of the theoretical risk of rubella infection to the foetus (CDC, 2000). Data collected by the CDC reveal the estimated risk to be 1.6% (CDC, 1998). However, no cases of congenital defects have been reported in the offspring of women who inadvertently received the vaccine in their first trimester of pregnancy (Dontigny *et al.*, 2008).

There is no evidence that rubella containing vaccines are teratogenic. CRS (Best *et al.*, 2004). However, as a precaution, MMR vaccine should not be given to women known to be pregnant. If MMR vaccine is given to adult women, they should be advised to guard against pregnancy for one month. Termination of pregnancy following inadvertent immunisation should not be recommended (Tookey *et al.*, 1991). The potential parents should be given information on the evidence of lack of risk from vaccination in pregnancy.

Rubella virus strain contained in MMR is grown in human diploid cell cultures, while the other 2 components, measles and mumps virus strains, are produced in chick embryo fibroblasts but do not contain significant amounts of egg white (ova albumin) cross-reacting proteins. MMR also contains hydrolyzed gelatin as a stabilizer and trace amounts of neomycin. Anaphylactic reaction to MMR is rare. Recent data indicate that allergic reactions are mostly caused by other components of the vaccine, such as gelatin and neomycin (Fox and Lack, 2003).

Children who developed a significant hypersensitivity reaction following the first dose of MMR vaccine should have serologic testing to determine immunity to the

component of the vaccines. If shown to be immune, they should not receive a second dose of the vaccine. If the second dose is necessary, these children should have adequate evaluation for possible serious reaction to the vaccine, and skin testing should be considered before administration of the vaccine. Measles Mumps and Rubella immunization is contraindicated in children who have had immediate anaphylactic reaction following previous administration of the vaccine. However, these patients require serologic testing to determine whether they are immune to the components of the vaccine. In case of history of anaphylactic reaction to topically or systematically administered neomycin, consultation with an allergist or immunologist is warranted. In addition, MMR should be administered only in settings where such reactions could be properly managed (Clark *et al.,* 2010).

Some of the adverse reactions to MMR include: Fever of 39.4°C (103°F) or higher, Rash, Mild lymphadenopathy, Joint pain, Arthralgia, Transient arthritis. Joint complaints occur approximately 7-21 days following MMR vaccination. Rare cases of transient peripheral neuritic symptoms, such as paresthesia and pain in the arms and legs, have been reported. Transient and benign thrombocytopenia within 2 months of immunization has been reported in 1 per 25,000-40,000 immunized children. Central Nervous System (CNS) manifestations have also been reported, but no causal relationship with rubella vaccine has been demonstrated. Adverse reactions are considerably less common after a second dose of MMR vaccine than after the first dose (Chen *et al.,* 1991).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study Area

The study was carried out in Kaduna State (Figure 3.1), one of the States located in North-Western Nigeria, with a population of 6,066,562 (NPC, 2006). It is characterized by a tropical climate with two seasons the rainy season (May to October), with a mean annual rainfall of 1092.8mm and the dry season which spans between November and April. The monthly mean temperature shows a maximum of 39.7°C in June and 13.8°C in December (Ukegbu, 2005). The State was one time administrative headquarters of the North during the colonial era. It consists of many commercial and manufacturing industries, and it is home to many institution of higher learning. All these in addition to its rapid urbanization have contributed to the diversity and integration of residents into the State.

The State consists of twenty–three (23) Local Government Areas with several districts and wards. The State is divided into three (3) senatorial zones: Northern (Zone 1), Central (Zone 2), and Southern (Zone 3). Zone 1 comprises of Zaria, Soba, Sabongari, Makarfi, Kudan, Ikara, Kubau and Lere LGAs. Zone 2 comprises of Birnin Gwari, Giwa, Chikun, Kaduna South, Kaduna North, Kajuru and Igabi LGAs. Zone 3 comprises of Zango-kataf, Jema'a, Kauru, Kaura, Kachia, Sanga, Jaba and Kagarko LGAs. Kaduna State has 739 local government health facilities, 29 secondary health care facilities and 5 tertiary hospitals (Abubakar *et al.*, 2013). Three hospitals were selected one from each of the Senatorial District, they are:

1. Hajiya Gambo Sawaba Memorial Hospital, Zaria City, representing Kaduna North.
2. Barau Diko General Hospital, representing Kaduna Central.
3. General Hospital, Kafanchan representing Kaduna South.



Figure 3.1: Kaduna State showing the Study Sites

Source: National Population Commission (2006).

Key

-Northern Senatorial District

-Central Senatorial District

-Southern Senatorial District

▲ Sample Collection Site

3.2 Study Population

The study was carried out among women of reproductive age (both pregnant and non-pregnant) attending the selected health care facilities in the respective senatorial districts.

3.3 Study Design

The study was cross-sectional, and it enrolled 360 women of reproductive age between 15-45 years attending the selected heath care facilities in Kaduna State who gave their consent. Purposive sampling technique was used in the choice of the hospitals. Subjects who consented were interviewed with a structured questionnaire (Appendix III) and data on demographics, risk factors, symptoms of the disease and knowledge about the disease were obtained. The subjects were screened for rubella IgG and IgM antibody using the RUB IgG (Lot Number: DA15-D1-251) and IgM ELISA (Lot Number: DA15-D1-229) kit respectively. Nested Reverse Transcription Polymerase Chain Reaction (RT-nPCR) was carried out on only one third of the IgM positive cases obtained by ELISA due to financial constraint.

3.4 Inclusion Criteria

The study included women of reproductive age (both pregnant and non-pregnant) between the age range of 15-45 years who consented and were attending the selected healthcare facilities in the different senatorial districts.

3.5 Exclusion Criteria

Women who did not fall within the stated age bracket of 15-45 years and those who did not give their consent were excluded from the study.

3.6 Ethical Considerations

Ethical approval (Appendix I) was obtained from the Medical and Scientific Committee of Kaduna State Ministry of Health (MOH/ADM/744/VOL.1/355). Informed consent (Appendix II) was also obtained from the respondent prior to collection of sample.

3.7 Sample Size Determination

For the purpose of the study, a prevalence of 93.1% as reported from a study conducted in Zaria, Nigeria (Olajide *et al*., 2015) was used to determine the sample size. Using the formula for cross-sectional studies the sample size was calculated as follows;

$N = \frac{Z^2Pq}{d^2}$ (Naing *et al.*, 2006)

Where;

N = number of samples

Z = 1.96 for 95% confidence interval

P = proportion of positive individuals (93.1%)

d = absolute precision and it is taken as 0.05

$q = 1 - p$

$N = \frac{1.96^2 \times 0.931(1 - 0.931)}{0.05^2}$

N = 98.71

A total of 360 samples were collected for this study. The calculated sample size is the minimum number of samples that can be used but the sample size was increased to 360 so as to increase the possibility of recovering the virus.

3.8 Data Collection

Questionnaire (Appendix III) was administered to women who consented prior to collection of samples and data on socio-demographics, awareness and knowledge of rubella, possible risk factors and clinical symptoms associated with the viral infection were obtained.

3.9 Collection and Processing of Samples

A total of 360 blood samples and 360 throat swab samples were collected respectively from the subjects.

3.9.1 Collection of Blood Sample

Exactly 5ml of blood was obtained from the subjects into an appropriately labelled sterile container free of anticoagulants or preservatives by venepuncture. Thereafter, the samples were transported in a cold box with frozen ice packs to maintain a condition of about 4°C - 8°C to the Laboratory, Department of Microbiology, Ahmadu Bello University Zaria where the analysis were carried out (Pennap *et al.,* 2009).

Serum samples were separated from blood cells by centrifugation at 2500 revolution per minutes (rpm) for 5 minutes. The serum was harvested using a sterile disposable pipette and transferred into labelled sterile cryovials and stored at -20°C until ready for analysis (Olajide *et al.,* 2015).

3.9.2 Collection of Throat Swab Sample

Throat swab samples were obtained from consenting patients by a Physician. The process involved firmly rubbing the back of the throat with sterile cotton swabs to dislodge epithelial cells. The swabs were placed in sterile viral transport medium (Phosphate Buffered Saline) in labelled screw-capped tubes and transported on ice packs to the

Department of Microbiology where the analysis was carried out. Prior to analysis, the throat swab samples were stored at -20^{o}C.

3.10 Rubella IgG ELISA

Rubella IgG ELISA which is an Enzyme immunoassay technique intended for use in the detection of IgG antibody to Rubella virus was used to diagnose subjects who have had previous exposure to the virus.

3.10.2 Reagent Preparations

All specimens and kit reagents were brought at room temperature (20-25˚C) and then mixed gently. Washing buffer (1x) was prepared by adding distilled or deionized water to 20x wash concentrate to a final volume of 1 litre.

3.10.3 Assay for IgG ELISA

The 360 serum samples were subjected to IgG ELISA. Rubella IgG ELISA kit (Lot Number: DA15-D1-251) was used and the procedure was carried out according to the manufacturer's instructions as stated below:

The desired numbers of coated strips to be used were placed into a holder and dilutions of 1:40 were prepared by adding 5µl of the test samples, negative control, positive control, and calibrators to 200µl of sample diluents after which it was mixed thoroughly. One hundred micro litres (100 µl) of diluted sera, calibrators, and controls were dispensed into the appropriate wells. For the reagent blank, 100 µl sample diluent was dispensed in 1A well position. The holder was tapped to remove air bubbles from the liquid after which it was mixed thoroughly. The plates were incubated for 30 minutes at room temperature after which liquid was removed from all the wells and the wells were washed repeatedly three times with washing buffer (Lezan, 2015).

One hundred micro litres (100 µl) of enzyme conjugate was dispensed into each well and incubated for 30 minutes at room temperature. The Enzyme conjugate was removed from the wells after incubation and the wells were washed repeatedly three times with washing buffer. One hundred micro litres (100 µl) of TMB Chromogenic Substrate was dispensed into each well and were incubated for 15 minutes at room temperature. After the third incubation, 100 µl of stop solution was added into each well to stop the reaction and the optical densities (O.D.) of each of the wells were read at 450 nm with a micro well reader (GF M3000; B Braun Scientific and Instrument, UK) (Lezan, 2015).

3.11 Rubella IgM ELISA

Rubella IgM ELISA which is an Enzyme immunoassay technique intended for use in the detection of IgM antibody to Rubella virus was used to ascertain subject with acute rubella infection.

3.11.1 Assay for IgM ELISA

Rubella IgM ELISA was conducted on the 360 serum samples using the Rubella IgM ELISA kit (Lot Number: DA15-D1-229) and the procedure was as described for Rubella IgG ELISA.

3.12 Assay for Nested RT-PCR

The primer sequences targeting the E1 gene of Rubella virus used for the nested RT-nPCR assay and their expected amplicon sizes are shown in Table 3.1.

Table 3.1: Primer sequences used in nested reverse transcription polymerase chain reaction (RT-nPCR) targeting the E1 gene of Rubella virus

Gene of Target	Primer	Primer sequence (5′-3′)	Expected Amplicons
Rubella virus E1 gene	Sense R1	CAA CAC GCC GCA CGG ACA AC	185bp
	Antisense R2	CCA CAA GCC GCG AGC AGT CA	
	Sense R3	CTC GAG GTC CAG GTC CTG CC	143bp
	Antisense R4	GAA TGG CGT TGG CAA ACC GG	

Source: Revello *et al.,* 1997

3.12.1 RNA Extraction Protocol

Total RNA extraction was carried out using QIAamp® viral RNA mini extraction kit and the procedures were carried out according to the manufacturer's instruction in which 30 Throat swab samples to be subjected to PCR were thawed at room temperature (15^oC-25^oC) and the samples were centrifuged at 25,000×g for 60 minutes, which the supernatant was carefully removed from the tubes, using a fine-tip transfer pipette leaving about 140µl of the sediment. Five hundred and sixty microliter (560µl) of prepared buffer AVL containing carrier RNA was added and incubated at room temperature (15^oC-25^oC) for 10 minutes. The tubes were spun to remove drops from inside the lids. Five hundred and sixty microliter (560µl) of ethanol (96-100%) was added to the sample, which was mixed by pulse vortexing for 15 seconds and tubes were briefly centrifuged to remove drops from the inside of the lid. Six hundred and thirty microliter (630µl) of the solution from the previous step was applied to the QIAamp Mini column in a 2ml collection tube without wetting the rim. The cap was closed and centrifuged at 6000×g (8000 rpm) for 1 minute. The Mini column was placed into a clean 2ml collection tube, and the tube containing the filtrate was discarded. The Mini column was carefully opened and the previous step was repeated.

The Mini column was carefully opened and 500µl of buffer AW1 was added. The cap was closed and centrifuged at 6000×g (8000 rpm) for 1 minute after which it was placed into a clean 2ml collection tube, and the tube containing the filterate was discarded. The Mini column was carefully opened and 500µl of buffer AW2 was added. The cap was closed and then centrifuged at full speed for 3 minutes. The Mini column was placed in a new 2ml collection tube and centrifuged at full speed for 1minute. The Mini column was placed in a clean 1.5ml microcentrifuge tube, the column was carefully opened and 65µl of buffer AVE equilibrated to room temperature was added. The cap was closed, incubated at

room temperature for 1minute and centrifuged at 8000rpm for 1 minute and the viral RNA was transferred into a clean 1.5ml screw cap tube.

3.12.2 Complementary Deoxyribonucleic Acid (cDNA) Synthesis Protocol

The cDNA synthesis protocol was carried out using One *Taq*® RT-PCR Kit and the procedures were as follows: Six microlitre (6µL) of the RNA sample and two microlitre (2µL) of the $d(T)_{23}$ making up a total volume of eight microlitre (8µL) were mixed in two sterile RNase-free microtubes. RNA was denatured at 70°C for 5 minutes, spun briefly and then placed on ice for 5 minutes to improve the yield of cDNA since the E1 region of Rubella virus RNA is rich in Guanine and Cytosine nucleotides.

To the tube containing 8 µL RNA and $d(T)_{23}$, 10µL of Molony Murine Leukaemia virus (M-MuLV) reaction mix and 2µL M-MuLV enzyme mix was added making up a total volume of 20µL. To the negative control tube containing 8µL RNA and $d(T)_{23}$, 10µL of M-MuLV reaction mix and 2µL of distilled water instead of enzyme mix was added making up a total volume of 20µL. The 20µL cDNA synthesis reactions were incubated at 42°C for one hour. The enzyme was inactivated at 80°C for 4 minutes. The reaction was diluted to 50µL with 30µL of distilled water for PCR.

3.12.3 PCR Amplification

Thirty micro litres (30µl) of the PCR mix containing 15pmol of forward primer R1 (Table 3.1) and 25pmol of reverse primer R2 (Table 3.1), 1 × PCR buffer, 10% dimethyl sulfoxide (DMSO) and 1.5µ of *taq* polymerase was prepared. This was then added to the RT mixture and Ten (10) cycles of 94°C for 45 seconds, 65°C for 30 seconds and 72°C for 30 seconds; 50cycles of 94°C for 30 seconds, 59.5°C for 40 seconds and

72°C for 45 seconds plus 2seconds for every subsequent cycle was amplified (Revello *et al.,* 1997).

The second round PCR mix contains 25pmol of each primer R3 and R4, 1× PCR buffer, 12.5mol of deoxynucleoside triphosphate, 10% dimethyl sulfoxide and 1.5µ *Taq* polymerase in a final volume of 50µl. In the second round, 1/100 of the first round product was further amplified for Thirty-five (35) cycles of 94°C for 30seconds, 58°C for 45seconds and 72°C for 45seconds and 15µl of the first and second round products were electrophoresed (Revello *et al.,* 1997).

3.12.4 Agarose Gel-electrophoresis

Four grams (4g) of Agarose powder was weighed and transferred into a sterile conical flask. Two Hundred millilitres (200ml) of 1× Tris Borate Ethylene diamine tetra Acetic acid Buffer (TBE) was added and the mixture was micro waved for 7 minutes to dissolve the agarose after which it was allowed to cool to about 45^{o}C. On cooling, five microlitres (5µl) of Ethidium bromide was added to the mixture and it was swirled gently.

The Gel casting tray to be used for the electrophoresis was assembled with the combs placed in position. The mixture was carefully poured into the casting tray and allowed to solidify at room temperature. The combs were carefully removed and the tray was transferred to the electrophoresis tank. The tank was filled to the gauge mark with running buffer (1×TBE). The first well was loaded with 5µl of 50bp DNA ladder, the second well was loaded with 10µl nuclease free water mixed with 5µl of 6×loading dye and other wells were loaded with 10µl genomic DNA samples mixed with 5µl of 6×loading dye. The voltage was set at 75V and the preparation was run for 45 minutes. The Gel tray was removed and transferred to the gel documentation unit. The gel was observed and the image documented in the computer for analysis of the image

CHAPTER FOUR

RESULT

Out of the 360 women screened by ELISA, 343 women tested positive for rubella IgG and 93 tested positive for rubella IgM antibodies. A prevalence of 95.3% and 25.8% was obtained for Rubella IgG and IgM antibodies as shown in Figures 4.1 and 4.2 respectively.

The Venn diagram showing the relationship between Rubella IgG and IgM among women of reproductive age in Kaduna State, Nigeria as shown in Figure 4.3 revealed that, 88(24.4%) women in the study population tested positive for both Rubella IgG and IgM antibodies. Only 12(3.3%) women in the study, tested negative to rubella IgG and IgM antibodies.

Plate I depicted the Agarose gel electrophoresis image showing PCR amplicons of rubella virus E1 gene size 143bp. Of the thirty (30) samples analysed by Nested RT-PCR, rubella virus E1 gene was detected in four (4) samples giving a prevalence of 13% by PCR.

The seroprevalence of Rubella IgG and IgM with respect to Senatorial District among women of Reproductive age as presented in Table 4.1 revealed that the Central Senatorial District had the highest number of women who tested positive for Rubella IgG antibodies with a prevalence of 96.7% while the Southern Senatorial District had the least number of women who tested positive with a prevalence of 93.3%. On the other hand, it was observed that the Northern Senatorial District had the highest prevalence of 38.3% and the Southern Senatorial District the least prevalence of 11.7% for rubella virus IgM antibodies.

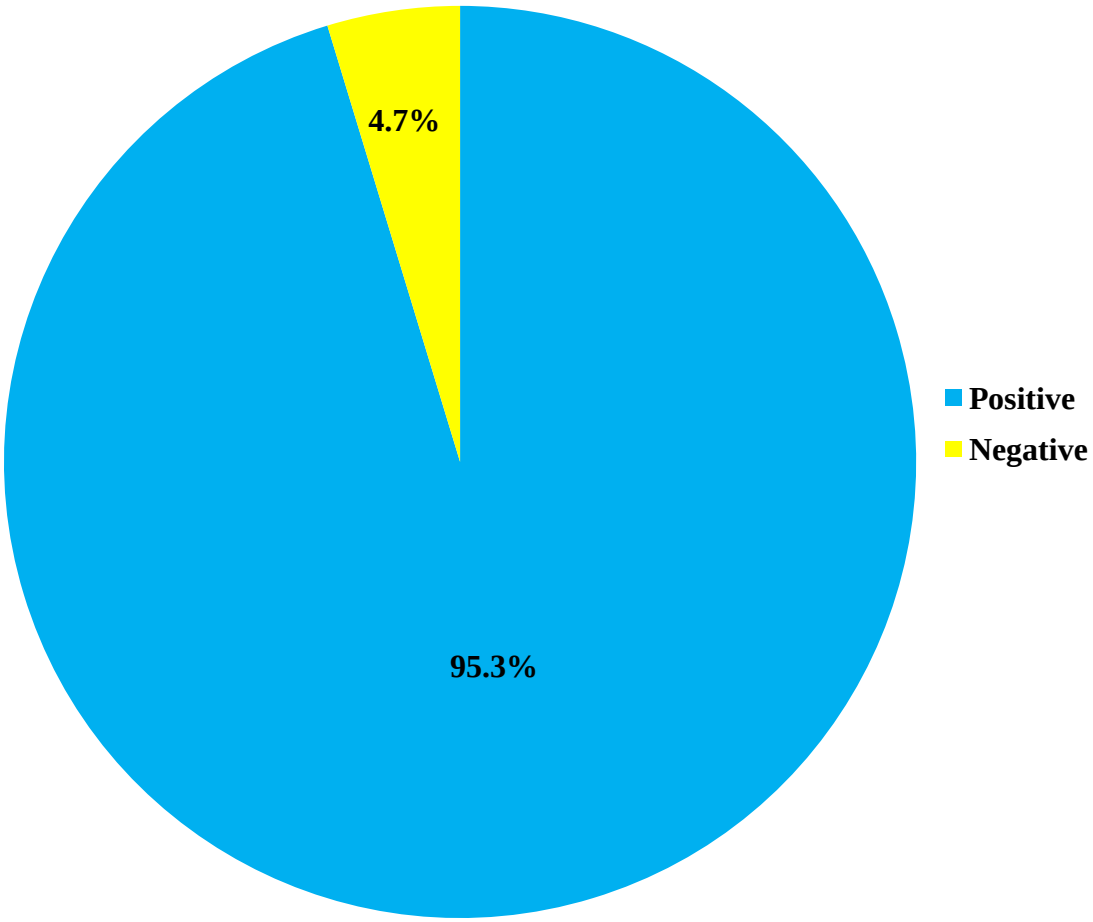


Figure 4.1: Seroprevalence of Rubella IgG among women of reproductive age

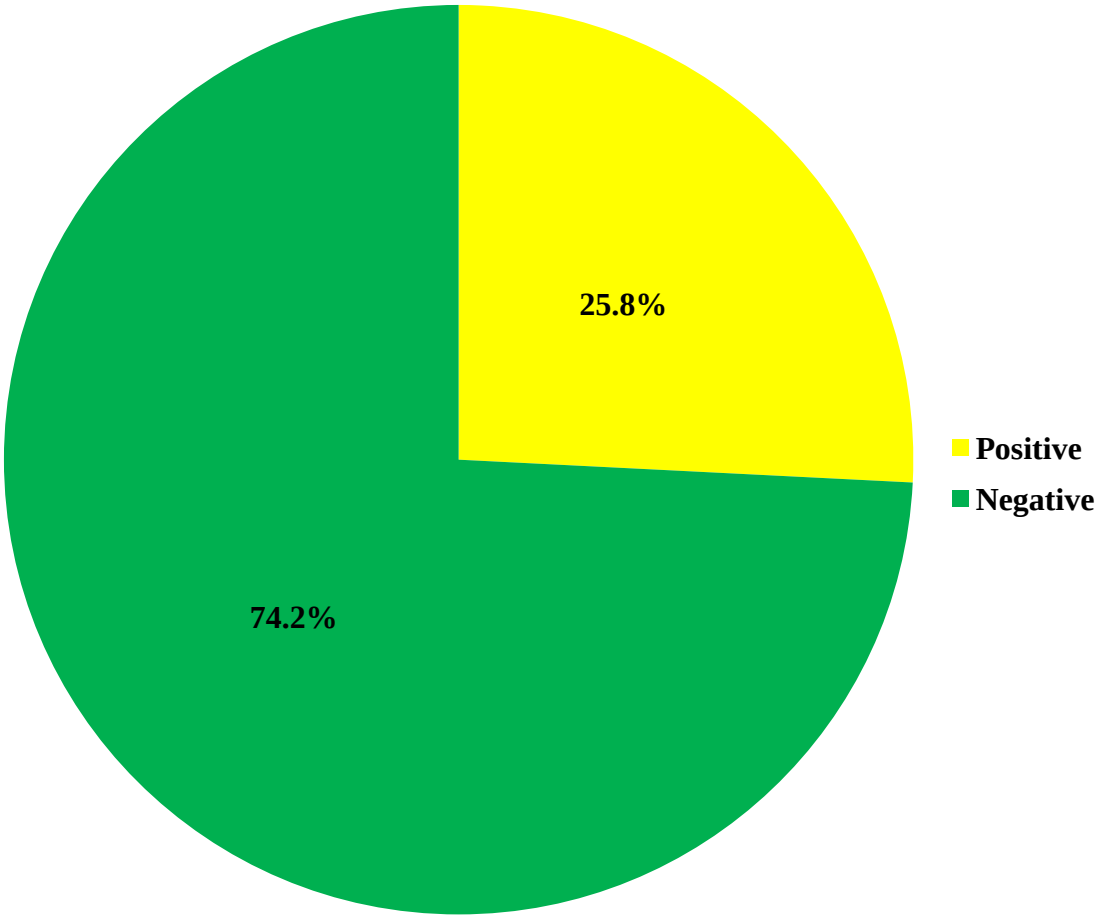


Figure 4.2: Seroprevalence of Rubella IgM among women of Reproductive Age

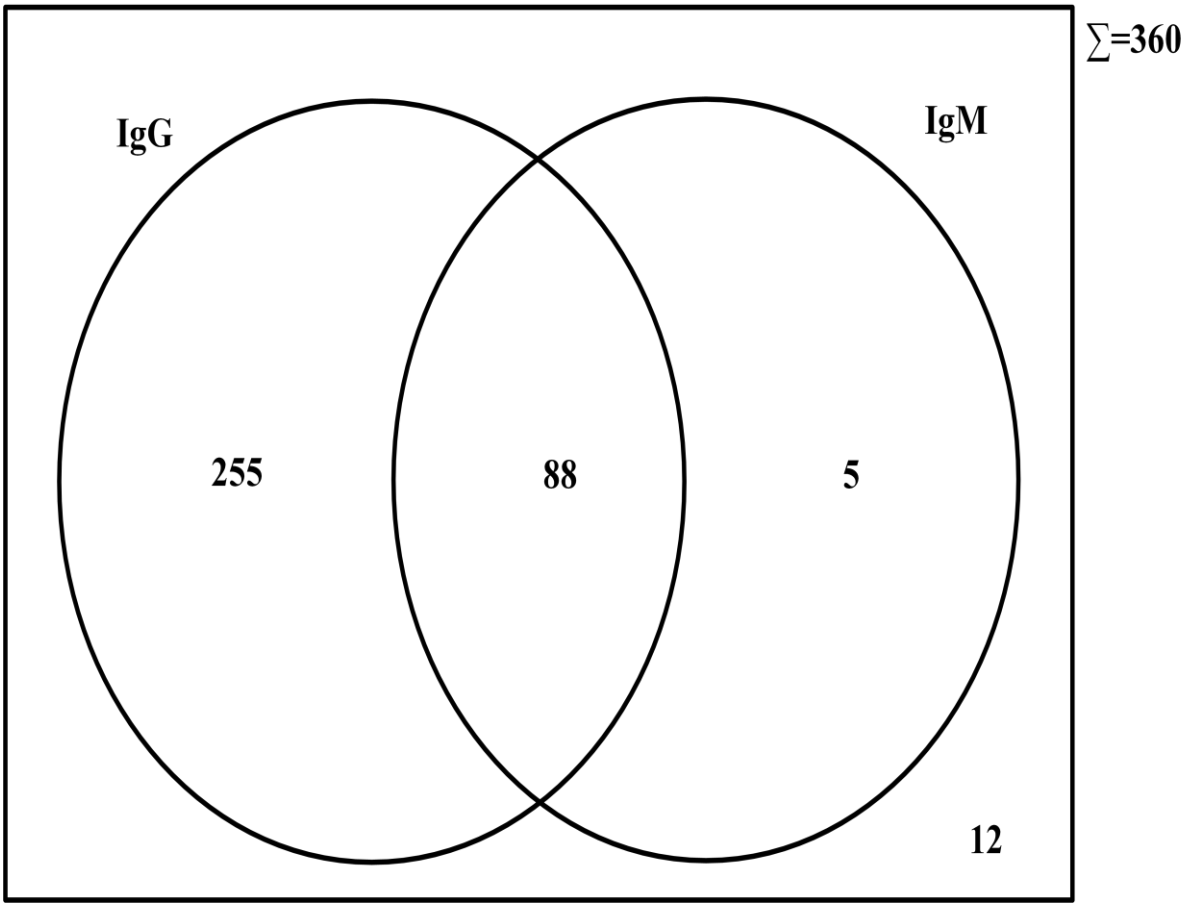


Figure 4.3: Venn diagram showing the relationship between rubella virus IgG and IgM antibodies among women of reproductive age

Key:

IgG positive = 255

IgM positive = 5

IgG and IgM positive = 88

IgG and IgM negative = 12

Sum Total = 360

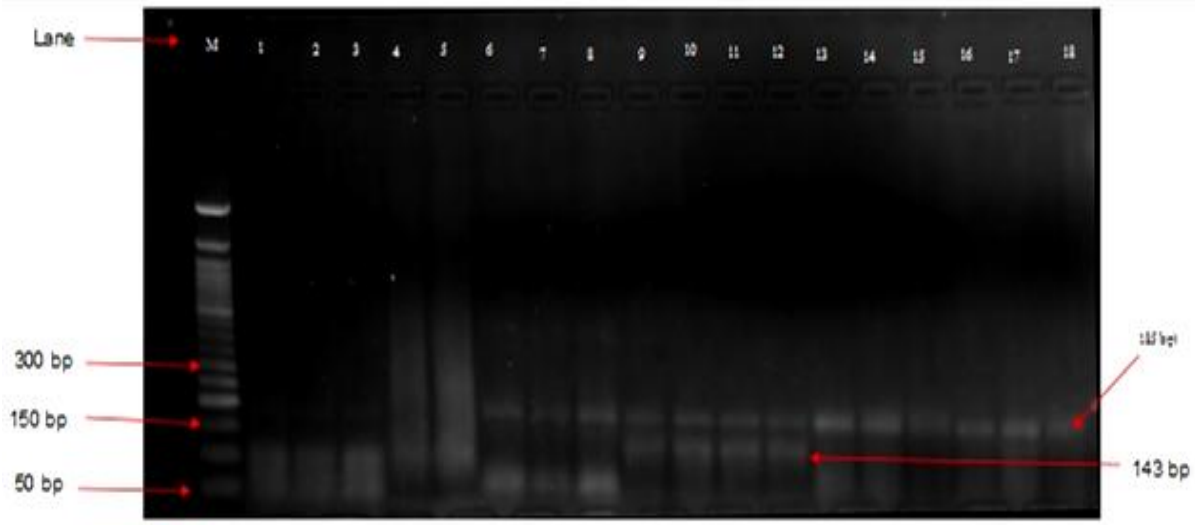


Plate I: Amplicons of Rubella virus E1 gene size 143bp lanes 1-18, Lanes M 50bp DNA ladder.

Key:

M: Molecular marker (50bp)

Lanes 9-12: Rubella virus E1 protein (size 143bp)

Table 4.1: Seroprevalence of Rubella IgG and IgM, with respect to Senatorial District

		Rubella IgG				Rubella IgM			
Senatorial District	**No Examined**	**No/% Positive**	**P value**	**χ^2**	**df**	**No/% Positive**	**P value**	**χ^2**	**df**
Zone 1	120	115(95.8)	0.448	1.605	2	46(12.8)	0.001	22.530	2
Zone 2	120	116(96.7)				33(9.2)			
Zone 3	120	112(93.3)				14(3.9)			
Total	**360**	**343(95.3)**				**93(25.8)**			

Key: df = degree of freedom, Zone 1 = Northern Senatorial District, Zone 2 = Central Senatorial District, Zone 3 = Southern Senatorial District, No = Number, P-value = Calculated Probability, χ^2 = Chi Square.

The seroprevalence of Rubella IgG and IgM antibodies among women of reproductive age with respect to their ages is presented in Table 4.2. Women who were within the age group of (40-45) years had the highest prevalence of 100% for Rubella IgG antibodies while women within the age group of (25-29) years had the least prevalence of 92.9%. On the other hand, women who were within the age group of (15-19) years had the highest prevalence of 31.8% while women within the age group of (20-24) years had the least prevalence of 19.6% for rubella IgM antibodies respectively.

Table 4.3 showed the seroprevalence of Rubella IgG and IgM with respect to the marital status of the study population. A higher prevalence (100%) for Rubella IgG was seen among subjects who were single while Subjects who married were had a lower prevalence (95.1%). A higher prevalence of 26.4% for Rubella IgM was observed among subjects who were married and a least prevalence of 9.1% was observed among singles.

The seroprevalence of Rubella IgG and IgM with respect to educational status of women of reproductive age as shown in Table 4.4 revealed the highest prevalence of 100% and 28.2% for Rubella IgG and IgM respectively of women with informal education while the least prevalence of 91.4% and 21.9% for Rubella IgG and IgM respectively were recorded in women with tertiary education.

Table 4.2: Seroprevalence of Rubella IgG and IgM, with respect to Age

Age (yrs)	No Examined	Rubella IgG				Rubella IgM			
		No/% Positive	P-value	χ^2	df	No/% Positive	P-value	χ^2	df
15-19	22	21(95.5)	0.821	2.196	5	7(31.8)	0.620	3.520	5
20-24	102	98(96.1)				20(19.6)			
25-29	99	92(92.9)				27(27.3)			
30-34	93	89(95.7)				28(30.1)			
35-39	31	30(96.7)				8(25.8)			
40-45	13	13(100)				3(23.1)			
Total	**360**	**343(95.3)**				**93(25.8)**			

Key: df = degree of freedom, No = Number, P: Calculated Probability, χ^2 = Chi Square

Table 4.3: Seroprevalence of Rubella IgG and IgM, with respect to Marital Status

		Rubella IgG			Rubella IgM			
Marital Status	**No Examined**	**No/% Positive**	**P-value**	**df**	**No/% Positive**	**P-value/OR**	**df**	**CI**
Married	349	332(95.1)	1.000	1	92(26.4)	0.301/3.580	1	0.452-28.351
Single	11	11(100)			1(9.1)			
Total	**360**	**343(95.3)**			**93(25.8)**			

Key: df = degree of freedom, No = Number, OR = Odds ratio, CI = Confidence Interval, P-value = Calculated Probability.

Table 4.4: Seroprevalence of Rubella IgG and IgM, with respect to Educational Status

		Rubella IgG				Rubella IgM			
Educational Status	**No Examined**	**No/% Positive**	**P - value**	χ^2	**df**	**No/% Positive**	**P - value**	χ^2	**df**
Primary	54	53(98.1)	0.094	6.396	3	15(27.7)	0.739	1.259	3
Secondary	163	156(95.7)				44(26.9)			
Tertiary	105	96(91.4)				23(21.9)			
Informal	38	38(100)				11(28.9)			
Total	**360**	**343(95.3)**				**93(25.8)**			

Key: df = degree of freedom, No = Number, P-value = Calculated Probability, χ^2 = Chi Square.

Table 4.5a and 4.5b revealed the seroprevalence of Rubella IgG and IgM respectively with respect to the pregnancy status of the women. Pregnant women had higher prevalence of 95.1% and 27% for Rubella IgG and IgM respectively as compared to their non pregnant counterparts. Rubella IgG and IgM antibodies were identified in all the three (3) trimesters of pregnancy as shown in Table 4.5a and 4.5b. Of the 345 pregnant women screened, 17 were in their first trimester, 169 in the second trimester and 162 in the third trimester of pregnancy. The highest prevalence (95.3%) for Rubella IgG was among women in their second trimester of pregnancy while women who were in their first trimester of pregnancy had the highest prevalence (35.3%) for rubella IgM antibodies.

Table 4.6a and 4.6b showed the seroprevalence of Rubella IgG and IgM with respect to the history of rubella. The highest prevalence (100%) for rubella IgG was among subjects who testified having a history of rubella, 96% in subjects who testified having a history of miscarriage and 95.3% among subjects who testified not having any history of still birth. The result for rubella IgM with respect to the history of the disease, stillbirth and miscarriage revealed the highest prevalence of 29.6% in subjects who testified not having a history of the disease, 26.5% among those who testified that they had a history miscarriage and 38.9% in those who testified that they had a history of still birth.

The seroprevalence of Rubella IgG and IgM with respect to some of the disease signs and symptoms are presented in Tables 4.7a and 4.7b respectively. High prevalence for rubella IgG antibodies was recorded in subjects who had fever (97%), rash (100%), swollen lymph node (100%) and joint pains (96%). On the other hand, the highest prevalence for rubella IgM was recorded in subjects who had fever (28.4%), no rash (26%), no swollen lymph node (25.9%) and joint pains (38%).

Table 4.5a: Seroprevalence of Rubella IgG with respect to Pregnancy Status

Pregnancy Status	No Examined	No/% Positive	P-value/ OR	χ^2	df	C I
Pregnancy						
Pregnant	345	328(95.1)	1.000/ 0.956	0.776	1	0.935- 0.978
Non pregnant	15	15(100)				
Trimester						
First	17	16(94.1)	0.894	0.611	2	
Second	167	161(96.4)				
Third	161	154(95.7)				

Key: OR = Odds Ratio, df = degree of freedom, CI = Confidence interval, No = Number P-value = Calculated Probability, χ^2 = Chi Square.

Table 4.5b: Seroprevalence of Rubella IgM with respect to Pregnancy Status

Pregnancy Status	No Examined	No/% Positive	P-value/ OR	χ^2	df	C I
Pregnancy						
Pregnant	345	93(27)	0.015/ 1.060	5.452	1	1.029 -1.091
Non pregnant	15	0(0)				
Trimester						
First	17	6(35.3)	0.004/ 13.098		2	
Second	167	56(33.5)				
Third	161	31(19.3)				

Key: OR= Odds Ratio, df = degree of freedom, CI=confidence interval, No = Number, χ^2 = Chi Square, P-value = Calculated Probability.

Table 4.6a: Seroprevalence of Rubella IgG with respect to History of the Disease

History of Rubella	No Examined	No/% Positive	P-value/ OR	χ^2	df	C I
Disease history						
Yes	7	7(100)	0.478	1.477	2	
No	228	215(94.3)				
Unknown	125	121(96.8)				
History of Miscarriage						
Yes	50	48(96)	0.603/ 1.220		1	0.271 -5.505
No	310	295(95.2)				
History of Still birth						
Yes	18	17(94.4)	0.590/ 0.834		1	0.104 -6.667
No	342	326(95.3)				

Key: OR = Odds Ratio, df = degree of freedom, CI = Confidence interval, No = Number, P-value = Calculated Probability, χ^2 = Chi Square.

Table 4.6b: Seroprevalence of Rubella IgM with respect to History of the Disease

History of Rubella	No Examined	No/% Positive	P-value/ OR	χ^2	df	C I
Disease history						
Yes	7	2(28.6)	0.472	1.503	2	
No	228	54(23.7)				
Unknown	125	37(29.6)				
History of Miscarriage						
Yes	50	11(22)	0.603/ 0.784	0.445	1	0.384 -1.603
No	310	82(26.5)				
History of Still birth						
Yes	18	7(38.9)	0.266/ 1.894	1.686	1	0.712 -5.040
No	342	86(25.1)				

Key: OR= Odds Ratio, df = degree of freedom, CI = Confidence interval, No = Number P-value = Probability, χ^2 = Chi Square.

Table 4.7a: Seroprevalence of Rubella IgG with respect to some Disease Signs and Symptoms

Signs and symptoms	No Examined	No/% Positive	P-value/ OR	df	C I
Fever					
Yes	67	65(97)	0.749/ 1.754	1	0.391- 7.858
No	293	278(94.9)			
Rash					
Yes	2	2(100)	1.000/ 0.994	1	0.986- 1.002
No	358	341(95.3)			
Swollen Lymph node					
Yes	1	1(100)	1.000/ 0.997	1	0.991 -1.003
No	359	342(95.3)			
Joint pains					
Yes	50	48(96)	1.000/ 1.224	1	0.271 -5.524
No	310	294(95.1)			

Key: OR = Odds Ratio, df = degree of freedom, CI = Confidence interval, No = Number, P-value = Calculated Probability.

Table 4.7b: Seroprevalence of Rubella IgM with respect to some Disease Signs and Symptoms

Signs and symptoms	No Examined	No/% Positive	P-value/ OR	df	C I
Fever					
Yes	67	19(28.4)	0.643/ 1.171	1	0.647 -2.120
No	293	74(25.3)			
Rash					
Yes	2	0(0)	1.000/ 1.008	1	0.997 -1.018
No	358	93(26)			
Swollen Lymph node					
Yes	1	0(0)	1.000/ 1.004	1	0.996 -1.011
No	359	93(25.9)			
Joint pains					
Yes	50	19(38)	0.054/ 1.946	1	1.039 -3.647
No	310	74(23.9)			

Key: OR = Odds Ratio, df = degree of freedom, CI = Confidence interval, No = Number, P-value: Calculated Probability.

The relationship between Rubella IgG and IgM with respect to immunity status as presented in Table 4.8 showed that subjects who claimed to have been vaccinated against the virus had the highest prevalence (100%) for Rubella IgG while those who have not had any vaccination had the least prevalence of 94.6%. On the contrary, for Rubella virus IgM Subjects who claimed they have not had rubella virus vaccination had a higher prevalence of 67.0% while those who claimed to have been vaccinated had a lower prevalence of 0%.

The relationship between Rubella IgG and IgM with respect to general knowledge about rubella is depicted by Table 4.9. No significant association was observed between Rubella IgG and IgM and knowledge about the virus. However, for rubella IgG the highest prevalence of 100% was observed among women who knew about rubella, 100% among women who had no knowledge about the mode of transmission of the virus, 96.2% among women who do not know whether the virus was contagious or not and 100% among those women who do not know if the foetus was at risk or not.

On the other hand, for Rubella IgM the highest prevalence of 21.1% was among women who do not know about rubella. Women who were not sure whether Rubella was contagious had the highest prevalence of 25.6%. With respect to the mode of transmission, women who were not sure of rubella's mode of transmission had the highest prevalence of 15.8% and also those women who were not sure about the risk of the virus to the foetus had the highest prevalence of 25%.

Table 4.8: Seroprevalence of Rubella IgG and IgM with respect to Vaccination status

		Rubella IgG				Rubella IgM			
Vaccination status	**No Examined**	**No/% Positive**	**P-value/ OR**	**χ^2**	**df**	**No/% Positive**	**P-value/OR**	**χ^2**	**df**
Vaccinated									
Yes	2	2(100)	0.533	1.257	2	0(0)	0.224	2.988	2
No	278	263(94.6)				67(24.1)			
Unknown	80	78(97.5)				26(32.5)			

Key: OR = Odds Ratio, df = degree of freedom, CI = Confidence interval, No = Number, P-value = Calculated Probability, χ^2 = Chi Square.

Table 4.9: Seroprevalence of Rubella IgG and IgM with respect to General knowledge about Rubella

Knowledge	No Examined	Rubella IgG				Rubella IgM			
		No/% Positive	P-value	χ^2	df	No/% Positive	P -value	χ^2	df
What is Rubella									
Yes	8	8(100)	0.593	1.044	2	1(0.3)	0.092	4.782	2
No	311	297(95.5)				76(21.1)			
Unknown	41	38(92.7)				16(4.4)			
Contagious									
Yes	9	8(88.9)	0.545	1.216	2	1(0.3)	0.137	3.980	2
No	8	8(100)				0(0)			
Unknown	343	327(95.3)				92(25.6)			
Mode of transmission									
Yes	8	6(75)	0.023	7.569	2	2(0.6)	0.998	0.004	2
No	131	126(96.2)				34(9.4)			
Unknown	221	211(95.5)				57(15.8)			
Foetus at risk									
Yes	11	9(81.8)	0.088	4.862	2	2(0.6)	0.649	0.865	2
No	7	7(100)				1(0.3)			
Unknown	342	327(95.6)				90(25)			

Key: df = degree of freedom, No = Number, P-Value = Calculated Probability, χ^2 = Chi Square.

CHAPTER FIVE

DISCUSSION

The seroprevalence of rubella IgG among women of reproductive age in Kaduna State, Nigeria obtained in this study was 95.3%. This implied that 95.3% of the population has detectable levels of Rubella IgG antibodies. The reasons for this high prevalence in the study population could be attributed to exposure and infection of the subjects with Rubella virus earlier in life before reaching childbearing age. Majority of the women in this study were not vaccinated because rubella vaccinations are not available in the country as such it can be deduced that the Rubella IgG prevalence obtained in this study was as a result of natural infection and not vaccination. The 95.3% Rubella IgG seroprevalence obtained in this study is in agreement with 93.1% carried out by Olajide *et al.* (2015) in Zaria, 97.5% by Mohammed *et al.* (2010) in Zaria, 88.6% by Fokunang *et al.* (2010) in Yaounde, Cameroun and 96.1% in Western region of Turkey (Tamer *et al.,* 2008). On the contrary, the prevalence is much higher than the 53% obtained by Onakewhor and Chiwuzie in Benin City (Onakewhor and Chiwuzie, 2011) and 7% obtained by Agbede *et al.* (2011). The reason for this contrast could be attributed to the differences in the immunity status of the study population and also the fact that rubella epidemics occurs every 5-9 years (WHO, 2000; Omer *et al.,* 2010).

Seroprevalence of Rubella IgM among women of reproductive age in Kaduna State, Nigeria as obtained in this study implies that about 25.8% of the study population have detectable levels of Rubella IgM antibodies which may indicate primary or an acute rubella infection since the presence of IgM antibodies is indicative of acute infection. The 25.8% IgM prevalence obtained in this study is higher than the 6.8% obtained in Abia State (Ogbonnaya *et al.,* 2012), 1.1% in Ilorin (Agbede *et al.,* 2011), 3.9% in Makurdi

(Pennap *et al.,* 2009), 0% in Jeddah, Saudi Arabia (Sharifa, 2014) but slightly lower than the 38.8% obtained in Zaria (Olajide *et al.,* 2015). The reason for this high prevalence could be due to a possible upsurge of the disease during the cause of the study which was not noticed due to the fact that: most subjects were asymptomatic, the disease is mild, self limiting or can be misdiagnosed (Tipple *et al.*, 2004; WHO, 2005; Dontigny *et al.,* 2008). The variation of the prevalence of this study with previous studies could be attributed to differences in assay methods and population dynamics.

More than Twenty percent (24.4%) of the Study population possessed both Rubella IgG and IgM antibodies. These groups of individuals are said to be re-infected because they d have not developed protective levels of Rubella IgG antibodies from previous exposure to rubella virus infection. A possible reason for this could be due to secondary infection or late primary infection. Maternal re-infection poses little threat to the foetus (Numazaki and Fujikawa, 2003). As in the case of primary infection, the gestational age at the time of re-infection influences the chance of foetal abnormalities (Lee and Bowden, 2000).

The percentage of women in the study population who were negative to Rubella IgG and IgM antibodies was 3.3%. This simply means that 3.3% of the women in this study do reach child bearing age without developing immunity against rubella and are therefore at risk of delivering a malformed baby when they contract the infection early in pregnancy. Possible reason for this 3.3% Rubella IgG and IgM seronegativity rate could be due to the fact that this group of persons have never being exposed to the virus or it could be that they have had exposure but because they lack receptor sites for the virus they did not come down with the disease. The susceptibility rate is low and this could be suggestive of a recent rubella outbreak or intense circulation of the virus in younger age groups (Barreto *et al.,* 2006). The 3.3% susceptible population obtained in this study is in

agreement with the report that approximately 3 to 23% of adults remain susceptible in various countries and areas (Su and Guo, 2002; Malakmadze *et al.,* 2004). It was also in agreement with the 4.2% susceptible population reported by Pennap *et al*. (2009) but contrast the report of Ghandhoke *et al.* (2005) that approximately 10% to 15% of women reached childbearing age without developing immunity against rubella virus and were at high risk of contracting infection during pregnancy. The susceptibility rate among women of reproductive age as shown by this study is low however, this does not rule out the risk of congenital rubella syndrome in the population as congenital rubella syndrome can still occur even when susceptibility levels are below 10% (WHO, 1999).

There was a wide disparity between the result obtained by screening with ELISA and PCR. The variations in the result of the PCR and IgM ELISA could be due to the time the sample was collected and the transport medium used.

No significant association was observed between rubella IgG seroprevalence and Senatorial district of the women ($p = 0.448$) However, the highest prevalence of 96.7% was reported in women from the Central Senatorial district (Zone 2) which could be due to the fact that the Central Senatorial District has a higher population density compared to the other two senatorial districts (NPC, 2006), as such it is very easy for spread of the virus since there is increased possibility of exposure due to overcrowding. A significant association ($p = 0.001$) was observed for Rubella IgM with the highest prevalence of 38.3% among women in the Northern Senatorial District (Zone 1). Though the Northern Senatorial District is less dense when compared to the Central Senatorial District, it has a higher population density when compared to the Southern Senatorial District (NPC, 2006). The possible reason therefore, for this observed prevalence could be attributed to increased contact with asymptomatic or symptomatic cases due to overcrowding. According to the report of Fokunang *et al.* (2010), overcrowding and ease with which the virus spreads

among unvaccinated population, could be the possible explanation for the high prevalence obtained in this study.

No significant association (p = 0.821, p = 0.620) was observed between Rubella IgG and IgM respectively with regards to the age of the subject. However, the highest prevalence of 100% for Rubella IgG was recorded among subjects between 40-45 years of age. It was also observed that the number of subjects who were immuned increased with increase in age which could be due to exposure risk with aging natural infection especially among female population who have not been vaccinated against rubella during their childhood. Longer exposure with an infectious environment could also be another reason for the increasing immunity. This result is in agreement with Kolawole *et al.* (2014) and Manga *et al.* (2014), who also reported high prevalence among women who were greater than 40 years but in contrast with Ekta *et al.* (2006) who reported high prevalence among women between age group 15 and 19 years. The reason for this disparity could be attributed to the fact that the subjects in the reported study had rubella vaccination during childhood as such the high prevalence of IgG in this age group could be attributed to the persistence of immune response to vaccination. The highest prevalence of 31.8% for Rubella IgM with respect to age was recorded among subjects within 15-19 years of age. This high prevalence among subjects between ages 15-19 years could be due to more frequent exposure of the younger age groups to Rubella. It was also observed that IgM seropositivity was increasing with age and then began to decline the reason for this decline, could be due to the fact that most of the subjects have already become immuned to the virus due to previous exposure.

In the present study, no significant associations (p = 0.905) was observed between Rubella IgG and the marital status of the women. However, the higher prevalence of 100% was reported among subjects who were single. This is in agreement with Bamgboye *et al.*

(2004) who reported the highest prevalence among singles. On the contrary, women who were married had the highest prevalence of 26.4% for Rubella IgM antibodies even though no significant association (p = 0.587) was observed. The reason for this high prevalence could be as a result of high level of contact/interactions at the family level which promotes rubella transmission (Arroya *et al.*, 1986). This is in agreement with the report of Ogbonnaya *et al.* (2012) who also observed the highest prevalence among married subjects.

The educational Status of the women revealed no statistical association with Rubella virus IgG and IgM respectively (p = 0.094, p = 0.739). However, the prevalence of Rubella IgG and IgM was high among women with informal education. The reasons could be due to poor knowledge of what the virus is, the mode of transmission and complications caused by the virus since they possess no formal education. The inadequate level of awareness creation by government and health bodies about this virus in the study population also has a role to play in the high prevalence among women with no form of formal education. This result obtained in this study is in contrast with the report of Olajide *et al.* (2015) who reported a high prevalence among educated females.

No significant association was observed between Rubella IgG and pregnancy status of the subjects (p = 1.000). However, the prevalence of Rubella IgG with respect to pregnancy status revealed a high prevalence in pregnant women. The reason for the high Rubella IgG prevalence could be attributed to natural infection acquired earlier in life. This is in agreement with Bushra *et al.* (2014*)* but in contrast with the report of Adam *et al.* (2013). On the other hand, a significant association (p = 0.015) was observed between Rubella IgM and pregnancy status of the women. The prevalence of Rubella IgM with respect to pregnancy status also revealed a high prevalence among women who were pregnant. This is in agreement with Barreto *et al.* (2006) and Bushra *et al.* (2014) who

reported a higher prevalence among pregnant women but in contrast with Singla *et al.* (2004) who reported a higher prevalence in non pregnant women.

The seroprevalence of rubella IgG and IgM with respect to trimesters of pregnancy revealed that majority of the women were in their second trimester and third trimester of pregnancy. No significant association was observed between Rubella IgG and trimester of pregnancy ($p = 0.894$). On the other hand significant observation was obtained between Rubella IgM and trimester of pregnancy. The prevalence of Rubella IgG is higher in the second trimester; though there is risk of congenital infection in the second trimester of pregnancy, the frequency of occurrence of congenital rubella syndrome is less likely compared to the first trimester. From the result obtained it could be deduced that women who were in their second trimesters of pregnancy were more immuned than women in other trimesters. This result is in agreement with the report of Olajide *et al.* (2015) but contrast the report of Agbede *et al.* (2011), Ogbonnaya *et al.* (2012) and Bushra *et al.* (2014) which had the highest prevalence in the first trimester. Women in their first trimester of pregnancy had the highest prevalence of 35.3% for Rubella IgM and a significant association ($p = 0.04$) was observed between Rubella IgM and trimester of pregnancy. Possible reasons for this result could be that women in this category are either more susceptible because they have never been exposed to the virus before now or they could be having a re-infection because they lack protective levels of Rubella IgG. Women infected in this trimester may be at risk of miscarriages and might give birth to children with congenital defects as it has been reported that the risk of miscarriage and congenital rubella syndrome are higher in the first trimester of pregnancy (Gabbe, 2002; CDC, 2012).

No significant association was observed between rubella IgG and disease history ($p = 0.478$), history of miscarriage ($p = 0.603$) and history of still birth ($p = 0.590$). This higher seropositivity observed in women indicated that infection with this virus could be

responsible for the miscarriages, and still birth in these subjects if it had been contracted in the first trimester of pregnancy. On the other hand, no significant association was observed between rubella IgM and disease history ($p = 0.603$), history of miscarriage ($p = 0.266$) and history of still birth ($p = 0.194$). This higher seropositivity of rubella IgM observed in women indicates that the miscarriages and stillbirth these subjects have had in the past could be due to infection with other teratogenic agents of the TORCH complex (Surpam *et al.*, 2006). Irrespective of the history of miscarriages and stillbirth in these subjects, women observed to show positive result for Rubella IgM could have miscarriages and give birth to babies with congenital rubella syndrome if it had been contracted in the first trimester of pregnancy (Lee and Bowden, 2000).

The relationship between Rubella IgG and some of the signs and symptoms associated with the disease such as fever, Rash, Swollen lymph node and joint pains revealed no statistical association between rubella IgG antibodies and disease signs and symptoms. On the other hand, no statistical association was observed between fever ($p = 0.643$), rash ($p = 1.000$) and swollen lymph node ($p = 1.000$) and Rubella IgM prevalence but a significant association was observed between joint pains ($p = 0.054$) and rubella IgM antibodies. The clinical signs and symptoms reported by some of the subjects are descriptive of rubella (Bukbuk *et al.*, 2002; Dontigny *et al.*, 2008), indicating that these signs and symptoms are associated with the disease (Brooks *et al.*, 2007). Also, rubella IgG and IgM antibodies were found in patients lacking some of the signs and symptoms indicating that the disease could be asymptomatic in some individuals (Dontigny *et al.*, 2008; Sotoodeh *et al.*, 2011).

The seroprevalence of Rubella IgG and IgM with respect to the immunity status of the subject showed no significant association but rubella IgG seroprevalence was seen to be higher in subjects who claimed to have been vaccinated, this indicates that vaccination

provides immunity and thus reduces the risk of acquiring rubella. The IgG seropositivity in subjects who have not had the Rubella virus vaccine could be as a result of natural infection with the virus. The seropositivity rate for Rubella IgM as shown in this study revealed that the higher prevalence was among subjects who were not vaccinated the possible reason for this could be lack of immunity either due to lack of previous exposure to the virus.

In terms of the prevalence of rubella virus IgG and IgM in the study population with respect to their knowledge about the virus, it can be deduced that basic knowledge and awareness about rubella virus, its mode of transmission and complications in the study population is low Since the knowledge about a disease and its mode of transmission is important in its prevention and control (Makemgue *et al.,* 2016), the low level of awareness observed in this study could have attributed to the high prevalence obtained. Reasons for this low level of awareness could be due to poor publicity by the government and health bodies about rubella. Similar result was obtained by Olajide *et al.* (2015) and Mohammed *et al.* (2010) who also reported in their studies that the level of awareness and knowledge about rubella was low.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Owing to the prevalence obtained in the study, rubella infection among women of reproductive age is common in the selected health facilities in Kaduna State, Nigeria.

The presence of Rubella virus E1 gene in the throat swab of some of the IgM positive samples as detected by Nested RT-PCR was an indication that the virus is responsible for the antibody reaction in subjects considered to be having an acute infection.

Some of the risk factors found to be associated with rubella seroprevalence included pregnancy and vaccination status. Disease symptoms such as fever, swollen lymph node and joint pains were found to be associated with the disease. Low level of awareness and poor knowledge about the virus also played an important role in the prevalence of the disease.

6.2 RECOMMENDATIONS

1. The Nigerian government should implement routine immunization of infants and women of reproductive age in Nigeria as rubella vaccination is key in the elimination of rubella and congenital rubella.
2. Government agencies and health care providers should educate the masses and create more awareness on rubella and the risk of contracting rubella in pregnancy.
3. Pregnant women should be routinely screened.
4. PCR testing should be carried out on all IgM positive cases so as to identify those who are truly positive.

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APPENDICES

Appendix I: Ethical Approval

MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/355 17th DEC, 2015

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

SEROPREVALENCE AND PCR DETECTION OF RUBELLA VIRUS AMONG WOMEN OF REPRODUCTIVE AGE IN KADUNA STATE,NIGERIA (AKUT DEBORAH)

Name of Principal Investigator:	**AKUT DEBORAH**
Address of Principal Investigator:	**Dept. Of Microbiology, Faculty of Science, A.B.U, Zaria.**
Date of receipt of Application	**30th Sept, 2015**
Date of Ethical Approval	**13th November, 2015**

This is to inform you that the Research described in the submitted Protocol, the Consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. BUTAWA NUHU N.
SECRETARY
FOR: CHAIRMAN

KADUNA STATE MINISTRY OF HEALTH
OPEN REGISTRY
DESPATCHED
SIGN ~~Hauwa~~ DATE 18/12/15

Independence Way, P.M.B 2014, Kaduna, Kaduna State-Nigeria
Tel: +234 (0) 818 407 8693 | Website: www.kdsg.gov.ng | Email: health@kdsg.gov.ng

Appendix II: INFORMED CONSENT FORM

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY ZARIA, NIGERIA.
INFORMED CONSENT FORM (ICF)

Serial No:**.....** ***Hospital No:*****.....** ***Age:*****.....**
Phone No:**.....**
Name of Investigator: Akut Deborah
Supervisory Team: Dr. A. B. Suleiman
Prof. S. E. Yakubu
Topic: Seroprevalence and PCR detection of rubella virus in women of reproductive age in Kaduna State, Nigeria.

INTRODUCTION

I am Akut Deborah a postgraduate student of Ahmadu Bello University Zaria currently carrying out my research work on Seroprevalence and PCR detection of rubella virus in women of reproductive age in Kaduna State, Nigeria. It is on this note that I seek your consent to participate in this research. The research will involve consenting women of reproductive age (pregnant and non- pregnant) attending the selected health facilities. For the purpose of this research both blood samples and throat Swab samples will be collected for the seroprevalence studies and PCR detection respectively.

PURPOSE OF THE RESEARCH

The purpose of this research is to assess the immunity status of women of reproductive age in Kaduna State, Nigeria to rubella virus and also to confirm the presence of an ongoing infection using molecular technique; PCR. The result that will be obtained from this Study will be useful in providing Epidemiological data on rubella which will be used to create awareness and sensitize healthcare administrators and providers and consequently make appropriate recommendations aimed at facilitating control.

RISK AND BENEFIT

The participants would not be exposed to any risk during the course of the research rather the research would ensure free Rubella virus screening to all consenting participants. PCR will be carried out on IgM positive samples to confirm the presence of the virus. Positive cases would be recommended to the right authorities for appropriate measures. All consenting Participants will be appropriately enlightened and sensitized on the socio-demographic and predisposing factors associated with the disease so as to prevent future occurrence of the disease.

CERTIFICATE OF CONSENT

I..... of .....
Hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that throat swab and blood samples will be collected. I therefore give this consent voluntarily without being subjected to any pressure.
Name of Participant:.....
Signature of Participant/thumb Print:.....
Date:.....

Statement by Witness

I have witnessed the detailed explanation of the study to the potential participant and the individual concerned has had the opportunity to ask questions. I confirm that the individual has given consent freely.
Name of Witness:.....
Signature of Witness/thumb Print:.....
Date:.....

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information about risk and benefits needed to make informed decision have been fully explained to the participant. The participant was given opportunity to ask questions so as to clear every existing doubt and all questions asked have been appropriately answered to the best of my ability. I confirm that the individual has not been coerced into giving consent and the consent has been given freely and voluntarily.
Name of Researcher:.....
Signature:.....
Date:.....

Appendix III: Research Questionnaire

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA, KADUNA STATE, NIGERIA.

RESEARCH QUESTIONNAIRE

Topic: "Seroprevalence and PCR detection of Rubella virus among women of reproductive age in kaduna, Nigeria".

Instruction: please ticks [√] as appropriate in the spaces provided and note that every bit of information given will be handled with maximum confidentiality. Thank you!

Patient's Serial No: _____

Code: _____

Local Govt. Area: _____

Name of Hospital: _____

1. Age group
   15-19[ ] 20-24 [ ] 25-29 [ ] 30-34 [ ] 35-39 [ ] 40-45 [ ]
2. Marital Status: Married [ ] single [ ] divorced [ ] others [ ]
3. Level of Education: Primary School [ ] Secondary School [ ] Tertiary Institution[ ] Non [ ]
4. Are you pregnant? Yes [ ] No [ ]

b. what's the Gestational age?

First Trimester [ ] Second Trimester [ ] Third Trimester [ ]

Disease History

5. Have you had Rubella virus infection before? Yes [ ] No [ ] Unknown [ ]
6. Have you had miscarriage before? Yes [ ] No [ ]
7. Have you had stillbirth before? Yes [ ] No [ ]

Disease Signs and Symptoms

8. Fever : Yes [ ] No[ ]
9. Rash: Yes [ ] No [ ]
10. Swollen Lymph nodes: Yes [ ] No [ ]
11. Joint pains: Yes [ ] No [ ]

Vaccination History

12. Have you had rubella vaccination before?
    Yes [ ] No [ ] Unknown [ ]
13. If not vaccinated reason_____

General Knowledge about Rubella

14. What is Rubella? _____
15. Is Rubella Contagious?
    Yes [ ] No [ ] Unknown [ ]
16. How is Rubella transmitted? _____
17. Is the foetus at risk?
    Yes [ ] No [ ] Unknown [ ]

Appendix IV: Summary of Result

RUBELLA IgG RESULT PLATE 1			
SAMPLE	ABSORBANCE	RUBELLA IgG IU/ml	RESULT
S2	4		Standard 1
S3	4		Standard 2
S4	4		Standard 3
S5	4		Standard 4
S6	0.204	2.2236	Negative
S7	0.318	3.4662	Negative
S8	4	43.6	Positive
S9	0.628	6.8452	Negative
S10	4	43.6	Positive
S11	3.74	40.766	Positive
S12	2.75	29.975	Positive
S13	2.805	30.5745	Positive
S14	3.598	39.2182	Positive
S15	2.868	31.2612	Positive
S16	2.942	32.0678	Positive
S17	2.621	28.5689	Positive
S18	4	43.6	Positive
S22	2.522	27.4898	Positive
S23	2.522	27.4898	Positive
S24	3.798	41.3982	Positive
S25	4	43.6	Positive
S26	3.352	36.5368	Positive
S27	3.798	41.3982	Positive
S28	3.352	36.5368	Positive
S29	4	43.6	Positive
S30	3.009	32.7981	Positive
S31	2.826	30.8034	Positive
S32	3.072	33.4848	Positive
S33	2.907	31.6863	Positive

SAMPLE	ABSORBANCE	RUBELLA IgG IU/ml	RESULT
S34	2.955	32.2095	Positive
S35	3.145	34.2805	Positive
S36	3.659	39.8831	Positive
S37	4	43.6	Positive
S38	3.452	37.6268	Positive
S39	3.659	39.8831	Positive
S40	3.452	37.6268	Positive
S41	3.659	39.8831	Positive
S42	3.007	32.7763	Positive
S43	2.981	32.4929	Positive
S44	3.133	34.1497	Positive
S45	3.133	34.1497	Positive
S46	3.065	33.4085	Positive
S47	3.065	33.4085	Positive
S48	3.848	41.9432	Positive
S49	3.475	37.8775	Positive
S50	3.683	40.1447	Positive
S51	3.848	41.9432	Positive
S52	3.339	36.3951	Positive
S53	3.339	36.3951	Positive
S54	3.284	35.7956	Positive
S55	3.057	33.3213	Positive
S56	3.284	35.7956	Positive
S60	3.621	39.4689	Positive
S61	3.621	39.4689	Positive
S62	3.784	41.2456	Positive
S63	3.621	39.4689	Positive
S64	3.784	41.2456	Positive
S65	3.416	37.2344	Positive
S66	3.033	33.0597	Positive

S67	3.033	33.0597	Positive
S68	3.1	33.79	Positive
S69	3.18	34.662	Positive
S70	3.1	33.79	Positive
S71	0.524	5.7116	Negative
S72	4	43.6	Positive
S73	4	43.6	Positive
S74	4	43.6	Positive
S75	4	43.6	Positive
S76	3.826	41.7034	Positive
S77	3.661	39.9049	Positive
S78	3.454	37.6486	Positive
S79	3.38	36.842	Positive
S80	3.263	35.5667	Positive
S81	3.263	35.5667	Positive
S82	3.215	35.0435	Positive
S83	3.661	39.9049	Positive
S84	3.444	37.5396	Positive
S85	3.204	34.9236	Positive
S86	4	43.6	Positive
S87	3.37	36.733	Positive
S88	3.444	37.5396	Positive
S89	3.204	34.9236	Positive
S90	2.97	32.373	Positive
S91	3.087	33.6483	Positive
S92	2.996	32.6564	Positive
S93	2.822	30.7598	Positive
S94	2.97	32.373	Positive
S95	3.024	32.9616	Positive

IgG RESULT PLATE 2			
SAMPLE	ABSORBANCE	RUBELLA IgG IU/ml	RESULT
S3	0.111	1.2099	Standard 1
S4	0.955	10.4095	Standard 2
S5	2.886	31.4574	Standard 3
S6	2.03	22.127	Standard 4
S7	4	43.6	Positive
S8	2.474	26.9666	Positive
S9	4	43.6	Positive
S10	4	43.6	Positive
S11	4	43.6	Positive
S12	3.594	39.1746	Positive
S13	2.463	26.8467	Positive
S14	2.463	26.8467	Positive
S15	1.975	21.5275	Positive
S16	2.438	26.5742	Positive
S17	2.463	26.8467	Positive
S18	2.489	27.1301	Positive
S19	2.372	25.8548	Positive
S20	2.415	26.3235	Positive
S21	2.393	26.0837	Positive
S22	2.315	25.2335	Positive
S23	2.655	28.9395	Positive
S24	2.686	29.2774	Positive
S25	2.776	30.2584	Positive
S26	2.941	32.0569	Positive
S27	2.812	30.6508	Positive
S28	2.812	30.6508	Positive
S29	2.776	30.2584	Positive
S30	2.85	31.065	Positive
S31	2.812	30.6508	Positive
S32	2.893	31.5337	Positive
S33	2.25	24.525	Positive

SAMPLE	ABSORBANCE	RUBELLA IgG IU/ml	RESULT
S34	2.714	29.5826	Positive
S35	2.812	30.6508	Positive
S36	2.924	31.8716	Positive
S37	1.925	20.9825	Positive
S38	2.971	32.3839	Positive
S39	3.025	32.9725	Positive
S40	1.718	18.7262	Positive
S41	3.025	32.9725	Positive
S42	2.971	32.3839	Positive
S43	2.924	31.8716	Positive
S44	2.971	32.3839	Positive
S45	2.971	32.3839	Positive
S46	3.055	33.2995	Positive
S47	3.055	33.2995	Positive
S48	2.999	32.6891	Positive
S49	2.999	32.6891	Positive
S50	2.951	32.1659	Positive
S51	2.975	32.4275	Positive
S52	3.054	33.2886	Positive
S53	2.999	32.6891	Positive
S54	2.975	32.4275	Positive
S55	3.054	33.2886	Positive
S56	3.026	32.9834	Positive
S57	2.951	32.1659	Positive
S58	2.975	32.4275	Positive
S59	2.951	32.1659	Positive
S60	3.026	32.9834	Positive
S61	2.873	31.3157	Positive
S62	2.895	31.5555	Positive
S63	3.026	32.9834	Positive
S64	2.968	32.3512	Positive
S65	2.996	32.6564	Positive
S66	2.873	31.3157	Positive
S67	2.942	32.0678	Positive
S68	2.895	31.5555	Positive
S69	2.942	32.0678	Positive
S70	2.942	32.0678	Positive
S71	2.996	32.6564	Positive
S72	3.09	33.681	Positive
S73	2.863	31.2067	Positive
S74	2.973	32.4057	Positive
S75	2.999	32.6891	Positive
S76	2.973	32.4057	Positive
S77	3.027	32.9943	Positive
S78	3.027	32.9943	Positive
S79	1.531	16.6879	Positive
S80	2.844	30.9996	Positive
S81	2.622	28.5798	Positive
S82	2.826	30.8034	Positive
S83	3.445	37.5505	Positive
S84	2.832	30.8688	Positive
S85	2.737	29.8333	Positive
S86	2.902	31.6318	Positive
S87	2.791	30.4219	Positive
S88	2.957	32.2313	Positive
S89	2.754	30.0186	Positive
S90	2.854	31.1086	Positive
S91	2.791	30.4219	Positive
S92	2.832	30.8688	Positive
S93	1.562	17.0258	Positive
S94	2.791	30.4219	Positive
S95	2.391	26.0619	Positive

RUBELLA IgG RESULT PLATE 3			
SAMPLE	ABSORBANCE	RUBELLA IgG IU/ml	RESULT
S3	0.111	1.2099	Standard 1
S4	0.955	10.4095	Standard 2
S5	2.886	31.4574	Standard 3
S6	2.03	22.127	Standard 4
S7	3.352	36.5368	Positive
S8	2.405	26.2145	Positive
S9	2.62	28.558	Positive
S10	4	43.6	Positive
S11	4	43.6	Positive
S12	2.148	23.4132	Positive
S13	1.964	21.4076	Positive
S14	2.225	24.2525	Positive
S15	2.064	22.4976	Positive
S16	2.397	26.1273	Positive
S17	2.125	23.1625	Positive
S18	2.419	26.3671	Positive
S19	2.337	25.4733	Positive
S20	2.552	27.8168	Positive
S21	2.552	27.8168	Positive
S22	2.467	26.8903	Positive
S23	2.467	26.8903	Positive
S24	2.669	29.0921	Positive
S25	2.6	28.34	Positive
S26	2.821	30.7489	Positive
S27	2.428	26.4652	Positive
S28	2.319	25.2771	Positive
S29	2.622	28.5798	Positive
S30	2.669	29.0921	Positive
S31	2.753	30.0077	Positive
S32	2.723	29.6807	Positive
S33	2.86	31.174	Positive
S67	2.783	30.3347	Positive
S68	3.061	33.3649	Positive
S69	2.898	31.5882	Positive
S70	2.971	32.3839	Positive
S71	2.047	22.3123	Positive
S72	2.352	25.6368	Positive
S73	1.05	11.445	Negative
S74	0.085	0.9265	Negative
S75	0.088	0.9592	Negative
S76	2.352	25.6368	Positive
S77	2.478	27.0102	Positive
S78	0.816	8.8944	Negative
S79	2.832	30.8688	Positive
S80	1.313	14.3117	Negative
S81	0.932	10.1588	Negative
S82	2.493	27.1737	Positive
S83	3.17	34.553	Positive
S84	2.778	30.2802	Positive
S85	2.487	27.1083	Positive
S86	1.937	21.1133	Positive
S87	2.652	28.9068	Positive
S88	1.853	20.1977	Positive
S89	2.042	22.2578	Positive
S90	2.838	30.9342	Positive
S91	2.86	31.174	Positive
S92	2.42	26.378	Positive
S93	1.75	19.075	Positive
S94	2.048	22.3232	Positive
S95	3.061	33.3649	Positive

RUBELLA IgG RESULT PLATE 4			
SAMPLE	ABSORBANCE	RUBELLA IgG IU/ml	RESULT
S3	0.111	1.2099	Standard 1
S4	0.955	10.4095	Standard 2
S5	2.886	31.4574	Standard 3
S6	4	43.6	Standard 4
S7	4	43.6	Positive
S8	4	43.6	Positive
S9	4	43.6	Positive
S10	4	43.6	Positive
S11	4	43.6	Positive
S12	2.156	23.5004	Positive
S13	2.216	24.1544	Positive
S14	2.623	28.5907	Positive
S15	2.156	23.5004	Positive
S16	2.312	25.2008	Positive
S17	2.341	25.5169	Positive
S18	3.134	34.1606	Positive
S19	2.76	30.084	Positive
S20	2.686	29.2774	Positive
S21	2.341	25.5169	Positive
S22	2.238	24.3942	Positive
S23	2.138	23.3042	Positive
S24	1.224	13.3416	Negative
S25	2.655	28.9395	Positive
S26	2.439	26.5851	Positive
S27	2.706	29.4954	Positive
S28	2.913	31.7517	Positive
S29	3.151	34.3459	Positive
S30	2.96	32.264	Positive
S31	3.522	38.3898	Positive
S32	3.151	34.3459	Positive
S33	2.96	32.264	Positive
S34	2.831	30.8579	Positive
S35	3.358	36.6022	Positive
S36	1.652	18.0068	Positive
S37	2.613	28.4817	Positive
S38	2.864	31.2176	Positive
S39	2.827	30.8143	Positive
S40	2.949	32.1441	Positive
S41	2.974	32.4166	Positive
S42	3.207	34.9563	Positive
S43	3	32.7	Positive
S44	3.028	33.0052	Positive
S45	2.926	31.8934	Positive
S46	3.058	33.3322	Positive
S47	3.028	33.0052	Positive
S48	2.094	22.8246	Positive
S49	1.525	16.6225	Positive
S50	2.959	32.2531	Positive
S51	2.896	31.5664	Positive
S52	3.033	33.0597	Positive
S53	3.198	34.8582	Positive
S54	3.091	33.6919	Positive
S55	3.061	33.3649	Positive
S56	3.406	37.1254	Positive
S57	2.959	32.2531	Positive
S58	3.159	34.4331	Positive
S59	3.289	35.8501	Positive
S60	1.625	17.7125	Positive
S61	2.442	26.6178	Positive
S62	2.511	27.3699	Positive
S63	1.978	21.5602	Positive
S64	3.005	32.7545	Positive
S65	3.102	33.8118	Positive
S66	3.624	39.5016	Positive

S67	2.783	30.3347	Positive
S68	3.061	33.3649	Positive
S69	2.898	31.5882	Positive
S70	2.971	32.3839	Positive
S71	2.047	22.3123	Positive
S72	2.352	25.6368	Positive
S73	1.05	11.445	Negative
S74	0.085	0.9265	Negative
S75	0.088	0.9592	Negative
S76	2.352	25.6368	Positive
S77	2.478	27.0102	Positive
S78	0.816	8.8944	Negative
S79	2.832	30.8688	Positive
S80	1.313	14.3117	Negative
S81	0.932	10.1588	Negative
S82	2.493	27.1737	Positive
S83	3.17	34.553	Positive
S84	2.778	30.2802	Positive
S85	2.487	27.1083	Positive
S86	1.937	21.1133	Positive
S87	2.652	28.9068	Positive
S88	1.853	20.1977	Positive
S89	2.042	22.2578	Positive
S90	2.838	30.9342	Positive
S91	2.86	31.174	Positive
S92	2.42	26.378	Positive
S93	1.75	19.075	Positive
S94	2.048	22.3232	Positive
S95	3.061	33.3649	Positive

RUBELLA IgM PLATE 1

SAMPLE	ABSORBANCE	RUBELLA IgM INDEX	RESULT
S3	2.988	3.333333333	Calibrator
S4	0.306	0.341365462	Negative
S5	0.663	0.739625167	Negative
S6	1.056	1.178045515	Positive
S7	0.815	0.909192325	Negative
S8	0.319	0.355867916	Negative
S9	0.922	1.028558679	Positive
S10	2.795	3.118027666	Positive
S11	0.418	0.466309683	Negative
S12	0.922	1.028558679	Positive
S13	1.454	1.62204373	Positive
S14	1.017	1.134538153	Positive
S15	0.211	0.235385988	Negative
S16	0.139	0.155064703	Negative
S17	0.775	0.864569389	Negative
S18	0.331	0.369254797	Negative
S19	0.13	0.145024543	Negative
S20	1.636	1.82507809	Positive
S21	1.273	1.420124944	Positive
S22	0.278	0.310129407	Negative
S23	0.479	0.534359661	Negative
S24	0.848	0.946006247	Negative
S25	0.843	0.94042838	Negative
S26	0.543	0.605756359	Negative
S27	0.621	0.692771084	Negative
S28	0.881	0.98282017	Negative
S29	0.889	0.991744757	Negative
S30	0.418	0.466309683	Negative
S31	0.433	0.483043284	Negative
S32	0.621	0.692771084	Negative
S33	1.237	1.379964302	Positive
S34	0.36	0.401606426	Negative

SAMPLE	ABSORBANCE	RUBELLA IgM INDEX	RESULT
S35	0.636	0.709504685	Negative
S36	1.075	1.19924141	Positive
S37	0.249	0.277777778	Negative
S38	1.438	1.604194556	Positive
S39	0.642	0.716198126	Negative
S40	0.911	1.016287372	Positive
S41	0.636	0.709504685	Negative
S42	0.994	1.108879964	Positive
S43	0.717	0.799866131	Negative
S44	0.894	0.997322624	Negative
S45	1.142	1.273984828	Positive
S46	0.545	0.607987506	Negative
S47	0.903	1.007362784	Positive
S48	0.91	1.015171798	Positive
S49	1.308	1.459170013	Positive
S50	1.041	1.161311914	Positive
S51	1.211	1.350959393	Positive
S52	0.787	0.87795627	Negative
S53	0.336	0.374832664	Negative
S54	1.895	2.114011602	Positive
S55	0.301	0.335787595	Negative
S56	0.883	0.985051316	Negative
S57	1.115	1.243864346	Positive
S58	0.86	0.959393128	Negative
S59	1.02	1.137884873	Positive
S60	1.244	1.387773315	Positive
S61	0.72	0.803212851	Negative
S62	2.315	2.582552432	Positive
S63	0.547	0.610218652	Negative
S64	0.431	0.480812137	Negative
S65	0.475	0.529897367	Negative
S66	0.728	0.812137439	Negative
S67	0.293	0.326863008	Negative
S68	0.959	1.069834895	Positive
S69	0.732	0.816599732	Negative
S70	0.53	0.591253905	Negative
S71	0.674	0.751896475	Negative
S72	1.576	1.758143686	Positive
S73	0.77	0.858991522	Negative

SAMPLE	ABSORBANCE	RUBELLA IgM INDEX	RESULT
S74	0.822	0.917001339	Negative
S75	0.457	0.509817046	Negative
S76	0.555	0.61914324	Negative
S77	0.453	0.505354752	Negative
S78	0.597	0.665997323	Negative
S79	0.411	0.458500669	Negative
S80	0.742	0.827755466	Negative
S81	0.295	0.329094154	Negative
S82	0.796	0.88799643	Negative
S83	1.113	1.241633199	Positive
S84	1.634	1.822846943	Positive
S85	1.799	2.006916555	Positive
S86	0.93	1.037483266	Positive
S87	0.438	0.488621151	Negative
S88	0.308	0.343596609	Negative
S89	0.377	0.420571174	Negative
S90	0.604	0.673806336	Negative
S91	0.727	0.811021865	Negative
S92	0.507	0.565595716	Negative
S93	0.468	0.522088353	Negative
S94	0.87	0.970548862	Negative
S95	0.519	0.578982597	Negative

RUBELLA IgM RESULT PLATE 2

SAMPLE	SAMPLE O.D	RUBELLA IgM INDEX	RESULT
S3	4	3.333333333	Calibrator
S4	0.392	0.326666667	Negative
S5	0.642	0.535	Negative
S6	1.195	0.995833333	Negative
S7	0.471	0.3925	Negative
S8	0.851	0.709166667	Negative
S9	0.372	0.31	Negative
S10	0.508	0.423333333	Negative
S11	1.002	0.835	Negative
S12	0.257	0.214166667	Negative
S13	0.248	0.206666667	Negative
S14	2.622	2.185	Positve
S15	0.166	0.138333333	Negative
S16	0.777	0.6475	Negative
S17	0.68	0.566666667	Negative
S18	0.669	0.5575	Negative
S19	0.486	0.405	Negative
S20	0.468	0.39	Negative
S21	0.682	0.568333333	Negative
S22	0.237	0.1975	Negative
S23	0.195	0.1625	Negative
S24	1.207	1.005833333	Positve
S25	0.38	0.316666667	Negative
S26	2.466	2.055	Positve
S27	0.677	0.564166667	Negative
S28	0.518	0.431666667	Negative
S29	0.579	0.4825	Negative
S30	0.559	0.465833333	Negative
S31	0.393	0.3275	Negative
S32	1.316	1.096666667	Positve
S33	0.012	0.01	Negative
S34	0.241	0.200833333	Negative
S35	0.702	0.585	Negative
S36	1.283	1.069166667	Positive
S37	0.505	0.420833333	Negative
S38	0.653	0.544166667	Negative
S39	0.325	0.270833333	Negative
S40	2.147	1.789166667	Positive
S41	0.313	0.260833333	Negative
S42	0.518	0.431666667	Negative
S43	0.361	0.300833333	Negative
S44	0.169	0.140833333	Negative
S45	1.591	1.325833333	Positive
S46	0.484	0.403333333	Negative
S47	0.296	0.246666667	Negative
S48	0.492	0.41	Negative
S49	0.965	0.804166667	Negative
S50	0.481	0.400833333	Negative
S51	3.029	2.524166667	Positive
S52	0.329	0.274166667	Negative
S53	0.275	0.229166667	Negative
S54	0.607	0.505833333	Negative
S55	0.651	0.5425	Negative
S56	0.444	0.37	Negative
S57	0.808	0.673333333	Negative
S58	0.348	0.29	Negative
S59	0.097	0.080833333	Negative
S60	1.195	0.995833333	Negative
S61	0.653	0.544166667	Negative
S62	0.648	0.54	Negative
S63	1.56	1.3	Positive
S64	0.229	0.190833333	Negative
S65	0.608	0.506666667	Negative
S66	0.677	0.564166667	Negative

SAMPLE	SAMPLE O.D	RUBELLA IgM INDEX	RESULT
S67	0.227	0.189166667	Negative
S68	0.413	0.344166667	Negative
S69	0.354	0.295	Negative
S70	0.618	0.515	Negative
S71	1.989	1.6575	Positive
S72	1.118	0.931666667	Negative
S73	1.629	1.3575	Positive
S74	0.353	0.294166667	Negative
S75	0.582	0.485	Negative
S76	1.067	0.889166667	Negative
S77	0.448	0.373333333	Negative
S78	0.491	0.409166667	Negative
S79	0.452	0.376666667	Negative
S80	0.451	0.375833333	Negative
S81	0.328	0.273333333	Negative
S82	0.217	0.180833333	Negative
S83	0.222	0.185	Negative
S84	2.651	2.209166667	Positive
S85	0.272	0.226666667	Negative
S86	0.908	0.756666667	Negative
S87	1.576	1.313333333	Positive
S88	0.897	0.7475	Negative
S89	0.516	0.43	Negative
S90	0.732	0.61	Negative
S91	0.372	0.31	Negative
S92	0.359	0.299166667	Negative
S93	0.405	0.3375	Negative
S94	0.207	0.1725	Negative
S95	0.219	0.1825	Negative

RUBELLA IgM RESULT PLATE 3

SAMPLE	SAMPLE OD	RUBELLA IgM INDEX	RESULT
S1	0.162		
S2	3.554		
S3	1.934		Calibrator
S4	1.386	1.155	Positive
S5	0.945	0.7875	Negative
S6	0.771	0.6425	Negative
S7	0.522	0.435	Negative
S8	1.452	1.21	Positive
S9	1.05	0.875	Negative
S10	0.525	0.4375	Negative
S11	0.571	0.475833333	Negative
S12	0.36	0.3	Negative
S13	0.251	0.209166667	Negative
S14	0.413	0.344166667	Negative
S15	0.729	0.6075	Negative
S16	0.514	0.428333333	Negative
S17	0.597	0.4975	Negative
S18	0.628	0.523333333	Negative
S19	0.358	0.298333333	Negative
S20	1.251	1.0425	Positive
S21	1.123	0.935833333	Negative
S22	1.225	1.020833333	Positive
S23	0.702	0.585	Negative
S24	0.514	0.428333333	Negative
S25	0.461	0.384166667	Negative
S26	1.083	0.9025	Negative
S27	0.466	0.388333333	Negative
S28	0.494	0.411666667	Negative
S29	0.458	0.381666667	Negative
S30	0.392	0.326666667	Negative
S31	0.958	0.798333333	Negative
S32	1.979	1.649166667	Positive
S33	0.923	0.769166667	Negative

SAMPLE	SAMPLE OD	RUBELLA IgM INDEX	RESULT
S34	0.983	0.819166667	Negative
S35	0.596	0.496666667	Negative
S36	0.369	0.3075	Negative
S37	0.5	0.416666667	Negative
S38	0.136	0.113333333	Negative
S39	0.278	0.231666667	Negative
S40	1.338	1.115	Positive
S41	0.625	0.520833333	Negative
S42	0.662	0.551666667	Negative
S43	0.468	0.39	Negative
S44	0.751	0.625833333	Negative
S45	1.638	1.365	Positive
S46	0.981	0.8175	Negative
S47	0.501	0.4175	Negative
S48	0.643	0.535833333	Negative
S49	0.794	0.661666667	Negative
S50	0.138	0.115	Negative
S51	2.629	2.190833333	Positive
S52	1.837	1.530833333	Positive
S53	0.756	0.63	Negative
S54	4	3.333333333	Positive
S55	3.847	3.205833333	Positive
S56	0.533	0.444166667	Negative
S57	0.951	0.7925	Negative
S58	0.848	0.706666667	Negative
S59	1.113	0.9275	Negative
S60	0.375	0.3125	Negative
S61	0.338	0.281666667	Negative
S62	0.151	0.125833333	Negative
S63	1.453	1.210833333	Positive
S64	0.577	0.480833333	Negative
S65	0.763	0.635833333	Negative
S66	0.606	0.505	Negative

SAMPLE	SAMPLE OD	RUBELLA IgM INDEX	RESULT
S67	0.724	0.603333333	Negative
S68	0.355	0.295833333	Negative
S69	0.169	0.140833333	Negative
S70	0.532	0.443333333	Negative
S71	0.237	0.1975	Negative
S72	0.897	0.7475	Negative
S73	0.212	0.176666667	Negative
S74	0.104	0.086666667	Negative
S75	0.525	0.4375	Negative
S76	0.487	0.405833333	Negative
S77	0.476	0.396666667	Negative
S78	0.275	0.229166667	Negative
S79	0.465	0.3875	Negative
S80	0.352	0.293333333	Negative
S81	0.35	0.291666667	Negative
S82	0.368	0.306666667	Negative
S83	1.645	1.370833333	Positive
S84	0.297	0.2475	Negative
S85	0.775	0.645833333	Negative
S86	0.066	0.055	Negative
S87	0.517	0.430833333	Negative
S88	0.52	0.433333333	Negative
S89	0.476	0.396666667	Negative
S90	0.621	0.5175	Negative
S91	0.156	0.13	Negative
S92	0.672	0.56	Negative
S93	0.763	0.635833333	Negative
S94	0.75	0.625	Negative
S95	1.052	0.876666667	Negative

RUBELLA IgM RESULT PLATE 4

SAMPLE	SAMPLE OD	RUBELLA IgM INDEX	RESULT
S3	2.841	3.333333333	Calibrator
S4	0.687	0.806054206	Negative
S5	0.801	0.939809926	Negative
S6	0.946	1.109937815	Positive
S7	1.325	1.554616919	Positive
S8	1.033	1.212014549	Positive
S9	0.55	0.645312683	Negative
S10	1.202	1.410301537	Positive
S11	1.321	1.549923736	Positive
S12	0.182	0.213539833	Negative
S13	2.408	2.825296257	Negative
S14	1.097	1.287105479	Positive
S15	0.175	0.205326763	Negative
S16	0.533	0.625366655	Negative
S17	1.547	1.815088584	Positive
S18	0.728	0.854159334	Negative
S19	0.962	1.128710548	Positive
S20	0.795	0.932770151	Negative
S21	1.103	1.294145254	Negative
S22	0.585	0.686378036	Positive
S23	0.338	0.396573976	Negative
S24	0.507	0.594860964	Negative
S25	0.51	0.598380852	Negative
S26	0.43	0.504517189	Negative
S27	0.568	0.666432008	Negative
S28	1.294	1.51824475	Positive
S29	1.401	1.643787399	Positive
S30	1.348	1.581602722	Positive
S31	0.638	0.748562713	Negative
S32	0.338	0.396573976	Negative
S33	1.097	1.287105479	Positive
S34	1.432	1.680159568	Positive

SAMPLE	SAMPLE OD	RUBELLA IgM INDEX	RESULT
S35	1.023	1.200281591	Positive
S36	0.392	0.459931949	Negative
S37	0.311	0.36489499	Negative
S38	0.426	0.499824006	Negative
S39	0.345	0.404787047	Negative
S40	0.84	0.985568462	Negative
S41	0.523	0.613633697	Negative
S42	1.035	1.21436114	Positive
S43	1.336	1.567523173	Positive
S44	0.571	0.669951895	Negative
S45	1.119	1.312917987	Negative
S46	0.65	0.762642262	Negative
S47	0.843	0.989088349	Negative
S48	0.608	0.713363839	Negative
S49	0.542	0.635926317	Negative
S50	1.078	1.264812859	Positive
S51	0.718	0.842426376	Negative
S52	0.271	0.317963159	Negative
S53	1.207	1.416168016	Positive
S54	0.319	0.374281356	Negative
S55	0.848	0.994954828	Negative
S56	1.104	1.29531855	Positive
S57	0.854	1.001994603	Positive
S58	2.532	2.970784935	Positive
S59	2.069	2.427548985	Positive
S60	0.234	0.274551214	Negative
S61	0.743	0.87175877	Negative
S62	1.012	1.187375337	Positive
S63	0.885	1.038366772	Positive
S64	0.427	0.500997301	Negative
S65	0.396	0.464625132	Negative
S66	0.333	0.390707497	Negative

SAMPLE	SAMPLE OD	RUBELLA IgM INDEX	RESULT
S67	0.54	0.633579725	Negative
S68	2.324	2.726739411	Positive
S69	2.642	3.099847472	Positive
S70	0.679	0.79666784	Negative
S71	2.92	3.426023701	Positive
S72	0.383	0.449372287	Negative
S73	0.536	0.628886542	Negative
S74	1.132	1.328170832	Positive
S75	0.652	0.764988854	Negative
S76	1.523	1.786929485	Positive
S77	0.356	0.4176933	Negative
S78	1.409	1.653173765	Positive
S79	0.55	0.645312683	Negative
S80	2.246	2.63522234	Positive
S81	0.739	0.867065587	Negative
S82	1.103	1.294145254	Positive
S83	0.638	0.748562713	Negative
S84	0.448	0.525636513	Negative
S85	0.295	0.346122257	Negative
S86	0.264	0.309750088	Negative
S87	0.619	0.726270093	Negative
S88	0.39	0.457585357	Negative
S89	0.774	0.90813094	Negative
S90	0.969	1.136923618	Positive
S91	1.01	1.185028746	Positive
S92	0.937	1.099378153	Positive
S93	0.937	1.099378153	Positive
S94	0.782	0.917517306	Negative
S95	0.762	0.89405139	Negative