بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Seroprevalence of Rubella Virus among Pregnant Women in Police Hospital (Khartoum State)

المسح المصلي لفيروس الحصبة الألمانية بين النساء الحوامل في مستشفى الشرطة(ولاية الخرطوم)

A dissertation submitted in partial fulfillment for the requirements of MSc in Medical Laboratory Science (Microbiology)

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الآيـــــــة

قال الله تعالى:

اقرأ باسمِ ربِّكَ الَّذي خلَق﴿1﴾خلَقَ الإنسانَ من علَق﴿2﴾اقرأ وربُّكَ الأكرم﴿3﴾الَّذي علَّمَ بالقلَمِ﴿4﴾علَّم الإنسانَ ما لم يعلم﴿5﴾

(صدق الله العظيم)

سورة العلق

DEDICATION

For my parents, my brothers, my sisters, my love, my friends and everyone, I offer my research…

Personally, I'd like to thank all those who have helped me with their advice and efforts …

I'd like also to thank all the microbiology staff in Sudan University.

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First and foremost, I would like to thank my merciful Allah for giving me strength and health to do this work.

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ABSTRACT

This study was carried out to determine the prevalence of rubella virus among pregnant women, in Police Hospital (Khartoum State), from April to May 2016. Ninety one blood specimens were collected from pregnant women and tested for the presence of rubella virus, by antibody detection using the solid- phase Enzyme-Linked Immune sorbent Assay.

Five ml of blood sample were collected from each pregnant woman and dispensed in sterile EDTA blood container. Plasma was obtained by centrifugation at 3000 g for 5 minutes. The plasma were examined for the presence of RV IgG and IgM antibodies using enzyme linked immune sorbent assay (ELISA).

This study showed that rubella virus was detected in 95.6% (87/91) of the total of patients. The rate of infection increase in the second trimester.

The severe symptoms and fatal outcome from rubella virus infection is due to early infection of fetus due to transplacental transmission in the first trimester of pregnancy that involve sensitive organs such as brain, heart, eye and ear. The Proper diagnosis helps to determine appropriate treatment. Use of vaccine is the most important preventive strategy.

المستخلص

أجريت هذه الدراسة لتحديد مدى إنتشار فيروس الحصبة الألمانية لدى النساء الحوامل في مستشفى الشرطة في ولاية الخرطوم خلال الفترة من أبريل إلى مايو 2016

تم جمع 91 عينة دم من النساء الحوامل وتم إختبارها لوجود فيروس الحصبة الألمانية بواسطة الكشف عن الجسم المضاد بإستخدام المرحلة الصلبة للاختبار المناعي المرتبط بالإنزيم.

تم جمع خمسة مل من عينة دم من كل النساء الحوامل وتم الحصول على البلازما بواسطة جهاز الطرد المركزي عند 3000 دورة لمدة 5 دقائق. تم فحص بلازما الدم لوجود الأجسام المضادة بإستخدام تقنية الأنزيم المناعي المرتبط (الأليزا).

و تم الكشف عن الجسم المضاد لفيروس الحصبة الألمانية في 95.6% (91/87) من مجموع المرضى

. وأظهرت أن أعلى نسبة إصابة كانت في المرحلة الثانية من الحمل

الأعراض الحادة والوفاة عادة ما تكون بسبب فيروس الحصبة الألمانية وتكون العدوى في حاله الإصابة المبكرة للجنين عن طريق نقل الفيروس عبر المشيمة في الشهور الأولى من الحمل وتشمل أعضاء حساسة مثل القلب ,العين , الأذن و المخ .

التشخيص السليم يساعد على تحديد العلاج المناسب , كما أن استخدام اللقاح هو أهم استراتجية وقائية .

ABBREVIATIONS

RV: Rubella Virus

CRS: Congenital Rubella Syndrome

HLA: Human Leukocyte Antigen

E: Envelope

EDTA: EthyleneDiamineTetraacetic Acid

ELISA: Enzyme Linked Immunosorbent Assay

IgG: Immunoglobulin Gamma

IgM: Immunoglobulin Mu

RT/PCR: Real Time, Polymerase Chain Reaction

RNA: Ribonucleic Acid

AF: Amniotic Fluid

RI: Replicative Intermediates

DsRNA: Double-Stranded RNA

RF: Replicative Forms

Ns: Non Structural

IU: International Unit

CVS: Chorionic Villi Sampling

FGR: Flue Gas Recirculation

TMB: Tetra Methyl Benzidine

SUST: Sudan University for Science and Technology

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CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Rubella, also known as German measles or three-day measles (Neighbors and Tannehill, 2010). It is a disease that caused by the rubella virus, the name "rubella" was derived from Latin, meaning little red. This disease is often mild and attacks often pass unnoticed. The disease can last one to three days. Children recover more quickly than adults. Infection of the mother by rubella virus during pregnancy can be serious; if the mother is infected within the first 20 weeks of pregnancy, the child may be born with congenital rubella syndrome (CRS), which entails a range of serious incurable illnesses. Miscarriage occurs in up to 20% of cases (Siegel *et al.*, 1971). Acquired (i.e. not congenital) rubella is transmitted via airborne droplet emission from the upper respiratory tract of active cases. The virus may also be present in the urine, feces and on the skin. There is no carrier state: the reservoir exists entirely in active human cases. The disease has an incubation period of 2 to 3 weeks (Richardson *et al*., 2001). The name rubella is sometimes confused with rubeola, an alternative name for measles in English-speaking countries; the diseases are unrelated. In some other European languages, like Spanish, rubella and rubeola are synonyms, and rubeola is not an alternative name for measles (Edlich *et al*., 2005).

Thus, in Spanish, "rubeola" refers to rubella and "sarampión" refers to measles. Rubella has symptoms that are similar to those of flu. Rubella can cause congenital rubella

syndrome in the newly born. The syndrome (CRS) follows intrauterine infection by the rubella virus and comprises cardiac, cerebral, ophthalmic and auditory defects (Santis *et al.*, 2006).

It may also cause prematurity, low birth weight, and neonatal thrombocytopenia, anemia and hepatitis. The risk of major defects or organogenesis is highest for infection in the first trimester. CRS is the main reason for development of a vaccine for rubella was developed (Frey, 1994).

The disease is caused by Rubella virus, a togavirus that is enveloped and has a single-stranded RNA genome (Forrest *et al*., 2002). Increased susceptibility to infection might be inherited as there is some indication that HLA-A1 or factors surrounding A1 on extended haplotypes are involved in virus infection or non- resolution of the disease

(Honeyman *et al*., 2014).

In children Rubella normally causes symptoms which last two days and include the following (Best, 2007), rash beginning on the face which spreads to the rest of the body, low fever of less than 38.3°C (101°F) and posterior cervical lymphadenopathy. In older to children and adults there is additional symptoms may be present including: swollen glands, coryza (cold like symptoms), aching joints (especially in young women) and serious problems can occur including brain infections and bleeding problems. RV specific IgM antibodies are present in people recently infected by Rubella virus but these antibodies can persist for over a year and a positive test result needs to be interpreted with

caution (Stegmann and Carey, 2002). The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis (Watson *et al*., 1998).

Early identification of these women by serologic testing might be used as part of a strategy to prevent some perinatal transmission of rubella viruses.

1.2. Rationale

Rubella virus is atypical viral pathogen that cause congenital infections in pregnant women and thereby cause, fetal or neonatal abnormalities with high fetal morbidity and mortality. The basic epidemiological information concerning these infections including their prevalence and some associated factors that increase the susceptibility of pregnant women to these infections is helpful to health planners, care providers and also for health promotion activities (Hamdan *et al*., 2011).

This virus is able to cross the placenta and infect the fetus causing fetal damage there by resulting in spontaneous abortion (miscarriage), stillbirth and wide range of malformations in newborns such as hearing loss, mental retardation, developmental delay, cerebral palsy, epilepsy, ocular abnormality, microcephaly, hydrocephaly, hydranencephaly (absence of the cerebral hemispheres), porencephaly (cavities in the brain), heart disease, cataract, intracranial calcification, microphthalmia, chorioretinitis, skin aplasia (failure of skin to develop), skin lesions, psychomotor retardation, abortion, stillbirth and congenital malformations. It may also cause prematurity, low birth weight, and neonatal thrombocytopenia, anemia and hepatitis. (Ross and Boppana, 2005; Atreya *et al*., 2004; Jones, 2003).

Evidence from several studies indicate that susceptible pregnant women are more to giving birth to infants with abnormalities caused by these virus in first time exposure than their counterparts who already have antibodies to these infections. Therefore, a study to determine the susceptibility levels among pregnant women and some associated factors increasing their susceptibility rate is inevitable. This study is therefore to determine prevalence of these virus among pregnant women in Police Hospital (Khartoum State) by using (ELISA) technique.

1.3. Objectives

1.3.1. General Objective

To determine the prevalence of rubella virus among pregnant women in Khartoum State.

1.3.2. Specific Objectives

1-To detect rubella virus IgG and IgM in plasma of pregnant women using ELISA.

2-To detect the frequency of abortion associated with RV among pregnant women.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2. Rubella

2.1. History

Rubella virus is a single stranded RNA virus of paramyxovirus group. It is a togavirus and the only member of the genus rubivirus and the cause of Rubella, a childhood disease commonly known as German measles. The disease was first described in the mid-eighteen century with Friedrich Hoffmann being the first to clinically describe the disease in 1740. This was later confirmed by de Bergen in 1752 and later again in1758 by Orlow of whom were all Germans (Wesselthoeft, 1949).

This disease was difficult to distinguish from measles and scarlet fever since they nearly produce similar clinical presentations until in 1814 where George Maton gave a more vivid description of the disease and therefore suggested that the disease should be considered different from that of measles and scarlet fever. The disease had its common name as "German measles" from the fact that all the early scientists who identified the disease were Germans (Best *et al*., 2005).

Henry Veale, an English Royal Artillery surgeon was the first to describe an outbreak of the disease in India and later in 1866 became the author of the now well-known name of the disease, "Rubella" (Lee and Scott, 2000; Ackerknecht and Erwin, 1982).

Alfred Fabian Hess, in 1914 based on some work on monkeys theorized that rubella is caused by a viral agent (virus). And this was later confirmed in 1935 by Hiro and Tosaka by passing the disease to children using a filter nasal washing from an acute case (Hess and Alfred, 1914).

In 1940, there was a widespread epidemic of rubella in Australia. Subsequently, ophthalmologist Norman McAllister Gregg found 78 cases of congenital cataracts in infants and 68 of them were born to mothers who had caught rubella in early pregnancy (Lee and Scott, 2000; Atkinson *et al*., 2007).

2.2. Classification

RV is classified as the only member of the genus Rubivirus within the family Togaviridae; the name "togavirus" is derived from the Latin "toga," meaning cloak or shroud, a reference to the virus envelope (Murphy *et al.,* 1995).

2.3. Structure and composition

The mature RV virion is a round or ovoid particle approximately 60 nm in diameter. The virion contains an electron-lucent spherical core composed of multiple copies of the RV capsid protein and a single copy of the viral RNA genome. The RV core is surrounded by a host-derived lipid bilayer containing 5- to 6-nm-long spikes which project from the virion surface; the spikes are composed of the E2 and E1 glycoproteins (Frey, 1994; Murphy, 1980).

2.3.1. Capsid Protein

The capsid protein is a non-glycosylated, phosphorylated, disulfide-linked homodimer with a reported molecular mass of 33 to 38 kDa (Frey, 1994; Marr *et al.,* 1991; Oker *et al*., 1983; Vaheri and Hovi, 1972).

The capsid protein contains clusters of proline and arginine residues, which have been postulated to be involved in binding to the RV genomic RNA to form the viral nucleocapsids (Bowden and Westaway, 1984; Frey, 1994).

2.4. Replication

Rubella virus is characterized by slow replication, which is reflected in the long viral latent period of 8 to 12 h (Bowden and Westaway, 1984; Frey, 1994).

During RV infection, four distinct viral RNA species can be detected, a single-stranded 40S RV genomic RNA (3.8 × 103 kDa) and a 24S subgenomic RNA (1.2 × 103 kDa) that corresponds to the 3′ one-third of the genomic RNA are present in infected cells (Hovi and Vaheri, 1970; Oker *et al*., 1984; Sedwick and Sokol, 1970).

Both contain a methyl7guanosine cap at the 5′ terminus and a polyadenylate tail at the 3′ terminus. In addition, viral replicative intermediates (RI) of 21S, representing partial double-stranded RNA (dsRNA), and viral replicative forms (RF) of 19 to 20S, representing full dsRNA, have been detected in RV-infected cells (Sedwick and Sokol, 1970; Wong *et al*., 1969).

During viral replication, the 40S RV genomic RNA serves as a messenger for the nonstructural (ns) proteins and as a template for the synthesis of a 40S negative-polarity RNA strand. The minus strand in turns acts as a template for the transcription of both the 40S RNA and the 24S RNA, (Frey, 1994).

Nascent 40S RNA is packaged with the RV capsid protein to form nucleocapsids. In terms of viral kinetics, both the RV 40S RNA and 24S RNA were detected at the end of the viral latent period, with viral structural proteins appearing 4 h later (Hemphill *et al*., 1988). Peak virus production occurs during the period from 36 to 48 h post infection.

One-step multiplication studies have shown that RV is unable to infect every cell at any specific time, irrespective of the titer of the input virus (Bowden and Westaway, 1989), Moreover, the proportion of cells infected by RV at any one time is cell type dependent. However, as infection proceeds, the entire culture eventually becomes infected. (Hemphill *et al*., 1988; Sedwick and Sokol, 1970; Wong *et al*., 1969).

2.5. Epidemiology

Rubella is a disease that occurs worldwide. The virus tends to peak during the spring in countries with temperate climates. Before the vaccine to rubella was introduced in 1969, 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 year old age group (Reef *et al*., 2002).

During the epidemic in the U.S. between (1962–1965), rubella virus infections during pregnancy were estimated to have caused 30,000 still births and 20,000 children to be born impaired or disabled as a result of CRS (Plotkin, 2001; Cooper, 1975)

Universal immunization producing a high level of herd immunity is important in the control of epidemics of rubella. (Danovaro *et al*., 2000)

In the UK, there remains a large population of men susceptible to rubella who have not been vaccinated. Outbreaks of rubella occurred amongst many young men in the UK in 1993 and in 1996 the infection was transmitted to pregnant women, many of whom were immigrants and were susceptible. Outbreaks still arise, usually in developing countries where the vaccine is not as accessible (Reef, 2006).

2.6. Transimmation

Rubella is transmitted via airborne droplets emission from the upper respiratory tract of active cases. The virus may also be present in the urine, feces and on the skin. There is no carrier state: the reservoir exists entirely in active human cases. The disease has an incubation period of 2 to 3 weeks (Richardson *et al*., 2001).

2.7. Pathogenesis and Immunity

The infection is acquired by inhalation of aerosols or nasopharyngeal secretion containing the virus. The virus then infects the cells of the upper respiratory tract and enters the cell by receptor-mediated endocytosis. It is believed that replication probably begins in the respiratory tract. From there the virus spread and replicates in the lymphoid

tissue of the upper respiratory tract, viraemia proceeds causing systemic infection after about 7 – 9 days and last until the appearance of antibody on about day 13 – 15 (Brooks *et al*., 2010). When viraemia has occurred, the virus spread to many organs including placenta where it can infect the fetus in pregnancy leading to congenital infection and it subsequent congenital rubella syndrome (CRS) (Coulter *et al*., 1999). Maternal immunity, either after vaccination or naturally derived, is generally protective against intrauterine rubella infection (Bullens *et al.*, 2000). Immune mothers usually transfer their antibodies to their offspring which protect them for about 4 – 6 months after birth (Brooks *et al*., 2010). A measure of antibody level of 10-15 international unit (IU) is universally considered to be a positive immunity status, i.e. one is immune (Mendelson *et al*., 2006). Like most infections, majority of the initial antibodies elicited are IgM which normally lasts for some week and has been used detecting recent rubella infection as well as congenital rubella (Best *et al*., 2005).

Even though it is not confirmative in itself. IgG is initially present in lower titer and rises with time while persisting throughout life. Majority of naturally infected victims develop life-long immunity while in vaccinated subjects immunity has been shown to be preventive against viraemia with protection usually lasting for more than 16 years, nevertheless few failure in vaccination have been reported where there is development of partial immunity and therefore protection offered wanes and last for about 5 to 8 years instead (Mendelson *et al*., 2006; Banatvala and Best, 1990). Immunity or past infection does not guarantee protection from reinfection. A study done in Italy followed

immunized subjects for 5 years to demonstrate an evidence of reinfection after vaccination and it was found that 9.8% of the subjects showed an indication of reinfection (Cusi *et al.,* 1993).

In maternal reinfections, some cases of CRS have resulted especially in maternal reinfection before the 12th week of pregnancy (Robinson *et al*., 1994). Even though the risk of CRS at this stage of the gestational period is very low (Best *et al*., 2005).

2.8. Clinical significance

2.8.1. Signs and symptoms

Rubella has symptoms that are similar to those of flu. However, the primary symptom of rubella virus infection is the appearance of a rash (exanthem) on the face which spreads to the trunk and limbs and usually fades after three days (that is why it is often referred to as three-day measles). Other symptoms include low grade fever, swollen glands (suboccipital& posterior cervical lymphadenopathy), joint pains, headache and conjunctivitis (Atreya *et al*., 2004), the facial rash usually clears as it spreads to other parts of the body. Other symptoms include low grade fever, swollen glands (sub occipital & posterior cervical lymphadenopathy), joint pains, headache, and conjunctivitis (Edlich *et al*., 2005)

The swollen glands or lymph nodes can persist for up to a week and the fever rarely rises above 38 °C (100.4 °F). The rash of German measles is typically pink or light red. The rash causes itching and often lasts for about three days. The rash disappears after a few

days with no staining or peeling of the skin. When the rash clears up, the skin might shed in very small flakes where the rash covered it. Forchheimer's sign occurs in 20% of cases, and is characterized by small, red papules on the area of the soft palate (Robert *et al*., 2006).

Rubella can affect anyone of any age and is generally a mild disease, rare in infants or those over the age of 40. The older the person is the more severe the symptoms are likely to be. Up to 60% of older girls or women experience joint pain or arthritic type symptoms with rubella (Robert and Jarrett, 2015).

2.8.2. Acute Rubella

Postnatal infection with RV is usually mild and frequently subclinical, (Banatvala and Brown, 2004; Dwyer *et al.*, 2001)

Symptoms, when present, typically include sore throat and low-grade fever, a maculopapular rash, lymphadenopathy, and, in some cases, conjunctivitis and/or arthralgia. The rash is first seen on the face and spreads in centripetal fashion. The lesions appear as distinct pink maculo-papules that fade rapidly over several days. A pronounced posterior cervical and suboccipital adenopathy is often present. However, these serious sequelae and death as a result of RV infection are rare. During course of the acquired infection and the accompanying immune response, the virus spread through respiratory secretions, and the mucosa of the upper respiratory tract and the nasopharyngeal lymphoid tissue serve as portals of virus entry as well as the initial sites for viral replication. Spread of virus via lymphatics or a transient viremia then seeds regional

lymph nodes. Local replication of virus in these nodes accounts for the posterior cervical and occipital nodal enlargement that typically appears 5 to 9 days before the onset of the rash. The incubation period (approximately 14 days) is followed by the appearance of virus in serum and the onset of viral shedding into the nasopharynx and stool, providing a source of spread to susceptible individuals. High levels of virus can be found in nasopharyngeal excretions, exceeding 105 tissue culture infectious dose 50 (TCID50) per 0.1 mL even in vaccinated individuals (Banatvala and Brown, 2004).

2.8.3. Congenital rubella syndrome

Although postnatal rubella is rarely associated with severe complications, infection in utero following transplacental transmission of virus from the mother has direct consequences for the developing fetus. These are reflected in a constellation of symptoms collectively called congenital rubella syndrome (Webster, 1998).

2.8.4. Pathogenesis of Congenital Rubella Syndrome

In general, maternal infection shortly before conception does not lead to intrauterine infection, (Enders *et al*., 1988).

However, when infection occurs after conception, the virus is present in placental villi approximately 10 days after the onset of rash in the mother and can be detected in the fetus after 20 to 30 days. Transplacental transmission occurs in up to 90% of cases during the first 8 weeks of gestation, falling to a low of 25% to 35% during the second trimester

and rising again near term (Banatvala and Brown, 2004; Garcia *et al*., 1985; Webster, 1998).

This fluctuating incidence of fetal infection is likely related to changes in the placenta during pregnancy. In early gestation, infection of the placenta causes scattered foci of necrotic syncytiotrophoblast and cytotrophoblast cells, as well as damage to the vascular endothelium, resulting in placental hypoplasia (Garcia *et al.,* 1985)

Infection at later stages is associated with multifocal mononuclear cell infiltrates in the placental membranes, cord, and decidua, along with vasculitis (Webster, 1998).

2.8.5. Risks associated with rubella infection in pregnancy

Maternal rubella infection can result in spontaneous miscarriage, fetal infection, stillbirth, or fetal growth restriction (Reef *et al*., 2000).

Congenital infection is most likely if the maternal infection occurs in the first 16 weeks of pregnancy, with congenital rubella syndrome occurring in all fetuses infected before the 11th week and in 35% of those infected at 13–16 weeks (Miller *et al*., 1982).

If infection occurs after 16 weeks of pregnancy, the risk of fetal damage is negligible.

Features of congenital rubella syndrome include cardiac defects, deafness, ocular defects, thrombocytopenic purpura, haemolytic anemia, enlarged liver and spleen, and inflammation of the meninges and brain (Sanchez *et al*., 2010).

Pneumonitis, diabetes, thyroid dysfunction and progressive panencephalitis are other late expressions of the syndrome (Weil *et al*., 1975; Cooper *et al*., 1995).

2.9. Diagnosis of rubella infection

Diagnosis of rubella in pregnant women is very important especially in suspected cases to rule a primary infection that has a high probability of resulting in CRS. The assessment of maternal primary infection usually relies on the detection of specific IgM antibodies to the rubella virus, seroconversion and or greater four folds rise in IgG antibodies. As in many other viral infections, IgM alone cannot provide an evidence of recent infection and therefore some, employs the use of IgG avidity testing to establish primary or recent infections. The test for IgM and IgG can be demonstrated serologically by the use ELISA. The laboratory methods used for virus detection are virus isolation in tissue culture or amplification of viral nucleic acids by RT/PCR. However, using those methods for detection of rubella virus in Amniotic fluid (AF) might be unreliable, particularly in (AF) samples due to low viral load. Thus, according to one opinion, detection of rubella virus in (AF) does not justify the risk of fetal loss following these invasive procedures (Alton and DeCherney, 1993),

While According to another opinion, laboratory diagnosis of fetal infection should combine a serological assay (detection of rubella specific IgM) with a molecular method (viral RNA detection) in order to enhance the reliability of the diagnosis (Tang *et al*., 2003). A recent study showed 83–95% sensitivity and 100% specificity for detection of Rubella virus in AF by RT/PCR (Mace *et al.,* 2004).

2.9.1. Diagnosis of Maternal Infection

Accurate diagnosis of acute primary rubella infection in pregnancy is imperative and requires serologic testing. Since an important number of cases are subclinical, Serology by ELISA to measure rubella-specific IgG and IgM is convenient, sensitive, and accurate. The presence of a rubella infection is diagnosed by: A fourfold rise in rubella IgG antibody titer between acute and convalescent serum specimens which is a positive serologic test for rubella-specific IgM antibody, a positive rubella culture (isolation of rubella virus in a clinical specimen from the patient (CDC, 2001).

Serologic studies are best performed within 7 to 10 days after the onset of the rash and should be repeated two to three weeks later. Viral cultures drawn from nasal, blood, throat, urine, or cerebrospinal fluid may be positive from one week before to two weeks after the onset of the rash. (CDC, 2001; Frey and Abernathy, 1993)

2.9.2. Diagnosis of Fetal Infection

There are small series reporting the usefulness of Rubella specific PCR on CVS for the prenatal diagnosis of intrauterine rubella infection (Bosma *et al.*,1995; HoTerry *et al* ., 1990) this technique has proved to be superior to assessment of amniotic fluid samples in one study (Tanemura *et al* ., 1996)

Because CVS is done at10 to12 weeks of gestation, it allows earlier detection than is possible with other samples, such as amniotic fluid taken at 14 to 16 weeks or fetal blood obtained at 18 to 20 weeks of pregnancy .Ultrasound diagnosis of CRS is extremely

difficult. Biometric data can aid in the diagnosis of FGR but is not a good tool for diagnosing CRS, given the nature of the malformations encountered. Any fetus presenting with FGR should be evaluated for congenital viral infections, including rubella (Ozsoylu *et al*., 1978)

2.10. Prevention and control

The Rubella in the absence of pregnancy usually presents a mild and self-limiting disease which usually resolves after some few weeks thereby resulting in lifelong immunity. There is usually the appearance of maculopapular rash about two to three weeks after first time exposure. The rash appears on the face and then spreads to the trunk and then to the extremities. There may also be other symptoms such as low-grade fever, sore throat, lymphadenopathy and general malaise (Lee and Scott, 2000).

However Some other complications such as arthritis and arthralgia may be seen in adults, surprisingly, these symptoms are more severe in adult females than in men Thrombocytopenic purpura and encephalopathy may be more severe complications in rubella infections (Brooks *et al*., 2010; Frey, 1994).

Rubella infections are prevented by active immunization programs using live, disabled virus vaccines. Two live attenuated virus vaccines, RA 27/3 and Cendehill strains, were effective in the prevention of adult disease. However their use in prepubertile females did not produce a significant fall in the overall incidence rate of CRS in the UK. Reductions were only achieved by immunization of all children (Dayan *et al*., 2006)

Screening for rubella susceptibility by history of vaccination or by serology is recommended in the United States for all women of childbearing age at their first preconception counseling visit to reduce incidence of congenital rubella syndrome (CRS) (Adam *et al* ., 2013)

2.11. Treatment

There is no specific treatment of rubella nevertheless management is directed towards symptoms so as to reduce discomfort. In the case of CRS in newborns, management focuses on dealing with the complications. The control of rubella has always the golden tool of prevention. Live attenuated vaccine have been in existence since 1969, this vaccine is available either as a single antigen or combined with measles and mumps vaccines. The primary purpose of the rubella vaccine however, is to prevent congenital rubella infections (Brooks *et al.,* 2010).

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER THREE

MATERIAL AND METHOD

3.1. Study Design

3.1.1 .Type of Study

Cross sectional hospital based study.

3.1.2. Ethical consideration

This study was approved by College of Graduated Studies in Sudan University of Science and Technology (SUST). Permission from hospital was applied and verbal consent was taken from patients involved in this study.

3.1.3. Study area

The study was conducted in Police Hospital, the practical part of this study was done in the Research Laboratory (Alwarif Medical Complex).

3.1.4. Study duration

This study was conducted during the period from April to May 2016.

3.1.5. Study population

Pregnant women with different ages and with and without history of miscarriage were included.

3.2. Sampling size

A total of ninety one blood samples (n=91) were collected from pregnant women.

3.3. Inclusion criteria

Pregnant women with and without abortion, pregnant women with different stages of pregnancy and different ages.

3.4. Exclusion criteria

Non pregnant women, male.

3.5. Data collection

Data were collected according to the questionnaire bellow in the appendix.

3.6. Methodology

3.6.1. Collection of blood samples

Blood samples were collected under direct medical supervision by medial vein puncture using 5 ml syringe into EDTA (Ethylenediaminetetraacetic acid) container.

3.6.2. Sample processing

Each blood sample was centrifuged at 3000 g for 5 minutes, then plasma was gently collected into plain container and stored at -20 °C until the serological analysis.

3.6.3. Sample analysis

The samples were analyzed for qualitative detection of Rubella (IgM and IgG) antibodies by commercially available enzyme–linked immunosorbent assay ‘ Rubella (IgM and IgG) ELISA kit (Foresight, Acon laboratories, Inc., 10125 Mesa Rim Road, San Diego, CA 92121, USA).

The assays were performed following the instructions of the manufacturer, According to the information included in the kit’s insert, the immunoassay used has (93.5%) sensitivity and (96.8%) specificity for IgM and (96.4 %) sensitivity and (99.9%) specificity for IgG.

3.6.4. Principles of RV ELISA

Principles of RV IgG

The RV IgG EIA test kit is a solid phase enzyme immunoassay based on indirect principle for qualitative detection of IgG antibodies in human serum or plasma. The micro well plate was coated with RV antigens. During testing, the specimen diluent and specimens were added to antigen coated micro well plate then incubated. If the specimens contain IgG antibodies to RV, it will bind to the antigens coated on the micro well plate to form immobilized antigen RV IgG antibody complexes. If the specimens do not contain IgG antibodies to RV, the complexes will not be formed. After initial incubation, the micro well plate was washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies were added to the micro well plate and then incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-RV

IgG antibody complexes present. After the second incubation, the micro well plate was washed to remove unbound materials. Substrate A (hydrogen peroxide) and substrate B (Tetra methyl benzidine) were added and then incubated to produce blue color indicating amount of RV IgG antibodies present in specimens. Sulfuric acid solution was added to micro well plate to stop the reaction producing a color change from blue to yellow. The color intensity was measured using micro well plate reader at 450nm.

Principles of RV IgM

The RV IgM EIA test kit is a solid phase enzyme immunoassay based on immunocapture principle for qualitative detection of IgM antibodies in human serum or plasma. The micro well plate was coated with Anti human IgM antibodies. During testing, the specimen diluent and specimens are added to antibody coated micro well plate and then incubated. If the specimens contain IgM antibodies to RV, it will bind to the antibodies coated on the micro well plate to form immobilized anti-human IgM antibody-rubella IgM antibody complexes. If the specimens do not contain IgM antibodies to RV, the complexes will not be formed. After initial incubation, the micro well plate was washed to remove unbound materials. The enzyme-conjugated Rubella antigens are added to the micro well plate and then was incubated. The enzyme-conjugated Rubella antigens will bind to the immobilized anti-human IgM antibody-rubella IgM antibody complexes present. After the second incubation, the micro well plate was washed to remove unbound materials. Substrate A (hydrogen peroxide) and substrate B (Tetra methyl benzidine) are added and then incubated to produce blue color indicating amount of RV

IgM antibodies present in specimens. Sulfuric acid solution was added to micro well plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of Rubella IgM antibodies present in the specimens, was measured with a micro plate reader at 450nm.

3.6.5. Procedure

All reagents and specimens were settled to reach room temperature, 100 µl of calibrator, positive control and negative control were added to their respective wells. 100 µl of sample diluents was added to each well except the blank, then 5 µl of sample was added.

The micro well plate was mixed gently and covered by plate sealer then incubated for 30 minutes at 37 °C. At the end of incubation the micro well plate was washed 5 times using diluted wash buffer.

100 µl of conjugate was added to each well except the blank, the plate was covered and incubated for 30 minutes at 37 °C. By the end of incubation period each well was washed 5 times with diluted wash buffer.

After washing 50 µl of substrate A and substrate B were added to each well including the blank, then the plate was covered and incubated for 10 minutes at 37 °C.

Finally 50 µl of stop solution was added to stop the reaction and the optical density was read at 450 nm within 30 minutes.

3.6.6. Quality control

Rubella IgM

Reagents and calibrators were checked for storage, stability and preparation before starting work.

Blank absorbance was < 0.050 and < 0.100 at 450 nm

Calibrator absorbance was > 0.150 and < 0.450 at 450 nm

Negative control absorbance was < 0.100 at 450 nm

Positive control absorbance was > 0.500 at 450 nm

Rubella IgG

Blank absorbance was < 0.05 at 450 nm

Calibrator 1 absorbance was < 0.100 at 450 nm

Calibrator 2 absorbance was > 0.200 and < 0.700 nm

Calibrator 3 absorbance was > Calibrator 2 and < Calibrator 4

Calibrator 4 absorbance was > 1.500 nm

3.6.6.1. Calculation of results

Rubella IgM

The results were calculated by relating each specimen absorbance to index value.

Cut-off value = absorbance of calibrator – Blank absorbance

Index value = Specimen absorbance / cut-off value

Rubella IgG

The results were calculated by relating each specimen absorbance to index value.

Cut-off value = absorbance of calibrator3 – Blank absorbance

Index value = Specimen absorbance / cut-off value

3.6.6.2. Interpretation of results

Interpretation rubella IgM

Positive more than 1.1nm

Negative less than 0.9 nm

Equivocal between {0.9-1.1} nm

<0.9 nm negative No significant IgM antibodies to RV were detected.

> 0.9 nm to <1.1 Equivocal The sample should be retested using a different method.

(1.1) nm positive Presumptive for the presence of IgM antibodies to RV.

In cases of Equivocal test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample.

Interpretation rubella IgG

Positive more than 1.1 nm

Negative less than 0.5 nm

Equivocal between {0.5-1.1} nm

<0.5 nm negative No significant IgG antibodies to RV were detected.

> 0.5 to <1.1 nm Equivocal the sample should be retested using a different method.

3.7. Data analysis

Data was analyzed using software program SPSS (Statistical Package for Social Sciences), Version 16, 0 computerized program.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

4.1. Results

A total of ninety one blood samples (n=91) were obtained from pregnant women in Police Hospital in Khartoum State. All specimens were examined for the presence of RV IgG and IgM antibodies using ELISA kit. The positive Prevalence of RV IgM and IgG among pregnant women were 87(95.6%),while the rest 4(4.4%) were negative for RV (Table 1 and Figrue1) The result showed that out of 91 blood samples investigated, 2(2. 2%) were positive for IgM RV, while the rest 89(97.8%) were negative (Table 2 and Figrue2). And the result showed that out of 87 blood samples investigated, 85 (97. 7%) were positive for IgG RV, while the rest 2(2. 2%) were negative (Table 3 and Figrue3). Out of 39/91 women with history of abortion 0(0%) were positive for IgM RV, while the rest 39(100%) were negative and 38 (97.43) were positive for IgG RV while the rest1 (2.6%) were negative (Table 4, 5 and Figrue4, 5). Moreover out of 52/91 women without history of abortion 2 (3.8%) were positive for IgM RV, while the rest 50(96.4%) were negative and 47 (98%) were positive for IgG RV, while the rest 1(2 %) were negative (Table4, 5 and Figrue4, 5). Negative RV IgM blood samples were distributed though pregnancy stage, 16/17(94 %) in the first trimester, 20/21(95 %) in second trimester and 53/53(100 %) in third trimester, Positive RV IgG blood samples were distributed though pregnancy stage, 16/17(94%) in the first trimester, 20/20 (100 %) in second trimester and 49/50(98 %) in third trimester, (Table 6, 7 and Figure 6,7). Negative RV IgM samples were distributed through the age groups, 32/32(100%) within (15 – 25), 47/49(96%)

within (26 – 36) and 10 /10(100%) within (37 – 47).Positive RV IgG samples were distributed through the age groups, 32/32(100 %) within (15 – 25), 43/45(95 %) within (26 – 36) and 10/10 (100 %) within (37 – 47) (Table 8, 9 and Figrue8,9).

Table 1. Prevalence of RV IgM and IgG among pregnant women

Result	No.	%
Positive	87	95.6
Negative	4	4.4
Total	91	100

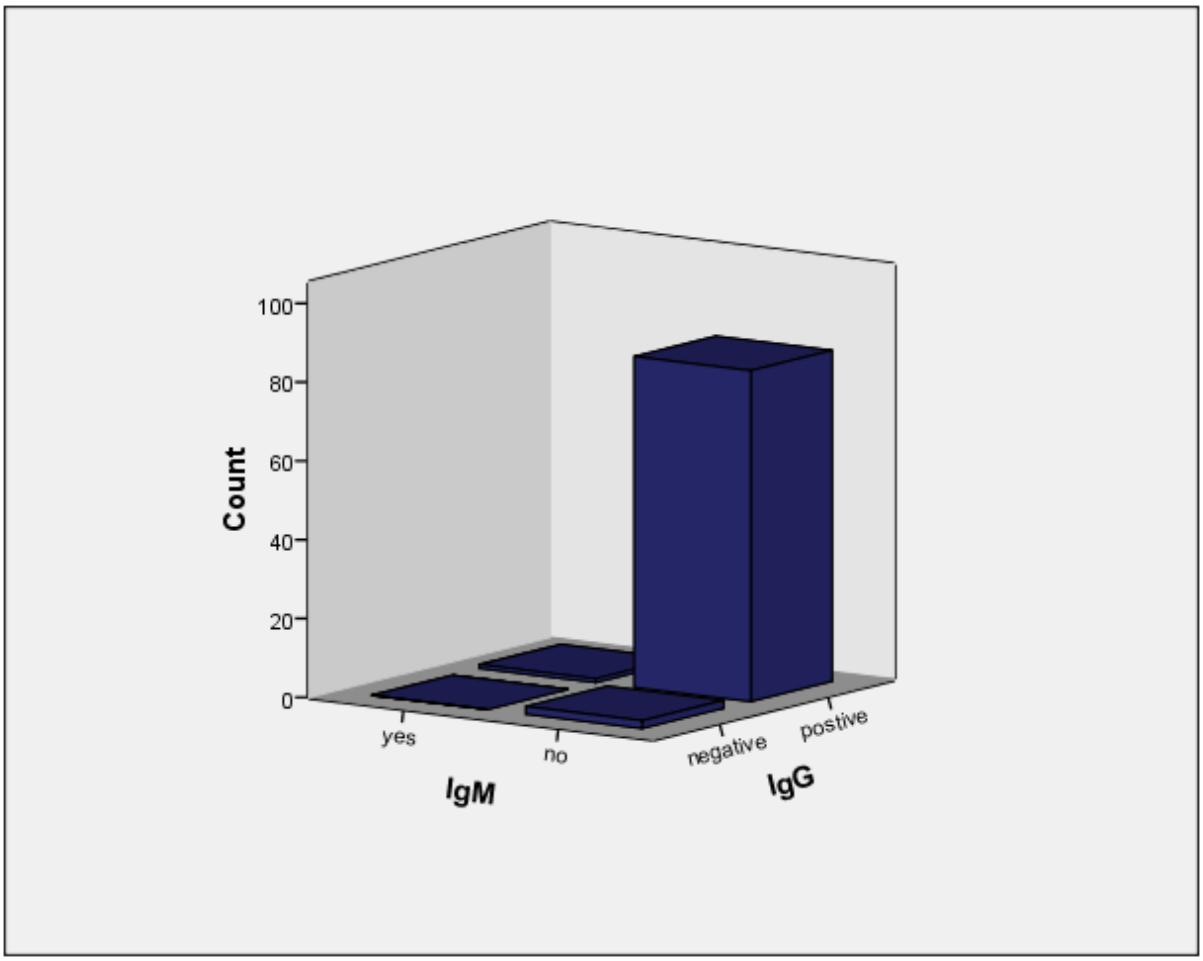

Figure 1 .Prevalence of RV IgM and IgG among pregnant women

Table 2. Prevalence of RV IgM among pregnant women

Result	No.	%
Positive	2	2.2
Negative	89	97.8
Total	91	100

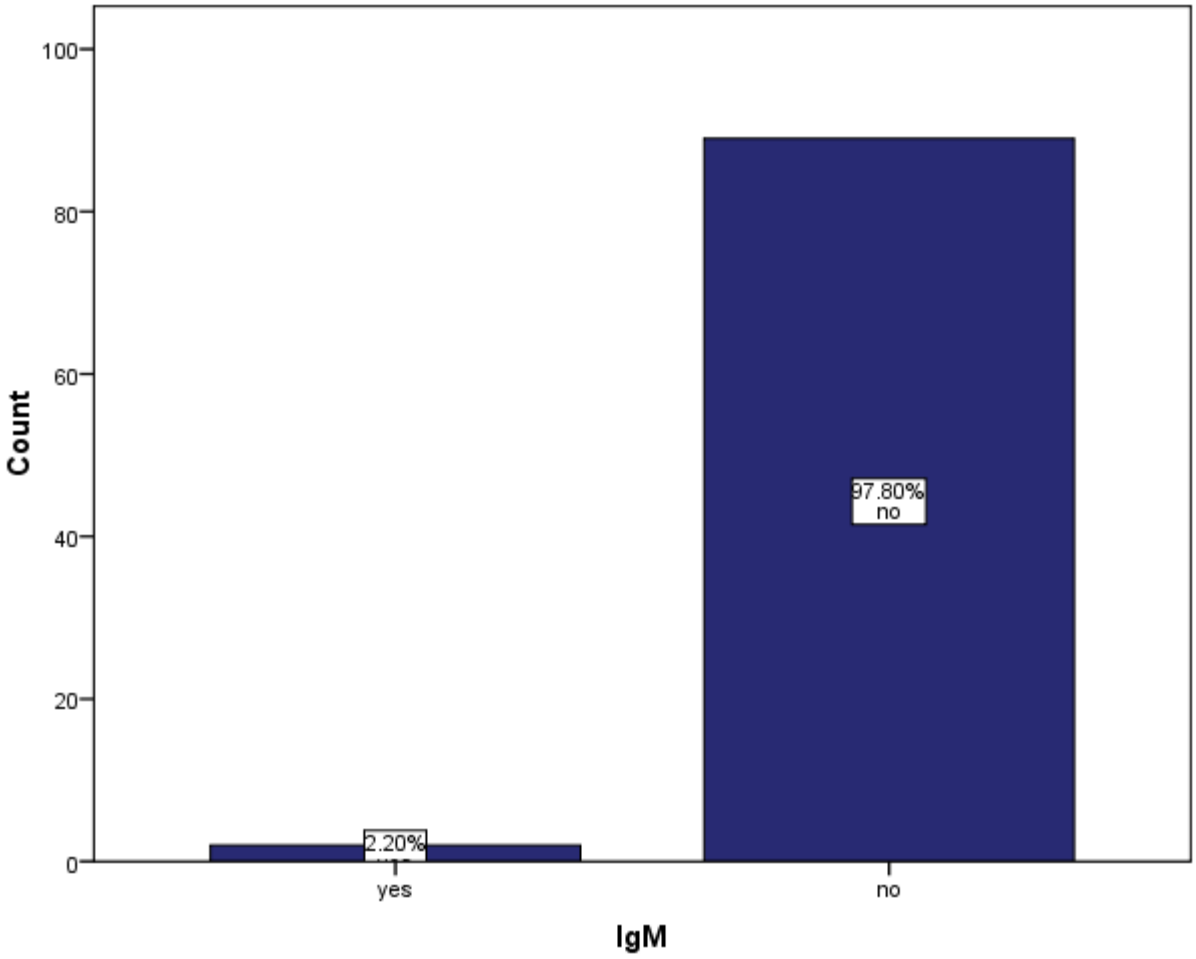

Figure 2. Prevalence of RV IgM among pregnant women

Table 3. Prevalence of RV IgG among pregnant women

Result	NO	%
Positive	85	97.7
Negative	2	2.2
Total	87	100

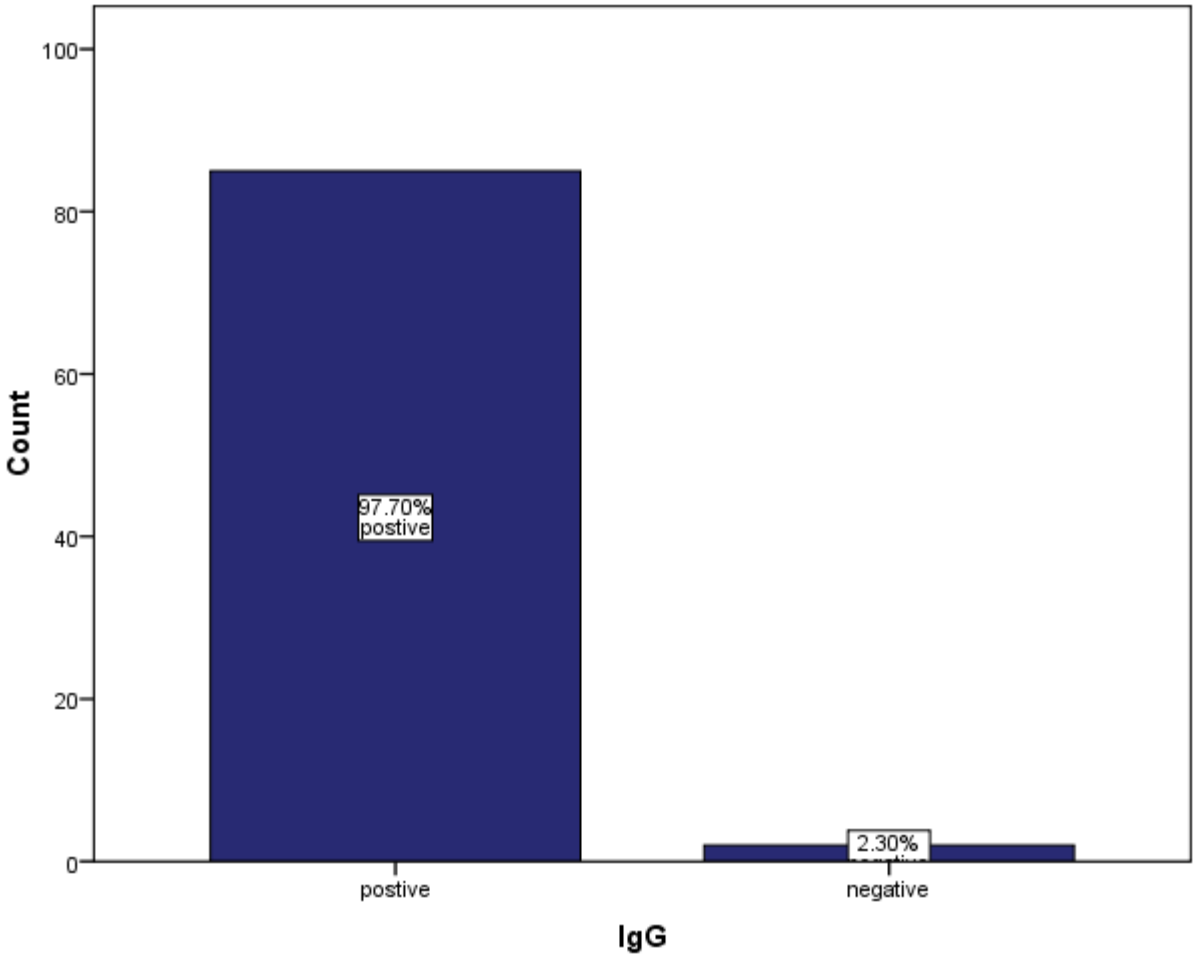

Figure 3. Prevalence of RV IgG among pregnant women

Table 4. Frequency of RV IgM according to history of abortion

Abortion	Result	No.	%
Yes (n=39)	Positive	0	0
	Negative	39	100
No (n=52)	Positive	2	3.8
	Negative	50	96.4
Total		91	100
p. value	.216		

In table (4) showed there is no statically significant association (p> 0.05) between history of abortion anti-rubella IgM +ve.

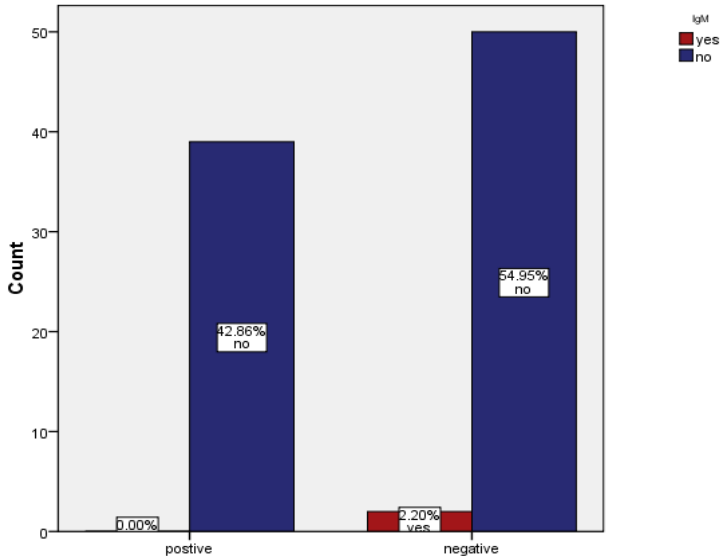

Figure 4. Frequency of RV IgM according to history of abortion

Table 5. Frequency of RV IgG according to history of abortion

Abortion	**Result**	**No.**	**%**
Yes (n=39)	Positive	38	97.4
	Negative	1	2.6
No (n=48)	Positive	47	98
	Negative	1	2
Total		87	100
p. value	.882		

In table (5) showed there is no statically significant association (p> 0.05) between history of abortion anti-rubella IgG +ve.

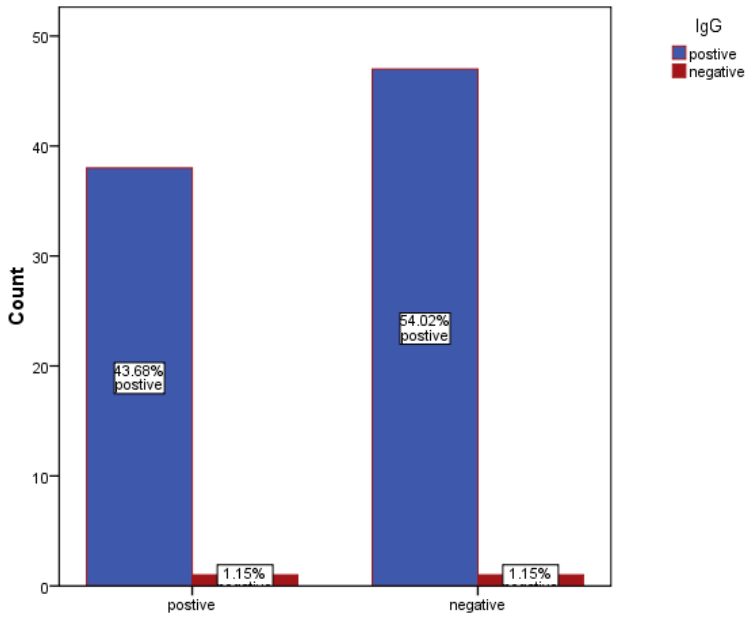

Figure 5. Frequency of RV IgG according to history of abortion

Table 6. Frequency of RV IgM according to gestational stages

Gestational stage	No.	Results	
		positive	Negative
First trimester	17	1(6%)	16(94%)
Second trimester	21	1(4.7)	20(95%)
Third trimester	53	0(0%)	53(100%)
Total	91	2	89
p.value	.406		

Table (6) showed there is no statically significant association (p >0.05) between the trimester and anti-rubella IgM +ve.

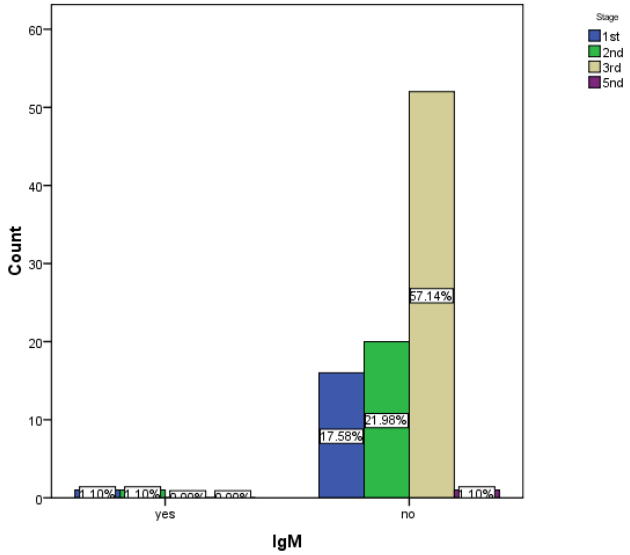

Figure 6. Frequency of RV IgM according to gestational stages

Table 7. Frequency of RV IgG according to gestational stages

Gestational stage	No.	Results	
		Positive	Negative
First trimester	17	16(94%)	1(6%)
Second trimester	20	20(100%)	0(0%)
Third trimester	50	49(98%)	1(2%)
Total	87	85	2
p. value	.687		

In table (7) showed there is no statically significant association ($p > 0.05$) between the trimester and anti-rubella IgG +ve.

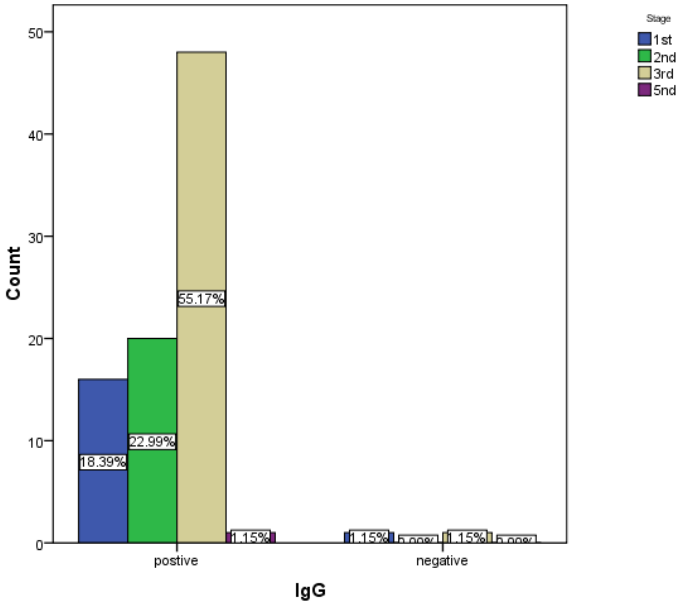

Figure 7. Frequency of RV IgG according to gestational stages

Table 8. Frequency of IgM according to age group

Age group	No.	Result	
		Positive	Negative
15 ─ 25	32	0(0%)	32(100%)
26 ─ 36	49	2(4%)	47(96%)
37─ 47	10	0(0%)	10(100%)
Total	91	2	89
p. value	.416		

In table (8) showed there is no statically significant association ($p > 0.05$) between Age group and anti-rubella IgM +ve.

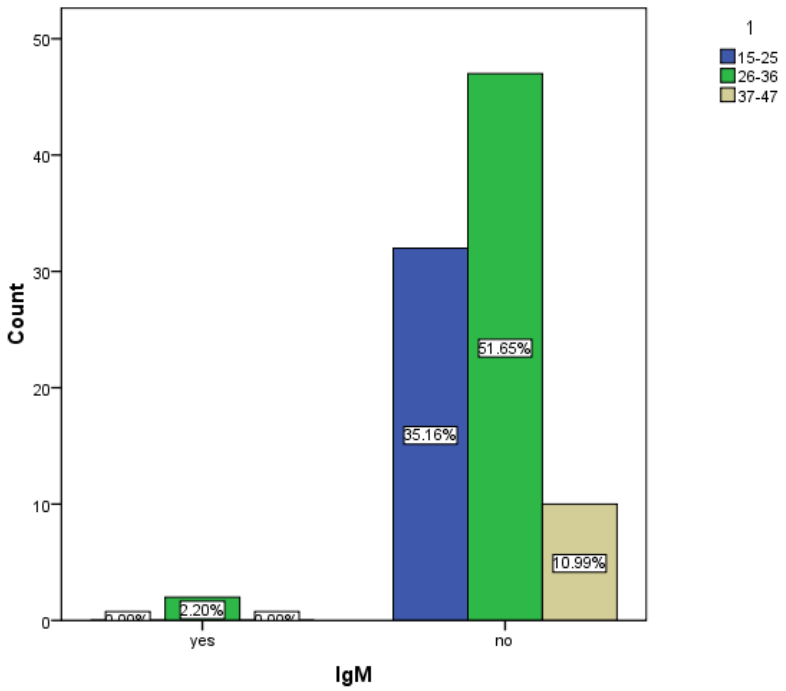

Figure 8. Frequency of IgM according to age group

Table 9. Frequency of IgG according to age group

Age group	No.	Result	
		positive	Negative
15 ─ 25	32	32(100%)	0(0%)
26 ─ 36	45	43(95%)	2(4.5%)
37─ 47	10	10(100%)	0(0%)
Total	87	85	2
p.value	.385		

In table (9) showed there is no statically significant association ($p > 0.05$) between Age group and anti-rubella IgG +ve.

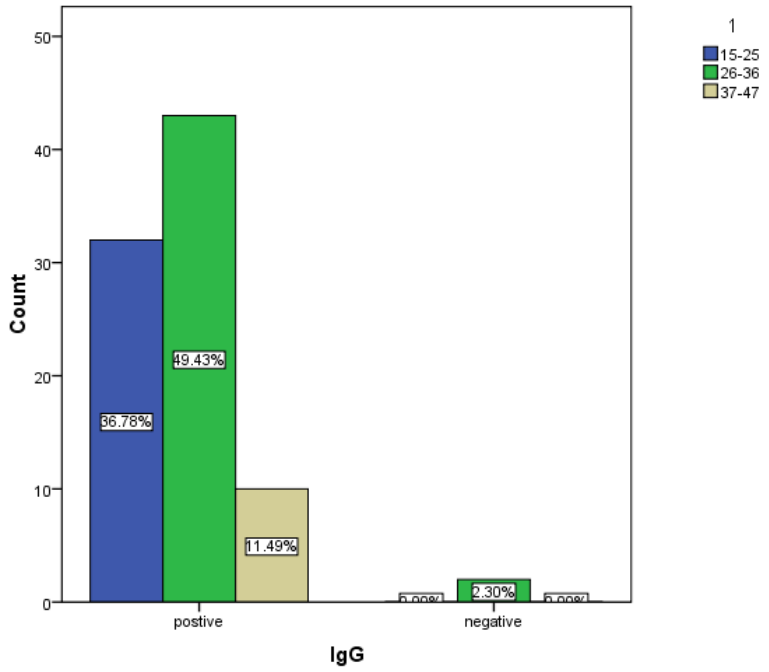

Figure 9. Frequency of IgG according to age group

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

5.1. Discussion

The high rate of morbidity and mortality caused by rubella especially to fetus of infected mothers due to congenital and neonatal infections with their consequent wide range of abnormalities and social as well as financial burdens to families and countries has made the screening of pregnant women an important research activity.

This study presents the most recent data on the frequency of Rubella in pregnant ladies in terms of age groups and stage of pregnancy and history of abortion, thereby for the prevention of rubella infection. The present study results revealed that 87(95. 6%) of pregnant women in this study were positive for both IgM antibody and IgG, when obtained comparable to results , in Khartoum by Adam *et al*., (2013) our result is similar to him. Who reported Rubella IgG antibodies in 95.1% among pregnant women. And also it is similar to study of (Antenatal screening for Toxoplasma gondii, Cytomegalovirus, rubella and Treponema pallidum infections in northern Benin, by De Paschale *et al*., (2014) No anti rubella IgM was detected by EIA screening and rubella IgG was (94%). But it is higher than results of the study obtained by Wafa *et al*., (2016) in Khartoum, who reported (0.7%) of pregnant women were IgM+ve (recent infection) and (89.4%) were IgG+ve (past infection). And also higher than the results reported in Western Sudan by Hamdan *et el*.,(2011) who reported of (65.3%)and it is higher than result of study in Benin city Nigeria by Onakewhor and Chiwuzie (2011) in which they found IgG

seroprevalence was 53%, The seroprevalence of 93.3% of rubella IgG antibodies was found among pregnant Saudi women by Ghazi *et al*., (2002) suggesting a successful vaccination campaign .Only (2.2 %) of respondents were observed to be susceptible or at risk of primary rubella infection since majority of respondents (97.7 %) showed evidence of past infection (had the IgG antibodies to the rubella virus) in that study. Some argue that since the disease is a childhood disease and the prevalence is high, it is better to allow its spread especially among children so that by the child-bearing age, most of them would have developed antibody that is capable of fully protecting them as well as reducing the number of babies born with CRS. This is a good measure for resource poor countries. However, 2.2 % of the respondents had IgM antibodies to the rubella virus but this may be are infection and not primary infection as all respondents with IgM also had IgG antibodies. This is less likely to result in congenital infection leading to congenital rubella syndrome (CRS) though some few cases of reinfection have been linked to CRS (Robinson *et al*., 1994).

This study disagree with a study in Italian women conducted by Gabutti *et al*., (2002) who reported 71.2% of pregnant women were positive for IgG antibody. This variation might be due to differences in sample size, study duration and techniques used for detection of the virus. We presume this high serofrequency indicates a high circulation of wild rubella virus in Khartoum. Similar studies in other Sudanese states would be important for informing a decision to introduce rubella vaccine to Sudan.

5.2. Conclusion

Prevalence of rubella seromarkers for previous infection is high.

Facilities for routine diagnosis and vaccination are lacking.

High prevalence rate was found in those women within the second trimester of gestation.

The level of infection is higher in those pregnant women without history of abortion than those aborted women.

5.3. Recommendations

1. Rubella virus IgG should be included in the listed tests for pre married girls in order to give vaccine to negative one.

2- Pre pregnant screening for antibodies to Rubella virus are recommended to be done as a routine practice that prevent fetus from infection by Rubella virus in uterus.

3. The diagnosis of primary maternal infection should be made by serological testing.

4. In a pregnant woman whom is exposed to rubella or whom develop signs or symptoms of rubella, serological testing should be performed to determine immune status and risk of congenital rubella syndrome

5. Providing universal infant immunization to decrease circulation of virus.

6. The diagnosis of infection should be made as soon as possible. Contact with rubella should be avoided throughout the first, second trimesters of pregnancy, even in IgG-positive pregnant women.

7. Unfortunately, there is no in utero treatment available for infected fetuses. Thus, prevention remains the best strategy to eliminate all cases of CRS.

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Foresight®

Rubella IgM EIA Test Kit
Package Insert

REF I231-1121 | English

An enzyme immunoassay (EIA) for the qualitative detection of IgM antibodies to Rubella in human serum or plasma.
For professional in vitro diagnostic use only.

INTENDED USE

The Rubella IgM EIA Test Kit is an enzyme immunoassay for the qualitative detection of IgM antibodies to Rubella in human serum or plasma. It is intended as an aid in the diagnosis of possible Rubella infection.

SUMMARY

Rubella is a small spherical enveloped RNA virus belonging to *Togaviridae* family. Most commonly known as the German or 3-day measles, the Rubella virus is spread through droplet infection resulting in mild contagious rash in children or young adults.
In childhood, the infection is self-limited, benign disease characterized by low-grade fever, headache, lymphadenopathy, arthralgia, and conjunctivitis. However, infection during pregnancy particularly in the first trimester can lead to spontaneous abortion, intrauterine infection causing fetal death, or congenital abnormalities. Congenital rubella depends on the time the infection occurs and may result in severe complications including deafness, ocular problems including cataracts and glaucoma, congenital heart disease and mental retardation. [1, 2] IgM antibodies against rubella are first produced reaching detectable levels within 2-3 days and peak 14-21 days after onset of symptoms which remain detectable over the next 4-8 weeks. Diagnosis of active or recent infection may be obtained by presence of IgM antibody in single early specimen. After several days, IgG antibodies appear after IgM response and peak 14-21 days later which then persist at varying levels for life. [3, 4] The presence of IgG antibodies to rubella is indicative of previous infection and presumptive immunity. [5, 6]
The Rubella IgM EIA Test Kit is an immunoassay for the qualitative detection of the presence of IgM antibodies to Rubella in serum or plasma specimen. The test utilizes purified Rubella antigens to selectively detect IgM antibodies to Rubella in serum or plasma.

PRINCIPLE

The Rubella IgM EIA Test Kit is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to Rubella in human serum or plasma. The microwell plate is coated with anti-human IgM antibodies. During testing, the specimen diluent and the specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to Rubella, it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-Rubella IgM antibody complexes. If the specimens do not contain IgM antibodies to Rubella, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated Rubella antigens are added to the microwell plate and then incubated. The enzyme-conjugated Rubella antigens will bind to the immobilized anti-human IgM antibody-Rubella IgM antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of Rubella IgM antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of Rubella IgM antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives which were found to be non-reactive for the HIV-1/HIV-2/HIV-O, Syphilis and HCV antibodies, as well as HBsAg. But no known test method can offer complete assurance that products derived from human blood will not

transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.

- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate, Calibrators and Controls. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5 M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The Rubella IgM EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity		
			96 wells/kit	480 wells/kit	48 wells/kit
	Rubella IgM Microwell Plate	Microwell plate coated with anti-human IgM antibodies	1 plate (96 wells/plate)	5 plates (96 wells/plate)	1 plate (48 wells/plate)
1	Rubella IgM Conjugate	Purified Rubella antigens bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL

2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 50 mL	5 x 50 mL	1 x 25 mL
2A	Specimen Diluent	Tris buffer; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
5	Stop Solution	0.5 M Sulfuric acid	1 x 8 mL	5 x 8 mL	1 x 4 mL
6	Rubella IgM Negative Control	Diluted human serum non-reactive for Rubella IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
7	Rubella IgM Cut-Off Calibrator	Diluted human serum weakly reactive for Rubella IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
8	Rubella IgM Positive Control	Diluted human serum highly reactive for Rubella IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
	Plate Sealers		3	15	3
	Package Insert		1	1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL for 96 wells/plate testing, or 625 mL for 48 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent) • Add 100 µL of Cut-Off Calibrator in wells D1 and E1. (Blue Reagent) • Add 100 µL of Positive Control in wells F1 and G1. (Red Reagent)	• B1 and C1: Add 100 µL Negative Control • D1 and E1: Add 100 µL Cut-Off Calibrator • F1 and G1: Add 100 µL Positive Control
2	• Add 100 µL of Specimen Diluent to assigned wells starting at H1. (Green Reagent) • Add 5 µL of specimen to assigned wells starting at H1. Then a color change from green to blue will occur to verify that the specimen has been added.	• Starting H1: Add 100 µL Specimen Diluent • Starting H1: Add 5 µL specimen

3	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
4	• Remove the Plate Sealer. • Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
5	• Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µL of Conjugate to each well except for the Blank well
6	• Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
7	• Repeat Step 4.	• Repeat Step 4
8	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
9	• Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min
10	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
11	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Negative Control, Cut-Off Calibrator, and Positive Control by referring to the table below.

Example of Cut-Off Calibrator Calculation

Item	Absorbance
Cut-Off Calibrator: Well D1	0.250
Cut-Off Calibrator: Well E1	0.260
Total Absorbance of Cut-Off Calibrator	0.250 + 0.260 = 0.510
Mean Absorbance of Cut-Off Calibrator	0.510/2 = 0.255

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm.
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Cut-Off Calibrator	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150 and < 0.450
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 0.500

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Cut-Off Calibrator. See an example of Cut-Off calculation below.

Item	Absorbance
Blank Absorbance: Well A1	0.001
Cut-Off Value: Mean Absorbance of Cut-Off Calibrator – Blank Absorbance	0.255 - 0.001=0.254

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the results by referring to the Interpretation of Results table below.

Item	Absorbance
Specimen: Well H1	0.812
Cut-Off Value	0.254
Index Value: Specimen/Cut-Off Value	0.812/0.254 = 3.197

Interpretation of Results - Qualitative

Results	Qualitative
	Index Value
Negative	< 0.9
Positive	> 1.1
Equivocal*	≥ 0.9 and ≤ 1.1

***NOTE**: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

LIMITATIONS

1. The Rubella IgM EIA Test Kit is used for the detection of IgM antibodies to Rubella in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.
4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a Positive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The Rubella IgM EIA Test Kit has correctly identified specimens of a mixed titer performance panel (PTR201, Boston Biomedica Inc). It has also been compared to a leading commercial Rubella IgM MEIA test using clinical specimens. The results show that the clinical sensitivity of the Rubella IgM EIA Test Kit is 93.5%, and the clinical specificity is 96.8%.

Rubella IgM EIA vs. Other MEIA

Method		Other MEIA		Total Results
	Results	Positive	Negative	
Rubella IgM EIA	Positive	43	3	46
	Negative	3	92	95
Total Results		46	95	141

Clinical Sensitivity: 93.5% (82.1-98.6%)*
Clinical Specificity: 96.8% (91.1-99.3%)*
Overall Agreement: 95.7% (91.0-98.4%)*
*95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 15 replicates of three specimens: a low positive, a medium positive and a high positive.
Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive and a high positive. Three different lots of the Rubella IgM EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	1.024	0.073	7.100	1.040	0.075	7.211
2	2.105	0.127	6.033	1.949	0.105	5.387
3	4.316	0.393	9.106	4.611	0.308	6.680

Interferences and Cross-Reactivity

Interferences are not observed up to concentrations of 0.6 mg/mL Oxalic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin, 1%

Methanol, and 1% Ethanol. Rheumatoid factors do not interfere with the test.
Cross-Reactivity are not observed in Syphilis, HBsAg, HIV, HCV, HCG, HSV1 IgG, HSV2 IgG, Toxo IgG, Rubella IgG, CMV IgG and CMV IgM positive specimens.

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Index of Symbols

	Consult instructions for use	Σ	Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	EC REP	Authorized Representative
Rubella IgM	Rubella IgM	LOT	Lot Number	2°C 8°C	Store between 2-8°C
Wash Buffer 25x	Wash Buffer (25x)	Substrate A	Substrate A	Substrate B	Substrate B
Calibrator Cut-Off	Cut-Off Calibrator	Conjugate	Conjugate	Control +	Positive Control
Microwell Plate	Microwell Plate	Control −	Negative Control	REF	Catalog #
Specimen Diluent	Specimen Diluent	Plate Sealer	Plate Sealer	Package Insert	Package Insert
		Stop Solution	Stop Solution		

ACON Laboratories, Inc.
10125 Mesa Rim Road,
San Diego, CA 92121, USA

CE 0123

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Number: 1150738902
Effective date:2015-07-31

Questionnaire for requirement of Master degree

Sudan University of Science and Technology, Khartoum

Faculty of Medical Laboratory Science - Department Of Microbiology

Prevalence of rubella virus among pregnant women

Khartoum state

ID number ( ) Date of collection.....

Name of patient.....

Age: ( )

Residence.....

Pregnant women

Gestational stage…….

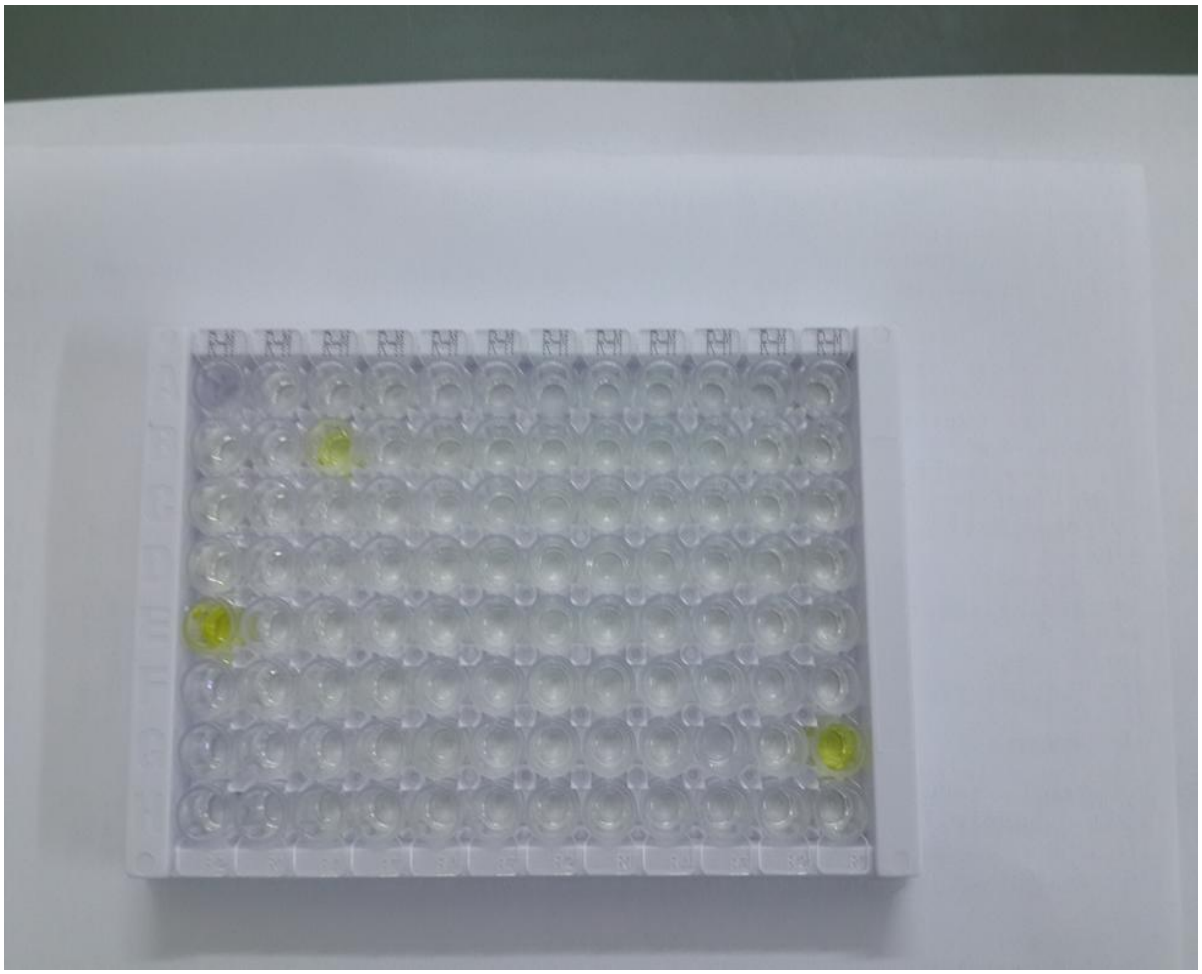
History of abortion.....

Laboratory diagnosis:

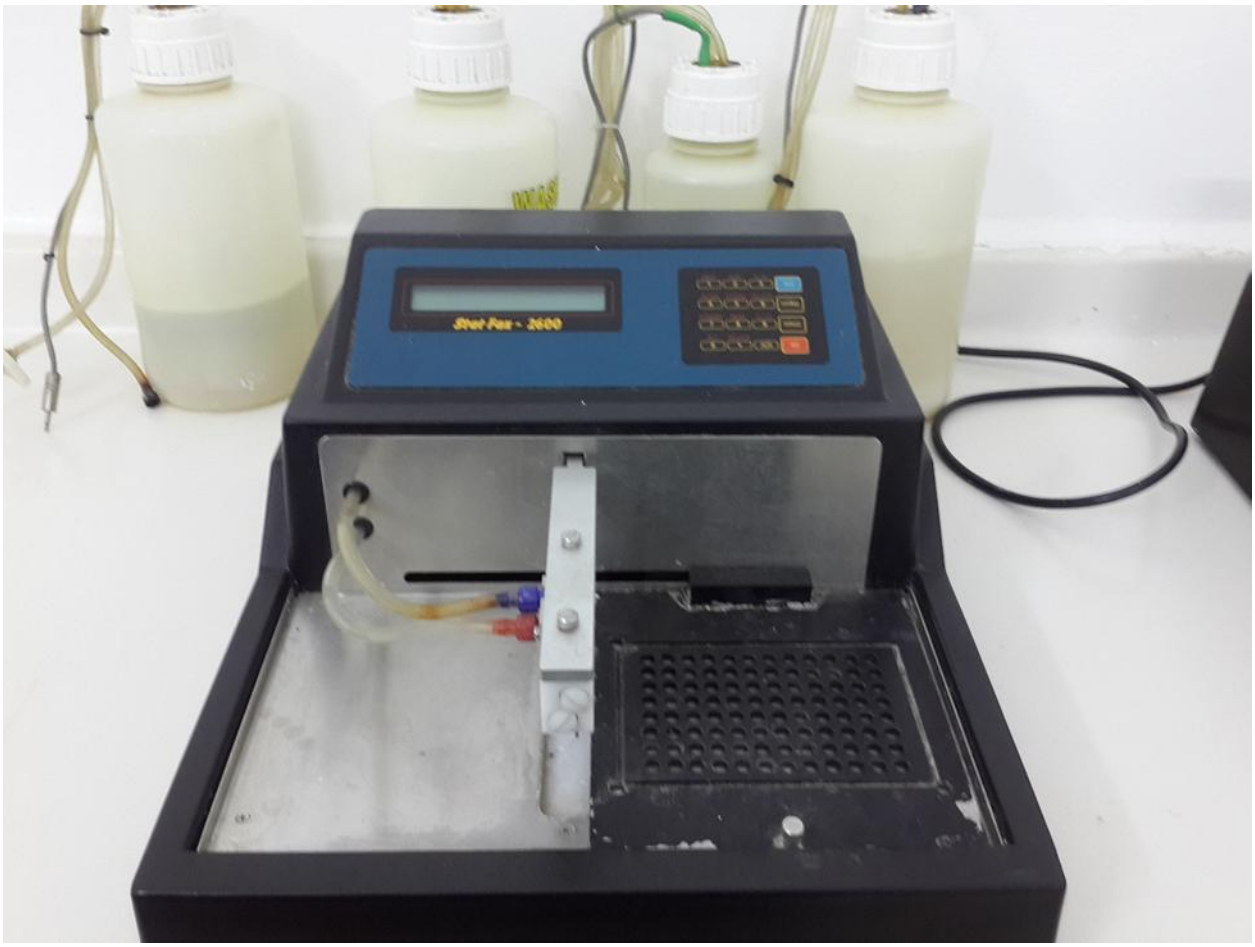
ELISA Result:

ELISA IgG ( ) IgM ( )

Micro titer EISA plate



ELISA Washer



ELISA Reader

Incubator