**Sudan University of Science and Technology**

**College of Graduate Studies**

# Sero-detection of Parvovirus B19 among Apparently Healthy Pregnant Women Attending Al Gadarif Teaching Hospital, Al Gadarif State

**الكشف المصلي للفيروس الصغير ب 19 وسط النساء الحوامل السليمات ظاهرياً اللآئي يترددن مستشفى القضارف التعليمي بولاية القضارف**

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# DEDICATION

إلي روح أبي الطاهرة

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First of all, thanks to **ALMIGHTY ALLAH** for blessing me with good health, strength and patience to carry out and complete this research work.

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## ABSTRACT

Parvovirus (B19V) infection in pregnant women is more common and major cause of clinical manifestation during the second trimester, and incidence of B19V infection is known to be main cause of outbreak during the period of winter .

This is a descriptive, cross- sectional hospital based study was aimed to detect B19V among apparently healthy pregnant women attending Al Gadarif Teaching Hospital, during the period from February 2020 to February 2021.

A total of 94 (n=94) blood specimens were collected to detect the presence anti-B19V IgM and IgG antibodies by Enzyme linked immune sorbent assay.

The participants ages ranged from 16 to 45 years with mean age of 31±7.5 SD

In relation to age groups; there were 3/5(60%) positive for anti-B19V IgG antibodies in age group 16-27 years, 2/5(40%) were positive for anti-B19V antibodies in age ranged from 36-45 years. There was no significant association between age and B19V infection (*P*=0.661).

About trimester; there were 3/5(60%) in second trimester, 1/5(20%) in the first trimester and 1/5(20%) in third trimester were found positive for anti-B19V antibodies. There was no statistical association between B19V infection and trimester (*P*=0.888).

In this study noted that; the highest frequency of anti-B19V antibodies among women with sickle cell anemia (3/5(60%)), lower in patients with thalassemia and iron deficiency anemias (1\5(20%) for each separately) and not detected among normal hematological status category. There was significant association between hematological status and B19V infection (*P*=0.001).

Furthermore; there were 4/5 (80%) had previous miscarriage and positive for anti- IgG-B19V and 1/5 (20%) hadn't previous miscarriage and positive for anti-B19V. There was significant relationship between previous miscarriage and B19V infection (*P*=0.001).

This study concluded that; anti- IgG-B19V and antibodies was detected in few apparently healthy pregnant women attending Al Gadarif Teaching Hospital.

## ملخص الأطروحة

تعد الإصابة بفيروس بارفو الصغير(ب19) في النساء الحوامل أكثر شيوعًا وسببًا رئيسيًا للمظاهر السريرية خاصة خلال الثلث الثاني من الحمل، ومن المعروف أن الإصابة بعدوى ب 19 هي أكثر شيوعاً خلال فترة الشتاء. هدفت هذه الدراسة الوصفية المقطعية المستعرضة المستندة إلى المستشفى للكشف عن عدوى فيروس ب19 بين النساء الحوامل السليمات ظاهرياً اللآتي يترددن على مستشفى القضارف التعليمي، خلال الفترة من فبراير2020 إلى فبراير2021. تم تضمين 94 (ن = 94) حامل سليمة ظاهرياً، وتراوحت أعمارهن من 16 إلى 45 عامًا بمتوسط عمر 31 ± 7.5 إنحراف معياري

لم يتم الكشف عن الأجسام المضادة من النمط IgM (0٪) بين المنتسبات في الدراسة ولكن كان هناك 94/5 (5.3٪) توجد لديهن أجسام مضادة من النمط IgG.

فيما يتعلق بالفئات العمرية؛ كانت هناك 5/3 (60٪) حالات موجبة للأجسام المضادة لـفيروس ب19 IgG في الفئة العمرية 16-27 سنة، 5/2 (40٪) كانت موجبة للأجسام المضادة لـفيروس ب19 في العمر تتراوح بين 36-45 سنة. لم يكن هناك إرتباط بين العمر وعدوى فيروس ب19 (القيمة الاحتمالية = 0.661). حول الفصل من الحمل كان هناك 5/3 (60٪) في الثلث الثاني من الحمل، و5/1 (20٪) في الثلث الأول من الحمل و1 /5 (20٪) في الثلث الثالث أعطو نتيجة إيجابية للأجسام المضادة لفيروس ب19. لم يكن هنالك إرتباط إحصائي بين عدوى فيروس ب19 ومراحل الحمل (القيمة الاحتمالية = 0.888). في هذه الدراسة لوحظ أنَّ؛ أعلى تواتر للأجسام المضادة لـفيروس ب 19 بين النساء المصابات بفقر الدم المنجلي (5/3 (60٪))، أقل في مرضى الثلاسيميا وفقر الدم بعوز الحديد ((1\ 50 (20٪) لكل منهما على حدة) ولم يتم إكتشافه بين فئة الحالة الدموية الطبيعية. كان هناك إرتباط بين الحالة الصحية الدموية وعدوى فيروس ب19 (القيمة الاحتمالية = 0.001). علاوةً على ذلك، كان هناك 5/4 (80٪) لديهن إجهاض سابق وأعطو نتيجة إيجابية لمضاد فيروس ب19 و5/1 (20٪) لم يكن لديهن الإجهاض السابق وإيجابيات للأجسام المضادة لفيروس ب19. كانت هناك علاقة معنوية بين الإجهاض السابق وعدوى فيروس ب 19 (القيمة الاحتمالية = 0.001).

خَلُصت هذه الدراسة إلى أنّ فيروس ب19 يوجد في عدد قليل من النساء الحوامل السليمات ظاهرياً اللآتي يترددن على مستشفى القضارف التعليمي.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| APCs | Antigen Presenting Cells |
| B19V | Parvovirus B19 |
| BCR | B cell Receptor |
| ELISA | Enzyme Linked Immunosorbent Assay |
| HF | Hydrops Fetalis |
| HLA | Human Leukocyte Antigen |
| IDA | Iron Deficiency Anemia |
| JIA | Juvenile Idiopathic Arthritis |
| NHS | Normal Hematological Status |
| ORFs | Open Reading Frames |
| PCR | Polymerase Chain Reaction |
| RA | Rheumatoid Arthritis |
| RBCs | Red Blood Cells |
| SPSS | Statistical Package for Social Science |
| TCR | T Helper Cells Receptor |
| WHO | World Health Organization |

# CHAPTER I

# 1. I NTRODUCTION

## 1.1.Introduction

Parvovirus B19 (B19V) is small, non-enveloped, single strand DNA virus and according to natural committee taxonomy of viruses it has been classified as a member of Parvoviridae family, genus Etrythrovirus (Maria *et al*., 2017).

However, B19V is consider to be global health problem affecting more than 60% of world population and epidemic outbreak occur most of the time in drying season (Zajkowska *et al*., 2015).

It is known as causative agent of Erythema infectiosum (fifth disease) which is a clinical manifestation of B19 infection during childhood (Brook *et al*., 2013).

The clinical manifestations of B19V infection among immunocompetant adults are vary according to infected individual's age, immunological status and hematological status (Gilber *et al*., 2004). So, it can be mild and self-limiting, but sever clinical manifestation usually occur when B19V infect pregnant women (Mirambo *et al*., 2017).

However, pregnant female how suffering from hematological disorders such as sickle cell anemia or thalassemia are more susceptible to B19V infection usually with poor pregnancy outcome (Bihatia *et al*., 2008 )

Prevalence of pregnant women infection caused by B19V are above 10% in epidemics period over the world (Zajkawska *et al.*, 2015). Moreover, more than 50% of African pregnant women are suffering of primary B19V infection (Elnifro *et al*., 2009).

B19V infection acquired or reactivated during pregnancy usually resulting in significant proportion of mortality and morbidity for both mother and infant (Zajkawska *et al.*, 2015).

Transmission of B19V from infected pregnant women to the embryo which she is carrying most through vertical route most of time occur when B19V infection acquired in the first three month of pregnancy, this rout of transmission resulting in mortal complication known as Hydropsfetalis (Mcmurrayh *et al*., 2017).

Third of viral infected pregnant women are lost their fetus due to B19V infection and miscarriages occur when B19Vinfection acquired in the first and second trimester (Mirambo *et al*., 2017).

The outcome among pregnant women is vary from asymptomatic infection to sever morbidity course which are differ from abortion and stillbirth to a fetal complications such as chronic anemia. However, those complications often controlled by infected female's age and time of pregnancy that B19V infection acquired (Enders *et al*., 2006).

## 1.2. Rationale

Because B19V are epidemic in most of the world. Infection course among pregnant female always result in a poor pregnancy outcome and high ratio of mortality and morbidity (Brooks *et al*., 2013).

More than 3% of pregnant women acquire B19Vinfection during their pregnancy (Mcmurrayh *et al*., 2017).

Infectious disease such as B19V infection during pregnancy and their epidemiological situation, infection outcome and infection among pregnant women are not accurate reported (Maria, 2017).

Concerning the above mentioned data, this study was designed among pregnant women attending Al Gadarif Teaching Hospital, Al Gadarif State and the obtained data may help to determine the frequency of B19V infection and the possible risk factors that may enhance the susceptibility of B19V infection. So, thus in order to setting a control approach to reduce the frequency of B19V infection and to minimize the severity of the clinical manifestations resulting from B19V infection during pregnancy.

## 1.3. Objectives

### 1.3.1.General objective

To detect B19V serologically among apparently healthy pregnant women attending Al Qadarif Teaching Hospital, Al Gadarif State.

### 1.3.2. Specific objectives

1.To detect anti-B19V IgM antibodies among pregnant women by (ELISA) .

2.To detect anti-B19V IgG antibodies among pregnant female by Enzyme linked immune sorbent assay (ELISA) technique.

3.Todeterminethe possible risk factors (e.g. age, trimester, previous miscarriage and hematological status) associated with B19V infection.

# CHAPTER II

# 2. LITRETURE REVIEW

## 2.1. Parvovirus B19

Parvovirus B 19V is a common causative agent for many human diseases and syndromes. However, the first time B19V has been described in 1975 by Cossart, since in a serum of healthy blood donor, the B19 name was originates from the coding of a serum sample, which was coded panel B number 19 (Norbeck *et al*., 2017).

Moreover, B19V virus is known to cause epidemics and pandemics out break among the world (Bernstein *et al*., 2012) affecting more than 60% of the world population (Challis *et al*., 2009). Although most of B19V infection are asymptomatic it has been reported to cause human death (Giorgio, 2010).

### 2.1.1. Classification

The B19V strains belonging to family Parvoviridae which display extensive genetic diversity (Cohen *et al*., 2015).

A taxonomic scheme was proposed to classify Parvoviridae family into tow subfamily which are Denovirinae which infect insect and Parvovirinae which infect vertebrates (Challis *et al*., 2009).

However, Parvovirinae subfamily comprise three genera according to their ability to efficiently replicas to (Dependovirus, Parvovirus, Erythrovirus). Although denpendoviruss has reported to cause a wide range of infection among mammalian, but no evidence of causing human infection (Woolf *et al*., 2015).

Parvovirus B19 was first classified as a member of parvovirus genera, but because replication only occurs in erythrocytes precursors, now B19V is classified as a member of erythrovirus genera (Zaki, 2008).

Three different B19V genotype have been recognized, with DNA variation of 1%-2%

However, those three genotypes are: genotype1, (prototype) genotype (LaLi-like) and genotype three (V9-like) In overall sequence those different genotypes are differ from each other by 10% (Mirambo *et al*., 2017).

However the prevalence of genotype 1 are higher than genotypes (1and 2) in western countries, genotypes (1and 2) has been reported to cause outbreaks in west of Africa and south America (Jonetzko *et al*., 2005).

### 2.1.2.Structure

Parvovirus B19 is the smallest non-enveloped ssDNA of 22-24 nm in diameter, with icosahedral nucleocapsid symmetry, which usually contain 60 capsomer (Singh, 2014).

Tow structural protein capsomers are represented the coat protein, those proteins are known as capsid protein, this capsid is encoded by over lapping of tow capsomers proteins which are (VP1 and VP2) (Jensen *et al*., 2011).

VP2 capsomer is accounting for more than 90% of total capsid protein with molecular mass 58, moreover, it recognized that VP1 capsomer is identical to VP2 capsomer with additional ≈ 220 amino acid presenting about 5% of total capsid protein with molecular mass 48 (Dickihson *etal*., 2006).

Although, structural protein are presenting a majority of B19V component, but non-structural protein are play a major role in B19V biological functions. Non- structural protein such NSI is not well and fully characterized, but it thought to possess sit-specific DNA-binding, DNA nicking, helices activities and transcription functions (Nicolay and Cootter, 2009). Moreover, it seems that NSI is resulted of transcription of a highly conversed nucleoside (triphosphate binding) which allow the NSI to play important role in different biological functions (Gilbert *et al*., 2005).

The liner ss DNA genome of B19V measuring 5kb, and containing about 5500 nucleotides, and the B19V genetics material is believe to package both negative DNA strand and positive DNA strand (Kelly *et al*., 2015).

The genome nucleotides are composed of an internal coding sequences which is flanked by terminal plandromic repeated sequence (Abiodun *et al*., 2013).

The B19V genome has two large open reading frames(ORF), it thought that the non-structural proteins encoded in by genes on the left side of B19V genome, and the tow capsid capsomer proteins (VP1 and VP2 ) are encoded in by on the right side of B19V genome (Woolf *et al*., 2015).

### 2.1.3. Replication

Because B19V cannot stimulate resting cells to initiate DNA synthesis so, it must infect only dividing cells, the replication mainly during the S phase (Watt *et al*., 2013) however, only primary erythroid progenitors in the bone marrow are known to be permissive for B19V infection (Elnifor *et al*., 2009).

The B19V life cycle is very much resembling those other non-enveloped DNA viruses. The replication steps are including B19V attachment to host cell via B19V

receptors, internalization of B19V inside the host cell, translocation of B19V genome to host cell nucleus, those steps are followed by B19V DNA replication and RNA transcription finally assembly of capsid and packaging of genome take place (Nicolay and Cootter, 2009). The attachment of B19V to the host is conducted by B19V agglutinin which thought to be engaged with erythroid progenitor receptor, the agglutinin is bind to Gb4cer (globoside) receptor and Ku80 co-receptor on the surface of erythroid progenitor cell (Haan *et al*., 2007). The receptor α5β1 is required in order to facilitate the internalization of B19V process via endocytosis, B19V replication is resulting lyses of host cell which lead to cell death (Maria *et al*., 2017).

**2.1.4. Epidemiology and transmission**

B19V is a global and common infectious pathogen in human, actually more than 15% of children become anti B19V IgG seropostive by the age of five and more than 60% are anti B19V IgG seropositive (Ballow *et al*., 2003). Moreover, incidence of recent infection are high among pregnant female, it have been reported that B19V can detected in blood of 1.5% of pregnant women with possible chance that pregnant women B19V infection has been acquired from pregnant women older children. Individual with hematological disorder such as sickle cell anemia and thalathemia are also as one of vulnerable population to B19V infection (Watt *et al*., 2013).

Incidence are also high among school teacher and individual how with close contact with children (Miller *etal* .,2016).

B19V infection occurs throughout the year as sporadic cases or outbreak, a seasonal change occurs in during late winter and early spring which usually appear as increased number of B19V clinical manifestation among children, epidemics outbreak may occur every 36 to 48 months( Nigro *etal*., 2000).

Transmission of B19V infection occur either through respiratory route, vertical transmission or prenatal route and respiratory route is the most common transmission route among the community (Watt *et al*., 2013), however, vertical transmission occur when B19V is transmitted from infected mother to fetus which she is carrying, this mode of transmission occur in one-third of anti B19V IgM seropostive pregnant women(Miller *etal* .,2016).

prenatally transmission occur when blood, blood product or bone marrow is transmitted from infected donor to non B19V infected recipient (Ballow *et al*., 2003).

Nosocomial acquired infection also been described frequently, it seems that the main source of B19V infection is patient suffering of a plastic crisis (Norbeck *et al*., 2017).

### 2.1.5. Pathogenisity

Reports has been described B19V as the causative agent for several disease with different clinical feature such as non- immune hydrous fetalis, transit a plastic crisis, erythema infectiosum (fifth disease), hepatitis, arthritis, and myocardities, most of this diseases are due to affinity of B19V to erythroid progenitor in the bone marrow. However it seems that B19V is own receptors for different type of cell including hepatic cell and cardiac cell (Haan *et al*., 2007).

After all 25% of B19V infected patients of both children and immunocompetant individual undergo a symptomatic infection (Schenk *et al*., 2009).

#### 2.1.5.1. Erythema infectiosum

Erythema infectiosum is the most B19V clinical manifestation, especially among children at their age from 4 years to 6 years. This disease initially presented as flu-like illness, prodromal symptoms appear after 14 days of infection acquired which is usually include mild fever, headache and nausea, those symptoms are followed 4 days later by slapped cheek rash that spread to trunk and limbs after 7 to 10 days of appearance of prodromal symptoms ( Nigro *et al*., 2000).

#### 2.1.5.2.Transient a plastic crisis

This syndrome occur when B19V attack red blood cell lines in the bone marrow causing RBCs cell membrane damage and cell plasia. This disease onset is minimal among healthy children and healthy adult (Leisi *et al*., 2016). however, very sever and serious complications occur when B19V infect individual how suffering from hematological disorders, clinical manifestations such as spherocytosis, pyruvate kinase deficiency and auto immune hemolytic anemia may be observed (Schenk *et al* .,2009).

Chronic suppression of bone marrow is reported mainly among immunocompromised patient and immundificiant this syndrome is known as pure red cell aplasia (Leisi *et al*., 2016).

### 2.1.5.3. Myocardities

Human B19V is linked to verity of heart problem (Schenk *et al*., 2009), it is the most etiological agent of viral myocardities resulting in a high rate of morbidity and mortality especially among children (Leisi *et al*., 2016).

Post B19V infection myocardities is a common clinical manifestation resulting in dilated cardiopathy (Bock, 2010), myocardities caused by B19V usually associated with acute lymphatic infiltration of the myocardium and myocellular necrosis (Nigro *et al*., 2000) this process is resulting in increasing of intestinal and perivasal fibrosis (Schenk *et al*., 2009)

Although B19V DNA has been detected in pericardium fluid and heart biopsy of but it seems that B19V doesn't infect cardiocytes so that the symptoms are believed to be caused due to immunological cross-reaction to epitopes that is shared between B19V and myocardium (Molina *et al*., 2013). Clinical manifestation caused by B19V such as Myocardities usually lead to heart failure due to myocellular damage (Brook *et al*., 2013).

### 2.1.5.4. Arthopathy

A previous study reported that; B19V DNA has been detected in synovial fluid and synovioum of more than 50% of individuals how suffering of arthopathy, infection caused by B19V may only presented as arthritis especially among middle age female (Takahashi *et al*.,2015).

However, rheumatoid arthritis (RA) is the most common joint manifestation presenting 90% of total B19V infected individuals, it seems that B19V targeting follicular dendritic cells other than synovial lining cell in the synovioum (Silasi *et al*., 2015).

Persistence B19V infection trigger RA factors resulting in non-septic arthritis, furthermore the persistence status vas infection resolution is believed that is conducted by HLA typing (Weissbrch *et al*., 2007).

Moreover, other related joint clinical manifestation due to B19V infection is reported such as Juvenile idiopathic arthritis (JIA), this syndrome is mainly effect population during the childhood period (Weissbrch *et al*., 2007).

### 2.1.5.5. Hydrops fetalis

Hydrops fetalis (HF) is defined as abnormal accumulation of fluid in interstitial space in at least of two or more compartment of fetal torso (peritoneal cavity, pleural, pericardium), in the normal status continuous circular movement between the lymph

and interstitial space in balance, a defect in this balance usually either resulted in local disorder or may developed into even more serious manifestation such as HF (Jonetzko *et al* .,2005)

Many studies presented the relation between B19V infection and HF, although HF is relatively rare disorder which caused by different etiological agents. Studies presented that B19V is responsible of more than 10% of this syndrome (Xu *et al*.,2003).

Moreover, it seems that fetus is affected when B19V infection acquired between the 11 week and 23 week of Pregnancy, HF start developing after 10 days of maternal infection (Bostic *et al*., 2000).

Fetus infected by B19V show sonogrophic sings or generalized edema with ascites and placental edema, hypnosis suggest that infecting and propagating of B19V with in the erythroid progenitor causing them lysed, this mechanism lead to severe anemia that resulting in hypoxia and carcinogenic heart failure (Bostic *et al*., 2000).

**2.1.5.6. Hepatitis**

Liver disease is one of clinical manifestation of B19V infection, the out com of such manifestation caused by B19V is differ from elevation of transaminase to more serious disease course such as acute hepatitis, fulminate liver failure and chronic hepatitis, hepatitis occur in more than 4% of B19V infected individual (Jonetzko *et al* .,2005)

However, acute and fulminate hepatic failure due to B19V infection mostly reported in children, moreover, in adult manifestation is seem to be less sever with exception of two categories which are immunocompromised and immundificiant patients (Bihatia *et al*., 2008).

Although most of B19V infection complication and manifestation are mainly depend upon patient hematological status, the outcome of liver disease doesn't appear to be correlate with under lining hemolytic abnormalities (Bernstein *et al*., 2011).

Chronic hepatitis caused by B19V is most common in individual suffering of lymphopenia, persistence status of B19V infection and chronic hepatitis is strongly linked to with extent of liver involvement, the severity of B19V chronic hepatitis is correlated with presence and absent of other hepatic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV) (Elnifor *et al*., 2009).

### 2.1.6. Immunity against B19V

Adaptive immunity play a vital role in immunity against B19V infection (Bernstein *et al* .,2011).

Both cellular and humeral immunity is involved in clearance of B19V infection in order to active CD4 helper T cells, and the antigen presenting cells (APCs) such as (dendritic cells and macrophages) present B19V epitopes to CD4 helper T cells in the primary lymphoid organs, the HLA class 11 present B19V epitopes to CD4 helper T cells T helper cell receptor (TCR) which expressed on the surface of CD4 helper T cells (Jonetzko *et al* .,2005)

Naïve B cells in the germinal center of secondary lymphoid organ undergo interaction with follicular dendritic cells, B cell expressed B cell receptor (BCR) on the cell surface, the BCR is mint to recognized B19V which displayed by follicular dendritic cells, engagement of BCR to B19V is mainly conducted by HLA class 11 which expressed on cell surface of dendritic cells. Those previous steps are followed by migration of B cells from the germinal center to T helper zone in order to be activated by CD4 T helper cells (Giorgio *et al* .,2010).

B cell activation signal is mediated by CD4 T helper cells. The provide signals is responsible of proliferation of B cell and from differentiation of B cell into either plasma cell which responsible of antibodies synthesis or differentiated into memory cells (Bostic *et al*., 2000).

Antibodies generated from plasma cell which matched B19V epitopes are aimed to neutralized B19V partials, this mechanism is known to be the dominant mechanism of clearing of B19V infection from the human body (Bihatia *et al*., 2008).

### 2.1.7. Laboratory diagnosis

#### 2.1.7. 1.Serology

Serological test are consider as reliable diagnose toll regarding detection of B19V infection, serological test can be performed by detecting anti-B19V (IgM-IgG) antibodies by using technique such as ELISA (Mestrovic *et al*., 2016)

Anti-B19V-IgM antibodies can be detected in circulation of B19V infected individual after 7-10 days of infection, more ever those antibodies can be detected in B19V patient plasma even after six month of infection resolution (Maria *et al*., 2017).

However, immune globulin G consider as indicator for previous exposure for B19V infection, comparing with IgM, IgG consider as slow rising, retching the pick after two to four weeks after acquiring the infection, moreover, immune globulin G can be

detected in B19V patient circulation tell one years after infection resolution (Mestrovic *et al.*, 2016).

**2.1.7.2. detection of B19V –DNA**

Detection of B19V–DNA in patient specimen consider the most sensitive and specific method to diagnose B19V recent infection, however this method is consider the most reliable method to diagnose B19V infection among immunocompromised patients, AIDS patient and pregnant women. However. This method can be performed using PCR technique (Maria *et al*., 2017).

**2.1.8. Treatment**

No antiviral drugs are mint to treat B19V infection, however, pooled immunoglobulin G is effective treatment for B19V persistent infection (Heegard and Brown, 2002).

Spontaneous resolution of infection is reported in most of cases. Pooled Intravenous Immunoglobulin G (IVIG) has been approved as effective treatment for B19V persistent infection in 66% of immune competent patient (Crane *et al*., 2014).

### 2.1.9. Vaccine

Although vaccine for B19V is continuously developed, but no vaccine is currently approved, recombinant human B19V vaccine (MEDI-491), this vaccine is composed of VP1 and VP2 proteins, those capside proteins carrying multi B19V epitopes that are able to stimulate immune system (Giorgio *et al* .,2010).

The MEDI-491 vaccine has successfully passed phase one and phase two of the clinical trial, its safety and immunogenicity has been ensured, all volunteers has developed neutralizing antibodies that were sustained through the study (Heegard and Brown, 2002).

Moreover, formulated (MEDI-491) with aluminum hydroxide was found to be cross phase one of the trail in doses1, 3, 10, 30, and 100, Mg in the healthy adult, however this vaccine is seem to be poorly immunogenic due to its falling to induce neutralizing antibodies (Crane *et al*., 2014).

## 2.2. Pregnancy

Pregnancy is term use to describe a fetus develops inside women uterus (Giorgio *et al* .,2010). Pregnancy is divided into three trimesters, three months for each trimester.

Many physiological changes occur in the period of pregnancy, comparing pregnant women by non-pregnant women: represented that pregnancy could be a major risk for infectious disease (Kaufmann *et al*., 2004).

Over 23 million of pregnancy are reported around the world and poor pregnancy outcome has been reported in about15% of pregnancy cases such as spontaneous pregnancy loss and still birth (Giorgio *et al* .,2010).

### 2.2.1.Viral infection during pregnancy

Viral infection during pregnancy has always consider as global health problem regarding the serious squeal that lay due such crisis (Heegard and Brown, 2002).

Last viral epidemics and pandemics outbreak has presented the vulnerability of pregnant women to viral infection, physiological and hormonal alteration that occur during pregnancy are responsible of immunological change that occur during pregnancy period (Nigro *et al*., 2000).

Immunological alteration such as suppression of T cell-mediated immunity is one of variable that facilitated viral infection during pregnancy (Ballow *et al*., 2003).

Hormonal changes such as that which occur in estrogen and progesterone level also seem to play a major role in alteration of immune system regulation and increase viral replication (Weissbrich *et al*., 2007).

Virus which own placenta receptor may invade those anatomical structure usually through hematognous rout (Haan *et al*., 2007).

Although most of viral infection seem to be mild and self-limiting among immunocompetant adults, but different squeals and complications are reported among pregnant women, a significant proportion of mortality and morbidity in both mother and her fetus are suggested to be caused due to viral infection (Schenk *et al*., 2009).

### 2.3. Previous studies

Zajkawska and his colleagues in (2015) in Bialystoh, found that; 26% (52\200) of pregnant women how participate in their study were anti-B19V positive.

In 2009 Elnifro and other in Tripoli, Libya found that ; 61% of pregnant women were positive for anti-B19V-IgG antibodies and 5% were positive for anti-B19V-IgM

Of women under study.

Adam *et al* (2015) in Khartoum, Sudan found that 57% of women were positive for B19V infection.

# CHAPTER III

## 3. MATERIALS AND METHODS

### 3.1 .Study design

This is descriptive, cross-sectional, hospital based study**.**

### 3.2 .Study area

This study was conducted in Al Gadarif Teaching Hospital, Al Gadarif State.

### 3.3 .Study duration

The study was carried out between February 2020 to February 2021.

### 3.4 .Study population

Pregnant women hwo attended Al Qadarif Teaching Hospital .

### 3.4.1.Inclusion criteria

Pregnant women with different age groups, race and educational level.

### 3.5 .Ethical considerations

Ethical approval to conduct this study was obtained from the Scientific Research Committee, Collage of Medical Laboratory Science, Sudan University of Science and Technology and Health Services Director in Al Gadarif Teaching Hospital. Consent was obtained from participants before collection of blood specimens (appendix-1.(

### 3.6 .Sample size

The total sample size was 94 (n=94) blood samples.

### 3.7 .Sampling technique

Non-probability, convenience sampling technique.

### 3.8 .Data collection

Data were collected through direct interview with each participant and Questionnaire was used as interview instrument (appendix-2.(

### 3.9 .Collection of blood specimens

After disinfection of vein puncture area, 3ml of venous blood withdrawal from each participant by vein-puncture technique. Specimens were allowed to clot then separation of serum was done using centrifuge apparatus at 3000 rpm for 10 minutes and separated sera was stored at -20˚C until performance of ELISA .

### 3.10 .Enzyme Linked Immunosorbent Assay (ELISA(

#### 3.10.1 .Detection of anti- B19V(IgM) antibodies

Indirect ELISA was used in order to detectanti-B19V–IgM antibodies which is recommended to detect B19V recent infection.

#### 3.10.1.1.Procedure

All samples and reagents were allowed to reach room temperature. The procedure was carried according to guidance of manufacturing (EUROIMMUN, Germany). In which 100µl of diluted patient's serum, calibrator, positive and negative controls were dispensed into microplate which was coated with B19V antigen. After incubating at room temperature for half an hour, washing was done for three consecutive times using working wash buffer.

Then 100µl of enzyme conjugate (peroxidase-lablled anti-human IgG) was added to all wells and incubated at room temperature for half an hour. Washing was done for three consecutive times with working wash buffer. Then100µl of chromogen\substrate solution was added into each well. The immune complex was incubated at room temperature for 15 minutes .

Last of all, the reaction was stopped by adding 100µl of sulphuric acid into each well. Finally optical density was read spectrophotometrically using ELISA reader at wave length of 450 nm with reference wavelength 620 nm and 650 nm within 30 minutes of adding sulphuric acid solution.

#### 3.10.1.2 .Calculation of cut-off value

Semi-quantitatively evaluation of result was done by calculating the ratio of extinction value of each sample over extinction value of the calibrator within 10 minutes after the stopping of the reaction .

#### 3.10.1.3 .Interpretation of the results

Positive result: samples with ratio equivalent or greater than 1.0 were considered positive .

Negative result: samples with ratio less than 0.8 were considered negative

Borderline: samples with ratio more than 0.8 and less than 1.0 were retested .

#### 3.10.2 .Detection of anti- B19V(IgG) antibodies

Indirect ELISA was used in order to detect anti-B19V–IgG antibodies which is recommended to detect previous B19Vinfectionor reinfection.

### 3.10.2.1.Procedure

All samples and reagents were allowed to reach room temperature. The procedure was carried according to guidance of manufacturing (EUROIMMUN, Germeny). In which 100µl of diluted patient's serum, calibrator, positive and negative controls were dispensed into microplate which is coated with B19V antigen. After incubating at room temperature for half an hour, washing was done for three consecutive times using working wash buffer.

Then 100µl of enzyme conjugate (peroxidase-labelled anti-human IgG) was added to all wells and were incubated at room temperature for half an hour. Washing was done for three consecutive times with working wash buffer. Then100µl of chromogen\ substrate solution was added into each well. The immune complex was incubated at room temperature for 15 minutes .

Last of all, the reaction was stopped by adding 100µl of sulphuric acid into each well. Finally optical density was read spectrophotometrically using ELISA reader at wavelength of 450 nm with reference wavelength 620 nm and 650 nm within 30 minutes of adding sulphuric acid solution.

### 3.10.2.2 .Calculation of cut-off value

Semi-quantitatively evaluation of result was done by calculating the ratio of the extinction value of each sample over extinction value of the calibrator within 10 minutes after the stopping of the reaction.

### 3.10.2.3 .Interpretation of the results

Positive result: samples with ratio equivalent or greater than 1.0 were considered positive .

Negative result: samples with ratio less than 0.8 were considered negative.

Borderline: samples with ration more than 0.8 and less than 1.0 were retested.

## 3.11 .Data analysis

Data were analyzed and presented using Statistical Package for Social Science (SPSS) software version 20.0 for windows.

Frequencies were presented in form of tables and figures and significant of differences was determined using chi-square test. Statistical significant was set at *p-value* $\leq 0.05$.

# CHAPTER IV

## 4. RESULTS

A total of 94 (n=94) apparently healthy pregnant women were included in this study and their age ranged from 16 to 45 years with mean age of 31±7.5 SD. Age was divided into three groups as follows: 51(54.4%) in age group 16- 27years, 25(26.5%) in age group between 28 to 35 years and 18(19.1%) in age group from 36 to 45 years. They were in different trimester of pregnancy, in which 20(21.2%) were in first trimester, 50(53.1%) in the second trimester and 24(25.5%) in the third trimester as shown in table 4.1.

In this study; different hematological status were reported among candidates, in which there were 78/94(82.9%) with normal hematological status (NHS) , 8/94 (8.5%) were suffering from iron deficiency anemia (IDA), 5/94 (5.3%) with sickle cell anemia and 2(2.1%) had thalassemia as described in table 4.1 .

Moreover, there were 12/94(12.7%) had previous miscarriage and other hadn't as exhibited in table 4.1.

With regard to type of detected antibodies; there were no detected IgM (0%) among precipitants but there were 5/94 (5.3%) IgG as presented in figure 2 and 3 respectively figure(4.2)

In relation to age groups; there were 3/5(60%) were positive for anti-B19V IgG antibodies in age group 16-27 years, 2/5(40%) were positive for anti-B19V antibodies in age ranged from 36-45 years and 0(0%) in age group from 28 to 35 years. There was no significant association between age and B19V infection (*P*=0.661) as explained in table (4.2.)

Furthermore; there were 4/5 (80%) had previous miscarriage and positive for anti-B19V and 1/5 (20%) hadn't previous miscarriage and positive for anti-B19V. There was significant relationship with previous miscarriage and B19V infection (*P*=0.001) (table 4.4.)

About trimester; there were 3/5 (60%) in second trimester, 1/5 (20%) in the first trimester and 1/5 (20%) in third trimester were found positive for anti-B19V antibodies. There was meningless association between B19V infection and trimester (*P*=0.888) (table 4.3.)

In this study noted that; the highest frequency of anti-B19V antibodies among women with sickle cell anemia (3/5(60%)), lower in patients with thalassemia and iron deficiency anemias ((1\5(20%) for each separately) and not detected among normal

hematological status category. There was significant association between hematological status and B19V infection (*P*=0.001) as presented in table( 4.5.)

**Table 4.1: Distribution of socio- demographic and clinical data among apparently healthy pregnant women.**

| (%Percentage ( | No. N=94 | Variable |
|---|---|---|
| **Age groups/ years** | | |
| 54.2% | 51 | 16-27 years |
| 26.5% | 25 | 28-35 years |
| 19.1% | 18 | 36-45 years |
| **Trimester** | | |
| 21.3% | 20 | First |
| 53.1% | 50 | Second |
| 25.3% | 24 | Third |
| **Hematological status** | | |
| 82.9% | 78 | NHS |
| 8.5% | 8 | IDA |
| 2.1% | 2 | Thalassemia |
| 6.2% | 6 | Sickle cell anemia |
| **Previous miscarriage** | | |
| 12.7% | 12 | Yes |
| 87.2% | 82 | No |

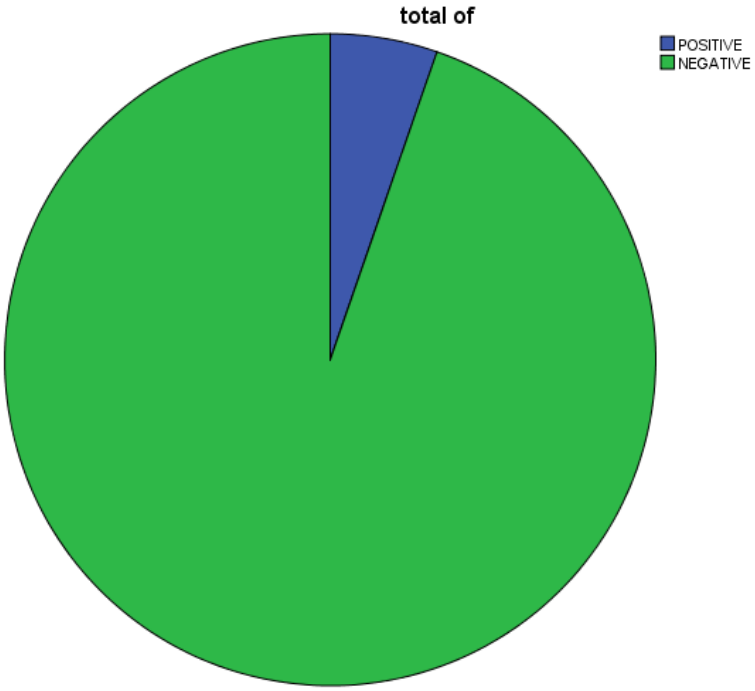


**Figure 4.2: Frequency of B19V antibodies among apparently healthy pregnant women**

**Table 4.2: Association between age groups and B19V infection among apparently healthy pregnant women**

| ***P.value*** | **Total** | **Age groups/years** | | | **Anti- B19V Antibodies** |
|---|---|---|---|---|---|
| | | 36-45 years | 28-35 years | 16-27 years | |
| 0.661 | 5(5.3%) | 2(2.1%) | 0(0.0) | 3(3.1%) | Positive |
| | 89(94.6%) | 16(17.2%) | (26.5%)25 | 48(51.5%) | Negative |
| | 94(100%) | 18(19.1%) | (26.5%)25 | 51(54.2) | Total |

**Table 4.3: Association between hematological status and B19V infection among apparently healthy pregnant women**

| ***P.value*** | **Total** | **Hematological status** | | | | **Anti- B19V Antibodies** |
|---|---|---|---|---|---|---|
| | | Sickle cell anemia | Thalassemia | IDA | NHS | |
| 0.001 | 5(5.3%) | 3(3.1%) | 1(1.0%) | 1(1.0%) | 0(0.00) | Positive |
| | 89(94%) | 3(3.1%) | 1(1.0%) | 7(7.4?%) | 78(82.9%) | Negative |
| | 94(100%) | 6(6.2\|) | 2(2.1%) | 8(8.5%) | 78(82.9%) | Total |

**Table 4.4: Association between previous miscarriage and B19V infection among apparently healthy pregnant women**

| *P.value* | Total | **Previous miscarriage** | | **Anti- B19V Antibodies** |
|---|---|---|---|---|
| | | No | Yes | |
| 0.001 | 5(5.3) | 1(1.0%) | 4(4.2%) | Positive |
| | 89(94.6%) | 81(86.1%) | 8(8.5%) | Negative |
| | 94(100%) | 82(87.2%) | 12(12.7%) | Total |

**Table 4.5: Association between the trimester of pregnancy and B19V infection among apparently healthy pregnant women**

| ***P.value*** | **Total** | **Trimester** | | | **Anti- B19V Antibodies** |
|---|---|---|---|---|---|
| | | Third | Second | First | |
| 0.888 | 5(5.3%) | 1(1.0%) | 3(3.1%) | 1(1.0%) | Positive |
| | 89(94.6%) | 23(24.4%) | 47(50%) | 19(20.2%) | Negative |
| | 94(100%) | 24(25.3%) | 50(53.1%) | 20(21.3%) | Total |

# CHAPTER V

# 5 .DISSCUSSIONS ,CONCLUSIONS AND RECOMMENDATION

## 5.1 .Discussion

In this study there were 5\94 (5.3%) apparently healthy pregnant women were anti-B19V positive which was similar to those obtained by Elnifro, *et al.* (2009) in Libya (5%) and dissimilar from those reported by Abiodun *et al.* (2013) in Nigeria (20%) and Watt *et al*. (2012) in UK (18%). The variation in results could be due to difference in sample size Also found that there were no apparently healthy pregnant women were anti-B19V IgM positive that was mismatched to that obtained by Adam *et al.* (2015) in Khartoum, Sudan (53%), and Zajkawsk *et al*. (2010) in Bialystok, Brazil (11% .(

Reason of the difference between results might of endemicity of virus, sample size and different diagnostic technique.

The frequency of anti-B19V IgG antibodies among participants was 5\94 (5.3%) which was disagreedfrom that reported by Abiodun *et al*. (2013) in Nigeria (20%) and Ziyaeyan *et al*. (2007) in Shiraz, Iran (62%). This variation may be resulted from differences in sample size.

The seropositivity of B19V is higher among pregnant women at age group 16-27 years (3\5 (60%)), 2\5(40%) of women belong to the age group 36-45 years were seropositive for B19V infection and no one found to be positive among the age group 28-35 years. However, there was no statistical association between age groups and B19V infection.

Furthermore, noted that; the high positivity rate of B19V infection among pregnant women during their second trimester (3\5(60%)), then came both the first and third trimester by 1\5(20%) for each. However, there was no statistical association between trimester and B19V infection.

Regarding to educational level; there were 3\5 (60%) women how complete their primary education, 1\5 (20%) had secondary school level and 1\5 (20%) illiterate were positive for anti-B19V antibodies. But no graduated women were positive for anti-B19V antibodies (0\5 (0.00%)). Moreover, there was a significant association between educational level and B19V infection.

About hematological status; found there were 3\5 (60%) of women were suffering of iron deficiency anemia, 1\5 (20%) had thalassemia and 1\94 (1.1%) with sickle cell anemia were positive for B19 antibodies and all normal hematological status were

negative (0\5 (0.00%). However, they was a statistical relationship between hematological status and B19V infection.

Moreover, found 4\94 (80%) of anti B19V positive antibodies had earlier miscarriages and 1\5 (20) hadn’t. Found there was a meaningful association between previous miscarriages and B19V infection.

## 5.2 .Conclusion

The finding of this study found that; the frequency of B19V infection is not high among pregnant women hwo attended Al Gadarif Teaching Hospital.

There was significant association between B19V infection and hematological status, educational level and previous miscarriages .

There was no significant relation between B19Vinfection and trimester of pregnancy and age group.

### 5.3 .Recommendations

Larger sample size with more sensitive and specific test (such as PCR) should be used in order to determine the rate of B19V infection accurately.

Screening program for B19V is highly recommended as part of routine test for pregnant women.

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**Appendix-1**

**Questionnaire**

**Sudan University of Since and Technology**

**College of Graduate Studies**

**Sero-detection of Parvovirus B19 among Pregnant Women Attending Al Qadarif Teaching Hospital, Al Gadarif State**

**ID. Number** ( )

**Age:** ( )

Second () Third ( ) **Trimester:** First ( )

**Medical history:**

**Previous miscarriage:** Yes ( ) No ( )

**Have any hematological disorders?** Yes ( ) No ( )

**If yes which type**? Sickle cell anemia ( ) thalassemia ( ) others ( )

**Laboratory investigation results:**

**ELISA results:**

Anti-B19V(IgM) antibodies: +ve( ) -ve( )

Anti-B19V(IgG) antibodies: +ve( ) -ve( )

## Appendix-1

## Informed consent

**جامعة السودان للعلوم والتكنولوجيا**

كلية الدراسات العليا

**وثيقة موافقة للمشاركة في بحث علمي**

**عنوان البحث:** الكشف المصلي عنالفيروس الصغير ب 19 لدى النساء الحوامل اللآتييحضرن مستشفى القضارف التعليمي بولاية القضارف

**مقدمة:** ينتشرالفيروس الصغير ب 19 في كافة المناطق الجغرافية. أكثر من 60% من سكان العالم يصابون بالفيروس عند الطفولة.

تعتبر النساء الحوامل من أكثر الفئات المجتمعية عرضة للكثير من الأمراض المعديةوتعتبر المضاعفات عند الحوامل النساء مضاعفات حادة: من بينها سقوط للحمل أو موت الجنين بالرحم المصاحبة لعدوى الفيروس الصغيرب 19.

**الهدف من الدراسة:** تهدف هذه الدراسة للكشف عن الفيروس الصغير ب 19 لدى النساء الحوامل اللآتييحضرن مستشفى القضارف التعليمي بولاية القضارف. حيث يمكن للمرأة الحامل السليمة ظاهرياً نقل المرض لأشخاصآخرين.

سوف تتطلب مشاركتك في هذه الدراسة إجراء بعض الإختبارات لعينة الدم التي لا يزيد مقدارها عن 5 مل. و ستفحص هذه العينات في معمل التراث بجامعة السودان للعلوم والتكنولوجيا- الجناح الغربي. وستحفظ العينات بالمعمل حتى إكتمال مشروع البحث.

**المخاطر**: قد يتعرض المشارك في البحث لألم بسيط نتيجة لوخز الحقن وسوف تتخذ كل تحوطات السلامة المعملية لأخذ العينة.

**البدائل** : البديل للمشاركة في الدراسة هو عدم المشاركة ولك كل الحرية المطلقة لإختيار المشاركة أو عدم المشاركة في هذه الدراسة.

**إنهاء المشاركة** : سيتم إنهاء المشاركة فى الدراسة إذا قررت الإنسحاب من الدراسة أو إذا قرر الباحث بأنك غير مستوفيه لشروط المشاركة فى البحث.

**المشاركة التطوعية** : المشاركة في هذه الدراسة طوعية وإذا قررت عدم المشاركة فإنك لن تتعرضي لأى مضايقات.

**السرية** : كمشاركة فى الدراسة ستكون هويتك ومحتويات الإختبارات المعملية سرية فى جميع المنشورات المتعلقة بنتائج الدراسة ويمكن الإطلاع عليها من قبل الباحثين ولجان الكلية فى حدود النظم والقوانين المطبقة بهذا الخصوص. هذه الدراسة بغرض الحصول على درجة الماجستير في الأحياء الدقيقة بكلية المختبرات الطبية. كل المعلومات المتحصلة سرية للغاية و عند الإنتهاء من الفحص المعملي سوف يتم إبلاغك عن النتائج.

الأشخاص الذين يمكن الإتصال بهم للإستفسارعن نتائج البحث: يمكن الإتصال بالباحث على رقم.

0922545636 الموبايل

**الجزء الثاني**

أنا ..... أوقع علي هذه الموافقة بعد ان شرح
.لي الباحث انني سأشارك في بحث علمي وأجاب علي كل تساؤلاتي بخصوص هذا البحث
.وبتوقيعي هذا أقر بأننى موافقة علي اخذ العينة (الدم) لغرض البحث

:المشاركة في البحث أو من يوقع عنها : الباحث

. الإسم : ..... الإسم: آلاء عثمان احمد حسن

.....: التوقيع أو البصمة

..... : التوقيع

..... : التاريخ :..... التاريخ

.....: صلة القرابة

(اذا كان الموقع غير المشاركة)

# Anti-Parvovirus B19 ELISA (IgG)
## Test instruction

| ORDER NO. | ANTIBODIES AGAINST | IG CLASS | SUBSTRATE | FORMAT |
|---|---|---|---|---|
| EI 2580-9601 G | Parvovirus B19 | IgG | Ag-coated microplate wells | 96 x 01 (96) |

**Indication:** The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the immunoglobulin class IgG against parvoviruses B19 in serum or plasma for the diagnosis of erythema infectiosum. Synonyms: megaloerythema, Sticker's disease, fifth disease.

**Application:** The determination of anti-parvovirus B19 antibodies of classes IgG and IgM, e.g. using ELISA, is after direct virus detection the most important method for diagnosis of a parvovirus B19 infection. Assessment of immunity against parvovirus B19 infection is of particular significance in pregnant women. Detection of virus-specific IgG antibodies together with negative IgM and negative direct virus detection indicate a past parvovirus B19 infection and existing immunity.

**Principles of the test:** The test kit contains microliter strips each with 8 break-off reagent wells coated with parvovirus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

**Contents of the test kit:**

| Component | Colour | Format | Symbol |
|---|---|---|---|
| 1. **Microplate wells coated with antigens** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use | --- | 12 x 8 | STRIPS |
| 2. **Calibrator 1** 100 IU/ml (IgG, human), ready for use | red coloured in decreasing intensity | 1 x 2.0 ml | CAL 1 |
| 3. **Calibrator 2** 25 IU/ml (IgG, human), ready for use | | 1 x 2.0 ml | CAL 2 |
| 4. **Calibrator 3** 5 IU/ml (IgG, human), ready for use | | 1 x 2.0 ml | CAL 3 |
| 5. **Calibrator 4** 1 IU/ml (IgG, human), ready for use | | 1 x 2.0 ml | CAL 4 |
| 6. **Positive control** (IgG, human), ready for use | blue | 1 x 2.0 ml | POS CONTROL |
| 7. **Negative control** (IgG, human), ready for use | green | 1 x 2.0 ml | NEG CONTROL |
| 8. **Enzyme conjugate** peroxidase-labelled anti-human IgG (rabbit), ready for use | green | 1 x 12 ml | CONJUGATE |
| 9. **Sample buffer**, ready for use | light blue | 1 x 100 ml | SAMPLE BUFFER |
| 10. **Wash buffer**, 10x concentrate | colourless | 1 x 100 ml | WASH BUFFER 10x |
| 11. **Chromogen/substrate solution** TMB/$H_2O_2$, ready for use | colourless | 1 x 12 ml | SUBSTRATE |
| 12. **Stop solution** 0.5 M sulphuric acid, ready for use | colourless | 1 x 12 ml | STOP SOLUTION |
| 13. **Test instruction** | --- | 1 booklet | |
| 14. **Quality control certificate** | --- | 1 protocol | |
| 15. **Protective foil** | --- | 2 pieces | FOIL |

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

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