Sudan University of Science and Technology

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Sero-Frequencey of Parvovirus B19 among Apparently Healthy Pregnant Women Attending Selected Antenatal Care Units in Khartoum State.

معدل الإنتشار المصلي لفيروس البارفو ب 19 وسط النساء الحوامل الأصحاء ظاهرياً اللآئي يحضرن وحدات مختارة لرعاية ما قبل الولادة في ولاية الخرطوم .

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قال تعالى :

(لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ) .

صدق الله العظيم

سورة البقرة

الاية (٢٨٦)

Dedication

To my beloved parents

To my brothers and sisters

To my teachers and myfriends

Thank you all

Acknowledgments

Firstly I would like to thank **ALLAH** for the guidance, strength, power of mind, protection andskills and giving me a healthy life.

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Abstract

Human parvovirus B19 infection can cause serious complications, especiallyamong pregnant women, patient with hematological disorders, immunocompromised and fetus,result in non-immune hydrops, intrauterine fetal death, and fetal death. This study aimedto detect thefrequency of B19V IgG andIgM antibodiesamong apparently healthy pregnant women attending selected antenatal care unitsin Khartoum state, Sudan. Blood specimens were collected during the period from September2018 toAugust 2019 from93 pregnant womentheir ages ranged from 15-45 years. Specimens were tested for the presenceof parvovirus B19IgGand IgMantibodies usingELISA technique. Out of the 93 participants, 8 (8.6%) werepositivefor IgM whereas 19 (20.4%) werepositive for IgGandthose aged 25-35yearshadthe highest frequency of IgM antibodies 7(7.5%) andIgG antibodies 13 (14%).There wassignificant association between age and sero-positivity of anti-parvovirus IgM antibodies (*p value*= 0.028)and IgG antibodies (*p value*= 0.034). There were no significant associationswith gravidity, history of blood transfusion, history of miscarriages with B19 infections.

In conclusion, the frequency of B19 virus IgM was lower (8.6%) than IgG (20.4%) amongapparently healthy pregnant women.

مستخلص الأطروحة

يمكن أن تسبب عدوى فيروسات البارفو19 ب البشرية مضاعفات خطيرة، لدى النساء الحوامل، والمرضى الذين يعانون من اضطرابات الدم، والذين يعانون من نقص المناعة وموت الجنين داخل و خارج الرحم. هدفت هذه الدراسة لتحديد تردد الاجسام المضادة النمط IgG وIgMلفيروس البارفو19، وسط النساء الحوامل اللائي يحضرن وحدات الرعاية الصحية المختارة في ولاية الخرطوم، السودان. تم جمع عينات الدم خلال الفترة من سبتمبر2018 إلى يوليو 2019 من 93 امرأة حامل سليمات ظاهريا تتراوح أعمارهن بين 15و45عامًا. تم اختبار جميع العينات لوجود الجسم المضاد النمط IgG وIgMلفيروس البارفو19ب باستخدام الامتزاز المناعي المرتبط بالانزيم. من بين 93 مشاركًا، كان8 (8.6٪) إيجابيين للجسم المضاد النمط IgM بينما19 (20.4٪) إيجابي لـ الجسم المضاد النمط IgGوالذين تتراوح أعمارهم بين 20 و35 عامًا كان لديهم أعلى معدل انتشار ل الجسم المضاد النمطIgM7 (7.5٪) والجسم المضاد النمط IgG13 (14٪). وكانهناكعلاقة معنوية بينالعمر والإيجابية المصلية للأجسام المضادIgM (القيمة الاحتمالية = 0.028) والأجسام المضادة النمطIgG (القيمةالاحتمالية = 0.034). في حين أنهلا توجد علاقة معنوية مع عدد مرات الحمل ,الاجهاض سابقا ,عدد مرات نقل الدممع الاصابه ب فيروس البارفو ب19.

في الختام , كان معدل انتشار الجسم المضاد النمط IgM أقل (8.6٪) من الجسم المضاد النمط IgGالجسم (20.4%) بين النساء الحوامل الاصحاء ظاهريا.

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List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
ASCT	Autologous Stem Cell Transplantation
B19V	Parvovirus B19
CD20	Cluster of differentiation 20
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
EM	Electron microscope
HIV	Human immunodeficiency virus
IF	Immunoflorescent
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVIG	Intravenous immunoglobulin therapy
KDa	Kilo Dalton
MD	Myelodysplastic
MEL	Melanin
mRNA	Messenger ribonucleic acid
NO.	Number
NSP	Non-structural Protein
PCR	Polymerase chain reaction
p-value	Probability value
QA	Quality assurance
RHR	Rolling hair pin
SPSS	Statistical Package of Social Science
V/V	Volume by volume
VP	Viral protein

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

1.1 Background:

Parvovirus B19 (B19V) is a small, non-enveloped DNA virus and belongs to the genus Erythrovirus of the family Parvoviridae (Brown, 2010).

Infection with Parvovirus B19 is common worldwide, most common in late winter or early spring and usually occurs through respiratory droplets, but can be transmitted by blood and blood-derived products and vertically from mother to fetus .Also iotrogenic transmission viacontaminated blood products, such as clotting factors .Also occurs (Enders *et al*., 2004; American Academy of Pediatrics, 2006;Elnifro *et al*., 2009).

Most persons with B19V infection are asymptomatic or exhibit mild, non-specific,cold-like symptoms that are never linked to the virus (Heegaard andBrown,2002).

When maternal infection occurs, maternal viremia reaches its peak approximately 1week after infection. Symptoms such as erythema infectiosum, mild fever, arthralgia and headache start approximately 10–14 days after infection in about 50% of infected women. At the time of the occurrence of IgM antibodies, presumably during the maternal peak viral load (day7), the risk of vertical transmission may be maximal and has been estimated to be around 25% (Nyman *et al*., 2002; De Haan*et al*., 2005).

Transplacental transmission of parvovirus B19 during pregnancy is one of the leading causes of non-immune fetal hydrops, spontaneous abortion or intrauterine fetal death (Skjoldebrand-Sparre *et al*., 2000).It has been reported that intrauterine fetal death and miscarriage occur more during the second trimester (Johansson *et al*., 2008, Silingardi *et al*., 2009).

1.2Rationale:

Up to 50% of women are susceptible to B19V infection, which may result in serious fetal complications during pregnancy,anemia,spontaneous abortion and hydrops fetalis(Alger,1997).

The transmission rate of maternal parvovirus B19 infection to the fetus is 17% to 33% (Harger *et al*., 1998).

Detection of early B19V infection will help in the early diagnosis and identify those at risk which can increase the possibility of the fetal survival.

Data about incidence and prevalence of B19 infection in Sudan is very limited therefore, this study aimed to provide more information about the sero-frequency of B19V infection.

1.3Objectives:

1.3.1General objective:

To detect the frequency of parvovirus B19 serologically in apparently healthy pregnant women attending selected antenatal care units in Khartoum State.

1.3.2Specific objectives:

1. To detect anti-B19V IgM antibodies in apparently healthy pregnant women using enzyme linked immune-sorbent assay (ELISA).
2. To detect anti-B19V IgG antibodies in apparently healthy pregnant women using enzyme linked immune-sorbent assay (ELISA).
3. To determine the possible risk factors associated with the infection including age, history of blood transfusion, history of miscarriage and gravidity.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

2.1Parvovirus B19:

2.1.1History:

Parvovirus B19 was discovered by chance in 1975 by Australian virologist Yvonne Cossart (Cossart *et al.*,1975;Sabella and Goldfarb, 1999).

It gained its name because it was discovered in well B19 of a large series of microplates(Cossart *et al.*,1975;Heegaard and Brown,2002).

Dependoviruses, the first *parvoviruses* to be discovered, were first isolated in the 1960s.*Parvovirus B19*, the first known parvovirus to infect humans that was discovered in London by Australian virologist Yvonne Cossart in 1974,who and her group were focused on hepatitis B and were processing blood samples when they discovered a number of "false positives" later identified as parvovirus B19. The virus is named for the patient code of one of the blood bank samples involved in the discovery diameter (Heegaard and Brown,2002).

2.1.2Taxonomy and classification:

Parvovirus is the common name applied to all the viruses in the *Parvoviridae* taxonomic family.

It is a member of the *Erythrovirus* genus of the *Parvoviridae*. Prior to 2014, it was also the name applied to a genus within the subfamily *Parvovirinae*, but this has been amended to genus *Protoparvovirus* to avoid confusion between taxonomic levels *Parvoviruses* that infect vertebrate hosts make up the subfamily *Parvovirinae*, while those that infect arthropods (currently only known to infect insects or shrimp) make up the subfamily *Densovirinae* (Cotmore *et al.*, 2014).

2.1.3 Viral Structure:

Parvoviruses are linear, non-segmented single-stranded DNA viruses, with an average genome size of 5000 nucleotides. *Parvoviruses* are among the smallest viruses and are 18–28 nm in diameter (Leippard *et al.,* 2007).

Icosahedral capsid consists of two structural viral proteins VP1 (83 kDa) and VP2 (58 kDa), which are identical except for 227 amino acids at the amino-terminal of the VP1-protein, the so-called VP1-unique region. Each capsid consists of a total of 60 capsomers, VP2 is the major capsid protein, and comprises approximately 95% of the total virus particle. VP1-proteins are incorporated into the capsid structure in a non-stochiometrical relation (based on antibody-binding analysis and X-ray structural analysis the VP1-unique region is assumed to be exposed at the surface of the virus particle (Landenberg *et al.,* 2006).

2.1.4 Genome organization:

The viral genome is 4–6 kilobases in length and terminates in imperfectly-palindromic hairpin sequences of ~120–500 nucleotides that exhibit genus-specific secondary structures, and can either be identical at the two ends of the genome (homotelomeric) or can differ in size, sequence and predicted secondary structure (heterotelomeric) (Stamenkovic *et al.*,2016).

At each end of the DNA molecule there are palindromic sequences which form "hairpin" loops. The hairpin at the 3'end serves as a primer for the DNA polymerase (Siegl and Cassinotti, 1998).

Three genotypes (with subtypes) have been identified (Molenaar-de Backer, *et al.*, 2012).

The Nucleotide substitution rate for total coding DNA has been estimated to be 1.03 (0.6-1.27) x 10^{-4} substitutions /site/ year. This rate is similar to that of other single stranded DNA viruses (Stamenkovic *et al.,* 2016).

All *parvoviruses* encode two major gene complexes: the non-structural (or Rep) gene that encodes the replication initiator protein (called NS1 or Rep) and the VP (or cap) gene, which encodes a nested set of ~2–6 size variants derived from the C-terminus of the single VP protein sequence (Cotmore and Tattersall, 2013).

Non-structural protein1(NS1) initiates and drives the viral "rolling hairpin" replication mechanism (RHR), which is a linear adaptation of the more-common "rolling-circle" replication strategy used by many small circular prokaryotic and viral replicons (Cotmore *et al.*,2014). RHR is a unidirectional mechanism that displaces a single, continuous DNA strand, which rapidly folds and refolds to generate a series of concatemeric duplex replication intermediates. Unit length genomes are then excised from these intermediates by the NS1 endonuclease (Cotmore *et al.*,2014), and packaged 3'-to-5' into preformed empty capsids driven by the SF3 helicase activity of NS1/Rep (King *et al.,* 2001).

2.1.5 Replication process:

Tropism may be mediated by the presence of a second, as yet types may mediate transplacetal infection, contribute to the rash of erythema infuctiosum, or lead to myocarditis, also the level of P antigen expression does not correlate with the efficiency of viral binding providing further evidence of the existence of a putative cellular co-receptor for facilitating entry of B19V into human cell (Weigel-Kelley *et al.,* 2003).

Before the start of any replication, *parvoviruses* must first enter the cell. An attachment to host receptors initiates clathrin-mediated endocytosis of the virion into the host cell. The virion consequently penetrates into the cytoplasm via permeabilization of host endosomal membrane, and reaches the nucleus (where uncoating occurs) via microtubular transport (Mestrovic, 2016).

These signals mediate the delivery of virion into the cell nucleus, where genome uncoating allows the establishment of viral DNA replication and transcription

complexes that rely predominantly upon the synthetic machinery of their host cell(Cotmore and Tattersall 2013;Cotmore and Tattersall , 2014).

Transcription of newly formed double-stranded DNA gives rise to viral mRNAs when host cell enters S-phase (or synthesis phase) of the cell cycle and are subsequently translated to produce viral proteins (Meštrović, 2016).

2.1.6Antigenic structure:

B19V was initially shown to agglutinate human red cell and it was hypothesized that the same agglutinin may act as host cell receptor on erythroid progenitor cells. Thus, the hemagglutinin was identified as the glycolipid globoside also known as blood group P antigen; P antigen is also present on megakaryocyte, endothelial cell and fetal myocytes (Heegaard and Brown,2002).

2.1.7 Epidemiology:

The yearly peak incidence of infection occurs during spring and epidemics occur every 4 years (Bosman *et al.*, 2002).

The prevalence of IgM antibodies directed against parvovirus B19 in the population ranges from 2 to 15% in children 1-5 years old , 15-60% in children 6-19% years old 30-60% in adult and more than 85% in the geriatric population (Heegard and Brown , 2002).

Outbreaks usually occur yearly, with larger epidemics every four to five years, and may last up to six months (De Jong*et al.*, 2011).

Most cases in pregnant women seem to occur in late spring and summer (Dijkmans *et al.*,2009).

2.1.8Transmission:

Acutely infected individuals have extremely high viral concentrations in blood and other body fluids such as saliva or urine. Since parvoviruses have no lipid capsule, their infectivity is unaffected by solvents and detergents (Modrow and Gärtner,2006).

Nosocomial transmission has been described infrequently (Miyamoto *et al*., 2000).

2.1.9Viral Pathogenesis:

Inhibition of hemopoiesis induced by globoside or p-antigen which occur in erythrocyte progenitor cell, but also, placental tissue,fetal myocardium and endothelial cell (Chisaka *et al*.,2003;Young and Brown, 2004).

Viral replication globocyte is binding site for B19 on the cell surface but it need α5β1 as a cellular co-receptor and functional activity of B1integrin for cell entry (Weigel-Kelly *et al*., 2003).

Recent data suggest that Ku80 auto antigen may also function as a co-receptor (Munakata *et al*., 2005).

The NSP1 protein of B19V is involved in inducing cell death both direct cell and injury and the induction of apoptosis contribute to this effect (Enders *et al*.,2004).

The virus infects the liver which is the main site of erythrocyte production in the embryo (Nyman *et al*.,2002).

2.1.10Pathogenesis of parvovirus B19 in pregnancy:

B19V has a propensity for infecting rapidly divided cells, particularly erythroblasts (Jeanne *et al*., 2001).

Between the third and sixth months of pregnancy, the fetal red blood cell mass increases thirty times, the risk of fetal complications depends largely upon the gestational age at the time of maternal infection with B19V. It seems that the highest risk for fetal loss is if maternal infection occurs during weeks 9–16 of

pregnancy, is reduced with infection in the second half of pregnancy, and rare if infection occurs in the last 2 months (Enders *et al*., 2004).

The riskof developing anemia if the fetus is infected by parvovirus B19 at the third trimester, the fetus is able to mount a more effective immune response to the virus, which may account for the decrease in fetal loss at this stage of pregnancy (Sukanya *et al*., 2006).

The erythroid lineage cells proved to be appropriate targets for B19V virus and that the infection could induce apoptosis of infected cells, which caused by the NS1 protein (Yaegashi, 2004).

2.1.11 Clinical presentations:

2.1.11.1Asymptomatic*:*

Up to 50% of non-pregnant women who develop parvovirus B1V infection, and up to 70% of infected pregnant women, will be asymptomatic (Chisaka *et al*.,2006; Lamont *et al*.,2011).

2.1.11.2Fifth disease:

Fifth disease or erythema infectiosum is only one of several expressions of B19V. The associated bright red rash of the cheeks gives it the nickname (slapped cheek syndrome) (Sabella and Goldfalb.,1999).

Any age may be affected, although it is most common in children aged six to ten years. It is so named because it was the fifth most common cause of a pink-red infection associated rash to be described by physicians (many of the others, such as measles and rubella, are rare now) (Lamont *et al*., 2011).

Once infected, patients usually develop the illness after an incubation period of four to fourteen days .The disease commences with high fever and malaise, when the virus is most abundant in the blood stream, and patients are usually no longer infectious once the characteristic rash of this disease has appeared (Servey *et al*., 2007).

Teenagers or young adults may develop Gloves and Socks Syndrome,typically occurs in young adults and presents as symmetric, painful erythema and edema of the feet and hands.The condition gradually progresses to petechiae and purpura and may develop into vesicles and bullae with skin sloughing(Santonja *et al*., 2011).

2.1.11.3 A plastic crisis:

Although most patients have a decrease of erythropoiesis during *parvovirus* infection, it is most dangerous in patients with pre-existing bone marrow stress, for example sickle cell anemia or hereditary spherocytosis, and are therefore heavily dependent on erythropoiesis due to the reduced lifespan of the red cells. This is termed "aplastic crisis" (also called reticulocytopenia) which treated with blood transfusion (Servey *et al*., 2007).

2.1.11.4 Infection in AIDS patients:

B19V is a cause of chronic anemia in individuals who have AIDS. It is frequently overlooked. Treatment with intravenous immunoglobulin usually resolves the anemia although relapse can occur. The *parvovirus* infection may trigger an inflammatory reaction in AIDS patients who have just begun antiretroviral therapy (Dijkmans *et al.*, 2009).

2.1.11.5Hydrops fetalis:

B19V infection in pregnant women is associated with hydrops fetalis due to severe fetal anemia, sometimes leading to miscarriage or stillbirth (Servey *et al*., 2007; Erga, *et al*., 2006)**.** The risk to the fetus will be reduced with correct diagnosis of the anemia (by ultrasound scans) and treatment (by blood transfusions). There is some evidence that intrauterine B19V infection leads to developmental abnormalities in childhood (Nagel *et al*., 2007).

If a fetus develops hydrops, ultrasound signs include ascites, skin edema, pleural and pericardial effusions, as well as placental edema. It is estimated that B19V infection accounts for 8% to 10% of non-immune hydrops, although some studies

found molecular evidence of parvovirus B19 in 18% to 27% of cases of non-immune hydrops (Royal College of Physicians of Ireland, 2014).

2.1.11.6Arthritis and arthralgia:

Arthralgia and arthritis are commonly reported in association with B19V infection in adults whereas erythema infectiosum is the main symptom observed in children. The occurrence of arthralgia coincides with the initial detection of circulating IgM and IgG antibodies against the viral structural proteins VP1 and VP2. B19Vinfection may affect the development of arthritis in adults and perhaps some children **(**Landenberg *et al*., 2006).

Women are approximately twice as likely as men to experience arthritis after B19V infection. Possibly up to 15% of all new cases of arthritis are due to B19V, and a history of recent contact with a patient and positive serology generally confirms the diagnosis (Corcoran and Doyle, 2004).

B19V arthritis doesn't progress to other forms of arthritis. Typically joint symptoms last 1–3 weeks, but in 10–20% of those affected; it may last weeks to months (Servey *et al*., 2007).

2.1.12Diagnosis of B19V:

2.1.12.1Serological tests:

Serologic examination of maternal blood is the first and most useful diagnostic test that should be performed as soon as possible once B19V infection is suspected during pregnancy. B19 IgG or IgM antibody detection now often performed by enzyme immune assay (ELISA), which tend to replace the immunofluorescent technique (IF) (Beersma *et al*., 2005).

B19V specific IgM antibodies become detectable in maternal serum within 7-10 days after infection, sharply peak at 10-14 days, and then rapidly decrease within 2 or 3 months (De Jong *et al*., 2006).

IgG antibodies will rise considerably more slowly and reach placenta at 4 weeks after infection, as a result, comparison of the IgG and IgM ELISA ratios can provide an indication of the actual stage of B19V infection. If IgM titre excess IgG titre, the B19V infection took place less than month ago, viral load level will be high and fetal complication, if absent, any still development (Beermsa *et al*., 2005).

2.1.12.2Viral Culture:

In all culture systems erythropoietin is require to maintain viral replication probably by supporting the rapid division of erthyroid progenitors. All systems are culture explants only and are not suitable for long turn cultured.HoweverB19V can also be propagated in a few specialized cell line: two magakaryoblastoid cell line, MB-02 and UT-7/EPO, and two human erythroid leukemia cell line, JK-1 and KU812EP6 (Heegaard and Brown 2002).

These lines have been usedto study mechanisms of replication and to develop neutralization (Bostic *et al*., 1999) and infectivity assays (Miyagawa *et al*., 1999).

2.1.12.3Diagnostic cytopathology:

The cytopathic effect of infection of erythroid progenitor cell with B19V, both inside the body and in cell lines, is manifest as giant peonormoblast (alternately referred to as lantern cell).First recognized in 1948 in the bone marrow of patient with diameter of 25-32m, large eosinophilic nuclear inclusion bodies, cytoplasmic vacuolization, and occasionally "dogear" projection may be observed. EM of cell reveals cytopathic ultra-cultural changes that include pseudopod formation, marginated chromatin, and virus particles in nucleus.

Although the presence of giant pronormoblasts in either bone marrow or peripheral blood is suggestive of B19V infection, their presence or absence should not be used alone to make a diagnosis of B19V infection. These cells are often absent in

patients with human immunodeficiency virus (HIV) infection or other chronic infections (Hagaared and Brown, 2002).

2.1.12.4 Direct hybridization:

Direct hybridization, usually as a slot blot or dot blot format, generally employs an almost-full-length viral DNA probe labeled with 32P, digoxigenin, or biotin to bind to DNA in clinical specimens (Mori *et al.*,1989).Results of the hybridization assay are readily quantifiable, with a detection limit of 10^5 genome copies/ml, and the hybridization assay will detect all known variants of B19V, including V9(Nyman *et al.*,2002).

2.1.12.5Viral DNA detection:

Nucleic acid amplification to detect B19V DNA is an extremely sensitive means (most published PCR assays are able to detect viral DNA at 1–100 copies/mL) to detect viral DNA in a sample.This method is especially useful in patients lacking an adequate antibody-mediated immune response, immunocompromised or immunosuppressed individuals, and fetuses. In such cases serological testing for B19V is unreliable (Jordan, 2001).

Using standard procedures, detection of B19V specific IgM in fetal blood hasa sensitivity of 29% compared to almost 100% for PCR(Enders *et al*.,2004; Beersma *et al*., 2005). However, low B19V DNA levels may persist for years after acute infection and therefore low-positive PCR results for B19V do not prove recent infection (Lindblom *et al*., 2005).

2.1.13 Treatment:

Intravenous immunoglobulin therapy (IVIG) therapy has been a popular alternative because doctors can administer it without stopping chemotherapy drugs like Melanin-Autologous Stem Cell Transplantation (MEL-ASCT) (Katragadda *et al.*, 2013). This is a large improvement over administering Rituximab. The monoclonal antibody against the CD20 protein has been shown to cause acute hepatitis (Yang

et al., 2011).However, it is important to note that IVIG therapy is not perfect as 34% of treated patients will have a relapse after 4 months (Crabol *et al*., 2012).

2.1.14 Prevention:

Because maternal exposure to B19V occurs before her child or any other contact has a rash or is otherwise symptomatic and considering that around 20% of children are asymptomatic, no reasonable strategy to avoid B19V exposure to pregnant women is apparent. Also, it would not be justified excluding pregnant women from the workplace during endemic periods, since the risk of occupational infection may be similar to or less than in the community or at home. Individual counseling of pregnant women, identified to be sero-negative, with a high-risk profile (school teachers, daycare workers) should be done to prevent unnecessary fetal death (Enders *et al*., 2004; Harger *et al*., 1998).

2.1.15 Vaccination:

Effective vaccines are available for animals, and it is likely that B19V infection can also be prevented. The recombinant immunogen that being developed as a vaccine for the human virus lacks DNA and is therefore non-infectious, empty capsids have been engineer to over express the highly immunogenic VP1, and a single dose of 2-*5mg* of empty capsid elicited neutralizing antibody responses in normal volunteer (Ballou *et al*., 2003) .

As with other vaccines, commercial interest rather than lack of efficacy or safety has limited the development of a B19V vaccine. Such a vaccine could prevent transient a Plastic crisis in patient with sickle cell disease or other hemolytic anemia and pure red-cell a plastic in some immune deficiency person, as well as hydrops fetalis, if seronegative women were inoculate early in pregnancy.

Chimerical viral capsid have been proposal as more general vehicles for the delivery of antigens B19V is especially attractive for this purpose, because the VP1 unique region can be entirely replaced with other protein sequences, allowing for

example, the presentation of conformation and functionally in fact enzyme on the surface of the empty viral capsids. This method is now being adapted for protection against an agent of bioterrorism: a domain of protective antigen of anthrax is being incorporated on *parvovirus B19* particle (Young and Brown, 2004).

Recently described a recombinant parvovirus B19 vaccine composed of VP1 and VP2 capsid proteins, which proved to be immunogenic and safe to use in human volunteers (Ballou *et al*., 2003).

2.2 Previous studies:

In Saudi Arabia presence of specific IgG antibodies was 46.6% and IgM was 2.25%positive for parvovirus B19 antibodies in tested women (Hani and Ghazi, 2007).

In central Nigeria, 27.5%participants had B19 IgG antibodies and 13.2% participants had parvovirus B19 IgM antibodies (Samuel *et al*., 2011).

In a population of Iranian pregnant women prevalence of parvovirus B19 IgG and IgM antibodies were 10.3% and 21.8% respectively (Keikha *et al*., 2006).

In Oyo state, Nigeria the seroprevelance of parvovirus B19 IgG antibodies was 20% and IgM antibodies was 4% (Abiodun *et al*., 2013).

In Mwanza,Tanzania the overall prevalence among pregnant women of parvovirus B19IgM antibodies was 32.8% while that prevalence of parvovirus B19 IgG antibodies was 55.0% (Mirambo *et al*., 2017).

In study conducted in Libya a neighboring country of Sudan, reported that 61% IgG and 5% IgM antibodies in pregnant women (Elnifro *et al.*, 2009).

A study in Khartoum revealed that 61.4% was positive for IgG and one subject(0.2%) was positive for IgM antibodies in pregnant women and IgG antibodies was significantly correlated with multigravidity(Adam *et al*.,2015).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1Study design:

This was analytical, descriptive, cross –sectional, hospital based study.

3.1.2 Study area:

The Study was conducted in antenatal care units of Chinese Friendship Hospital, Bashaier Hospital and Alzahraa clinic center in Khartoum State, Sudan.

3.1.3 Study duration:

The study was conducted during the period from September 2018 to August2019

3.1.4 Study population:

The study subjects included in this study were apparently healthy pregnant women who attended to antenatal care units in Khartoum State.

3.1.4.1 Inclusion criteria:

The study included women who were apparently healthy pregnant with different age and trimesters, attended the antenatal care clinics.

3.1.4.2 Exclusion criteria:

The exclusion criteria were symptomatic pregnant women and pregnant women with symptoms similar to the B19V infection.

3.1.5 Ethical considerations:

Ethical approval to conduct this study was obtained from Scientific Research Committees, College of Medical Laboratory Science, Sudan University of Science and Technology .All participants were informed verbally about the study and consented before enrolled.

3.1.6 Sample Size:

A total of 93 pregnant women (n=93) were enrolled in this study

3.1.7 Method of data collection:

Data were collected from apparently healthy pregnant women by direct interview (questionnaire) from each participant (Appendix 1).

3.1.8 Sampling technique:

This study is based on non-probability, convenience sampling technique.

3.1.9 Laboratory processing:

3.1.9.1 Specimen collection:

The vein puncture technique was used for collection and the suitable vein was selected, then skin was cleaned by 70% (v/v) ethanol.Sterile5 ml syringe was used to collect 5 ml of blood, and then the blood was dispensed in sterile plain blood container (without anticoagulant). Blood samples were allowed to clot and then centrifuged at 3000 rpm for 5-10 minutes to obtain serum. Then, the obtained sera were preserved at -20ºC until the serological analysis.

3.1.9.2Enzyme linked immunosorbent assay (ELISA):

ELISA technique was used to detect anti-parvovirus B19 antibodies IgM and IgG classes.

Detection of IgM antibodies indicates for recent infection while IgG antibodies for past infection or vaccination(Cohen and Buckley,1988).

3.1.9.2.1Principle:

The ELISA test kit (EUROIMMUN) provides a semi-quantitative *in- vitro* assay for human antibodies of the IgM and IgG class against parvovirus B19 in serum or plasma; contain microtiter strip wells coated with *parvovirus* antigens. In the case of positive samples, specific IgM and IgG antibodies (also IgA) will bind to the antigens. Detection of the bounds antibodies using an enzyme-labelled anti-human IgM and IgG (enzyme conjugate) is catalyzing a color reaction.

3.1.9.2.2 Procedure:

The reagents and samples were allowed to reach room temperature (+ 18^{o} C to +25 ^{o} C) and the samples were diluted 1 to 20.Then 100 µl of the calibrator, positive control, negative controls and diluted specimen were added into their respective wells of the 96-wellmicrotiterplate.The finished test plate was incubated for 60 minutes at 37^{o} C ± 1 ^{o} C after covering with the protective foil .The productive foil was removed and emptying the wells and the plate was washed 3 times using 300 µl of working strength diluted wash buffer for each wash .The washing buffer was left in each well for dispose of 30 to 60 seconds per washing cycle, after washing thoroughly all liquid from the microplates was disposed by tapping it on absorbent paper with the opening facing downwards to remove all residual wash buffer.100 µl of enzyme conjugate (peroxidase-labelled anti human IgM) was added into each of the microplate wells and the plate was incubated for 30 minutes at room temperature for IgM detection. Also 100 µl of enzyme conjugate (peroxidase-labelled anti human IgG) was added into each of the microplate wells and the plate was incubated for 30 minutes at room temperature for IgG detection. Then washedas described above.

100 µl of chromogen/substrate solution was added into each microplate wells and incubated for 15 minutes at room temperature and protected from direct sunlight. Then 100 µl of stop solution was added into each microplate in the same order and at the same speed as the chromogen/substrate solution was introduced.

3.1.9.2.3 Calculation and interpretation of the result:

Photometric measurement of the color intensity made at wave length of 450 nm within 30 minutes of adding the stop solution.

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive and those below as negative.

Results can be evaluated semi quantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

Extinction of the control or patient sample ÷ Extinction of calibrator = ratio

EUROIMMUN recommends interpreting of results as follows:

Ratio <0.8= negative

Ratio≥0.8 to <1.1= borderline

Ratio≥1.1= positive

Evaluation information: for duplicate determinations the main of the two values should be taken. If the two values deviate substantially from one another the sample should be resulted.

Diagnosis can be secured by the determination of the titer change in to serum samples taken at an interval of at least 7 days and analyzed in parallel.

For diagnosis, the clinical symptoms of the patient should always be taken in to account along with the serological results.

3.1.10 Data analysis:

Statistical analysis of collected data was performed using Statistical Package for Social Science (SPSS) version 16. Statistical significance and difference from control and test values was evaluated.

Data expressed in form of tables and chi-square test was performed in the analysis as association between classified variables .*p value* as ≤ 0.05 was considered to be statistically significant.

3.1.11 Quality control and management:

Blood was collected with care and adequate safety precautions to ensure test results are reliable. Quality Assurance (QA) and standard Operating System were followed for all hematological and immunological and microbiological tests to achieve validity and reliability of test results.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

A total of 93 apparently healthy pregnant women who attended to selected antenatal care units in Khartoum State during the period from September 2018 to July August were enrolled in this study to determine the frequency of B19V.

Their age range between 15-45 years and the majority of them 50 (53.8%) were at age between 20-35 years (Table 1).

Regarding the gestational age, 17(18.3%) were at the first trimester, 40(43%) were at the second trimester and36 (38.7%) were at the third trimester (Table 2).

Antibody detection of parvovirus B19 8 (8.6%) were positive for IgM and 19 (20.4%) were positive for IgG as showed in Table3.

Pregnant women aged 20 -35 years had the highest frequency of IgM antibodies 7 (7.5%) and IgG antibodies 13 (14%), but pregnant women aged more than 35 years had the lowest prevalence of IgM antibodies 0 (0.0%) and IgG antibodies 5 (5.4%) and there was association between age and sero-positivity of anti-parvovirus IgM(*p value*= 0.028) and IgG antibodies (*p value* = 0.034)as illustrated in Table 4.

Most of pregnant women had anti-parvovirus B19 IgM antibodies were within third trimester 4 (4.3%) and for IgG antibodies were in the second trimester 8 (8.6%) and no association between gestational age and sero-positivity of anti-parvovirus IgM (*p value*= 0.542)and IgG antibodies (*p value* = 0.086)(Table 5).

Concerning gravidity pregnant women with multigravida had anti parvovirus B19 IgM antibodies 6(6.5%) and IgG antibodies 15 (16.1%) and there was no association between gravidity age and sero-positivityof anti- parvovirus IgM (*p value* = 0.576) and IgG antibodies (*p value* = 0.289)(Table 6).

Parvovirus sero-positivity among pregnant women with history of miscarriage was 2 (2.2%) anti parvovirus B19 IgM antibodies and 3 (3.2%) had IgG antibodies and

there was no association between history of miscarriage and sero-positivity of anti-parvovirus IgM (*p value* = 0.548) and IgG antibodies (*p value* = 0.370)(Table 7). Most of pregnant women had history of blood transfusion 8 (8.6%) were positive for anti- parvovirus B19 IgM antibodies and 18 (19.3%) for IgG antibodies andthere was no association between history of blood transfusion and sero-positivity of anti-parvovirus IgM(*p value*= 0.835) and IgG antibodies (*p value* =0.369)(Table 8).

Table (1): The Distribution of age group among apparently healthy pregnant women:

Age groups	Frequency	Percentage
Less than 25 Years	12	12.9%
25-35Years	50	53.8%
More than 35 Years	31	33.3%
Total	93	100%

Table (2): The Distribution of gestational age among apparently healthy pregnant women:

Trimester	Frequency	Percentage
First	17	18.3
Second	40	43.0
Third	36	38.7
Total	93	100%

Table (3):The Percentage of anti-Parvovirus B19 IgM and IgG antibodies among apparently healthy pregnant women:

Serological tests / Result	Anti-parvovirus IgM	Anti-parvovirus IgG
Positive	8 (8.6%)	19 (20.4%)
Negative	85 (91.4%)	74 (79.6%)
Total	93 (100%)	93 (100%)

Table (4): The association between age group and IgM, IgG results among apparently healthy pregnant women:

Serological tests		Age group (No. (%)				*p-value*
		Less than 25 years	20-35 years	More than 35 years	Total	
IgM Result	Positive	1**(**1.1%)	7 (7.5%)	0 (0.00)	8(8.6 %)	* 0.028
	Negative	11(11.8%)	43(46.3%)	31(33.3%)	85(91.4%)	
Total		12(12.9%)	50 (53.8)	31 (33.3%)	93(100%)	
IgG Result	Positive	4 (4.3%)	13 (14%)	2 (2.1%)	19(20.4%)	* 0.034
	Negative	8(8.6%)	37 (39.8%)	29 (31.2%)	74(79.6%)	
Total		12(18.3%)	50(43.0%)	31(38.7%)	93(100%)	

Table (5): The association between trimester and IgM, IgG results among apparently healthy pregnant women:

Serological tests		Trimester				*p-value*
		First trimester	Second Trimester	Third Trimester	Total	
IgM Result	Positive	2 (2.1%)	2 (2.1%)	4 (4.3%)	8 (8.6%)	0.542
	Negative	15(16.1%)	38 (40.9%)	32 (34.4%)	85(91.4%)	
Total		17(18.2%)	40 (43.1%)	36 (38.7%)	93 (100%)	
IgG Result	Positive	6 (6.4%)	8(8.6%)	5 (5.4%)	19(20.4%)	0.086
	Negative	11(11.9%)	32 (34.4%)	31 (33.3%)	74(79.6%)	
Total		17(18.3%)	40(43.0%)	36 (38.7%)	93(100%)	

Table (6): The association between gravidity and IgM, IgG results among apparently healthy pregnant women:

Serological tests		Gravidity		Total	*p-value*
		Primigravida	Multigravida	No.	
IgM result	Positive	2(2.1%)	6(6.5%)	8(8.6%)	0.576
	Negative	25(26.9%)	60(64.5%)	85(91.4%)	
Total		27(29.0%)	66(80.0%)	93(100%)	
IgG Result	Positive	4(4.3%)	15(16.1%)	19(20.4%)	0.289
	Negative	23(24.7%)	51(54.9%)	74(79.6%)	
Total		27(29.0%)	66(70.9%)	93(100%)	

Table (7): The association between history of miscarriage and IgM, IgG results among apparently healthy pregnant women:

Serological tests		History of miscarriage		Total	*p-value*
		Yes	No	No.	
IgM Result	Positive	2(2/.2%)	6(6.4%)	8(8.6%)	0.548
	Negative	18(19.3%)	67(72.1%)	85(91.4%)	
Total		20(21.5%)	73(78.5)	93(100%)	
IgG Result	Positive	3(3.2%)	16(17.2%)	19(20.4%)	0.370
	Negative	17(18.4%)	57(61.2%)	74(79.6%)	
Total		20(21.4%)	73(78.4%)	93(100%)	

Table (8): The association between history of blood transfusion and IgM, IgG results among apparently healthy pregnant women:

Serological tests		History of blood transfusion		Total	*p-value*
		Yes	No	No.	
IgM Result	Positive	8(8.6%)	0(0.0%)	8(8.6%)	0.835
	Negative	83(89.2%)	2(2.2%)	85(91.4%)	
Total		91(97.8%)	2(2.2%)	93(100%)	
IgG Result	Positive	18(19.3%)	1 (1.1%)	19(20.4%)	0.369
	Negative	73(78.5%)	1(1.1%)	74(79.6%)	
Total		91(97.8%)	2(2.2%)	93(100%)	

CHAPTER V

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

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DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion:

About 35-45% of women of childbearing age do not possess protective IgG antibodies against B19 (Jon *et al*., 2006). The incidence of acute B19 infection in pregnancy is approximately 1-2% in endemic periods, but in epidemic periods the infection rate may rise to >10% (Trotta *et al*.,2004).

The frequency of B19V IgM antibodies detected in this study was 8 (8.6%) which interestingly close to other studies carried out in Libya by Elnifro *et al*.(2009) 5.3% , Nigeria 13.2%bySamuel*et al*.(2011) and in Iran (10.3%) by Keikha *et al*. (2006).

This study was higher than the previous reported study done in Sudan by Adam *et al*.(2015) which found that the seropositive was 0.2% that may be due the large sample size, long duration between the two studies (since 2008),different type of ELISA Kits and B19V infection is difficult to prevent since the infection is frequently asymptomatic and exposure is common during epidemics.

In Saudi Arabia, the prevalence of IgM was.25 % (Ghazi, 2007), that was lower than this study and may be due to the demographic and geographical variation season.

The frequency of B19V IgG antibodies detected in this study was (20.4 %), which matched too their studies reported by Samuel *et al*.(2011) in Nigeria27.5%, 21.8% in Iran by Keikha *et al*. (2006).

It was lower than the previous reported study done in Sudan by Adam and his colleagues (2015) which found that the seropositive was 61.4% and may be due to the large sample size (500).

In pregnant Saudi women; Ghazi (2007), found IgG was 46.6% which is higher than that found in the current study and may be due to the large sample size (1200), demographic and geographical variations season.

The majority of the study population 50 (53.8%) were at age range 20-35 years and the frequency of IgM and IgG antibodies were increased with age and there was significant association between IgM and IgG results and age, which matched with previous study in Saudi Arabia done by Ghazi (2007).

Women in their third trimester of pregnancy were had the highest frequency of IgM antibodies 4 (4.3%) followed by those in their first 2 (2.1%) and second 2 (2.1%) trimesters but women in their second trimester of pregnancy had the highest rate of IgG antibodies 8 (8.6%) followed by those in their first 6(6.4%) and third 5(5.4%) trimesters, that was agreed with other previous study done in Nigeria by Samuel *et al*.(2011) which found that the highest rate of IgG antibodies among pregnant women in their second trimester 42 (31.1%)followed by those in their first trimester 30 (26.3%) and third trimester13(12.5%) and may be due to in this study most of the pregnant women present in third trimester.

The frequency of B19V antibodies was higher in pregnant women with multigravida in which 6 (6.5%) were IgM and 15 (16.1%) were IgG positive, but there was no significant association which harmonized with Adam *et al*. (2015) in Sudan, and there was no significant association between gravidity and sero-positivity..

Sero-positivity of anti-B19V IgG antibodies among pregnant women that had history of blood transfusion was 18(19.3%) that was lower than the previous reported study done in Nigeria by Samuel *etal*.(2011) which found that the seropositive was 12 (30.8%).

There was no significant association between miscarriage, history of chronic or infectious disease and seroopsitively of IgM and IgG anti - B19V antibodies.

5.2 CONCLUSIONS:

The frequency of IgM(recent infection) was lower (8.6%) and IgG (past infection)was slightly increased (20.4%).

There was significant association between sero-positivity of IgM and IgG antibodies among apparently healthy pregnant women and age and there was insignificant association between B19V infection and others (gravidity, history of miscarriage ,history of blood transfusion).

5.3 RECOMMENDATIONS:

- More accurate tests must be used to confirm the infection such as antigen detection methods.
- Routine screening for B19 IgM antibodies for all women of childbearing age and subsequent clinical management of positive cases.
- Like other blood-transmissible viral infections, it recommends that routine screening for B19V IgM antibodies should be made especially for donors.
- Further studies concerning B19V with large number of participants.
- Public awareness of B19V must be increased.

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APPENDICES

Appendix (1)

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Sero-Frequencey of parvovirus B19 among Apparently Healthy Pregnant Women Attending Selected Antenatal Care Units in Khartoum State.

معدل الإنتشار المصلي لفيروس البارفو ب 19 وسط النساء الحوامل الأصحاء ظاهريا اللآئي يحضرن وحدات مختارة لرعاية ما قبل الولادة في ولاية الخرطوم .

ID. number ( )

Age: a.Less than 25 years ( ) b. 25 -35years ( ) c. more than 35 years ( )

Gestation stage:

a. First trimester ( ) b. Second trimester ( ) c. Third trimester ( )

Gravidity: a. primagravida ( ) b. multigravida ( )

History of miscarriage: a. yes ( ) b. no ( )

History of blood transfusion: a. yes ( ) b. no ( )

Investigation results:

Anti-B19V IgM:	+ve		-ve	
Anti-B19V IgG:	+ve		-ve	

Anti-Parvovirus B19 ELISA (IgM)
Test instruction

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

Indication: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the immunoglobulin class IgM 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. Virus-specific IgM antibodies occur at the earliest around ten days after contact with the virus and often drop below the detection limit after a few weeks. A positive IgM result together with evidence of IgG seroconversion and/or viral DNA is proof of an acute infection.

Principles of the test: The test kit contains microtiter 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 IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (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** (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. **Positive control** (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. **Negative control** (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. **Enzyme conjugate** peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. **Sample buffer** containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
7. **Wash buffer** 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. **Test instruction**	---	1 booklet	
11. **Quality control certificate**	---	1 protocol	
12. **Protective foil**	---	2 pieces	FOIL

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

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C and +8°C and protected from contamination, unless stated otherwise below.
The thermostat adjusted ELISA incubator must be set at +37°C ± 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ☼. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrator and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors of class IgM from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM-negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with green coloured sample buffer. For example, add 10 µl sample to 1.0 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing. Incubate the mixture for at least **10 minutes** at room temperature (+18°C to +25°C). Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analysed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls are ready for use, do not dilute them.

EUROIMMUN Medizinische Labordiagnostika AG

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 microtiter 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

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.
The thermostat adjusted ELISA incubator must be set at +37°C ± 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by votexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 3** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1 to 4** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual test performance cover the reagent wells with the protective foil. For automated test performance follow the recommendations of the instrument manufacturer.
Incubate for **60 minutes at +37°C ± 1°C**.

Washing:
Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.