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Seroprevalence of TORCH Infection among Pregnant Women with Recurrent Pregnancy Loss in Sana'a City, Yemen

A graduation Project Submitted to Faculty of Medicine and Health Sciences as a partial Fulfillment for Requirement of Bachelor's Degree in Laboratory Medicine

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Abstract

The TORCH infections are important groups of organisms which are initially inapparent, asymptomatic, and difficult to diagnose on the clinical ground during pregnancy but have the potential to cause bad obstetrical outcome in the form of congenital anomalies, oligohydramnios, FGR (fetal growth restriction), IUFD (Intrauterine fetal death), recurrent pregnancy loss (RPL), and stillbirth.

This study was focusing on the prevalence of TORCH infection problems for assessment the effectiveness of screening programs among pregnant women with recurrent abortion in Sana'a city-Yemen.

A total of 200 Blood samples were collected from pregnant women with recurrent pregnancy loss whom attending government and private clinics and health centers in Sana'a city Yemen, of whom 159 subjects had TORCH infection, the rest 41 showed non infected.

All samples were tested for TORCH routine screen test using Immunochromatography (cassette) test and confirmed by ELISA technique.

All samples were tested for ABO to detect their Blood grouping and Rh typing.

Individual data were collected in a pre-designed questionnaire, including, clinical data, demographic data, and some important risk factors.

Study results showed that; IgM to *Toxoplasma gondii* was positive in 31 (**19.5%**), IgM to herpes simplex virus 1,2 (HSV1/HSV2) was recorded in 25 (**15.7**%), IgM to rubella virus was positive in 11 (**6.9**%), while IgM to cytomegalovirus was recorded in only 2 (**1.3**%) of all cases.

Although the age of pregnant was ranged from 18 to 50 years but the highest infection was noted in age group 21-30 years (**47.7**%) followed by age group 31-40 years (**31.4**%).

The majority of women were of blood group O (61.6%), followed by blood group A (24.5%), while blood groups B and AB were less frequent (8.8% and 5.1%, respectively) and (**87.4**%) of women were Rh-positive and (**12.6**%) were Rh negative.

Regarding to the medical risk factors and recurrent pregnant loosing (RPL), the results showed that; History of recurrent miscarriage in the family was a risk factor for in 16.9% , animals in the family was a risk factor for toxoplasmosis (11.5%), medications using during pregnancy was 18.5%, uterine disorder was 15.6%, non-vaccination against rubella was (23.5%), blood transfusion 8.6% and Own handicapped children were 5.4% among the tested females.

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Dedication

To

We have no valuable and sufficient words to express our feeling and thanks, but we would to lovingly dedication this research to our respective parents and families, to the hundreds of people in community who are in pursuit of a healthy living, to our university, doctors, friends, and all supporters who were a backbone to us.

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List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
aCGH	Array Comparative Genomic Hybridization
BMI	Body Mass Index
CDC	Centers for Disease Control
cDNA	Complementary Deoxyribonucleic acid
CI	Confidence intervals
CMV	Cytomegalovirus
CCMV	Congenital Cytomegalovirus
CRS	Congenital Rubella Syndrome
HSV	Herpes simplex virus
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
DRG	Dorsal root ganglia
EBV	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
FISH	Fluorescence In Situ Hybridization
Ig	Immunoglobulin
IBD	Inflammatory bowel diseases
Kb	Kilobase(s)
ml	Milliliter
mRNA	Messenger RNA
MTOC	Microtubule- organizing center
MLPA	Multiplex Ligation-dependent Probe Amplification
ng	Nano gram
NB	Newborn
NK	Natural killer
nm	Nano meter
OT	Ocular toxoplasmosis

OD	Optical density
OR	Odd ratio
p	Probability value
RPL	recurrent pregnancy loss
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT-PCR	Reverse transcriptase
SNP	Single nucleotide polymorphisms
SD	Stander Division
SNHL	sensorineural hearing loss
SPSS	Statistical Package for the Social Sciences
T. gondii	*Toxoplasma gondii*
TE	Toxoplasmic encephalitis
TNF-α	Tumor necrosis factor alpha
UC	Ulcerative colitis
USA	United State of America
Ml	Micro-liter
VCR	Vaccination Coverage Rates
χ^2	Chi-square

Chapter 1

Introduction

1-Introduction

The term TORCH refers to a collection of illnesses that can cause catastrophic congenital abnormalities or severe fetal problems if contracted during pregnancy, particularly before 20 weeks. The acronym TORCH was coined by immunologist Andre Nahmias. TORCH is an acronym which stands for toxoplasmosis, other agents, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV) infection. The majority of TORCH infections cause mild maternal illness, but the fetal consequences are serious. Treatment of maternal infection frequently has no impact on fetal outcomes (**Pradeep *et al,* 2015; Kumar *et al*, 2018**).

TORCH includes: (T) toxoplasmosis, (O) various agents, the most common being chickenpox, syphilis, parvovirus B19, and, more recently, Zika virus, (R) rubella, (C) cytomegalovirus, and (H) herpes simplex virus. The TORCH infections are important groups of organisms which are initially inapparent, asymptomatic, and difficult to diagnose on the clinical ground during pregnancy but have the potential to cause bad obstetrical outcome in the form of congenital anomalies, oligohydramnios, FGR (fetal growth restriction), IUFD (Intrauterine fetal death), recurrent pregnancy loss (RPL), and stillbirth (**Singh *et al,* 2010; Kumar *et al,* 2018**).

Early detection of maternal sickness and fetal monitoring once the condition has been identified are critical. Knowledge of these diseases can help clinicians counsel moms on preventive strategies to avoid these infections, as well as parents on the possibility of bad fetal outcomes when these infections are present (**Rajani, 2018**).

Diagnosis based on the clinical presentation is difficult. Thus, the diagnosis of these infections largely depends on serological evidence. Utilizing TORCH panel for screening may help prevent many of these potential birth defects, as some of the TORCH infections can be effectively treated if the mother is diagnosed early in her pregnancy. Detection of IgM antibody against toxoplasma, rubella, HSV, and CMV is the best diagnostic modality for these infections. A national screening program for TORCH infections is lacking in INDIA (**Kumar *et al,* 2018**).

1.1 Justification

Based on previous studies, the prevalence of TORCH infection has heightened the problems among pregnant women in Yemen. Thus, continuous monitoring of the magnitude and trend of TORCH in blood is important for assessment of the effectiveness of screening programs. It might also be related directly to the prevalence of the disease in the community. Therefore, the current study aimed to determine the prevalence of TORCH Infection among pregnant women with recurrent abortion in Sana'a City, Yemen and the present study was conceived to provide an update and insightful information regarding prevalence of the common TORCH and risk factors at government and private clinics for investigation the recurrent pregnancy loss.

Chapter 2

Literature Review

2. Literature Review

2.1 Recurrent miscarriage (recurrent pregnancy loss)

2.1.1 Defination and introduction

Up to 5% of women are thought to experience recurrent miscarriages, also known as recurrent pregnancy loss (RPL), which is the spontaneous loss of two to three pregnancies. Medical societies vary in the precise number of gestational weeks and pregnancy losses used to define RPL (**Giouleka *et al*, 2023**). Despite genetic testing and a comprehensive review, the precise cause of pregnancy loss is typically unknown. Nearly half of RPL cases have a chromosomal aberration (i.e., aneuploidy) as their cause. Numerous risk factors, such as genetic and parental factors (such as chromosomal abnormalities, advanced maternal age, and sperm DNA fragmentation), congenital and acquired anatomical conditions, lifestyle factors (such as stress, alcohol, caffeine, and cigarette smoking), endocrine disorders, thrombophila (clotting disorders), immunological factors, and infections, have been linked to RPL(**Giouleka *et al*, 2023**).

After two consecutive miscarriages, the American Society of Reproductive Medicine advises a comprehensive evaluation; however, other medical societies may have different recommendations. Depending on the risk factors, a variety of tests and imaging examinations can be used to evaluate RPL. These range from cytogenetic studies, blood tests for clotting disorders, hormone levels, diabetes screening, thyroid function tests, sperm analysis, antibody testing, and imaging studies. Treatment is typically tailored to the relevant risk factors and test findings. RPL can have a substantial influence on the psychological well-being of couples and has been connected with greater levels of despair, anxiety, and stress. Therefore, it is advised that healthcare professionals take into account

proper screening and management (such as pharmaceuticals and counseling services) (**PCAS, 2012; Giouleka *et al*, 2023**).

2.1.2 Epidemiology of RPL

Pregnancy loss, also referred to as spontaneous abortion, occurs in up to 25% of pregnancies. Recurrent pregnancy loss occurs less frequently, with an estimated 5% of women experiencing two consecutive pregnancy losses and only 1% experiencing three or more (**van Dijk *et al,* 2020**). In addation, recurrent pregnancy loss (RPL), defined as two pregnancy losses prior to 20 weeks from the last menstrual period, occurs in 1–3% of all couples trying to conceive. Based on available data, there is consensus that women should not undergo extensive evaluation after a single first trimester or early second trimester pregnancy loss, given that these are relatively common and sporadic events with only a modestly increased risk of recurrence. In prospective studies, the risk of pregnancy loss increases with each loss from approximately 11% amongst nulligravidae to approximately 40% after three or more losses (**Magnus *et al*, 2019**).

2.1.3 Etiology and risk factors of (RPL)

About half of repeated pregnancies have an unknown etiology. Parental and genetic variables (chromosomal abnormalities, sperm DNA fragmentation, advanced maternal age), anatomical problems, lifestyle factors, endocrine disorders, thrombophilia (bleeding disorders), immunological issues, and infections are among the risk factors linked to RPL. In around half of instances, the precise cause of recurrent pregnancy loss remains unexplained despite careful assessment for these risk factors (**Ng *et al,* 2021; Lei *et al,* 2022**).

2.1.4 Parental and genetic factors associated with RPL

2.1.4.1 Advanced maternal age

Maternal age is connected with higher risk of miscarriage with a rate of 50% in women over 40 years of age. This higher chance of pregnancy loss can be ascribed to the higher occurrence of trisomies, a chromosomal disorder, reported in women over the age of 35 (**PCAS, 2012**).

1.1.4.2 Chromosomal abnormalities

In almost half of instances, chromosomal abnormalities in the fetus are the most common cause of recurrent pregnancy loss. These include numerical aberrations, also known as aneuploidies (trisomies, monosomy X, and triploidy), and structural aberrations (chromosomal inversions, insertions, deletions, and translocations). Cytogenetic tests including FISH, MLPA, aCGH, SNP array, and karyotyping—a test that examines the number and shape of chromosomes—can identify these (**Smits *et al,* 2020**). According to some study, women who experience three or more pregnancy losses are less likely to develop RPL, and chromosomal abnormalities are more common in spontaneous pregnancy loss than in recurrent pregnancy loss. In about half of cases, chromosomal abnormalities in the fetus are the most common cause of recurrent pregnancy loss. These include numerical aberrations, also known as aneuploidy (triploidy, monosomy X, and triploidy), and structural aberrations (chromosomal inversions, insertions, deletions, and translocations) (**Lei *et al,* 2022**). Cytogenetic tests including FISH, MLPA, aCGH, SNP array, and karyotyping—a test that examines the number and shape of chromosomes can identify these aberrations (**Smits *et al,* 2020**). According to some studies, women who have

had three or more pregnancy losses are less likely to have recurrent pregnancy loss, and chromosomal abnormalities are more common in spontaneous pregnancy loss than in recurrent pregnancy loss (**Lei *et al,* 2022**).

2.1.4.3 Paternal Factors

Recurrent pregnancy loss may be caused by male factors, according to new research. According to a comprehensive review, RPL may be linked to sperm DNA fragmentation, which is characterized as breaks in the sperm cells' DNA strand. They discovered that men with RPL had greater rates of SDF and other sperm metrics (i.e., less sperm, motility, or ejaculation volume). There is no proof that RPL is correlated with alcohol consumption, smoking, BMI, or paternal age (**Inversett *et al,* 2023**). Therefore, SDF testing is advised in cases of infertility or repeated pregnancy loss according to the European Association of Urology Guidelines on Sexual and Reproductive Health (**Minhas *et al,* 2021**).

2.1.4.4 Anatomical conditions

Fifteen percent of women who have had three or more recurrent miscarriages have an anatomical reason for not being able to carry the pregnancy to term. The structure of the uterus has an impact on the ability to carry a baby to term. Anatomical differences are common and can be congenital or acquired (**Alfirevic *et al,* 2017**).

2.1.4.5 Congenital uterine malformations

Unicornuate, septate, bicornate, didelphic, and arcuate uteri are examples of congenital uterine abnormalities. Although there is a correlation with pregnancy loss, it is unknown how uterine anomalies that exist from birth relate to RPL. The Müllerian tract is disrupted

throughout development, which results in several anatomical abnormalities. About 12.6% of RPL instances have them, with patients with septate (44.3%), bicornuate (36%), and arcuate (25.7%) uteri having the highest rates. A number of imaging tests, such as MRI, ultrasonography, and hysterosalpingography, can show these structural uterine anomalies (**PCAS, 2012**).

2.1.4.6 Acquired uterine malformations

Other structural uterine irregularities such as uterine fibroids, polyps, and adhesions (also known as Asherman's syndrome) have a less definite link with recurrent pregnancy loss. Cervical weakening has been demonstrated to lead to premature pregnancy loss resulting in miscarriages or preterm deliveries. An estimated 8% of women who experience repeated losses in the second trimester are thought to have cervical insufficiency as a contributing factor (**Alfirevic *et al,* 2017)**

2.1.4.7 Torch Infection

2.1.4.7.1 Toxoplasmosis

- **Introduction**

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii* (**Al-Malki *et al,* 2021**). This species belongs to the phylum Apicomplexa intracellular coccidian parasite (**Delgado *et al,* 2022**). This parasite is usually spread by animals, such as domestic pets like dogs, cats, and birds, as well as livestock including pigs, cows, goats, and sheep (**Stelzer *et al,* 2019**). This parasite is present all over the world (**Ait Hamou *et al,* 2021**). It is a parasite with characteristics resembling the malariacausing disease (**Spalenka *et al,* 2018**) Despite infecting a major fraction of the global population, *T. gondii* rarely results in clinically significant disease (**Mose *et al,* 2020**). Toxoplasmosis can be fatal during

pregnancy if it affects the fetus, baby, or those with impaired immune systems (**Pawełczyk *et al,* 2022**). The majority of toxoplasmosis cases in immunocompetent adults are benign or subclinical (**Layton, 2023**). Individuals with immunodeficiency and congenital versions of the illness have the most severe symptoms (**Avelino *et al,* 2014**).

T. gondii is transmitted to humans by three principal routes (**Figure 2.1**). First, humans can acquire *T. gondii* by eating raw or inadequately cooked infected meat, especially pork, mutton, and wild game, or uncooked foods that have come in contact with infected meat. Second, humans can inadvertently ingest oocysts that cats have passed in their feces, either from a litter box or from soil (e.g., soil from gardening, on unwashed fruits or vegetables, or in unfiltered water) (**Almashhadany *et al,* 2024**). Third, women can transmit the infection transplacentally to their unborn fetus **Cerutti *et al,* 2020**). Women infected with *T. gondii* before conception rarely transmit the parasite to their fetus, but those who become acutely infected or have reactivation of *T. gondii* during pregnancy (i.e., because of immunosuppression) can transmit the organism transplacentally. The risk of congenital disease is lowest (10 to 25 percent) when maternal infection occurs during the first trimester and highest (60 to 90 percent) when maternal infection occurs during the third trimester. However, congenital disease is more severe when infection is acquired in the first trimester. The overall risk of congenital infection from acute *T. gondii* infection during pregnancy ranges from approximately 20 to 50 percent (**Dawson *et al,* 2020; Woldesenbet and Harito, 2023**).

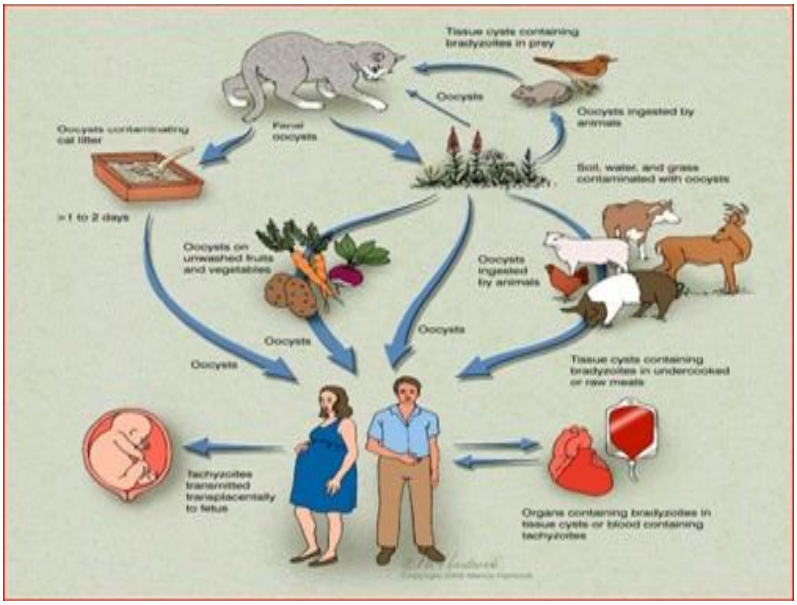

Figure 2.1. Pathways for Toxoplasma gondii infection. The feline intestinal tract is the only source for the production of T. gondii oocysts. Transmission to humans usually occurs through the ingestion of oocysts from contaminated sources (e.g., soil, cat litter, garden vegetables, water) or the ingestion of tissue cysts in undercooked meat from infected animals. Although fetal infection most often occurs after acute T. gondii infection in a pregnant woman, it also can occur after the reactivation of latent infection in an immunocompromised pregnant woman (**Woldesenbet and Harito, 2023)**

- **Clinical Symptoms**

The majority of immunocompetent people will not have symptoms for the remainder of their life, although both competent and immunocompetent people can get the condition, particularly retinochoroiditis (**Abdul Basit *et al,* 2018**). Infected individuals may experience asymptomatic cervical lymphadenopathy during acute systemic infection, as well as signs and symptoms that resemble infectious mononucleosis, such as myalgia, sore throat, myocarditis, maculopapular rash, fever, meningitis, polymyositis, muscle pain, malaise, pneumonia, and psychomotor disorders (**Layton *et al,* 2023**).

- **Congenital Toxoplasmosis**:

Congenital toxoplasmosis in infants may be asymptomatic or result in retinochoroiditis and CNS damage (**Li *et al,* 2021**). Congenital toxoplasmosis is estimated to impact 1-10 out of every 10,000 live births in the United States, 770 live births in Brazil, and 3,000 live births in France (**Furtado *et al,* 2012**). The burden of this condition in the Netherlands, however difficult to assess, is around 620 disability-adjusted people per year, mostly because of retinal disease and fetal death (**Kortbeek *et al,* 2009**). This burden is comparable to that of the ubiquitous and common foodborne pathogen Salmonella spp. (**Bintsis *et al,* 2017**). The infection is usually more severe if transmitted early in pregnancy, but the risk of vertical transmission is higher in the latter stages of pregnancy. (**Chaudhry *et al,* 2014**).

The infection's most visible sign is when it affects a pregnant woman, which raises the risk of abortion, stillbirth, live births with deformities including hydrocephalus or microcephalus, motor difficulties, retinal and brain damage, and symptoms of mental illnesses (**McAuley *et al,* 2014**). Ultrasonography can be used to detect and diagnose cerebral calcifications (**Saade *et al,* 2019**). Clinical signs and symptoms include epilepsy and delayed mental development, which are similar to those of other complex congenital disorders such as rubella, CMV, and the herpes simplex virus (**Flegr *et al,* 2015**).

- **Laboratory Diagnosis**

Diagnostic examination is critical for early detection, transmission prevention, and determining treatment choices for infected pregnant women (**Pomares *et al,* 2016**). Diagnostic testing, which is not regularly conducted, includes maternal serum collection, early fetal ultrasonography, and amniocentesis (**Peyron *et al,* 2019**). Maternal examinations are performed periodically in high-risk settings, particularly in European countries (**Chaudhry *et al,* 2014**). Given that food intake has been demonstrated as a

substantial contributor to maternal infection, cultural practices including food preparation and consumption are a significant feature impacting the current transmission process (**Al-Adhroey *et al,* 2019**). However, in order to prevent congenital infection, pregnant women who are in the high-risk category for getting toxoplasmosis must be examined getting toxoplasmosis must be examined (**Bollani *et al,* 2022**). The initial diagnostic procedure for identifying both present and historical infections is serological testing (**Villard *et al,* 2016**). Serum markers identify the presence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in maternal blood samples (**Calderaro *et al,* 2008**). These antibody markers are indicative of *T. gondii* infection (**Liu *et al,* 2015**). There is no sign of infection in the mother if IgG and IgM markers are not found (**Montoya *et al,* 2008**).

Persistent infection was found if the test revealed a positive IgG antibody marker with a negative IgM signal (**Liu *et al,* 2015**). Acute infection is characterized by a significant rise in serum IgG antibody titer, which is typically at least four times the baseline level (**Landry *et al,* 2016**). In nations that use serum testing, blood samples will be collected for screening even if the mother tests negative. (**Guegan *et al,* 2021**). This screening technique allows for early diagnosis in the mother and acts as a starting point for therapy and prevention before the fetus suffers seroconversion (**Villard *et al,* 2016**). Amniocentesis can be performed on pregnant individuals whose toxoplasmosis tests are positive. (**Findal *et al,* 2017**) *T. gondii* parasite detection in amniotic fluid is required for the amniocentesis-based prenatal diagnosis of congenital toxoplasmosis (**Giannoulis *et al,* 2008**). Polymerase chain reaction (PCR) fluid testing is a prominent diagnostic method (**de Oliveira Azevedo *et al,* 2016**). The positive predictive value of PCR testing is approaching 100 percent (**Remington, 2004**). Historically, fetal blood samples obtained during cordocentesis were regarded as the gold standard of diagnostic testing. Cordocentesis is no longer used due to the increased fetal risk compared to amniocentesis (**Findal *et al,* 2017**).

Fetal ultrasonography was used in recent diagnostic trials (**Chaudhry *et al,* 2014**). Ultrasound is advised for women suspected or diagnosed with an acute infection (**Montoya *et al,* 2008**). Ultrasound can detect intracranial irregularities, including hydrocephalus, ventriculomegaly, and intracerebral calcifications (**Galvan-Ramírez *et al,* 2012**). Ultrasonography can detect splenomegaly, congenital nephrosis, and ascites in addition to toxoplasmosis (**McLeod *et al,* 2009**).

- **Treatment**

To treat toxoplasmosis, dihydropteroate synthetase and dihydrofolate reductase inhibitors are recommended (**Hopper *et al,* 2019**). Pyrimethamine is a dihydrofolate reductase inhibitor that has been shown to effectively cure this illness (**Ben-Harari *et al,* 2017**). This infection is also treated with drugs such as trimethoprim and sulfamethoxazole (**Rajapakse *et al,* 2013**). Almost all prescription drugs are more effective against the tachyzoite stage than cysts containing bradyzoites (**Dunay *et al,* 2018**). Cysts stay latent in the retina or uvea, and bradyzoite reactivation is common in immunocompetent patients (**Cerutti *et al,* 2020**). However, oocyst eradication with a reliable treatment has not been proven. To treat toxoplasmosis, which can sometimes injure the eyes, use pyrimethamine combined with clindamycin or sulfadiazine (**Konstantinovic *et al,* 2019**). Pyrimethamine and sulfadiazine are regarded to be effective in cases of immune system impairment. Sulfadiazine combined with pyrimethamine is an alternate treatment (**Castaño *et al,* 2022**). Medications such as pyrimethamine and trimethoprim-sulfamethoxazole are also recommended for treating this illness. (**Madi *et al,* 2012**). In order to prevent parasite infections of host cells in immunocompromised hosts, vaccination is also advised (**Barros, 2021**). Recombinant antitoxoplasma antibodies stop tachyzoite proliferation or early parasite invasion (**Ferra *et al,* 2020**). Although effective therapy has been reported, drug safety, duration of therapy, and drug potency are major concerns in the treatment of

Toxoplasma infections (**Alday *et al,* 2017**). Spiramycin is helpful for expecting mothers up until the 16th week of pregnancy, and different combinations of sulfadiazine, pyrimethamine, and folinic acid are advised (**Damar Çakırca *et al,* 2023**). Spiramycin is frequently used to reduce mother-to-child transmission (**Montoya *et al,* 2021**). Pyrimethamine and sulphadiazine are used to treat neonatal infections (**Prasil *et al,* 2023**). However, the drug's efficacy remains unknown. Pyrimethamine, sulfadiazine, and corticosteroids have been shown to be particularly effective in treating ocular toxoplasmosis (**Jasper *et al,* 2017**).

2.1.4.7.2 Rubella virus

- **Introduction**

Rubella is caused by a virus in the *Togaviridae* family, genus *Rubivirus*. It is a benign disease characterized by a macular rash accompanied by low fever, joint pain, pharyngitis and cervical adenopathies. The infection can in some cases be asymptomatic, although it can become severe when it occurs during pregnancy (**Lambert *et al,* 2015**). The teratogenic properties of the rubella virus were first discovered in Australia in 1941 by Gregg who associated the occurrence of rubella during pregnancy with the presence of congenital cataracts (**Bukase *et al,* 2018**).

The most common form of transmission is through direct contact with droplets with the respiratory secretions of infected people. In addition, transmission can also be Congenital (**karacan *et al,* 2014; Yeshwondm *et al,* 2016**). The severity of fetal infection is related to the time of pregnancy in which maternal infection occurs, being more sever in the period of organogenesis (1st trimester of pregnancy), due to the high tropism of the virus to fetal tissues (**Yeshwondm *et al,* 2016**). *Rubella* infection in pregnant women can cause devastating results, such as spontaneous abortion, fetal death and birth defects (**Rasti *et al,* 2016**). If the primary infection is contracted in the first three months, the likelihood

of the appearance of "Congenital Rubella Syndrome (CRS)" is high (**Karacan *et al,* 2014; Rasti *et al,* 2016**). A newborn (NB) with CRS may present major malformations (cataracts, congenital glaucoma, heart disease, deafness, microcephaly, retinopathy), minor type (purple, thrombocytopenia, jaundice splenomegaly in the first 24 hours of life) or present with jaundice asymptomatic condition at birth, in which later clinical manifestations such as partial deafness or psychomotor delay may appear (**Karacan *et al,* 2014**).

CRS remains a public health problem in a significant number of countries. Therefore, global health experts encourage the use of rubella vaccination, with the main objective of preventing CRS (**MARTINEZ-Quintana *et al,* 2015; Winter *et al,* 2018**). *Rubella* is a vaccine-preventable infection and is considered potentially eradicable. As a result of the vaccination program in many high-income countries and in some low- and middle-income countries, the estimated number of CRS cases has declined globally from around 119000 cases in 1996 to around 105000 cases in 2010 (**Vynnycky *et al,* 2016; Tamirat *et al,* 2017**). The scale vaccination program in the Americas and Europe has managed to dramatically reduce or eliminate both the virus and CRS. In contrast, the highest risk of CRS is found in countries where the rubella-containing vaccine (VCR) has not been introduced in the national immunization program or vaccination coverage is low (**Tamirat *et al,* 2017**).

There is a growing recognition of the importance of going beyond national analyzes and considering heterogeneity within countries to assess public health interventions with VCR (**Plotkin *et al,* 2014; Winter *et al,* 2018**). High acceptance can disrupt endemic rubella transmission and prevent CRS cases, as demonstrated by the World Health Organization (WHO) (**Su *et al,* 2018; Winter *et al,* 2018**). In contrast, countries in the WHO regions of Southeast Asia and Africa, which have been the slowest to add VCR to their national vaccination programs, have the highest incidence of CRS, suffering 84% of

the estimated 105.000 global incidence cases of CRS in 2010 (**Vynnycky *et al,* 2016; Winter *et al,* 2018**).

- **Clinical manifestations**

Rubella is characterized by a diffuse maculopapular and punctiform rash, which begins on the face, scalp and neck, and subsequently spreads to the entire body. Low fever and the presence of retroarticular, cervical and occipital lymphadenopathy, which usually precede the rash (5 to 10 days) are signs that contribute to the differential diagnosis in relation to other rash diseases (**lambert *et al,* 2015; Getahum *et al,* 2016**). In general, a prodromal period is not observed in children with rubella. Adolescents and adults may present prodromes with low fever, headache, generalized pain (arthralgia and myalgia), conjunctivitis, runny nose and cough. About 25% to 50% of rubella virus infections are subclinical. Viremia occurs for about 7 days before the rash appears which disappears when the humoral immune response begins to develop (**Grangeot-keros *et al,* 2014; Getahum *et al,* 2016**).

The main concern represented by *Rubella* is its teratogenicity, with maternal infection in early pregnancy leading to CRS in children (**George *et al,* 2019**). There is more than an 80% risk of birth defects when viral infection is acquired in the first 12 weeks of pregnancy (1st trimester) (**Boncoiran *et al,* 2018**). The time when the infection occurs during pregnancy can influence the outcome. The earlier in pregnancy the maternal infection occurs, the more severe the damage to the fetus. The risk of fetal infection and the severity of congenital abnormalities decreases after the first trimester; after 17 weeks of gestation, the risk of developing any defects is low (**Boncoiran *et al,* 2018**).

The effects of *Rubella* infection in pregnancy are unpredictable, ranging from normal birth, spontaneous abortion, death shortly after birth or even birth with simple or combined abnormalities, such as damage to the central nervous system, leading to delayed physical

growth and mental, microcephaly, encephalitis, hepatomegaly, cardiac malformations, pneumonia, eye and hearing defects (**Yamamota *et al,* 2017**). Insulin-dependent diabetes mellitus commonly occurs as a late sequel to CRS, and defects such as deafness may not be detected initially. CRS cases have been reported after maternal reinfection, although this appears to be a rare phenomenon (**Grant *et al,* 2018**). As with primary rubella infection, gestational age at the time of reinfection influences the likelihood of fetal abnormalities. No case of *Rubella* reinfection that causes CRS has been reported after 12 weeks (**Kaushik *et al,* 2018**).

- **Laboratory Diagnosis**

Diagnosis of *Rubella* virus infection is generally done using the serological tests. IgM antibodies usually develop within 4–5 days after the onset of the infection and may remain positive up to 6 months and rarely for a much longer duration (**Singh, 2011; McLeane *et al,* 2013**). The serological diagnostic algorithm is by and large similar to Toxoplasmosis, except that false anti-Rubella IgM positivity can also be seen in recently vaccinated cases. Therefore, history of vaccination is most crucial to interpret the test results. IgG avidity test plays very important role in pinpointing the time of infection (**Singh, 2011)**. The Enzyme-Linked Immunosorbent Assay (ELISA) is sensitive, widely available and relatively easy to perform, and can also be modified to measure IgM antibodies. Rubella 2019. During prenatal care, it is recommended to request maternal serology for rubella in the first consultation, with the aim of knowing the immune status of the pregnant woman (**Hubschen *et al,* 2017**).

For amniotic fluid, CSF and other body fluids, PCR methods are available, but only few laboratories do these molecular tests due to easy availability and high sensitivity of serological methods. Although virus isolation is a diagnosis of rubella infection, viral cultures are laborious and therefore are not done in many laboratories; they are generally

not used for the routine diagnosis of *Rubella*. However, viral isolation is an extremely valuable epidemiological tool and should be used in all suspected cases of rubella or CRS (**Bouthry *et al,* 2014**).

- **Treatment**

Currently, there is no specific treatment for *Rubella*. The signs and symptoms presented must be treated according to the symptomatology and appropriate therapy. The most important thing, however, will be to promote control and minimize possible sequelae in case of teratogenic effects, with interdisciplinary monitoring (**Hui *et al,* 2017).**

2.1.4.7.3 Human cytomegalovirus

- **Introduction**

Cytomegalovirus (CMV) is a double-stranded DNA virus and is a member of the *Herpesviridae* family. Infection with CMV is ubiquitous, infecting approximately half of the population in high-income countries by adulthood and nearly everyone by an early childhood in low- and middle-income countries (**Manicklal *et al,* 2013**). CMV infection passes undetected in healthy children and adults. However, several high-risk groups, including immunocompromised organ transplant recipients, hematopoietic stem cell transplant recipients, and individuals infected with human immunodeficiency virus (HIV), are at risk of developing life-threatening and sight-threatening CMV disease (**Buxmann *et al,* 2017**). CMV is also a major cause of morbidity and occasional mortality in neonates. In recent years, it has become evident that congenital CMV (cCMV) infection is the most common congenital viral infection, with an estimated birth prevalence of 0.2–6% in industrialized countries (**Kalser *et al,* 2017**), while limited studies in developing countries (Asia, Africa, Latin America) have shown a prevalence ranging from 0.6% to 6.1% (**Kenneson and Cannon, 2007**). cCMV contributes to a high burden of disease and is the

leading non-genetic cause of sensorineural hearing loss (SNLH) and an important cause of neurodevelopmental disabilities in children (**Lanzieri *et al,* 2014**).

Intrauterine CMV transmission may occur in mothers without pre-existing immunity who first acquired CMV infection in pregnancy (primary infection) or in women with pre-existing antibodies to CMV either by reactivation of a previous maternal infection or by the acquisition of a different viral strain (non-primary infection) (**Barton *et al,* 2020**). The risk of intrauterine transmission is highest when primary infection occurs during pregnancy, with a higher rate of vertical transmission in mothers with older gestational age at infection, while the risk of adverse fetal effects significantly increases if fetal infection occurs during the first half of pregnancy (**Enders *et al,* 2011**).

An important determinant of cCMV is the prevalence of maternal CMV infection in the population. Among low seroprevalence populations, one-half to three-quarters of all congenital infections among newborns are due to non-primary infection during pregnancy, whereas in populations with high maternal seroimmunity, almost all congenital infections occur as a result of non-primary infection. Therefore, countries with high seroprevalence have high rates of congenital infection, even though the risk of infecting the fetus is higher in cases of primary infection (**Singh and Pandit, 2004**). Moreover, some progress has been made in treating symptomatic newborns with cCMV. This narrative review aims to explore the latest developments in the diagnosis and treatment of congenital CMV infection (**Chiopris *et al,* 2020**).

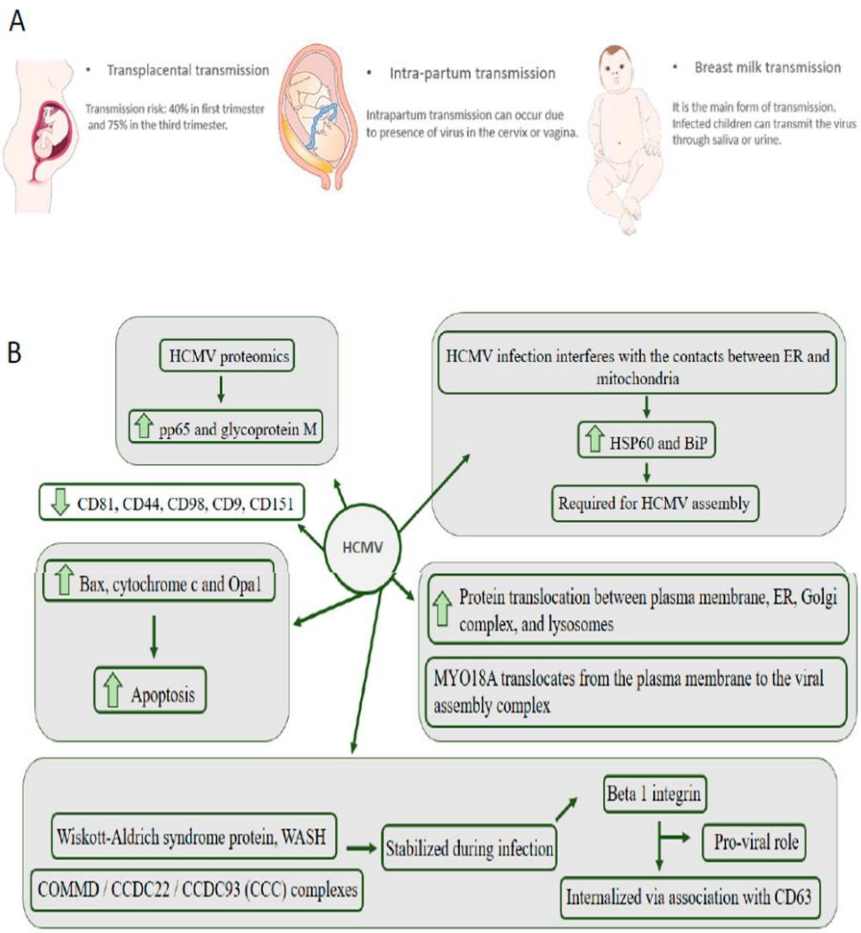

Figure 2. Main forms of maternal–fetal transmission of cytomegalovirus (**A**). Main findings indicated by proteomic studies that explore the proteome of human cytomegalovirus (HCVM) infection (**B**) (**Macedo-da-Silva *et al*, 2020)**.

- **Clinical Manifestation**

Congenital infection may be classified as symptomatic or asymptomatic, even though there is no clear definition of these two classifications in the literature. In general, moderately to severely symptomatic cCMV is defined as infants who are infected and who have multiple manifestations or central nervous system (CNS) involvement; mildly symptomatic cCMV is defined as infants who have one or two isolated manifestations that

are mild and transient; asymptomatic cCMV with isolated sensorineural hearing loss (SNHL) is defined as infants who have no apparent clinical symptoms other than hearing loss; asymptomatic cCMV is defined as infants who have no apparent abnormalities at birth and have normal hearing (**Foulon *et al,* 2008**). Among congenitally-infected infants, approximately 10–15% have signs and symptoms of the disease at birth, and approximately half experience long-term sequelae (**Thigpen, 2020**). Among asymptomatic infants with cCMV, an estimated 10–15% will develop long-term sequelae. The most common long-term sequela is hearing loss (**Goderis *et al,* 2014**).

CCMV infection is the leading non-genetic cause of SNHL in children across studies. Among infants who develop CMV-related SNHL, hearing loss may be present at birth or may have delayed onset, occurring throughout the first several years of life. Approximately 50% of children with SNHL experience further deterioration or progression of their loss during childhood, and the degree of hearing loss may fluctuate in up to half of the infants (**Dahle *et al,* 2000**). Therefore, it is important that all infants with cCMV infection, irrespective of their clinical presentation at birth, receive serial audiological monitoring throughout the first years of life to promptly detect possible SNHL to proceed with non-pharmacological interventions that can reduce the functional impairment resulting from hearing loss, significantly improving the receptive and expressive language and the social-emotional development of the affected child (**Chiopris *et al,* 2020**).

Furthermore, cCMV is the leading viral cause of neurodevelopmental delay, with a large proportion of symptomatic infants suffering from some degree of psychomotor and cognitive disabilities and with visual impairment presenting in up to half of the symptomatic infants. As many affected children require significant ongoing care and special therapeutic and educational services, the economic burden associated with congenital CMV infection is substantial (**Lim and Lyall, 2017**).

- **Laboratory Diagnosis**

Due to very high seroprevalence of CMV in general population, diagnosis and especially the interpretation of test results is very complex and not straight forward as in the case of Rubella. The commonest method for the diagnosis of maternal infection is CMV IgM positive or very high IgG antibody titers (**Singh *et al,* 2014**). The presence of IgM antibodies in the foetal or cord blood is a very sensitive and specific test for the detection of congenital CMV infection. The CMV avidity test is also available to determine if the infection is recently acquired. A low avidity indicates recent infection and high avidity indicates past infection. Highly sensitive and most specific PCR methods are available, but only few laboratories do these molecular tests due to easy availability and high sensitivity of serological methods (**Batra *et al,* 2020**).

- **Treatment**

Ganciclovir or valganciclovir for symptomatic neonates

Symptomatic neonates are given antiviral drugs. Oral valganciclovir 16 mg/kg 2 times a day for 6 months decreases viral shedding in neonates with congenital CMV and modestly improves hearing and developmental outcomes at 12 and 24 months of age (**Revello *et al,* 2014**). In utero treatment of affected fetuses with acyclovir or valacyclovir may also improve neonatal outcomes, but large studies are lacking. The main toxicity of treatment is neutropenia (**Kimberlin *et al,* 2015**).

2.1.4.7.4 Herps viruses

- **Introduction**

Herpes Simplex viruses (HSV) belong to a Herpesviridae and consists of two viruses, HSV-1 and 2, which resemble each other at the molecular level and also in their clinical manifestations (**Munawwar and Singh, 2016**). It has been classically described that HSV-1 causes lesions above the belt while HSV-2 causes lesions below the belt.

However, recent studies (**Ayoub *et al,* 2019**) show changing pattern of clinical manifestations especially in HIV infected individuals. The risk of transmission from pregnant women to an infant in individuals with primary genital herpes is 33–50% whereas in recurrent maternal infection is only 1–3%. As per the WHO 2012 report (**Looker *et al,* 2015**), nearly 417 million people worldwide were seropositive for HSV-2. A recent study published in India shows that HSV-1 and HSV-2 infections are prevalent in 40% and 25.9% of adult males (**Munawwar *et al,* 2018**).

- **Clinical Symptoms**

Primary infection of HSV-1 usually spreads in the system of the host without showing visible symptoms. But when the immune system initiates suppression of the virus, the symptoms vary from children to adults e.g. formation of blisters around lips orfever usually occurs among children whereas adults may have sore throats or swelling of cervical lymphnode (**Opstelten and Neven, 2008**). Symptoms may cling to the patients for up to a couple of weeks and change form through a range of phases. Initially, itching and the inflammation occur at thesite of infection which may wind upthe formation of blisters or mini-papules around the site of infection (**Emmert, 2000**). Subsequently, them in i-papules fuse and for the larger blister which is highly itchy and aching either. Thereafter, renewal of the skin takes place beneath the scrabies that result in the formation of Meier complex later on which feel pain and highly itchy as well (**Johnston and Sladden, 2005**).

Recurrent viruses are sometimes highly critical to be observed because,they can be asymptomatic for weeks to months and the symptoms they createinitially after infection,like itching or blister formation,are sometimes ignored by the patient sconfusing with some allergic reactions or other minor infections. Moreover, furtherstudy is needed regarding the characteristics and genetics of HSV-1 to achievev much more precise observation of the clinical representation (**Johnston and Sladden, 2005**).

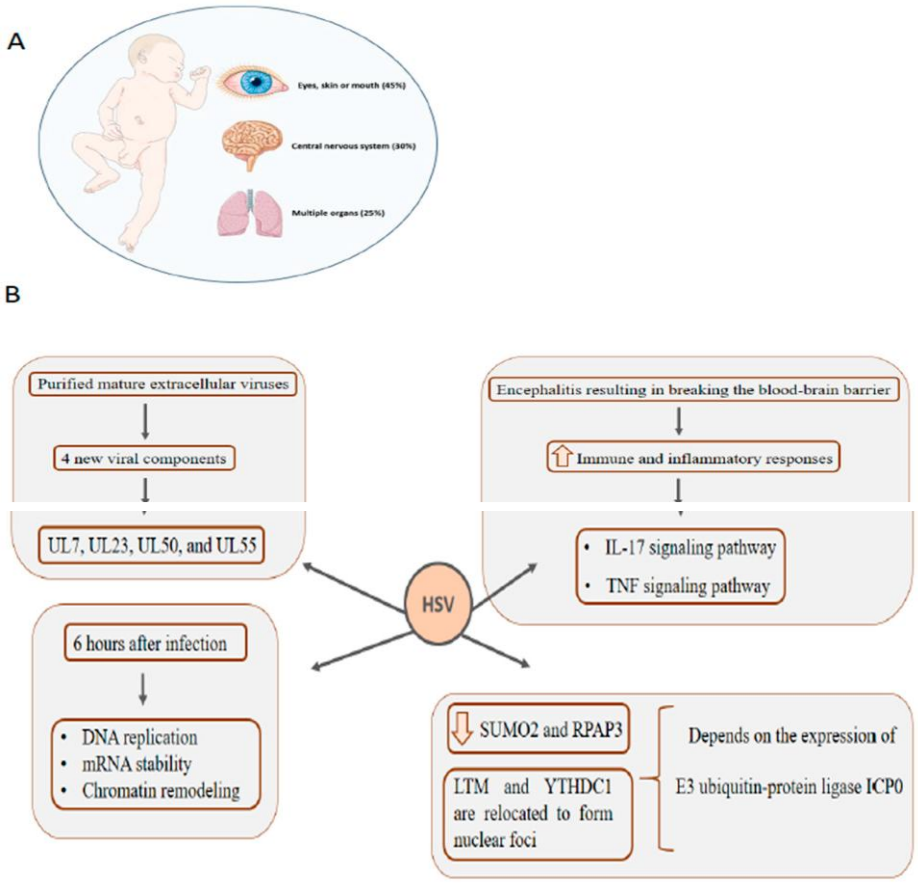

Figure 4. Congenital HSV infection can affect multiple organs, the central nervous system (CNS) and eyes, mouth and skin of newborns. Medical treatment is essential to avoid severe and irreversible damage (**A**). Main findings indicated by proteomic studies that explore the proteome of HSV infection (**B**) (**Macedo-da-Silva *et al*, 2020)**.

- **Laboratory Diagnosis**

Nearly 90% of the women with genital herpes due to HSV- 1 or HSV-2 are underdiagnosed as they are asymptomatic or their symptoms are incorrectly attributed to other vulvovaginal disorders (**Cowan *et al*, 2003**). Thus, a negative maternal history of HSV does not dissuade the clinicians from considering the possibility of neonatal herpes in infants with compatible signs and symptoms. All individuals with HSV-2 seropositivity are at high risk of transmitting infection during sexual activity, labour, delivery and intimate contact (**Deka *et al*, 2006**). Thus, all women should be screened serologically for HSV-1

and 2 antibodies and women with genital lesions at the time of delivery must be counselled for caesarean delivery, antiviral suppressive therapy and minimal invasive intrapartum procedures. Single-plex and multiplex PCR methods are available which can detect and differentiate HSV-1 and HSV-2 but only few laboratories do these molecular tests (**Batra *et al,* 2020**).

- **Treatment**

As a persistent virus, HSV1 causes life time latency in the host and recurrent labial ulcer lesions occur whenever the virus is reactivated by several factors (eg. radiation, stress, etc). The virus cannot be cured, however proper treatments can lower the degree of the labial lesion and oral infection caused by the viral population. The approaches for preventive measures are aimed to suppress the viral DNA replication either by the admission of nucleo side an alogs (eg. Acyclovir or ACV, Acyclovir monophosphate, Acyclovir triphosphate valacyclovir, penciclovir) or helic aseprimase inhibitors (eg. Amenamevir. Pritelivir) (**Mohseni *et al*, 2018**).

2.1.4.8 Lifestyle factors of recurrent pregnancy loss

Lifestyle variables are typically not recognized as particular causes of RPL, although they have been linked to an increased risk of miscarriage in general. These include stress, BMI, alcohol consumption, caffeine consumption, and cigarette smoking (**PCAS, 2012**).

A- Smoking and recurrent pregnancy loss

B- Caffeine and recurrent pregnancy loss

C- Alcohol Use and recurrent pregnancy loss

D- BMI and recurrent pregnancy loss

E- Stress and recurrent pregnancy loss

F- Endocrine disorders and recurrent pregnancy loss

G- Luteal phase defect and recurrent pregnancy loss

H- Thrombophilia and recurrent pregnancy loss

I- Immune factors and recurrent pregnancy loss

J- Antiphospholipid syndrome and recurrent pregnancy loss

K- Thyroid antibodies and recurrent pregnancy loss

L- Increased uterine NK cells and recurrent pregnancy loss

M- Male-specific minor histocompatibility and recurrent pregnancy loss

N- Infection and recurrent pregnancy loss

2.1.4.9 Assessment of pregnancy

Nowadays, transvaginal ultrasonography is the main technique used to evaluate the health of an unborn child. The following tests are typically carried out on non-pregnant patients who are assessed for recurrent pregnancy loss. After two or three miscarriages, parental chromosomal testing (karyogram) is typically advised. Blood tests are conducted for diabetes, thyroid, ovarian, and thrombophilia and TORCH infection (**Giakoumelou *et al,* 2016**).

2.1.4.10 Treatment of RPL

Treatment should be tailored to the likely cause of recurrent pregnancy loss if it can be identified. Anticoagulants appear to raise the live birth rate among pregnant women with a history of recurrent miscarriages in those with antiphospholipid syndrome and perhaps congenital thrombophilia, but not in those with unexplained recurrent miscarriages. According to one study, "after appropriate antibiotic treatment (**Cicinelli *et al,* 2014**),

fertility was restored in many women with chronic endometritis." According to study, some prenatal counseling and psychological support may increase the likelihood of a successful pregnancy for women who experience unexplained recurrent pregnancy loss. According to certain studies, early prenatal ultrasonography and psychological assistance "gives'success rates' of between 70% and 80%" for these patients (**Brigham *et al,* 1999)**.

Every further loss, however, raises the mother's physical and mental dangers and decreases her chances of a successful pregnancy. For women who experience unexplained recurrent pregnancy loss, aspirin has little effect on preventing repeated miscarriages. There is no evidence that immunotherapy is helpful. In vitro fertilization combined with preimplantation genetic diagnosis may be able to identify embryos with a lower risk of future pregnancy loss, which would subsequently be transferred, even if therapy may not be available in some chromosomal conditions. However, maternal-fetal tolerance imbalances are not improved by in vitro fertilization (**Wong *et al,* 2014**).

2.1.4.11 Psychological Impact of RPL

Experiencing pregnancy loss can have a significant and sometimes prolonged psychological impact, including higher levels of anxiety, stress, and depression. There is evidence that women who experience recurrent pregnancy loss in particular may be more severely affected (**Inversetti *et al,* 2023**). Grief is a normal and expected response to pregnancy loss. However, prolonged and intense grief can be extremely distressing and detrimental to an individual's mental health. This is particularly noticeable in women who have developed maladaptive coping mechanisms after a miscarriage, have isolated themselves due to cultural and societal stigma, or have received inadequate social support from medical providers, partners, families, and other personal relationships (**Ho *et al,***

2022). In heterosexual couples, men also experience grief as a result of pregnancy loss and have reported feeling obligated to ignore their feelings in order to support their partner. The psychological effects of recurrent pregnancy loss on the emotional and mental well-being of the father have not been widely studied, however, there is emerging research exploring this further. According to a recent meta-analysis comparing the psychological impact of men and women with a history of recurrent anorexia nervosa, women were found to have higher levels of moderate to severe depression, stress, and anxiety than women without recurrent anorexia nervosa and men with recurrent anorexia nervosa (**Inversetti *et al,* 2023**).

Given the impact that RPL can have on the mental health and psychological well-being of couples, mental health assessment, anxiety/depression screening, and treatment may be considered (**Inversetti *et al,* 2023**). There is also emerging research suggesting that untreated depression and depressive symptoms can lead to negative outcomes in future pregnancies such as preterm birth and low Apgar scores (**Vlenterie *et al,* 2021**). Consequently, there has been an increase in the use of antidepressants (i.e., selective serotonin reuptake inhibitors) during pregnancy over the past few years with a prevalence of 1–8%. This decision should be made under the guidance of a healthcare provider given the potential teratogenicity and adverse effects of antidepressants on the fetus (**Vlenterie *et al,* 2021**). For those who have recurrent pregnancy loss, psychological care may involve counseling and other supportive services in addition to psychotherapy. According to the Guidelines for Medical Professionals Providing Care to the Family Experiencing Perinatal Loss, Neonatal Death, SIDS, or other Infant Death, women who received bereavement counseling after a pregnancy loss reported 50% less despair than those who did not receive this intervention, suggesting that these women were better able to cope (**Ho *et al,* 2022**).

2.1.4.12 Prognosis in RPL

Recurrent miscarriages alone are linked to an odds ratio of roughly two for the later development of coronary artery disease, as well as an increased risk of cardiovascular complications, ovarian cancer, and all-cause mortality of 44%, 86%, and 150% for women who have had one, two, or three miscarriages, respectively. In subsequent pregnancies, women with a history of repeated miscarriages are susceptible to preeclampsia (**Oliver-Williams *et al,* 2012).**

2.2 Objectives of the study

2.2.1 General Objective of this study was:

To Determine the prevalence of TORCH Infection among pregnant women with recurrent abortion in Sana'a City, Yemen.

2.2.2 Specific Objectives

The specific objectives of this study were to:

1. Determine the prevalence of IgM and IgG of TORCH among pregnant women with recurrent abortion.
2. Determine the risk factors among pregnant women with recurrent abortion.
3. Determine the type of ABO blood grouping and RH factors among pregnant women with recurrent abortion
4. Investigate the relation between risk factors and IgM TORCH infection.

Chapter 3

Subjects & Methods

3. Subjects and Methods

3.1 Subjects

3.1.1 Study design

This study was a cross sectional study.

3.1.2 Study area and design

A cross-sectional study was carried out among 200 women with recurrent pregnancy loss in Sana'a City from October 1st, 2024, to the end of December 2024 whom attending government and private clinics for investigation the recurrent pregnancy loss. The laboratory works was performed at the Emirates International University and Department of serology in AL-Zahrawi Laboratories Centre, Sana'a city, Yemen.

3.1.3 Sample size

The sample size was statistically calculated for a cross sectional study by the Epi Info statistical program version 26 (CDC, Atlanta, USA). According to the following facts: 200 pregnant women with recurrent abortion cases.

3.1.4 Data analysis

Individual data were collected in a pre-designed questionnaire, including, clinical data, demographic data, risk factors of RPL and laboratory results.

3.2 Methods

3.2.1 Specimen collection

Five ml of venous blood was collected from each individual into EDTA tube, whole blood was used for blood group test. The specimens in EDTA tube was centrifuged at 3500 rpm for five minutes. plasma was separated from each sample Eppendorf tubes and stored at - 20°C until tested for TORCH test.

3.2.2 Laboratory analytical methods

3.2.2.1 Immunochromatography (cassette) test

- **Principle**

Rapid test is antibodies lateral Flow Chromatography Immunoassay of qualitative detection of antibodies (IgM and IgG) in human plasma for TORCH. Mf (SAFECARE-Bio-TECH) (**A ppendix 3**).

(SAFECARE- Bio-TECH)

3.2.3 ELISA kit

Confirmatory test for positive result for TORCH test.

- **Principle**

plasma was analyzed for IgG (Immunoglobulin G) and IgM (Immunoglobulin M) antibodies against TORCH agents using a commercially available ELISA kit (For **cobas e** 411 analyzer: test number 550] following the manufacturer's instruction (**COBAS, COBAS E, ELECSYS and PRECICONTROL are trademarks of Roche. INTRALIPID is a trademark of Fresenius Kabi AB.© 2021, Roche Diagnostics).**

μ-Capture test principle. Total duration of assay: 18 minutes.

· 1st incubation: 10 μL of sample are automatically prediluted 1:20 with Diluent Universal. Biotinylated monoclonal anti-human IgM-specific antibodies and Toxoplasma-specific recombinant antigen are added and react with anti-Toxoplasma- IgM antibodies present in the sample to form a complex.

· 2nd incubation: After addition of ruthenium-labeleda) TORCH (Toxo-Rubella-CMV-HSV) specific antibodies and streptavidin-coated microparticles the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

· The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

・Results are determined via a calibration curve which is instrument specifically generated by 2‐point calibration and a master curve provided via the reagent barcode or e‐barcode.

・Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration (Method Appendix 2).

a) Tris(2,2'-bipyridyl) ruthenium (II)-complex (Ru(bpy)) (**Appendix 3**).

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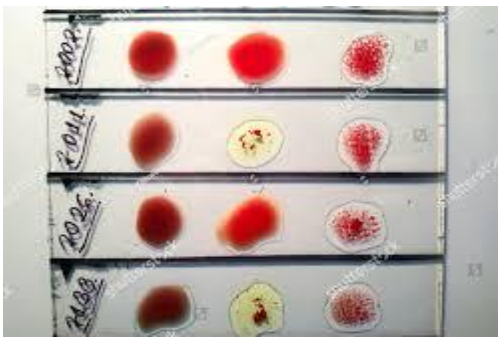
3-2-2 Blood group test

Principle

Anti-A, Anti-B and Anti-AB reagents (here under referred to as ABO reagents) are used for qualitative in-vitro determination of human blood groups of the ABO system to determine the blood type. Anti-D reagent is used for the qualitative determination of Rhesus factor on human blood groups. These reagents are intended to be used in slide and

tube methods. The test procedure is based on agglutination principle, where red cells possessing the antigen agglutinate in the presence of the corresponding antibody indicating that the result is positive. The test is considered negative when no agglutination appears. Anti-D is not colored. The procedure is based on agglutination principle, where red cells' possessing the antigen agglutinates in the presence of the corresponding antibody in the reagent indicating that the result is positive. The test is considered negative when no agglutination appears (**ATLAS Medical GmbH, Ludwig-Erhard Ring 3, 15827, Blankenfelde-Mahlow, Germany**) (**A ppendix 3**).



(ATLAS Medical GmbH, Ludwig-Erhard Ring 3, 15827, Blankenfelde-Mahlow, Germany)

3.3 Statistical analysis

All data was collected then analyze using statistical software (SPSS Version 26; SPSS Inc, Chicago, USA). The quantitative data will be expressed as mean values, Standard deviation (SD) when the data was normal distributed or expressed as median and range when the data was normal distributed. The qualitative data was expressed as percentages, chi-square (χ^2) test will be used for comparison of two variables to determine the *p value* and odd ratio (OR) was used with 95% confidence interval. *P* values <0.05 will be considered statistically significant. All test was performed with a 95% statistically significant or confidence interval (CI).

3.4 Ethical considerations

The study proposal was approved by the Ethical Committee of the Faculty of Medicine and Health Sciences, international Emirate University, Yemen.

Chapter 4

Results

4. Results

The study was considered a cross sectional study. A total of 200 blood samples were collected from pregnant women with a history of recurrent abortion in Sana'a city Yemen, of whom 159 subjects had TORCH infection, the rest 41 showed non infected.

All samples were tested for TORCH routine screen test for pregnant women.

Table 4.1: The distribution of TORCH infection among pregnant women with recurrent abortion in Sana'a city, Yemen

TORCH Infection	Number	Percentage
Infected	**159**	**79.5**
Non-infected	**41**	**20.5%**
Total	**200**	**100**

Table 4.1 The distribution of TORCH infection among pregnant women with recurrent abortion in Sana'a city, Yemen. The infected women with TORCH was 1591 (79.5%) and non-infected was 41 (20.5%).

Table 4.2: The rate of different infectious causes of TORCH among pregnant women with recurrent abortion in Sana'a city, Yemen

Causes	IgG		IgM	
	Number	Percentage	Number	Percentage
Toxoplasmosis	39	24.5	31	19.5
Rubella virus (RV)	30	18.9	11	6.9
Cytomegalovirus (CMV)	41	25.8	2	1.3
Herpes simplex virus 2 (HSV2)	41	25.8	25	15.7
Total	151	94.97	69	43.4

Table 4.2 shows the incidence of different infectious causes of TORCH among 159 pregnant women with recurrent miscarriage in Sana'a city, Yemen. IgM to Toxoplasma gondii was positive in 31 (19.5%) of the women with RPL, IgM to rubella virus was positive in 11 (6.9%), while IgM to cytomegalovirus was recorded in only 2 (1.3%). In addition, IgM to herpes simplex virus 2 (HSV2) was recorded in 25 (15.7%) of the total patients.

Table 4.3: Age distribution of pregnant women with recurrent abortion in Sana'a city, Yemen

Characters	Number	Percentage
Age in Years		
Less than 21 years	**21**	**13.2**
21 - 30 years	**76**	**47.7**
31 – 40 years	**50**	**31.4**
More than 40 years	**12**	**7.5**
Mean	**29.7 years**	
SD	**7.9**	
Median	**29**	
Mode	**30**	
Min to Max	**18-50**	
Total	**159**	

Table 4.3 shows the age distribution of 159 pregnant women with recurrent abortion in Sana'a city, Yemen. The mean age of RPL women was 29.7 years with SD equal to 7.9 years, and ranged from 18 to 50 years. Most women with RPL was in age group 21-30 years counting 47.7% followed by age group 31-40 years counting 31.4%.

Table 4.4: The distribution of blood group among pregnant women with recurrent abortion in Sana'a city, Yemen

Blood groups	Number	Percentage
A	**39**	**24.5**
B	**14**	**8.8**
AB	**8**	**5.1**
O	**98**	**61.6**
Total	**159**	**100**

Table 4.4 shows the distribution of blood group and Rh factor among 159 pregnant women with recurrent miscarriage in Sana'a city, Yemen. The majority of women were of blood group O (61.6%), followed by blood group A (24.5%), while blood groups B and AB were less frequent (8.8% and 5.1%, respectively).

Table 4.5: The distribution of Rhesus factor among pregnant women with recurrent abortion in Sana'a city, Yemen

Blood groups	Number	Percentage
Rhesus factor		
Positive	**139**	**87.4**
Negative	**20**	**12.6**
Total	**159**	**100**

Table 4.5 shows the distribution of Rh factor among 159 pregnant women with recurrent miscarriage in Sana'a city, Yemen. Regarding Rh factor, 87.4% of women were positive while only 12.6% were Rh negative.

Table 4.6: The distribution of risk factors for pregnant women with recurrent pregnancy loss **(RPL)** in Sana'a city, Yemen

Risk Factors	Number	Percentage
Family recurrent abortion history	**41**	**16.9**
Animals in the house hold	**28**	**11.5**
Medicine during pregnancy	**45**	**18.5**
Uterus disorder	**38**	**15.6**
Non-vaccinated for Rubella	**57**	**23.5**
Blood transfusion history	**21**	**8.6**
Own handicapped children	**13**	**5.4**

Table 4.6 shows the distribution of risk factors for pregnant women with recurrent pregnancy loss (RPL) in Sana'a city, Yemen. History of recurrent miscarriage in the family was a risk factor for RPL in 16.9% of women, animals in the family was a risk factor for toxoplasmosis (11.5%), use of medications during pregnancy was a risk factor for RPL in 18.5%, uterine disorder was a risk factor for RPL in 15.6% of women in our study, non-vaccination against rubella (23.5%), history of blood transfusion as a source of viral infection leading to RPL was very common with 8.6% of total females, and Own handicapped children were 5.4% among the tested females.

Table 4.7: Associated risk factors for Toxoplasmosis (IgM) in pregnant women with recurrent pregnancy **loss (RPL)** women in Sana'a city, Yemen

Risk Factors	Positive Toxoplasmosis (IgM) 31/159		OR	CI	χ^2	*p*
	No.	%				
Family recurrent abortion history n=41	9	22	1.2	0.4-2.6	0.06	0.79
Animals in the house hold n=28	3	10.7	1.4	0.35-5.7	0.24	0.6
Medicine during pregnancy n=45	14	31.1	2.6	1.2-6	**5.6**	**0.01**
Uterus disorder n=38	9	23.7	**1.8**	0.8-4.1	1.7	0.19
Non-vaccinated for Rubella n=57	15	26.3	1.7	0.8-3.8	1.9	0.17
Blood transfusion history n=21	2	9.5	0.36	0.08-1.6	1.8	0.17
Own handicapped children n=13	6	23.1	**4.5**	1.4-14.4	**7.2**	**0.007**
Total	58/159 (36.5)					

OR Odds ratio= >1(there is a risk), **CI** Confidence interval, χ^2 Chi-square= >3.8 (**significant**) and *p* Propagability Value= <0.05 (**significant**).

Table 4.7 shows the associated risk factors for toxoplasmosis in recurrent pregnancy loss **(RPL)** women in Sana'a city, Yemen. There was no significant association between Animals in the house hold and toxoplasmosis infection in which odds ratio was 1.4, with CI equal to 0.35-5.7 with *p* value equal to 0.6, but there was significant association between medicine during pregnancy and toxoplasmosis infection in which odds ratio was 2.6, with CI equal to 1.2-6 with *p* value equal to 0.01.and but there was significant association between Own handicapped children and toxoplasmosis infection in which odds ratio was 4.5, with CI equal 1.4-14.4 with *p* value equal to 0.007.

Table 4.8: Associated risk factors for Rubella (IgM) in pregnant women with recurrent pregnancy loss **(RPL)** women in Sana'a city, Yemen

Risk Factors	Positive Rubella IgM 11/159		OR	CI	χ^2	*p*
	No.	%				
Family recurrent abortion history n=41	3	7.3	1	0.2-3.9	0.001	0.99
Animals in the house hold n=28	2	7.1	1.0	0.2-3.9	0.001	0.99
Medicine during pregnancy n=45	1	2.2	0.21	0.02-1.7	2.4	0.11
Uterus disorder n=38	3	7.9	1.0	0.2-3.5	0.001	0.99
Non-vaccinated for Rubella n=57	2	3.5	0.3	0.07-1.6	1.9	0.16
Blood transfusion history n=21	2	9.5	1.4	0.28-7.1	0.18	0.67
Own handicapped children n=13	2	15.4	2.6	0.5-13.5	1.4	0.23
Total	11/159 (6,9)					

OR Odds ratio= >1(there is a risk), **Cl** Confidence interval, χ^2 Chi-square= >3.8 (**significant**) and p Propagability Value= <0.05 (**significant**).

Table 4.8 shows the risk factors associated with rubella (IgM) in pregnant women with recurrent pregnancy loss (RPL) in Sana'a City, Yemen. There was no significant association between blood transfusion and current rubella infection with an odds ratio (**OR**) of 1.4 with 0.28–7.1, χ^2 = 0.18, p value 0.67. Non-vaccination against rubella was not a risk factor for rubella IgM.

Table 4.9: Associated risk factors for Herpes simplex virus 2 (HSV2) (IgM) in pregnant women with recurrent pregnancy loss (RPL) women in Sana'a city, Yemen

Risk Factors	Positive HSV2 (IgM) 25/159		OR	CI	χ^2	*p*
	No.	%				
Family recurrent abortion history n=41	4	9.8	0.45	0.14-1.4	1.8	0.16
Animals in the house hold n=28	6	21.4	1.5	0.5-4.2	0.6	0.44
Medicine during pregnancy n=45	3	6.7	0.27	0.07-0.98	4.5	**0.03**
Uterus disorder n=38	9	23.6	1.8	0.75-4.7	1.9	0.17
Non-vaccinated for Rubella n=57	8	14.04	0.7	0.3-1.8	0.41	0.51
Blood transfusion history n=21	8	38.1	4.1	1.5-11.3	8.2	**0.004**
Own handicapped children n=13	3	23.1	1.6	0.4-6.3	0.43	0.5
Total	25/159 (15.7)					

OR Odds ratio= >1(there is a risk), **CI** Confidence interval, χ^2 Chi-square= >3.8 (**significant**) and p Propagability Value= <0.05 (**significant**).

Table 4.9 shows the risk factors associated with herpes simplex virus 2 (HSV2) (IgM) infection in women with recurrent pregnancy loss (RPL) in Sana'a City, Yemen. History of blood transfusion was a significant risk factor for HSV2 infection with an odds ratio (**OR**) of 4.1, with a range of 1.5–11.3 (**CI**), with a chi-square ($\chi^{2)}$ of 8.2 and a significant p-value of 0.004.

Chapter 5

Discussion

5. Discussion

TORCH is the most widely recognized pathogen of eugenics in the world. During pregnancy, the immunity of pregnant women decreases due to the changes of endocrine system, especially the weakening of T lymphocyte immune function, which is prone to the primary infection of TORCH or the potential recurrence of virus activation. Most of them cause mild maternal morbidity, but have serious fetal consequences, such as abortion, malformation and stillbirth (**Wang *et al,* 2019**).

The cross-sectional study investigated the prevalence of infectious outcomes in women with RPL with different numbers of previous pregnancy losses. Overall, we found a high rate of IgM infectious agents indicative of current infection while the majority of women with RPL may have other undiagnosed causes such as abnormal test results for parental structural chromosomal abnormalities, uterine anomalies, Antiphospholipid syndrome, hereditary thrombophilia and thyroid disorders.

The present study revealed that 79.5% of pregnant women with recurrent pregnancy loss (RPL) in Sana'a city, Yemen, were infected with TORCH pathogens, indicating a high prevalence and potential association with miscarriage. This figure aligns with findings from neighboring countries; for instance, a study in Iraq by ALSAADY *et al*, which reported *Toxoplasma gondii* was seropositivity among pregnant women 31.25%, (**ALSAADY *et al,* 2021**), and similarly, a study in Iraq's Anbar region, which reported 22% CMV IgM and 54% IgG seropositivity in high-risk pregnancies, supporting the association between TORCH infections and pregnancy complications (**Al-Hakami *et al,* 2020: Manjunathachar *et al,* 2020**).

Although, a large proportion had no known cause. Therefore, the latest RPL guidelines (**ESHRE, 1983**) recommend screening for antiphospholipid antibodies after two pregnancy losses. Thyroid screening and uterine anatomy assessment are recommended for

RPL, but there is no recommendation after the number of pregnancy losses. Parental stratification is not routinely recommended. As the chance of finding an abnormality is very low, it should only be considered after individual risk assessment. Given the weak association between RPL and inherited thrombophilias and the lack of available evidence-based treatment, inherited thrombophilia screening is not routinely recommended in couples with RPL (**ESHRE, 1983**).

In terms of specific pathogens, the most common IgG-positive infections were cytomegalovirus (CMV) and toxoplasmosis, both at 25.8% and 24.5% respectively, suggesting past exposure. IgM positivity, indicating active or recent infections, was highest for *Toxoplasma gondii* (19.5%), followed by HSV1/HSV2 (15.7%), which are likely contributors to RPL. These findings are consistent with recent studies in similar settings, where toxoplasmosis has been identified as a leading contributor to spontaneous abortion and fetal anomalies in India (**Surpam *et al,* 2006)**, in Pakistan (**Khan *et al,* 2023**), in Iraq (**ALSAADY *et al,* 2021**) and in India **(Manjunathachar *et al,* 2020**) , and different to those of studies in Asmara, Eritrea, Turkey and Iraq, which reported the IgG seropositivity is found to be lower compared to the other studies (**Adgoy *et al,* 2020; Mohymen *et al,* 2020**). Several studies confirm a strong link between acute toxoplasmosis and early pregnancy losses (**Petersen *et al,* 2010**). **In Iran, Rasti *et al,* (2016)** demonstrated that co-infections with TORCH agents significantly increase the risk of spontaneous abortions. Additionally, a study in Central India revealed 61.1% of high-risk pregnancies tested positive for TORCH IgM, particularly rubella virus, reaffirming the role of these pathogens in fetal loss. Although local data in Yemen are limited, a recent study in Aden linked elevated CMV IgG/IgM titers to recurrent miscarriages. This supports the need for integrated TORCH screening in reproductive healthcare. Given Yemen's proximity and

sociomedical similarities with countries reporting high TORCH prevalence, targeted intervention is necessary.

In the current study, the mean age of RPL women was 29.7 years with SD equal to 7.9 years, and ranged from 18 to 50 years. Most women with RPL was in age group 21-30 years counting 47.7% followed by age group 31-40 years counting 31.4%. This finding is similar to previous studies in European countries where the average age of the female at first live birth is approximately 30 years, and as the female age increases, the risk of fetal aneuploidy increases. Therefore, fetal aneuploidy is often the cause of RPL, especially in women over 36 years of age (**Stephenson *et al,* 2002; Marquard *et al,* 2010; Ahmed *et al,* 2022**). The decision on when to start investigations should be based on the female age, previous pregnancy loss, as well as other maternal conditions such as apparent autoimmune or thrombotic diseases, family history, and the results of karyotyping of the miscarriage tissue, if performed. It should also be the result of joint decision-making by the physician and the couple with the commitment of available resources. Consideration should also be given to individualized diagnostic tests, where some tests can be performed and others omitted (**Bernardi *et al,* 2012**).

Blood group O was the most prevalent (61.6%), though no statistically significant link has been established between ABO blood groups and TORCH susceptibility in recent meta-analyses (**Zhou *et al.,* 2022**). Regarding Rh factor, 87.4% were Rh-positive, suggesting Rh incompatibility may not be a predominant contributor to RPL in this population.

Toxoplasmosis showed no significant associations with the handling of raw meat or the presence of cats in houses. certain risk factors (p=0.6). This result was similar to that reported by **ALSAADY *et al,* (2021)** which reported no relationship with the handling of raw meat or the presence of cats in houses. The study's data indicated a statistical

relationship between medication use during pregnancy (p=0.01) and having disabled children (*p*=0.007) with IgM positivity.

Analysis of potential risk factors showed that non-vaccination against rubella (23.5%), use of medication during pregnancy (18.5%), and uterine abnormalities (15.6%) were frequent among affected women. Notably, use of medication during pregnancy was significantly associated with toxoplasmosis ($p = 0.01$), while a history of blood transfusion was a significant risk factor for HSV1/HSV2 infection ($p = 0.004$). This highlights the need for stringent infection screening and safer transfusion protocols.

Importantly, non-vaccination against rubella did not significantly correlate with rubella IgM positivity, underscoring the need for further investigation into vaccine efficacy or potential reinfection with mutant strains (**WHO, 2024**). Furthermore, a history of having handicapped children was significantly associated with toxoplasmosis ($p = 0.007$), possibly reflecting congenital transmission.

We suggest that future research should focus on designing a dynamic prediction model for couples experiencing recurrent pregnancy loss. A dynamic model has the advantage of allowing adjustment for changes in the underlying data over time (**Van den *et al*, 2013**). In this model, age, previous pregnancy losses, and other risk factors for recurrent pregnancy loss, such as viral and parasitic infections as well as spontaneous abortion syndrome, can be incorporated. If treatment possibilities exist for the risk factors (i.e. spontaneous abortion syndrome), correction should be applied. Using this prediction model, the chance of a live birth can be estimated more accurately. The prediction model can also be used to send a positive message to couples experiencing anxiety and depression after pregnancy loss, as this phenomenon is high in Yemen.

Chapter 6

Conclusions & Recommendations

6. Conclusion and Recommendation

6.1 Conclusion

The following can be concluded from this study results:

- In this study, the prevalence of TOURSH infection was high among women with RPL.
- Most women with RPL was in age group 21-30 years.
- *Toxoplasma gondii* and *HSV1/2* were the most frequently detected active infections, indicating ongoing risk.
- Risk factors such as medication use during pregnancy, having disabled children, and blood transfusion history showed significant associations with TORCH infections.
- The difference in prevalence in other factors such as uterine abnormalities and APS is likely to be the cause of pregnancy loss. We cannot rule out a lower prevalence of chromosomal abnormalities, hereditary thrombophilia and thyroid disorders after testing after pregnancy loss, but these conditions were not investigated in the women tested in this study. It should be emphasized that further studies are urgently needed on the predictive value of test results used in the RPL cohort. There is currently no evidence-based treatment available in the majority of cases when test results are abnormal.

6.2 Recommendation:

From this outcome, the following can be recommended:

1. Increase the sample size in future studies to enhance the statistical power and generalizability of findings across different regions of Yemen.
2. To implement routine TORCH screening for women with a history of recurrent pregnancy loss in clinical practice, especially in high-prevalence areas such as Sana'a City.
3. Employ advanced diagnostic techniques such as:

- Quantitative ELISA and Polymerase Chain Reaction (PCR)

4. Future studies should include comprehensive screening for all potential causes of recurrent pregnancy loss, including parental chromosomal abnormalities, uterine anomalies, antiphospholipid syndrome, hereditary thrombophilia, and thyroid disorders.
5. Future research should focus on designing a dynamic prediction model for couples experiencing recurrent pregnancy loss. A dynamic model has the advantage of allowing adjustment for changes in the underlying data over time.
6. National health policies should support the integration of cost-effective diagnostic protocols for TORCH infections into maternal health care services.
7. Health authorities should enhance awareness programs and preventive strategies targeting TORCH infections among women of reproductive age.
8. There is an urgent need for further research to determine the clinical significance and predictive value of TORCH serological testing in the context of recurrent miscarriage.

Chapter 7

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Appendices

Consent form

I .....agree to participate in this research project. the purpose of this study is to learn more about the TORCH infection in Sana'a city -Yemen. I know that he/she will be one of the volunteers who will be studied. The information that will be obtained in this study will help physicians to determine the most effective public measures for prevention, being a volunteer or refusal to participate in this study will have no effect upon me or my family regarding access to routine medical care. The risk associated with being volunteer is absent. Laboratory results and information that I will give will be confidential and is used only for medical research purposes. I understand that my /my family name will not be used in any scientific publications and that i/my family privacy will be strictly maintained. I may withdraw from this study at any time without any loss of health care privileges.

استمارة الموافقة

انا .....سميره علي احمد البدش.....اوافق على المشاركة في هذا البحث.

اعلم بأني سأكون احد المتطوعين في هذه الرسالة وانه سيتطلب مني الاجابة على بعض الاسئلة الصحية .

وبالرغم من أنني قد لا أستفيد من هذه الدراسة بشكل مباشر الا ان المعلومات التي سيتم الحصول عليها ستساعد الاطباء على تحديد التدابير الاكثر فعالية.

كما اعلم بانه ليس هناك اي مخاطر من كوني متطوع في هذه الرسالة كما ان النتائج ستكون سريه ولن تستخدم الا للأغراض الطبية المعتادة. واعلم ان اسمي لن يستخدم في أي منشور علمي وسيتم الحفاظ على هذه الخصوصية ويحق لي الانسحاب من هذه الدراسة في اي وقت.

Signature of Volunteer

Signature of project investigator

الجامعة الإماراتية الدولية
قسم المختبرات الطبية

استبيان طبي

Seroprevalence of TORCH Infection among Pregnant Women with Recurrent Pregnancy Loss in Sana'a City, Yemen

الرقم التسلسلي (98)

الاسم: — العمر: 36

رقم الهاتف: — الوظيفة : ربة بيت

هل توجد قرابة بين الزوج والزوجة؟

☐ نعم ☑ لا

نوع فصيلة الدم:

☑ O ☐ AB ☐ B ☐ A

العامل الرايزيسي :

☑ موجبة ☐ سالبة

هل يوجد أطفال؟

☐ 1 ☑ 2 ☐ 3 ☐ أكثر ☐ لا يوجد

هل تم إجهاض من قبل؟

☑ نعم ☐ لا

كم مرة ..... مرتين .....

هل تم إجهاض من قبل في العائلة؟

☐ نعم ☑ لا

هل يوجد حيوانات أليفة في البيت؟

☐ نعم ☑ لا

ما هو الحيوان؟ .....

هل كنتي تتناولين أي نوع من الأدوية خلال فترة الحمل؟

☑ نعم ☐ لا

هل لديك أطفال معاقين أو متشوهين خلقيا أو لديهم أمراض اخرى منذ الولادة؟

☑ نعم ☐ لا

ماهي الامراض .....

ما هو ترتيب الطفل المعاق؟

☐ الاول ☑ الثاني

هل تعانين من أمراض متعلقة بالرحم؟

☑ نعم ☐ لا

هل توجد أمراض اخرى وراثية في العائلة او امراض مزمنة؟

☐ نعم ☑ لا

ماهي الأمراض؟ .....

Medical Laboratory Test Results

Test	Toxo	RV	CMV	HSV
IgG	−	−	−	−
IgM	+	−	−	−

ATLAS ANTI-A, ANTI-B, ANTI AB and ANTI-D SLIDE AND TUBE TESTS

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C Store at 2- 8°C

INTENDED USE

Anti-A, Anti-B and Anti-AB reagents (here under referred to as ABO reagents) are used for qualitative in-vitro determination of human blood groups of the ABO system to determine the blood type. Anti-D reagent is used for the qualitative determination of Rhesus factor on human blood groups.

These reagents are intended to be used in slide and tube methods.

INTRODUCTION & PRINCIPLES

ATLAS ABO reagents are prepared from In-Vitro culture supernatants of hybridized immunoglobulin-secreting mouse cell lines. The reagents are diluted with phosphate buffer containing sodium chloride, EDTA and bovine albumin to give reagents that are optimized for use in tube and slide procedures. Anti-A is colored with acid blue (patent blue) dye, Anti-B is colored with acid yellow (tartrazine) dye, and Anti-AB is not colored. The test procedure is based on agglutination principle, where red cells possessing the antigen agglutinate in the presence of the corresponding antibody indicating that the result is positive. The test is considered negative when no agglutination appears.

ATLAS Anti-D reagent is prepared from carefully blended human monoclonal IgM and IgG. Anti-D is suitable for slide and tube test procedures. The reagent will directly agglutinate Rh D positive cells, including majority of variants (but not DVI) and a high proportion of weak D (Du) phenotypes. The reagent will agglutinate category DVI and low grade weak D (Du) phenotypes by the indirect anti-globulin techniques. Anti-D reagent is diluted with a sodium chloride solution, sodium phosphate solution and bovine albumin (sodium caprylate free). Anti-D is not colored. The procedure is based on agglutination principle, where red cells' possessing the antigen agglutinates in the presence of the corresponding antibody in the reagent indicating that the result is positive. The test is considered negative when no agglutination appears.

MATERIALS

MATERIALS PROVIDED

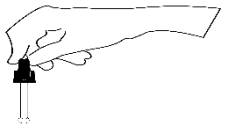
- ABO grouping reagent: Anti-A (10 ml/vial), Anti-B (10 ml/vial), Anti-AB (10 ml/vial).
- Anti-D IgG/IgM Blend reagent (10 ml/vial).

MATERIALS NEEDED BUT NOT PROVIDED

- Plastic test tube or glass.
- Isotonic saline solution (0.9%) NaCl).
- Applicator sticks.
- Centrifuge (100-1200 g for tube test).
- Timer.
- Incubator
- Anti-Human Globulin Reagent (can be ordered from Atlas Medical).
- White or transparent glass slide.

PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- The test is for well trained professional healthy user not for lay user.
- These reagents are derived from animal and human sources, thus, appropriate care must be taken in the use and disposal of these reagents, as there are no known test methods that can guarantee absence of infectious agents.
- Do not use reagents if it is turbid or contain particles as this may indicate reagent deterioration or contamination.
- Protective clothing should be worn when handling the reagents.
- The reagents contain 0.1% Sodium Azide which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.
- The reagents should be used as supplied and in accordance to the procedure mentioned below. Don't use beyond expiration date.
- Avoid cross contamination of reagents or specimens.
- Visible signs of microbial growth in any reagent may indicate degradation and the use of such reagent should be discontinued.
- Don't use these reagents if the label is not available or damaged.
- Do not use dark glass slide.
- Don't use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Wash hands and the test table top with water and soap once the testing is done.
- Hemolyzed blood sample should not be used for testing.
- The test should be performed at room temperature in a well-lit area with very good visibility.
- Failure to follow the procedure in this package insert may give false results or safety hazard.
- Close the vial tightly after each test.
- The reagent is considered toxic, so don't drink or eat beside it.
- If spillage of reagent occur clean with disinfectant (disinfectant used could be irritable so handle with care).
- The dropper should be held in vertical position (see the illustration below), or it may lead to inaccurate volume of the reagent.



STORAGE CONDITIONS

- The reagents should be stored refrigerated between 2 - 8°C.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

REAGENT PREPRATION

- The reagents are intended for use as a supplied, no prior preparation or dilution of the reagent is required.
- All reagents should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

- Blood collected with anticoagulant (EDTA, ACD/CPD, sodium citrate, or heparin) can be used for antigen typing.
- The specimens should be tested as soon as possible after collection. If testing is delayed, the specimens should be stored at 2- 8 ºC, Sample must be retained to room temperature prior to analysis. (Testing should be carried out within five days of collections).
- Blood collection is to be done with great care.

PROCEDURES

A.DIRECT METHOD IN A TUBE AT ROOM TEMPERATURE

1. Bring reagents and samples to room temperature (18-25°C).
2. Prepare a 5% suspension of red blood cells in isotonic solution.
3. Using the vial dropper, transfer a drop (40 µl ± 10 µl) of each reagent into a separate and appropriately marked tube.
4. Add 50 µl of red blood cells suspension.
5. Shake to homogenize the mixture, then centrifuge at 500 g for 1 minute.
6. Read macroscopically while gently shaking the tubes so as to detach the red blood cell pellet.
7. Note the appearance of any agglutination.

B. ANTIGLOBULIN INDIRECT METHOD for ANTI-D

1. After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37°C for 15 minutes.
2. Wash the red blood cells twice with isotonic saline (NaCl 0.9%) solution and discard the last washing liquid.
3. Add (40 µl ± 10 µl) of ANTI-HUMAN GLOBULIN to the tube. Mix and centrifuge at 120 g for 1 minute.
4. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
5. Read the reaction immediately.

C. SLIDE PROCEDURE

1. Bring reagents and samples to room temperature (18-25°C).
2. Using the wax pen divide the slide into appropriate numbers of divisions.
3. Using the provided dropper, place one drop (40 µl ± 10 µl) of each reagent onto its correspondent division on the slide.
4. Add 25 µl of the whole blood cell next to each drop of reagents.
5. Mix the reagent and the cells using a clean stirring stick over an area with a diameter of approximately 20-40 mm.
6. Hold the slide and gently rock the slide for 1 minute and observe macroscopically for any agglutination.
7. Read the reaction immediately.

READING THE RESULT

POSITIVE: If Agglutination appears.

NEGATIVE: If no agglutination is observed.

Use the below table to determine the blood group:

Result of each reaction				ABO
Anti-A	Anti-B	Anti-AB	Anti-D	Group
+	-	+	+	A+
+	-	+	-	A-
-	+	+	+	B+
-	+	+	-	B-
+	+	+	+	AB+
+	+	+	-	AB-
-	-	-	+	O+
-	-	-	-	O-

Stability of the reactions

- ABO Blood Grouping Tube tests should be read immediately following centrifugation.
- Delay in reading and interpreting results may lead to inaccurate results.

PROCEDURE LIMITATION

1. False positive/ negative results may occur due to:
   - Contamination from test materials.

- Improper storage, wrong cells concentration, inaccurate incubation time or temperature.
- Improper or excessive centrifugation.
- Deviation from the recommended technique.

2. Blood samples of weak A or B subgroups may give rise to false negative results or weak reactions.
3. Weaker reactions may be observed with stored blood than with fresh blood.
4. ABO antigens are not fully developed at birth, weaker reactions may therefore occur with cord or neonatal red cells.
5. ABO blood grouping interpretation on individuals greater than 6 months old should be confirmed by testing serum or plasma of the individual against group A and group B red cells (reverse grouping). If the results obtained with the serum do not correlate with the red cell test, further investigation is required.
6. Return the kit to the agent if it does not function properly.

DIAGNOSTIC PERFORMANCE CHARACTERISTICS

Atlas Blood Grouping Reagents were compared with competitive CE marked devices and the results showed:

Analytical sensitivity: 100%
Analytical specificity: 100%
Precision: 100%
Accuracy: 100%

QUALITY CONTROL

The reactivity of all blood grouping reagents should be confirmed by testing known positive and negative red blood cells on each day of use.

To confirm the specificity and sensitivity, ATLAS blood grouping reagents and ATLAS Anti-D should be tested with antigen-positive and antigen-negative red blood cells.

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PPI1428A01
Rev F (22.03.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry

Elecsys Toxo IgM

cobas®

REF	Σ	SYSTEM
		MODULAR ANALYTICS E170
04618858 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 530
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 95

Intended use

Immunoassay for the in vitro qualitative determination of IgM antibodies to *Toxoplasma gondii* in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Toxoplasmosis is a relatively common infection caused by the protozoan parasite Toxoplasma gondii.

The infection is mainly acquired by ingestion of food or water contaminated by mature oocysts shed by cats or by undercooked meat containing tissue cysts.[1,2,3,4] Infection can also be transmitted congenitally if a woman is newly infected during, or just prior to pregnancy, and also via organ transplant or blood transfusion from an infected donor.[4]

Primary, acute infection in healthy individuals is mostly mild or even asymptomatic and is followed by life-long latency.[3,4] Reactivation of a latent Toxoplasma infection can occur as a result of immunosuppression (e.g. in organ transplant recipients, patients with cancer or HIV) and can be associated with high morbidity and mortality.[3,4] Reactivated disease in immunocompromised hosts frequently presents with brain lesions, especially in patients with advanced HIV-related immunosuppression.[3,4,5]

Primary maternal Toxoplasma infection occurring during pregnancy may have significant implications for the fetus as the parasite can be transmitted across the placenta.[3,6] The majority of infants with congenital infection do not present clinical symptoms at birth but may develop severe sequelae later in life such as chorioretinitis, intellectual and psychomotor disabilities, visual and hearing impairment.[3,6,7,8] The fetal infection rate increases with gestational age but, the risk of severe clinical manifestations is higher in the case of early maternal infection.[3,6,7,8]

Early drug therapy in acute infection during pregnancy can prevent congenital damage or ameliorate the severity of clinical manifestations.[6,7]

The diagnosis of Toxoplasma infection is most commonly made by the detection of anti-Toxoplasma-specific IgG and IgM antibodies.[3,4,9]

Detection of Toxo IgM antibodies is presumptive of an acute or recent Toxoplasma infection.[3,4,9]

The determination of Toxo IgG antibodies is used to assess the serological status of T. gondii infection and is indicative of a latent or acute infection.[4,9]

The diagnosis of the acute acquired infection during pregnancy is established by a seroconversion or a significant rise in antibody titers (IgG and/or IgM) in serial samples.[8,9]

Test principle

µ-Capture test principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample are automatically prediluted 1:20 with Diluent Universal. *T. gondii*-specific recombinant antigen labeled with a ruthenium complex[a)] is added. Anti-Toxo IgM antibodies present in the sample react with the ruthenium-labeled *T. gondii*-specific recombinant antigen.
- 2nd incubation: Biotinylated monoclonal h-IgM-specific antibodies and streptavidin-coated microparticles are added. The complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as TOXIGM.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Toxoplasma-Ag~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 9 mL:
Toxoplasma-antigen labeled with ruthenium complex > 1 mg/L; MES[b)] buffer 50 mmol/L, pH 6.0; preservative.

R2 Anti-h-IgM-Ab~biotin (black cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-h-IgM antibody (mouse) > 500 µg/L; HEPES[c)] buffer 50 mmol/L, pH 7.2; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

c) HEPES = [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid

TOXIGM Cal1 Negative calibrator 1 (white cap), 2 bottles of 0.67 mL each:
Human serum, negative for anti-Toxo IgM; preservative.

TOXIGM Cal2 Positive calibrator 2 (black cap), 2 bottles of 0.67 mL each:
Anti-Toxo IgM (human) approximately 130 U/mL (Roche units) in human serum; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious. All products derived from human blood (TOXIGM Cal1, TOXIGM Cal2) are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The serum containing anti-Toxo IgM (TOXIGM Cal2) was sterile filtrated.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[10,11]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit are ready-for-use and are supplied in bottles compatible with the system.

cobas e 411 analyzer: The calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzer, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles

(CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	2 weeks or 12 weeks if stored alternately in the refrigerator and on the analyzers (up to 84 hours)

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_3-EDTA and Na-citrate plasma.

Criterion: Mean recovery of positive samples within 80-120 % of serum value.

Stable for 3 weeks at 2-8 °C, 3 days at 25 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 6 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be subsequently altered with additives (biocides, anti-oxidants or substances that could possibly change the pH of the sample) in order to avoid erroneous findings.

Pooled samples and other artificial material may have different effects on different assays and thus may lead to discrepant findings.

Centrifuge samples containing precipitates and thawed samples before performing the assay. Lyophilized samples, heat-inactivated samples and samples and controls stabilized with azide (up to 1 %) can be used.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See “Reagents – working solutions” section for reagents.

- 2 x 2 bottle labels

Materials required (but not provided)

- REF 04618866190, PreciControl Toxo IgM, 16 x 0.67 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against a Roche standard. The units have been selected arbitrarily.

Calibration frequency: Calibration must be performed once per reagent lot using TOXIGM Cal1, TOXIGM Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

- as required: e.g. quality control findings outside the defined limits
- more frequently when this is required by pertinent regulations

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (TOXIGM Cal1): 400-2500
Positive calibrator (TOXIGM Cal2): 4500-35000

Quality control

For quality control, use PreciControl Toxo IgM.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of TOXIGM Cal1 and TOXIGM Cal2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

Interpretation of the results

Results obtained with the Elecsys Toxo IgM assay can be interpreted as follows:

Non-reactive: < 0.8 COI

Indeterminate: ≥ 0.8 - < 1.0 COI

Reactive: ≥ 1.0 COI

Samples with a cutoff index < 0.8 are non-reactive in the Elecsys Toxo IgM assay.

Samples with a cutoff index between ≥ 0.8 and < 1.0 are considered indeterminate. The sample should be retested. In case the result is still indeterminate, a second sample should be tested e.g. within 2-3 weeks. Samples with a cutoff index ≥ 1.0 are reactive in the Elecsys Toxo IgM assay.

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present in the sample.

The anti-Toxoplasma IgM results in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay and reagent methods.

Limitations - interference

A negative Toxo IgM test result, also in combination with a positive Toxo IgG result, does not completely rule out the possibility of an acute infection with *Toxoplasma gondii:*

- Individuals at the early stage of acute infection may not exhibit detectable amounts of Toxo IgM antibodies. In some of these individuals an indeterminate or low positive result with the Elecsys Toxo IgG assay may be found and indicate an early acute infection. A second sample should be tested e.g. within 2 weeks. The detection of Toxo IgM and/or a significant increase of the Elecsys Toxo IgG antibody titer in the second sample supports the diagnosis of acute Toxoplasma infection.
- In some individuals Toxoplasma IgM-specific antibodies may revert to non-reactive levels within few weeks after infection with *T. gondii.*

The detection of IgM antibodies against *T. gondii* in a single sample is not sufficient to prove an acute Toxoplasma infection since elevated IgM antibody levels may persist even for years after initial infection.[12,13] Further tests or a combination of test methods should be done for clarification.[1,14,15,13] A significant increase of the Toxo IgG antibody titer from a first to a second sample taken e.g. within 2 weeks may support the diagnosis of acute Toxoplasma infection.

If a treatment is prescribed early enough, antibody production may not increase. IgG and IgM levels may remain low and can coexist for years.

Elecsys Toxo IgM results should be used in conjunction with Toxoplasma-specific IgG results, the patient's medical history, clinical symptoms and other laboratory tests.

The results in HIV patients, in patients undergoing immunosuppressive therapy, or in patients with other disorders leading to immune suppression, should be interpreted with caution.

Specimens from neonates, cord blood, pretransplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.

The assay is unaffected by icterus (bilirubin < 684 µmol/L or < 40 mg/dL), hemolysis (Hb < 1.24 mmol/L or < 2 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Mean recovery of positive samples within ± 20 % of serum value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 3720 IU/mL.

The high-dose hook effect does not lead to false-negative results in the Elecsys Toxo IgM assay.

In vitro tests were performed on 18 commonly used pharmaceuticals and in addition on spiramycine, sulfadiazine, folinic acid and pyrimethamine. No interference with the assay was found.

As with many µ-capture assays an interference with unspecific IgM is observed. Increasing amounts of unspecific IgM may lead to a decrease in the recovery of positive samples with the Elecsys Toxo IgM assay.

In rare cases, interference due to extremely high titers of antibodies to immunological components, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, human sera and controls (repeatability n = 21, intermediate precision n = 10); intermediate precision on MODULAR ANALYTICS E170 analyzer was determined in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60). The following results were obtained:

cobas e 411 analyzer						
	Repeatability			**Intermediate precision**		
Sample	Mean COI[d)]	SD COI	CV %	Mean COI	SD COI	CV %
HS[e)], negative	0.109	0.002	2.2	0.103	0.006	5.4
HS, positive	1.37	0.021	1.5	1.33	0.034	2.5
HS, positive	3.78	0.067	1.8	3.70	0.171	4.6
PC[f)] Toxo IgM 1	0.120	0.002	1.6	0.118	0.005	4.1
PC Toxo IgM 2	1.35	0.015	1.1	1.29	0.043	3.3

d) COI = cutoff index

e) HS = human serum

f) PC = PreciControl

Elecsys Toxo IgM

cobas®

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, negative	0.107	0.002	1.8	0.103	0.002	1.9
HS, positive	1.33	0.011	0.9	1.36	0.023	1.7
HS, positive	3.86	0.034	0.9	3.83	0.061	1.6
PC Toxo IgM 1	0.116	0.002	1.6	0.117	0.002	1.7
PC Toxo IgM 2	1.30	0.015	1.2	1.31	0.032	2.4

Method comparison

In study 1 the performance of the Elecsys Toxo IgM assay was determined by testing a total of 826 fresh and frozen samples at two sites in comparison to a commercially available Toxoplasma IgM test.

In study 2 the Elecsys Toxo IgM assay was compared to another commercially available Toxoplasma IgM assay by testing 400 fresh and frozen samples. In both studies all specimens with initially discordant results were re-tested. Resolution of repeatedly discordant samples was done by avidity testing. 51 specimens with indeterminate results in one of the assays were excluded from the final calculation of relative sensitivity and specificity.

Relative sensitivity and specificity after resolution

Study	N	Relative sensitivity %	Lower confidence limit %	Relative specificity %	Lower confidence limit %
1	785	95.3 (162/170)	91.7	98.9 (595/602)	97.8
2	390	98.8 (83/84)	94.4	99.7 (294/295)	98.4

Study 1: Of 21 samples which were initially discordant negative with the Elecsys Toxo IgM assay, 11 samples revealed a high avidity test result, 2 samples were found negative with Toxo ISAGA IgM. 7 discordant negative samples revealed a low avidity test result, 1 sample was found positive with Toxo ISAGA IgM. 5 samples which were discordant positive with the Elecsys Toxo IgM assay revealed a high avidity test result, 2 samples were from individuals without Toxoplasma infection.

Study 2: Of 12 samples which were initially discordant negative with the Elecsys Toxo IgM assay, 11 samples revealed a high avidity test result. 1 sample revealed a low avidity test result. 1 sample which was discordant positive with the Elecsys Toxo IgM assay was from an individual without Toxoplasma infection.

Analytical specificity

455 potentially cross reacting samples were tested with the Elecsys Toxo IgM assay and a comparison Toxo IgM assay comprising specimens:

- containing antibodies against HAV, HBV*, HCV, HIV, CMV, EBV*, HSV, VZV, Rubella, Treponema pallidum, Malaria**, Amebiasis, Chlamydia and Gonorrhea
- containing autoantibodies (AMA*, ANA) and elevated titers of rheumatoid factors
- after vaccination against HBV and Influenza

An overall agreement of 99.1 % (446/451) was found in these specimens using the Elecsys Toxo IgM assay and the comparison test. 444 samples were found concordantly negative and 2 samples were found positive. 4 samples were found indeterminate either by the Elecsys Toxo IgM assay or the comparison test.

* 1 discordant sample in each of these groups

** 2 discordant samples

Seroconversion panels

In two studies seroconversion samples obtained during pregnancy screening were tested with the Elecsys Toxo IgM assay in comparison to two different commercially available Toxo IgM assays.

In 24 seroconversion panels comprising 83 samples at the first site, the Elecsys Toxo IgM assay detected 64 samples from 66 samples which were found positive using a comparison test. 2 discordant negative sera were follow-up samples, taken more than 8 weeks after infection.

In 29 seroconversion panels (including 92 samples) at the second site, the Elecsys Toxo IgM assay detected 67 samples from 74 samples which were found positive by a second comparison test. 2 discordant negative sera from the very early phase of infection were also negative by another comparison test. In two panels (comprising 3 and 2 serial bleeds from the very early phase of infection) IgM was not detected, however seroconversion could be demonstrated by the Elecsys Toxo IgG assay.

In both panels discordant negative results for several samples were also found by two other commercial Toxoplasma IgM assays.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used

Elecsys Toxo IgM

cobas®

REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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