CHAPTER-I

1. INTRODUCTION

TORCH, as an acronym, stands for *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV) and Herpes Simplex Virus (HSV). This acronym has become one of the most recognized in the field of neonatal/perinatal medicine (Johnson, 2006). TORCH infections also pose a threat to immunosupressed patients including HIV/AIDS patients, cancer patients undergoing chemotherapy, transplant recipients etc.

The usual way in which the fetus is infected is by transplacental spread after maternal infection in which the organism circulates in the mother's blood (Klein and Remington, 2001). Because of their relatively low virulence, the organisms involved seldom lead to fetal death beyond the earliest stages of embryogenesis. The fetus is especially susceptible to infection during the first trimester which is the period when most complex events in embryogenesis take place and also the immature fetus lacks the immunologic mechanisms necessary to completely eliminate an infecting organism and during the perinatal period (Klein and Remington, 2001; Mims *et al*, 2001).

Clinical evidence of infection may be seen at birth, soon afterward, or not until years later. The infection can also lead to the late onset of the disease in what appears to be a "normal" newborn e.g., the development of vision-threatening chorioretinitis in an adolescent with congenital toxoplasmosis. Progressive tissue destruction is seen in infections caused by Rubella, HSV, CMV and *Toxoplasma* as the infective agents continue to survive and replicate in the tissues for months or years after initial infection. The sequelae of these diseases can also progress over time, e.g., hearing loss that is secondary to rubella infection can progress or develop even after years of normal hearing (Boyer and Boyer, 2004).

Caused by the protozoan, *Toxoplasma gondii,* toxoplasmosis is most frequently acquired orally by eating raw meat or exposure to infected cat feces (Boyer, 2000).

Infections with *Toxoplasma gondii* in humans are usually asymptomatic or in the form of mild febrile illness. Primary infection in pregnant women may result in congenital toxoplasmosis while infection in immunocompromised subjects like AIDS patients may cause potentially fatal *Toxoplasma* encephalitis (Mohan *et al*, 2002). Acute infection acquired after birth may be asymptomatic but frequently results in the chronic persistence of cysts within the host tissues (Kasper, 2003). Congenitally infected infants develop chorioretinitis that can lead to blindness, obstructive hydrocephalus, and intracranial calcifications that are associated with mental retardation, seizure activity, and motor and developmental delays (Boyer and Boyer, 2004).

Rubella or German measles is an infectious disease caused by the rubella virus which is usually transmitted by droplets from the nose or throat that others breathe in. It can also pass through a pregnant woman's bloodstream to infect her unborn child (Hirsch, 2006). Up to 20% of maternal infections occurring in the first eight weeks' of gestation result in miscarriage, spontaneous abortion, or stillbirth. Those fetuses infected before 11 weeks have multiple organ damage while those after 11 to 12 weeks are more likely to have only deafness and/or retinopathy (Best and O'Shea, 1989).

Cytomegalovirus (CMV) is the most frequent cause of congenital infection in humans (Enders, 1998). About 10 to 20% of infected infants may suffer sensorineural hearing loss, ocular damage, or impairment of cognitive and motor function (Fowler *et al*, 1993). In addition to the transplacental route, CMV can be transmitted at delivery via the maternal genital tract, during the postpartum period in breast milk, and in transfused blood products. CMV is easily spread in daycare centers and in families with young children. The organism can cause significant illness by endogenous reactivation among immunosuppressed individuals, including transplant recipients (Boyer and Boyer, 2004). In addition to intrauterine growth restriction, over 70% of symptomatic infants have evidence of CNS involvement: microcephaly, lethargy, hypotonia, optic atrophy, decreased hearing, and intracranial calcifications. Such infants have a mortality rate of 12% by six months of age (Hicks *et al*, 1993).

HSV is ubiquitous virus, infecting the majority of the world's population early in life (Ogilvie, 1997). A fascinating attribute of HSV is its ability to enter a quiescent state and establish a lifelong latent infection in sensory neurons that innervate the site of primary productive infection. Latent infection forms a reservoir of virus for recurrent infection, disease and transmission to other individuals. HSV type 1 (HSV-1) is usually associated with primary infections of the orofacial area and latent infection of the trigeminal ganglion, while HSV-2 is usually associated with genital infections and latent infection in sacral ganglia. Although both primary and recurrent infections are usually self-limited, HSV can cause serious diseases such as neonatal disseminated herpes, viral encephalitis and blinding keratitis (Whitley, 2001). Also, genital herpes infection has been associated with an increased risk for HIV infection (Stamm *et al*, 1988; Wald and Link, 2001).

Most of the TORCH infections cause mild maternal morbidity, but have serious fetal consequences, and treatment of maternal infection frequently has no impact on fetal outcome. Therefore, recognition of maternal disease and fetal monitoring once disease is recognized are important (Stegmann and Carey, 2002).

Routine screening of pregnant women at the first prenatal visit for TORCH antibody titers (IgG and IgM) should be done because IgG frequently persists once patients have been exposed to the pathogen and IgM is produced during acute infection (Johnson, 2006). The increased use of polymerase chain reaction (PCR) amplification of a sample of amniotic fluid and ultrasound during pregnancy are more recent advancements that support earlier case finding which is important because the mother can be treated to prevent fetal infection (Boyer and Boyer, 2004).

Nepal, being a developing country, has people who are less health conscious. Most of the people in our country do not have access to safe drinking water, hygienic food and proper sanitation practices. Moreover, most of the people are not financially capable to have a routine health check-up. In such context, diseases such as TORCH infections are not given much importance and this inattention may ultimately lead to serious complications in pregnant women, infants, children and immunosupressed patients. Therefore, early detection and treatment of TORCH infections and preventive measures have to be carried out in order to reduce the incidence of later occurring life threatening consequences.

As very few studies have been carried out in the past to find out the burden of TORCH infections in our country, the results obtained from the present research may help to bring awareness about TORCH infections and their preventive measures in the general population.

The present study was conducted among various types of patients visiting National Public Health Laboratory (NPHL) suspected of TORCH infections. The main objective of this study was to determine the seroprevalence of TORCH infections among the suspected patients of different age groups and gender; and to correlate this data with different disease conditions. In this study, IgM antibodies present in the serum of suspected patients to be tested were detected by Enzyme Linked Immunosorbent Assay (ELISA) which reveals recent infection.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To study the seroprevalence of TORCH infections among the patients visiting National Public Health Laboratory.

2.2 SPECIFIC OBJECTIVES

- To study the seroprevalence of TORCH infections among different types of patients of different age group and gender visiting NPHL.
- To study the correlation of *Toxoplasma* infection with different disease conditions of patients.
- To find out the correlation of *Toxoplasma* infection with meat eating habit and cat rearing practices.
- To study the correlation of Rubella virus infection with different disease conditions of patients.
- To study the correlation of Cytomegalovirus infection with different disease conditions of patients.
- To study the correlation of Herpes simplex virus infection with different disease conditions of patients.

CHAPTER-III

3. LITERATURE REVIEW

3.1 INTRODUCTION

3.1.1 *Toxoplasma*

Toxoplasma gondii is an obligate intracellular parasite, which causes toxoplasmosis in humans. The parasite probably is the only protozoan, whose all the stages (tachyzoite, tissue cyst and oocyst) are infectious for human. It is found inside the reticuloendothelial cells and other nucleated cells (muscle and intestinal epithelium) of the host (Parija, 2004). It is a species of parasitic protozoa whose definitive host is cat but can also be carried by the vast majority of other warm-blooded animals including human. It belongs to the Apicomplexa and is the only known member of the genus *Toxoplasma*. It causes the disease toxoplasmosis which is usually minor and self-limiting but can have serious or even fatal effects, particularly in cats, or for a foetus when first contracted during pregnancy. Infants born to mothers who became infected with *Toxoplasma* for the first time during or just before pregnancy and persons with severely weakened immune systems, such as those with AIDS, those taking certain types of chemotherapy, and persons who have recently received an organ transplant are under risk. In these people, illness may result from an acute *Toxoplasma* infection or reactivation of an infection that occurred earlier in life (The Analyst, 2006; Wikipedia, 2006).

Tachyzoites, tissue cysts and oocysts are the important morphological stages of the parasite. Tachyzoites are the actively proliferating trophozoites, which are found in any organ but most commonly in the brain, skeletal muscle, and heart muscle during the acute stage of infection. Intracellular infection can occur in all mammalian cells except anuclear erythrocytes. Once inside a cell, they multiply within a vacuole by a process known as endodyogeny. Intracellular multiplication continues until host cells lyse or a tissue cyst is formed. In an immunocompetent host, tachyzoites are eliminated and tissue cysts form. Tissue cysts are the extremely resistant resting forms of the parasite most commonly found in the brain and in skeletal and cardiac muscle during chronic stage of the infection. These cysts contain slowly growing trophozoites known as bradyzoites. Each cyst contains hundreds of bradyzoites. Oocysts are shed only by the members of the cat family (Parija, 2004; Sciammarella, 2002).

Life cycle: The Life cycle of *Toxoplasma gondii* has two phases. The sexual part of the lifecycle takes place only in members of the Felidae family (domestic and wild cats), the definitive host of *Toxoplasma gondii*. The asexual life cycle can take place in any warm-blooded animal, like other mammals (including felines) and birds (Wikipedia, 2006).

Asexual cycle: Human and other intermediate hosts acquire infection by ingestion of water and food contaminated with infectious sporulating oocysts or tissue cysts present in the raw or undercooked meat (mutton, pork, etc.) from another intermediate host, or transplacentally (Parija, 2004).

Sporozoites from the oocysts and bradyzoites from the tissue cysts invade the intestinal mucosa and in epithelial cells multiply as tachyzoites. In the cells, tachyzoites continue to multiply and may spread locally to mesenteric lymph nodes by invading new host cells. They also spread to distant extra-intestinal organs (e.g. brain, eye, liver, spleen, heart, skeletal muscle and placenta of pregnant mother) by invading lymphatics and blood. With the development of immunity, many of the tachyzoites are destroyed in visceral organs and acute infection is resolved. Some tachyzoites may still persist and continue to grow and develop into large tissue cysts in the brain, heart muscles and also in the skeletal muscles. These tissue cysts, which contain hundreds of bradyzoites, remain viable for years, thus retaining the potential for their re-activation. The immunosupression of the host causes reactivation of the cyst and renewed infection in the host. The infection in human is dead end (Parija, 2004).

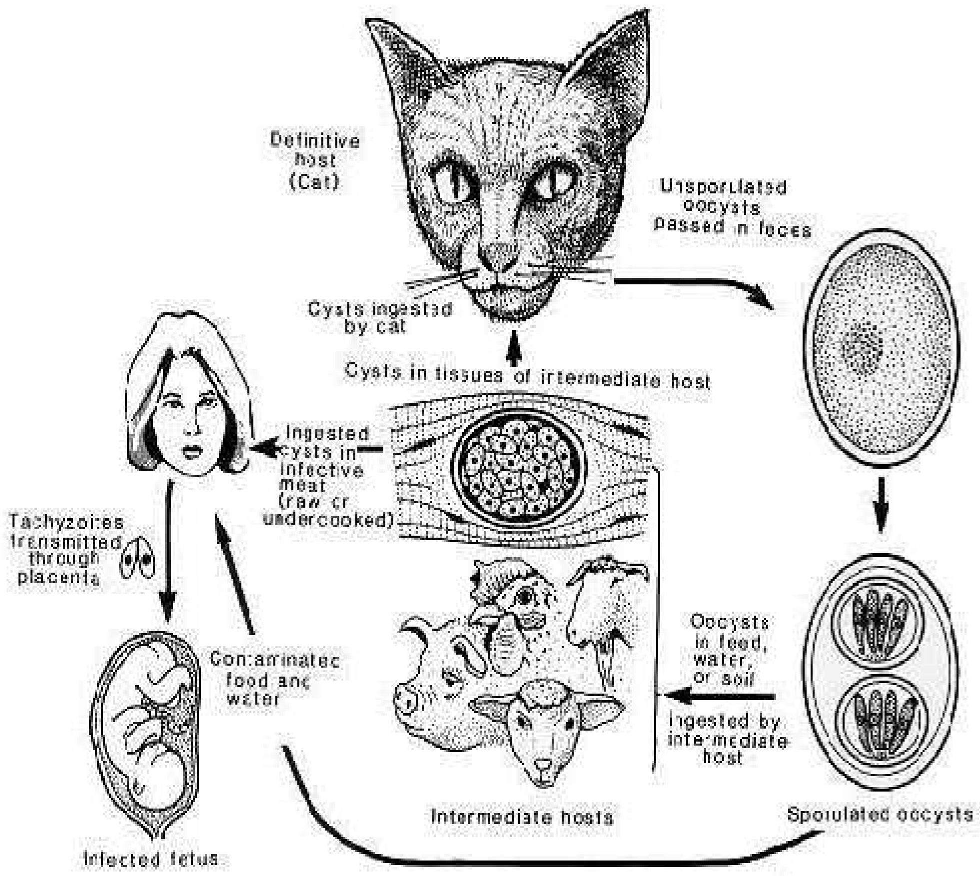

Figure 1: Life cycle of *Toxoplasma gondii* (Dubey, 1986).

Sexual cycle: Cats acquire infection by their predatory habit of feeding on infected mice, which harbour the tissue cysts or by being fed raw meat of domesticated animals containing these cysts (Parija, 2004). Cats acquire *Toxoplasma* by ingesting any of three infectious stages of the organism. Fewer than 50 percent of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts.

The bradyzoites released in the small intestine penetrate the mucosal epithelial cells of the small intestine in which, they undergo several cycles of asexual generation before the sexual cycle begins. The sexual cycle ends with the production of zygote which is surrounded by a thin but remarkably resistant and rigid wall to form an oocyst. The oocysts are then released into the lumen of intestine by rupture of the host cells.

Millions of these oocysts, which are non-infectious and non-sporulated, are excreted in faeces of cats daily, for a period of 1-3 weeks. In the environment, the sporogony occurs in oocysts for up to 21 days. During sporogony two sporocysts, each containing two sporozoites is formed in each oocyst in the next 3-4 days at room temperature. With sporulation, these oocysts become infective and remain infectious for more than one year in warm, humid environments. Man acquires infection by ingesting these sporulating oocysts and the cycle is repeated (Parija, 2004).

Pathogenesis: Bradyzoites released from the tissue cysts are resistant to the effect of pepsin and they penetrate the intestinal epithelial cells and multiply in the intestine. Within enterocytes, the parasites undergo morphological transformation, giving rise to invasive tachyzoites. These tachyzoites induce a parasite-specific secretory IgA response. Tachyzoites infect any nucleated cell, where they multiply and lead to cell destruction and production of necrotic foci surrounded by inflammation. The clinical picture is determined by the extent of injury especially to vital and vulnerable organs such as the eye, heart, and adrenals. *Toxoplasma gondii* does not produce a toxin; necrosis is caused by intracellular multiplication of tachyzoites (Kasper, 2003; Subauste, 2006). By about the third week after infection, *Toxoplasma gondii* tachyzoites begin to disappear from visceral tissues and may localize as tissue cysts in neural and muscular tissues. *Toxoplasma* tachyzoites may persist longer in the spinal cord and brain because immune responses are less effective in these organs. In immunosuppressed patients, rupture of a tissue cyst may result in renewed multiplication of bradyzoites into tachyzoites, and the host may die from toxoplasmosis (Dubey, 1988).

Immune responses: Development of both the antibody and cell-mediated immunities (CMI) significantly alter the course of *Toxoplasma* infection and its clinical manifestations and resolve the acute infection in the immunocompetent hosts.

Toxoplasma specific IgM antibodies are first to appear, hence their detection is suggestive of acute infection. The IgG antibodies appear late but are present in the circulation for a longer period as in chronic infection. The CMI, through activated macrophages and monocytes, is suggested to play an important role in conferring resistance to re-infection as well as in the development of initial resistance in toxoplasmosis, possibly in co-operation with humoral antibodies (Parija, 2004).

Clinical manifestations: Toxoplasmosis in human may occur as congenital, acquired, or ocular infections in the immunocompetent hosts or in the immunocompromised hosts (Parija, 2004).

Congenital Toxoplasmosis: It occurs when a non-immune susceptible woman becomes infected during pregnancy, leading to transplacental transmission of *T. gondii* to the fetus (Parija, 2004). The proportion of fetuses that become infected increases but the clinical severity of the infection declines as gestation proceeds (Kasper, 2003). Nearly 17% of babies of mothers infected during first trimester develop congenital toxoplasmosis and this may lead to still birth or abortion. Nearly 65% of babies suffer from congenital toxoplasmosis by infection during last trimester of pregnancy but symptoms are mild and babies may be asymptomatic at the time of birth (Parija, 2004).

Up to 90% of children born with congenital toxoplasmosis have no symptoms early in infancy, but a large percentage will show signs of infection months to years later. Some are born prematurely or are unusually small at birth. Other signs and symptoms may include: fever, swollen lymph nodes, jaundice, an unusually large or small head, rash bruises or bleeding under the skin, anemia, enlarged liver or spleen. Some babies with congenital toxoplasmosis have brain and nervous system abnormalities that cause: seizures, limp muscle tone, feeding difficulties, hearing loss, mental retardation (Homeier, 2005). Prenatally acquired *T gondii* often infects the brain and retina and can cause a wide spectrum of clinical disease. Mild disease may consist of slightly diminished vision, whereas severely diseased children may exhibit a classic tetrad of signs: retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcifications. Ocular disease is the most common sequela (Dubey, 1988).

Immunosuppression resulting from human immunodeficiency virus (HIV) infection or therapies for malignancies, organ transplantation, and lymphoproliferative disorders can result in the reactivation of latent *T. gondii* infection. Women with reactivated *T. gondii* infection can transmit the organism transplacentally (Remington *et al*, 2001). Maternal-fetal transmission of *T. gondii* can also occur in HIV-infected pregnant women who are chronically infected with *T. gondii*, although the risk of transmission is low (no more than 4%) (European Collaborative Study and Research Network on Congenital Toxoplasmosis, 1996; Minkoff *et al*, 1997). The risk of transmission may be higher in severely immunocompromised HIV-infected women with CD4 T-cell counts of <100/µl who are not receiving prophylaxis (Minkoff *et al*, 1997).

Acquired toxoplasmosis: It may be of the following 3 types:

Toxoplasmosis in immunocompetent host: Acute toxoplasmosis is asymptomatic in 80%-90% of healthy hosts and is symptomatic in only 10%-20% of cases. Lymphadenopathy is the classical clinical sign. The deep cervical lymph nodes are most commonly affected. Less frequently, supraclavicular, sub occipital, axillary and inguinal lymph nodes are also involved. This is associated with sore throat, fever, malaise, night sweats and myalgias. The condition is also associated with a maculopapular skin rash that does not affect the palms and soles. Ocular manifestations such as retinochoroiditis are present in 10% of cases. In some cases, retroperitoneal and mesenteric lymphadenopathy with abdominal pain may be present (Parija, 2004). Rare complications in the normal immune host include pneumonia, myocarditis, encephalopathy, pericarditis, and polymyositis. Symptoms associated with acute infection usually resolve within several weeks, although the lymphadenopathy may persist for some months (Kasper, 2003).

Toxoplasmosis in the non-AIDS immunocompromised host: This condition is observed in immunosupressed patients receiving immunosuppressive therapy for malignancies and persons receiving bone marrow and solid organ transplantations. The condition may be a newly acquired disease or reactivation of cysts in a chronic infection. Central nervous system is mainly affected in 50% of patients. The condition manifests as encephalitis, meningoencephalitis, myocarditis and pneumonitis. Hemiparesis, seizures, mental status changes and visual changes are the common clinical symptoms (Parija, 2004).

Toxoplasmosis in HIV/AIDS patients: Toxoplasmosis associated with HIV infection is typically caused by reactivation of a chronic infection and manifests primarily as toxoplasmic encephalitis. This disease is an important cause of focal brain lesions in HIV-infected patients (Luft and Remington, 1992). Characteristically, toxoplasmic encephalitis has a subacute onset with focal neurologic abnormalities frequently accompanied by headache, altered mental status, and fever (Levy and Bredesen, 1988; Navia *et al*, 1986; Renold *et al*, 1992). The most common focal neurologic signs are motor weakness and speech disturbances. Patients can also present with seizures, cranial nerve abnormalities, visual field defects, sensory disturbances, cerebellar dysfunction, meningismus, movement disorders, and neuropsychiatric manifestations (Gray *et al*, 1989). Diffuse toxoplasmic encephalitis should be considered in patients with anti-*T. gondii* immunoglobulin G (IgG) antibodies and CD4 T-cell counts of $<100/\mu l$ who present with unexplained neurologic disease (Subauste, 2006).

HIV-infected patients may develop extracerebral toxoplasmosis with or without concomitant encephalitis. Ocular and pulmonary diseases are the most common presentations. Patients with chorioretinitis present with blurred vision, scotoma, pain, or photophobia (Rabaud *et al*, 1994). Ophthalmologic examination reveals multifocal, bilateral lesions that typically are more confluent, thick, and opaque than those caused by cytomegalovirus (CMV) (Mansour, 1997). Pulmonary toxoplasmosis is the condition that occurs mostly in AIDS patients with CD4 counts less than 50 cells/cubic mm. The condition manifests as pneumonitis with prolonged febrile illness, cough and dyspnoea. Along with toxoplasmic pneumonitis, extrapulmonary manifestations may be present in about 50% of cases (Parija, 2004). A highly lethal syndrome of disseminated toxoplasmosis that consists of fever and sepsis like syndrome with hypotension, disseminated intravascular coagulation, elevated lactic dehydrogenase, and pulmonary infiltrates has been described in HIV-infected patients (Oksenhendler *et al*, 1990; Rabaud *et al*, 1994).

Ocular toxoplasmosis: Focal necrotizing retinochoroiditis is the hall mark of ocular toxoplasmosis. This accounts for nearly 35% of cases of chorioretinitis in children and adults. The majority of cases occur as a consequence of congenital infection. The retinochoroiditis usually is unilateral in acquired toxoplasmosis while it is bilateral in congenital toxoplasmosis. Blurred vision, photophobia, pain, and scotoma are the symptoms (Parija, 2004). In most cases, the infection causes inflammation of a small patch of retina, which spontaneously resolves, usually without being recognized. A localized pigmented scar involving the retina and the underlying tissue (the choroid) develops, which contains the *Toxoplasma* organism in an inactive, encysted form. In most cases, these chorioretinal scars are visually inert, and cause no significant visual abnormality. Rarely, the scarring process may involve the central portion of the retina (the macula), causing significant visual impairment. Occasionally, the encysted *Toxoplasma* organisms may cause a reactivation of the infection (Kasper, 2003).

3.1.2 Rubella

Rubella, commonly known as German measles or 3-day measles, is an infection that primarily affects the skin and lymph nodes. It is caused by the rubella virus which is an enveloped RNA virus that belongs to the family Togaviridae. Rubella virus shares the structural properties and mode of replication with other toga viruses. Unlike other togaviruses, rubella virus has no known invertebrate host and therefore it is not transmitted by arthropod (Banatvala and Best, 1998; Chakrobarty, 2003; Hirsch, 2006). The virion has a mean diameter of 58 nm with a 30 nm core. The core is surrounded by a lipoprotein envelope with surface spikes 5-8 nm in length. The virion is pleomorphic, owing to the delicate non-rigid nature of the envelope (Banatvala and Best, 1998).

Pathogenesis: Rubella infection occurs through the mucosa of the upper respiratory tract. Initial viral replication probably occurs in the respiratory tract, followed by the multiplications in the cervical lymph nodes. Viraemia develops after 7-9 days that spreads the virus throughout the body and lasts until the appearance of antibody on about day 13-15. Incubation period ranges from 12-23 days. The development of antibody coincides with the appearance of the rash, suggesting an immunological basis for the rash. After the rash appears, the virus remains detectable only in the nasopharynx, where it may persist for several weeks. During viraemic phase, Rubella virus is able to cross the placental barrier and can replicate in differentiating cells of the embryo which may result in teratogenic effects. The fetal cells are not destroyed by the virus and only their rate of growth is reduced resulting in fewer numbers of cells in affected organs (Brooks *et al*, 2004; Chakrobarty, 2003).

After infection in early pregnancy, rubella induces a generalized and persistent virus infection in the fetus which may result in multisystem disease. Tondury and Smith (1966) suggested that rubella virus enters the fetus via the chorion, in which it induces necrotic changes in the epithelial cells as well as in the endothelial lining of the blood vessels; the damaged endothelial cells are desquamated into the lumen of the vessel and then transported as virus-infected ‘emboli’ into the fetal circulation to settle in and infect various fetal organs. If retardation of cell division occurs during the critical phase of organogenesis, it is likely to result in congenital malformations (Banatvala and Best, 1998).

Immune responses: Rubella-specific IgG, IgM and IgA responses develop rapidly after the onset of rash. Rubella-specific IgG persists for life, but may decline to low levels in old age. Rubella-specific IgM usually appears within 4 days of onset of rash and persists for 4-12 weeks. Specific IgM may sometimes persist for up to one year after both naturally acquired infection and rubella immunization. Serum and nasopharyngeal IgA responses are detectable for at least 5 years after infection. MHC class I-restricted CD8+ cytotoxic T lymphocytes have also been demonstrated in rubella-immune individuals (Banatvala and Best, 1998).

Clinical manifestations:

Postnatally acquired infection: In young children the onset of illness is usually abrupt. Such constitutional symptoms as fever and malaise may be present for a day or two before onset of the rash but they usually subside rapidly after its appearance. Older children and adults may experience more pronounced constitutional symptoms 3-4 days before the rash appears, and during this prodromal phase an exanthem consisting of erythematous pinpoint lesions on the soft palate may be present. The exanthema is usually discrete, in the form of pinpoint maculopapular lesions. It appears first on the face and spreads rapidly to the rest of the body; lesions on the body may coalesce. The rash usually persists for about 3 days, occasionally longer, but may be fleeting. Patients may complain of tender lymph nodes when or just before the rash appears.

Rubella is rarely associated with severe complications. Encephalitis may occur in approximately 1 in 10,000 cases, but in general the prognosis is good. Very occasionally, rubella is associated with thrombocytopenia, which may result in purpuric rash, epistaxis, haematuria and gastrointestinal bleeding. Symptoms generally develop as the rash subsides and vary in severity from mild stiffness of the small joints of the hands to a frank arthritis with severe pain, joint swelling and limitation of movement. The duration of these symptoms is usually about 3 days but occasionally they may persist for up to a month (Banatvala and Best, 1998).

Congenitally acquired infection: The pathogenesis of transient lesions is not understood, but they are usually present only during the first few weeks of life, do not recur and are not associated with the development of permanent sequelae. Intrauterine growth retardation resulting in low birth weight but at a normal gestational age is among the commonest of the transient features. A petechial or purpuric rash is also common, particularly among infants whose mothers had had maternal rubella in early pregnancy. These infants may have other anomalies such as congenital heart and eye defects, although they may not always be apparent at birth (Banatvala and Best, 1998).

Whether maternal rubella induces fetal damage that causes intrauterine death or the birth of a malformed infant depends on the gestational age at which maternal rubella occurs, although other factors may also be involved. Maternal rubella may result in spontaneous abortion in up to 20% of cases when maternal infection is acquired during the first 8 weeks of pregnancy. It is now known that 75-100% of infants born to mothers infected in the first trimester will be congenitally infected and most of those infected will have associated defects. When maternal rubella is acquired during the first 8 weeks of pregnancy- the critical phase of organogenesis- cardiac and eye defects are likely to occur. However, because organogenesis is complete by 12 weeks and in more mature fetuses responses may limit or terminate infection, such infants rarely have severe or multiple anomalies. Deafness is usually the sole clinical manifestation of fetal infection occurring between 13 and 16 weeks (Banatvala and Best, 1998).

3.1.3 Cytomegalovirus

Cytomegalovirus (CMV), which was initially isolated from patients with congenital cytomegalic inclusion disease, is now recognized as an important pathogen in all age groups. In addition to inducing severe birth defects, CMV causes a wide spectrum of disorders in older children and adults, ranging from an asymptomatic, subclinical infection to a mononucleosis syndrome in healthy individuals to disseminated disease in immunocompromised patients. The virus is associated with the production of characteristic enlarged cells- hence the name *cytomegalovirus* (Hirsch, 2003). It is a major cause of multiorgan disease in immunocompromised patients, the severity of disease being related to the degree of immunosupression (Britt, 1998).

CMV is a member of the β-herpesvirus group and has doublestranded DNA, a protein capsid, and a lipoprotein envelope. Like other herpesviruses, CMV demonstrates icosahedral symmetry, replicates in the cell nucleus, and can cause either a lytic and productive or a latent infection (Hirsch, 2003). CMV has the largest genetic content of the human herpes viruses. One, a cell surface glycoprotein, acts as an Fc receptor that can nonspecifically bind the Fc portion of the immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins (Brooks *et al,* 2004).

Pathogenesis: Primary infection with CMV may be acquired at any time possibly from conception onwards (Ogilvie, 1997). Congenital CMV infection can result from either primary or reactivation infection of the mother. However, clinical disease in the fetus or newborn is almost exclusively related to primary maternal infection (Hirsch, 2003*)*. Cytomegalovirus preferentially invades the salivary glands and, in infants, the germinal matrix, but can infect other organ cells with development of characteristic cytoplasmic or nuclear inclusion bodies. Timing of the maternal infection and fetal development with hematogenous transplacental transmission is felt to play a critical role in the pathogenesis (Stagno *et al,* 1986), as disruption from the inclusion viral material may cause cell damage versus cell death and subsequent sequelae (Chriss-Price *et al*, 1986).

Primary infection in late childhood or adulthood is often associated with a vigorous T lymphocyte response that may contribute to the development of a mononucleosis syndrome. The hallmark of such infection is the appearance of atypical lymphocytes in the peripheral blood; these cells are predominantly activated CD8+ T lymphocytes. Polyclonal activation of B cells by the virus contributes to the development of rheumatoid factors and other autoantibodies during CMV mononucleosis. Once acquired by symptomatic or asymptomatic primary infection, CMV persists indefinitely in tissues of the host. Transmission following blood transfusion or organ transplantation is due to silent infections in these tissues. Chronic antigenic stimulation in the presence of immunosupression (for example, following tissue transplantation) appears to be an ideal setting for CMV activation and CMV-induced disease (Hirsch, 2003*)*.

Immune responses: The host response to primary CMV includes IgM, IgG and T cell responses. CMV early genes transactivate other viral and cellular genes and this may be an important interaction with HIV, leading to the production of HIV from latently infected cells. Because CMV infects mononuclear cells, there is a degree of immunosupression associated with the acute infection. Cell-mediated responses are crucial to control of CMV, as shown by the serious consequences of disseminated infections in those deficient in effector cell functions (Ogilvie, 1997).

Clinical manifestations:

Congenital CMV infection: Congenital CMV is usually only a risk when the mother develops a CMV infection for the first time during pregnancy. This is asymptomatic in 95% of infected babies, but around 15% of these will go on to show sensorineural deafness or intellectual impairment later. Some babies with congenital CMV may be born with obvious problems such as: prematurity, low birth weight, lung problems, growth retardation, hepatosplenomegaly, jaundice and thrombocytopenia. The 5% symptomatic infants have ‘cytomegalic inclusion body disease’. Central nervous system involvement is the significant problem; microcephaly, encephalitis and retinitis may be noted at birth (Ogilvie, 1997; Southern Cross Healthcare, 2004).

Perinatal CMV infection: The newborn may acquire CMV at the time of delivery by passage through an infected birth canal or by postnatal contact with maternal milk or other secretions. Approximately 40 to 60% of infants who are breast-fed for longer than 1 month by seropositive mothers become infected. The great majorities of infants infected at or after delivery remain asymptomatic. Poor weight gain, adenopathy, rash, hepatitis, anemia, and atypical lymphocytosis may also be found, and CMV excretion often persists for months or years (Hirsch, 2003).

CMV mononucleosis: The most common clinical manifestation of CMV infection in normal hosts beyond the neonatal period is a heterophil antibody-negative mononucleosis syndrome. Although the syndrome occurs at all ages, it most often involves sexually active young adults. Prolonged high fevers, sometimes accompanied by chills, profound fatigue, and malaise, myalgias, headache, and splenomegaly are frequent. Less commonly observed are interstitial or segmental pneumonia, myocarditis, pleuritis, arthritis, and encephalitis. The excretion of CMV in urine, genital secretions, and/or saliva often continues for months or years (Hirsch, 2003).

CMV infecton in the immunocompromised patients: In recipients of kidney, heart, lung and liver transplants, CMV induces a variety of syndromes, including fever and leucopenia, hepatitis, pneumonitis, esophagitis, gastritis, colitis and retinitis. The period of maximal risk is between 1 and 4 months after transplantation, although retinitis may be a later complication. The transplanted organ is particularly vulnerable as a target for CMV infection. CMV is recognized as an important pathogen in patients with advanced HIV infection, in whom it often causes retinitis or disseminated disease, particularly when peripheral-blood CD4+cell counts fall below 50 to 100/µl. Syndromes produced by CMV in the immunocompromised host often begin with prolonged fever, malaise, anorexia, fatigue, night sweats and arthralgias or myalgias. Liver function abnormalities, leucopenia, thrombocytopenia, and atypical lymphocytosis may be observed during these episodes. The development of tachypnea, hypoxia, and unproductive cough signals respiratory involvement. Gastrointestinal CMV involvement includes ulcers of the esophagus, stomach, small intestine, or colon that may result in bleeding or perforation. CMV meningoencephalitis and CMV retinitis are seen in patients with advanced AIDS (Hirsch, 2003).

3.1.4 Herpes simplex virus

Herpesvirus hominis, or herpes simplex virus (HSV), is one of the most common agents infecting humans of all ages. The virus occurs worldwide and produces a variety of illnesses, including mucocutaneous infections, infections of the CNS, and occasionally infections of the visceral organs. Infections in children can include neonatal disease, mucocutaneous infections during childhood and adolescence, and serious disease in individuals who are immunocompromised. Genital HSV infection in older adolescents and adults is a major public health problem, having markedly increased in prevalence in the last 3 decades. Neonatal HSV infection is a disease with high morbidity and mortality rates (Alter, 2006). There are two types of HSV: HSV-1 and HSV-2 which share many common characteristics (Chakrobarty, 2001).

HSV has structure similar to other Alphaherpes viruses. They are approximately 150 nm in diameter, contains icosahedral capsid made of 162 capsomers, double-stranded DNA genome and are surrounded by a lipid envelope derived from the nuclear membrane of host cell (Chakrobarty, 2001). Projecting from the trilaminar lipid envelope are spikes of viral glycoproteins (Ogilvie, 1997).

Pathogenesis: The biologic properties of HSV that control the course of infection are neuroinvasiveness (the ability of the virus to invade the brain), its neurotoxicity (its ability to multiply and destroy the brain), and its latency (its ability to remain in a nonreplicating form in the dorsal root ganglia of the CNS) (Alter, 2006). The virus replicates actively in skin or mucosal vesicular lesions. The incubation period varies between 5-6 days. After primary infection the virus travels by retrograde intra-axonal flow to sensory root ganglia which innervate the area of infection. They settle within the neurons in the sensory ganglia, either the trigeminal ganglion (HSV-1) or the sacral ganglion (HSV-2), where it remains latent. No viral particles are produced during latency. After the initial nonspecific inflammatory response to primary infection, specific antibody response occurs in a few days, followed by a cellular immune response in the second or third week. In persons with cellular immune defects, primary HSV infection can result in life-threatening disseminated disease. In recurrence, the virus travels back down the nerve, causing lesions at the same spot each time (Alter, 2006; Chakrobarty, 2001).

Immune responses: Both antibody-mediated and cell-mediated reactions are clinically important. Multiple cell populations, including natural killer cells, macrophages, a variety of T lymphocytes, and lymphokines play a role in host defenses against HSV infections. Maximum protection usually requires the activation of multiple T cell subpopulations, including cytotoxic T cells and T cells responsible for delayed hypersensitivity. The latter cells may confer protection by the antigen-stimulated release of lymphokines (e.g., interferons), which may have a direct antiviral effect and may activate and enhance a variety of specific and nonspecific effector cells.

Clinical manifestations: The clinical manifestations and course of HSV infection depend on the anatomic site involved, the age and immune status of the host, and the antigenic type of the virus. Primary HSV infections with either HSV-1 or HSV-2 are frequently accompanied by systematic signs and symptoms, involve both mucosal and extramucosal sites, and have a longer duration of symptoms, a longer duration of virus isolation from lesions, and a higher rate of complications than recurrent episodes of disease. Both viral subtypes can cause genital and oral-facial infections, and the infections caused by the two subtypes are clinically indistinguishable (Corey, 2003).

Oral-facial infections: Gingivostomatitis and pharyngitis are the most frequent clinical manifestations of first-episode HSV-1 infection, while recurrent herpes labialis is the most frequent clinical manifestation of reactivation HSV infection. Clinical symptoms and signs, which include fever, malaise, myalgias, inability to eat, irritability, and cervical adenopathy, may last from 3-14 days. Lesions may involve the soft and hard palate, gingival, tongue, lip and facial area (Corey, 2003).

Genital infections: Both types of HSV can infect the genital tract. Genital infection may be acquired by auto-inoculation from lesions elsewhere on the body, but most often results from intimate sexual contact, including orogenital contact. The lesions are vesicular at first but rapidly ulcerate. In the male, the glans and shaft of the penis are the most frequent sites of infection, while in the female, the labia and vagina or cervix may be involved. Lesions may spread to surrounding skin sites (Ogilvie, 1997).

Skin infections: Herpetic whitlow is the HSV infection of finger that may occur as a complication of primary oral or genital herpes by inoculation of virus through a break in the epidermal surface or by direct introduction of virus into the hand through occupational or some other types of exposure. Clinical signs and symptoms include the abrupt onset of edema, erythema, and localized tenderness of the infected finger (Corey, 2003). Eczema herpeticum is a severe form of cutaneous herpes that may occur in children. Vesicles resembling those of chickenpox may appear, mainly on already eczematous areas. Extensive ulceration results in protein loss and dehydration, and viraemia can lead to disseminated disease with severe, even fatal, consequences (Ogilvie, 1997).

Eye infections: There may be simply conjunctivitis, or keratoconjunctivitis associated with corneal ulceration. Typically, branching or dendritic cornel ulcers are found, and recur resulting in corneal scarring and impairment of vision (Corey, 2003).

Central and peripheral nervous system infections: HSV Encephalitis is an acute febrile illness most often caused by HSV-1 (Chakrobarty, 2001). The infection has a high mortality rate and significant morbidity in survivors of the acute necrotizing form (Ogilvie, 1997). HSV Meningitis is most often caused by HSV-2, is usually mild and symptoms resolve on their own (Chakrobarty, 2001).

Neonatal HSV infections: A rare but very serious infection, untreated neonatal herpes has a case fatality rate exceeding 60%, with half of the survivors severely damaged (Ogilvie, 1997). Neonatal infection is usually acquired perinatally. Congenitally infected infants have been reported. In most series, 30% of neonatal HSV infections are due to HSV-1 and 70% to HSV-2 (Corey, 2003). Virus dissemination to internal organs is the most serious complication, in which the infant shows signs of general sepsis, including fever, poor feeding and irritability. Pneumonia and jaundice develop, with or without signs of meningitis or encephalitis. Early antiviral therapy is the key to survival with minimal morbidity (Ogilvie, 1997).

3.2 LABORATORY DIAGNOSIS

Diagnosis of *Toxoplasma*:

- Serological tests.
- Demonstration of tachyzoites in blood, body fluids, tissue specimens, bronchoalveolar lavage etc.
- Demonstration of tissue cysts in lymph nodes and other tissues sections obtained either by biopsy or autopsy.

- PCR performed on amniotic fluid for the diagnosis of fetal *T. gondii* infection (Parija, 2004; TSL-PAMF, 2006).

Diagnosis of Rubella:

- Serological tests.
- Isolation of the virus in cell culture.
- Detection of rubella-specific IgM in cord serum or serum samples obtained in early infancy, isolation of Rubella virus or detection of viral RNA by RT-PCR in specimens (such as pharyngeal swabs) taken from infants during early infancy for the diagnosis of CAR in infants.
- Testing fetal blood samples obtained by fetoscopy for rubella-specific IgM after 22-23 weeks of gestation, virus isolation from amniotic fluid or by tests of chorionic villus samples or amniotic fluids for viral RNA using RT-PCR for prenatal diagnosis of CAR in fetuses (Banatvala and Best, 1998; Chakrobarty, 2003).

Diagnosis of Cytomegalovirus:

- Serological tests.
- Culture of virus from specimens obtained from urine, throat swabs, bronchial lavages and tissue samples to detect active infection.
- PCR testing to monitor the viral load of CMV-infected patients (Wikipedia, 2006).

Diagnosis of Herpes Simplex Virus:

- Serological tests.
- Isolation of the virus in tissue cultures.
- Microscopic examination of smear made from scrapings from base of vesicle stained by toludine blue which reveals Tzank cells.
- Immunofluorescent staining of infected tissue culture cells.
- Polymerase chain reaction (PCR).

- Lumbar puncture (LP) with submission of CSF for the Gram staining, bacterial culture and other analyses for the evaluation of patients with encephalitis.
- Brain biopsy to diagnose HSV CNS disease (Alter, 2006; Chakrobarty, 2001).

Serodiagnosis of TORCH infections: Serodiagnosis is based primarily on detection of TORCH-specific antibodies in the serum. ELISA (Enzyme-linked immunosorbent assay) is now widely used for detecting both IgG and IgM antibodies (Parija, 2004). Other tests include various fluorescence assays, indirect haemagglutination and latex agglutination (Wikipedia, 2006). In acute infection, IgG and IgM antibody levels generally rise within one to two weeks of infection (Montoya and Remington, 2000). IgG titers peak within 1-2 months after infection but remain elevated for life. The presence of elevated levels of specific IgG antibodies indicates that infection has occurred but does not distinguish between recent infection and infection acquired in the distant past. Detection of specific IgM antibodies has been used as an aid in determining the time of infection. The absence of anti-TORCH IgM virtually excludes recent infection in immunocompetent patients. This issue is important in the evaluation of pregnant women because congenital transmission of TORCH agents in immunocompetent women occurs almost exclusively when infection is acquired during gestation (Subauste, 2006; Wilson and McAuley, 1999; Wilson *et al*, 1997).

3.3 THE GLOBAL SCENARIO

Toxoplasma is the parasite found throughout the world, with greater presence in regions with warmer climates. Infection with the parasite occurs among all age groups, and as a consequence, serologic evidence of it increases with increasing age. It is thought that between 30% and 60% of the world's population are infected. The incidence of infection is highly specific to each nationality with ranges such as 22% infected in the UK to over 88% in France, probably due to a high consumption of raw and lightly cooked meat (Wikipedia, 2006). In the U.S. NHANES III national probability sample, 22.5% of 17,658 persons >12 years of age had *Toxoplasma*-specific IgG antibodies, indicating that they had been infected with the organism (Wikipedia, 2006). In contrast, in Asian countries, low prevalences of *T. gondii* infection were found in a Korean study (Song *et al*, 2005), and a Vietnamese study (Buchy *et al*, 2003) (0.8% and 11.2%, respectively). While prevalences as high as 41.8% to 55.4% in pregnant women have been reported in Indian (Singh and Pandit, 2004; Akoijam *et al*, 2002), Malaysian (Nissapatorn *et al*, 2003) and Nepalese (Rai *et al*, 1998) populations. For its part, a Sudanese study showed that 34.1% of the pregnant women studied had anti-*T. gondii* antibodies (Elnahas *et al*, 2003). Similarly, a study performed in New Zealand revealed a 33% prevalence of anti-*T. gondii* antibodies (Morris and Croxson, 2004). In the American continent, a study performed in south Brazil revealed that 74.5% of the pregnant women studied had anti-*T. gondii* IgG antibodies (Spalding *et al*, 2005).

Rubella is a major cause of birth defects among the TORCH group of agents causing congenital anomalies (Chakravarti and Jain, 2006). The mean incidence of CRS per 100,000 live births is estimated to be significantly lower in the eastern Mediterranean region (77.4, range 0-212) and higher in the Americas (175, range 0-598). On the other hand, the 1996 CRS mean estimate for developing countries was approximately 110,000, ranging from 14,000 to 308,000 cases (Cutts and Vynnycky, 1999). A review of the epidemiology of clinical rubella in the Perm region of the Russian Federation from 1979-97 showed that the incidence was about 220 cases per 100,000 population. Congenital rubella syndrome (CRS) accounted for 15% of birth defects and for about 3.5 cases of CRS per 1000 live births per year. Surveys of the seroepidemiology of rubella infection revealed that the susceptibility rate among pregnant women (i.e. rubella virus antibody haemagglutination-inhibition (HAI) assay titres < 10) was 16.5% (Semerikov *et al*, 2000).

Human cytomegalovirus (HCMV) is a ubiquitous virus worldwide, which causes a wide variety of clinical manifestations, the most severe occurring in immunocompromised hosts. Seropositivity for this virus increases with age, ranging from 40 to 100 percent depending upon geography and socioeconomic status (Tremblay, 2006). CMV infects between 50% and 85% of adults in the United States by 40 years of age. CMV infection is more widespread in developing countries and in areas of lower socioeconomic conditions. It causes the most birth defects in industrialized countries out of all the herpes viruses. The incidence of primary CMV infection in pregnant women in the United States varies from 1% to 3% (Wikipedia, 2006). An estimated 0.2-2.4% of worldwide live-born infants acquire the virus perinatally (Berge *et al*, 1990). Only 10% of these will demonstrate classic signs of illness at birth (Bale, 1994).

Infections caused by herpes simplex viruses (HSV) types 1 and 2 are common throughout the world, with considerable variation from country to country and within population groups (Smith and Robinson, 2002). In the United States, HSV-2 seroprevalence increases from about 20-30% in patients aged 15-29 years to 35-60% in patients aged 60 years. Genital HSV infection in pregnant women is common. Approximately 22% of pregnant women are infected with HSV-2. In pregnant women, the prevalence of HSV excretion from the genital tract at term is estimated to be 0.3-1.9%. However, the incidence of neonatal infection is estimated to be 1 in 2000-5000 births (Alter, 2006). Studies in selected populations, considered to be at high risk for the acquisition of sexually transmitted infections (STIs), have shown that HSV-2 occurs in 13–75% of patients attending STI clinics (Cowan *et al*, 1994; Cunningham *et al*, 1993; Hashido *et al*, 1998; Langeland *et al*, 1998; Van de Laar *et al*, 1998), 24–87% of men who have sex with men (Cowan *et al*, 1994; Dukers *et al*, 2000; Russell *et al*, 2001; Stamm *et al*, 1988; Van de Laar *et al*, 1998), and 74–98% of female sex workers (Limpakarnjanarat *et al*, 1999; Nzila *et al*, 1991).

Both herpes simplex viruses (HSV) types I and type 2 can cause genital herpes. The prevalence of the HSV-2 antibody among women in the United States is 26%, although genital herpes has been diagnosed in only a small proportion (10-25%) of individuals with HSV-2 antibodies. Herpes simplex virus type I is becoming a more frequent cause of genital herpes, especially among young women. Overall, HSV-I seroprevalence in the U S is estimated to be 67% (ACOG practice bulletin, 2004).

TORCH panel

In a serosurvey conducted in Southern Africa to determine the prevalence of antibodies to rubella, herpes simplex 2 and cytomegalovirus in pregnant women and in neonates, blood samples were collected from 917 women attending the Obstetrics and Gynaecology Department at Ga-Rankuwa Hospital during a one year period. Each woman presented with an unfavourable outcome to pregnancy. Blood was also obtained from 99 newborn babies who were jaundiced, or who died within a few days of birth or who showed gross congenital abnormalities. IgM antibodies to cytomegalovirus (CMV), Herpes simplex virus type 2 (HSV-2) and rubella virus were determined by commercial ELISA. CMV was found to be the most prevalent infection in both groups of women (19.2 pc) and in the babies (24.2 pc) indicating the importance of this virus in intra-uterine infection in this community. Rubella and HSV-2 infection were identified in the population sample but seemed to play a much less significant role than CMV (Bos *et al*, 1995).

Seroprevalences for toxoplasmosis, rubella, and cytomegalovirus were evaluated among 211 pregnant women residing in the Cotonou area of France. One hundred and thirteen women (53.6%) had *Toxoplasma* antibodies and 181 (85.8%) rubella antibodies. Among the 205 (97.2%) women with cytomegalovirus antibodies, 6 presented recent or current infection (Rodier *et al*, 1995).

A cross-sectional, sero-epidemiological survey of the prevalence of antibodies to TORCH agents in pregnant Thais during various stages of gestation performed in Thailand revealed an overall rate of 13-15 percent having antibodies to *Toxoplasma gondii*; 85-87 percent, to rubella ; 79-81 percent, to herpes simplex virus (HSV); 100 percent, to cytomegalovirus (CMV). Although a tendency was noted towards an increase of antibody detection to each TORCH agent as gestation progressed, a statistically significant increase in antibodies titer and specific IgM antibody was found with regard to CMV (Taechowisan *et al*, 1997).

A prospective study designed in India to detect the seroprevalence of IgM antibodies to *Toxoplasma gondii*, rubella virus and cytomegalovirus and IgG antibodies to herpes simplex virus type 1 and 2 included 120 pregnant women presenting to the antenatal clinic. Out of these 120 women 112 (93.4%) had evidence of one or more infections. Prevalence of IgG antibodies to HSV was 70%. Seropositivities for toxoplasmosis, rubella and CMV respectively were 11.6, 8.3 and 20.8% (Kaur *et al,* 1999).

In a study, 247 women with different complications of pregnancy were screened at the time of delivery for infections like *Toxoplasma, Rubella* and cytomegalovirus (CMV). One hundred and forty two women with normal outcome of pregnancy served as controls. Specific IgM due to these agents were determined in the sera using commercial diagnostic kits. Results of the study showed *Toxoplasma* (13.1%), Rubella (6.5%) and CMV (5.8%). Adverse outcome was seen among those seropositive for *Toxoplasmosis* and *Rubella*. CMV showed no association with adverse outcome of pregnancy (Yasodhara *et al*, 2001).

To determine the seroprevalence rates of IgG to common TORCH agents in pregnant Saudi women using indirect ELISA, a total of 926 samples of sera were tested in Makkah, Kingdom of Saudi Arabia for antibodies to TORCH agents known to cause serious congenital infections: *Toxoplasma gondii*, rubella, cytomegalovirus (CMV), herpes simplex viruses (HSV-1 and HSV-2). *Toxoplasma* IgG antibodies were detected in 35.6%, CMV total IgG antibodies were found in 92.1%, rubella IgG antibodies in 93.3%, HSV-1 IgG antibodies in 90.9% and HSV-2 IgG in 27.1%. (Ghazi *et al,* 2002).

Immunoglobulin M antibodies using m-capture ELISA for S-TORCH agents (Syphilis, tested by VDRL) were analysed in Lucknow, UP in 47 sera from women with recurrent spontaneous abortions (RSA). Results compared with 29 age matched normal pregnant women. S-TORCH positivity in RSA group was 31.9% and nil in the control group ($p < 0.005$). Present study demonstrates a strong association between IgM antibodies to S-TORCH agents in women with history of RSA (Kishore *et al,* 2003).

Over a one-year period 380 serum samples were collected from pregnant women having bad obstetric history attending antenatal clinic to determine the prevalence of *Toxoplasma*, Rubella, CMV and HSV-II infection in pregnant women by demonstrating the presence of IgM and IgG antibodies by ELISA test. It was found that IgM antibodies were positive in 40 (10.52%) for *Toxoplasma*, 102 (26.8%) for Rubella, 32 (8.42%) for CMV and 14 (3.6%) for HSV-II. IgG antibodies were positive in 160 (42.10%) for *Toxoplasma*, 233 (61.3%) for Rubella, 346 (91.05%) for CMV 145 (33.58%) for HSV-II (Turbadkar *et al*, 2003).

To review the indications and value of TORCH testing, cases with confirmed maternal or fetal infections were studied during nearly a 10-year period in U.K. Four hundred and sixty-two maternal TORCH tests were performed. Of those, TORCH tests were also performed on fetal samples (amniotic fluid or fetal blood) in 67 cases. Fourteen fetal tests without maternal testing were identified; making the total number of patients tested 476. There were 11 cases of maternal CMV infection (2.3%), 10 cases of fetal CMV infection, and none of the other viruses (Abdel-Fattah *et al*, 2005).

Over a nine months period seropositivity of *Toxoplasma*, rubella, CMV, and HSV infections (TORCH) in 20 pregnant women in Kumaon region of Nainital with bad obstetric history were demonstrated by the presence of IgM and IgG antibodies by ELISA method. It was found that, IgM antibodies were positive in 4 cases (20%) for *Toxoplasma*, 4 cases (28.6%) for rubella and 4 cases (26.7%) for CMV and HSV each. IgG antibodies were positive in 11cases (55%) for *Toxoplasma*, 10 cases (66.6%) for rubella, 14 cases (93%) for CMV and 11 (73%) for HSV (Thapliyal *et al*, 2005).

Toxoplasma

Central nervous system (CNS) toxoplasmosis is an important infectious complication of AIDS which requires prolonged treatment. In a study done in AIDS patients, antibody to *Toxoplasma gondii* was found in 130 out of 411 patients with AIDS (32%). Of these, CNS toxoplasmosis developed in 31 (24%). By survival analysis, the estimated probability of ever developing CNS infection in antibody-positive individuals was 28%, occurring in 26% of patients within 2 years of the onset of AIDS. All patients with HIV infection should be tested for antibody to *T. gondii* and monitored for any neurologic change (Grant *et al*, 1990).

Two hundred and seventy nine sera (age group 13-50 years) were tested for anti*Toxoplasma* IgG/IgM antibodies by ELISA techniques in a study done in India. Sera were obtained from (i) 165 (100 men/65 women) healthy adult voluntary blood donors (HIV, HBsAg, VDRL negative); (ii) 89 consecutive HIV/AIDS patients (82 men/7 women); and (iii) 25 patients (HIV negative: 12 men/13 women) treated for cerebral Tuberculoma or Neurocysticercosis during this study from January 1996-June 1997. The overall seroprevalence was 30.9% (51/165) in the immunocompetent adult: 34% (34/100) men and 26.2% (17/65) in women. In HIV infected hosts the seroprevalence was 67.8% (56/82 men, 04/07 women). The seroprevalence was 20.5% (8/39), 32.8% (22/67), 34.8% (16/46) and 38.4% (5/13) in the 2nd, 3rd, 4th and 5th decades respectively in healthy adults. In HIV/AIDS patients, 69% (29/42) in the 3rd and 70.6% (24/34) in 4th decade were seropositive. The risk of cerebral Toxoplasmosis (encephalitis-02, granuloma-24) was 43.3%. The seroprevalence was 28% in group iii. Anti-toxo IgM was negative in all (Meisheri *et al*, 1997)

In a study conducted in India 200 uveitis cases and 100 controls were serologically analysed for *Toxoplasma* antibodies using indirect fluorescent antibody test (IFAT-IgG, IgM) and enzyme linked immunosorbent assay (ELISA IgG, IgM). *Toxoplasma* seropositivity of 32% in cases and 4% in controls was established. IHA, IFAT, ELISA detected 20%, 18% and 32% cases as seropositive respectively, IFAT being most specific (100%) and ELISA most sensitive (41.37%). Insignificant change in antibody titre was observed in sequential samples of seropositive cases. Highest seropositivity was in 16-25 years age group with no sex preponderance (Jain *et al*, 1998).

In a study in Srinagar (Kashmir) involving 2371 women with recurrent abortions and 310 women with neonatal deaths tested for IgM antibody against *Toxoplasma*, 1260 (53.14%) and 215 (69.35%) were tested positive respectively. One hundred and twenty-two women with recurrent abortions and 55 women with neonatal deaths who had tested positive for IgM antibody were followed during subsequent pregnancy and were treated with spiramycin; 115 (94.26%) in current abortion group and 35 (63.64%) in neonatal death group delivered normal babies (Zargar *et al*, 1999).

A screening based on identifying IgG and IgM specific antibodies was performed to estimate the frequency of *Toxoplasma gondii* infections in 2016 pregnant women and their children during the year 2000 in Warsaw. Children born by infected mothers were examined serologically and observed in terms of congenital abnormality. There were 1294 (64.19%) seronegative; 722 (35.81%) were infected before pregnancy (the presence of IgG antibodies). Five women with previous seronegative results (0.29%) were diagnosed as having primary infection during pregnancy. Congenital infection, confirmed serologically, was recognized in 3 newborn infants (Niemiec *et al*, 2002).

In a study done to investigate the prevalence of toxoplasmosis in subjects from rural, urban and urban slum populations of Chandigarh, serum samples from 500 subjects from each group were collected and anti*Toxoplasma* IgM and IgG was detected by conventional micro ELISA technique using soluble *Toxoplasma gondii* tachyzoite antigen. Overall 5.4% subjects were positive for IgM while 4.66% showed IgG anti*Toxoplasma* antibodies. Amongst the three groups, significantly higher number of subjects in slum area (7.8%) showed IgM antibodies as compared to urban and rural areas (4.2% each). There was no significant difference in IgG positivity between three study areas. Prevalence of T. gondii specific IgG antibodies was significantly higher amongst females of both slum (7.31%) and rural area (8.44%) as compared to the males (2.85% and 3.27% respectively) in the same areas ($p<0.05$) and also to females of the urban area (2.98%, $p<0.05$). Prevalence of IgM antibodies was significantly higher ($p<0.05$) in females in the slum area (10.5%) as compared to females in the urban area (2.55%). In both urban and slum areas, highest IgM seropositivity was observed in age group 6-12 years (10% and 13.3% respectively), while in the rural area the highest IgM seropositivity was seen in the age group > or = 5 years (17.7%) (Mohan *et al*, 2002).

In a study carried out in Malaysia on 200 pregnant women, the overall seroprevalence of toxoplasmosis was found to be 49%, in which 39%, 4% and 6% for anti-*Toxoplasma* IgG, IgM and both anti-*Toxoplasma* IgG and IgM antibodies, respectively. Differences found in *Toxoplasma* seroprevalence rates among the races were significant: the highest rate was in the Malays (55.7%), followed by the Indian (55.3%) and the Chinese (19.4%) (P<0.05) populations. An increase in *Toxoplasma* seroprevalence with increasing parity was detected (P<0.05). Women with no children had a prevalence of 39.7%, while women with one or more than two children had a prevalence of 44.2% and 62.9%, respectively (Nissapatorn, 2003).

To determine the prevalence of IgG and IgM antibody to *T. gondii* in pregnant Auckland women, five hundred serum samples submitted for routine antenatal blood tests were tested for IgG and IgM antibodies to *T. gondii*. One hundred consecutive serum samples were tested from five age groups: <20, 21-25, 26-30, 31-35, >36 years. One hundred and sixty three (33%) women had IgG antibody to *T. gondii* and 12 (2.4%) also had IgM antibody (Morris and Croxson, 2004).

To determine the prevalence and risk factors for *T. gondii* infection in Guatemalan children, in 1999 and 2003 a survey was carried out in which caretakers and children were serologically tested using Platelia Toxo IgG TMB enzyme immunoassay kits. In 1999, of 532 children six months to two years old, 66 (12.4%) were antibody positive. In 2003, in 500 children 3–10 years old antibody prevalence increased from 24% to 43% at age five years then leveled off. By multivariate analysis, drinking well water (relative risk [RR] = 1.78, 95% confidence limit [CL] = 1.00, 3.17, P = 0.05) and not cleaning up cat feces (RR = 2.06, 95% CL = 1.00, 4.28, P = 0.05) increased the risk of *T. gondii* seropositivity. Most *T. gondii* infections in children from these villages occurred by age five, but half were still not infected by adolescence. Therefore, it was found that it is important to educate girls entering child-bearing age about the risks of acute *T. gondii* infection and the local risk factors for infection (Jones *et al*, 2005).

To determine the seroprevalence and risk factors for toxoplasmosis among pregnant women in Jordan, sera from 280 pregnant women after the first antenatal visit were tested for *Toxoplasma* IgG antibodies using an indirect fluorescent antibody during the period January 2000-May 2001. Seroprevalence gradually increased with age, from 31.7% at 15-24 years to 90.0% at 35-45 years. Regression analysis showed that seroprevalence of toxoplasmosis is positively correlated with age and residence. Consumption of undercooked meat and contact with soil were significant risk factors (Jumaian, 2005).

Toxoplasma gondii IgG antibody seroprevalence was studied in two different populations of 219 HIV-infected patients and 144 apparently healthy individuals (AHIs). Clinical toxoplasmosis was assessed among the HIV-infected patients. Antibodies to T. gondii were detected in 85 (38.8%, 95% CI: 32.36%-45.26%) of the HIV-infected patients and in 30 (20.8%, 95% CI: 14.20%-27.46%) of the AHIs. Among the AHIs, males represented 22.0% of infections compared to females (20.0%) and individuals within age group 21-30 years accounted for the highest prevalence of 33.3% (95% CI: 11.56%-55.10%). Assessment of epidemiological factors showed higher seroprevalence of *Toxoplasma* antibodies among those who eat rodents (29.6%) and those who constantly have contact with the soil (21.2%). Among the HIV-infected, individuals 31-40-years-old had the highest *T. gondii* seroprevalence (36.5%) (Uneke *et al*, 2005).

Three hundred and forty three women seeking prenatal care in a public hospital of Durango City in Mexico were examined for *T. gondii* infection. All women were tested for anti-*T. gondii* IgM and IgG antibodies. Socio-demographic, clinical and behavioural characteristics from each participant were also obtained. Twenty one out of the 343 (6.1%) women had IgG anti-*T. gondii* antibodies. None of the 343 women had IgM anti-*T. gondii* antibodies. Multivariate analysis using logic regression showed that *T. gondii* infection was associated with living in a house with soil floor (adjusted OR = 7.16; 95% CI: 1.39–36.84), residing outside of Durango State (adjusted OR = 4.25;

95% CI: 1.72–10.49), and turkey meat consumption (adjusted OR = 3.85; 95% CI: 1.30–11.44) (Alvarado-Esquivel *et al*, 2006).

A study was performed to evaluate the seroprevalence of toxoplasmosis among the inhabitants of rehabilitation centers of northern Iran. A total of 336 serum samples (161 males, 175 females) were examined for the IgG antibodies by indirect immunofluorescense technique. Among 336 sera, 77.4% showed seropositivity by IFAT. The positive rates of males and females were 77.6% (125/161) and 80% (140/175), respectively (Sharif, 2006).

Prevalence of antibodies against *Toxoplasma* gondii was studied in 534 pregnant women and 40 domestic cats in Grenada, West Indies. Antibodies (IgG) for T. gondii were sought in human sera by an enzyme-linked immunosorbent assay and in cat sera by using the modified agglutination test (MAT). Antibodies were found in 57 % of pregnant women. Seroprevalence increased with age; 51% of 15- to 19-yr-old women (100 total) had antibodies versus 60% of 20- to 24-yr-old women (127 total). Antibodies to T. gondii (MAT, 1:25 serum dilution) were found in 35% of cats; titers were 1:25 in 7 cats, 1:50 in 4 cats, and 1:500 in 3 cats. Epidemiological data suggested that the ingestion of food or water contaminated with oocysts was an important mode of transmission of T. gondii to women (Asthana *et al*, 2006).

Rubella

A serosurvey of rubella was carried out in Kinshasa (Zaire) by haemagglutination inhibition and IgM assay among 106 newborn infants (91% positive); 101 suckling infants aged 9-18 months (32.7% positive); 100 children aged 2-4 (58% positive); and 100 young girls 9-11 (68% positive), while 93% of mothers showed the presence of protective antibodies (Omanga *et al*, 1991).

In a serosurvey conducted in southern Italy, serum titers of anti-rubella antibodies were measured in 4,424 babies and children (aged 0-15 years) and in 2,362 females of childbearing age by a microhemagglutination-inhibition technique. Sera were screened for IgM antibodies with an ELISA; positive sera were titrated in a capture immunoenzymatic test. The incidence of serological positive response, high at birth and in the first 6 months of life (65.0%), declined in the older age-groups (60.2% from 6 to 12 months, 57.0% from 1 to 2 years, 54.2% from 2 to 3 years, and 55.2% from 3 to 6 years). Over 6 years, the incidence increased progressively (63.9% from 6 to 9 years and 76.1% from 9 to 15 years). In females aged 15-45 years the seronegativity rate was 8.6% (Leogrande, 1993).

A study that demonstrates prevalence of sensorineural hearing loss due to rubella in Saudi children found positive IgM antibody against rubella virus in the blood of 23 out 1,054 (2.2%) children (age ranged between 12 months and 14 years). 15 out of 23 infected children were found to have bilateral sensorineural hearing loss. Hearing impairment was bilateral in all cases, profound in 1, moderate to severe in and mild in 5 (Zakzouk and al-Muhaimeed, 1996).

A documented case of rubella reinfection during pregnancy was reported in a previously vaccinated woman with residual antibody titer to rubella of 15 IU/ml in Israel. The reinfection occurred following an exposure to rubella virus (contact with 6-year-old daughter with clinical rubella) between the 7th and 10th week of pregnancy which resulted in transmission of the virus to the fetus. Umbilical cord blood drawn by cordocentesis was found to be strongly positive for rubella IgM antibody. After termination of the pregnancy rubella virus was isolated in cell culture from fetal tissues (Aboudy *et al*, 1997).

In a study in Manipal, India, which includes a total of 342 infants suspected of having congenital infections from January 1991-December 1993, 52 (15.2%), were found to be positive for IgM antibodies to rubella virus. The commonest clinical presentation in infants with IgM antibodies to rubella virus was bilateral congenital cataract and hepatosplenomegaly (Ballal and Shivananda, 1997).

Indirect ELISA assay was used to test 1193 sera for rubella IgG and IgM antibodies in a seroepidemiological survey conducted in Shiraz, Islamic Republic of Iran involving three age- and gender-differentiated sample populations in Shiraz: 203 children aged 2-7 years, 255 paired mothers and neonates (cord blood) and 480 women aged 14-70 years. Seropositivity among women aged 14-70 years was 96.2%. No IgM positive case was found among the 255 tested cord blood samples. Seropositivity among the 203 children was 97.0% (Doroudchi *et al*, 2001).

In a study in Bangladesh, a total of 198 hearing-impaired children and 200 children without hearing problems were studied. Blood samples were collected from both mothers and children; sera were subjected to ELISA for anti-rubella IgG. Rubella antibody was detected in 74% of the hearing-impaired children and in 18% of those with normal hearing: this finding correlated with the presence of rubella antibody in the mothers (67%) of rubella seropositive hearing-impaired children. In contrast, rubella antibody was observed in only 14% of the mothers of the children without hearing problems. Consistent with the presence of antibody, 41% of the seropositive mothers who had hearing-impaired children gave a history of fever and rash during early pregnancy. This study indicated a strong association between rubella infection and hearing impairment in children (Rahman *et al*, 2002).

To determine the prevalence of rubella antibodies and age of exposure to rubella among schoolgirls in Yemen, the sera samples of 323 female students (age range 11-21 years; mean age 16.26 +/- 1.89 years) drawn from three schools in Sana'a were screened for rubella IgG antibodies using ELISA and, if negative, for IgM in order to exclude the possibility of recent exposure. Of 323 sera, 296 (91.64%) were positive for rubella IgG. All IgG negative sera were also IgM negative. The prevalence of rubella IgG among Yemeni schoolgirls was found to be high, with most becoming immune between the ages of 11 and 21 years (Sallam *et al*, 2003).

In an investigation carried out in northwestern Brazil, during a large rubella outbreak from April 1 to December 31, 2000, 391 confirmed rubella cases were reported. The incidence among persons ages 12 to 19 years (3.3 per 1000 population) was increased 3.7-fold relative to children ages 1 to 4 years (95% confidence interval, 2.4 to 5.8). Of

21 infants with suspected CRS cases, 17 (91%) were tested for rubella-specific antibodies, of whom 7 were IgM-positive and 5 had confirmed CRS. The peak incidence of confirmed CRS (4.3 per 1000) was in March 2001, 7 months after the outbreak peak, with an annualized incidence of 0.6 per 1000 (Lanzieri *et al*, 2003).

In a study in India, paired sera of 146 babies with suspected intra uterine infection and their mothers from lower socioeconomic strata was tested for IgM antibodies by commercially available Enzyme immunoassay (EIA) kits. It was seen that out of 146-paired samples evaluated, 15-paired samples (10.27%) were positive for IgM antibodies. The transmission rate of rubella virus from mother to child when the mother was infected was around 55.55% according to this study. CRS prevalence of 10.27% among symptomatic infants is significant as a large majority of rubella infection remains undetected and hence the actual burden of the disease may be higher (Chakravarti and Jain, 2006).

Cytomegalovirus

In a study carried out in China, Human cytomegalovirus (HCMV)-IgG, IgM antibodies were detected by indirect ELISA in 103 serum specimens from women with history of abnormal pregnancy. The results showed that the positive rates of HCMV-IgG and IgM of abnormal pregnant women were 90.29% and 13.59% respectively. The positive rate of HCMV-IgM in abnormal pregnancies was higher than that of normal pregnancies (4.07%, $P < 0.001$) (Xu, 1991).

Clinical data and samples of blood for detecting CMV antibodies were obtained from 716 pregnant women in China of whom, 6.84% and 95.61% had CMV IgM and CMV IgG antibody, respectively. Statistic analysis showed that high positivity rates of CMV IgM antibody were correlated with lower socioeconomic status, first pregnancy at less than or equal to 22 or greater than or equal to 29 years of age, and history of abortion, especially natural abortion. Positivity rates of CMV IgM antibody were higher in medical workers and peasants ($P < 0.05$) (Guo, 1992).

To determine risk factors responsible for primary CMV infection in Taiwan, samples of blood for antibody to CMV were obtained from 362 children aged 4 to 12 years: 58% were found to be positive for anti-CMV IgG antibody and 0.6% for anti-CMV IgM antibodies. Logistic regression analysis showed that seropositivity correlated with age, method of delivery, duration of breast feeding, and younger age of mother. Since the majority of pregnant women were seropositive in Taiwan, two of the major sources of primary CMV transmission are infected breast milk and the infected genital tract (Shen *et al,* 1992).

Perinatal cytomegalovirus (CMV) infection was studied, using method of CMV-IgM ELISA, in 256 pregnant women at different periods and in the cord blood of 84 babies born to CMV positive mothers in a study in China. Results showed that in 42 cases at early and midtrimester pregnancy, 17 were CMV-IgM positive with an infection rate of 40.48%. Among the 214 women at late pregnancy, 84 were positive (39.25%). There was a higher prevalence of perinatal morbidity, neonatal asphyxia, malformation, intrauterine death, and poor obstetrical outcome in the CMV positive mothers as compared with the CMV negative group ($P < 0.01$) (Yang *et al* ,1994).

Seroprevalence of cytomegalovirus (CMV) of pregnant women was examined in Japan for 18 years since 1980 with complement-fixing antibody (CF) and specific IgG antibody. CF seropositive rate decreased gradually from 93.2% to 66.7%. CMV-IgG Seropositive rates were 87.4% in 1985, and 75.2% in 1996 to 1997 (Hoshiba *et al,* 1998).

In a study carried out in Japan, the prevalence of CMV IgG antibody was determined in 573 pregnant women in the first trimester. The overall prevalence of CMV IgG antibody was 77.5%. The rate of seropositivity was 67.7% in women < 25 yr, and increased with age to 85.7% in women 40 yr (Nishimura *et al*, 1999).

Pregnant women (60) with and without serological evidence of active CMV infection were followed until delivery to detect the incidence and types of overt congenital CMV infection in neonates in Mosul, Iraq. CMV-IgM was detected in cord blood samples of six (10%) overtly sick infants (with different congenital malformations) born to mothers with active CMV infection. Central nervous system abnormalities were detected in all six cases (two with microcephaly and four with hydrocephaly) (al-Ali *et al*, 1999).

In 2002, sera from 1047 pregnant women were tested by enzyme immunoassay for CMV IgG to determine the sero-prevalence of cytomegalovirus (CMV) IgG antibody in pregnant women in Ireland and assess individual risk factors for prior acquisition of CMV. Only 30.4% (204/670) of Irish women were CMV antibody positive compared to 89.7% (322/359) of non-Irish women ($p < 0.001$). Non-Irish women were mostly from Sub-Saharan Africa, Eastern Europe and Asia. Lower socio-economic group and increasing number of children were significant independent predictors of CMV sero-positivity among Irish pregnant women ($p < 0.05$) (Knowles *et al*, 2005).

A diagnostic algorithm utilizing immunoglobulin G (IgG), IgM, and IgG avidity was used to prospectively screen serum from 600 pregnant women enrolled from two groups: < or =20 weeks gestation (n = 396) or >20 weeks gestation (n = 204) in a study in Australia. PCR testing of urine and/or blood was performed on all seropositive women (n = 341). The majority (56.8%) of women were CMV IgG seropositive, with 5.5% being also CMV IgM positive. In the IgM-positive women, 1.2% had a low-avidity IgG, indicating a primary CMV infection and a high risk of intrauterine transmission. Two infants with asymptomatic CMV infection were born of mothers who had seroconverted in the second trimester of pregnancy. Women at high risk of intrauterine transmission of CMV were identified at all stages of gestation. Baseline, age-stratified CMV serostatus was established from 1,018 blood donors. Baseline seropositivity from a blood donor population increased with age from 34.9% seroprevalence at less than 20 years of age to 72% seroprevalence at 50 years of age (Munro *et al*, 2005).

In a study in India, 500 women of childbearing age belonging to different socioeconomic class were screened for the presence of IgM antibodies against CMV infection by ELISA. Among these were 70 pregnant women, positive for CMV specific

IgM antibodies, whose newborns were also tested for the same. IgM positivity was found to be 5.4% (27/500) while 2.2% (11/500) gave equivocal results. There was an increasing trend in IgM positivity with age, decreasing socioeconomic status and increasing parity. Prevalence rate was more in women from rural as compared to those of urban area. Among the pregnant women, in the higher income group, 4 (14.28%) had CMV specific IgM antibodies as compared to 9 (21.43%) of low income. Congenital infection occurred more often (14.28 vs. 7.14%) in infants in low income group. Signs and symptoms compatible with acute CMV infection were found in 7.69% (1/13) women and 27.78% (5/18) newborns (Chakravarty *et al*, 2005).

A study was performed in Canada to determine the proportion of CMV-seropositive female educators in the day care setting and to identify associated risk factors. Sera collected from 473 female educators from 81 day care centers in Montréal, Canada, analyzed by enzyme-linked immunosorbent assay gave CMV seroprevalence of 57%. Significant risk factors for CMV seropositivity were (i) increasing age ($OR_{5\text{-yr}}$ = 1.19; 95% CI = 1.05–1.35), (ii) low-income country of birth (OR = 10.23; 95% CI = 2.64–39.50) or middle-income country of birth (OR = 4.99; 95% CI = 2.39–10.40), (iii) having ≥2 children of their own (OR = 1.98; 95% CI = 1.19–3.31) and (iv) child-to-educator ratio >6 (18–35 months old) in a day care center (OR = 1.87; 95% CI = 1.25–2.81) (Joseph *et al*, 2005).

Herpes simplex virus

In a 10-year retrospective review of mucocutaneous infection by human herpesvirus 1 (HHV1) and human herpesvirus 2 (HHV2) carried out in Malaysia, a total of 504 specimens were tested by direct immunofluorescence (IF) and by virus isolation; 198 samples from patients with oral lesions and 306 from patients with genital lesions. HHV1 was found to be responsible for 98.4% of oral lesions whereas HHV2 was the cause of 83.6% of all genital lesions (Hooi *et al*, 2002).

To estimate the age and sex specific seroprevalence of HSV-1 and HSV-2 infections in selected populations in Brazil, Estonia, India, Morocco, and Sri Lanka, serum samples were collected from various populations including children, antenatal clinic attenders, blood donors, hospital inpatients, and HIV sentinel surveillance groups. 13,986 serum samples were tested using type specific HSV-1 and HSV-2 antibody assay, 45.0% from adult females, 32.7% from adult males, and 22.3% from children. The prevalence of HSV-1 varied by site ranging from 78.5%-93.6% in adult males and from 75.5%-97.8% in adult females. In all countries HSV-1 seroprevalence increased significantly with age ($p<0.001$) in both men and women. The prevalence of HSV-2 infection varied between sites. Brazil had the highest age specific rates of infection for both men and women, followed by Sri Lanka for men and Estonia for women, the lowest rates being found in Estonia for men and India for women. In all countries, HSV-2 seroprevalence increased significantly with age ($p<0.01$) and adult females had higher rates of infection than adult males by age of infection (Cowan *et al*, 2003).

A retrospective review of genital herpes simplex virus (HSV) isolates collected in a university student health service from 1993 to 2001 (n = 499) showed that an increasing proportion of isolates were HSV-1 rather than HSV-2. HSV-1 accounted for 78% of all genital isolates in this population by 2001, compared with 31% of isolates in 1993. The proportion of newly diagnosed genital herpes infections resulting from HSV-1 increased from 31% in 1993 to 78% in 2001 ($P <0.001$, linear trend $P <0.001$). HSV-1 was more common in females than males, but increases were noted for both sexes. HSV-1 was more common in persons aged 16 to 21 than in persons aged 22 or older (Roberts *et al*, 2003).

Serum samples from 1016 children, 794 first trimester antenatal women, and 1036 blood donors (462M, 574F) were tested for HSV IgG antibodies by type-specific HSV-1 and HSV-2 assays in a study in Estonia. High seroprevalence rates of HSV-1 among children, pregnant women and blood donors were found. HSV-2 infection was not detected among boys. Gender differences in HSV-2 seroprevalence rates among people of reproductive age were observed: higher rates were recorded among pregnant women (23%) and female blood donors (21%), compared to 11% among male blood donors.

HSV-1 seroprevalence was high in adults. HSV-2 seroprevalence was higher among females than males and increased substantially with age (Uuskula, 2004).

According to a retrospective study of cases of genital herpes seen at a STD Control clinic in Singapore over a 1-year period, out of 324 cases of genital herpes, 153 (47.2%) were first-episode and 171 (52.8%) were recurrent HSV infections. There were 259 males and 65 females; their mean age was 35.2 years (range, 17 to 75 years). Of the 241 cases with positive culture results, 28 (11.6%) tested positive for HSV-1 and 213 (88.4%) were positive for HSV-2. HSV-1 accounted for 19.3% of first-episode and 4.7% of recurrent infections. HSV-2 accounted for 80.7% of first-episode and 95.3% of recurrent infections. Cultures taken from lesions < or = 4 days, between 5 and 7 days and > 7 days of onset were positive in 79.2%, 75.7% and 75% of cases, respectively (Theng and Chan, 2004).

Suligoi and colleagues conducted a retrospective longitudinal study among 345 Italian adolescents tested for anti-HSV-1 and anti-HSV-2 on samples collected at 11 and 17 years of age. At 11 years of age, the HSV-1 prevalence was 51.6% and the HSV-2 prevalence was 2.6%; when 17 years old, these rates increased to 61.4% and 4.9%, respectively. The HSV-1 incidence was 1.6 per 100 person-years and was higher among females. The HSV-2 incidence was 0.4 per 100 person-years with no gender differences (Suligoi *et al*, 2004).

In a study performed in France, a total of 4410 subjects chosen at random were serotyped for HSV-1 and HSV-2 in order to determine the prevalence of clinically probable genital herpes and the relationship between serotype and clinical expression.

Seroprevalences of HSV-1 and HSV-2 were 65.6% and 15.5%, respectively. Prevalence of clinically probable genital herpes was 11.8%, identified in 11.1% of HSV-1-positive subjects and 26.8% of HSV-2-positive subjects, with a lower prevalence in those coinfected with both virus types. Coinfection with HSV-1 appeared to protect against symptom expression in subjects infected with HSV-2 (Malvy *et al,* 2005).

To determine risk factors for HSV acquisition among at risk pregnant women a study was undertaken in USA. A total of 3192 couples enrolled; 22% included women at risk for HSV-1 or HSV-2. Among 582 HSV-1 seronegative women with HSV-1 seropositive partners, 14 acquired HSV-1. Having a partner with a history of oral herpes was associated with HSV-1 acquisition and accounted for 75% of incident infections. Among 125 HSV-2 seronegative women with HSV-2 seropositive partners, 17 acquired HSV-2. Duration of partnership of 1 year or less was associated with HSV-2 acquisition and accounted for 63% of incident infections (Gardella *et al*, 2005).

A cross-sectional study was conducted involving 2082 serum samples of 725 adults, 300 pregnant women, 200 blood donors, 483 sex workers and 110 patients with genital warts and 264 hotel staff in Istanbul, Turkey. All serum samples were assessed for HSV-1 and HSV-2 IgG antibodies using an HSV-type specific ELISA. The prevalence of HSV-2 and HSV-1 antibodies was 4.8 and 85.3% in sexually active adults; 5.5 and 96% in blood donors; 5 and 98% in pregnant women, 17.3 and 93.6% in patients with genital warts; 8.3 and 97.3% in hotel staff; and 60% and 99% in sex workers (Dolar *et al*, 2006).

In a study carried out in Norway, sera were collected from Tanzanian children and young persons from 1 to 20 years old, with at least 100 individuals in each age group. Antibodies against HSV-1 and HSV-2 were detected by Western blot method. Type-specific antibodies were also analyzed by two noncommercial ELISA methods. The prevalence of HSV-1 antibodies increased gradually from 73% for the age group of 1 to 4 years to 92% for the age group of 17 to 20 years. The prevalence of HSV-2 antibodies was unexpectedly high, as 15% of the children were infected by the age of 8 years, with the incidence increasing gradually to 40% in the age group of 17 to 20 years (Kasubi *et al*, 2006).

3.4 NEPALESE SCENARIO

The antibody positive rates among Nepalese, in a community, to Herpes simplex virus (HSV) and Cytomegalovirus (CMV) were studied by using complement fixation test to measure the antibodies. An 80% positive rate of anti-HSV antibodies was found in early childhood (1-4 years) that further increased with age (96.1% positive in greater than 15 years age). Antibody against CMV was positive in all the subjects studied (Kubo *et al*, 1991).

In a study carried out in Nepal, a total of 302 serum samples collected from Chitawan (159) and Mustang (143) districts of Nepal were tested for anti-*Toxoplasma* antibody using micro-latex agglutination (MLA) and ELISA methods. An overall positive rate was found to be 57.9%. The positive rate in Chitawan was significantly higher (64.1%) (<1,000 m altitude) compared to that in Mustang (51.0%) (>3,000 m altitude) ($p < 0.05$). Females in Chitawan showed significantly higher positive rate (71.2%) compared to males (56.9%) ($p < 0.05$). On the contrary, though insignificantly, males showed higher positive rate (57.9%) compared to that of females (43.3%) in Mustang. Almost equal positive rate was observed among males in both study area. Females in Chitawan showed significantly higher (71.2%) positive rate compared to their counterparts in Mustang (43.3%) ($p < 0.001$). A slight increase in positive rate with age was observed in Chitawan while in Mustang a decreasing trend was noticed. Ethnically though statistically not significant, Indo-Aryans showed a higher positive rate (69.2%) compared to the positive rate shown by Tibeto-Burmans (63.1%) in Chitawan while the reverse was true in Mustang (Tibeto-Burmans: 53.8% and Indo-Aryans: 38.4%). Interestingly, 2.9% and 1.3% of MLA positive samples showed *Toxoplasma* IgM antibody. None of the IgM positive samples were positive for toxoplasmic antigens (Rai *et al*, 1994).

In a study carried out to ascertain the seroprevalence rate in different geographical areas in Central and Western Regions in Nepal, a total of 1,237 serum samples collected from Nuwakot (217), Kathmandu valley (402) and Chitawan (159) districts in Central Region, and Mustang (143), Surkhet (64) and Banke (252) districts in Western Region in Nepal were included. *Toxoplasma* antibodies were detected by micro-latex agglutination (MLA) and IgM-ELISA methods. The seropositive rate in Central and Western Regions were found to be 48% and 49%, respectively; with an overall positive rate of 48 percent. Districtwise, the seropositive rate in Nuwakot, Kathmandu valley, Chitawan, Mustang, Surkhet and Banke districts were 38, 46, 64, 51, 67 and 44%, respectively. Interestingly, the relatively newly inhabited Surkhet district in Western Region and Chitawan district in Central Region showed significantly higher seropositive rate compared with those of two other districts in the respective Regions ($p < 0.05$). Ethnically, Tibeto-Burmans showed higher seropositive rates in Central Region ($p > 0.05$). In contrast, Indo-Aryans showed higher seropositive rate in Western Region ($p > 0.05$). Age related increase in seropositivity was observed only in Central Region. One percent of *Toxoplasma* antibody positive samples also showed *Toxoplasma* IgM antibody positivity (Rai *et al*, 1996).

In a study done in Nepal, sera from randomly selected 345 pregnant Nepalese women aged 16-36 years and 13 women with bad obstetric history (BOH) were tested for the presence of *Toxoplasma* antibodies using microlatex agglutination (MLA) and ELISA methods. The overall prevalence was 55.4% (191/345). Prevalence was slightly higher (59.0%) in older age-group (27-36 years) compared with younger age-group (16-26 years) (52.2%). No significant difference in antibody prevalence in women belonging to two different ethnic-groups (Tibeto-Burmans 57.8%, Indo-Aryans 52.7%) was observed ($p>0.05$). MLA antibody titer ranged from 1:16 to 1:2,048. Over three-fourth of the women showed either high (1:510 or over) or low (1:16 or 1:32) antibody titer. Three percent (6/191) of MLA antibody positive subjects had *Toxoplasma* IgM antibodies by IgM-ELISA. All six IgM antibody positive pregnant women had MLA antibody titer of over 1:510. Of the total 13 women with BOH, 5 (38.5%) had *Toxoplasma* antibodies of which 2 (40.0%) were positive for *Toxoplasma*-IgM antibodies (Rai *et al*, 1998).

In a serosurvey of *Toxoplasma gondii* infection associated with meat eating habits of locals in Nepal carried out in apparently healthy subjects ($n = 404$) living in Achham ($n = 215$) and Dang ($n = 189$) districts in western Nepal, the overall seroprevalence was found to be 65.3% with no significant difference in the two districts (Achham: 66.9% and Dang: 63.5%) included ($p = 0.546$). Females and the Indo-Aryan ethnic-group showed marginally higher prevalence compared with their male ($p = 0.545$) and Tibeto-

Burman (p = 0.075) counterparts. The majority of the infections were found to have occurred during childhood. The frequency of meat eating in western and eastern regions differed greatly (p = 0.000) with the people in the eastern region being frequent meat eaters than those in the western region. About one-third of the subjects, all Indo-Aryans, in the western region had the raw meat eating habit but none in the eastern region. Approximately 7.0% of households in both western and eastern regions were found to keep cats. A typical role of meat eating habits of people in the high *Toxoplasma* seroprevalence in Nepal was demonstrated from these findings (Rai *et al*, 1999).

In a study on seroprevalence of *Toxoplasma* infections in 272 patients with ocular diseases (uveitis, retinochoroiditis), malignancy (including leukemia), women with bad obstetric history (BOH) and others (patients with fever, lymphadenitis and encephalitis), *Toxoplasma* antibodies were detected by microlatex agglutination and IgM ELISA techniques. Overall, 50.7% (138/272) patients included in this study had *Toxoplasma* antibodies, out of which 5.7% (8/138) had IgM antibodies. Patients with malignancy had highest positive rate [68.7% (22/32)] followed by group of others. Of the different groups, women with BOH had 2^{nd} highest *Toxoplasma* IgM positive rate of 25.0% (2/8) (Rai *et al*, 2003).

A total of 61 TORCH suspected women aged 15-45 years were studied in a study that was conducted to evaluate the seroprevalence of TORCH infections in Nepalese women of childbearing age. Seroprevalence frequencies of IgG antibody were 0%, 0%, 50%, 58.53% and 65.85% for Toxoplasmosis, Rubella, CMV, HSV-1 and HSV-2 respectively. Similarly, seroprevalence frequencies of IgM antibody were 5%, 3.63%, 5% and 0% for *Toxoplasma*, Rubella, CMV and HSV respectively (Kafle *et al*, 2004).

CHAPTER-IV

4. MATERIALS AND METHODS

The present study was conducted in the Immunology section of National Public Health laboratory, Teku, the national reference laboratory. The study was carried out from May to September 2006. During this period, a total of 276 blood samples from patients suspected of TORCH infections were collected and processed according to the standard laboratory protocols.

4.1 MATERIALS

All the materials required for present work are listed in the Appendix-II.

4.2 METHODS

4.2.1 Data Collection: Data collection was done from each patient by interview through questionnaire given in appendix I. Clinical history (name, age, sex, signs and symptoms, past history of TORCH test, meat eating habit and presence/absence of domestic cats) of patients were collected. Female patients were also asked about marital status, pregnancy, gravida and number of miscarriage or stillbirth, if any.

4.2.2 Specimen collection and storage: Following aseptic precautions, blood specimens were collected by vein puncture from each patient and were kept in a labelled, clean and dry test tube. The blood samples in the tubes were allowed to clot for 30 minutes at room temperature. Blood specimens were then centrifuged for serum separation and separated serum was kept in other labelled tube. The sera were then refrigerated at 2-8º C until tested. Few serum specimens for TORCH tests referred from different hospitals situated at Kathmandu valley were also received directly at NPHL. Same protocol was followed to those specimens also. TORCH tests were performed once a week.

4.2.3 Enzyme Linked Immunosorbent Assay (ELISA) Procedure: ELISA techniques for the detection of IgM antibodies to TORCH agents (*Toxoplasma*, Rubella, Cytomegalovirus and Herpes simplex virus) were performed. Steps involved in ELISA were common to all TORCH agents and were as follows:

Reagent Preparation: All the reagents were brought to room temperature and mixed gently before use. Frozen specimens were also thawed and homogenized. Wash buffer was diluted 20 times with fresh distilled water.

Serum dilution: Patient's sera were diluted using dilution buffer provided with the kit. For this purpose, 10 µl of each of the serum sample was mixed thoroughly with 1 ml of dilution buffer. Diluted samples were incubated for about 5-10 minutes prior to further processing.

Samples/Controls loading: The required numbers of microtiter strips were taken and placed into a microstrip-holder. Diluted sera, positive and negative controls (100 µl each) were dispensed into the appropriate wells. The holder was tapped gently to remove air bubbles from the liquid and to mix the samples. Microtiter strips were then covered with adhesive strips and incubated for 30 minutes at room temperature.

Washing: At the end of incubation period, adhesive strips were removed and the contents of the wells were aspirated off into 5% sodium hypochlorite solution. Diluted wash buffer was then dispensed to each well and was aspirated off after 30 second soak time. Washing was repeated for 4 times. Remaining liquid in the wells was then removed by tapping the plate upside down on tissue paper.

Addition of conjugate: After washing, 100 µl of enzyme conjugate was dispensed to each well. Microtiter strips were then covered with adhesive strips and incubated for 30 minutes at room temperature.

Addition of substrate: Washing procedure similar as above was carried out for 4 times**.** 100 µl of Substrate Reagent [3,3',5,5'-tetramethylbenzidin (TMB)] was then dispensed to each well. Microtiter strips were then covered with adhesive strips and incubated in dark for 15 minutes at room temperature.

Stopping the reaction: Finally, the reaction was stopped by adding 100 µl of stop solution (Sulphuric acid) and mixed carefully.

Reading: The absorbance were measured as soon as possible or within 30 seconds after terminating the reaction by using ELISA microtiter plate reader (HUMAREADER) at the wavelength 450 nm using a reference wavelength of 630-690 nm.

Safe disposal: Used microtiter strips and pipette tips were first treated with Sodium hypochlorite solution and then discarded in the plastic containers in sealed condition. Test tubes and other glasswares used were washed and then sterilized by autoclaving.

Calculation: Calculations and Interpretations of results were done (Appendix-III).

4.2.4 Data analysis: Data were statistically analysed using Chi-square test (Appendix-IV).

CHAPTER-V

5. RESULTS

Two hundred and seventy six blood samples were collected from patients visiting NPHL for TORCH tests and the samples were processed at the Immunology section of NPHL.

Table 1: Age and gender wise distribution of TORCH suspected patients

Age group	**Male**		**Female**		**Total**	
	No.	**Percentage**	**No.**	**Percentage**	**No.**	**Percentage**
0-10	13	15.11	10	5.26	23	8.33
11-20	6	6.98	19	10.00	25	9.06
21-30	32	37.20	125	65.79	157	56.88
31-40	25	29.06	32	16.84	57	20.65
41-50	6	6.98	2	1.05	8	2.90
51-60	2	2.32	2	1.05	4	1.45
61-70	1	1.16	0	0	1	0.36
71-80	1	1.16	0	0	1	0.36
Total	**86**		**190**		**276**	

As shown in table 1, the highest number of patients 157 (56.88 %) belonged to the age group 21-30 years followed by 57 (20.65%) to 31-40 years and so on. The highest number of female patients 125 (65.79%) were found in the age group 21-30 years followed by 32 (16.84%) in 31-40 years. Similarly, the highest number of male patients 32 (37.20%) were found in the age group 21-30 years followed by 25 (29.06%) in 31-40 years.

Table 2: Gender wise distribution of patients according to the TORCH test performed

Test performed	Total patients	Male	Female
TORCH profile	190	31	159
Toxoplasma	25	8	17
Rubella	2	1	1
Cytomegalovirus	2	1	1
Herpes simplex virus	52	43	9
Toxoplasma and CMV	4	2	2
Rubella and CMV	1	0	1
Total	**276**	**86**	**190**

Distribution of patients requesting for different types of TORCH tests is shown in table 2. Among 190 female patients, 159 were tested for TORCH profile, 17 for *Toxoplasma* and so on. And, among 86 male patients, 43 were tested for HSV, 31 for TORCH profile and so on.

Table 3: Gender wise distribution of total TORCH tested cases and test result

Gender	Total patients	Positive		Negative	
		No.	%	No.	%
Male	86	14	16.28	72	83.72
Female	190	50	26.32	140	73.68
Total	**276**	**64**	**23.19**	**212**	**76.81**

Of the total 276 blood samples, 86 were from male patients and 190 were from female patients as shown in table 3. Out of 86 male patients, 14 (16.28%) and out of 190 female patients, 50 (26.32%) were positive to one or more TORCH agents.

TORCH test result	No. of patients	Male	Female
Single infection	46	12	34
Multiple infection	18	2	16
No infection	212	72	140
Total	**276**	**86**	**190**

Table 4: Pattern of TORCH test results

As shown in table 4, out of 276 patients, 46 (12 male and 34 female) were found to be infected with single TORCH agent and 18 (2 male and 16 female) with multiple TORCH agents.

Table 5: Gender wise distribution of TORCH (IgM) test results

S. N.	Test performed	Total no. of suspected cases	Gender	No. of cases	No. of positive cases	Seropositivity %
1.	ELISA test for *Toxoplasma* (IgM)	219	Male	41	2	4.88
			Female	178	28	15.73
2.	ELISA test for Rubella (IgM)	193	Male	32	3	9.37
			Female	161	6	3.73
3.	ELISA test for Cytomegalovirus (IgM)	197	Male	34	5	14.70
			Female	163	18	11.04
4.	ELISA test for Herpes simplex virus (IgM)	242	Male	74	7	9.46
			Female	168	20	11.90

As shown in table 5, 4.88% of male and 15.73% of female patients among 219 suspected cases were positive to *Toxoplasma* (IgM); 9.37% of the male and 3.73% of the female patients among 193 suspected cases were positive to Rubella (IgM); 14.70% of the male and 11.04% of the female patients among 197 suspected cases were positive to CMV (IgM) and 9.46% of the male and 11.90% of the female among 242 suspected cases were positive to HSV (IgM).

Table 6: Seropositivity percentage of TORCH agents in patients with different disease conditions

S. N.	**Patients with different disease conditions**	**Seropositivity Percentage of**			
		***Toxoplasma* (IgM)**	**Rubella (IgM)**	**Cytomegalo Virus (IgM)**	**Herpes simplex virus (IgM)**
1.	Female with BOH	15.43 (25/162)	4 (6/150)	9.33 (14/150)	11.33 (17/150)
2.	Male (Husbands of female with BOH)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/8)
3.	Infants with congenital infections or those born to female with BOH	0 (0/20)	0 (0/20)	27.78 (5/18)	5.55 (1/18)
4.	Patients suffering from ocular infection	30 (3/10)	14.28 (1/7)	14.28 (1/7)	14.28 (1/7)
5.	Patients suffering from HIV/AIDS	33.33 (2/6)	16.67 (1/6)	33.33 (2/6)	16.67 (1/6)
6.	Patients with genital infections	-	-	-	12.76 (6/47)
7.	Patients with other symptoms such as fever, joint pain, fainting attacks, paralysis etc	0 (0/13)	25 (1/4)	12.5 (1/8)	12.5 (1/8)

As shown in table 6, female patients with BOH were found to have the highest seropositivity of *Toxoplasma* (15.43%) as compared to the other TORCH agents. Similarly, infants were found to have the highest seropositivity of CMV (27.78%); patients suffering from ocular infection were found to have the highest seropositivity of *Toxoplasma* (27.78%); patients suffering from HIV/AIDS were found to have the equal seropositivity of *Toxoplasma* and CMV (33.33%) and so on. Among the patients with different disease conditions, *Toxoplasma*, CMV and HSV were found to have the highest seroprevalence rates in HIV/AIDS patients. And, the highest seroprevalence rate of Rubella was found in the patients with other symptoms such as fever etc.

Table 7: Prevalence of *Toxoplasma* infection according to the age group and sex

Age group	Total patients suspected of *Toxoplasma*	Male			Female		
		Suspected cases	Positive cases	Positive %	Suspected cases	Positive cases	Positive %
0-10	20	12	0	0	8	0	0
11-20	24	5	0	0	19	7	36.84
21-30	124	5	0	0	119	16	13.45
31-40	42	12	1	8.33	30	5	16.67
41-50	6	5	1	20.00	1	0	0
51-60	1	0	0	0	1	0	0
61-70	1	1	0	0	0	0	0
71-80	1	1	0	0	0	0	0
Total	**219**	**41**	**2**		**178**	**28**	

As given in table 7, a total of 219 patients were tested for *Toxoplasma* (IgM). The highest number of suspected cases was 119 from the age group 21-30 in case of female that was 12 from the age groups 0-10 and 31-40 in case of male. Positivity rate was highest (36.84%) in the age group 11-20 years among females and in 41-50 (20.00%) years among males.

Table 8: Correlation of *Toxoplasma* infection with different disease conditions

S. N.	Patients with different disease conditions	Total no. of suspected cases of *Toxoplasma*	Male		Female	
			Suspected cases	Positive cases	Suspected cases	Positive cases
1.	Female with BOH	162	-	-	162	25
2.	Male (Husbands of female with BOH)	8	8	0	-	-
3.	Infants with congenital infections or those born to female with BOH	20	12	0	8	0
4.	Patients suffering from ocular infection	10	5	0	5	3
5.	Patients suffering from HIV/AIDS	6	6	2	-	-
6.	Patients with other symptoms such as fever	13	10	0	3	0
	Total	**219**	**41**	**2**	**178**	**28**

The study included different types of patients with different disease conditions, who were subjected to ELISA test (IgM) for *Toxoplasma*. As given in table 8, among 162 female patients with BOH, 25 were positive to *Toxoplasma* (IgM) and among 10 patients suffering from ocular infection, 3 were positive to *Toxoplasma* (IgM).

Table 9: Correlation of *Toxoplasma* infection with meat eating habit

Eating habit	**No. of suspected patients of age >10**	**Positive cases**	**Seropositivity %**
Vegetarian habit	22	1	4.54
Non vegetarian not having pork	152	21	13.00
Non vegetarian having pork	25	8	32.00
Total	**199**	**30**	

Among 199 *Toxoplasma* suspected cases of age greater than 10 years, 22 were vegetarian, 152 were non-vegetarian not having pork and 25 were non-vegetarian having pork as shown in table 9. Highest positivity (32%) was observed among non-vegetarian patients having pork followed by others.

Table 10: Correlation of *Toxoplasma* infection with domesticated cats

Domestication of cat	**No. of suspected patients of age >10**	**Positive cases**	**Seropositivity %**
Have cat as pet	10	4	40.00
Do not have cat as pet	189	26	13.76
Total	**199**	**30**	

Among 199 *Toxoplasma* suspected cases of age greater than 10 years, 10 patients were having cat as pet and 189 were not having cat as pet at their home as shown in table 10. Higher positivity (40%) was observed among patients having cat as pet followed by those not having cat as pet.

Table 11: Prevalence of Rubella infection according to the age group and sex

Age group	Total patients suspected of Rubella	Male			Female		
		Suspected cases	Positive cases	Positive %	Suspected cases	Positive cases	Positive %
0-10	19	11	1	9.09	8	0	0
11-20	17	2	0	0	15	1	6.67
21-30	113	3	0	0	110	5	4.54
31-40	36	9	1	11.11	27	0	0
41-50	6	5	1	20.00	1	0	0
51-60	0	0	0	0	0	0	0
61-70	1	1	0	0	0	0	0
71-80	1	1	0	0	0	0	0
Total	**193**	**32**	**3**		**161**	**6**	

As given in table 11, a total of 193 patients were tested for Rubella (IgM). The highest number of suspected cases was 110 from the age group 21-30 in case of female that were 11 from the age group 0-10 in case of male. Positivity rate was highest (6.67%) in the age group 11-20 years among females and in 41-50 years (20.00%) among males.

Table 12: Correlation of Rubella infection with different disease conditions

S. N.	Patients with different disease conditions	Total no. of suspected cases of Rubella	Male		Female	
			Suspected cases	Positive cases	Suspected cases	Positive cases
1.	Female with BOH	150	-	-	150	6
2.	Male (Husbands of female with BOH)	8	8	0	-	-
3.	Infants with congenital infections or those born to female with	18	10	0	8	0
4.	Patients suffering from ocular infection	7	4	1	3	0
5.	Patients suffering from HIV/AIDS	6	6	1	-	-
6.	Patients with other symptoms such as fever	4	4	1	-	-
	Total	**193**	**32**	**3**	**161**	**6**

The study included different types of patients with different disease conditions, who were subjected to ELISA test (IgM) for Rubella. As given in table 12, among 150 female patients with BOH, 6 were positive to Rubella (IgM); among 7 patients suffering from ocular infection, 1 was positive; among 6 patients suffering from HIV/AIDS, 1 was positive and among 4 patients with other symptoms such as fever 1 was positive to Rubella (IgM).

Table 13: Prevalence of Cytomegalovirus (CMV) infection according to the age group and sex

Age group	Total patients suspected of CMV	Male			Female		
		Suspected cases	Positive cases	Positive %	Suspected cases	Positive cases	Positive %
0-10	18	10	1	10.00	8	4	50.00
11-20	19	2	1	50.00	17	2	11.76
21-30	113	4	0	0	109	10	9.17
31-40	38	10	2	20.00	28	2	9.14
41-50	7	6	1	16.67	1	0	0
51-60	0	0	0	0	0	0	0
61-70	1	1	0	0	0	0	0
71-80	1	1	0	0	0	0	0
Total	**197**	**34**	**5**		**163**	**18**	

As given in table 13, a total of 197 patients were tested for CMV (IgM). The highest number of suspected cases was 109 from the age group 21-30 in case of female that were 10 from the age groups 0-10 and 31-40 years in case of male. Positivity rate was highest (50.00%) in the age group 0-10 years among females and in 11-20 years (50.00%) among males.

Table 14: Correlation of Cytomegalovirus (CMV) infection with different disease conditions

S. N.	Patients with different disease conditions	Total no. of suspected cases of CMV	Male		Female	
			Suspected cases	Positive cases	Suspected cases	Positive cases
1.	Female with BOH	150	-	-	150	14
2.	Male (Husbands of female with BOH)	8	8	0	-	-
3.	Infants with congenital infections or those born to female with BOH	18	10	1	8	4
4.	Patients suffering from ocular infection	7	4	1	3	0
5.	Patients suffering from HIV/AIDS	6	6	2	-	-
6.	Patients with other symptoms such as fever, joint pain	8	6	1	2	0
	Total	**197**	**34**	**5**	**163**	**18**

The study included different types of patients with different disease conditions, who were subjected to ELISA test (IgM) for Cytomegalovirus. As given in table 14, among the 150 female patients with BOH, 14 were positive to Cytomegalovirus (IgM); among the 18 infants, 5 were positive; among 6 patients suffering from HIV/AIDS, 2 were positive; among 7 patients suffering from ocular infection, 1 was positive; and among 4 patients with other symptoms, 1 was positive to Cytomegalovirus (IgM).

Table 15: Prevalence of Herpes simplex virus (HSV) infection according to the age group and sex

Age group	Total patients suspected of HSV	Male			Female		
		Suspected cases	Positive cases	% of positive cases	Suspected cases	Positive cases	% of positive cases
0-10	20	10	0	0	10	2	20.00
11-20	18	3	1	33.33	15	6	40.00
21-30	142	30	3	10.00	112	9	8.03
31-40	50	22	1	4.54	28	3	10.71
41-50	7	5	1	20.00	2	0	0
51-60	3	2	1	50.00	1	0	0
61-70	1	1	0	0	0	0	0
71-80	1	1	0	0	0	0	0
Total	**242**	**74**	**7**		**168**	**20**	

As given in table 15, a total of 242 patients were tested for HSV (IgM). The highest number of suspected cases was 112 from the age group 21-30 in case of female that were 30 from the age group 21-30 years in case of male. Positivity rate was highest (40.00%) in the age group 11-20 years among females and in 51-60 years (50.00%) among males.

Table 16: Correlation of Herpes simplex virus infection with different disease conditions

S. N.	Patients with different disease conditions	Total no. of suspected cases	Male		Female	
			Suspected cases	Positive cases	Suspected cases	Positive cases
1.	Female with BOH	148	-	-	148	17
2.	Male (Husbands of female with BOH)	8	8	0	-	-
3.	Infants with congenital infections or those born to female with BOH	18	10	0	8	1
4.	Patients suffering from ocular infection	7	4	1	3	0
5.	Patients suffering from HIV/AIDS	6	6	1	-	-
6.	Patients with genital infections	47	41	5	6	1
7.	Patients with other symptoms such as fever, fainting attacks, paralysis	8	5	0	3	1
	Total	**242**	**74**	**7**	**168**	**20**

The study included different types of patients with different disease conditions, who were subjected to ELISA test (IgM) for Herpes simplex virus (HSV) as given in table 16. Among 148 female patients with BOH, 17 were positive to HSV (IgM); among 47 patients with genital infections, 6 were positive to HSV (IgM) and 1 patient was positive to HSV (IgM) from each of the group of infants, patients suffering from ocular infection, patients suffering from HIV/AIDS and patients with other symptoms.

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

TORCH infections are unique in their pathogenesis and have potentially devastating clinical manifestations (Boyer and Boyer, 2004). These infections, acquired in utero, can be severe enough to cause fetal loss or can result in intrauterine growth restriction, prematurity, or chronic postnatal infection. The degree of severity is dependent on the gestational age of the fetus when infected, the virulence of the organism, the damage to the placenta, and the severity of maternal disease (Klein and Remington, 2001).

Congenital toxoplasmosis remains an important cause of blindness, although avoiding exposure to cats and uncooked meat can prevent it. The incidence of congenital rubella has been lowered due to vaccination. While cytomegalovirus remains the most common cause of congenital infection even in the developed countries, the possibility of effective treatment with Ganciclovir has emerged from recent studies. In neonatal herpes, selective use of cesarean delivery and antiviral therapy can decrease incidence and improve outcomes (Boyer and Boyer, 2004).

The present study was conducted among the patients visiting NPHL to determine the seroprevalence of antibodies (IgM) against the agents causing TORCH infections. Two hundred and seventy six blood samples were collected from the suspected patients and subjected to Enzyme Linked Immunosorbent Assay (ELISA).

Out of 276 patients requesting for different panels of TORCH tests, 164 patients were female with bad obstetric history (BOH) ranging from 19 to 39 years of age, 8 patients were male partners of the females with BOH, 20 patients were infants suspected of congenital infections or those born to the females with BOH, 6 were HIV/AIDS patients, 10 patients were suffering from eye infection, 47 patients were suffering from genital infections and 20 patients were suffering from other symptoms such as fever, joint pain, fainting attacks, headache, paralysis etc.

The highest number of female patients 125 (45.29 %) were found in the age group 21-30 followed by 32 (11.59%) in the age group 31-40. This is probably because of the fact that females of these age groups are of childbearing age and are screened for antibodies against TORCH infection agents at their prenatal visits.

In the present study on the seroprevalence of antibodies (IgM) against the agents causing TORCH infections, an overall prevalence of 13.70% to *T. gondii*, 4.66% to Rubella virus, 11.67% to CMV and 11.16% to HSV was found. *Toxoplasma* (IgM) was found to have the highest seroprevalence among the four TORCH agents because in addition to congenital transmission, *Toxoplasma* is also transmitted orally by eating raw meat or exposure to infected cat faeces.

Out of total 276 TORCH infections suspected serum samples, 76.81% (212/276) samples showed negative result and 23.19% (64/276) samples showed positive result. Out of 64 positive patients, 46 were found to be infected with single TORCH agent and 18 with multiple TORCH agents. Similarly, out of 18 patients with multiple infections, 16 were female. Among these 18 patients with multiple infections, 2 female patients with bad obstetric history were positive to all the four TORCH agents under study.

Of the total 276 TORCH suspected cases, 86 (31.16%) were male and the rest 190 (68.84%) were female. Though statistically insignificant, females showed higher positive rate of 26.32% (50/190) as compared to 16.28% (14/86) in males.

Seroprevalence percentages of IgM antibodies were 15.43% (25/162) for *T. gondii*, 4.00% (6/150) for Rubella, 9.33% (14/150) for CMV and 11.49% (17/148) for HSV in the female patients with BOH. In a similar study carried out in India in women with different complications of pregnancy, seroprevalence percentages of *Toxoplasma* (13.1%), Rubella (6.5%) and CMV (5.8%) was found (Yasodhara *et al*, 2001). In another study carried out in Mumbai, India in 380 pregnant women with BOH, it was found that, IgM antibodies were positive in 40 (10.52%) for *Toxoplasma*, 102 (26.8%) for Rubella, 32 (8.42%) for CMV and 14 (3.6%) for HSV-II (Turbadkar *et al*, 2003). A higher seroprevalence to TORCH infection is also accounted in a study conducted in 20 pregnant women with BOH in Nainital, India (Thapliyal *et al*, 2005). However, lower seroprevalence of IgM were found in a study that was conducted to evaluate the seroprevalence of TORCH infections in TORCH suspected Nepalese women of childbearing age (Kafle *et al*, 2004).

Seroprevalence of IgM antibodies were 0.00% (0/20) for *Toxoplasma*, 0.00% (0/18) for Rubella, 27.78% (5/18) for CMV and 5.55% (1/18) for HSV in the infants. Among the four TORCH agents under study, CMV was found to have the highest seroprevalence (27.78%) rate in the infants. This may be because of the fact that in addition to the placental route, CMV can be transmitted at delivery via the maternal genital tract, during the post parturm period in breast milk and transfused blood products.

Similarly, seroprevalence of IgM antibody were found to be 33.33% (2/6) for *Toxoplasma*, 16.67% (1/6) for Rubella, 33.33% (2/6) for CMV and 16.67% (1/6) for HSV in the HIV/AIDS patients. Thus, from our study, *Toxoplasma* and CMV were found to be more prevalent opportunistic infectious agents than Rubella and HSV in the HIV/AIDS patients. However, this cannot be statistically defined because of small sample size of HIV/AIDS patients.

Out of 219 suspected cases of *Toxoplasma*, 41 were male and 178 were female. Among males and females, 4.88% (2/41) and 15.73% (28/178) were positive to *Toxoplasma* (IgM) respectively. Seroprevalence of *Toxoplasma* was highest (29.17%) in the age group 11-20 in this study. Overall, 13.70% (30/219) patients included in this study had *Toxoplasma* IgM antibodies. In a study done in 272 patients with ocular diseases, malignancy (including leukemia), women with bad obstetric history (BOH) and others (patients with fever, lymphadenitis and encephalitis), 5.70% had *Toxoplasma* IgM antibodies (Rai *et al*, 2003).

Of the different groups of patients under our study, HIV/AIDS patients were found to have highest *Toxoplasma* (IgM) positive rates [33.33% (2/6)]. This result is in accordance with the results [32% (130/411)] obtained in a similar study done by Grant *et al* (1990). However, the seroprevalence of *Toxoplasma* (IgG/IgM) was found to be higher (67.8%) in HIV infected hosts in a similar study carried out in Bombay (Meisheri *et al*, 1997).

In the present study, patients with ocular infections had *Toxoplasma* IgM positive rates of 30.00% (3/10) which correlates with the study done in India using ELISA (IgG/IgM) in 200 uveitis cases (Jain *et al*, 1998).

Female patients with bad obstetric history had *Toxoplasma* IgM positive rates of 15.43% (25/162) which is comparable with the findings of a study done by Rai *et al* (2003). However, a higher *Toxoplasma* IgM seroprevalence (41.47%) was found in a study done in Kashmir, India, in 285 women with repeated abortions (Zargar *et al*, 1998).

An increase in *Toxoplasma* seroprevalence with increasing gravida was detected in female patients with BOH. *Toxoplasma* IgM prevalence was found to be 11.11% in women with 1 gravida; 14.54% in women with 2 gravida and 18.30% in women with more than 2 gravida. This results correlates to the finding of Nissapatorn *et al* (2003) in Malaysia in which an increase in *Toxoplasma* seroprevalence with increasing parity was detected ($P<0.05$).

Among 199 *Toxoplasma* suspected cases of age greater than 10 years, 4.54% (1/22) among the vegetarian, 13.00% (21/152) among the non-vegetarian not having pork, and 32.00% (8/25) among the non-vegetarian patients having pork were found to be *Toxoplasma* (IgM) positive. Statistically significant association of *Toxoplasma* infection with meat eating habit ($p<0.05$) was found. This results correlates to the finding of Rai *et al* (1999). By this fact, a typical role of meat eating habits of people in the high *Toxoplasma* seroprevalence in Nepal was demonstrated.

Moreover, statistically significant association of *Toxoplasma* infection with domesticated cats ($p<0.05$) was found. Among 199 *Toxoplasma* suspected cases of age greater than 10 years, 40.00% (4/10) among the patients having cat as pet and 13.76% (26/189) patients not having cat as pet were found to be *Toxoplasma* (IgM) positive.

This results correlates to the finding of Jones *et al* (2005). By this fact, a typical role of domesticated cats in the high *Toxoplasma* seroprevalence was demonstrated.

Out of 193 suspected cases of Rubella, 32 were male and 161 were female. Among males and females, 9.37% (3/32) and 3.73% (6/161) were positive to Rubella (IgM) respectively. Seroprevalence of Rubella was highest (5.89%) in the age group 11-20 in this study. Overall, 4.66% (9/193) patients included in this study had Rubella IgM antibodies. This lower seroprevalence rate of Rubella (IgM) as compared to other TORCH agents may be due to Rubella vaccination during the childhood.

Of the different groups of patients under our study, patients with symptoms such as fever, joint pain etc. were found to have highest Rubella (IgM) positive rates [25% (1/4)]. In the present study, patients suffering from ocular infection had Rubella positive rate of 14.28% (1/7) and HIV/AIDS patients had positive rate of 16.67% (1/6).

In our study, female patients with bad obstetric history (BOH) had Rubella (IgM) positive rate of 4.00% (6/150). However, a higher Rubella (IgM) seropositivity of 10.38% was found among 183 women presented with history of adverse pregnancy outcome in a study in Amritsar, India (Singla *et al*, 2003).

Out of 197 suspected cases of Cytomegalovirus (CMV), 34 were male and 163 were female. Among males and females, 14.70% (5/34) and 11.04% (18/163) were positive to CMV (IgM) respectively. Seroprevalence of CMV was highest (27.78%) in the age group 0-10 in this study. Overall, 11.67% (23/197) patients included in this study had CMV IgM antibodies.

Of the different groups of patients under our study, HIV/AIDS patients were found to have highest CMV (IgM) positive rates [33.33% (2/6)]. In the present study, patients suffering from ocular infection had CMV positive rate of 14.28% (1/7) and patients with other symptoms such as fever, joint pain etc had positive rate of 12.50% (1/8).

In the present study, female patients with bad obstetric history (BOH) was found to have CMV (IgM) positive rate of 9.33% (14/150) and infants with various problems suspected of congenital infection or those born to female with BOH was found to have CMV (IgM) positive rate of 27.78% (5/18). In a similar study carried out in Southern Africa, CMV (IgM) positive rate of 19.20% was found in female patients with BOH and that of 24.20% was found in the infants (Bos *et al*, 1995). In another study carried out in China in women with history of abnormal pregnancy, the positive rate of CMV-IgM was 13.59% (Xu, 1991). In a similar study carried out in 96 samples from symptomatic babies in the age group of few days to 6 months exhibiting different congenital anomalies in India, the positive rate of CMV-IgM was 18.75% (Gandhoke *et al*, 2006). High seroprevalene of CMV can also be seen in other studies carried out in India (Abraham *et al*, 1999; Chakravarty *et al*, 2005 and Thapaliyal *et al*, 2005)

Out of 197 suspected cases of Herpes simplex virus (HSV), 74 were male and 168 were female. Among males and females, 9.46% (7/74) and 11.90% (20/168) were positive to HSV (IgM) respectively. Seroprevalence of HSV was highest (38.89%) in the age group 11-20 in this study. Overall, 11.16% (27/242) patients included in this study had HSV IgM antibodies.

Of the different groups of patients under study, female patients with bad obstetric history (BOH) were found to have HSV (IgM) positive rate of 11.49% (17/148). In a similar study carried out in Turkey in the sera of 73 mothers who had different kinds of obstetrical problems like abortus, stillbirth, prematurity, postmaturity and intrauterine development retardation, HSV-1 and HSV-2 IgM seropositivities were found to be 9.6% (7/73) and 8% (6/73) respectively (Cengiz *et al*, 1993).

Similarly, infants with various problems suspected of congenital infection or those born to female with BOH had HSV (IgM) positive rate of 5.55% (1/18), patients suffering from ocular infection had positive rates of 14.28% (1/7), HIV/AIDS patients had positive rate of 16.67% (1/6), patients suffering from genital infections had positive rate of 12.76% (6/47) and patients with other symptoms such as fever, fainting attacks, paralysis, etc had positive rate of 12.50% (1/8).

6.2 CONCLUSION

The study was carried out to determine the seroprevalence of TORCH (IgM) among suspected patients visiting NPHL, Teku. Out of total 276 TORCH suspected serum samples, 23.19% (64/276) samples showed positive result. An overall seroprevalence of 13.70% to *T. gondii*, 4.66% to Rubella virus, 11.67% to CMV and 11.16% to HSV was found in the study. Seroprevalence percentages of 15.43% (25/162) to *T. gondii*, 4.00% (6/150) to Rubella, 9.33% (14/150) to CMV and 11.49% (17/148) to HSV were found in the female patients with BOH.

Highest seroprevalences of *Toxoplasma*, Rubella and HSV were found in the age group 11-20 years whereas that of CMV was found in the age group 0-10 years. Among the patients with different disease conditions, the highest seroprevalence of *Toxoplasma*, CMV and HSV were found to be in HIV/AIDS patients whereas the highest seroprevalence of Rubella was found in the patients with other symptoms (fever, joint pain etc). The study revealed that there is no statistically significant difference on the seropositivity of TORCH agents among male and female patients ($P > 0.05$). Statistically significant associations of *Toxoplasma* infection with meat eating habit and with domesticated cats ($P<0.05$) was found.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. The study was conducted among the patients suspected of TORCH infections visiting National Public Health Laboratory, Teku in collaboration with Central Department of Microbiology, Tribhuvan University, from May-September 2006.
2. During the study period, a total of 276 patients were studied out of which 86 were male and 190 were female. Out of 86 male patients, 14 (16.28%) were found to be positive and out of 190 female patients, 50 (26.32%) were found to be positive to one or more TORCH agents.
3. Out of 276 patients, 46 (12 male and 34 female) were found to be infected with single TORCH agent, 18 (2 male and 16 female) with multiple TORCH agents and 212 were found to be negative to all TORCH agents under study.
4. Among 276 patients under study, 164 patients were female with bad obstetric history (BOH), 20 patients were infants (2 days to 10 months old) with various problems suspected of congenital infection or those born to female with BOH, 8 patients were male who were the husbands of females patients with BOH, 15 were patients with other symptoms such as fever, joint pain, fainting attacks, paralysis etc., 10 were patients suffering from various eye problems and 6 were HIV/AIDS patients.
5. Among 219 (41 male and 178 female) suspected cases of *Toxoplasma*, 4.88% (2/41) of male and 15.73% (28/178) of female patients were positive to *Toxoplasma* (IgM).
6. Among 193 (32 male and 161 female) suspected cases of Rubella, 9.37% (3/32) of the male and 3.73% (6/161) of the female patients were positive to Rubella (IgM).
7. Among 197 (34 male and 163 female) suspected cases of Cytomegalovirus, 14.70% (5/34) of the male and 11.04% (18/163) of the female patients were positive to CMV (IgM).

8. Among 242 (74 male and 168 female) suspected cases of Herpes simplex virus, 9.46% (7/74) of the male and 11.90% (20/168) of the female patients were positive to HSV (IgM).
9. The overall seroprevalence rates of IgM antibody were 13.70% to *T. gondii*, 4.66% to Rubella virus, 11.67% to CMV and 11.16% to HSV.
10. Associations of *Toxoplasma* infection with both meat eating and cat rearing habits of patients were found to be statistically significant ($P<0.05$).

7.2 RECOMMENDATIONS

1. All women of childbearing age should be tested for antibodies against TORCH infection agents. Especially, pregnant women and women with bad obstetric history should be screened for TORCH titers (IgG and IgM). Prenatal and newborn screening should be employed to identify and treat congenital infections.
2. Immunosupressed patients including HIV/AIDS patients, cancer patients undergoing chemotherapy, transplant recipients etc. should be routinely tested for TORCH infection agents and treated timely in order to avoid serious complications.
3. Prenatal and newborn screening should include not only the causative agents of TORCH infections but also the causative agents of other congenital infections such as Varicella Zoster Virus (VZV), *Treponema pallidum*, Hepatitis B Virus (HBV), Human immunodeficiency virus (HIV), Parvo virus, *Chlamydia trachomatis*, Mycoplasma, Group B Streptococci, etc.
4. More advanced molecular diagnostic methods such as Polymerase chain reaction (PCR) should be used.
5. Early detection and treatment of TORCH infections and preventive measures including awareness of the routes of transmission have to be carried out in order to reduce the incidence of later occurring life threatening consequences. Vaccines where available, should be affordable.
6. As this study was confined to NPHL, Teku, it does not necessarily reveal the total picture of the whole country, therefore this type of study should be carried out in hospitals and laboratories as well as in the communities of different parts of our

country in order to obtain information regarding geographical, ethnic and gender wise variation of seroprevalence of TORCH infection.

7. Seroprevalence of *Toxoplasma* should be determined in various ethnic groups of our country especially those with raw meat eating habit and in the communities with people of low socioeconomic profile.
8. There are some limitations in our study. In our study, seroprevalence of only IgM antibody againt the TORCH infection agents is determined. Hence it is recommended that the study should be carried out to determine seroprevalence of both IgG and IgM antibodies against the TORCH infection agents.

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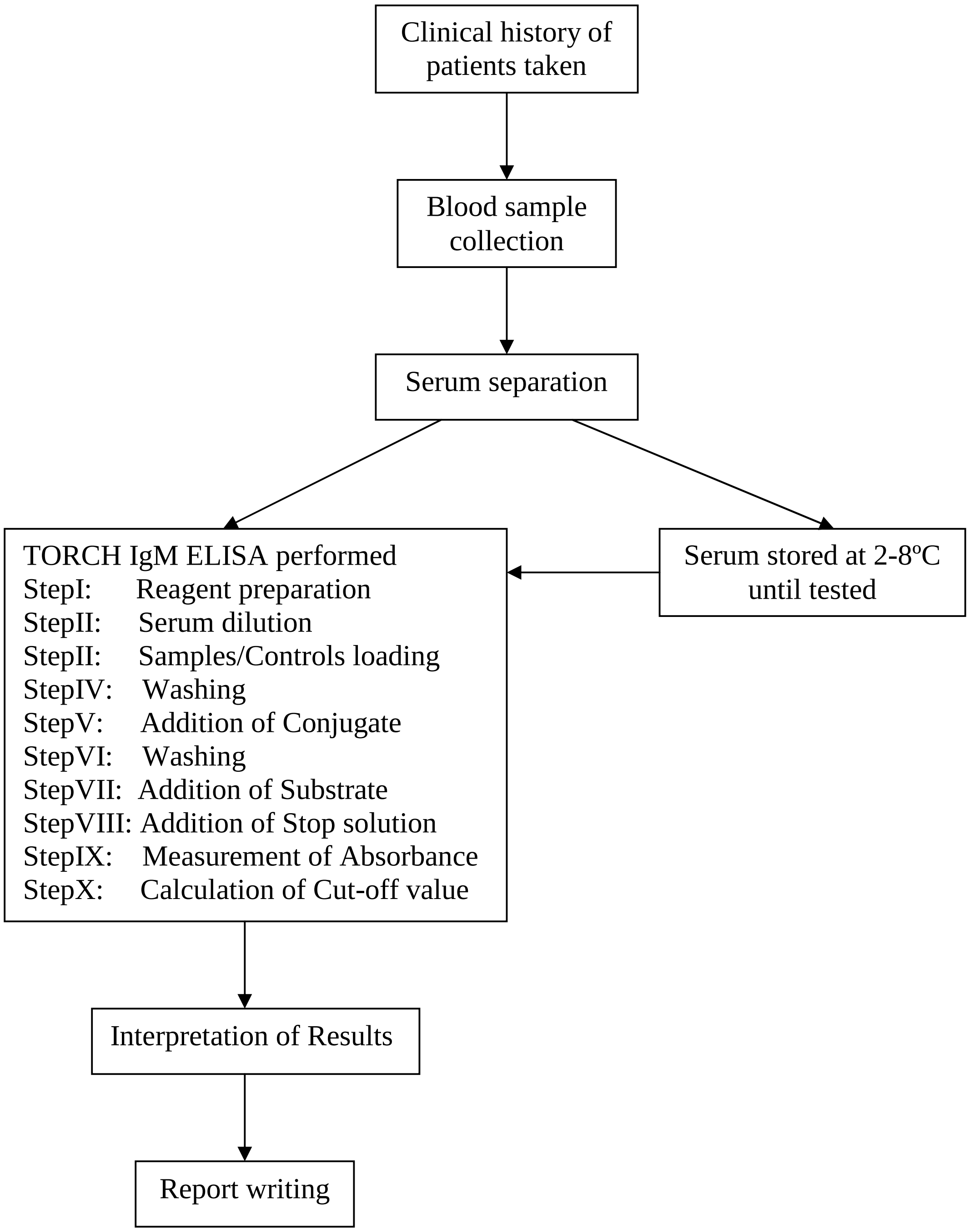
Clinical history of patients taken
Blood sample collection
Serum separation
TORCH IgM ELISA performed
StepI: Reagent preparation
StepII: Serum dilution
StepII: Samples/Controls loading
StepIV: Washing
StepV: Addition of Conjugate
StepVI: Washing
StepVII: Addition of Substrate
StepVIII: Addition of Stop solution
StepIX: Measurement of Absorbance
StepX: Calculation of Cut-off value
Serum stored at 2-8ºC until tested
Interpretation of Results
Report writing

APPENDIX-I

QUESTIONNAIRE

Clinical Profile:

Name: ………………………………………………………… **Lab No:**
Age: ………………………...
Sex: Male ☐ Female ☐
Address: …………………………………………………….
Date: ……………………………………………………
Test requested: ……………………………………………………

Clinical History:

Symptoms: ………………………………………………………………………
Meat eating habit:
Vegetarian ☐ Non-Vegetarian not having pork ☐
Non-Vegetarian having pork ☐
Presence of cat at home:
Yes ☐ No ☐
Past history of TORCH test:
Yes ☐ No ☐
If Yes, When? …………………………………

To be filled in case of female patients only:

Marital Status: Married ☐ Unmarried ☐
Age at marriage: …………. years
Age at first child birth: ……years
Pregnancy: Yes ☐ No ☐
Week of Gestation …………
Gravida: …………………
No. of Abortion/ Miscarriage/ Stillbirth: ……………….

Result:

	Positive	**Negative**
Toxoplasma Antibody (IgM):		
Rubella Antibody (IgM):		
Cytomegalovirus Antibody (IgM):		
Herpes Simplex Virus Antibody (IgM):		

APPENDIX –II

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. Materials provided with the TORCH IgM kits (Human, Germany)

Microtiter Strips coated with purified *Toxoplasma* antigens

Microtiter Strips coated with purified Rubella virus antigens

Microtiter Strips coated with purified CMV antigens

Microtiter Strips coated with purified HSV antigens

Toxoplasma IgM Negative control and Positive Control

Rubella IgM Negative control and Positive Control

CMV IgM Negative control and Positive Control

HSV IgM Negative control and Positive Control

Toxoplasma Anti-IgM Conjugate

Rubella Anti-IgM Conjugate

CMV Anti-IgM Conjugate

HSV Anti-IgM Conjugate

Dilution Buffer IgM

Washing Solution

TMB Substrate Reagent

Stop Solution

Adhesive Strips

NOTES: The **general purpose reagents:** Dilution Buffer IgM, Washing Solution, Substrate Reagent and Stop Solution are interchangeable between different lots and kits. All other reagents are **specific for the individual package lot** and must not be interchanged with other lots.

B. Equipments

Centrifuge	Gemmy (Taiwan)
Refrigerator	Videocon (India)
Vortex mixer	Gemmy (Taiwan)
Incubator (25ºc)	Gallenkamp

Autoclave	Stermite (Japan)
Hot air oven	Memmert (Japan)
ELISA washer (Humawash manual)	Human (Germany)
Human ELISA Plate Reader	Human (Germany)
Printer	Epson (Indonesia)
Water Distillation Plant	India
Micropipettes (1000µl, 500µl, 100µl)	Biohit (Finland)

C. Miscellaneous

Syringe, Pipette tips, Test tubes, Test tube markers, Towel paper, Cotton, Distilled water, Measuring cylinder, Lysol, Plastic containers.

APPENDIX-III

CALCULATION AND INTERPRETATION OF RESULTS

Calculation: In the ELISA tests for the detection of IgM antibodies to *Toxoplasma*, Rubella virus, Cytomegalovirus and Herpes simplex virus, **Cut-off value (COV)** is calculated by the formula:

Cut-off value (COV) = Negative control (NC) + 0.2 × Positive control (PC)

The test run may be considered valid provided that the following criteria are met:

NC ≤ 0.250

PC ≥ 0.400

PC: NC ≥ 3

Interpretation of Results:

In case of *Toxoplasma*,

Absorbance of patient at 450 nm ≥ COV + 15%: anti-Toxo-IgM-Ab-positive

Absorbance of patient at 450 nm < COV + 15%: anti-Toxo-IgM-Ab-negative

In case of Rubella virus (RV),

Absorbance of patient at 450 nm ≥ COV + 15%: anti- RV-IgM-Ab-positive

Absorbance of patient at 450 nm < COV + 15%: anti- RV-IgM-Ab-negative

In case of Cytomegalovirus (CMV),

Absorbance of patient at 450 nm ≥ COV + 15%: anti-CMV-IgM-Ab-positive

Absorbance of patient at 450 nm < COV + 15%: anti-CMV-IgM-Ab-negative

In case of Herpes simplex virus (HSV),

Absorbance of patient at 450 nm ≥ COV + 15%: anti-HSV-IgM-Ab-positive

Absorbance of patient at 450 nm < COV + 15%: anti-HSV-IgM-Ab-negative

Note: Due to physiological and analytical variations patients results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later.

APPENDIX-IV

DATA ANALYSIS (CHI-SQUARE TEST)

A. Association of presence of TORCH infections in male and female patients

	Presence of TORCH infections	Absence of TORCH infections	Total
Male Patients	14	72	86
Female Patients	50	140	190
Total	**64**	**212**	**276**

Test statistic is χ^2

Ho: There is no significant association of presence of TORCH infections in male and female patients.

H_1: There is significant association of presence of TORCH infections in male and female patients.

From $\chi^2=\Sigma (0\text{-}E)^2/E$, we find $\chi^2=3.409$

Thus χ^2 cal (3.409) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 1 i.e., 3.841

Hence, Ho is accepted i.e. there is no significant association of presence of TORCH infections in male and female patients.

B. Association of *Toxoplasma* infection with meat eating habit

Meat Eating habit	*Toxoplasma* Positive cases	*Toxoplasma* Negative cases	Total no. of suspected patients of age >10
Vegetarian habit	1	21	22
Non vegetarian not having pork	21	131	152
Non vegetarian having pork	8	17	25
Total	**30**	**169**	**199**

Test statistic is χ^2

Ho: There is no significant association of *Toxoplasma* infection with meat eating habit.

H_1: There is significant association of *Toxoplasma* infection with meat eating habit.

From $\chi^2 = \Sigma (0-E)^2/E$, we find $\chi^2 = 7.687$

Thus χ^2 cal (7.687) > χ^2 tab at $\alpha = 0.05$ and degree of freedom = 2 i.e., 5.99

Hence, Ho is rejected and the test is significant i.e. there is significant association of *Toxoplasma* infection with meat eating habit.

C. Association of *Toxoplasma* infection with domestication of cat

Domestication of cat	***Toxoplasma* Positive cases**	***Toxoplasma* Negative cases**	**No. of suspected patients of age >10**
Have cat as pet	4	6	10
Do not have cat as pet	26	163	189
Total	**30**	**169**	**199**

Test statistic is χ^2

Ho: There is no significant association of *Toxoplasma* infection with domestication of cat

H_1: There is significant association of *Toxoplasma* infection with domestication of cat

From $\chi^2 = \Sigma (0-E)^2/E$, we find $\chi^2 = 5.158$

Thus χ^2 cal (5.158) > χ^2 tab at $\alpha = 0.05$ and degree of freedom = 1 i.e., 3.841

Hence, Ho is rejected and the test is significant i.e. there is significant association of *Toxoplasma* infection with domestication of cat.

APPENDIX-V

TOXO IgM

ELISA Test for the Detection of IgM Antibodies to *Toxoplasma Gondii* in Human Serum

Package Size

[REF]	51109	96 Tests	Complete Test Kit
[IVD]			

Intended Use

The TOXO IgM ELISA is intended for the detection of Immunoglobulin M (IgM) class antibodies to *Toxoplasma gondii* in human serum.
Toxoplasma infects nearly all mammalians and birds. It is the most widely distributed of all intracellular parasites. Humans become infected through contamination with feces or uncooked meat, or through direct inoculation via blood transfusions or congenital transmission.
Pregnant women who acquire toxoplasmosis during the first trimester have a 25% risk of fetal transmission resulting in spontaneous abortions, stillborns, or severe disease. Sixty five percent of infants born to women infected during the third trimester have subclinical infection with ultimately 85% developing chorioretinitis or neurological sequelae.

Principle **- Classic EIA -**

The HUMAN TOXO IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with purified *Toxoplasma gondii* membrane antigens (TOXO-Ag). In the first incubation step corresponding specific antibodies (TOXO-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to **prevent rheumatoid factor (RF) interference and competition from specific IgG** present in the specimen.
At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the TOXO-IgM-Ab concentration in the specimen.
The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HUMAREADER or ELISYS line). Results for patient samples are obtained by comparison with a cut-off value.

Reagents and Contents

[MIC]	12	**Microtiter Strips** (in 1 strip holder)	
		(Code TOX M)	
		8-well snap-off strips	
		coated with *Toxoplasma gondii* membrane antigen	
[NC]	2.5 ml	**TOXO IgM Negative Control** (green cap)	
		ready for use, human	
[PC]	2.5 ml	**TOXO IgM Positive Control** (red cap)	
		ready for use, human	
[DIL-M]	100 ml	**Dilution Buffer IgM** (blue cap)	
5111		ready for use, coloured green	pH 6.5 ± 0.2
		Phosphate buffer	10 mmol/l
		NaCl	8 g/l
		Albumin	10 g/l
		Anti-human-IgG (goat)	
[CON]	12 ml	**Anti-IgM Conjugate** (white cap)	
		ready for use, coloured red	
		Anti-human IgM (rabbit), peroxidase-conjugated	
[WS]	50 ml	**Washing Solution** (white cap)	
5102		Concentrate for about 1000 ml	pH 7.2 ± 0.2
		Tris buffer	10 mmol/l
		NaCl	8 g/l
[SUB]	15 ml	**Substrate Reagent** (black cap)	
5103		ready for use, colourless to bluish	pH 3.7 ± 0.2
		3,3', 5,5'-tetramethylbenzidin (TMB)	1.2 mmol/l
		Hydrogen peroxide	3 mmol/l
[STOP]	15 ml	**Stop Solution** (red cap)	
5104		Sulphuric acid, ready for use	0.5 mol/l
	2	**Adhesive Strips**	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.
All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.
[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.
After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC] (Code: TOX M)

- sealed in an aluminium bag with a desiccant,
- must be at room temperature before opening,
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use. Reagents not in use should always be stored at 2...8°C.

Notes

The **general purpose reagents** [DIL-M] 5111, [WS] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits. For **IgM tests** use only IgM dilution buffer [DIL-M] 5111.
All other reagents are **specific for the individual package lot** and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution [WASH]

- dilute [WS] 5102 1+20 with fresh deionised water, e.g. 50 ml [WS] 5102 + 1000 ml = 1050 ml.
- Stability: **up to 60 days at 15...25°C**.

Specimen

Serum
Do not use highly lipemic or hemolysed specimens.
Specimens may be stored for 7 days at 2...8°C or longer at -20°C.
Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.
P2: Do not use reagents that could be contaminated or look or smell different than usual.
P3: Record specimens and controls carefully on the spread sheet supplied with the kit.
P4: [MIC] - select the required number of Microtiter Strips.
P5: Run duplicates for controls. Pipette controls and specimen **on the bottom** in the microwells.
P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.
P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.
P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].
P9: [DIL-M] – turbidity after addition of the sample has no influence on the results.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add WASH to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with WASH. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, **remove remaining liquid** by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.				
Sample Preparation: Dilute the patient's sera 1+100 with DIL-M 5111, e.g. 10 µl serum + 1 ml DIL-M 5111, mix thoroughly (see P9). **Incubate diluted samples at least 5 min. prior to further processing.** Diluted samples can be stored up to 24 h at 2...8°C before testing. Controls are ready for use.				
Step 1	**Well [µl]**			
	A1 Blank	**B1/C1** NC	**D1/E1** PC	**F1...** Sample
NC in duplicate	--	100	--	--
PC in duplicate	--	--	100	--
Diluted samples	--	--	--	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 2				
CON	--	100	100	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 3				
SUB 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
STOP 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HUMAREADER) using the substrate blank in well A1.				
Measure the absorbance at **450 nm** as soon as possible or **within 30 min.** after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Calculation of Control Values and Cut-off

Mean absorbance values of NC in wells B1 and C1 **(MNC)** and PC in wells D1 and E1 **(MPC)** are calculated according to:

$$\mathbf{MNC} = \frac{A_{450}(B1) + A_{450}(C1)}{2} \;;\quad \mathbf{MPC} = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value **COV = MNC + 0.2 x MPC**

The test run may be considered valid provided that the following criteria are met:

1. **Substrate blank in well A1 < 0.150**
2. **MNC ≤ 0.250**
3. **MPC ≥ 0.400**
4. **MPC : MNC ≥ 3**

Interpretation of Results

A_{450} (patient) ≥ COV + 20%: **anti-TOXO-IgM-Ab-positive**
A_{450} (patient) < COV - 20%: **anti-TOXO-IgM-Ab-negative**

Due to physiological and analytical variations patient results lying 20% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Samples from patients with infectious mononucleosis may give an equivocal or low positive result. This may be due to a reactivation of Toxo IgM antibody production. The possibility of an infectious mononucleosis infection should therefore be investigated before interpretation of results.

If an ELISA reader is not available a visual interpretation of results is possible:

- The substrate blank in well A1 should appear colourless.
- A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the NC wells B1/C1.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-toxom.pdf or
www.human-de.com/data/gb/vr/el-toxom.pdf

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.
The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

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APPENDIX-VI

RUBELLA IgM

ELISA Test for the Detection of IgM Antibodies to *Rubella Virus* in Human Serum

Package Size

REF	51108	96 Tests	Complete Test Kit
IVD			

Intended Use

The RUBELLA IgM ELISA is intended for the detection of Immunoglobulin G (IgM) class antibodies to *Rubella virus* (RV) in human serum.

Rubella virus is the cause of German measles, usually a mild exanthem, often subclinical. However, when acquired *in utero*, *Rubella virus* can cause the congenital rubella syndrome, and lead to fetal demise, cataracts, malformation, deafness, and mental retardation.

In pregnant women (first trimester) the detection of Rubella-specific IgM antibody strongly supports a diagnosis of congenital infection.

Principle - Classic EIA -

The HUMAN RUBELLA IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived *Rubella virus* antigens (RV Ag). In the first incubation step corresponding specific antibodies (RV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to **prevent rheumatoid factor (RF) interference and competition from specific IgG** present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the RV-IgM-Ab concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HUMAREADER or ELISYS line). Results for patient samples are obtained by comparison with a cut-off value.

Reagents and Contents

MIC	12	**Microtiter Strips** (in 1 strip holder)	
		(Code RUB M)	
		8-well snap-off strips	
		coated with purified RV antigens	
NC	2.5 ml	**RUBELLA IgM Negative Control** (green cap)	
		ready for use, human	
PC	2.5 ml	**RUBELLA IgM Positive Control** (red cap)	
		ready for use, human	
DIL-M	100 ml	**Dilution Buffer IgM** (blue cap)	
5111		ready for use, coloured green	pH 6.5 ± 0.2
		Phosphate buffer	10 mmol/l
		NaCl	8 g/l
		Albumin	10 g/l
		Anti-human-IgG (goat)	
CON	12 ml	**Anti-IgM Conjugate** (white cap)	
		ready for use, coloured red	
		Anti-human IgM (rabbit), peroxidase-conjugated	
WS	50 ml	**Washing Solution** (white cap)	
5102		Concentrate for about 1000 ml	pH 7.2 ± 0.2
		Tris buffer	10 mmol/l
		NaCl	8 g/l
SUB	15 ml	**Substrate Reagent** (black cap)	
5103		ready for use, colourless to bluish	pH 3.7 ± 0.2
		3,3', 5,5'-tetramethylbenzidin (TMB)	1.2 mmol/l
		Hydrogen peroxide	3 mmol/l
STOP	15 ml	**Stop Solution** (red cap)	
5104		Sulphuric acid, ready for use	0.5 mol/l
	2	**Adhesive Strips**	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

STOP irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

MIC (Code: RUB M)

- sealed in an aluminium bag with a desiccant.
- must be at room temperature before opening.
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use. Reagents not in use should always be stored at 2...8°C.

Notes

The **general purpose reagents** DIL-M 5111, WS 5102, SUB 5103, STOP 5104 are interchangeable between different lots and kits. For **IgM tests** use only IgM dilution buffer DIL-M 5111.

All other reagents are **specific for the individual package lot** and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

***Working Wash Solution* WASH**

- dilute WS 5102 1+20 with fresh deionised water, e.g. 50 ml WS 5102 + 1000 ml = 1050 ml.
- Stability: **up to 60 days at 15...25°C**.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. **Freeze and thaw once only**. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: MIC - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen **on the bottom** in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: SUB – incubate in the dark. SUB initiates a kinetic reaction, which is terminated by STOP.

P9: DIL-M – turbidity after addition of the sample has no influence on the results.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add WASH to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with WASH. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, **remove remaining liquid** by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.				
Sample Preparation: Dilute the patient's sera 1+100 with DIL-M 5111, e.g. 10 µl serum + 1 ml DIL-M 5111, mix thoroughly (see P9). **Incubate diluted samples at least 5 min. prior to further processing.** Diluted samples can be stored up to 24 h at 2...8°C before testing. Controls are ready for use.				
Step 1	**Well [µl]**			
	A1 Blank	**B1/C1** NC	**D1/E1** PC	**F1...** Sample
NC in duplicate	--	100	--	--
PC in duplicate	--	--	100	--
Diluted samples	--	--	--	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 2				
CON	--	100	100	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 3				
SUB 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
STOP 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HUMAREADER) using the substrate blank in well A1.				
Measure the absorbance at **450 nm** as soon as possible or **within 30 min.** after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Calculation of Control Values and Cut-off

Mean absorbance values of NC in wells B1 and C1 **(MNC)** and PC in wells D1 and E1 **(MPC)** are calculated according to:

$$\mathbf{MNC} = \frac{A_{450}(B1) + A_{450}(C1)}{2}\ ;\quad \mathbf{MPC} = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value **COV = MNC + 0.2 x MPC**

The test run may be considered valid provided that the following criteria are met:

1. **Substrate blank in well A1 < 0.150**
2. **MNC ≤ 0.250**
3. **MPC ≥ 0.400**
4. **MPC : MNC ≥ 3**

Interpretation of Results

A_{450} (patient) ≥ COV + 15%:	**anti-RV-IgM-Ab-positive**
A_{450} (patient) < COV -15%:	**anti-RV-IgM-Ab-negative**

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Samples from patients with infectious mononucleosis may give a positive result. This may be due to reactivation of Rubella IgM antibody production. The possibility of an infectious mononucleosis condition should therefore be investigated before interpretation of results.

If an ELISA reader is not available a visual interpretation of results is possible:

* The substrate blank in well A1 should appear colourless.
* A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the NC wells B1/C1.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-rubm.pdf or
www.human-de.com/data/gb/vr/el-rubm.pdf

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Literature

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EL-RUBM
INF 5110601 GB
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APPENDIX-VII

CMV IgM

ELISA Test for the Detection of IgM Antibodies to *Cytomegalo Virus* in Human Serum

Package Size

REF	51103	96 Tests	Complete Test Kit
IVD			

Intended Use

The CMV IgM ELISA is intended for the detection of Immunoglobulin G (IgM) class antibodies to *Cytomegalo virus* (CMV) in human serum.

CMV infections occur worldwide. About 50% of the general population is seropositive by the third decade of life. CMV is often a sexually transmitted disease, but may also be aquired by blood transfusions or via saliva, feces, urine, or milk.

During pregnacy the fetus may be infected by CMV, and while the majority appear healthy at birth, up to 25% of these asymptomatically infected infants will show developmental disorders later (deafness, mental retardation). In normal adults infection with CMV is usually asymptomatic, but when symptoms do occur, the most common form is mononucleosis. Typically, the patient presents with fever, chills, myalgia, and headache. Often the physician must rely on serological tests to distinguish CMV infection from other infections and clinical syndromes which have similar symptoms.

Principle - **Classic EIA** -

The HUMAN CMV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived CMV antigens (CMV Ag). In the first incubation step corresponding specific antibodies (CMV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to **prevent rheumatoid factor (RF) interference and competition from specific IgG** present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the CMV-IgM-Ab concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HUMAREADER or ELISYS line). Results for patient samples are obtained by comparison with a cut-off value.

Reagents and Contents

MIC	12	**Microtiter Strips** (in 1 strip holder) (Code CMV M) 8-well snap-off strips coated with CMV antigen (cell culture derived)	
NC	2.5 ml	**CMV IgM Negative Control** (green cap) ready for use, human	
PC	2.5 ml	**CMV IgM Positive Control** (red cap) ready for use, human calibrated against Paul-Ehrlich-Institute (PEI) reference material: (Titer of ready for use positive control: 1:100 dilution of the PEI ref. preparation within a range of ± 10%)	Titer: 1:100 ± 10 %
DIL-M 5111	100 ml	**Dilution Buffer IgM** (blue cap)	
		ready for use, coloured green	pH 6.5 ± 0.2
		Phosphate buffer	10 mmol/l
		NaCl	8 g/l
		Albumin	10 g/l
		Anti-human-IgG (goat)	
CON	12 ml	**Anti-IgM Conjugate** (white cap) ready for use, coloured red Anti-human IgM (rabbit), peroxidase-conjugated	
WS 5102	50 ml	**Washing Solution** (white cap)	
		Concentrate for about 1000 ml	pH 7.2 ± 0.2
		Tris buffer	10 mmol/l
		NaCl	8 g/l
SUB 5103	15 ml	**Substrate Reagent** (black cap)	
		ready for use, colourless to bluish	pH 3.7 ± 0.2
		3,3', 5,5'-tetramethylbenzidin (TMB)	1.2 mmol/l
		Hydrogen peroxide	3 mmol/l
STOP 5104	15 ml	**Stop Solution** (red cap)	
		Sulphuric acid, ready for use	0.5 mol/l
	2	**Adhesive Strips**	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

STOP irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

MIC (Code: CMV M)

- sealed in an aluminium bag with a desiccant
- must be at room temperature before opening
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use.
Reagents not in use should always be stored at 2...8°C.

Notes

The **general purpose reagents** DIL-M 5111, WS 5102, SUB 5103, STOP 5104 are interchangeable between different lots and kits. For **IgM tests** use only IgM dilution buffer DIL-M 5111.

All other reagents are **specific for the individual package lot** and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution WASH

- dilute WS 5102 1 + 20 with fresh deionised water, e.g. 50 ml WS 5102 + 1000 ml = 1050 ml.
- Stability: **up to 60 days at 15...25°C**.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. **Freeze and thaw once only**. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.
P2: Do not use reagents that could be contaminated or look or smell different than usual.
P3: Record specimens and controls carefully on the spread sheet supplied with the kit.
P4: MIC - select the required number of Microtiter Strips.
P5: Run duplicates for controls. Pipette controls and specimen **on the bottom** in the microwells.
P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.
P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: SUB – incubate in the dark. SUB initiates a kinetic reaction, which is terminated by STOP.
P9: DIL-M – turbidity after addition of the sample has no influence on the results.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.
W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add WASH to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.
W2: In case of automatic washers fill and prime with WASH. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).
W3: After washing, **remove remaining liquid** by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.

Sample Preparation:
Dilute the patient's sera 1 + 100 with DIL-M 5111, e.g. 10 µl serum + 1 ml DIL-M 5111, mix thoroughly (see P9).
Incubate diluted samples at least 5 min. prior to further processing.
Diluted samples can be stored up to 24 h at 2...8°C before testing.
Controls are ready for use.

Step 1	Well [µl]			
	A1 Blank	B1/C1 NC	D1/E1 PC	F1... Sample
NC in duplicate	--	100	--	--
PC in duplicate	--	--	100	--
Diluted samples	--	--	--	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 2				
CON	--	100	100	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 3				
SUB 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
STOP 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HUMAREADER) using the substrate blank in well A1.				
Measure the absorbance at **450 nm** as soon as possible or **within 30 min.** after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Calculation of Control Values and Cut-off

Mean absorbance values of NC in wells B1 and C1 **(MNC)** and PC in wells D1 and E1 **(MPC)** are calculated according to:

$$\mathbf{MNC} = \frac{A_{450}(B1) + A_{450}(C1)}{2}\ ; \quad \mathbf{MPC} = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value **COV = MNC + 0.2 x MPC**

The test run may be considered valid provided that the following criteria are met:

1. **Substrate blank in well A1 < 0.150**
2. **MNC ≤ 0.250**
3. **MPC ≥ 0.400**
4. **MPC : MNC ≥ 3**

Interpretation of Results

A_{450} (patient) ≥ COV + 15%: anti-CMV-IgM-Ab-positive
A_{450} (patient) < COV -15%: anti-CMV-IgM-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Samples from patients with EBV-induced infectious mononucleosis may give an equivocal or low positive result (sample to cut-off ratio < 1.75), possibly as a result of a reactivation of CMV IgM antibody production caused by EBV-induced B-cell proliferation.

If an ELISA reader is not available a visual interpretation of results is possible:

- The substrate blank in well A1 should appear colourless.
- A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the NC wells B1/C1.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via
www.human.de/data/gb/vr/el-cmvm.pdf or
www.human-de.com/data/gb/vr/el-cmvm.pdf

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.
The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!
(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Literature

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EL-CMVM
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APPENDIX-VIII

HSV IgM

ELISA Test for the Detection of IgM Antibodies to *Herpes Simplex Virus* in Human Serum

Package Size

REF	51126	96 Tests	Complete Test Kit
IVD			

Intended Use

The HSV IgM ELISA is intended for the detection of Immunoglobulin M (IgM) class antibodies to *Herpes Simplex virus* in human serum.

HSV infections occur world-wide. The incubation period following exposure to the virus is about 1 week (range 2-26 days). Clinical symptoms include herpes genitalis and labialis.

HSV infection in new-borns (less then 6 weeks of age) is frequently devastating. Untreated, the mortality exceeds 65%. Many who survive exhibit developmental disabilities. Infection occurs most commonly during delivery as the neonate passes through the infected birth channel.

Principle - Classic EIA -

The HUMAN HSV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived *Herpes Simplex virus* antigens (HSV-Ag). In the first incubation step corresponding specific antibodies (HSV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to **prevent rheumatoid factor (RF) interference and competition from specific IgG** present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the HSV-IgM-Ab concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HUMAREADER or ELISYS line). Results for patient samples are obtained by comparison with a cut-off value.

Reagents and Contents

MIC	12	**Microtiter Strips** (in 1 strip holder)	
		(Code HSV M)	
		8-well snap-off strips	
		coated with HSV antigen (tpye 1 and type 2)	
NC	2.5 ml	**HSV IgM Negative Control** (green cap)	
		ready for use, human	
PC	2.5 ml	**HSV IgM Positive Control** (red cap)	
		ready for use, human	
DIL-M	100 ml	**Dilution Buffer IgM** (blue cap)	
5111		ready for use, coloured green	pH 6.5 ± 0,2
		Phosphate buffer	10 mmol/l
		NaCl	8 g/l
		Albumin	10 g/l
		Anti-human-IgG (goat)	
CON	12 ml	**Anti-IgM Conjugate** (white cap)	
		ready for use, coloured red	
		Anti-human IgM (rabbit), peroxidase-conjugated	
WS	50 ml	**Washing Solution** (white cap)	
5102		Concentrate for about 1000 ml	pH 7.2 ± 0,2
		Tris buffer	10 mmol/l
		NaCl	8 g/l
SUB	15 ml	**Substrate Reagent** (black cap)	
5103		ready for use, colourless to bluish	pH 3.7 ± 0.2
		3,3', 5,5'-tetramethylbenzidin (TMB)	1.2 mmol/l
		Hydrogen peroxide	3 mmol/l
STOP	15 ml	**Stop Solution** (red cap)	
5104		Sulphuric acid, ready for use	0.5 mol/l
	2	**Adhesive Strips**	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

STOP irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

MIC (Code: HSV M)

- sealed in an aluminium bag with a desiccant,
- must be at room temperature before opening,
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The **general purpose reagents** DIL-M 5111, WS 5102, SUB 5103, STOP 5104 are interchangeable between different lots and kits. For **IgM tests** use only IgM dilution buffer DIL-M 5111.

All other reagents are **specific for the individual package lot** and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution WASH

- dilute WS 5102 1+20 with fresh deionised water, e.g. 50 ml WS 5102 + 1000 ml = 1050 ml.
- Stability: **up to 60 days at 15...25°C**.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. **Freeze and thaw once only**. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: MIC - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen **on the bottom** in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: SUB – incubate in the dark. SUB initiates a kinetic reaction, which is terminated by STOP.

P9: DIL-M – turbidity after addition of the sample has no influence on the results.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add WASH to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with WASH. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, **remove remaining liquid** by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.

Sample Preparation:
Dilute the patient's sera 1+100 with DIL-M 5111, e.g. 10 µl serum + 1 ml DIL-M 5111, mix thoroughly (see P9).
Incubate diluted samples at least 5 min. prior to further processing.
Diluted samples can be stored up to 24 h at 2...8°C before testing.
Controls are ready for use.

Step 1	A1 Blank	B1/C1 NC	D1/E1 PC	F1... Sample
NC in duplicate	--	100	--	--
PC in duplicate	--	--	100	--
Diluted samples	--	--	--	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 2				
CON	--	100	100	100
MIC cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
WASH	350	350	350	350
Step 3				
SUB 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
STOP 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HUMAREADER) using the substrate blank in well A1.				
Measure the absorbance at **450 nm** as soon as possible or **within 30 min.** after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

(Well [µl])

Calculation of Control Values and Cut-off

Mean absorbance values of NC in wells B1 and C1 **(MNC)** and PC in wells D1 and E1 **(MPC)** are calculated according to:

$$\mathbf{MNC} = \frac{A_{450}(B1) + A_{450}(C1)}{2}; \quad \mathbf{MPC} = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value **COV = MNC + 0.2 x MPC**

The test run may be considered valid provided that the following criteria are met:

1. **Substrate blank in well A1 < 0.150**
2. **MNC ≤ 0.250**
3. **MPC ≥ 0.400**
4. **MPC : MNC ≥ 3**

Interpretation of Results

A_{450} (patient) ≥ COV + 15%: **anti-HSV-IgM-Ab-positive**
A_{450} (patient) < COV -15%: **anti-HSV-IgM-Ab-negative**

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Due to reactivation of latent infections with other viruses, related to the Herpes viridae family, positive HSV IgM results may occur in sera from patients with such infections. The possibility of infections with other members of the Herpes viridae family should therefore be investigated before interpretation of results.

If an ELISA reader is not available a visual interpretation of results is possible:

* The substrate blank in well A1 should appear colourless.
* A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the NC wells B1/C1.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-hsvm.pdf or
www.human-de.com/data/gb/vr/el-hsvm.pdf

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.
The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!
(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Literature

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APPENDIX-IX

VERTICAL TRANSMISSION OF TORCH INFECTIONS: RELATIVE FREQUENCY OF CONGENITAL AND PERINATAL INFECTIONS ACCORDING TO PATHOGEN

Pathogen	Congenital	Perinatal
Toxoplasma gondii	+++	+
Rubella virus	+++	+
Cytomegalovirus	+	+++
Herpes Simplex Virus	+	+++

Note: ++++ = most frequent, + = least frequent.

Source: Boyer and Boyer, 2004

APPENDIX-X

RECOMMENDATIONS FOR THE PREVENTION OF TORCH INFECTIONS

A. Prevention of Toxoplasmosis

Three principal interventions are presently used to reduce morbidity and mortality from congenital toxoplasmosis:

a) Education about how to prevent infection (especially during pregnancy) to adolescents and women in the preconception period;

b) Prenatal and newborn screening to identify and treat congenital infection; and

c) Animal rearing and production methods designed to reduce *T. gondii* contamination of meat (Ross *et al*, 2006).

Recommendations for the prevention of toxoplasmosis are

- To prevent toxoplasmosis and other food borne illnesses, food should be cooked to safe temperatures. A food thermometer should be used to measure the internal temperature of cooked meat to ensure that meat is cooked all the way through. Beef, lamb, and veal roasts and steaks should be cooked to at least 145 F, and pork and ground meat should be cooked to 160 F before eating. Whole poultry should be cooked to 180 F in the thigh to ensure doneness.
- Fruits and vegetables should be peeled or thoroughly washed before eating.
- Cutting boards, dishes, counters, utensils, and hands should always be washed with hot soapy water after they have contacted raw meat, poultry, seafood, or unwashed fruits or vegetables.

- Pregnant women should wear gloves when gardening and during any contact with soil or sand because cat waste might be in soil or sand. After gardening or contact with soil or sand, hands should be washed thoroughly.
- Pregnant women should avoid changing cat litter if possible. If no one else is available to change the cat litter, use gloves, then wash hands thoroughly. Change the litter box daily because *Toxoplasma* oocysts require several days to become infectious. Pregnant women should be encouraged to keep their cats inside and not adopt or handle stray cats. Cats should be fed only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.
- Health education for women of childbearing age should include information about meat-related and soil borne toxoplasmosis prevention. Health-care providers should educate pregnant women at their first prenatal visit about food hygiene and prevention of exposure to cat feces.
- The government and the meat industry should continue efforts to reduce *Toxoplasma* in meat (Hughes *et al*, 2000).

B. Prevention of Rubella

- Rubella can be prevented by a rubella vaccine. The rubella vaccine is usually given as a combined measles-mumps-rubella (MMR) inoculation. Children should receive the MMR vaccine between 12 and 15 months of age, and again between 3 and 6 years of age (DoctorNDTV Team, 2004).
- The prevention of congenital rubella obviously is dependent upon adequate early immunization, resulting in a high prevalence of immunity in women of childbearing age. If there is any doubt that they are immune, women should be screened for rubella immunity at the beginning of pregnancy. Contact isolation is required for neonates suspected to have congenital rubella (Boyer and Boyer, 2004).
- Pregnant women who are not immune should avoid anyone who has the illness and should be vaccinated after delivery so that they will be immune during any future pregnancies (Hirsch, 2006).

C. Prevention of Cytomegalovirus (CMV) infection

Recommendations for pregnant women:

- Throughout the pregnancy, practice good personal hygiene, especially handwashing with soap and water, after contact with diapers or oral secretions (particularly with a child who is in day care).
- Women who develop a mononucleosis-like illness during pregnancy should be evaluated for CMV infection and counseled about the possible risks to the unborn child.
- Laboratory testing for antibody to CMV can be performed to determine if a women has already had CMV infection.
- Recovery of CMV from the cervix or urine of women at or before the time of delivery does not warrant a cesarean section.
- The demonstrated benefits of breast-feeding outweigh the minimal risk of acquiring CMV from the breast-feeding mother.
- There is no need to either screen for CMV or exclude CMV-excreting children from schools or institutions because the virus is frequently found in many healthy children and adults.

Recommendations for individuals providing care for infants and children:

- Female employees should be educated concerning CMV, its transmission, and hygienic practices, such as handwashing, which minimize the risk of infection.
- Pregnant women working with infants and children should be informed of the risk of acquiring CMV infection and the possible effects on the unborn child.
- Routine laboratory testing for CMV antibody in female workers is not recommended, but can be performed to determine their immune status (Wikipedia, 2006).

D. Prevention of Herpes simplex virus (HSV) infection

- Herpes infections can be prevented by avoiding direct contact with sores or ulcers of someone who has an active herpes infection - either on the mouth or on the genitals.
- Teens that are sexually active should properly use a latex condom during sexual activity, but even condoms will not completely eliminate the risk of spreading genital

herpes while there are active lesions. The only surefire way to prevent genital herpes is abstinence (Homeier, 2005).

- Daily maintenance treatment with oral antiviral agents reduces the frequency of HSV recurrences and viral shedding (Alter, 2006).
- Prevention of neonatal HSV requires the prevention of acquisition of HSV in the third trimester of pregnancy. Identification of women or couples susceptible to acquisition of HSV in pregnancy through serologic screening is receiving increasing attention, and such screening is being used with increasing frequency (Corey, 2003).
- Exposure of the infant at birth can be avoided if delivery by caesarean section is performed in the early stage of labour, but this is only to be recommended when lesions are present in the mother or virus has been demonstrated at that time (Ogilvie, 1997).