

recomLine TORCH Screening IgG

recomLine TORCH Screening IgM

Line immunoassay based on whole cell lysate and recombinant antigens for the determination of IgG and IgM antibodies against *Toxoplasma gondii*, rubella virus, cytomegalovirus, and herpes simplex virus in human serum or plasma. The test is based on the test principle of ELISA.

1. General aspects, intended use

The MIKROGEN *recomLine* TORCH Screening IgG and *recomLine* TORCH Screening IgM assays are qualitative *in-vitro* tests for the detection of IgG and IgM antibodies against *Toxoplasma gondii*, rubella virus, cytomegalovirus (CMV), and herpes simplex virus types 1 and 2 (HSV-1/2).

1.1 MIKROGEN *recomLine* TORCH Screening IgG assay

For the determination of the immune status for *Toxoplasma gondii*, rubella virus, CMV, and HSV-1/2, whole cell lysate antigens are used in the IgG and IgM assays.

A special feature of the MIKROGEN *recomLine* TORCH Screening IgG assay is the use of additional recombinant antigens on the test strip. These antigens enable differentiated diagnosis in the first step analysis (screening).

Interpretation of the MIKROGEN *recomLine* TORCH Screening IgG test follows a two-band strategy (eight pathogen-specific antigen bands per test strip). In the first step, the presence or the absence of specific antibodies to an infectious agent is detected (major band).

- The ancillary band specific for *T. gondii* (p30) enables exclusion of a primary infection during the last 3 months if a positive reactivity is detectable.
- The ancillary band specific for cytomegalovirus (gB2) enables exclusion of a primary infection during the last 6 – 8 weeks if a positive reactivity is detectable.
- The ancillary band specific for rubella virus (rubella vaccination band) enables the assessment of protective or non-protective immunity. This band is adjusted according to the guidelines of the World Health Organization (WHO) and it nearly corresponds with an anti-rubella virus IgG antibody titer of ≥ 15 IU/ml. The intensity of the rubella major band is proportional to the activity of rubella-specific IgG antibodies present in a sample. Interpretation of the rubella major band is carried out through comparison with the intensity of the rubella vaccination band.

Please note that the interpretation of an IgG test result can only be made in combination with an IgM test result and the anamnesis, respectively.

- The ancillary band specific for the HSV type 2 (gG2) allows identification of infections caused by the HSV type 2 (the most common causative agent of genital herpes).

1.2 MIKROGEN *recomLine* TORCH Screening IgM assay

The diagnostic information presented by the *recomLine* TORCH Screening IgG assay is supported by the MIKROGEN *recomLine* TORCH Screening IgM assay. The test strips contain one band per infectious agent (four pathogen-specific whole cell or virus lysate antigen bands per test strip), which enables the identification of specific IgM class antibodies to *T. gondii*, rubella virus, CMV, and HSV types 1 and 2, respectively. Sensitivity of the *T. gondii*-specific cell lysate antigen band is enhanced by an early phase antigen ROP1c [36].

2. Congenital diseases

2.1 Toxoplasmosis

Toxoplasmosis is caused by *Toxoplasma gondii*, a protozoan parasite with cats as the primary hosts. Sexual reproduction takes place only in the primary host, leading to the excretion of infectious oocytes, which in cat feces are a source of infection for humans. Since *T. gondii* persists in pigs, goats and other mammals, humans can also be infected through the consumption of raw or insufficiently heated meat [1, 2].

Toxoplasmosis is the most frequent parasitic disease in the central Europe. The seroprevalence is correspondingly high in the general population and increases with age (by approximately one percent per year of life). However, there may be considerable local differences in the prevalence varying from less than 10% (e.g. Scandinavian countries) to almost 80% (e.g. France) The incidence rate of congenital toxoplasmosis in the central Europe is approximately 5 to 6 per 1,000 live births [1, 2].

The course of infection of an immunocompetent individual with *T. gondii* is usually asymptomatic, rarely with non-specific flu-like symptoms, such as slight fever, fatigue, aches, and swollen lymph nodes. The initial infection results in a lifelong persistence of the parasite and confers a lifelong immunity to further toxoplasmosis infections [1].

One of the appalling consequences of *T. gondii* infections in humans occurs when women contract the infection during gestation and pass the parasite to their unborn child. The diaplacental transmission of *T. gondii* can cause devastating disease in the fetus and the newborn (congenital toxoplasmosis) [1, 2]. Serious sequelae of congenital toxoplasmosis include decreased vision or blindness as well as mental and psychomotoric retardation (encephalitis followed by severe neurological disorders).

The likelihood and severity of an intrauterine infection depends on the time of infection during the pregnancy. Frequency of transplacental transmission of *T. gondii* increases as the pregnancy progresses; the estimated transmission rate in the 1st trimester is 14% in comparison to 59% in the 3rd trimester. Fortunately, the rate of severe sequelae and stillbirth as well as the severity of the clinical manifestations among infected infants decreases as the gestation progresses; the estimated rate of severe sequelae among infected infants accounts for 41% in the 1st trimester in comparison to nearly 0% in the 3rd trimester. The clinical manifestations of congenital toxoplasmosis range from subclinically infected children to severe sequelae. Drug therapy can be indicated and initiated if a primary infection is confirmed in the course of the pregnancy. With an appropriate therapy, severity of indications and later complications will be reduced by a considerable margin [1].

Toxoplasmosis is also a significant cause of morbidity and mortality in immunodeficient patients, including bone marrow and organ transplant recipients, AIDS patients and those with lymphoproliferative or hematologic disorders [1, 2].

2.2 German Measles

The rubella virus, a togavirus of the genus rubivirus, is an enveloped single-stranded RNA virus, which was first isolated by Parkman, Weller, and Neva in 1962.

An infection manifests as "German measles" (in the recent past one of the most common children's diseases). Rubella virus enters the body via inhalation and infects cells of the respiratory tract. Viremia occurs normally 5 to 7 days after the exposure. Primary contact with the virus induces an immune response and results in long-lasting immunity.

An infection is characterized by unspecific flu-like symptoms (e.g. low-grade fever, headache, fatigue, sore throat) and is in approximately 50% of cases accompanied by a discrete rash in the form of pinpoint macular lesions, which may later coalesce. A number of postnatal infections proceed subclinically; approximately 40 % (children) to 60 % (adults) of rubella infections proceed without the characteristic rash.

Post-infectious complications of immunocompetent individuals include reactive arthritis of the finger, hand, elbow, and ankle joints. The arthritic symptomatology can persist in adults, especially in women, for up to 3 weeks. Further rarely observed complications are myocarditis, neuritis, otitis, and bronchitis (occasionally) as well as rubella encephalitis (very rarely).

In developed countries, rubella infections have been effectively combated by practice of universal childhood vaccination programs. Nonetheless, rubella has not vanished totally, and in many developing countries rubella immunization has not been uniformly implemented.

An anti-rubella IgG antibody titer of 10-15 IU/ml is indicative of protective immunity. However, in rare cases, despite of vaccination, wild strains of rubella virus may cause an infection. Therefore, all suspected contacts with rubella in the early pregnancy should be investigated.

A primary rubella infection during the first trimester of pregnancy assumes considerable clinical concern. Although intrauterine transmission of the virus to the fetus may occur at any time during gestation, transmission is associated particularly at this stage with the congenital rubella syndrome. The congenital rubella syndrome is characterized by persistent infection of the fetus and may lead to multisystemic disease and severe sequelae of the fetus. The classical symptoms include cardiac and hepatosplenic anomalies, blindness, and deafness as well as mental and growth retardation [3]. Ca 10-20% of congenitally infected infants die within the first year of life.

2.3 Cytomegalovirus Infections

The human cytomegalovirus (CMV) is an enveloped DNA virus, which belongs to the family of herpes viruses. CMV is distributed in the human population worldwide. This lifelong persisting virus is transmitted by smear infections, by direct contact of the mucosa (e.g. sexual activities, breastfeeding, infection of the newborn in the birth canal), by iatrogenic transmission (e.g. blood transfusion or transplantation) or by intrauterine transmission (i.e. congenital infection).

Generally, a primary infection in immunocompetent patients is asymptomatic or accompanied by mild, unspecific flu-like symptoms. The incubation period is four to eight weeks. Following a primary infection of a seronegative individual, CMV establishes latency in the host. The latent virus can be reactivated by immunosuppression or other stimuli, which might result in a nonprimary CMV infection (i.e. reactivation).

The high variability of clinical manifestations depends on the age and the immunocompetence of the subject. In immunocompromised individuals such as transplant recipients, AIDS and tumor patients, CMV infection may lead to severe and life-threatening illness.

Primary as well as reactivated CMV infections of pregnant women may result in fetopathy. The risk of sequelae is higher in the case of primary infection than during the reactivation of the virus. If seroconversion occurs during the pregnancy, virus transmission to the fetus occurs in 40% of the cases. Up to 15% of the affected infants will have symptoms at birth or will suffer of disabilities, such as mental or developmental retardation, or loss of hearing. Late manifestations of the disease can be observed in 10 to 15% of the infected children. [4, 5].

2.4 Herpes Simplex Disease

The herpes simplex viruses (HSV) belong to the most widespread human pathogens in the world [6, 7]. Herpes simplex viruses have been characterized into two distinct serotypes: HSV type 1 (HSV-1) and HSV type 2 (HSV-2) with different clinical manifestations [6, 8].

A HSV infection can result from a direct contact with infected secretions from either symptomatic or asymptomatic host. During a primary infection, herpes simplex viruses infect the epithelial cells of mucosa, in which they replicate and can cause the characteristic inflammations with blister formation [7]. For transmission of the virus, a direct contact with tissue or secretions containing the virus is required [6, 9].

In the later phase of the infection, the virus advances into the sensory nerves and the ganglia where they persist lifelong (latency) [6]. Under conditions that are favorable for the virus, e.g. UV radiation as well as stress and hormonal effects, they can be reactivated and cause a secondary infection (recurrence). Since recurrent infections may be subclinical, asymptomatic virus shedding is a significant reservoir for virus transmission. Infection with HSV-1 does not protect against infections with HSV-2 [6, 8, 9].

The two HSV serotypes generally prefer different sites of infection, but in principle they can infect any area of the skin [6]. In this respect, HSV-1 is primarily responsible for infections in the area of head (tongue, mouth, lips, pharynx, and eyes). It typically causes diseases such as *herpes labialis* (herpes simplex infection of the lips). HSV-2 is mainly responsible for diseases in the genital and anal area, such as *herpes genitalis* (genital herpes) [6, 8]. Severe rare manifestations include HSV-associated encephalitis and *herpes corneae* [6, 8].

One of the most serious sequelae of genital herpes is the neonatal herpes (*herpes neonatorum*). *Herpes neonatorum* is triggered by an infection of the newborn with HSV at birth, usually through contact with the infected maternal genital secretions [6, 8]. HSV-2 is responsible for approximately 75% of the cases and HSV-1 for 25% [10]. *Herpes neonatorum* occurs in three known forms: 1) disease of the skin, the eyes and the mouth, 2) encephalitis, and 3) a generalized infection [6]. Without therapy, mortality for untreated infants who develop dissemination exceeds 60% [6]. The risk of congenital infection is particularly high with a primary maternal *herpes genitalis*, above all shortly before birth [10]. The likelihood of lateral virus transmission during maternal recrudescence is considerably lower [10]. In addition, both the risk of a HSV primary infection and the occurrence of relapses increase during pregnancy [10]. Most mothers who transmit HSV to their children are asymptomatic at delivery [11]. This emphasizes the importance of the serological screening of the mothers during pregnancy.

3. Diagnostics of congenital diseases

3.1. Toxoplasmosis

Because the signs and symptoms of toxoplasmosis are non-specific and because most cases are subclinical, the diagnosis of toxoplasmosis is mainly based on serological tests, such as Sabin-Feldmann Dye test, differential agglutination tests, enzyme-linked immunosorbent assays (ELISA), and immunoblots. The Sabin-Feldmann Dye test, introduced in 1948, is still the gold standard in toxoplasmosis diagnostics due to its high level of sensitivity. However, the test is complicated and cost intensive and is therefore now rarely used except as a reference method [12].

The prospective serological testing is especially recommended for individuals at risk for serious outcomes resulting from a *T. gondii* infection, such as seronegative pregnant women or seronegative women contemplating pregnancy. As the risk of an infection of the unborn child via transplacental transmission is generally limited only to the seronegative women who acquire a primary infection during the gestation, assessment of the immune status against *T. gondii* is of high importance.

T. gondii-specific IgG antibodies are produced throughout life after primary infection. Detection of IgG antibodies in a sample at any titer proves only that the infection has been acquired at some time in the recent or distant past. Therefore, one diagnostic criteria for an acute toxoplasmosis is the avidity, i.e. binding efficiency of the *T. gondii*-specific IgG serum antibodies [1, 13, 14, 15]. This test procedure relies on the maturation cycle of the IgG antibodies. Anti-*T. gondii* IgG antibodies in the early-phase of an infection show low binding efficiency, i.e. low avidity, while antibodies generated during a past infection have a highly effective binding capacity, i.e. high avidity.

IgM antibodies are detected in individuals with a recently acquired infection. However, they may persist for one year or longer. An acute *T. gondii* infection is rarely accompanied with very low or no detectable levels of IgM antibodies (so-called IgM low- / non-responders). In addition, if an adequate drug therapy is initiated in the early phase of the infection, the antibody titers, especially the IgM titer, diminish rapidly. False-positive reactions may be found in chronically infected individuals or in some patients who have never been infected by *T. gondii*. In such individuals, tests for the specific IgG remain negative.

The diagnostic finding using the MIKROGEN *recomLine* TORCH Screening IgG assay is based on the two antigen strategy. In the first step, the general immunological status is confirmed using the major antigen band composed of the *T. gondii* whole cell lysate antigen. The obtained information is amended by the ancillary antigen band composed of the *T. gondii*-specific recombinant antigen p30. As an IgG antibody response against this antigen can generally be detected only after 3 months of the primary infection, p30 antigen allows an estimation of the time of the infection. However, as the IgG response specific for p30 may not be present in all infected individuals or it may diminish or disappear during the time, the absence of p30-specific signal cannot be used to diagnose an acute toxoplasmosis.

The diagnostic finding of toxoplasmosis using the MIKROGEN *recomLine* TORCH Screening IgG assay is amended by the MIKROGEN *recomLine* TORCH Screening IgM assay. The *T. gondii*-specific antigen band is a composition of highly purified whole cell lysate antigen and an early phase antigen ROP1c [36]. Detectable levels of *T. gondii*-specific IgM antibodies, especially in the absence of *T. gondii*-specific IgG response, may be an indication for a primary or a recent infection, and should always be reanalyzed and confirmed by requesting a follow-up sample (10 to 14 days later) or using a confirmatory assay.

For clarification and certification of unclear or diagnostically conspicuous toxoplasmosis findings, the MIKROGEN *recomLine* Toxoplasma IgG [avidity] and the *recomLine* Toxoplasma IgM assays provide valuable diagnostic tools. These test systems are based on highly specific recombinant antigens and enable a comprehensive diagnostic finding based on the parallel determination of the specific IgG and IgM antibodies as well as the avidity of the IgG antibodies.

3.2. German measles

A clinical diagnosis of Rubella infection should, in all cases, be supported by the serological evidence, as the unspecific symptoms of rash and arthritis may be caused by a variety of other agents. Serological diagnosis of a rubella infection can only be considered in accordance with the clinical history, epidemiological data, and other data available to the attending physician.

A clear distinction is made between routine diagnostics (i.e. routine screening) and diagnosis with medical indication. The standard diagnostic procedure of rubella is represented by the hemagglutination inhibition test (HAIT). In the absence of clinical symptoms, HAIT titers of 1:32 and higher are reckoned as protective against rubella infections (i.e. indication of an protective immunity to the virus). However, the HAIT assay does not differentiate between the anti-rubella virus antibodies of IgG and IgM class. As a consequence, acute rubella infections, which are characterized by an elevated IgM titer, can be overseen [16].

As an alternative or a supplementary test method for the assessment of protective immunity against rubella infections, an ELISA assay specific for antibodies of the IgG class can be implemented. According to the recommendation of the World Health Organization (WHO), an IgG antibody titer of ≥ 15 IU/ml is considered as an indication of the protective immunity [17]. In routine diagnostics, an anti-rubella IgG antibody concentration of 15 - 25 IU/ml is regarded as being in the borderline range, meaning that the protective immunity can not be confirmed. In addition, anti-rubella IgG EIA tests can be used as supplementary tests, if other test systems (such as the hemagglutination inhibition test) do not assess a clear diagnostic statement (i.e. ambiguous test results).

An elevated IgM antibody titer against rubella virus can be indicative of a primary infection. The antibodies of the IgM class can be detected for 1 to 2 months post primary infection, but may persist in detectable levels for up to one year. In addition, detectable levels of anti-rubella IgM antibodies might be present after reinfection with the rubella virus or after vaccination. False positive test results may occur due to cross-reactivity or as a result of polyclonal stimulation by e.g. Epstein-Barr virus, CMV, or Parvovirus B19 infections. As a consequence, detectable anti-rubella IgM titer alone does not accurately predict the risk of a congenital infection.

A test results indicative of an anti-rubella IgM antibody titer should always be confirmed; the test should be repeated 4 to 12 weeks later with a new sample (i.e. follow-up control) or the specimen tested with a different assay. As a primary rubella infection during the gestation may lead to multisystemic disease and severe damage of the fetus, the confirmatory testing is of particular importance during pregnancy. In addition to the maternal testing, prenatal diagnostics, e.g. analysis of the fetal blood by polymerase chain reaction (PCR), should be conducted in specialized laboratories.

The MIKROGEN *recomLine* TORCH Screening IgG assay enables an estimation of the immune status (i.e. due to vaccination or past infection) against rubella infections. The ancillary band specific for rubella virus (rubella vaccination band; denoted as "Ru-co" on the IgG test strips) corresponds to an IgG antibody titer of ≥ 15 IU/mL. The intensity of the rubella major band is proportional to the activity of rubella-specific IgG antibodies present in a sample. Interpretation of the rubella major band (composed of whole virus lysate) is carried out through comparison with the intensity of the rubella vaccination band.

The diagnostic finding using the MIKROGEN *recomLine* TORCH Screening IgG assay is amended by the MIKROGEN *recomLine* TORCH Screening IgM assay. Detectable levels of rubella-specific IgM antibodies may be an indication for a primary or a recent infection and should always be reanalyzed and confirmed by requesting a follow-up sample (10 to 14 days later) or using a confirmatory assay.

As primary rubella virus infections may be characterized by an elevated anti-rubella virus IgG titer, prospective screening up to 17th week of gestation and determination of the time of infection (e.g. through confirmation of an anti-E2 IgG response or avidity testing) is highly recommended also in the absence of detectable levels of anti-rubella virus IgM antibodies.

For clarification of unclear or diagnostically conspicuous rubella findings, the MIKROGEN *recomBlot* Rubella IgG provides a valuable diagnostic tool. This immunoblot test system is based on the highly specific rubella antigens: dimeric form of the rubella structure proteins E1-E2, capsule protein c, E1, and E2. A positive result for the E2 band enables exclusion of primary infections during the last three months [18].

3.3 Cytomegalovirus infections

The currently used diagnostic methods for CMV infections are based either on detection of CMV-specific antibodies, detection of viral DNA (e.g. PCR), or detection of virus components (e.g. isolation of the virus particles or the pp65 antigen). The method used will be chosen case-specifically in accordance with the clinical history. The chosen method depends on the previous findings and the specific problems of each case [19].

Serological testing is recommended for individuals at risk and required for the following cases in particular:

- Detection of a primary infection in immunocompetent patients
- Determination of the immune status of donors and recipients of allografts
- Determination of the immune status of blood donors
- Diagnostics in pregnancy

The standard screening for CMV specific antibodies is usually performed by an ELISA. These assays provide a general statement of the humoral immune response against the virus. However, a distinct differentiation between a primary or a past infection cannot always be obtained [20, 21].

Classical CMV serology is based on the finding that IgM antibodies are produced during the acute and the late phases of a primary CMV infection, whereas the corresponding IgG response is of longer duration. Therefore, detectable levels of CMV-specific IgM antibodies can supply evidence for an acute infection, while an IgG response in the absence of IgM antibodies can be an indication of a long past infection. However, the CMV-specific IgM antibodies may persist for months. In addition, an acute CMV infection can rarely be accompanied with very low or no detectable levels of IgM antibodies (so-called IgM low- / non-responders). Due to variability of the individual immune responses and the frequently occurring aberrant serological courses (i.e. persistence, reactivation, or non-responders), the classical procedure may lead to a wrong or misleading diagnostic finding.

The classical serology can be supplemented by determination of the avidity of the CMV-specific IgG antibodies. During the first weeks following a primary infection, low avidity antibodies (i.e. antibodies with low binding efficiency) are produced. The maturation of IgG antibody avidity over the time can be used as a diagnostic criteria [22, 23].

The diagnostic finding of CMV infections using the MIKROGEN *recomLine* TORCH Screening IgG assay is based on the two antigen strategy. In the first step, the general immunological status is confirmed using the major antigen band composed of the CMV whole cell lysate antigen. The obtained information is amended by the ancillary antigen band composed of the CMV-specific recombinant antigen gB2. An IgG antibody response against this antigen is generally detectable only 6 to 8 weeks after a primary infection. However, as the IgG response specific for gB2 may not be present in all infected individuals or may diminish or disappear, the absence of gB2-specific signal cannot be used to diagnose an acute cytomegalovirus infection.

The diagnostic finding using the MIKROGEN *recomLine* TORCH Screening IgG assay is amended by the MIKROGEN *recomLine* TORCH Screening IgM assay. Detectable levels of CMV-specific IgM antibodies, especially in the absence of CMV-specific IgG response, may be an indication for a primary or a recent infection, and should always be reanalyzed and confirmed by requesting a follow-up sample (10 to 14 days later) or using a confirmatory assay.

For clarification and certification of unclear or diagnostically conspicuous cytomegalovirus findings, the MIKROGEN *recomBlot* CMV IgG [avidity] and the *recomBlot* CMV IgM assays provide valuable diagnostic tools. These test systems are based on highly specific recombinant antigens (IE1, p150, CM2, p65, gB1, and gB2) and enable comprehensive diagnostics based on the parallel determination of the specific IgG and IgM antibodies as well as the avidity of the IgG antibodies [22, 23].

3.4 Herpes Simplex Virus Disease

Many individuals infected with the herpes simplex viruses are not diagnosed properly. This is mostly due to ambiguous clinical characteristics or because the patients only exhibit subclinical symptoms or are entirely symptom-free (through asymptomatic shedding) [9, 25, 26]. Consequently, 50% to 90% of cases of primary infections with the herpes simplex viruses are clinically unapparent [10].

The frequency of relapses varies from one individual to another. They are usually less pronounced and of shorter time than the primary infections [10, 25]. Genital herpes caused by HSV type 1 leads to relapses less frequently than genital herpes caused by HSV type 2, rendering the prognosis of the course of infection different in each case [26, 25].

The clinical diagnosis of HSV infections is supported by virological or serological methods. The most reliable diagnosis is achieved by direct viral isolation followed by the typing of the viral isolate. However, this method is time consuming and requires tissue sections that contain infectious virus particles (preferably from acute lesions) [11, 27]. A further disadvantage is the high frequency of false negative results [11, 25]. Immunofluorescence tests (IFT) and PCR are further methods that are available for the direct detection of the pathogen [28].

In the absence of active lesions, the viral infections can be indirectly confirmed by serological methods [27]. During an acute HSV infection, specific antibodies of the IgG, IgM, and IgA class are present [8]. In contrast to many other pathogens, no statement can be made about the phase of infection on the basis of the various antibody classes [11, 29].

Most serological assays for assessing the HSV immune status are based on native whole cell lysate antigens. However, due to a high homology between the HSV type 1 and type 2, assays based on the viral lysates cannot distinguish between these two serotypes [29]. Glycoprotein G (gG) is currently the only known antigen whose homologues of HSV-1 (gG-1) and HSV-2 (gG-2) exhibit sufficient divergence to rule out cross-reactions [30]. According to the current state of technology, a type-specific diagnosis is only guaranteed by the assays based on glycoprotein G [11, 29].

Diagnosis of an HSV infection using the MIKROGEN *recomLine* TORCH Screening IgG assay is based on the two antigen strategy. In the first step, the general immune status is confirmed using the major antigen band composed of the HSV-1 and HSV-2 whole cell lysate antigens. The obtained information is amended by the ancillary antigen band composed of the HSV type 2-specific recombinant antigen gG2. However, as the IgG response specific for gG2 may not be present in all infected individuals or it may diminish or disappear during time, the absence of gG2-specific signal cannot be solely used to diagnose a HSV type 2 infection.

A diagnostic finding using the MIKROGEN *recomLine* TORCH Screening IgG assay can be amended by the MIKROGEN *recomLine* TORCH Screening IgM assay. Detectable levels of HSV-specific IgM antibodies, especially in the absence of HSV-specific IgG response, may be an indication for a primary or recurrent infection. The test results indicative of a HSV type 2 infection (IgG) as well as of primary or recurrent HSV infection (IgM) should always be reanalyzed and confirmed using a confirmatory assay.

The MIKROGEN *recomLine* HSV-1 & HSV-2 IgG assay provides a tool for differentiation of HSV-1 and HSV-2 infections. This assay implements recombinant gG-1 (HSV-1) and gG-2 (HSV-2) in combination with native viral lysate antigens (HSV-1/2) and thus enables estimation of the HSV immune status.

4. Test principle

The *recomLine* TORCH Screening IgG and *recomLine* TORCH Screening IgM assays are qualitative *in-vitro* immuno assays for the detection of IgG and IgM antibodies against *Toxoplasma gondii*, rubella virus, cytomegalovirus, and herpes simplex virus. The assays are based on the test principle of ELISA.

These two test systems are composed of purified native and recombinant antigens, which are applied onto nitrocellulose membrane strips. The test strips are numbered and packaged.

The MIKROGEN *recomLine* TORCH Screening IgG and IgM assays are processed in two stages. In the first stage, the patient specimen are diluted and incubated with individual test strips. In the second stage, the bound immune complexes are visualized by a peroxidase (POD) labeled secondary antibody (directed either against the human IgG or IgM antibodies). After addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution, a colored precipitate is formed and the immobilized antigen-antibody complexes are visualized. The obtained signals (colored bands) can be classified to the individual infectious agents according to the position of the bands on the test strips.

The MIKROGEN *recomLine* TORCH Screening IgG and the *recomLine* TORCH Screening IgM test results (the patterns of colored bands) are interpreted individually for each infectious agent, i. e. for *T. gondii*, rubella virus, CMV, as well as for HSV types 1 and 2 (HSV-1/2) and HSV type 2 (HSV-2), respectively.

Each test encloses a control band for the test validity (i.e. the reaction control), a control band for the antibody class (the anti-human IgG conjugate control or the anti-human IgM conjugate control, respectively) and a threshold control band (the cut-off control). If a specimen fails to react with any of the control bands, the test result must be considered invalid.

The three control bands are arranged side-by-side at the upper end of each test strip:

1. The reaction control band directly below the strip number. All specimens must show a high, clearly positive reactivity with this control band (dark blue coloring).
2. The conjugate control band (IgG or IgM, respectively). This band is used as a control for the corresponding immunoglobulin class. For instance, if the test strip is intended to detect serum antibodies of the IgG class and the IgG conjugate is correctly added, the IgG conjugate control band shows a clearly defined blue coloring.
3. The cut-off control band. The reference band for controlling of the staining process and the interpretation of the test results. The color intensity of this band provides a threshold value for the interpretation of the antibody reactivity as positive, borderline or negative.

5. Package contents

The MIKROGEN *recomLine* TORCH Screening IgG and the *recomLine* TORCH Screening IgM test kits contain sufficient materials to perform 20 determinations.

Each reagent set contains:

WASHBUF A 10 X	100 ml	Wash buffer A (10-fold concentration) for serum dilution, conjugate dilution, and washes contains phosphate buffer, NaCl, KCl, detergents, MIT (0.1%) and Oxypyron (0.2%)
SUBS TMB	40 ml	Substrate solution Tetramethylbenzidin (TMB, ready-to-use) Contains H ₂ O ₂
MILKPOW	5 g	Skim milk powder
INSTRU	1	Instructions for use
EVALFORM	1	Evaluation form

5.1 The *recomLine* TORCH Screening IgG assay

Additionally to the components listed under point 5 each reagent set contains:

TESTSTR	2	Test tubes with 10 consecutively numbered test strips (e.g. LTOG 01) coated with whole cell or virus lysate antigens specific for <i>T. gondii</i> , rubella virus, cytomegalovirus, and herpes simplex virus types 1 and 2 as well as with recombinant antigens specific for <i>T. gondii</i> (p30), CMV (gB2), and HSV type 2 (gG2).
CONJ IgG	500 µl	Anti-human IgG conjugate (green screw cap, 100-fold concentration) from rabbit, contains NaN ₃ (<0.1%), MIT (<0.1%), Chloracetamide (<0.1%)

5.2 The recomLine TORCH Screening IgM assay

Additionally to the components listed under point 5 each reagent set contains:

TESTSTR	2	Test tubes with 10 consecutively numbered test strips (e.g. LTOM 01) coated with whole cell or virus lysate antigens specific for <i>T. gondii</i> , rubella virus, cytomegalovirus, and herpes simplex virus types 1 and 2
CONJ IgM	500 µl	Anti-human IgM peroxidase conjugate (purple screw cap, 100-fold concentration) from rabbit, contains NaN ₃ (<0.1%), MIT (<0.1%), Chloracetamide (<0.1%)

6. Additional reagents and accessory equipment required

Material required, but not supplied:

- Incubation trays (can be obtained from MIKROGEN GmbH)
- De-ionized (DI) or distilled water
- Blunt tipped forceps or tweezers
- Platform rockers or Horizontal reciprocating shaker
- Vortex mixer or equivalent
- Vacuum pump or equivalent
- Clean volumetric flasks or graduated cylinders, 50-mL and 1000-mL
- 20-µL and 1000-µL pipette with disposable tips
- 10-mL pipette or dispenser
- Timer
- Paper towels or absorbent paper
- Protective gloves
- Disposal basin or sink
- Biohazard waste container

7. Information on test and reagents

7.1 Warnings and Precautions

- ☞ For *in vitro* diagnostic use only
- ☞ The test must be performed by well-trained and authorized personnel
- ☞ All blood products should be treated as potentially infectious
- ☞ The test stripes are produced with inactivated native whole cell or viral antigens. The reagents have been inactivated. We are aware of no specific hazards associated with the product. Since no procedure can guarantee absolute sterility of the products, when handling the reagent the same precautions should be taken as when working with any similar potentially infectious material.
- ☞ After adding patient or control specimen, the stripes should be considered potentially infectious and handled accordingly.
- ☞ Suitable single-use gloves must be worn during the entire test procedure.
- ☞ Reagents contain antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), Oxypyron, Chloracetamide, and hydrogen peroxide. Avoid ingestion and contact with skin or mucosa. Sodium azide can form explosive azides if it comes into contact with heavy metals such as copper or lead.
- ☞ All aspirated liquids should be collected. All solution containers should contain a disinfectant for inactivating pathogenic human viruses and other pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with suitable disinfectants or autoclaved at +121°C for at least 1 hour. The concentrations and times specified by the manufacturer must be observed.
- ☞ Use incubation trays only once.
- ☞ Handle strips carefully with a plastic forceps.
- ☞ Do not substitute or mix reagents from other manufacturers

- ☞ Use only protocol described in this user manual. Incubation times or temperatures other than those specified may give erroneous test results.

7.2 Handling information

- ☞ Store reagents before and after use at +2°C-8°C, **do not freeze**.
- ☞ Identical reagents (according to the printed symbols) in all *recomLine*- and *recomBlot* kits can be used irrespective of parameter or batch.
- ☞ A quality guarantee can only be given up to the expiry date on the packages.
- ☞ Temper all components before starting the test to +18°C-25°C (room temperature) for at least 30 minutes. Both the test and the incubation procedures are carried out at room temperature.
- ☞ Mix the concentrated reagents and patient specimen well before use. Avoid foaming.
- ☞ Do not open the tube containing the test strips until shortly before the use to avoid condensation of water. The remaining stripes must be left in the tube and stored further at +2°C-8°C (reclose the tube tightly, test strips must not become moist before testing!).
- ☞ The strips are consecutively numbered and marked with the shortcut of the respective test (i.e. LTOG or LTOM).
- ☞ Protect all kit components from direct sun light.
- ☞ In case of substantial modifications of the product or the instructions for use, the application of the test might differ from the purpose intended by MIKROGEN.
- ☞ Cross-contamination of patient specimens or conjugates can cause erroneous test results. Add patient specimens, test stripes, and conjugate solution carefully. Avoid cross-contamination of adjoining incubation tray wells. Decant carefully.
- ☞ During the test procedure, the liquids must flow freely over the entire length of the test strips.
- ☞ For automation solutions refer MIKROGEN for details.
- ☞ Before starting the test interpretation, please take into account the "Notes for test interpretation and test limitations" (Chapter 11.4)

7.3 Preparation of solutions

7.3.1 Preparation of the ready-to-use wash buffer A

This buffer is used for both the serum and conjugate dilution as well as for the washing steps.

Prior to dilution, the volume of wash buffer A (WPA) required for the corresponding number of tests (for the serum and the conjugate dilution as well as for the subsequent washing steps) must be determined.

The skim milk powder is first dissolved in the wash buffer A concentrate. This mixture is then filled up with deionized water to the final volume (dilution: 1+9). See Table 1 for the volumes required. Volumes for numbers of test strips not listed in the table 1 have to be determined by calculation.

Ready-to-use wash buffer A is slightly turbid and scentless.

Ready-to-use wash buffer A can be stored at +2°C-8°C for up to four weeks. The stored buffer must be well mixed before use.

Table 1: The volumes of Wash buffer A per number of test strips used

Test strips used*	Skim milk powder	Wash buffer A concentrate	Deionized water	Ready-to-use wash buffer A
1	0,1 g	2 ml	18 ml	20 ml
2	0,2 g	4 ml	36 ml	40 ml
3	0,3 g	6 ml	54 ml	60 ml
5	0,5 g	10 ml	90 ml	100 ml
10	1 g	20 ml	180 ml	200 ml
15	1,5 g	30 ml	270 ml	300 ml
20	2 g	40 ml	360 ml	400 ml
25	2,5g	50 ml	450 ml	500 ml
50	5 g	100 ml	900 ml	1000 ml

* The volumes are calculated without the dead volume. Depending on the handling (manual or automatic processing) prepare Wash buffer A solution for additional 1 to 3 stripes.

7.3.2 Preparation of the conjugate solutions

The conjugate solutions are to be prepared immediately before use. It is not possible to store the ready-to-use conjugate solutions.

One part of the anti-human IgG or the anti-human IgM conjugate concentrate is diluted with 100 parts of the ready-to-use wash buffer A (1+100).

The volumes used are listed in table 2. The volumes for numbers of test strips not listed in the table 2 have to be determined by calculation.

Table 2: Volumes required for the anti-human IgG and the anti-human IgM conjugate dilutions

Test strips used *	100-fold anti-human IgG or anti-human IgM conjugate concentrate	Ready-to-use wash buffer A
1	20 µl	2 ml
2	40 µl	4 ml
3	60 µl	6 ml
5	100 µl	10 ml
10	200 µl	20 ml
15	300 µl	30 ml
20	400 µl	40 ml
25	500 µl	50 ml

* The volumes are calculated without the dead volume. Depending on the handling (manual or automatic processing) prepare conjugate solution for additional 1 to 3 stripes.

7.3.3 Substrate solution

The TMB substrate solution is ready-to-use. Bring to room temperature (+18°C-+25°C) before starting the staining reaction.

Avoid contamination of the unused substrate solution by nonsterile pipette tips etc. at all costs, since this may affect the assay sensitivity.

7.4 Storage and stability

Store reagents at +2°C-+8°C before and after use. Do not freeze!

Ready-to-use wash buffer A can be stored at +2°C-+8°C for up to four weeks.

The conjugate solutions are always to be freshly prepared.

8. Sample material

The sample material can be human serum or plasma that is separated from the coagulum shortly after sampling. A microbial contamination of the sample has to be avoided at all costs. Insoluble substances are to be removed from the sample prior to incubation by the means of centrifugation.

The use of heat-treated, icteric, haemolytic, lipaemic or turbid specimens is not recommended.

Important!

If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at +2°C-+8°C. Longer storage of the samples is possible at -20°C or colder. Repeated freezing and thawing of the samples is not recommended because this may affect the quality of the results.

9. Test procedure

9.1 General

The reproducibility of the results depends to a great extent on consistent washing of the test strips. The washing frequencies described under 9.3 should therefore always be maintained.

9.2 Incubation of samples

One well in the incubation tray (see point 6) is required per sample to be tested. **2 ml** of the ready-to-use Wash buffer A (see table 1) is pipetted into each well. Then one test strip is carefully dipped into each of the wells filled with the ready-to-use Wash buffer A using the plastic forceps. The strip number must face upwards.

Important!

The strip must be completely wet and immersed in the ready-to-use Wash buffer A.

Record the tube and the strip numbers used on the evaluation sheet.

Adding of the samples:

Pipet **20 µl** of an undiluted sample (human serum or plasma) into the appropriate well (corresponding to the **dilution of 1+100**).

Add the sample at one end of the immersed strips into the ready-to-use Wash buffer A and mix immediately by shaking the tray carefully.

Record the sample numbers and the immunoglobulin class used (i.e. IgG or IgM) on the evaluation sheet.

Cover the incubation tray with the plastic lid and incubate for **1 hour** at room temperature while shaking gently.

Important!

Make sure the incubation solutions are not carried over into other wells; be particularly careful to avoid splashing when opening and closing the lid.

9.3 Washing

1. Following incubation, the plastic lids are carefully removed from the incubation trays.
2. The serum dilution is carefully aspirated from the individual wells.

Important!

When the solutions have been aspirated from a well, the pipette tip has to be changed or rinsed well with deionized water after each aspiration procedure to prevent cross-contamination of the different samples.

In the case of automatic processing, the references and instructions of the equipment manufacturer have to be considered.

3. Pipet **2 ml** of the ready-to-use Wash buffer A into each well. Place the incubation tray on the shaker and wash while shaking gently for **5 minutes**. The ready-to-use Wash buffer A is aspirated after the washing procedure.
4. Carry out the washing step described under point 3 a total of **three times**.

9.4 Incubation with the anti-human IgG or IgM peroxidase conjugate

After washing the strips, place **2 ml** of the appropriately prepared **conjugate solution** (see table 2) into each well and incubate for **45 minutes** while shaking gently at room temperature, whereby the incubation tray is covered with the plastic lid.

9.5 Washing

The conjugate solutions are aspirated from the wells and the strips are washed as described in point 9.3. Avoid cross-contamination of the different conjugate solutions!

9.6 Staining reaction

Add **1.5 ml TMB substrate solution** into each well and incubate for **8 minutes** under observation while shaking gently at room temperature.

9.7 Stopping of the staining reaction

1. After the TMB solution is aspirated, wash the test strips briefly **three times with deionized water**.
2. Use a plastic forceps to remove the test strips carefully from the water and place them between 2 layers of absorbent paper to dry for about 2 hours at room temperature. Subsequently, the test strips are adhesively attached to the enclosed evaluation sheets and the results are recorded.
3. The test strips should be stored protected from exposure to light.

10. Summary of the test procedure

1.	bring all reagents to room temperature
2.	deposit the test strips in 2 ml ready-to-use Wash buffer A and take care that they are completely immersed in the buffer solution
3.	pipette 20 µl of the sample
4.	incubate at room temperature while shaking gently for 1 hour
5.	wash on the shaker three times with 2 ml of ready-to-use Wash buffer A for 5 minutes each time
6.	add 2 ml appropriately prepared conjugate solution
7.	incubate at room temperature while shaking gently for 45 minutes
8.	wash on the shaker three times with 2 ml of ready-to-use Wash buffer A for 5 minutes each time
9.	add 1.5 ml of the TMB substrate solution, incubate while shaking at room temperature for 8 minutes
10.	wash the test strips at least three times with deionized water
11.	dry the test strips between 2 layers of absorbent paper for 2 hours and read off the result

11. Evaluation of the test results

11.1 Evaluation of the band intensity

1. On the enclosed evaluation sheet, record the date, the batch number and the tube number along with the antibody class detected.
2. Enter the sample identification number on the evaluation sheet.
3. Attach the corresponding test strips with a glue stick into the corresponding fields on the evaluation sheet. To do this, place the test strips with the reaction control band on the marking lines. Then use clear adhesive tape to attach the test strips to the marking line. Important! Do not place the adhesive tape over the control bands on the test strips. Complete sticking of the test strip with glue or adhesive tape leads to aberrations of the coloring.

- Identify the bands on the developed test strips with the help of the printed control strip on the evaluation sheet and record the obtained bands in the evaluation sheet (see table 3). Identify the bands on the test strips separately for the respective immunoglobulin classes.

The cut-off control band is the reference band for controlling of the staining process and the interpretation of the test results. The color intensity of this band provides a threshold value for the interpretation of the antibody reactivity as positive, borderline, or negative.

Evaluation of the band intensities specific for *T. gondii*, CMV, and HSV-1/2 is described in table 3.

Evaluation of the rubella major band is carried out through comparison with the intensity of the rubella vaccination band (i.e. Ru-co). Evaluation of the rubella major band is described in table 4. The rubella-specific IgM response is evaluated according to the general interpretation schema described in table 3.

Table 3: Intensity of the *T.gondii*-, CMV-, and HSV-specific bands in relation to the cut-off band.

Color intensity of a band	Intensity	Interpretation
no reaction	-	negative
lower than that that of the cut-off band	±	negative
same intensity as that of the cut-off band	+	positive
higher intensity (higher than that of the cut-off band)	++	positive

Table 4: Intensity of the rubella major band in relation to the rubella vaccination band (Ru-co).

Color intensity of a band	Intensity	Interpretation
no reaction	-	negative
lower than that of the rubella vaccination band	±	negative
same intensity as that of the rubella vaccination band	+	borderline
higher intensity (higher than that of the rubella vaccination band)	++	positive

Important!

The obtained band patterns in the *recomLine* TORCH Screening IgG and the *recomLine* TORCH IgM assays may show varying intensities. It is possible that the bands on the *recomLine* TORCH IgG test strips show more intense and darker coloring than the antigen bands on the *recomLine* TORCH IgM test strips.

11.2 Control results

The test results are valid and can be evaluated only if the following criteria are met:

- Reaction control band (upper line): The reaction control band shows a pronounced, dark coloring.
- Conjugate class control (second band): The corresponding conjugate control band shows a clear dark coloring.
- The cut-off control (third band): The cut-off control band shows a weak, but clearly evident coloring.

11.3 Test results and test interpretation

To ensure a reliable and simple test evaluation procedure, different guidelines and references were implied [1, 16, 31, 32, 33, 34, 35]. The pathogen-specific interpretation of the test results is described in table 5.

Analysis and interpretation of the test results is assessed separately for each pathogen. The test result for each pathogen-specific antigen will be obtained relative to the cut-off control by comparison of the coloring of the cut-off control band with the reactivity (i.e. color intensity) of the antigen band (see tables 3 and 4)

Before starting the test interpretation, please take into account the "Notes for test interpretation and test limitations" (Chapter 11.5).

Table 5: Pathogen-specific interpretation of the test results

Toxoplasma gondii				
IgG		IgM		
Lysate	p30	Lysate / Rop1c	Interpretation	Recommendation
negative	negative	negative	seronegative no immunity	prospective monitoring and follow up control in 8 to 12 weeks
negative	negative	positive	suspicious of seroconversion (primary infection)	further clarification: a) confirmation of the positive IgM result (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgM) b) follow up control in 10 to 14 days
positive	negative	positive	suspicious of recent infection	further clarification: a) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgG plus avidity) b) confirmation of the positive IgM result (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgM) c) follow up control in 10 to 14 days
positive	positive	positive	suspicious of recent infection infection more than 3 months ago	after 12 weeks of gestation further clarification is highly recommended: a) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgG plus avidity) b) confirmation of the positive IgM result (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgM)
positive	positive	negative	suspicious of past infection infection more than 3 months ago	in general no further diagnostics necessary but after 12 weeks of gestation further clarification is highly recommended: a) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgG plus avidity) in the case of clinical indication, clinical suspicion of an IgM low responder or a delayed IgM response further testing is highly recommended
positive	negative	negative	suspicious of past infection time of infection not determinable	in general no further diagnostics necessary but after 12 weeks of gestation further clarification is highly recommended: a) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomLine</i> Toxoplasma IgG plus avidity) in the case of clinical indication, clinical suspicion of an IgM low responder or a delayed IgM response further testing is highly recommended
negative	positive	not specified	no diagnostic statement possible	atypical test result it is highly recommended to request a follow-up sample or repeat the test with another test system

Rubella Virus				
IgG		IgM		
Rubella vaccination band	Lysate	Lysate	Interpretation	Recommendation
positive	negative	negative	seronegative no immunity	prospective monitoring and follow up control in 4 to 6 weeks
positive	negative	positive	suspicious of no protective immunity suspicious of primary infection	further clarification: a) it is highly recommended to question the clinical history of the patient (vaccination in the past? Contact with persons suffering of an acute rubella infection? Rubella specific symptoms recently?) b) follow up control up to the 17 th week of gestation c) confirmation of the positive IgM result with other test method
positive	equivalent or lower as the rubella vaccination band	positive	suspicious of no protective immunity suspicious of primary infection	further clarification: a) it is highly recommended to question the clinical history of the patient (vaccination in the past? Contact with persons suffering of an acute rubella infection? Rubella specific symptoms recently?) b) follow up control up to the 17 th week of gestation c) confirmation of the positive IgM result with other test method d) determination of the time of infection (e.g. MIKROGEN <i>recom</i> Blot Rubella IgG or avidity testing with another method)
positive	equivalent or lower as the rubella vaccination band	negative	suspicious of no protective immunity	further clarification: follow up control up to the 17 th week of gestation in the case of clinical indication, clinical suspicion of an IgM low responder or a delayed IgM response further testing is highly recommended
positive	more intense as the rubella vaccination band	positive	suspicious of protective immunity suspicious of recent infection	further clarification: a) it is highly recommended to question the clinical history of the patient (vaccination in the past? Contact with persons suffering of an acute rubella infection? Rubella specific symptoms recently?) b) confirmation of the positive IgM result with other test method c) determination of the time of infection (e.g. MIKROGEN <i>recom</i> Blot Rubella IgG or avidity testing with another method)
positive	more intense as the rubella vaccination band	negative	suspicious of protective immunity suspicious of past infection	in general no further diagnostics necessary determination of the time of infection (e.g. MIKROGEN <i>recom</i> Blot Rubella IgG or avidity testing with another method) in the case of clinical indication, clinical suspicion of an IgM low responder or a delayed IgM response further testing is highly recommended
negative	negative or positive	not specified	no diagnostic statement possible	invalid test result it is highly recommended to request a follow-up sample or repeat the test with the <i>recom</i> Line TORCH Screening IgG assay or with another test system

Cytomegalovirus				
IgG		IgM		
Lysate	gB2	Lysate	Interpretation	Recommendation
negative	negative	negative	seronegative no immunity	prospective monitoring and follow up control in 4 to 6 weeks
negative	negative	positive	suspicious of seroconversion (primary infection)	further clarification: a) follow up control in 10 to 14 days b) confirmation of the positive IgM result (e.g. MIKROGEN <i>recomBlot</i> CMV IgM)
positive	negative	positive	suspicious of recent infection time of infection not determinable	further clarification: a) follow up control in 10 to 14 days b) confirmation of the positive IgM result (e.g. MIKROGEN <i>recomBlot</i> CMV IgM) c) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomBlot</i> CMV IgG plus avidity)
positive	positive	positive	suspicious of recent infection infection more than 6 to 8 weeks ago	after 6 to 8 weeks of gestation further investigation is necessary. a) confirmation of the positive IgM result (e.g. MIKROGEN <i>recomBlot</i> CMV IgM) b) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomBlot</i> CMV IgG plus avidity)
positive	positive	negative	suspicious of past infection infection more than 6 to 8 weeks ago	in general no further diagnostics necessary but after 6 to 8 weeks of gestation further investigation is recommended: a) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomBlot</i> CMV IgG plus avidity) in the case of clinical indication, clinical suspicion of an IgM low responder or a delayed IgM response further testing is highly recommended
positive	negative	negative	suspicious of past infection time of infection not determinable	in general no further diagnostics necessary but after 6 to 8 weeks of gestation further investigation is recommended: a) avidity testing for determination of the time of infection (e.g. MIKROGEN <i>recomBlot</i> CMV IgG plus avidity) in the case of clinical indication, clinical suspicion of an IgM low responder or a delayed IgM response further testing is highly recommended
negative	positive	not specified	no diagnostic statement possible	atypical test result it is highly recommended to request a follow-up sample or repeat the test with another test system

Herpes simplex virus				
IgG		IgM		
Lysate	gG2	Lysate	Interpretation	Recommendation
negative	negative	negative	seronegative no immunity	prospective monitoring and follow up control 2 to 3 weeks before delivery
negative	negative	positive	positive for HSV-1/2 IgM suspicious of seroconversion (primary infection)	further clarification: a) in case of clinical symptoms (e.g. blisters, lesions): confirmation of the infection status by PCR or cell culture b) confirmation of the positive IgM result with other test method c) follow up control in 10 to 14 days
positive	negative	positive	positive for HSV-1/2 IgG and IgM suspicious of a HSV-1/2 infection no type specific differentiation possible suspicious of a recent or reactivated infection	further clarification: a) in case of clinical symptoms (e.g. blisters, lesions) confirmation of the infection status by PCR or cell culture b) confirmation of the HSV type (e.g. MIKROGEN recomLine HSV-1 and HSV-2 IgG)
positive	positive	positive	positive for HSV-1/2 IgG and IgM suspicious of a HSV type 2 infection suspicious of a recent or reactivated infection	further clarification: a) in case of clinical symptoms (e.g. blisters, lesions) confirmation of the infection status by PCR or cell culture b) confirmation of the HSV type (e.g. MIKROGEN recomLine HSV-1 and HSV-2 IgG)
positive	positive	negative	positive for HSV-1/2 IgG and HSV type 2 IgG suspicious of a HSV type 2 infection	further clarification: a) in case of clinical symptoms (e.g. blisters, lesions) confirmation of the infection status by PCR or cell culture b) confirmation of the HSV type (e.g. MIKROGEN recomLine HSV-1 and HSV-2 IgG)
positive	negative	negative	positive for HSV-1/2 IgG suspicious of a HSV-1/2 infection no type specific differentiation possible	further clarification: a) in case of clinical symptoms (e.g. blisters, lesions) confirmation of the infection status by PCR or cell culture b) confirmation of the HSV type (e.g. MIKROGEN recomLine HSV-1 and HSV-2 IgG)
negative	positive	not specified	no diagnostic statement possible	atypical test result a) in case of clinical symptoms (e.g. blisters, lesions) confirmation of the infection status by PCR or cell culture b) it is highly recommended to request a follow-up sample or repeat the test with some other test system

Test strip interpretation software: recomScan

The *recomScan* software is designed for support of the interpretation of the **recomLine TORCH** test strips.

You will receive further information on request from MIKROGEN.

Warning:

Please do not use the automated interpretation without taking into account the „Notes for Interpretation of the test results and test limitations“ mentioned below.

11.4 Notes for interpretation of the results and test limitations

The tests are indicated for screening of expectant mothers or women contemplating pregnancy for aiding in the presumptive diagnosis of infectious agents with impact on the neonatal outcome. The performance of the assays has not been evaluated for use in a pediatric population, for neonatal screening or for testing of immunocompromised patients.

Ambiguous test results or test results indicative of an acute infection must be confirmed by a confirmatory assay, such as the MIKROGEN *recomLine* Toxoplasma IgG (avidity), the MIKROGEN *recomLine* Toxoplasma IgM, the MIKROGEN *recomBlot* Rubella IgG, the MIKROGEN *recomBlot* CMV IgG (avidity), the MIKROGEN *recomBlot* CMV IgM, or the MIKROGEN *recomLine* HSV-1 & HSV-2 IgG. Depending on the clinical symptoms, a follow-up control should be requested and further diagnostic methods applied, respectively.

The human immune response to an infection is highly variable. The fact that in many cases the specific IgM antibodies are detectable for years after primary infection, renders the interpretation of the serological findings difficult.

For interpretation of the test results, IgG findings must always be considered in combination with IgM findings.

For all test interpretations, especially of weak positive results, it is important to include all clinical record data available. Close cooperation between the laboratory and the physician in charge is recommended. It is strongly recommended to confirm unclear or inconsistent test results with some other test system.

As with other serological tests, negative test results do not rule out the diagnosis of toxoplasmosis, cytomegalovirus infections, German measles, or herpes simplex disease. The time required to seroconvert following primary infection varies within the individual; the specimen may have been drawn prior to the appearance of detectable antibodies. When appropriate, the test should be repeated 4 to 12 weeks later or the specimen should be tested with a different assay.

For pregnant women with negative test results for *Toxoplasma gondii*, rubella virus, and CMV, a follow-up control should be requested on a regular basis. For HSV, a new serum sample should be taken and retested 2 to 3 weeks before delivery.

The use of heat-treated, icteric, haemolytic, lipaemic or turbid specimens is not recommended.

The specimens with microbial contamination should not be tested with these assays.

The assay performance may be influenced by autoantibodies (e.g. ANA/ENA) or rheuma factors (RF) in a specimen. In addition, unspecific IgM reactivity may occur due to cross-reactivity with antibodies to other viruses of the Herpes group or due to unspecific polyclonal stimulation of B lymphocytes caused by a primary virus infection or virus reactivation (e.g. Epstein-Barr Virus, CMV, human Parvovirus B19).

Dark test strips: Some patient sera may cause a dark, continuous or patterned coloration over the entire nitrocellulose strip (e.g. samples from patients with milk protein allergies). Various different factors in the human specimen are responsible for this effect. Evaluation of these strips is usually feasible in a very restricted sense only. The corresponding sample should in any case be tested using other test methods.

12. Clinical Results

12.1. TOXOPLASMA GONDII

12.1.1. Diagnostic sensitivity and specificity

	recomLine TORCH Screening IgG	recomLine TORCH Screening IgM
	% (n)	% (n)
Diagnostic Sensitivity	100% (74/74)	100% (36/36)
Diagnostic Specificity	93.2% (55/59)	100% (12/12)

12.1.2. Diagnostic sensitivity and specificity of the *T. gondii* p30 antigen

	recomLine TORCH Screening IgG
	% (n)
Diagnostic Sensitivity	100% (53/53)
Diagnostic Specificity	88.9% (16/18)

MIKROGEN recomLine Toxoplasma IgG (Avidity) was used as reference

12.1.3. Relative specificity of the *T. gondii* p30 antigen with a seronegative population

	recomLine TORCH Screening IgG
	% (n)
Relative Specificity	100% (285/285)

12.1.4. Relative agreement

	recomLine TORCH Screening IgG	recomLine TORCH Screening IgM
	% (n)	% (n)
Positive Agreement	94.9% (130/137 ²)	80.0% (20/25 ³)
Negative Agreement	94.3% (148/157)	94.7% (251/265)

The determination of the positive and the negative agreement was made with a legally marketed EIA

²Nine samples with a "borderline" test result (EIA) were regarded as samples with a "positive" test result

³Three samples with a "borderline" test result (EIA) were regarded as samples with a "positive" test result

12.1.5. Seroprevalence

	recomLine TORCH Screening IgG		recomLine TORCH Screening IgM	
	Seropositive % (n)	Seronegative % (n)	Seropositive % (n)	Seronegative % (n)
	Blood donors	41.0% (41/100)	59.0% (59/100)	3.0% (3/100)
Pregnant women	22.9% (22/96)	77.1% (74/96)	3.1% (3/96)	96.9% (93/96)

The seroprevalence of *T. gondii* infections in Germany accounts approximately for 50%. The seroprevalence among women in childbearing age is presumably lower (ca. 26-54%). Seroprevalence differs among different populations and age groups.

12.2. RUBELLA VIRUS

12.2.1. Diagnostic sensitivity and specificity

	<i>recomLine TORCH</i> Screening IgG	<i>recomLine TORCH</i> Screening IgM
	% (n)	% (n)
Diagnostic Sensitivity	96.4% (80/83)	86.4% (19/22)
Diagnostic Specificity	100% (9/9)	96.0% (73/76)

12.2.2. Relative agreement

	<i>recomLine TORCH</i> Screening IgG	<i>recomLine TORCH</i> Screening IgM
	% (n)	% (n)
Positive Agreement	95.7% (243/254)	86.4% (19/22)
Negative Agreement	97.6% (41/42)	96.1% (124/129)

The determination of the positive and the negative agreement was made with a legally marketed EIA

12.2.3. Seroprevalence

	<i>recomLine TORCH</i> Screening IgG			<i>recomLine TORCH</i> Screening IgM	
	Seropositive % (n)	Borderline ² % (n)	Seronegative % (n)	Seropositive % (n)	Seronegative % (n)
Blood donors	78.0% (78/100)	15.0% (15/100)	7.0% (7/100)	2.0% (2/100)	98.0% (98/100)
Pregnant women	89.6% (86/96)	7.3% (7/96)	3.1% (3/96)	5.2% (5/96)	94.8% (91/96)

The estimated seroprevalence of rubella immunity (i.e. natural infections and vaccination) in Germany ranges from 87% to 99%. The seroprevalence differs among different populations and age groups.

²Detectable amounts of anti-rubella virus IgG antibodies, but suspicion of no protective immunity to rubella virus

12.3. CYTOMEGALOVIRUS

12.3.1. Diagnostic sensitivity and specificity

	recomLine TORCH Screening IgG		recomLine TORCH Screening IgM	
	% (n)		% (n)	
Diagnostic Sensitivity	100% (78/78)		100% (26/26)	
Diagnostic Specificity	100% (132/132)		100% (24/24)	

12.3.2. Diagnostic sensitivity and specificity of the CMV gB2 antigen

	recomLine TORCH Screening IgG	
	% (n)	
Diagnostic Sensitivity	96.8% (92/95)	
Diagnostic Specificity	100% (103/103)	

MIKROGEN recomBlot CMV IgG (Avidity)) was used as reference

12.3.3. Relative specificity of the CMV gB2 antigen with a seronegative population

	recomLine TORCH Screening IgG	
	% (n)	
Relative Specificity	99.0% (202/204)	

12.3.4. Relative agreement

	recomLine TORCH Screening IgG		recomLine TORCH Screening IgM	
	% (n)		% (n)	
Positive Agreement	100% (132/132)		89.5% (34/38 ²)	
Negative Agreement	100% (66/66)		97.9% (141/144)	

The determination of the positive and negative agreement was made with a legally marketed EIA

²Two samples with a "borderline" test result (EIA) were regarded as samples with a "positive" test result

12.3.5. Seroprevalence

	recomLine TORCH Screening IgG		recomLine TORCH Screening IgM	
	Seropositive	Seronegative	Seropositive	Seronegative
	% (n)	% (n)	% (n)	% (n)
Blood donors	34.0% (34/100)	66.0% (66/100)	0% (0/100)	0% (0/100)
Pregnant women	56.3% (54/96)	43.7% (42/96)	0% (0/100)	0% (0/100)

The seroprevalence of cytomegalovirus infections in Germany accounts approximately for 64%. The seroprevalence differs among different populations and age groups.

12.4. HERPES SIMPLEX VIRUS

12.4.1. Diagnostic sensitivity and specificity

	recomLine TORCH Screening IgG	recomLine TORCH Screening IgM
	% (n)	% (n)
Diagnostic Sensitivity	100% (69/69)	--- ²
Diagnostic Specificity	100% (16/16)	98.7% (74/75)

²There were not a sufficient number of defined anti-HSV IgM positive samples present to determine the diagnostic sensitivity. An anti-HSV IgM titer does not allow differentiation between an acute and a recurrent infection. For serological diagnosis, only the type-specific antibody tests based on the glycoprotein G (gG2) have acceptable accuracy.

12.4.2. Diagnostic sensitivity and specificity of the HSV type 2 gG2 antigen

	recomLine TORCH Screening IgG
	% (n)
Diagnostic Sensitivity	100% (10/10)
Diagnostic Specificity	100% (75/75)

12.4.3. Relative specificity of the HSV gG2 antigen with a seronegative population

	recomLine TORCH Screening IgG
	% (n)
Relative Specificity	95.1% (98/103)

12.4.4. Relative agreement

	recomLine TORCH Screening IgG	recomLine TORCH Screening IgM
	% (n)	% (n)
Positive Agreement	99.5% (194/195 ²)	47.6% (10/21 ³)
Negative Agreement	84.0% (63/75)	97.4% (265/272)

The determination of the positive and the negative agreement was made with a legally marketed EIA

²Five samples with a "borderline" test result (EIA) were regarded as samples with a "positive" test result

³Twelve samples with a "borderline" test result (EIA) were regarded as samples with a "positive" test result

12.4.5. Seroprevalence

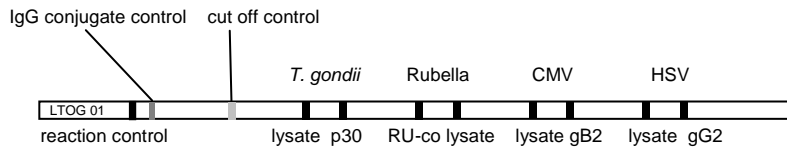
	recomLine TORCH Screening IgG		recomLine TORCH Screening IgM	
	Seropositive % (n)	Seronegative % (n)	Seropositive % (n)	Seronegative % (n)
Blood donors	80.0% (80/100)	20.0% (20/100)	1.0% (1/100)	99.0% (99/100)
Pregnant women	87.5% (84/96)	12.5% (12/96)	3.0% (3/100)	93.0% (93/100)

The seroprevalence of herpes simplex virus type 1 and type 2 infections in Germany accounts approximately for 83%. The seroprevalence differs among different populations and age groups.

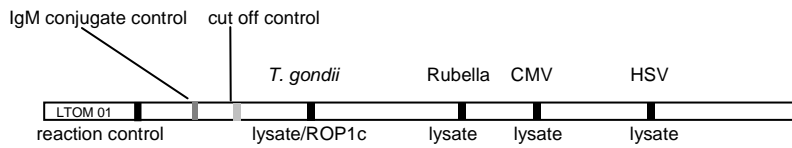
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recomLine TORCH Screening IgG



recomLine TORCH Screening IgM



14. Explanations of the symbols

	Contains sufficient for < n > tests amount of tests	Inhalt ist ausreichend für < n > Ansätze Anzahl der Ansätze
EVALFORM	Evaluation form	Auswertebogen
INSTRU	Instructions for use	Gebrauchsinformation
	Consult instructions for use	Gebrauchsinformation beachten
CONT	Contains	Inhalt, enthält
IVD	in vitro diagnostic device	In vitro Test
LOT	Batch code	Chargen-Nummer
	Do not freeze	Nicht einfrieren
REF	Catalogue number	Bestell-Nummer
	Use by expiry date	verwendbar bis Mindesthaltbarkeitsdatum
	Temperature limitation Store between x°C and y°C	Lagerung bei x°C bis y°C

recomLine TORCH Screening IgG		Artikel-Nr./ Article No.:	6472
recomLine TORCH Screening IgM		Artikel-Nr./ Article No.:	6473
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