EUROLINE Anti-TO.R.C.H. Profile (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DN 2410-1601-4 M DN 2410-6401-4 M	separate: Toxoplasma gondii, ROP1 (T. gondii), rubella virus, CMV, HSV-1, HSV-2	IgM	Ag-coated immunoblot strips	16 x 01 (16) 64 x 01 (64)

Indications: The EUROLINE test kit provides a qualitative in vitro assay for human antibodies of the immunoglobulin class IgM to 5 different TO.R.C.H. antigens, i.e. **Toxoplasma gondii, rubella virus, CMV, HSV-1 and HSV-2** in serum or plasma for the diagnosis of infections.

Application: The EUROLINE Anti-TO.R.C.H. Profile (IgG and IgM), based on highly purified recombinant and native antigens, allows screening for specific antibodies against the pathogens Toxoplasma gondii, rubella virus, CMV, HSV-1 and HSV-2 with one test strip. The detection of antibodies of classes IgG and IgM provides useful information for determining the immunity status, assessing the risk in pregnancy and performing continuative monitoring during prenatal care.

Principles of the test: The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, the immunoblot strips are incubated with diluted patient samples. In the case of positive samples, the specific IgM antibodies (also IgA and IgG) will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format 1601	Format 6401	Symbol
1. Test strips coated with antigens: Toxoplasma gondii, ROP1 (T. gondii), rubella virus, CMV, HSV-1 and HSV-2	16 strips	4 x 16 strips	STRIPS
2. Positive control (IgM, human), 50x concentrate	1 x 0.04 ml	4 x 0.04 ml	POS CONTROL 50x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgM (goat), 10x concentrate	1 x 3 ml	4 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 50 ml	2 x 100 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	4 x 30 ml	SUBSTRATE
6. IgM-sample buffer (with absorbent) containing IgG/RF absorbent, ready for use	1 x 30 ml	4 x 30 ml	IgM-SAMPLE BUFFER
7. Incubation tray	2 x 8 channels	---	
8. Test instruction	1 booklet	1 booklet	

LOT Lot description — CE 0197 — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

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

Waste disposal: Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels
ZD 9898-0144 Incubation tray with 44 channels
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under:
ZD 2410-0101-4 M Visual evaluation protocol EUROLINE Anti-TO.R.C.H. Profile (IgM).

If the packaged reagents are visibly damaged, do not use the test kit.
Please ask EUROIMMUN AG about automation concepts.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips the package should be sealed tightly and stored at +2°C to +8°C.

- **Positive control:** The control is a 50x concentrate. For the preparation of the working strength control the amount required should be removed from the bottle using a clean pipette tip and diluted 1:51 with IgM-sample buffer containing IgG/RF absorbent. Example: add 30 µl of control to 1.5 ml of IgM sample buffer and mix thoroughly. The working strength control should be used at the same working day.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the working strength enzyme conjugate the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with the working strength universal buffer. For 1 test strip dilute 0.15 ml anti-human IgM concentrate with 1.35 ml working strength universal buffer. The working strength enzyme conjugate should be used on the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the working strength universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 2.0 ml universal buffer (10x concentrated) should be diluted with 18.0 ml deionised or distilled water. The working strength universal buffer should be used on the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

- **IgM sample buffer:** Sample buffer containing IgG/RF absorbent, ready for use, for the dilution of the samples and the positive control.

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

Preparation and stability of the patient samples

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

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

Sample dilution: See next chapter for more details. The **patient samples** for analysis are diluted **1:51** with the **ready for use IgM sample buffer** using a clean pipette tip. For example, add 30 µl of sample to 1.5 ml IgM-sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Preparation of serum or plasma samples

Introduction: Before a patient's serum is tested for specific antibodies of the IgM class against TO.R.C.H. antigens, antibodies of class IgG must be removed by ultracentrifugation, chromatography or immunoabsorption. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent that specific IgG displace IgM from the antigen, which would lead to false negative IgM test results.

Functional principle: The IgM-sample buffer containing IgG/RF absorbent contains an anti-human IgG antibody preparation from goat. IgG of a serum or plasma sample is bound with high specificity by these antibodies. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

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

Notes: Antibodies of the class IgG may not be analysed with this mixture. It is possible to check the efficacy of the IgG/RF absorbent by performing an IgG test in parallel with the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.

Incubation

Blocking: Fill the channels of the incubation tray according to the number of serum samples to be tested with 1.5 ml working strength diluted universal buffer each. Remove the required amount of test strips from the packaging using a pair of tweezers and place them one by one in the channels containing the buffer (Make sure that the surface of the test strips is not damaged!). The number on the test strip should be visible.
Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.

Sample incubation: (1st step) Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip.
Incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.

Wash: Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.

Conjugate incubation: (2nd step) Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase conjugated anti-human IgM) into each channel.
Incubate for **30 minutes** at room tem-perature (+18°C to +25°C) on a rocking shaker.

Wash: Aspirate off the liquid from each channel. Wash as described above.

Substrate incubation: (3rd step) Pipette 1.5 ml substrate solution into the channels of the incubation tray.
Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Stop: Aspirate off the liquid from each channel and wash **3 x 1 minute** each strip with deionised or distilled water.

Evaluate: Place test strip on the evaluation protocol, air dry and evaluate.

For automated incubation with the **EUROBlotMaster** select the program **Euro03 ToRCHM EL30**.

For automated incubation with the **EUROBlotOne** select the program **Euro03**.

EUROLINE Anti-TO.R.C.H. Profile (IgM)

Incubation protocol

Blocking
Pipette 1.5 ml working strength universal buffer into the incubation channel and insert strip

15 min — Shake

1. Step: Sample incubation
Aspirate off, pipette 1.5 ml of diluted serum sample (1:51) into the incubation channel

30 min — Shake

Washing
Aspirate off, wash 3 x 5 min with 1.5 ml working strength universal buffer each

2. Step: Conjugate incubation
Aspirate off, pipette 1.5 ml working strength enzyme conjugate into the incubation channel

30 min — Shake

Washing
Aspirate off, wash 3 x 5 min with 1.5 ml working strength universal buffer each

3. Step: Substrate incubation
Aspirate off, pipette 1.5 ml substrate into the incubation channel

10 min — Shake

Stop
Aspirate off, rinse three times with 1.5 ml distilled water

Evaluation
EUROLineScan (digital)

Evaluation and interpretation of the results obtained by the EUROLINE Anti-TO.R.C.H. Profile (IgM)

Handling: For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan.** Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **test** into EUROLineScan is **TORCH_M4**.

If a visual evaluation is to be performed in individual cases, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 2410-0101-4 M.

Note: A correctly performed test for class IgM antibodies against different TO.R.C.H. antigens is indicated by a positive reaction of the control band and the IgM band. If one of these bands shows a very weak reaction or none at all, the result must not be used for evaluation.

Antigens and their arrangement on the strips:

Antigens:

Toxoplasma gondii: Lysates of sonicated and gamma irradiated Toxoplasma gondii tachyzoites.

Toxoplasma gondii, ROP1:
Highly purified recombinant protein ROP1.

Rubella virus: Highly purified rubella virus glycoproteins. The antigen source is provided by inactivated cell lysates of Vero cells infected with the “HPV-77” strain of rubella virus.

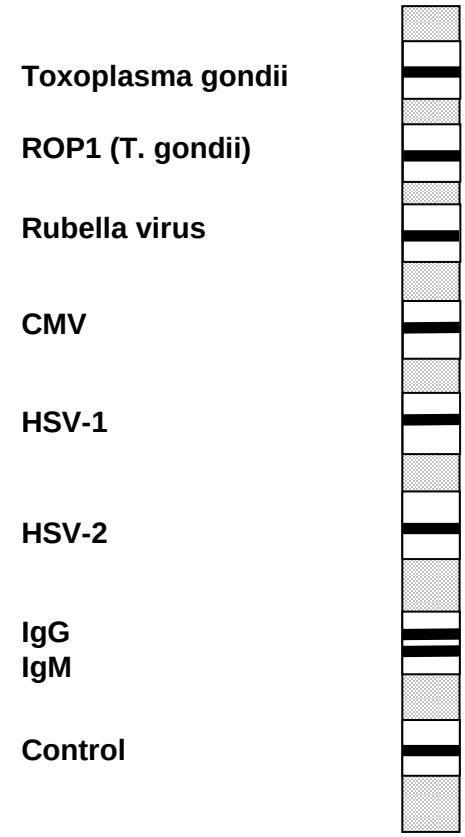
CMV:
Highly purified recombinant protein p52.

HSV-1:
Affinity-purified glycoprotein C1.

HSV-2:
Affinity-purified glycoprotein G2.

Control bands:
IgG or IgM

Control: Incubation control indicating a correctly performed incubation.



Antibodies of class IgM against TO.R.C.H antigens

It is recommended that the results of the bands be classified according to the signal intensity:

Signal	Result	
No signal or very weak band	o	Negative
Weak band	(+)	Borderline
Medium to strong band	+	Positive

For a borderline result of IgM antibodies against Toxoplasma gondii at least one of the bands, Toxoplasma gondii lysate or ROP1, must show a borderline reaction.
For a positive result of IgM antibodies against Toxoplasma gondii at least one of the bands, Toxoplasma gondii lysate or ROP1 (T. gondii), must show a positive reaction.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided.
The lower detection limit is the mean value of a tenfold determination of an analyte-free sample plus three times the standard deviation and is, on average, EUROLineScan intensity value 2. This intensity value corresponds to a negative result.

Inter- and intra-assay variation: The inter-assay variation and intra-assay variation were determined by multiple analyses of characterised samples over several days. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml for haemoglobin, of 20 mg/ml for triglycerides and of 0.4 mg/ml for bilirubin showed no effect on the analytical results of the present EUROLINE.

Specificity: The EUROLINE specifically detects IgM class antibodies directed against Toxoplasma gondii, rubella virus, CMV, HSV-1 and HSV-2. No cross-reactions have been found.

Note: The detection of antibodies of class IgM against two or more TO.R.C.H. antigens in one serum sample is extraordinary and should be confirmed with an independent test method.

Sensitivity and specificity: Precharacterised quality assessment samples and sera from 150 blood donors were investigated for the following antibodies using the EUROLINE.

Antibodies against	n	Sensitivity	Specificity	n	Prevalence
Toxoplasma	24	100%	100%	150	1.3%
ROP1 (T. gondii)	25	88.9%	100%	150	1.3%
Rubella virus	24	100%	100%	150	0%
CMV	25	100%	100%	150	0%
HSV-1	25	100%	100%	150	0%
HSV-2	31	100%	100%	150	0.7%

Cross reactivity: The cross reactivity was determined using a representative selection of antibodies against infectious agents. Results obtained for parameters with the highest relevance in the assessment of the cross reactivity are given as examples in the following table.

ELISA Antibodies against	pos (n)	EUROLINE Anti-Toxoplasma IgM positive
Rubella IgM	34	0%
CMV IgM	29	0%
HSV-1 IgM	9	0%
HSV-2 IgM	45	0%
Parvovirus B19 IgM	23	0%
VZV IgM	16	0%

ELISA Antibodies against	pos (n)	EUROLINE Anti-ROP1 IgM positive
Rubella IgM	26	0%
CMV IgM	18	0%
HSV-1 IgM	7	0%
HSV-2 IgM	38	0%
Parvovirus B19 IgM	20	0%
VZV IgM	10	0%

ELISA Antibodies against	pos (n)	EUROLINE Anti-Rubella IgM positive
Toxoplasma IgM	23	0%
CMV IgM	13	0%
HSV-2 IgM	14	0%
Parvovirus B19 IgM	2	0%
VZV IgM	5	0%

ELISA Antibodies against	pos (n)	EUROLINE Anti-CMV IgM positive
Toxoplasma IgM	28	0%
Rubella IgM	29	0%
HSV-1 IgM	8	0%
HSV-2 IgM	40	0%
Parvovirus B19 IgM	20	0%

Samples that were positive for a specific parameter in ELISA showed no reactivity with the respective other parameters in the EUROLINE test. Thus, cross-reactions can be excluded.

Correlation analyses: Investigation of 246 sera in relation to the CE-registered reference methods Anti-Toxoplasma gondii ELISA and Anti-CMV ELISA (test systems from EUROIMMUN AG) yielded the following specificities and sensitivities for detection of the corresponding antibodies. Borderline samples were not included in the evaluation.

		Anti-Toxoplasma gondii ELISA (IgM)		
n = 246		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgM)	positive	21	3	12
	borderline	6	3	16
	negative	3	4	178

Specificity: 93.7%
Sensitivity: 87.5%

		Anti-CMV ELISA (IgM)		
n = 246		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgM)	positive	17	2	2
	borderline	3	1	3
	negative	6	6	206

Specificity: 99.0%
Sensitivity: 73.9%

Investigation of 206 sera in relation to the CE-registered reference method EUROIMMUN Anti-Rubella virus Glycoprotein ELISA yielded the following specificity and sensitivity. Borderline samples were not included in the evaluation.

		Anti-Rubella Virus Glycoprotein ELISA (IgM)		
n = 206		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgM)	positive	9	0	4
	borderline	1	0	5
	negative	2	0	185

Specificity: 97.9%
Sensitivity: 81.8%

Clinical significance

Toxoplasma gondii:
The pathogen of toxoplasmosis is the sporozoon Toxoplasma gondii. It was first found in the gundi, an African rodent. However, the main host animal is the cat, in which after peroral infection the parasite lives in intestinal cells and causes oocysts to develop (sexual development cycle). During the asexual development cycle, the Toxoplasma parasites develop in the brain, muscle, liver, spleen and other organs of a warm-blooded animal, where they become encapsulated. Humans are generally infected perorally by ingestion of oocysts (contained in the faeces of infected cats) or from meat products (the raw flesh of infected animals contains cysts with viable trophozoites). Toxoplasma can also be transmitted diaplacentally when a pregnant woman is infected for the first time.

Toxoplasmosis acquired post-natally generally proceeds inapparently. In this case, cysts containing trophozoites, which can persist for years and can sustain immunity, form in the tissue. Depending on the organ manifestation, the symptoms of the disease include fever, lymph-adenopathy, encephalitis, chorioretinitis, myositis, myocarditis, pneumonia, hepato-spleno-megaly and exanthema. Specific antibodies have even been detected in the brain. In the case of immunologically challenged patients (recipients of transplants, tumour patients, HIV-infected patients), a primary infection with Toxoplasma or the reactivation of a toxoplasmosis can lead to life-threatening illness.

After an intrauterine infection in the first trimester, with the placenta and embryo being severely affected, a rejection of the foetus occurs. An infection in the second or third trimester results in foetal symptoms which vary depending on the timepoint of infection, the dose of the infection and the immune status of mother and foetus. In the foreground are: hepatosplenomegaly, pneumonia, myocarditis, purpura, hydrocephalus, chorioretinitis, oedema of the optic nerve, intracerebral calcification.

Rubella:
The pathogenic agent of rubella is the rubella virus, which is present worldwide. The disease is transmitted via aerosols and is already contagious during the two to three-week incubation period. The symptoms include headaches, characteristic lymph node swellings, and often a typical blotchy rash. The majority of infections occur between the ages of 5 and 14 years, whereby about 40 to 50% of cases proceed subclinically. A rubella infection leads to life-long immunity. An infection spread of 80 to 90% is assumed for adults in central Europe. This means that 10 to 20% of women of child-bearing age are not immune.

Rubella virus transmitted diaplacentally causes the highest rate of embryonic deformities, through infection in the form of a rubella embryopathy. This leads to serious disorders, such as cataract, inner ear damage, heart defects, and microcephaly, particularly in the first three months of pregnancy. In many countries, an acute rubella infection is considered to be a medical indication for termination of pregnancy.

For this reason, the serological analysis of antibodies against the three most important rubella structure proteins (E1, E2, C), which can be detected two to three days after the onset of the exanthema, is of particular importance in pregnant women. In case of a negative result, an unknown immunisation status or the presence of specific IgM, passive immunisation can be given within 7 days after exposition in the early stage of pregnancy.

Various inoculation strategies have been employed worldwide to prevent rubella infections. Since active immunisation is well tolerated, vaccination programs aim to protect all young persons before puberty using a two-stage rubella vaccination.

CMV:
The majority of cytomegalovirus (CMV) infections proceed inapparently. The disease can manifest itself in almost all organs, in the foreground are, however, hepatitis and pneumonia, which are accompanied by a prolonged fever. Of importance is connatal CMV infection, which causes damage in particular to the liver, the spleen and the central nervous system. About 1% of all fetal cases are infected in utero and exhibit IgM class antibodies against CMV.

Antibodies against CMV can be detected in the serum of nearly all patients after the disease has taken its course. Life-long immunity is generally acquired. The prevalence in adults is 80%. Past CMV infections can be reactivated if the immunological defence mechanisms are weakened. In the case of immunologically challenged seronegative patients, such as tumour patients and recipients of transplants, passive immunisation with specific immunoglobulin concentrates is frequently indicated. Such patients and infants (particularly premature children), should not be given blood products from CMV-infected (CMV-antibody positive) blood donors.

HSV-1/HSV-2:
Herpes simplex is a disease which causes the formation of blisters on the skin and mucous membranes. It is prevalent worldwide and normally proceeds blandly. Complications result when internal organs are also affected and start to necrotise. Herpes simplex virus 1 (HSV-1) prefers the area of the mouth and nose, herpes simplex virus 2 (HSV-2) the genitals. Provocable relapses are typical.

Antibodies against HSV-2 can be detected in the serum of nearly all patients after the disease has taken its course. More than 90% of the population are infected with HSV-1, whereas antibodies against HSV-2 can only be found in 7 to 20%. However, the antibodies are not able to prevent a relapse or re-infection. In primary HSV-2 infections, cephalalgia and neck stiffness are frequent symptoms, whereas meningitis is rare. If newborns are infected with HSV-2 passing the birth canal, blisters of the skin but also on the mouth and eyes are frequently observed, often accompanied by hepatomegaly, splenomegaly, loss of kidney function, icterus and encephalitis. Without treatment the disease is fatal in most cases.

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