

Anti-Chlamydia trachomatis ELISA (IgG)

Test instruction




ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2191-9601 G	Chlamydia trachomatis	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides semiquantitative or quantitative in vitro determination of human antibodies of the immunoglobulin class IgG against Chlamydia trachomatis in serum or plasma to support the diagnosis of trachoma, infections of the urogenital tract and lymphogranuloma venereum.

Application: By determination of antibodies against the species-specific Chlamydia trachomatis MOMP antigen (major outer membrane protein), persisting or chronic Chlamydia trachomatis infections can be reliably diagnosed and clearly delimited by differential diagnosis from infections with other species of Chlamydia. In acute and peripherally localised Chlamydia trachomatis infections, however, the direct detection of the pathogen (e.g. PCR) is the method of choice.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified Chlamydia trachomatis antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
<div> <div> <div>LOT</div> <div>Lot description</div> </div> <div> <div>IVD</div> <div>In vitro diagnostic medical device</div> </div> <div>  <div>0197</div> </div> <div> <div>  <div>Storage temperature</div> </div> <div>  <div>Unopened usable until</div> </div> </div> </div>			

Modifications to the former version are marked in grey.



Preparation and stability of the reagents

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

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.
- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ☀. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready for use.

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

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

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

Preparation and stability of the patient samples

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

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

Sample dilution: Patient samples are diluted **1:101** in sample buffer.

For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

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



Incubation

For **semiquantitative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)

Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.

Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

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

Note: Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.

Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.

Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.

Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.

Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:

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

Measurement:

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



Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.

Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).

The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the **extinction of the control or patient sample** over the **extinction of the calibrator 2**. Calculate the ratio according to the following formula:

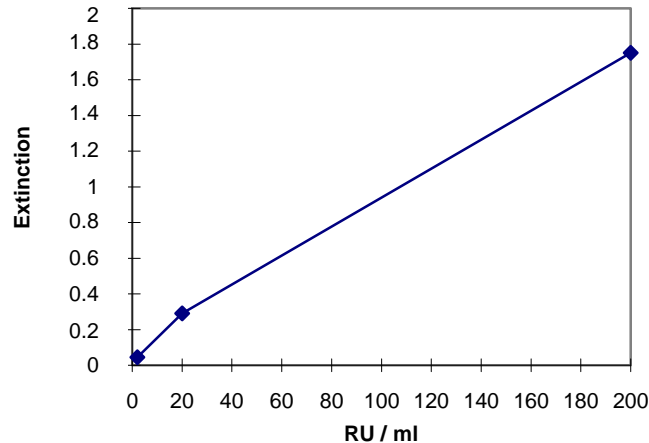
$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	negative
Ratio ≥0.8 to <1.1:	borderline
Ratio ≥1.1:	positive



Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction readings measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the extinction of calibrator 1 (corresponding to 200 RU/ml), the result should be reported as ">200 RU/ml". It is recommended that the sample be retested in a new test run at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by factor 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	negative
≥16 to <22 RU/ml:	borderline
≥22 RU/ml:	positive

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant IgG titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.



Test characteristics

Calibration: As no international reference serum exists for antibodies against *Chlamydia trachomatis*, the calibration is performed in relative units (RU/ml). The calibration is performed with internal reference sera, which were used for evaluation of the test system.

For every group of tests performed, the extinction readings of the calibrators and the relative units and/or ratio values determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The reagent wells are coated with MOMP antigen (major outer membrane protein) which is a transmembrane protein and the major part of the outer membrane of the elementary bodies. Protein purification starts with BGM cells infected with *Chlamydia trachomatis* of serotype K.

Linearity: The linearity of the Anti-*Chlamydia trachomatis* ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The Anti-*Chlamydia trachomatis* ELISA (IgG) is linear at least in the tested concentration range (2 RU/ml to 177 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-*Chlamydia trachomatis* ELISA (IgG) is 0.6 RU/ml.

Cross reactivity: The quality of the antigen used ensures high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-*Chlamydia trachomatis* ELISA (IgG). For this ELISA there is no known cross reactivity with other *Chlamydia pneumoniae* positive samples.

Antibodies against	n	Anti- <i>Chlamydia trachomatis</i> ELISA (IgG) positive
Adenovirus	12	0%
<i>Chlamydia pneumoniae</i>	10	0%
CMV	12	0%
EBV-CA	12	0%
<i>Helicobacter pylori</i>	12	0%
HSV-1	12	0%
Influenza A virus	12	0%
Influenza B virus	12	0%
Measles virus	12	0%
Mumps virus	12	0%
<i>Mycoplasma pneumoniae</i>	12	0%
Parainfluenza pool	12	0%
Rubella virus	12	0%
RSV	12	0%
Toxoplasma	12	0%
VZV	12	0%



Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

<i>Intra-assay variation, n = 20</i>		
Sample	Mean value (RU/ml)	CV (%)
1	8	9.2
2	65	8.2
3	137	3.2

<i>Inter-assay variation, n = 4 x 6</i>		
Sample	Mean value (RU/ml)	CV (%)
1	7	10.6
2	62	9.5
3	139	6.5

Sensitivity and specificity: 69 clinically pre-characterised patient samples (INSTAND) were investigated with the EUROIMMUN Anti-Chlamydia trachomatis ELISA (IgG). The sensitivity amounted to 100%, with a specificity of 97,0%. Borderline results were not included in the calculation.

n = 69		INSTAND		
		positive	borderline	negative
EUROIMMUN Anti-Chlamydia trachomatis ELISA (IgG)	positive	24	0	1
	borderline	0	0	2
	negative	0	1	41

Prevalences: For different sample panels the following prevalences could be determined:

Panel	Number of samples	Prevalence (IgG)
Patients with pos. direct determination for C. trachomatis	100	67.0%
Risk group (prostitutes)	134	42.5%
Patients with reactive arthritis	54	27.8%
Pregnant women	200	12.5%
Healthy blood donors I	200	8.0%
Healthy blood donors II	200	4.0%

Reference range: The levels of anti-Chlamydia trachomatis antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 13.4% of the blood donors were anti-Chlamydia trachomatis positive (IgG).

Clinical significance

The infectious agent Chlamydia trachomatis belongs to the human pathogenic Chlamydia genus, together with Chlamydia pneumoniae and Chlamydia psittaci. It is one of the smallest intracellular, gram-negative bacteria. It subsists as an energy parasite on the ATP of infected cells. Around 700 million people are infected worldwide, with approximately 50 million new infections taking place each year. In the USA the prevalence of mainly asymptomatic C. trachomatis infections in 16- to 25-year-old women is 22%, and in Western Europe 2.7% (in Italy) to 8% (in Iceland) according to the WHO. The disease is transmitted by contact with infected humans.

C. trachomatis is the pathogenic agent of non-gonorrheal urethritis, lymphogranuloma venereum, trachoma, inclusion conjunctivitis, neonatal pneumonia and Reiter's syndrome.



Sexually transmitted non-gonorrheal urethritis caused predominantly by *C. trachomatis* serotypes D to K is nowadays the most frequent sexually transmitted disease. The bacteria live mostly in the cells of the urethra, in men also in the prostate and the seminal vesicles and in women in the cervix or oviducts (salpinx). Infections proceed asymptotically in around 50% of men and 70 to 80% of women. If symptoms develop they are urethritis, epididymitis and prostatitis in men, and in women urethritis, cervicitis and salpingitis/adnexitis with itching, pain and discharge. Chronic infections of the inner female organs lead to sterility in many cases. In Germany, more than 100,000 women suffer from Chlamydia-caused infertility. Secondary infertility in men has also been shown. There is an evident connection between acute *C. trachomatis* infections during the first three months of pregnancy and early abortions, premature deliveries or stillbirths (32nd to 34th week of pregnancy).

Lymphogranuloma venereum (lymphogranuloma inguinale, lymphopathia venera, Durand Nicolas Favre disease) is caused by *C. trachomatis* serotypes L1, L2 and L3. It is a rare venereal disease which occurs worldwide but mainly in tropical areas. Approximately 40% of men and 70% of women become infected after sexual contact with an infected person.

In tropical regions, *C. trachomatis* leads to trachoma (serotypes A, B, Ba and C), an eye infection of varying severity which is also known as trachomatous conjunctivitis, granular conjunctivitis or Egyptian ophthalmia. It is caused by direct contact between the mucous membranes of the eye, nose and mouth or may be transmitted by the mutual use of towels or washcloths. The first symptoms of severe conjunctivitis occur after an incubation period of 5 to 12 days. Around 400 million people suffer from trachoma, which is the most frequent cause of blindness worldwide (trachoma blindness).

The disease must be differentiated from an infection with *C. trachomatis* serotypes D to K, which causes paratrachoma in adults, also known as acute suppurative inclusion conjunctivitis or swimming pool conjunctivitis. It is generally transmitted through bathing water.

In newborns, particularly premature infants, prenatally or perinatally transmitted *C. trachomatis* causes conjunctivitis (ophthalmia neonatorum) and pneumonia (serotypes D to K). The latter is noticeably often accompanied by pneumothorax and lifelong health problems.

In 1 to 3% of cases, an urogenital infection with *C. trachomatis* is followed by reactive arthritis (Reiter's disease with the triad urethritis, conjunctivitis and arthritis). This is an oligoarthritis which predominantly affects the lower extremities, particularly the knee and ankle joints, causing local swelling. The distal interphalangeal joints and the spine (inflammatory back pain) are also frequently involved.

In reactive arthritis *C. trachomatis* occurs as a metabolically active agent in the joints. Due to the persisting infection, Chlamydia antigens such as major outer membrane protein (MOMP) and lipopolysaccharide (LPS) are continuously produced, stimulating and sustaining an inflammatory process and the production of antibodies. The immune response is an intra-articular production of anti-*C. trachomatis* IgG. Type-specific serological test methods using MOMP as the target antigen allow a reliable diagnosis.

Despite distinct clinical symptoms, *C. trachomatis* antibodies are not necessarily produced in the serum in localised processes. IgM antibodies are not formed in all cases of florid infection. An increase in IgG titer is also not always found. In problem cases, it is therefore useful to determine the presence of Chlamydia in infectious secretions using direct immunofluorescence, or to determine the specific genetic sequences using PCR, although this is often unsuccessful in cases where the infection has taken place some time ago. Nevertheless PCR is often used for the detection of first infections, in particular as part of the screening programmes that are offered to young women in various countries in order to prevent asymptomatic ascending Chlamydia infections from causing sterility. The test results show that on average 10.9% of the young women investigated (up to age 24) were infected but asymptomatic. The ethnic breakdown was: white 18.1%, black Caribbean 9.9%, black African 15.2%, black British/other 5.9%, Asian subcontinent 6.7%, Chinese/other Asian 6.4% and other ethnic groups 14.9%.

The fastest and most reliable investigation of specific antibodies in infections with *C. trachomatis*, even with low antibody titers, is obtained with MIF (microimmunofluorescence) as "gold standard", IIFT (indirect immunofluorescence test) and ELISA (enzyme linked immunosorbent assay) using MOMP as the target antigen and taking into consideration different serotypes. In the MIF assay purified elementary bodies of the species *C. trachomatis* are used and the lipopolysaccharide (LPS) antigen common to all three species (*C. trachomatis*, *C. pneumoniae* and *C. psittaci*) is inactivated. This minimises cross reactions.



Studies throughout Europe show that these methods are suited to confirming *C. trachomatis*-induced infertility in women and men by the determination of *C. trachomatis* specific serum IgA and IgG antibodies. *C. trachomatis* specific IgA and IgG antibodies are frequently found in women who have had a premature delivery or a stillbirth, mostly in connection with high IgM titers. Recognised medical centres therefore recommend *C. trachomatis* screening for both parents before pregnancy.

Diagnosed Chlamydia infections can generally be cured with various antibiotics within 7 days, even during pregnancy. In reactive arthritis, a long-term, differentiated treatment is required, which acts locally and systemically.

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