Version 22-04/24

User's Manual

Chlamydia IgG ELISA Kit

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Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Chlamydia IgG ELISA is quantitative and qualitative tests for detection of human antibodies in serum or plasma directed against genus specific human Chlamydia-antibodies. For sale in the U.S. for Research Use Only. Not for use in diagnostic procedures.

General Description

The order Chlamydiales currently has one family, the Chlamydiaceae, containing two genera, Chlamydia and Chlamydophila. Three human pathogenic species, Chlamydia trachomatis, Chlamydophila pneumoniae and Chlamydophila psittaci are belonging to these genera. Nearly all birds and mammals can be infected with C. psittaci, whereas man is the main host of C. trachomatis. Workers on poultry farms are in addition at high risk of C. psittaci infection which may cause a severe psittacosis disease. The different Chlamydia species are transmitted via different routes of infection: C. pneumoniae via aerosol transmission, C. trachomatis via direct contact or smear infection, C. psittaci via inhalation of fecal droppings, feather dust or smear infection. Chlamydia are coccoide, gram-negative bacterial pathogens which are metabolically deficient in their ability to synthesize ATP and thus have an obligate intracellular life cycle. During an infection with Chlamydia two characteristic cell forms occur: the highly infectious elementary bodies and the non-infectious intracellular reticular bodies which are able to propagate.

Principles of Testing

Microtest plates are coated with antigens. This constitutes the solid phase. Sample is added to the plates and any antibodies specific for the antigen present will bind to the solid phase. After removal of unbound material, anti-human IgG, IgA or IgM conjugated to an enzyme (alkaline phosphatase) is allowed to react with the immune complex. After removal of excess conjugate by washing, an appropriate substrate (paranitrophenylphosphate) is added, with which the conjugated enzyme reacts producing a colored derivative of the substrate. The color intensity is proportional to the level of specific antibody bound and can be quantified photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with 8 antigen coated single wells (altogether 96), 1 frame, the coating material is inactivated
2. Standard serum (ready-to-use): Human serum in phosphate buffer with protein; negative for anti-HIV-Ab, anti-HBs-Ag (Hepatitis B-Virus-surface antigen) and anti-HCV-Ab; preservative: < 0.1 % sodium azide colouring: Amaranth O. 2×2 mL
3. Negative control serum (ready-to-use): Human serum in phosphate buffer with protein; negative for anti-HIV, anti-HBs (Hepatitis B Virus-surface antigen) and anti-HCV; preservative: < 0.1 % sodium azide colouring: Lissamin green V. 2 mL
4. Anti-human-IgG-conjugate (ready-to-use): Anti-human-IgG from goat (polyclonal), conjugated to alkaline phosphatase, stabilized with protein stabilization solution preservative: 0.01 % methylisothiazolone, 0.01 %

bromnitrodioxane. 13 mL

5. Washing solution concentrate (sufficient for 1 litre): Sodium chloride solution with Tween 20, 30 mM Tris, preservative: < 0.1 % sodium azide. 33.3 mL
6. Dilution buffer: Phosphate buffer with protein and Tween 20; preservative: < 0.1 % sodium azide, 0.01 g/l Bromphenol blue sodium salt. 2×50 ml
7. Stopping solution: 1.2 N sodium hydroxide. 15 ml
8. Substrate (ready-to-use): Para-nitrophenylphosphate, solvent free buffer, preservative: < 0.1 % sodium azide, (Substrate in unopened bottle may have a slightly yellow coloring. This does not reduce the quality of the product!). 13 ml

Materials Required But Not Supplied

1. common laboratory equipment
2. photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
3. incubator 37°C
4. moist chamber
5. distilled water

Storage

1. microtiter strips (antigen), after opening at 2-8°C in closed aluminum bag with desiccant. Strips which are not used must be stored in the press-seal bag of aluminum compound foil under dry and airtight conditions! 4 weeks
2. control sera /standard sera, after opening at 2-8°C, until expiry date; 24 months from date of production
3. conjugate, ready-to-use solution, at 2-8°C, Avoid contamination (sterile tips!). until expiry date 28 months from date of production
4. dilution buffer, after opening at 2-8°C(24 months). Discard cloudy solutions! unopened (until expiry date; 36 months from date of production)
5. washing solution, concentrate after opening at 2-8°C (until expiry date), working dilution at 2-8°C (2 weeks), working dilution at room temperature (1 weeks). Bottles used for the working dilution should be cleaned regularly, discard cloudy solutions.
6. substrate, ready-to-use solution at 2-8°C, protected from light! Avoid contamination (sterile tips!) Discard when solution turns yellow (extinction against distilled water > 0.25). Until expiry date 24 months from date of production
7. stopping solution, after opening at room temperature, until expiry date

Specimen Collection And Preparation

1. Sample preparation and storage

Lipaemic, hemolytic or icteric samples should only be tested with reservations although inour testing no negative influence has been found. Obviously contaminated samples (serum or plasma) should not be tested

due to the risk of wrong results. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples.

Samples must not be thermally inactivated.

a. Sample preparation

Before running the test, samples must be diluted in dilution buffer (V1 + V2) as follows:

$V_1 + V_2 = 1+20$	add	10 µl	sample
	each to	200 µl	dilution buffer

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

2. Sample storage

The stoppered samples can be stored in a refrigerator up to 7 days at 2-8°C. Extended storage is possible at ≤ -20°C.

Avoid repeated freezing and thawing of samples.

Diluted samples can be stored at 2-8°C for one week.

Plate Preparation

Microtest strips in frame are packed with desiccant in an aluminum bag. Take unrequired cavities out of the frame and put them back into the press-seal bag. Close press-seal bag carefully to ensure airtight conditions.

Reagent Preparation

Control sera / standard sera

Control and standard sera are ready-to-use and must not be diluted any further. They can be used directly for the test run. For each test run and for each test system - independent of the number of microtest strips to be used - control and standard sera must be included. The cut-off-control should be set up in duplicate. With the quantitative tests the standard serum should also be set up in duplicate. Do not treat control sera with Rf-absorbent.

Anti-human-IgG-AP-conjugate (ready-to-use)

Please do not mix up conjugates from different kits. They are optimized for each lot. Avoid contamination of ready-to-use conjugates (please pour sufficient for test into a secondary container to avoid repeatedly pipetting from the original bottle).

Washing solution

Dilute washing buffer concentrate (V1) 1:30 with distilled water to a final volume of V2.

Example:

buffer concentrate (V_1)	final volume (V_2)
33.3 ml	1000 ml
1 ml	30 ml

Dilution buffer for samples (ready-to-use)

Substrate (ready-to-use)

To avoid contamination use gloves. For pipetting substrate solution use sterile tips only!

Stopping solution (ready-to-use)

Assay Procedure

1. Place the required number of cavities in the frame and prepare a protocol sheet.
2. Add each 100 µl of diluted sample or ready-to-use controls into the appropriate wells of microtest strips. Spare one well for substrate blank, e.g.:

IgG quantitative	
well A1	substrate blank
well B1	negative control
well C1	standard serum
well D1	standard serum
well E1	sample 1....

3. Sample incubation for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber
4. After incubation wash all wells with washing solution (by automated washer or manually):

   aspirate or shake out the incubation solution

   fill each well with 300 µl washing solution

   aspirate or shake out the washing buffer

   repeat the washing procedure 3 times (altogether 4 times!)

   dry by tapping the microtest plate on a paper towel
5. Addition of conjugate: Add 100 µl of IgG-conjugate (ready-to-use) to the appropriate well (except substrate blank)
6. Conjugate incubation for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above)
8. Addition of substrate: Add 100 µl substrate solution (ready-to-use) to each well (including well for substrate blank!)
9. Substrate incubation for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.
10. Stopping of the reaction: Add 100 µl stopping solution to each well, shake microtest plate gently to mix.
11. Read optical density: Read OD within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

***Please note, that under special working-conditions internal laboratory adaptations of the incubation times could be necessary.**

Calculation

1. Single-point quantification with the 4PL method

Optimized assignment of extinction signals to quantitative values is guaranteed by using non-linear functions, which adjust a sigmoide curve without any further transformation to OD-values.

Determination of antibody concentrations with the SERION ELISA classic is carried out by the logistic-log-model (4 PL; 4 parameter) which is ideal for exact curve-fitting. It is based on the formula:

$$OD = A + \frac{D - A}{1 + e^{B(C - \ln conc.)}}$$

The parameters A, B, C, and D are representative for the exact shape of the curve:

1. lower asymptote → parameter A
2. slope of the curve → parameter B
3. turning point → parameter C
4. upper asymptote → parameter D

For each lot the standard curve is evaluated by CD in several repeated test runs under optimal conditions. Time consuming and cost intensive construction of the standard curve by the user is not necessary.

For evaluation of antibody concentrations a lot specific standard curve as well as a lot specific evaluation table is included with each test kit. Appropriate evaluation software is available on request.

To compensate for normal test variations and also for test run control a standard serum is used in each individual test run. For this control serum a "reference value" with a validity range is determined by the quality control of the producer. Within this range a correct quantification of antibody concentration is ensured. Since the standard serum is not necessarily a positive control, the value of the standard serum may be borderline or negative in some ELISA tests.

2. Criteria of validity

a. the substrate blank must be OD < 0.25

b. the negative control must be negative

c. quantitative ELISA: the mean OD-value of the standard serum must be within the validity range, which is given on the lot specific quality control certificate of the kit (after subtraction of the substrate blank!)

d. qualitative ELISA: the mean OD-value of the positive control must be within the validity range, which is given on the lot specific quality control certificate of the kit (after subtraction of the substrate blank!)

e. the variation of OD-values may not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

3. Calculation Chlamydia IgG quantitative

Non-automated evaluation

For the test evaluation a standard curve and an evaluation table are included in the test kit so that the obtained OD-values may be assigned to the corresponding antibody activity.

The reference value and the validity range of the standard serum is given on the evaluation table (quality control certificate).

The blank (A1) must be subtracted from all OD-values prior to the evaluation.

Method 1: Qualitative Evaluation

To fix the cut-off ranges please multiply the mean value of the measured standard-OD with the numerical data of the certificate of quality control (see special case formulas), e.g.:

OD = 0.502 x MW(STD) with upper cut-off

OD = 0.352 x MW(STD) with lower cut-off

If the measured mean absorbance value of the standard serum is 0.64, the range of the cutoff is in between 0.225-0.321.

Method 2: Continuous determination of antibody activities using the standard curve.

So called interassay variations (day to day deviations and laboratory to laboratory deviations) are compensated by multiplication of the current measured value obtained with a sample with the correction factor F. This factor is calculated as follows:

$$F = \frac{\text{OD-reference value (of standard serum)}}{\text{OD-current value (of standard serums)}}$$

The procedure is necessary to adjust the current level of the test of the user with the lotspecific standard curve.

First, daily deviations have to be corrected by calculating a factor (correction factor F):

a. The mean of the two OD-values of the standard serum has to be calculated and checked that it is within the given validity range.

b. Calculation of the factor "F": the given reference value is divided by the mean of the OD-value of the standard serum:

F = reference OD-value standard serum / mean OD-value standard serum.

c. All measured values of samples are multiplied by "F".

d. Antibody activities in IU/ml or U/ml can be determined from the standard curve with the corrected values.

Precautions

The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

All kit reagents and human specimens should be handled carefully, using established good laboratory practice.

This kit contains human blood components. Although all control- and cut-off-sera have been tested and found negative for HBs-Ag-, HCV- and HIV-antibodies, they should be considered potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimen. Wash hands thoroughly afterwards.

Samples and other potentially infectious material should be decontaminated after the test run.

Reagents should be stored safely and be unaccessible to unauthorized access e.g. children.

Stopping solution: corrosive (C); cause acid burn (R34) use safety glasses, gloves and laboratory coat while handling!