Version 30-04/25

User's Manual

Human Chlamydia pneumoniae IgG EIA Kit

REF **DEIA1726**

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

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PRODUCT INFORMATION

Intended Use

CD's Chlamydia pneumoniae IgG test is developed for the detection of IgG antibodies specific to Chlamydia pneumoniae in human serum or plasma.

The kit is semiquantitative allowing comparison of paired samples. The change of antibody level is an aid for the diagnosis of acute Chlamydia pneumonia infection.

The test is recommended to be run and interpreted in parallel with the CD's Chlamydia pneumoniae IgA and IgM EIA kits.

General Description

Since the description in 1986 of Chlamydia pneumoniae as a pathogen (1) it has become recognized as a common infectious agent all over the world. C. pneumoniae is primarily a respiratory tract pathogen that causes approximately 10-20% of community acquired pneumonia in adults and children, and 10-20% of acute bronchitis in adults (2, 3, 4). It also causes sinusitis, primary pharyngitis, and may trigger for asthma (5). Most of infections with this micro-organism are in fact subclinical and asymptomatic and only rarely cause on overt disease (3). Chronic infection with C. pneumoniae has been suggested as a factor in the development of atherosclerosis (6, 7).

Seroepidemiologic studies (8, 9, 10, 11) in different populations suggest that the seroprevalence increases sharply in young children and adolescence. After adolescence the seroprevalence continues to increase and may achieve almost complete saturation for IgG and IgA-class antibodies in the senescence (11).

To date, most investigations have relied on serologic diagnosis. Early studies have been performed with a complement fixation (CF) test which is genus-specific and is more likely to be positive in initial infection than during reinfection (8). The microimmunofluorescence (MIF) method is species-specific, but requires a skilled interpreter and is not suitable for automation and high volume testing. Those technical problems are avoided with the EIA methods developed by CD, providing easy, fast and objective tests.

Principles of Testing

The principle of the Chlamydia pneumoniae IgG EIA kit is based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as a marker enzyme. The assay proceeds according to the following reactions.

1. Chlamydia pneumoniae IgG antibodies from the patient sample bind to Chlamydia pneumoniae antigen attached to the polystyrene surface of the microplate wells.
2. Residual patient sample is removed by washing and horseradish peroxidase conjugated anti-human IgG (sheep) is added.
3. Unbound conjugate is washed off and a colorless enzyme substrate (H_2O_2) containing the chromogen (TMB, Tetramethylbenzidine, a non-mutagenic chromogen for horseradish peroxidase) is added. The enzyme reaction with the chromogen results in a colored end product.
4. The color formation reaction is terminated by adding acid (H_2SO_4). The color intensity is directly proportional to the concentration of Chlamydia pneumoniae antibodies in a patient sample.

Reagents And Materials Provided

Note:

- Reagents are stored between 2°C and 8°C.

- The expiration date is printed on each component label and on the package. Do not use reagents after the expiration date.

- Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB-substrate solution, the latter one is packaged in non-transparent plastic vials for protection.

1. **MICROSTRIPS** 12 X 8 wells

Coated Microstrips.

2. **SAMPLE DILUENT** 100 mL

Phosphate buffered saline with additives, a blue coloring reagent, and 0.05% Bronidox as preservative.

3a . **CALIBRATOR** (EIU=130) 1.0 mL

Diluted human serum with 0.05% Bronidox as preservative and a red coloring reagent. Potential biohazardous material.

3b . **BORDERLINE CONTROL** 1.0 mL

Diluted human serum with intermediate amount of the antibodies, containing 0.05 % Bronidox as a preservative and a red coloring reagent. Potential biohazardous material.

3c . **POSITIVE CONTROL** 1.0 mL

Diluted human serum with high amount of the antibodies, containing 0.05 % Bronidox as a preservative and a red coloring reagent. Potential biohazardous material.

4. **CONJUGATE** 30 mL

Buffered salt solution with additives, a red coloring reagent, horseradish peroxidase conjugated antihuman IgG (sheep) with 0.1% N-Methylisothiazolone as preservative.

5. **TMB-SUBSTRATE SOLUTION**, ready to use 18 mL

Citrate buffered solution of 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide with additives and 0.01% Kathon CG as preservative.

6. **STOPPING SOLUTION** 25 mL

0. 45 M H_2SO_4

7. **WASHING SOLUTION** 100 mL

Concentrated citrate buffered saline, with additives, and 0.05 % Bronidox as preservative.

INCUBATION COVERS 2 pcs

REAGENT BASINS 6 pcs

Materials Required But Not Supplied

1. Distilled or deionized water, preferably sterile.

2. Graduated cylinders for reagent dilution.
3. Vials to store the diluted reagents.
4. Precision pipettes (one channel eg. 0.5-10 µL, 5-50 µL, 20-200 µL, 100-1000 µL ranges and multi-channel 50-300 µL)
5. Paper towels or absorbent paper.
6. Timer, 60 min range.
7. Microplate incubator
8. Microplate photometer, 450 nm
9. Microplate washer (not compulsory)
10. Sodium hypochlorite solution, free available chlorine 50-500 mg/L.
11. Disposable gloves.

Storage

Reagents are stored between 2°C and 8°C.

Specimen Collection And Preparation

Serum and plasma samples should be refrigerated (4°C) after collection or, if the test cannot be performed within 1 week, frozen (-20°C or preferably -70°C)

Samples should not be repeatedly frozen and thawed.

Do not use sodium azide as preservative because it inactivates horseradish peroxidase.

Heat activation of samples(+56°C, 30 min) may cause non-specific results.

Microbially contaminated, grossly hemolyzed or hyperlipemic samples may give erroneous results.

Long storage of samples (frozen over one year) may cause the formation of lipid aggregates. These aggregates may cause a non-specific result.

Chlamydia pneumoniae IgG, IgA and IgM EIAs can be performed from serum and plasma (EDTA, Li-heparin and Na-Citrate), however paired samples should be collected in the similar way.

Serum and EDTA plasma and heparin plasma results are comparable while results obtained with citrate plasmas may be lower due to the dilution of the plasma with the anticoagulant.

Reagent Preparation

1. Coated Microstrips: Ready for use, 6 months *
2. Sample diluent: Ready for use, 6 months *
3. Calibrator and controls: Ready for use, 6 months *
4. Conjugate: Ready for use, 6 months *
5. TMB-Substrate solution: Ready for use, 6 months *

Discard unused reagent from the reaction basin. A deep blue color present in the substrate solution indicates

that the solution has been contaminated and must be discarded.

6. Stopping solution: Ready for use, 6 months *
7. Washing solution concentrate (10x): 6 months *

Dilute the concentrate (vial 7) 1+9 (1:10) with distilled water, 1 month at 4°C or 1 week at room temperature

Once the microplate foil-package is opened it should be resealed tightly with the desiccant: fold the opened end a few times and seal air-tightly with tape over the whole length of the opening. The stability of the opened reagents is the maximum only if they are stored properly at 2°C to 8°C. High environmental temperature and contamination may decrease the stability.

Assay Procedure

PRELIMINARY PREPARATIONS:

- Bring the reagents and Microstrips to room temperature (20°C-25°C) before starting the assay.

- Prewarm the incubator to 37°C.

Prestep: Dilute the samples 1:101 in sample diluent

STEP I

1. Reserve 2 empty wells for the blank.
2. First pipette 10 µL of diluted samples
3. Then pipette in duplicate 10 µL of ready for use calibrator (vial 3a) and controls (vials 3b and 3c)
4. Pipette 100 µL of sample diluent into each well
5. Cover the plate and incubate 1 hr (±5 min) at 37°C (±1°C)
6. Wash 5 x 300-400 µL/well

STEP II

1. Add 100 µL conjugate solution (vial 4) into each well
2. Cover the plate and incubate 1 hr (±5 min) at 37°C (±1°C)
3. Wash 5 x 300-400 µL/well

STEP III

1. Add 100 µL TMB-substrate solution (vial 5) into each well
2. Incubate 30 min at RT (20°C-25°C) in dark

STEP IV

1. Add 100 µL stopping solution (vial 6) into each well
2. Measure the absorbances immediately at 450 nm /reference 620 nm (590 -690 nm)

NOTE:

The use of an 8-channel pipette device is recommended for improved efficiency and precision.

1. Dilute the sample 1:101 in sample diluent (5 µL sample and 500 µL of sample diluent).

Use of duplicates is preferable especially for the calibrator. **Mix well.**

The prediluted samples are stable at 4°C for at least 2 weeks. **Do not dilute calibrators or controls.**

2. **Avoid contamination:** When removing aliquots from the reagent vials, use aseptic technique to avoid contamination.

Use a new pipette tip for each sample. Pour needed amount of the **sample diluent, conjugate and TMB-substrate solutions into a disposable basin for reagents** (6 pieces supplied with the kit). **Discard any unused solutions; do not pour it back to the vials.**

Do not touch the walls of the wells with pipette tips when adding TMB-substrate.

3. Washing may be performed manually or with a washer. Washing solution is recommended to stay in wells for 15-30 seconds during each cycle. After the washing step tap the inverted microplate a few times on the paper towel.
4. The absorbance of the reagent blank has to be measured to check that it falls within the Quality Control values.

Calculation

Calculation of the Results

The results are expressed as enzyme immunounits (EIU). The kit is calibrated and scaled such a way that the EIUs correspond to the inverted titers of the CD's C. Pneumoniae IgG MIFA.

Use the formula for calculations:

EIU sample = 130 x (A sample - A blank)/(A cal - A blank)

where

A sample = absorbance of the sample

A blank = absorbance of the Blank

A cal = absorbance of the calibrators

Expression of results in EIU-values

Sample	Mean A at 450 nm	EIU
Blank	0.070	
Calibrator	0.759	
Borderline control	0.280	40
Positive control	1.186	211
Sample 1	0.250	34
Sample 2	0.977	171

Acceptance criteria

The results of the run are accepted when:

Expected absorbance units:

Blank: ≤ 0.150

Calibrator: 0.400–1.200

Expected EIU values:

Borderline control: 20-60

Positive control: 150-270

*) The reagent blank absorbance has already been subtracted from these values.

Interpretation Of Results

Negative: EIU < 30

Equivocal: 30 ≤ EIU ≤ 45

Positive: EIU > 45

Chlamydia pneumonia IgG EIA allows discrimination between acute and non-acute infections on the basis of seroconversion between paired serum samples (the 2nd sample taken in average after 2-4 weeks).

Acute infection:

When EIU values are below 130, a 1.5-fold or larger increase of EIU value with paired samples assayed in the same run indicates seroconversion.

When EIU values are above 130, a 1.3-fold or larger increase of EIU value with paired samples assayed in the same run indicates seroconversion.

CD's Chlamydia pneumoniae IgA and IgM EIAs provide additional information for the diagnosis of acute Chlamydia pneumoniae infection. In primary acute infection IgM response may be detected already in the first serum sample, while the IgG response develops more slowly, especially if the patient has received antibiotics against Chlamydia infections.

Reinfection is typically characterized by a rapid IgG and IgA response.

Non-acute infection:

Stable or decreasing levels of IgG and/or IgA with negative or equivocal IgM may indicate one of the following: past infection, recent infection, cured condition or persistent infection.

Performance Characteristics

Comparison of the four serological methods for detection of acute C. pneumoniae infection

	MIF (%) in-house	EIA1 (%) competitive	EIA2 (%) competitive	EIA(%) CD
Sensitivity	93/106 (88)	92/106 (87)	97/106 (92)	102/106 (96)
Specificity	133/134 (99)	132/134 (99)	127/134 (95)	133/134 (99)
PV pos	93/94 (99)	92/94 (98)	97/104 (93)	102/103 (99)
PV neg	133/146 (91)	132/146 (90)	127/136 (93)	133/137 (97)

The study shows that out of the 106 cases that were interpreted as acute infection by at least 2 methods, competitor's EIA 1 misinterpreted 14 cases, competitor's EIA2 9 cases, whereas CD's EIAs only 4 cases.

Specificity

Sixteen available Chlamydia trachomatis IgG positive but Chlamydia pneumoniae IgG negative sera (confirmed by the CD MIFA IgG) did not show cross-reactivity.

Paired samples (n = 20) from infants and a few adults with proven by isolation Bordetella pertussis infection were analyzed in the EIA method. The cases were interpreted as negative for ongoing Chlamydia pneumoniae infection.

Reproducibility

Within-run reproducibility

Samples	Dilution replicates	EIU	CV%
Sample 1	10	31.7	4.1
Sample 2	10	100.4	3.6
Sample 3	10	119.2	4.7

Between-run reproducibility

Samples	Dilution replicates	Operators	Total runs	EIU	CV%
Sample 1	4	5	10	30	15.4
Sample 2	4	5	10	53	10.9
Sample 3	4	5	10	87	15.0
Sample 4	4	5	10	145	8.4

Summary of the evaluation studies

Paired serum samples collected during the outbreak of C. pneumoniae epidemics in 1995 in Sweden were analyzed for the seroconversion. The rate of seroconversion detected by CD methods was compared to the respective rates by a competitor's EIA's. an in-house MIFA and an in-house complement fixation methods. Seroconversion of IgG and IgA values and/or positive IgM was interpreted as an acute C. pneumoniae infection by CD methods.

Originally, the samples were grouped as:

- positive pairs, meaning acute primary or reinfection (n = 106)

- negative pairs, meaning no infection or past infection (n = 134)

Precautions

For in vitro diagnostic use only.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL:

All human materials used in the preparation of the calibrators/controls in the kit have been tested for the presence of the antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as Hepatitis B surface antigen (HBsAg) and found to be non-reactive. Because no test method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent, these calibrators and controls as well as samples should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood sample in the Centers for Disease Control/National Institutes for Health Manual, "Biosafety in Microbiological and Biomedical Laboratories" 2007 (14).

Discard all materials and samples as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/L free available chlorine. Allow 30 minutes for decontamination to be completed. Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal.

NOTE:

- Reusable glassware must be disinfected, washed out and rinsed free of detergents.

- Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite. Stopping solution (vial 6) contains 0.45 M sulfuric acid, avoid contact with skin and eyes.

- Wear disposable gloves while handling samples and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.

- Do not mix or interchange plates, controls, calibrators or conjugates from different lots of this product. Do not interchange vial caps.

- Once the assay has been started, all subsequent steps should be performed without interruption. Do not let the wells dry once the assay has been started.

- Do not reuse a strip of the microplate even if some wells were not used.

- Accurate and precise pipetting, as well as following the exact time and temperature requirements, is essential.

Limitations

Because no single method leads to the definitive diagnosis, the results of the present method should be interpreted in conjunction with the clinical condition, epidemiological situation and other laboratory methods.

A sample obtained during the acute phase of infection, when only IgM antibodies are present, may be negative by the determination of IgG and/or IgA antibodies. In some occasions acute infection will not induce antibody response (12). Non-responders are, however, rare.

The possible interference of the rare cases of Chlamydia psittaci infections cannot completely be ruled out. However, since both infections require similar medication, this limitation does not lead to mistreatment of the patient.

The present method is optimized against the CD's Chlamydia pneumonia IgG MIF. Since the MIF methods in general are subjective, and variable readings are obtained in different laboratories, **there is no systematic correlation between CDs EIU values and laboratories' in-house or other commercial MIF methods.**

The samples with absorbance higher than of the Positive control should be prediluted more than 1:101 (e.g. 1:201 ... 1:401 ... 1:801) to obtain results in the linear portion of the curve. The calculated EIU result is multiplied by predilution factor (e.g. 2… 4 or 8). **It is important that paired samples are diluted and assayed simultaneously.**

It is recommended that the assay is performed by qualified and trained laboratory technician.

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