Product no. 61 08 380
Product no. 61 08 382

Instructions for use

For *in vitro* diagnostic use only

Chlamydia pneumoniae IgG / IgM Micro-IF Test kit

An indirect microimmunofluorescence test for detecting antibodies against *Chlamydia pneumoniae* in human serum.

Product no. 61 08 380 (20x21well slides)
Product no. 61 08 382 (20x12well slides)

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INTENDED USE

Ani Labsystems' Chlamydia pneumoniae IgG/IgM micro-IF test has been developed for the detection of antibodies against *C. pneumoniae* in human serum.

INTRODUCTION

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). Epidemic cycles of *C.pneumoniae* infection depends on the density of the population, and was reported to occur in 4-7 years' intervals (8).

To date most investigations have relied on serologic diagnosis, using modifications of a microimmuno-fluorescence (MIF) test (8). Early studies have been performed with a complement fixation (CF) test, which has been used for many years for the detection of psittacosis. This test is genus-specific and is more likely to be positive in initial infection than during reinfection (8).

All three C*hlamydia* species known to cause human infections, *C. pneumoniae, C. trachomatis* and *C. psittaci*, have species and subspecies specific antigens as their surface components. In addition they share a major genus specific lipopolysaccharide (LPS) antigen.

C. pneumoniae elementary bodies (EBs), which are the infective cell forms of *C. pneumoniae*, are used as antigen in Ani Labsystems´ C. pneumoniae IgG/IgM micro-IF test. C. *trachomatis* and *C. psittaci* antigens (EBs) are included in the test as controls.

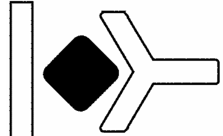
In order to diminish cross-reactions between different *Chlamydia* species the immunological activity of chlamydial LPS in *C. pneumoniae* and *C. trachomatis* antigens has been specially reduced. As LPS in *C. psittaci* antigen has not been subjected to any treatment, this antigen serves as a control for both LPS- and *C. psittaci* positive sera.

For a reliable diagnosis of *C. pneumoniae* infection it is recommended that antibodies of the main immunoglobulin classes, IgG, IgM and IgA, in paired serum samples should be determined. In a study with adult pneumonia patients it has been shown, that one fifth of *C. pneumoniae* diagnoses would have been missed without IgA determinations (12). In literature IgA has been considered a marker of chronic chlamydial infection (13, 14, 15). Along with the Chlamydia pneumoniae IgG/IgM micro-IF test Ani Labsystems also produces Chlamydia pneumoniae IgA micro-IF test kit (product no. 61 08 390 or 61 08 392).

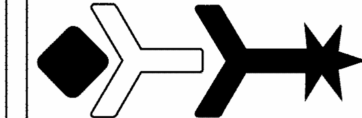
PRINCIPLE OF THE TEST

This test is based on an indirect detection of IgG/IgM antibodies against *C. pneumoniae* using fluorescein isothiocyanate (FITC) as the marker compound.

When present in patient serum, *C. pneumoniae* antibodies ( ) will combine with *C. pneumoniae* antigens ( ), attached to the glass surface ( ) of a microscopic slide:



The residual patient sample is removed by washing and fluorescein conjugated anti-human antibodies are added:



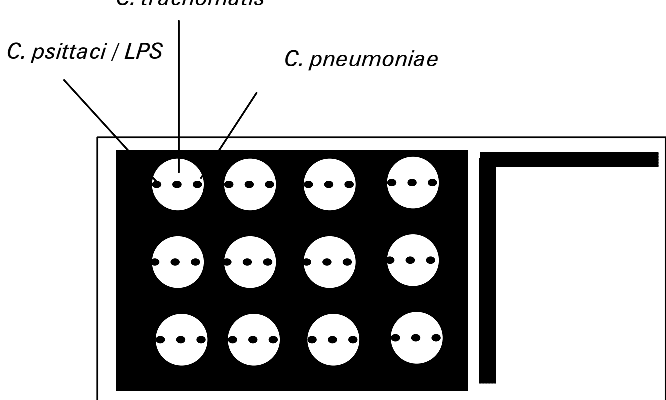
The slide is washed and green fluorescence is detected microscopically.

KIT CONTENTS

- For *in vitro* diagnostic use only.
- Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully.
- Each kit contains 20 microscopic slides with 21 wells (Product no. 61 08 380) or 12 wells (Product no. 61 08 382) dotted with three chlamydial antigens (*C. pneumoniae*, *C. trachomatis* and *C. psittaci*).
- Reagents are sufficient for testing 100 sera (20 x 21 well slides) or 55 sera (20 x 12 well slides) for both IgG and IgM (three dilutions for IgG and one dilution for IgM). The amounts of reagents for kits containing 20 x 12 well slides are indicated below in the parenthesis.
- Store the reagents and the slides between +2°C and +8°C. Avoid excessive exposure to light because FITC-conjugates are light sensitive.
- The expiration date is printed on each component and on the package.
- **Once opened, the components must be sealed tightly!**

1. ANTIGEN DOTTED SLIDES 20 x 21 well slides, (20 x 12 well slides)

Microscopic slides dotted with inactivated *C. pneumoniae*, *C. trachomatis* and *C. psittaci* elementary bodies.



Note: When viewed through the microscope, the antigens are usually in reversed order as microscopes usually reverse the image (see page 6).

2. SAMPLE DILUENT, 50 ml
Phosphate buffered saline, pH 7.4 ± 0.2, proprietary additives and 15 mM sodium azide as preservative.

3a. *C. pneumoniae* IgG POSITIVE CONTROL (HUMAN) , 0.30 ml
Cap color: **black.**
Diluted human serum, proprietary additives and 15 mM sodium azide as preservative.

3b. *C. pneumoniae* IgM POSITIVE CONTROL (HUMAN), 0.30 ml
Cap color: **red.**
Diluted human serum, proprietary additives and 15 mM sodium azide as preservative.

4. *C. pneumoniae* NEGATIVE CONTROL (HUMAN) , 0.30 ml
Cap color: **white.**
Diluted human serum, proprietary additives and 15 mM sodium azide as preservative.

5a. ANTI-HUMAN IgG-FITC-CONJUGATE (RABBIT) 2 x 2.5 ml (1 x 2.5 ml)
Cap color: **black.**
FITC conjugated anti-human IgG, 0.001 % Evan's Blue counterstain, proprietary additives and 15 mM sodium azide as preservative.

5b. ANTI-HUMAN IgM-FITC-CONJUGATE (RABBIT) , 1.5ml
Cap color: **red.**
FITC conjugated anti-human IgM, 0.001 % Evan's Blue counterstain, proprietary additives and 15 mM sodium azide as preservative.

6. MOUNTING FLUID, 7 ml
Glycerol, proprietary additives and 15 mM sodium azide as preservative.

EXTRA DROPPER BOTTLE (for mounting fluid, 3 ml), 1 set
1 transparent dropper bottle
1 plug
1 cap (yellow)

COVER SLIPS (for microscopic slides), 30 pcs

REAGENT PREPARATION

Reagent	Preparation	Stability of opened reagents (+2°C to +8°C)
1 Antigen dotted Slides	Ready for use **Slide foil-package must be prewarmed to room temperature before opening**	3 months **Slide foil-package must be immediately air-tightly resealed with desiccant**
2 Sample diluent	Ready for use	8 months
3a *C.pneumoniae* IgG Positive Control	Ready for use	8 months
3b *C.pneumoniae* IgM Positive Control	Ready for use	8 months
4 *C. pneumoniae* Negative Control	Ready for use	8 months
5a Anti-human-IgG- FITC-Conjugate	Ready for use	8 months
5b Anti-human-IgM- FITC-Conjugate	Ready for use	8 months
6 Mounting fluid	Ready for use	8 months

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- 0.01M Phosphate buffered saline (PBS), pH 7.4.
- Micropipettes of different sizes (eg. one-channel eg. 5-50 μl, 20-200 μ, 100-1000 μ ranges and multi-channel 50-300 μl)
- 96-well microdilution plates
- Moist incubation chamber (A wad of wet paper towels or absorbent paper in a plastic box).
- Paper towels or absorbent paper.
- Timer, 30 min range.
- Incubator, +37°C.
- Laboratory dishes (plastic or glass).
- Carrier and racks for microscopic slides.
- Confirmation reagent for confirmation of IgM positive samples, e.g. Ani Labsystems IgG blocking reagent (code 6106020).
- Fluorescence microscope with filter system for fluorescein isothiocyanate (FITC), i.e. maximum excitation wavelength 490 nm, mean emission wavelength = 520-530 nm.
- The immersion oil for microscopy having nd 1.516 (non-fluorescence) eg. Olympus.

PRECAUTIONS

For *in vitro* diagnostic use only.

Warning - Potential biohazardous material

Microscopic slides have been dotted with inactivated *C. pneumoniae*, *C. trachomatis* and *C. psittaci* elementary bodies, but because the used inactivation method cannot offer complete assurance that the infectious agents are absent, these antigen dotted slides must be handled as potential infectious material.

Each donor unit used in the preparation of the control sera in the kit has been tested for the presence of the antibody to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as for hepatitis B surface antigen (HBsAg) and found to be non-reactive. Because no test method can offer complete assurance that HIV, hepatitis B and C virus, or other infectious agents are absent, these controls as well as specimens should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”, 1999.

The conjugate contains Evan’s Blue dye, which is a possible carcinogen. Contact with skin should be avoided even though the concentration of the dye is very low.

Never pipette by mouth. Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully. Avoid splashing or forming aerosols.

Take care in discarding the reagents containing sodium azide. Azides are reported to react with lead and copper in plumbing to form compounds that may detonate upon percussion. When disposing of solutions containing sodium azide, flush with large volumes of water. Please refer to precautions and decontamination procedures as outlined by the National Institution for Occupational Safety and Health (16).

Discard all materials and specimens as if capable of transmitting infection (17, 18 19, 20). 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.

Note: Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.

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 (17, 18, 19, 20). Reusable dishes must be disinfected, washed out and rinsed free of detergents.

Reagents should be stored between +2°C and +8°C. Storage of reagents and samples in self-defrosting freezers is not recommended. Avoid unnecessary exposure to light, because the FITC-conjugate is light sensitive.

Do not use reagents after the expiration date printed on each component and on the package.

Do not mix or interchange reagents from different lots. Cross contamination of reagents or samples could cause erroneous results. Do not interchange vial caps. Use a new pipette tip for each sample.

Optimal results will be obtained by strict adherence to the test protocol. Accurate and precise pipetting are essential. Once the assay has been started, all subsequent steps should be performed without interruption.

SPECIMEN COLLECTION AND HANDLING

Blood is collected aseptically by venipuncture, and the blood is allowed to clot in a sterile tube. The serum is separated by centrifugation. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are refrigerated (+4°C) upon collection or, if the test cannot be performed within 48 hours, frozen (-20°C).

Samples should not be repeatedly frozen and thawed.

Long storage of the serum (over one year in a frozen stage) and repeatedly performed freezing and thawing may cause lowering of the IgM antibody level. The IgG antibody level should not be affected by these procedures.

Microbially contaminated, grossly haemolyzed, icteric or hyperlipemic sera may give erroneous results.

TEST PROCEDURE

OUTLINE OF THE PROCEDURE

STEP 1
Add 10 µl serum dilutions and one drop of undiluted control sera on slides.

Incubate in a moist chamber at +37°C:
IgG slides for 30 minutes and IgM slides for 3 hours. Procede with the IgG slides during the IgM incubation.

Wash slides and let dry completely

STEP 2
Add one drop of the conjugate

Incubate slides at +37°C for 30 minutes in a moist chamber

Wash slides and let dry completely

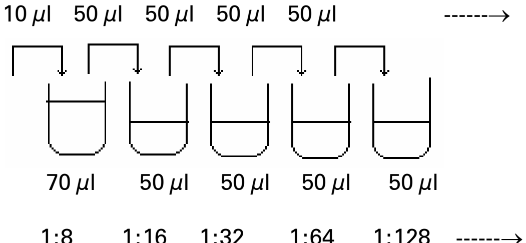
STEP 3
Add the mounting fluid on slides and place cover slips.

Read slides using fluorescence microscope.

PRELIMINARY PREPARATIONS

– Allow the reagents and patient sera to reach room temperature.
– Allow the slides to reach room temperature before the foil-package is opened to prevent condensation. Take the needed amount of slide foil-packages out of the kit box to room temperature about 30 minutes **before opening the foil-packages**
– Put the slides not needed immediately back into the small plastic slide-box and into the foil-package with the dry bag, and **close the foil-package tightly** .
– Prepare a moist incubation chamber
– Before use mix well the reagents by inverting the vials.
– Handle the slides with care to avoid scratching of the antigen dots.

PREPARATION OF SERUM DILUTIONS



1. Prepare 1:8 dilutions of serum samples in sample diluent (70 µl of sample diluent + 10 µl of serum). Use the first wells of rows 1 to 12 on a 96-well

microdilution plate. Mix thoroughly when pipetting. Incubate 10 minutes at room temperature.

Note: Do not dilute the controls.

Note: Before testing IgM antibodies, IgG antibodies are recommended to be blocked to exclude false positive results due to rheumatoid factor (RF). Dilutions are made as follows: dilute IgM samples 1:8 in IgG blocking reagent (e.g. Ani Labsystems' product no. 61 06 020), incubate at RT for 15 minutes and then dilute1:2 in sample diluent (= 1:16, final dilution). See also page 9.

If IgG blocking reagent is used, separate serum dilutions are needed for IgG test. It is also possible to confirm IgM-reactive samples afterwards with the IgG blocking reagent.

2. For each two-fold serum dilution pipet 50 μl of sample diluent in a microdilution plate well. Prepare desired dilutions (50 μl diluted serum +50 μl sample diluent) starting from 1:8 dilution.

If you cannot continue the test procedure right after preparing the serum dilutions, cover the microplate tightly with a plastic sheet and store it at +4°C. The serum dilutions should be used within 24 hours.

PIPETTING OF SERUM DILUTIONS AND CONTROLS ON SLIDES

1. The microscopic slides have wells with three chlamydial antigen dots placed in every well. Before pipetting, it is advised to mark the frosted ends of the slides with pencil.

In one test run two wells are needed for the controls: *C. pneumoniae* positive control and a negative control.

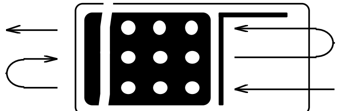
2. Pipet 10 μl of serum dilutions and add a drop of positive and negative control serum to their places by gently touching the slide with the drop falling from the tip of the bottle.

The IgG and IgM determinations should be made on separate slides.

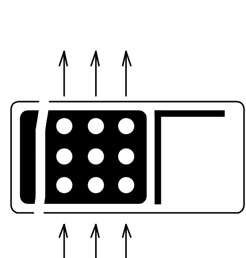
Following serum dilutions are recommended for determination of different immunoglobulin classes:

IgG 1:32, 1:128, 1:512
IgM 1:16

Microscopic reading of the slides will be easier if you pipet serum samples in the following order:



or



To avoid mistakes start pipetting from the last well of the slide. You do not have to change the pipette tip within dilutions of one serum if you start pipetting from the highest dilution (e.g. 1:512).

INCUBATION

Place the slides in a moist chamber, but avoid tilting the slides. Incubate the IgG slides for 30 minutes and the IgM slides for 3 hours at +37°C.

The procedure for the IgG slides is continued during the IgM incubation.

WASHING THE SLIDES

Fill four dishes with 0.01 M PBS, pH 7.4 and two dishes with distilled water. The volume in every dish should be enough to cover the slides in a slide carrier completely.

Step 1.

Rinse all the slides separately in the first PBS dish by lifting and lowering the slides a few times. Gently tap the slide against a wad of paper towels to remove excess liquid. Place the slide in a slide carrier.

Step 2.

Step 2 can be performed alternatively by using method A or B.

Method A
Place the carrier without delay into the second PBS dish. Lift and lower the carrier about 20 times. Take the carrier out of the dish and place it to the next PBS dish. Repeat until you have gone through all the four PBS dishes and the two dishes with distilled water. Gently tap the carrier a few times against a wad of paper towels.

Method B
Place the carrier without delay into the second PBS dish for 10 minutes. Take the carrier out of the dish and gently tap it a few times against a wad of paper towels. After that place the carrier into distilled water dish. Rinse all the slides in the water by lifting and lowering the carrier gently about 10 times.

Step 3

Take the slides out of the carrier, tap them against paper towels and let them dry properly (preferably in an upright position at +37°C).

Note: Washing the slides is critical and should be performed with care.
Note: Make sure that the slides are completely dry after the washing

ADDING THE CONJUGATE

1. Add anti-human IgG-FITC or anti-human IgM-FITC conjugate to the wells of corresponding slides by gently touching the slide with the drop falling from the tip of the bottle. A drop size roughly equal to that of serum dilutions (10 μl) will be enough.

   Note: Do not let the drop fall freely from the dropper bottle.

2. Place the slides in a moist chamber and incubate at +37°C for 30 min. (Maximum incubation time is 35 min.)

3. Wash and dry the slides as after serum incubation.

ADDING THE MOUNTING FLUID

Use a dropper bottle specially provided in the kit for the mounting fluid. Place the slide on soft paper and add 4-5 drops of mounting fluid evenly over the wells. Place a cover slip carefully on the slide. Try to avoid bubbles. After the mounting fluid has spread out evenly, put the slide in an upright position and let soft paper absorb excess fluid. (The amount of mounting fluid is optimal when the cover slip sticks onto the slide when you lift it up.)

If necessary, slides can be stored in dark between +2°C and +8°C for 24 hours. However, storing may lead to fading of the fluorescence to some extent, and consequently to lower serum titers.

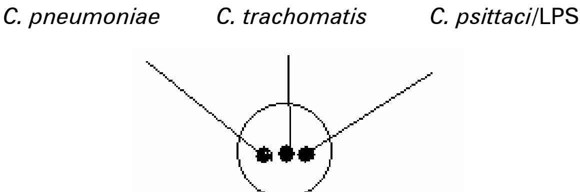
MICROSCOPY

Recommended combination for routine reading of samples is 10x oculars with 50x objective with oil having nd 1.516 (non-fluorescence; 500x magnification). For confirmation and closer examination of elementary bodies a combination of 10x oculars and 100x objective with oil (1000x magnification) is recommended.

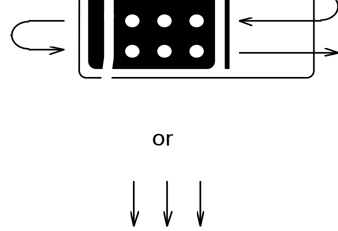
Fluorescent intensity and endpoint titers depend on the type of microscope, microscope optics and fluorescence light source condition.

When the frosted end of the slide is on the right side, the three antigen micro-dots will show up under the microscope as follows (because a microscope usually reverses the image):

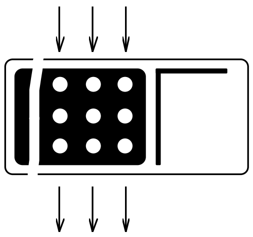
C. pneumoniae *C. trachomatis* *C. psittaci*/LPS



It is recommended to read the slide in the following order, starting from the lowest dilution(e.g.1:32):



or



RESULTS

Examples of the positive and negative reactions are shown in the separate *C. pneumoniae* interpretation chart (enclosed).

POSITIVE REACTION

C. pneumoniae

In positive reactions *C. pneumoniae* fluorescent elementary bodies are all the same size, stained evenly (Fig 1) and seem to be “coin-shaped” or “ring shaped”. Only few fluorescent particles are indicating unspecific reaction - density of the EBs should be similar to the positive control dot.

Positive IgM antibodies may show more uneven and patchy reaction (Fig 2). This type of reaction is considered positive also for IgG at the very beginning of the immune response development against C. pneumoniae, but the pattern will get even with time. A second sample is needed to detect a titer rise.

The *C. pneumoniae* positive controls show the fluorescence intensity that is considered to be strong positive.

<u>*C. trachomatis*</u>

Positive *C. trachomatis* antigen reaction resembles that of *C. pneumoniae.*

<u>*C. psittaci/LPS*</u>

Positive LPS reaction does not resemble specific chlamydial reactions because elementary bodies are covered with LPS. Also the typical reaction of very small fluorescent particles can be seen (Fig 4).

NEGATIVE AND NON-SPECIFIC REACTION

The reaction is considered to be negative for *C. pneumoniae*, if no distinct elementary bodies are seen in the *C. pneumoniae* antigen dot (Fig 5).

Titers less than 1:32 in IgG and less than 1:16 in IgM suggest that the patient does not have infection.

In chlamydial infections antibodies against LPS will appear first. A sample taken just after the onset of the infection will most likely contain antibodies against LPS, but species-specific antibodies have not had enough time to develop.

If the serum sample contains high levels of antibodies against chlamydial LPS, the *C. psittaci*/LPS antigen will show a strong positive reaction and the *C. pneumoniae* and/or *C. trachomatis* antigen dots may show fluorescent green reaction with no elementary bodies (Fig 6). This kind of diffuse fluorescence is in most cases due to LPS, which in spite of the reduced activity reacts with sera with high LPS antibody levels. Sometimes the greenish LPS reaction disappears with higher serum dilutions, and elementary bodies can be seen. If not, a second serum after 2-4 weeks is needed to find out if any specific antibodies have been formed.

INTERPRETATION OF THE RESULTS

C. PNEUMONIAE

<u>The positivity threshold titer:</u>
- IgG $\geq$ 1: 32
- IgM $\geq$ 1:16

With properly timed paired serum samples (2-4 weeks), the micro-IF method allows discrimination between acute and non-acute infection on the basis of IgG seroconversion and/or IgM positivity.

<u>Acute infection:</u>
- Fourfold titer change of IgG
- IgM titer $\geq$ 1: 16
- IgG titer $\geq$ 1:512 (suggestive)

In primary acute infection IgM response may be detected already in the first serum sample, while the IgG and IgA responses develop more slowly, especially if the patient has received antibiotics against chlamydia infections. Reinfection is typically characterised by a rapid IgG and IgA response.

Ani Labsystems' Chlamydia pneumoniae IgA micro-IF test provides additional information for the diagnosis of acute *Chlamydia pneumoniae* infection.

<u>Non-acute infection:</u>
Stable or decreasing end point titers of IgG and/or IgA with negative IgM may indicate one of the following: past infection, recent infection, cured condition or persistent infection.

C. TRACHOMATIS

The dot with *C.trachomatis* antigen serves as a negative control to facilitate correct judgement regarding *C.pneumoniae* seropositivity. Some samples may be reactive with *C.trachomatis* antigen, however due to long persistence of antibodies no clinical judgement should be drawn based on these results.

C. PSITTACI

The dot with *C.psittaci* antigen in which LPS is not removed serves as a control for seropositivity to chlamydial lipopolysaccharide (LPS).

CHLAMYDIAL INFECTION GUIDE

	Antigen		
	C. pneumoniae	***C. trachomatis***	***C. psittaci* / LPS**
***C.pneumoniae* infection**	+	no or steady IgG or IgA	+/-
Suspect of *C. trachomatis* infection	no or steady IgG or IgA	+	+/-
Early *Chlamydia* infection or suspect of *C. psittaci* inf.	no or steady IgG or IgA	no or steady IgG or IgA	+

PERFORMANCE CHARACTERISTICS

EVALUATION STUDIES

Paired (n=16) and single (n=99) serum samples from 115 patients with suspected chlamydial infection were tested with Ani Labsystems' Chlamydia pneumoniae IgG/IgM and IgA micro-IF tests and with non-commercial Chlamydia pneumoniae IgG, IgM and IgA micro-IF tests used at the Department of Virology, Haartman Institute, University of Helsinki, Finland.

The principle of these non-commercial C.pneumoniae micro-IF tests was similar to that of Ani Labsystems' tests,

except that untreated *Chlamydia pneumoniae* elementary bodies were used as the antigen. At least fourfold titer change (IgG, IgA, IgM) and/or a positive IgM test result (IgM titer > 1:16) with the non-commercial comparison method indicated a current *C.pneumoniae* infection.

Summary of the results is presented in Table 1.

Table 1. Summary of the evaluation results of Ani Labsystems' Chlamydia pneumoniae micro-IF tests.

		Ani Labsystems' micro-IF test		
		+	–	N
Non-commercial micro-IF test	+	30 *)	0	30
	–	3 **)	82 **)	85
	N	33	82	115

*) 28 of these samples were IgM-positive in both assays. One of these IgM positive cases showed also a fourfold IgG titer change in both assays. Six of these IgM-positive samples had high IgG titers (≥1:512) in the comparison method; IgG titers of these specimens in Ani Labsystems' C. pneumoniae micro-IF test was as follows: ≥1:512 (n=2), 1:256 (n=2), 1:128 (n=1) and 1:32 (n=1).
2 of these samples were considered to be positive due to the fourfold IgA titer change in both assays.

**) 3 samples which were considered to be positive in Ani Labsystems assay (IgG titer ≥ 512) and 2 samples out of 82 negative samples were only suspected to be positive (IgG titer ≥ 512) in the non-commercial assay.

Paired (n=3) and single (n=29) serum samples from 32 patients with serologically diagnosed or suspected *Chlamydia pneumoniae* infection were tested with Ani Labsystems' Chlamydia pneumoniae IgG/IgM and IgA micro-IF tests and with another commercial IF-test for the detection of IgG-, IgM- or IgA-glass antibodies against *Chlamydia pneumoniae* in human serum. Summary of the results is presented in Table 2.

Table 2. Summary of the evaluation results of Ani Labsystems' Chlamydia pneumoniae micro-IF tests.

		Ani Labsystems' micro-IF test		
		+	–	N
Commercial micro-IF test	+	25 *)	0	25
	–	2	5	7
	N	27	5	32

*) 25 samples were IgM-positive in both assays. One of these IgM positive cases showed also a fourfold IgG titer change in both assays. Two of these IgM-positive samples had also high (>1:512) IgG titers in Ani Labsystems' C. pneumoniae micro-IF test; IgG titers of these specimens in the commercial C. pneumoniae micro-IF test was as follows: ≥1:512 (n=1), 1:256 (n=1).

Ani Labsystems' Chlamydia pneumoniae IgM micro-IF test was also evaluated against a commercial assay by another laboratory. The sensitivity of Ani Labsystems' IgM test was 86 % (6/7) versus competitor's sensitivity of 57 % (4/7). Reader to reader agreement of Ani Labsystems' IgM test was 97 % versus competitor's agreement of 74%.

WITHIN-RUN REPRODUCIBILITY
Within-run reproducibility was tested by using 4 sera with variable levels of Chlamydia specific antibodies. Each serum was diluted individually 10 times. The overall within-run reproducibility was excellent:
-in Chlamydia pneumoniae IgG MICRO-IF test each of the four sera gave the same result in 10 parallel samples resulting in reproducibility of 100 % (40/40),
-in Chlamydia pneumoniae IgM MICRO-IF test each of the four sera gave the same result in 10 parallel samples resulting in reproducibility of 100 % (40/40),
-in Chlamydia pneumoniae IgA MICRO-IF test each of the four sera gave the same result in 10 parallel samples resulting in reproducibility of 100 % (40/40)

BETWEEN-RUN REPRODUCIBILITY
Between-run reproducibility was tested with 4 samples representing variable levels of Chlamydia specific antibodies. The samples were tested by 3 operators in total of 10 consecutive runs. Each run was performed in quadruplicates. For each quadruplicate sample dilutions were prepared separately. Microscopic reading of the samples was performed by one operator.
-In Chlamydia pneumoniae IgG MICRO-IF test 144 out of 160 samples (90 %) in different runs gave the same result.
-In Chlamydia pneumoniae IgM MICRO-IF test 152 out of 160 samples (95 %) in different runs gave the same result
-In Chlamydia pneumoniae IgA MICRO-IF test 147 out of 160 samples (92 %) in different runs gave the same result

LOT-TO-LOT REPRODUCIBILITY
Lot-to-lot reproducibility was followed in Chlamydia pneumoniae IgG, IgM and IgA MICRO-IF tests by using sample which was titrated to the endpoint of corresponding Chlamydia pneumoniae antibodies.
-In IgG MICRO-IF test the titer of sample was within same dilution in 20 lots out of 23 (87 %)
-In IgM MICRO-IF test the titer of sample was within same dilution in 40 lots out of 45 (89 %).
-In IgA MICRO-IF test the titer of sample was within same dilution in 39 lots out of 44 (89 %).

CROSS-REACTIVITY
No cross-reactivity has been found with the following micro-organisms:

Cytomegalovirus
Epstein-Barr virus
Toxoplasma gondii
Bordetella pertussis
Mycoplasma pneumoniae

LIMITATIONS OF THE PROCEDURE

– A negative test result does not exclude the possibility of exposure to or infection with *Chlamydia pneumoniae*

– The presence of rheumatoid factor (IgM-class antibody against human IgG) may lead to false positive IgM reaction. That is why positive IgM results should be confirmed after absorbing IgG antibodies with e.g. Ani Labsystems IgG blocking reagent (cat. no 610 6020). On the other hand, the presence of *C.pneumoniae* specific IgG antibodies may directly interfere detecting IgM antibodies. Use of reagent for inactivation of IgG (e.g. above mentioned IgG blocking reagent) usually makes the IgM reaction stronger and easier to read.

– Long storage of the serum (over one year in a frozen stage), heat inactivation and repeated freezing and thawing may cause lowering of the antibody level.

– Deteriorated serum samples may cause lowering of the antibody level and may hamper the interpretation.

– Because the interpretation of the titer is by microscopy and thus subjective to individual interpretation, the microscopic slides should be read by an experienced person.

– The diagnosis of *C. trachomatis* or *C. psittaci* infection is not recommended to be made with this test, but the results can be considered indicative.

– Results should be considered in the context of all available clinical and laboratory data.

TROUBLE SHOOTING

Fluorescence seems to be dim.

-Do not leave the slide foil-package open. It must be tightly closed with the desiccant, because moisture may weaken the reaction.
-Insufficient incubation time for the conjugate might cause dim fluorescence. Always put the timer on only when you have pipetted the last slide.
-The conjugate is sensitive to light, so avoid unnecessary exposure to light after adding the conjugate.
-Make sure that the mounting fluid supplied in the kit is used.
-Check that you are using the 50x objective in the microscope.

Part of the antigen dot is loosened and washed off.

-Avoid moisture. Make sure that the slides reach the room temperature before opening the foil package, and immediately reseal the foil-package tightly with the desiccant
-Make sure that the pipette tip does not touch the antigen while pipetting samples and that the cover slip is not moved after placed on the slide.
-Try the more gentle method B in washing

Sample spreads out over the slide.

-Make sure that the slides stay dry until the samples are pipetted (see above the advice on avoiding moisture). Put the slides into the moist chamber only after pipetting the samples.

-Add the sample in the middle of the well, avoiding the red coating

Conjugate spreads out over the slide.

-Make sure that the slide, also the red coating, is completely dry after the washing, before dispensing the conjugate

-Remember in washing to always have rinsing with distilled water after PBS. Residual PBS may crystallize and cause the floating of the conjugate.
-Add conjugate in the middle of the well, avoiding the red coating

Slides look “dirty” with a greenish cover.

-The reason is most likely insufficient washing after serum or conjugate incubation. Keep the slides in the last PBS dish for 10 minutes.

Fluorescent (non-chlamydial) particles are seen on the slide.

-This is usually due to excess conjugate which has not been washed off completely.

The focusing of the slides is difficult.

-Try to avoid air bubbles when placing the cover slip on the slide.

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RELATED PRODUCTS AND ORDER INFORMATION:

Product number	Product description
61 08 380	C. pneumoniae IgG/IgM MIF (20x21 well slides)
61 08 382	C. pneumoniae IgG/IgM MIF (20x12 well slides)
61 08 390	C. pneumoniae IgA MIF (20x21 well slides)
61 08 392	C. pneumoniae IgA MIF (20x12 well slides)
61 08 384	C.pneumoniae MIF slides (5x21 wells)
61 11 300	Chlamydia pneumoniae IgG EIA
61 11 310	Chlamydia pneumoniae IgA EIA
61 11 320	Chlamydia pneumoniae IgM EIA
61 06 020	IgG blocking reagent

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