RIDASCREEN® Chlamydophila pneumoniae

REF  K2811 (IgA)
     K2821 (IgG)
     K2831 (IgM)
1. Intended use

For in vitro diagnostic use. The RIDASCREEN® Chlamydophila pneumoniae tests are
enzyme immunoassays for the determination of IgA, IgG or IgM antibodies against
Chlamydophila (C.) pneumoniae in human serum.
The tests should be used for confirmation purposes when there is a suspected case
of infection with C. pneumoniae or for clarifying the immune status.

2. Summary and explanation of the test

In the group of the Chlamydia, there are three human pathogens: Chlamydia
trachomatis, Chlamydia psittaci and Chlamydophila pneumoniae.
Until a few years ago, it was generally thought that Chlamydia pneumoniae was a
subspecies of Chlamydia psittaci. The existence of this third Chlamydia species was
established in 1989. More recent investigations have led to the formation of the
separate genus, Chlamydophila. Depending on factors which are specific to the host,
Chlamydophila pneumoniae can give rise to acute diseases of the upper respiratory
system, bronchi and lung. Research results suggest that Chlamydophila pneumoniae
plays a role in asthma, lung diseases and other chronic disorders. Chlamydophila
psittaci is a pathogen which is very common among birds and mammals. With
humans, it occurs only rarely and usually causes respiratory disorders.
The detection of antibodies is the preferred method of diagnosis for C. pneumoniae
infection. Antibodies which are highly specific against Chlamydophila pneumoniae
are detected by using species-specific COMP antigens (COMP = complexes of outer
membrane proteins).

3. Test principle

A coating of purified COMP antigens is applied to a microwell plate. Antibodies in the
patient samples bind to the antigens and are determined during the second
incubation step by using enzyme-labelled anti-human antibodies (the conjugate). The
enzyme converts the colourless substrate (H₂O₂/TMB) to a blue end product. The
enzyme reaction is stopped by adding sulphuric acid and the colour of the mixture switches from blue to yellow at the same time. The final measurement is carried out at 450 nm on a photometer using a reference wavelength 620 nm.

4. Reagents provided

Tab. 1: Reagents provided (The reagents in a kit are sufficient for 96 determinations)

<table>
<thead>
<tr>
<th></th>
<th>K2811 IgA</th>
<th>K2821 IgG</th>
<th>K2831 IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plate</strong></td>
<td>96 det.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diluent</strong></td>
<td>25 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Wash</strong></td>
<td>50 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control IgA +</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control IgG +</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control IgM +</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control IgA −</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control IgG −</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control IgM −</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cut Off IgA</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cut Off IgG</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cut Off IgM</strong></td>
<td>500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate IgA</strong></td>
<td>15 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate IgG</strong></td>
<td>15 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conjugate IgM</strong></td>
<td>15 ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Microwell plate; 12 microwell strips (can be divided) in strip holder; coated with COMP antigens of C. pneumoniae

Sample buffer, ready for use; blue coloured phosphate-buffered; contains protein stabilisors, Neolone and Bronidox

Wash buffer, (20 x concentrate); phosphate buffer, contains tween 20 and proclin

Positive control IgA, human serum; contains Neolone and Bronidox

Positive control IgG, human serum; contains Neolone and Bronidox

Positive control IgM, human serum; contains Neolone and Bronidox

Negative control IgA, human serum; contains Neolone and Bronidox

Negative control IgG, human serum; contains Neolone and Bronidox

Negative control IgM, human serum; contains Neolone and Bronidox

Cut-off control IgA, human serum; contains Neolone and Bronidox

Cut-off control IgG, human serum; contains Neolone and Bronidox

Cut-off control IgM, human serum; contains Neolone and Bronidox

Anti-human IgA conjugate, ready for use; peroxidase conjugated antibodies in orange-coloured buffer solution, contains Neolone and Bronidox

Anti-human IgG conjugate, ready for use; peroxidase conjugated antibodies in orange-coloured buffer solution, contains Neolone and Bronidox

Anti-human IgM conjugate, ready for use; peroxidase conjugated antibodies in orange-
5. Storage instructions

- The test kit must be stored at 2 - 8 °C and can be used until the expiry date printed on the label.
- The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 - 8 °C.
- For all other reagents, the expiry date indicated on the packaging applies for storage at 2 - 8 °C
- After the expiry date, the quality guarantee is no longer valid.
- The aluminium bag containing the microwell plate must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must be returned to the aluminium bag immediately and stored between 2 and 8 °C.
- The reagents also must not be allowed to become contaminated and the colourless substrate must be protected from exposure to direct light.

6. Reagents required but not provided

6.1. Reagents
- distilled or deionised water
- **Vircell ELISA SORBENT (Ref. Vircell S001)** (see Section 9.3.)

6.2. Accessories
- Incubator or moist chamber at 37 °C
- Test tubes
- Vortex mixer
- Micropipettes for 10 - 100 µl and 100 - 1000 µl capacities
- Measuring cylinder (1000 ml)
- Stop clock
- Microplate washer or multichannel pipette
- Microplate reader (450 nm, reference wavelength 620 nm)
- Filter paper (laboratory towels)
- Waste container containing 0.5 % sodium hypochlorite solution
7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat or drink in areas where samples or test reagents are being used.

For further details, see the safety data sheets (SDS) at [www.r-biopharm.com](http://www.r-biopharm.com).

The control sera (positive control, negative control and cut-off control) in the kit have been tested for HIV- and HCV-Ab as well as HbsAg with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.

Hydrogen peroxide (substrate) can cause burns. Handle with care. If the skin is contaminated with the stop reagent, rinse it off with water.

The stop reagent contains 0.5 M sulphuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the stop reagent, rinse it off with water.

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121 °C for at least 1 hour.

**Note:** To prevent the formation of poisonous gases, any liquid waste containing stop reagent must be neutralized before adding it to hypochlorite solution.

8. Collection and storage of specimens

The test has been developed for testing human serum samples. After blood collection, the blood should be separated from blood clots as soon as possible in order to prevent haemolysis. The samples must be stored cold or frozen until they are tested. Repeated freezing and thawing of the samples and microbial contamination must be prevented at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid samples can lead to false results.

**Tab. 2:** Specimen storage

<table>
<thead>
<tr>
<th>undiluted Serum oder Plasma</th>
<th>2 - 8 °C</th>
<th>-20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>&gt; 1 week</td>
<td></td>
</tr>
</tbody>
</table>
9. Test procedure

9.1 General information

All reagents and the microwell plate must be brought to room temperature (20 - 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage between 2 and 8 °C.

Take only the volume of reagents that is needed for test procedure. Do not return reagents back into vials as this may lead to contamination.

The microwell strips cannot be used more than once. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

The sample buffer, wash buffer, substrate and stop reagent are not test specific; they can also be used for other RIDASCREEN® ELISA for determining antibodies against Chlamydia.

9.2 Preparing the wash buffer

1 part wash buffer concentrate [Wash] is mixed with 19 parts distilled water. In order to do this, place 50 ml of the concentrate in a 1000 ml measuring cylinder and make up the solution to 1000 ml with distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37°C. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 - 8 °C.

9.3 Specimen preparation

Dilute the serum samples and the controls with sample buffer [Diluent] 1:21 before starting the test. Carry out the dilution directly on the microwell plate. The cut-off control must be determined in duplicate. The controls which correspond to the determination (IgA, IgG or IgM) must be used.

- A1 Negative control
- B1 Cut off control
- C1 Cut off control
- D1 Positive control
- E1, F1 Patient sample 1, 2, usw.

After placing a sufficient number of cavities for controls and the samples in the strip holder, pipette 100 µl sample buffer into each of the microwells in the plate for the IgG determination and then add 5 µl sample or control. The plate should be vibrated briefly for mixing:

IgG:

sample and controls -> 100 µl [Diluent] + 5 µl sample or control

For IgA and IgM determinations, the samples in the plate should be subjected to IgG absorption [e.g. with the Vircell ELISA SORBENT (Ref. Vircell S001)] and only then adjusted with the sample buffer to the dilution required in the test.
When using the **Vircell ELISA SORBENT**, proceed as follows:

IgA/IgM:
- controls -> 100 µl Diluent + 5 µl control sample
- sample -> 25 µl **Vircell ELISA SORBENT** + 5 µl sample + 75 µl Diluent

**Note:** Controls must not be absorbed.

**9.4 First incubation**
Incubate the plate for 45 minutes at 37 °C in an incubator or moist chamber. During this process, the bottoms of the wells must not be in contact with heat-conductive materials (such as metals or moist paper). The microwell plate must be covered during incubation.

**Note:** The microwell plate must not be placed in a cold incubation container reaching 37 °C during incubation. The temperature of the container must be adjusted to 37 °C beforehand.

**9.5 Washing**
The wells must be emptied into a waste container containing hypochlorite solution for disinfection. Then knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 5 times using 300 µl diluted wash buffer each time. Make sure that the wells are emptied completely by knocking them out on an unused part of the absorbent paper after each wash.

**When using a microplate washer, make sure that the machine is correctly adjusted to the type of plate being used. After washing, knock out the plate onto clean absorbent paper in order to remove any residual moisture.**

**9.6 Second incubation**
Add 100 µl of the conjugate **Conjugate IgA**, **Conjugate IgG** or **Conjugate IgM** to each of the corresponding wells. Next, cover the plate and incubate it at 37 °C for 30 minutes (see Section 9.4).

**9.7 Washing**
Wash 5 times in accordance with Section 9.5.

**9.8 Third incubation**
Add 100 µl substrate **Substrate** to each well. After this, cover the plate and incubate at room temperature for 20 minutes, protected from light. Then, stop the reaction by adding 50 µl stop reagent **Stop** to each well. After mixing carefully (by lightly tapping the side of the plate), measure the absorbance at 450 nm (reference wavelength 620 nm) in a plate photometer. Calibrate the zero against air. The measurement must be carried out within one hour after adding the stop reagent.
Note: When using a moist chamber, the underside of the microwell plate must be wiped off to remove condensation water before the measurement is carried out.

10. Quality control – indications of instability or expiration of reagents

For quality control purposes, positive, negative and cut-off controls must be used each time the test is carried out. The cut-off control is determined in duplicate and the average value calculated from the two measurements. The test has been carried out correctly when the extinction values (O.D.) satisfy the following criteria:

**Tab. 3:** Quality control criteria

<table>
<thead>
<tr>
<th>OD</th>
<th>Negative control</th>
<th>Cut off control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.5</td>
<td>&gt; 0.55</td>
<td>&gt; 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 1.5</td>
<td></td>
</tr>
</tbody>
</table>

If the values differ from those required, if the reagent is turbid or the substrate has turned blue before adding to the wells, it may indicate that the reagents have expired. If the stipulated values are not met, the following points must be checked before repeating the test:
- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please contact your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1 Calculation of the specimens index

1. The average absorbance is calculated for the cut-off control.
2. The sample index is obtained by dividing the absorbance for the sample by the calculated average value.

For example:
- Cut-off control 1 OD = 0.821
- Cut-off control 2 OD = 0.865
- Average value = 0.843
- Specimens OD = 1.508

Specimen index = \( \frac{1.508}{0.843} = 1.789 \)
11.2 Interpretation of results

Tab. 4: Analysis of specimen index

<table>
<thead>
<tr>
<th>Specimen index</th>
<th>negative</th>
<th>equivocal</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.9</td>
<td>0.9 – 1.1</td>
<td>&gt; 1.1</td>
</tr>
</tbody>
</table>

12. Limitations of the method

RIDASCREEN® Chlamyphila pneumoniae ELISA are highly specific in detecting IgA, IgG or IgM antibodies against C. pneumoniae. The test cannot be used to derive a relationship between the determined extinction and occurrence of serious clinical symptoms. The test is not suitable for locating the site of infection. The results obtained must always be interpreted in combination with the clinical picture and other diagnostic methods (such as pathogen isolation).

A negative result does not rule out an infection, since the serum can be sampled too early for the antibodies to be detectable. In this case, where an infection is suspected for clinical reasons the test should be repeated on a second sample of the serum collected two to three weeks later.

Two consecutive samples of sera should always be collected from a patient and subjected to serological testing in parallel in order to improve the quality of the diagnosis. The progress of the titre is important for interpreting the findings.

The prevalence of infection with Type C. pneumoniae among the population is very high. The frequency of finding the antibodies is therefore also high. However, the titres of antibodies which are there as a result of earlier infections are relatively low. IgM antibodies are regularly found in the case of primary infections with C. pneumoniae. High IgG titres are available three to six weeks after the onset of disease. With reinfection, it is usually the case that no IgM antibodies can be found whereas IgA and IgG antibody titres increase very rapidly.

Positive IgG findings with newborn children must be interpreted carefully since they can be caused by maternal antibodies. The detection of IgM antibodies is more helpful for children younger than six months.

IgG titres which are very high may lead to false negative results during IgA and IgM determinations. In addition to this, IgM determinations can lead to false positive results due to rheumatoid factors. This can be prevented by absorption of the serum before carrying out the test (see Section 9.3.).
13. Performance characteristics

Tab. 5: Inter-assay variance (n = 10)

<table>
<thead>
<tr>
<th>Inter-assay variance</th>
<th>IgA CV</th>
<th>IgG CV</th>
<th>IgM CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>3.76 %</td>
<td>3.07 %</td>
<td>3.97 %</td>
</tr>
<tr>
<td>Negative control</td>
<td>18.19 %</td>
<td>12.49 %</td>
<td>13.29 %</td>
</tr>
<tr>
<td>Cut off control</td>
<td>7.88 %</td>
<td>5.18 %</td>
<td>5.59 %</td>
</tr>
</tbody>
</table>

Tab. 6: Intra-assay variance (n = 10)

<table>
<thead>
<tr>
<th>Intra-assay variance</th>
<th>IgA CV</th>
<th>IgG CV</th>
<th>IgM CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>2.93 %</td>
<td>2.43 %</td>
<td>1.56 %</td>
</tr>
<tr>
<td>Negative control</td>
<td>16.21 %</td>
<td>11.60 %</td>
<td>10.35 %</td>
</tr>
<tr>
<td>Cut off control</td>
<td>6.13 %</td>
<td>4.27 %</td>
<td>4.16 %</td>
</tr>
</tbody>
</table>

Tab. 7: Sensitivity and specificity in comparison to the microimmuno-fluorescence test (MIF)

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>100</td>
<td>178</td>
<td>61</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97 %</td>
<td>100 %</td>
<td>91 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>94 %</td>
<td>83 %</td>
<td>98 %</td>
</tr>
</tbody>
</table>

In addition, the IgG test examined 22 samples from patients infected with *Chlamydia trachomatis* or other pathogens causing similar clinical pictures to *C. pneumoniae* (*R.conorii, L. pneumophila, C. burnetii, M. pneumoniae*). In the IgA test 18 such samples were examined (no *R.conorii*) and in the IgM test 12 samples. In addition, 2 rheumatoid factor positive samples were tested for IgM. No cross-reactions or influence on the ELISA results were observed in the sera tested.
14. Version history

<table>
<thead>
<tr>
<th>Version number</th>
<th>Section and name</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015-11-01</td>
<td>Release version</td>
</tr>
<tr>
<td>2018-08-15</td>
<td>General revision</td>
</tr>
<tr>
<td>2018-09-20</td>
<td>Adaptation CE symbol (Identification no. Notified Body)</td>
</tr>
<tr>
<td>2019-12-16</td>
<td>Revision 6.1. Reagents</td>
</tr>
<tr>
<td></td>
<td>Revision 9.3. Specimen preparation</td>
</tr>
</tbody>
</table>

15. Explanation of symbols

General symbols

- [IVD] In-vitro diagnostics
- [!] Observe the instruction for use
- [LOT] Lot number
- [!] Use before
- [!] Storage temperature
- [REF] Article number
- [!] Number of tests
- [!] Date of manufacture
- [!] Manufacturer
**Test-specific symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plate</strong></td>
<td>Microtiter plate</td>
</tr>
<tr>
<td><strong>Diluent</strong></td>
<td>Sample dilution buffer</td>
</tr>
<tr>
<td><strong>Wash</strong></td>
<td>Wash buffer 20x</td>
</tr>
<tr>
<td>Control IgA +</td>
<td>Positive control IgA</td>
</tr>
<tr>
<td>Control IgG +</td>
<td>Positive control IgG</td>
</tr>
<tr>
<td>Control IgM +</td>
<td>Positive control IgM</td>
</tr>
<tr>
<td>Control IgA −</td>
<td>Negative control IgA</td>
</tr>
<tr>
<td>Control IgG −</td>
<td>Negative control IgG</td>
</tr>
<tr>
<td>Control IgM −</td>
<td>Negative control IgM</td>
</tr>
<tr>
<td>Cut Off IgA</td>
<td>Cut off control IgA</td>
</tr>
<tr>
<td>Cut Off IgG</td>
<td>Cut off control IgG</td>
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<tr>
<td>Cut Off IgM</td>
<td>Cut off control IgM</td>
</tr>
<tr>
<td>Conjugate IgA</td>
<td>Conjugate IgA</td>
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<tr>
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<td>Conjugate IgG</td>
</tr>
<tr>
<td>Conjugate IgM</td>
<td>Conjugate IgM</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td>Substrate</td>
</tr>
<tr>
<td><strong>Stop</strong></td>
<td>Stop solution</td>
</tr>
</tbody>
</table>
16. References


