**Instructions for Use**

**Coxiella burnetii (Q-Fever) Phase 1 IgG ELISA**

Enzyme immunoassay for the qualitative determination of IgG-class antibodies against Coxiella burnetii in human serum.

**REF** RE58851

**Σ** 96

2-8°C

**EU:** IVD

**U.S.:** For research use only. Not for use in diagnostic procedures.
1. INTRODUCTION

The Rickettsia Coxiella burnetii, a worldwide distributed pathogen, is responsible for the disease known as Q fever. The small gram-negative, obligate intracellular bacterium reproduces in the digestive system of ticks (Dermacentor marginatus) and placental trophoblasts. As a result, tick faeces and afterbirths of infected mammals (particularly sheep) are highly infectious. Occupational groups with direct contact to farm animals are at particular risk of infection. In this group, antibodies against Coxiella burnetii can be detected in 30 to 70%. Infection usually results from inhalation of contaminated aerosols, especially during dry summer months.

The incubation time is around two to four weeks. Clinical symptoms are developed in 30 to 50%; the remaining individuals (50 to 70%) reveal subclinical or non-specific symptoms. Influenza-like symptoms are often evident. In about 50% of cases an atypical, interstitial pneumonia develops. Less frequently, the infection results in a hepatitis. In a few cases, the acute phase may be complicated by meningoencephalitis, myocarditis, or pericarditis. If not treated, the pathogen persists in 1 to 11% of all cases in a variety of organs, which ends after months or years, in a chronic infection. Chronic courses often lead to an endocarditis (especially patients with heart valve disease) and/or granulomatous hepatitis. About 65% of chronic Q-fever cases are lethal.

Following a primary infection, antibodies directed against the phase 2 antigen are produced during the acute phase of Q fever disease. IgM antibodies appear approximately after two weeks followed by IgG within two months post infection. While IgM antibodies can be detected up to three months post infection, IgG is frequently detectable for up to five years. Only when an infection enters the chronic stage, IgA and IgG directed against the C. burnetii phase 1 antigen appear. These antibodies are particularly significant when diagnosing Q fever endocarditis. Due to the fact, that IgM antibodies directed against phase 1 antigen are not present after a longer time period, there is no sense of IgM detection during a chronic course. Rheumatoid factors are significantly increased in the chronic phase.

Due to the lack of characteristic clinical symptoms of acute and chronic Q fever infection, diagnosis is based primarily on serologic techniques. The use of ELISA test systems is recommended by the WHO due to its high sensitivity and specificity and the possibility to perform a differential analysis of the antibody response.

Following diseases should be considered for differential diagnosis: Chlamydia infections, Mycoplasma pneumoniae-infections, Legionella pneumophila and Legionella micdadei infections, Virus-pneumonias and Leptospirosis.

2. INTENDED USE

The ELISA is intended for the qualitative determination of IgG class antibodies against Coxiella burnetii phase 1 in the early stages of infection in human serum.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgG-class antibodies to Coxiella burnetii is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with phase 1 antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured Coxiella burnetii-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of phase 1-specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.
4. MATERIALS

4.1. Reagents supplied

- **C. burnetii phase 1 Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with phase 1 antigens; in resealable aluminium foil.
- **IgG Sample Diluent ***:** 1 bottle containing 100 mL of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle containing 50 mL of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **C. burnetii anti-IgG Conjugate***:** 1 bottle containing 20 mL of peroxidase labelled rabbit antibody to human IgG; coloured blue, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **C. burnetii IgG Positive Control***:** 1 bottle containing 2 mL; coloured yellow; ready to use; red cap.
- **C. burnetii IgG Cut-off Control***:** 1 bottle containing 3 mL; coloured yellow; ready to use; green cap.
- **C. burnetii IgG Negative Control***:** 1 bottle containing 2 mL; coloured yellow; ready to use; blue cap.

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foil
- 1 Test protocol

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Pipe stand
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2-8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (18-25 °C) before starting the test run!

6.1. Coated snap-off strips

The ready to use breakapart snap-off strips are coated with phase 1 antigen. Store at 2-8 °C. **Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8 °C; stability until expiry date. After first opening stability until expiry date when stored at 2-8 °C.**

6.2. C. burnetii anti-IgG Conjugate

The bottle contains 20 mL of a solution with anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use. Store at 2-8 °C. **After first opening stability until expiry date when stored at 2-8 °C.**
6.3. Controls
The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1 % Kathon and has to be stored at 2...8 °C. **After first opening stability until expiry date when stored at 2-8 °C.**

6.4. IgG Sample Diluent
The bottle contains 100 mL phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2-8 °C. **After first opening stability until expiry date when stored at 2-8 °C.**

6.5. Washing Solution (20x Conc.)
The bottle contains 50 mL of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+19; e.g. 10 mL Washing Solution + 190 mL fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. **After first opening stability until expiry date when stored at 2-8 °C.**

6.6. TMB Substrate Solution
The bottle contains 15 mL of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8 °C, away from the light. **The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until expiry date when stored at 2-8 °C.**

6.7. Stop Solution
The bottle contains 15 mL 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2-8 °C. **After first opening stability until expiry date.**

7. SPECIMEN COLLECTION AND PREPARATION
Use human serum samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2-8 °C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70 °C). If samples are stored frozen, mix thawed samples well before testing. **Avoid repeated freezing and thawing.**

7.1. Sample Dilution
Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 μL sample and 1 mL IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE
Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300 μL to 350 μL to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the substrate blank,
1 well (e.g. B1) for the negative control,
2 wells (e.g. C1+D1) for the cut-off control and
1 well (e.g. E1) for the positive control.

**It is recommended to determine controls and patient samples in duplicate, if necessary.** Perform all assay steps in the order given and without any appreciable delays between the steps.
A clean, disposable tip should be used for dispensing each control and sample. 
Adjust the incubator to 37 °C ± 1 °C.

1. Dispense 100 µL controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 °C ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   *Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100 µL C. burnetii anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature. Do not expose to direct sunlight.**
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
    *Any blue colour developed during the incubation turns into yellow.*
    *Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in U by 2.*
11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

**8.2. Measurement**
Adjust the ELISA Microwell Plate Reader to zero using the **substrate blank in well A1.**

*If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!*

*Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.*

*Dual wavelength reading using 620 nm as reference wavelength is recommended.*

Where applicable calculate the **mean absorbance values** of all duplicates.

**9. RESULTS**

### 9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance Value < 0.100.
- **Negative Control** in B1: Absorbance Value < 0.200 and < cut-off
- **Cut-off Control** in C1 and D1: Absorbance Value 0.150 – 1.300
- **Positive Control** in E1: Absorbance Value > cut-off.

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

### 9.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

*Example: OD Cut-off control 0.39 + 0.37 OD Cut-off Control = 0.76 / 2 = 0.38
Cut-off = 0.38*
9.3. Interpretation of Results
Samples are considered **POSITIVE** if the absorbance value is higher than 10 % over the cut-off.
Samples with an absorbance value of 10 % above or below the cut-off should not be considered as clearly positive or negative

->grey zone
It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.
Samples are considered **NEGATIVE** if the absorbance value is lower than 10 % below the cut-off.

9.3.1. Results in Units

\[
\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{Units}
\]

Example:

\[
\frac{1.786 \times 10}{0.38} = 47 \text{ U (Units)}
\]

Cut-off: 10 U
Grey zone: 9-11 U
Negative: <9 U
Positive: >11 U

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

<table>
<thead>
<tr>
<th>Inter-Assay</th>
<th>n</th>
<th>Mean (U)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivocal serum</td>
<td>5 (2)</td>
<td>9.62</td>
<td>2.4</td>
</tr>
<tr>
<td>Positive serum</td>
<td>6 (2)</td>
<td>27.4</td>
<td>3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>n</th>
<th>Mean (OD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive serum</td>
<td>15</td>
<td>0.57</td>
<td>5.7</td>
</tr>
<tr>
<td>Positive serum</td>
<td>15</td>
<td>1.50</td>
<td>4.7</td>
</tr>
</tbody>
</table>

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is > 90 %.

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 88 %.

10.4. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

**Note:** The results refer to the groups of samples investigated; these are not guaranteed specifications.
11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

**WARNING:** In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!

**WARNING:** Sulfuric acid irritates eyes and skin. Keep out of reach of children. Upon contact with eyes, rinse thoroughly with water and consult a doctor!

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. BIBLIOGRAPHY

SCHEME OF THE ASSAY
Coxiella burnetii (Q-Fever) Phase 1 IgG-ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all specimens and controls on a result sheet. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th>Substrate blank (e.g. A1)</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Cut-off control</th>
<th>Sample (diluted 1+100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive control</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
</tr>
<tr>
<td>Cut-off control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
</tr>
<tr>
<td>Sample (diluted 1+100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit

**Incubate for 1 h at 37 °C**

Wash each well three times with 300 µL of washing solution

Conjugate          | 100 µL | 100 µL | 100 µL | 100 µL | 100 µL

Cover wells with foil supplied in the kit

**Incubate for 30 min at room temperature**

Wash each well three times with 300 µL of washing solution

TMB Substrate      | 100 µL | 100 µL | 100 µL | 100 µL | 100 µL

**Incubate for exactly 15 min at room temperature in the dark**

Stop Solution      | 100 µL | 100 µL | 100 µL | 100 µL | 100 µL

Photometric measurement at 450 nm (reference wavelength: 620 nm)
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.-Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθµός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Λοτ n.: / Αριθµός-Παραγωγή:</td>
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</table>


<table>
<thead>
<tr>
<th>CONC</th>
<th>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
</tr>
</tbody>
</table>

| IVD | In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση. |

| Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης. |

| Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση. |

| Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται µακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου. |

| Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους: |

| Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός: |

| Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή! |

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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**LIABILITY**: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.