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

RUO

REF DECOX1G0600

Σ 96

**For Research Use Only – Not for Use in Diagnostic Procedures**



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## 1. INTENDED USE

The Coxiella burnetii (Q-Fever) Phase 1 IgG ELISA is intended for the determination of IgG class antibodies against Coxiella burnetii (Q-Fever) Phase 1 in human serum or plasma (citrate, heparin).

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## 2. PRINCIPLE OF THE ASSAY

The immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

## 3. MATERIALS

### 3.1. Reagents supplied

- 1) **[SORB] [MT] Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Coxiella burnetii (Q-Fever) Phase 1 antigens; in resealable aluminium foil.
- 2) **[SAM] [DIL] IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- 3) **[STOP] [SOLN] Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- 4) **[WASH] [SOLN] [20x] Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- 5) **[ENZ] [CONJ] Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- 6) **[SUB] [TMB] TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- 7) **[CAL] [C] Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- 8) **[CAL] [B] Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- 9) **[CAL] [A] Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 11.1

### 3.2. Materials supplied

- 1 Cover foil
- 1 Instructions for use (IFU)

### 3.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

#### 4. STABILITY AND STORAGE

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

#### 5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20...25 °C) and mix them before starting the test run!

##### 5.1. Microtiterplate

The break-apart snap-off strips are coated with Coxiella burnetii (Q-Fever) Phase 1 antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

##### 5.2. WASH SOLN 20x

Dilute WASH SOLN 20x 1 + 19; e. g. 10 mL WASH SOLN 20x + 190 mL distilled water. The diluted buffer (WASH SOLN 1x) is stable for 5 days at room temperature (20...25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

##### 5.3. SUB TMB

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. SUB TMB should be colourless or could have a slight blue tinge. If SUB TMB turns into blue, it may have become contaminated and should be thrown away.

#### 6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

##### 6.1. Sample Dilution

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

## 7. ASSAY PROCEDURE

Please read the instructions for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instructions for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of **WASH SOLN 1x** from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample. Adjust the incubator to  $37 \pm 1$  °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour  $\pm$  5 min at  $37 \pm 1$  °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 **WASH SOLN 1x**. Avoid overflows from the reaction wells. The interval between washing and aspiration 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 important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL **SUB TMB** into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL **STOP SOLN** into all wells in the same order and at the same rate as for the **SUB TMB**, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the **STOP SOLN**.

### 7.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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 standard/control and sample.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

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

## 8. RESULTS

### 8.1. Run Validation Criteria

In order for an assay run to be considered valid, these instructions for use have to be strictly followed and the following criteria must be met:

- **Substrate Blank:** Absorbance value < **0.100**
- **Negative Control:** Absorbance value < **0.200** and < **Cut-off**
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value > **Cut-off**

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

### 8.2. Results

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

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43 Cut-off = 0.43

#### 8.2.1. Results in Units [U]

Sample (mean) absorbance value x 10 =  
[Units = U] Cut-off

Example:  $\frac{1.591 \times 10}{0.43} = 37$  U (Units)

### 8.3. Evaluation of Results

|          |   |
|----------|---|
| 10 U     | Cut-off   |
| > 11 U   | Antibodies against the antigen are present.   |
| 9 – 11 U | Antibodies against the antigen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. |
| < 9 U    | The sample contains no antibodies against the antigen.  |

## 9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

### 9.1. Precision

**Intraassay**            **n**            **Mean (E)**            **CV (%)**

#1                      24                      0.303                      6.75

#2                      24                      0.601                      7.03

#3                      24                      1.380                      11.19

**Interassay**            **n**            **Mean (NTU)**            **CV (%)**

#1                      12                      21.41                      4.84

#2                      12                      47.55                      6.23

#3                      12                      2.13                      8.84

### 9.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100% (95% confidence interval: 86.77% - 100%).

### 9.3. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100% (95% confidence interval: 59.04% - 100%).

**9.4. Interferences**

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

**9.5. Cross Reactivity**

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

**10. LIMITATIONS OF THE PROCEDURE**


Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

**11. PRECAUTIONS AND WARNINGS**


- 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.
- For research use only.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or Microtiterplates 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 standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

**11.1. Safety note for reagents containing hazardous substances**

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 3.1).  
Therefore, the following hazard and precautionary statements apply.

|   |                |           |  |
|---|----------------|-----------|--|
|  | <b>Warning</b> | H317      | May cause an allergic skin reaction.                     |
|   |                | P261      | Avoid breathing spray                                    |
|   |                | P280      | Wear protective gloves/ protective clothing.             |
|   |                | P302+P352 | IF ON SKIN: Wash with plenty of soap and water.          |
|   |                | P333+P313 | If skin irritation or rash occurs: Get medical advice/   |
|   |                | P362+P364 | Take off contaminated clothing and wash it before reuse. |

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to 3.1).  
Therefore, the following hazard and precautionary statements apply.

|   |                |                    |  |
|---|----------------|--------------------|--|
|  | <b>Warning</b> | H315               | Causes skin irritation.  |
|   |                | H319               | Causes serious eye irritation  |
|   |                | P280               | Wear protective gloves/ protective clothing.   |
|   |                | P302+P352          | IF ON SKIN: Wash with plenty of soap and water.  |
|   |                | P305+P351<br>+P338 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
|   |                | P337+P313          | If eye irritation persists: Get medical advice/attention.  |

Further information can be found in the safety data sheet.

**11.2. 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.

**ABBREVIATIONS**

|      |  |
|------|--|
| CMIT | 5-chloro-2-methyl-4-isothiazolin-3-one |
| MIT  | 2-methyl-2H-isothiazol-3-one           |

**SUMMARY OF TEST PROCEDURE****SCHEME OF THE ASSAY**

Coxiella burnetii (Q-Fever) Phase 1 IgG














**Test Preparation**

Prepare reagents and samples as described.  
Establish the distribution and identification plan for all samples and standards/controls.  
Select the required number of microtiter strips or wells and insert them into the holder.

**Assay Procedure**

|  | Substrate Blank (A1) | Negative Control | Cut-off Control | Positive Control | Sample (diluted 1+100) |
|--|----------------------|------------------|-----------------|------------------|------------------------|
| Negative Control   | -                    | 100 µL           | -               | -                | -                      |
| Cut-off Control  | -                    | -                | 100 µL          | -                | -                      |
| Positive Control   | -                    | -                | -               | 100 µL           | -                      |
| Sample (diluted 1+100)   | -                    | -                | -               | -                | 100 µL                 |
| Cover wells with foil supplied in the kit<br><b>Incubate for 1 h at 37 ± 1 °C</b><br>Wash each well three times with 300 µL of <b>WASH SOLN 1x</b>               |                      |                  |                 |                  |                        |
| Conjugate  | -                    | 100 µL           | 100 µL          | 100 µL           | 100 µL                 |
| <b>Incubate for 30 min at room temperature (20...25 °C)</b><br>Do not expose to direct sunlight<br>Wash each well three times with 300 µL of <b>WASH SOLN 1x</b> |                      |                  |                 |                  |                        |
| <b>SUB TMB</b>   | 100 µL               | 100 µL           | 100 µL          | 100 µL           | 100 µL                 |
| <b>Incubate for exactly 15 min at room temperature (20...25 °C) in the dark</b>  |                      |                  |                 |                  |                        |
| <b>STOP SOLN</b>   | 100 µL               | 100 µL           | 100 µL          | 100 µL           | 100 µL                 |
| Photometric measurement at 450 nm (reference wavelength: 620 nm)   |                      |                  |                 |                  |                        |

**SYMBOLS USED WITH DEMEDITEC ASSAYS**

| Symbol  | English                            | Deutsch                                      | Française  | Espanol                                       | Italiano                            |
|---|------------------------------------|--|--|---|-------------------------------------|
|  | European Conformity                | CE-Konformitäts-kennzeichnung                | Conforme aux normes européennes                        | Conformidad europea                           | Conformità europea                  |
|  | Consult instructions for use       | Gebrauchsanweisung beachten                  | Consulter les instructions d'utilisation               | Consulte las Instrucciones                    | Consultare le istruzioni per l'uso  |
|  | In vitro diagnostic device         | In-vitro-Diagnostikum                        | utilisation Diagnostic in vitro                        | Diagnóstico in vitro                          | Per uso Diagnostica in vitro        |
|  | For research use only              | Nur für Forschungszwecke                     | Seulement dans le cadre de recherches                  | Sólo para uso en investigación                | Solo a scopo di ricerca             |
|  | Catalogue number                   | Katalog-Nr.                                  | Référence  | Número de catálogo                            | No. di catalogo                     |
|  | Lot. No. / Batch code              | Chargen-Nr.                                  | No. de lot   | Número de lote                                | Lotto no                            |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze                  | Contenu suffisant pour "n" tests                       | Contenido suficiente para <n> ensayos         | Contenuto sufficiente per "n" saggi |
|  | Note warnings and precautions      | Warnhinweise und Vorsichtsmaßnahmen beachten | Avertissements et mesures de précaution font attention | Tiene en cuenta y advertencias y precauciones | Annoti avvisi e le precauzioni      |
|  | Storage Temperature                | Lagerungstemperatur                          | Température de conservation                            | Temperatura de conservacion                   | Temperatura di conservazione        |
|  | Expiration Date                    | Mindesthaltbarkeitsdatum                     | Date limite d'utilisation                              | Fecha de caducidad                            | Data di scadenza                    |
|  | Legal Manufacturer                 | Hersteller                                   | Fabricant  | Fabricante                                    | Fabbricante                         |
| <i>Distributed by</i>   | Distributed by                     | Vertrieb durch                               | Distribution par                                       | Distribución por                              | Distribuzione da parte di           |
|  | Version                            | Version                                      | Version  | Versión                                       | Versione                            |
|  | Single-use                         | Einmalverwendung                             | À usage unique   | Uso único                                     | Uso una volta                       |