DRG® Coxiella burnetii (Q-Fever) Phase 1 IgG (EIA-5188)

As of 27 July 2010 rm (Vers. 1.1) **IVD**

Please use only the valid version of the package insert provided with the kit.

1 INTRODUCTION

Q-Fever is a disease that results from infection with small, polymorph and gram-negative bacteria called Coxiella burnetii. After an outbreak in Brisbane, Australia, the responsible organism was isolated and named Coxiella burnetii in honour of Dr. Herald Rae Cox and Sir Frank Burnet. New molecular research demonstrated a close relationship to Legionella. The zoonosis Q-Fever is found everywhere except New Zealand (no data available). There is an extensive reservoir (mainly ticks) of C. burnetii. Ticks are an important vector of the pathogen in the transmission between domestic and wildlife animals. But the ticks are unimportant in the direct infection of humans. Cattles, sheep and goats are usually the source of transmission of this microorganism to humans. However cats, dogs and rabbits are also important in this regard. In most instances humans become infected with Coxiella burnetii following inhalation of contaminated aerosols (respiratory tract). The incubation period for Q-Fever in humans is about 2 weeks. The resulting illness can be divided into acute and chronic varieties. During the acute phase of illness antibodies to the phase II-antigen are formed. Anti phase-I antibodies in high titers are typical for a chronic disease.

In areas where Q-Fever is endemic, 12% or more of the population have antibodies to C. burnetii. Most of the infections are subclinical or undiagnosed.

The acute infection shows symptoms of high fever, shivers, muscle pain and headache. Later on more severe diseases such as pneumonia or hepatitis can occur. Infections during pregnancy can lead to an abort or premature birth. Approximately 1% of all infections become chronic. The most frequent organ manifestation in Q-Fever is endocarditis.

Diagnostic methods:

Complement binding reaction is still used

IFT (immuno fixation test)

ELISA

Cell culture

PCR

IFT as well as ELISA differentiate between different antibody classes (IgG/ IgM/ IgA).

Acute phase of Q-Fever

IgM specific to phase 2 after 2-3 weeks

IgG approximately 2 months after infection

Chronic phase of Q-Fever

From 6 weeks up to 4 months after infection phase 1 IgG and IgA antibodies can be detected.

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
1 distribution and identification plan

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 °C - 8 °C.

6 REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20 °C - 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 °C - 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 °C - 8 °C; stability until expiry date.*
After first opening stability until expiry date when stored at 2 °C - 8 °C.

6.2 C. burnetii anti-IgG Conjugate

The bottle contains 20ml 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 °C - 8 °C.
After first opening stability until expiry date when stored at 2 °C - 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 °C - 8 °C.
After first opening stability until expiry date when stored at 2 °C - 8 °C.

6.4 IgG Sample Diluent

The bottle contains 100 ml phosphate buffer, anti-human-IgG, 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 °C - 8 °C. *After first opening stability until expiry date when stored at 2 °C - 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 °C - 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 °C - 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 °C - 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 °C - 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 °C - 8 °C; otherwise they should be aliquoted and stored deep-frozen (-20 °C to – 70 °C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*
Heat inactivation of samples is not recommended.

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

8.1 Test Preparation

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° ± 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±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 >5sec. 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 DU by 2.
11. Measure the absorbance of the specimen at 450/620nm 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 – 1.30.**
- **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: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 =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

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

Example: $\frac{1.786 \times 10}{0.38} = 47$ *DU (Units)*

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

10 SPECIFIC PERFORMANCE CHARACTERISTICS

10.1 Precision

Inter-assay	n	Mean (DU)	Cv (%)
Greyzone serum	5 (2)	9.62	2.4
Positive serum	6 (2)	27.4	3.5

Intra-assay	n	Mean (OD)	Cv (%)
Greyzone serum	15	0.57	5.7
Positive serum	15	1.5	4.7

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!

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WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the 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.

SCHEME OF THE ASSAY

Test preparation

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

Assay procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut-off control	Sample (diluted 1+100)
Negative control	-	100µl	-	-	-
Positive control	-	-	100µl	-	-
Cut-off control	-	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well three times with 300µl of washing solution					
Conjugate	-	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution					
TMB Substrate	100µl	100µl	100µl	100µl	100µl
Incubate for exactly 15 min at room temperature in the dark					
Stop Solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					