MONOSCREEN Ab ELISA

Coxiella burnetii – phase I

ELISA Kit for serodiagnosis of Q Fever
Indirect test for blood sera, plasma and milk
Diagnostic test for cattle and small ruminants
Monowell

I - INTRODUCTION

Q fever affects human beings, cattle, sheep, and goats in particular. The aetiological agent, *Coxiella burnetii*, is a Gram-negative intracellular bacterium that multiplies in macrophage phagolysosomes. *Coxiella burnetii* can occur in two antigenic forms, namely, a pathogenic Phase I that is isolated from infected people or animals and an avirulent Phase II that is obtained *in ovo* or *in vitro*. The two forms of infection – acute and chronic – have different serological profiles. During the acute phase of the disease, the titres of IgG antibody against Phase II antigens are elevated, whereas during the chronic phase of the disease the titres of IgG antibody against Phase I and Phase II antigens are high. In cows, ewes, and goats Q fever is associated with late-term abortions and reproductive problems such as premature births, dead or weakened foetuses, metritis, and infertility. Nevertheless, the serological responses and isolation of the bacterium in a given species are not necessarily correlated with the disease's clinical expression. Serotests are appropriate for herd screening, but may be difficult to interpret for an individual subject.

II – PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by phase I antigenic extract from *Coxiella burnetii* cells. The test blood sera and plasma are diluted in the dilution buffer. The milk samples are used undiluted. Samples are added to the plate which is then incubated and washed. The conjugate, protein G peroxidase-labelled, is added to the wells. The plate is incubated a second time at 21°C +/- 3°C. After the second incubation, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific *Coxiella burnetii* immunoglobulins are present in the test sera or milk the conjugate remains bound to the microwell that contains the bacterial antigen and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitised by the bacterial antigen.

III - COMPOSITION OF THE KIT

- **Microplates** Two 96-well microtitration plates (24 strips of 8 wells) sensitised by phase I antigenic extract from *Coxiella burnetii* cells.
- **Washing solution**: One 100-ml bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until all crystals have disappeared. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.
- **Dilution buffer**: One 50-ml bottle of 5x colored and concentrated buffer for diluting the blood sera, plasma and the conjugate. The bottle's content is to be diluted with distilled or demineralised water. If a deposit forms at the bottom of the receptacle filter the solution on Whatman filter paper.
- **Conjugate**: One bottle of Protein G horseradish peroxidase-labelled.
- **Positive reference**: One bottle of positive serum. Store this reagent between +2°C and +8°C.
- **Negative reference**: One bottle of negative serum. Store this reagent between +2°C and +8°C.
- **Tracer:** One bottle of tracer.The tracer is a reference sample that can be used to check the intra-laboratory reproducibility of the kit's batch.
  Intra-laboratory reproducibility: Degree of agreement between the results of reiterated tests on the same sample with an identical technical protocol in a given laboratory under variable working conditions.
- **Single component TMB** One 25-ml bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. **This solution is ready to use.**
- **Stop solution**: One 15-ml bottle of the 1 *M* phosphoric acid stop solution.

	BIO K 404/2
Microplates	2
Washing solution	1 X 100 ml (20 X)
Colored Dilution buffer	1 X 50 ml (5 X)
Conjugate	1 X 0,5 ml (50 X)
Positive serum	1 X 0,5 ml (1 X)
Negative serum	1 X 0,5 ml (1 X)
Tracer	1 X 0,5 ml (1 X)
Single component TMB	1 X 25 ml (1 X)
Stop solution	1 X 15 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, microplates for dilution, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional).

V - PRECAUTIONS FOR USE

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.

- The stop solution contains 1 *M* phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

1- Bring all components to 21°C +/- 3°C before use. Remove the microplate from its wrapper.

2- DILUTION OF SAMPLES

2.1- Blood sera and plasma preparation
The blood serum and plasma samples must be diluted 1:100. Avoid using haemolysed samples or those containing coagulum.

2.1.1- Dilution in tubes
Distribute 990 µl aliquots of dilution buffer, prepared as instructed in the section "Composition of the Kit", to 5 or 10 ml tubes. Add 10 µl aliquots of the samples to each of these tubes and mix briefly on a mechanical stirrer (final dilution: 1:100).

2.1.2- Dilution on a microplate
Distribute 20 µl aliquots of each of the samples to the microwells of a dilution plate. Add 180 µl of dilution buffer. Mix five times by pumping and surging or orbital agitation (dilution: 1:10). Distribute 90 µl aliquots of dilution buffer to the wells of the kit's microplate. Transfer 10 µl of the 1:10 prediluted samples. Mix five times by pumping and surging or orbital agitation (final dilution: 1:100).

2.2- Dilution of the kit's reference sera (positive and negative controls) and tracer
The positive and negative sera and the tracer must be diluted 1:100. Do these dilutions in one step in a tube (see Point 2.1.1.) or in two steps on a dilution microplate (see Point 2.1.2.).

2.3-Milk preparation
Centrifuge at 4000 g for 20 minutes. Take up the middle layer of liquid by means of a glass Pasteur pipette inserted through the upper layer of cream, taking care not to touch the underlying cell sediment.
Use undiluted skimmed milk samples in the wells.

3- Distribute the samples (blood serum, plasma, or milk) using 100 µl per well. One well per sample. Positive serum in well A1, Negative serum in well A2, tracer in well A3, Sample 1 in well A4, and so on. Cover with a lid and incubate the plate at 21°± 3°C for one hour.
4- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, dispose of the microplate's contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation two more times, taking particular care to avoid bubble formation in the wells. After these three rinses, go on to the next step. Using a plate washer (whether automatic or manual) is also recommended. However, the depth of the needles' immersion must be set so as not to disturb the layer of reagents adsorbed to the bottom of each well.
5- Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.250 ml of diluent). Add 100 µl of the diluted conjugate solution to each well. Cover the plate with a lid and incubate for 1 hour at 21°C +/- 3°C.

6- Wash the plate as described in step 4 above.
7- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.
8- Incubate for 10 minutes at 21°C +/- 3°C protected from the light and uncovered. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
9- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.
10-Read the optical densities in the microwells using a plate reader and a 450 nm filtre. Results must be read fairly soon after the stopping solution has been added since the chromogen may cristallize in wells with strong signals and thereby distort the data.

VII – INTERPRETING THE RESULTS

The test can be **validated** only if the difference between the optical density readings of the positive control serum and negative control serum (OD positive serum - OD negative serum) at ten minutes is greater than 1,000 and the negative serum yields an optical density that is lower than 0,400.

Calculate each serum's, plasma's or milk's coefficient by means of the following formula:

$$\text{Sample's Coeff.} = \frac{\text{OD sample - OD negative serum}}{\text{OD positive serum - OD negative serum}} \times 100$$

Blood sera and plasma :
A sample is negative if its coefficient is less than 60%.
A sample is positive if its coefficient is greater than or equal to 60%.

Milk :
A sample is negative if its coefficient is less than 50%.
A sample is positive if its coefficient is greater than or equal to 50%.

VIII – ORDERING INFORMATION

Monoscreen AbELISA *Coxiella burnetii* – phase I : 2 x 96 tests BIO K 404/2