

MVD-Enfer *Chlamydia abortus* specific ELISA

01H14 (186 Tests)

Test for the *in vitro* detection of antibodies to *Chlamydia abortus* in sheep

For *in vitro* veterinary diagnostic use only

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Enfer Scientific complies with the quality system standard ISO9001:2008

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1.0 General Information

Ovine enzootic abortion (OEA) (also referred to as Enzootic Abortion of Ewes (EAE) or ovine chlamydiosis), resulting from infection of sheep with the Gram-negative bacterium *Chlamydia abortus*, is of major importance worldwide. The disease is a major health and welfare issue for the global livestock industry with large economic losses amounting to many millions of pounds annually. It is difficult to identify infection in individual animals within a flock, making the disease difficult to control. The first sign of infection is often the appearance of dead or alive but weak lambs in the last few weeks of pregnancy with visibly diseased placentas and a vaginal discharge of infective fluid, which can last for several days. *Chlamydia abortus* can also infect cattle, pigs and horses but disease outbreaks are much more sporadic and less frequent in these species. Infection is zoonotic and can thus be passed from animals to humans, in whom it can cause spontaneous abortion and, in rare cases, be potentially life-threatening for pregnant women.

Over the last 50 years the serological diagnosis of *Chlamydia abortus* infection has been based mainly on the complement fixation test (CFT), which lacks both sensitivity and specificity because of cross-reactive antibodies to other Gram-negative bacteria, including another common chlamydial pathogen of sheep, *Chlamydia pecorum*. In studies published by the Moredun Research Institute^{1,2} the POMP90-3 antigen gives high sensitivity (96.8%) and shows no cross reaction with sera from *C. pecorum* infected animals or from EAE-free flocks (100% specificity).

2.0 Intended Use

The MVD-Enfer *Chlamydia abortus*-specific ELISA – is an indirect ELISA for the detection of antibodies to POMP90-3 in sheep serum. This test is specific for antibodies to *C. abortus* and does not detect antibodies to *C. pecorum*. The kit is intended for *in vitro* veterinary diagnostic use and research purposes only.

3.0 Principle of the Procedure

The MVD-Enfer *Chlamydia abortus* specific ELISA and is an indirect enzyme immunoassay based on the sequential addition of ovine serum to an antigen coated plate. Antibodies specific to the bacteria form complexes with the immobilized antigen. A rabbit anti-sheep IgG conjugate labelled with HRPO (Horseradish peroxidase) is added, forming an antigen-antibody-conjugate-peroxidase complex. A solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxidase is then added; the colour is read spectrophotometrically at 450nm. The amount of conjugate bound, and hence the colour in the wells, is directly proportional to the amount of antibodies to POMP90-3 in the test serum¹.

4.0 Reagents

Reagent pack 01H14 contains sufficient material for 186 tests in single wells. The reagent pack is stored at 2-8°C. Note the storage conditions for individual components.

Kit Contents	Storage Conditions
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Antibody Capture Plate Microtitre plates (96 wells) coated with <i>Chlamydia abortus</i> -specific antigen.	2 x 96-well (in 8-well strips) 2-8°C (in sealed in foil pouches)
20X Wash Buffer 20X Concentrate	1 x 100ml 2-8°C
Chlamydia Sample Diluent Ready to use	2 x 100ml 2-8°C

Chlamydia Conjugate Diluent Ready to use	1 x 100ml 2-8°C
Concentrate Conjugate Undiluted rabbit anti-sheep HRPO antibody conjugate.	1 x 0.1ml 2-8°C
TMB Concentrate 3,3',5,5',-tetramethylbenzidine (TMB) pink solution.	1 x 15ml 2-8°C
TMB Diluent Colourless solution of hydrogen peroxide.	1 x 15ml 2-8°C
Stop Solution Contains Sulphuric acid	1 x 30ml 2-8°C
Chlamydia Negative Control Undiluted Sheep serum containing preservative	1 x 0.1ml -15-20°C
Chlamydia Positive Control Undiluted Sheep serum containing preservative	1 x 0.1ml -15-20°C

5.0 Materials and Equipment required but not provided

- Microplate Incubator thermostated at 37°C ± 2°C.
- Device for the delivery and aspiration of wash solution.
- High quality deionised, distilled, or reverse osmosis water.
- Precision single channel and multichannel micropipettes of appropriate volume and disposable tips.
- Reagent dispensing trays and microplate cover seals.
- Glass or polypropylene containers for dilution of the concentrate conjugate/other reagents.
- Polypropylene tubes/plates for dilution of the samples.
- Microplate reader for measurement at 450nm with reference filter of 690nm.

6.0 Warnings and Precautions

- 6.1** Follow the instructions and do not modify the test procedure or substitute reagents from other manufacturers. Do not use the reagents beyond the stated expiry date and do not intermix components from different kit lots.
- 6.2** Please refer to the manufacturer's safety data sheets and the product labelling for information on potentially hazardous components.
- 6.3** Care should be taken to prevent contamination of kit components.
- 6.4** Use a new pipette tip for each sample.

- 6.5** Allow the reagents to adjust to room temperature (RT) (+18°C to 30°C). Immediately after use, return all reagents to their appropriate storage conditions.
- 6.6** Avoid cross contamination of the TMB solution with the diluted conjugate solution. Do not pour unused TMB solution back into the TMB bottles.
- 6.7** Do not allow plates to sit for more than 3 minutes between wash steps and the addition of reagents.
- 6.8** Do not expose the substrate solution to strong light or oxidizing agents.
- 6.9** All reagents must be prepared in either clean glass, or polypropylene bottles. Care must be taken to avoid cross contamination of reagents. Use separate dispensing trays for each reagent.
- 6.10** All unused biological materials should be disposed according to the local, regional and national regulations.

7.0 Preparation of Reagents

Component	Storage of Prepared Component
Working Strength Wash Buffer (1X) 1. Dilute the 20X Wash Buffer, 1/20 in deionised water. Prepare approximately 500ml per plate by adding 25ml of 20X Wash Buffer to 475ml deionised water and mix thoroughly.	1 month at RT (+15 to 25°C) or at 2-8°C
Working Strength Conjugate 1. Prepare only the required volume for the number of tests to be carried out. 2. Dilute the Concentrate Conjugate in Conjugate Diluent to 1:12000. 3. Mix by inversion.	Use within 1hr of preparation
TMB Working Solution 1. Prepare only the required volume for the number of tests to be carried out. 2. Add a volume of TMB Diluent to an equal volume of TMB Concentrate in either a clean glass or plastic vessel.	Store TMB solution at 2-8°C or +15 to 25°C, away from sunlight and use within two days of preparation.

8.0 Sample & Control Preparation

Bring all samples to room temperature prior to testing. All samples and controls must be added to the capture plate at approximately the same time, therefore use of a transfer/master plate is recommended to add samples and controls to first, and then transfer to the capture plate.

- 8.1** Specimens containing precipitate may yield inconsistent test results and such specimens must be clarified prior to testing.
- 8.2** Ensure the serum sample is mixed thoroughly before addition to the Chlamydia Sample Diluent.
- 8.3** The Chlamydia Negative Control, Chlamydia Positive Control and samples are prepared to a 1:150 dilution by adding for example 5µl of the serum to 0.750ml of the Chlamydia Sample Diluent and mix by inversion.

9.0 Test Protocol

- 9.1** Prepare the samples and controls as described above (use of transfer/master plate is recommended).

- 9.2 The Chlamydia Negative Control is dispensed into one well and the Chlamydia Positive Control is dispensed into two wells.
- 9.3 All samples are tested in singlicate.
- 9.4 Label each strip with a number. Transfer 100µl of the controls and samples into the appropriate wells of the coated plate and cover the plate/strips with a cover seal.
- 9.5 Incubate the plate/strips for 60 minutes at 37 ± 2°C.
- 9.6 Wash the wells 6 times with 250-300µl of 1X wash buffer with a 3 second soak. Ensure that all wells are completely filled, then completely emptied. Do not adjust the recommended washing steps. Inadequate washing can give incorrect results.
- 9.7 Dry by inversion on absorbent paper.
- 9.8 Add 100µl of working strength conjugate to each well. Seal the plate/strips and incubate the plate/strips for 30 minutes at 37 ± 2°C.
- 9.9 Wash the wells 6 times with 250-300µl of 1X wash buffer with a 3 second soak and dry by inversion on absorbent paper.
- 9.10 Add 100µl of the TMB substrate solution to each well of the plate/strips and cover the plate/strips with a cover seal and incubate the plate/strips for 5 minutes at 37±2°C.
- 9.11 Remove the microplate cover seal, and add 100µl of Stop solution to each of the wells. Shake for 10 seconds on the reader or manually by tapping the sides of the plate.
- 9.12 Within 30 minutes read the absorbance at 450nm using a reference filter of 690nm. Blank the instrument on air (no plate in the carriage).

10.0 Results

10.1 Calculations

Calculate the mean absorbance (OD) of the Chlamydia Positive Control. All OD values for the test samples as well as the Chlamydia Negative Control are related to the OD value of the Chlamydia Positive Control as follows:

$$PP = \frac{(\text{OD Sample} - \text{OD Negative Control})}{(\text{OD}_{\text{av}} \text{ Positive Control} - \text{OD Negative Control})} \times 100$$

10.2 Validation of Test Performance

The control results must be validated before the sample results can be interpreted. The control values should fall within the following limits;

Control	Acceptance Criteria (OD)
Negative	<0.4
Positive (corr)	1.000-2.000

Should any of these criteria not be fulfilled, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.

10.3 Interpretation of Results

Samples with a PP less than or equal to 20% are considered **Negative** for the presence of *C. abortus* antibodies.

Samples with a PP greater than 20% and less than 30% are considered **Doubtful** and must be retested before deciding on a result.

Samples with a PP greater than or equal to 30% are considered **Positive** for the presence of *C. abortus* antibodies.

11.0 Limitations of the Procedure

Enfer Scientific complies with the quality system standard ISO9001:2008. As with any biological test, this test may give a false positive or a false negative result owing to local conditions. A test should be interpreted in the context of all available clinical, historical, and epidemiological information relevant to the animal(s) under test. Any change or modification of the procedure might affect the results. *Responsibility for test interpretation and consequent animal husbandry decisions rests solely with the user and any consulting veterinarian and appropriate animal health advisors or authorities. Enfer Scientific accepts no responsibility for any loss or damage, howsoever caused, arising out of the interpretation of test results.*

12.0 Disclaimer and Reservation of Rights

Enfer Scientific gives no warranty of any kind, whether expressed or implied, in regard to the carrying out of the MVD-Enfer assay or for the stability and storage of the MVD-Enfer kit, or for the procedure used. Without prejudice to the foregoing, Enfer Scientific disclaims all responsibility for merchantability and fitness for use after it leaves Enfer Scientific. Enfer Scientific shall not be liable, under any circumstances, for damages, direct or consequential.

13.0 References

- 13.1 Longbottom D, Fairley S, Chapman S, Psarrou E, Vretou E, Livingstone M. Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with recombinant protein fragment of the polymorphic outer membrane protein POMP90 of *Chlamydophila abortus*. J Clin Microbiol. 2002 Nov; 40(11):4235-43.
- 13.2 Wilson K, Livingstone M, Longbottom D. Comparative evaluation of eight serological assays for diagnosing *Chlamydophila abortus* infection in sheep. Vet Microbiol. 2009 Mar 16;135 (1-2):38-45. doi: 10.1016/j.vetmic.2008.09.043. Epub 2008 Sep 16.