4. REAGENTS PROVIDED

Each kit contains sufficient materials for 96 determinations or 44 serum specimens assayed in duplicate plus controls. The shelf life of the kit is as indicated on the outer box label.

4.1. Oxoid Mycoplasma hyopneumoniae ELISA CONTENTS

Instructions For Use.

Microtitre Plate

One clear plastic 96 well Microtitration Plate of 12, 8 microwell strips coated with M. hyopneumoniae antigen. A resealable plastic pouch is provided for storage of unused microwells.

White Strips

One white plastic microplate of 12, 8 well strips – uncoated. The white microwells are used for preparing 1:10 dilutions of specimens and control sera.

Sealing Tape

Two sheets of sealing tape

Sample Diluent

18μL buffer containing antimicrobial agent and coloured dye. May contain a harmless precipitate.

Positive Control Serum

200μL reference porcine serum containing antibodies to M. hyopneumoniae. Contains an antimicrobial agent.

Negative Control Serum

200μL reference porcine serum negative for antibodies to M. hyopneumoniae. Contains an antimicrobial agent.

Conjugate

12μL of peroxidase conjugated mouse monoclonal antibody to M. hyopneumoniae in a buffer containing detergent, protein, coloured dye and an antimicrobial agent.

Wash Buffer Concentrate

250mL Wash Buffer Concentrate (x25): Tris buffered solution containing antimicrobial agent and detergent.

Substrate

25mL Tris buffered solution containing antimicrobial agent and detergent.

Stop Solution

25mL Stop Solution. 0.46mol/L sulphuric acid

Safe handling precautions must be observed. Dilute with distilled water to 0.4mol/L.

5. ADDITIONAL REAGENTS

5.1. REAGENTS

Positive Control Serum

Sample Diluent

Positive Control Serum

Negative Control Serum

Conjugate

Wash Buffer Concentrate

Substrate

Stop Solution

5.2. PREPARATION, STORAGE AND RE-USE OF KIT COMPONENTS

In order to ensure optimal kit performance, it is important that all unused kit components are stored according to the following instructions.

4.2.1 Antigen Coated Microwells

Open the plate pouch by cutting along the seal. Place unused microwells in the resealable plastic bag with the desiccant, carefully seal the bag and store at 2-8°C. Microwells may be used for up to 12 weeks after initial opening, provided they are stored in this manner.

4.2.2 Sample Diluent

Ready to use. Store unused Sample Diluent at 2-8°C.

4.2.3 Positive Control Serum

Ready to use. Store unused Positive Control Serum at 2-8°C.

4.2.4 Negative Control Serum

Ready to use. Store unused Negative Control Serum at 2-8°C.

4.2.5 Wash Buffer Concentrate

Provided x25 concentrated. Dilute Wash Buffer by adding 1 part of Wash Buffer concentrate to 24 parts of fresh deionised or distilled water. There is sufficient concentrate to prepare 100mL working strength Wash Buffer for 4 washes of each microwell strip. Prepare working strength Wash Buffer as required on the day of use (See Section 7.2.7). Store remaining Wash Buffer Concentrate at 2-8°C.

Do not store unused working strength Wash Buffer for subsequent use (See Section 7.2.7).

4.2.6 Substrate

Ready to use. Store unused Substrate at 2-8°C, protected from light.

4.2.7 Stop Solution

Ready to use. Store unused Stop Solution at 2-8°C.

5. ADDITIONAL REAGENTS

5.1. REAGENTS

5.1.1 Stop Solution

Clean absorbent paper (onto which microwells can be taped dry)

Precision pipettes, adjustable multichannel pipettes and disposable tips to deliver 50μL, 1,000μL, and 1–5mL.

Waste discard container with suitable fresh disinfectant

Automated plate washer (optional) or suitable equipment for washing 8 microwell strips

Spectrophotometer or ELA plate reader capable of reading 96 microwell plate of 8 microwell strips at an absorbance of 450nm with a reference at 620-650nm

7. PRECAUTIONS

Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

7.1. SAFETY PRECAUTIONS

7.1.1 The Stop Solution contains sulphuric acid (0.46mol/L).

Avoid eye and skin contact by wearing protective clothing and eye protection

7.1.2 Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area

7.1.3 Do not pipette materials by mouth

7.1.4 Wear disposable gloves while handling clinical specimens and always wash hands after working with potentially infectious materials

7.1.5 Dispose of all clinical specimens and reagents in accordance with local legislation

7.1.6 Safety data sheet available for professional user on request

7.2. TECHNICAL PRECAUTIONS

7.2.1 Components must not be used after the expiry date printed on the labels. Do not mix or interchange different batches/lots of reagents.

7.2.2 The reagents are provided at fixed working concentrations. Test performance will be adversely affected if reagents are modified or stored under conditions other than those detailed in Section 4.2.

Avoid contamination of reagents.

7.2.3 Use separate disposable pipettes or pipette tips for each specimen or control in order to avoid cross contamination of either specimens, controls or reagents which could cause erroneous results.

Store deionised or distilled water for dilution of concentrated reagents in clean containers to prevent microbial contamination. Microwells can not be re-used.

7.2.4 Do not store unused working strength Wash Buffer for subsequent use. When not in use Wash Buffer reservoirs should be rinsed with deionised or distilled water and left to dry.

7.2.5 Manual or automated washing equipment must be free of microbial contamination, be correctly calibrated and maintained according to the manufacturer’s instructions.

7.2.6 Do not use substrate showing a blue colour prior to its addition to the microwells.

7.2.7 Protect Substrate from light.

8. COLLECTION OF SPECIMENS

Collect serum specimens according to routine method.

9. TEST PROCEDURE

PLEASE REFER TO SECTION 7.2 TECHNICAL PRECAUTIONS BEFORE PERFORMING TEST PROCEDURE.

9.1. SAMPLE PREPARATION

NOTE: It is helpful to load samples into the white microwells in the location corresponding to that which will be used to run the assay as this allows a multichannel pipette to be used to transfer samples directly from the white plate to the coated plate.

Locate the required number of white microwell strips in the frame provided, according to the number of samples to be tested. Two white microwells are required for each specimen. In addition allow two microwells each for the Positive Control serum and
9.2. ASSAY PROCEDURE

9.2.1 Specimen Preparation
Locate the required number of clear monoclonal antigen coated microwells into the microwell holder. Transfer 100μL of each specimen/control from the white microwells to the corresponding clear microwell. Cover the clear microwells and incubate for 90 minutes at 20–30°C without shaking.

9.2.2 Conjugate Addition
Add 100μL of Conjugate to each microwell. Do not mix.
Incubate the microwells containing samples and Conjugate at 20–30°C for 10 minutes without shaking.

9.2.3 Washing the Microwells
The microwells should be washed using freshly prepared working strength Wash Buffer (see Section 4.2.5).

The washing technique is critical to the test performance and should be carried out so as to ensure complete filling (with a minimum of 350μL of working strength Wash Buffer) and emptying of the microwells.

Four wash cycles are essential, by either automated or manual washing techniques, which should include 2 minute soak period during the second wash or a total of 2 minute soak period during the complete cycle.

Manual Washing
If washing microwells manually, aspirate or shake out the contents of the microwells and using freshly prepared Wash Buffer, ensure complete filling and emptying of microwells. Between each wash step remove all remaining Wash Buffer by tapping the inverted microwells on to clean absorbent material. Manual washing efficiency is further ensured if the Wash Buffer is delivered at an angle so as to produce a vortex in the microwells. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of Wash Buffer.

Automated Washing
Automated washers should be programmed to complete 4 wash cycles and to incorporate the equivalent of 2 minutes soaking time during the complete washing cycle. Washers must be correctly calibrated to ensure complete filling and emptying of microwells between each wash.

After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of Wash Buffer.

9.2.4 Substrate Addition
Add 100μL of Substrate to each microwell.

9.2.5 Second Incubation
Incubate the microwells on the plate shaker at 20–30°C without shaking for 10 minutes.

9.2.6 Stopping the Reaction
Add 100μL of stop solution to each microwell. Ensure thorough mixing in the microwells. The coloured product is stable for 30 minutes. Do not expose to direct sunlight as photobleaching of the coloured product may occur.

9.3. READING THE TEST RESULTS
The microwells should be read photometrically within 30 minutes after addition of the Stop Solution. Mix the contents of the microwells and read the absorbance of each microwell using a suitable spectrophotometer or EIA plate reader set at 450nm. Ensure that the bottoms of the microwells are clean before reading and check that no foreign matter is present in the microwells. The reader should be blanked on air (ie with no plate in the carriage) before the plate is scanned.

Alternatively if the spectrophotometer or EIA plate reader allows for the use of a reference wavelength (at 620 to 650nm), dual wavelength reading should be performed which will eliminate any potential interference caused by aberrations, such as dirt or marks, on the optical surface of the microwells.

9.4. SUMMARY OF ASSAY FOR THE DETERMINATION OF ANTIBODIES TO M. HYOPNEUMONIAE

Ensure all reagents reach room temperature (20–30°C) before use.
Add 150μL Sample Diluent to white microwells
Add 150μL sample/control to appropriate sample/control white microwells
Transfer 100μL of prepared sample/control to appropriate clear microwells
Incubate 30 minutes at 20–30°C
Add 100μL of Conjugate to all microwells
Incubate 15 minutes at 20–30°C
Wash (4)
Add 100μL of Substrate
Incubate 10 minutes at 20–30°C
Add 100μL of Stop Solution
Read absorbance at 450nm

10. QUALITY CONTROL AND INTERPRETATION OF TEST RESULTS
If the following quality control criteria are not met, test results may be invalid and the assay procedure should be repeated.

10.1. BUFFER CONTROL
As detailed in Section 9.1 (Sample Preparation), at least two buffer control microwells must be included in each assay.
Calculate the mean OD value for the buffer control wells – ODbuffer control
ODbuffer control must be greater than 0.750 absorbance units and less than 2.500 absorbance units.
The expected value is between 1.000 and 2.000 absorbance units.

10.2. POSITIVE CONTROL
As detailed in Section 9.1 (Sample Preparation), at least two Positive Control microwells must be included in each assay.
Calculate the mean OD value for the Positive Control control wells – ODcontrol
ODcontrol must be less than 50% of ODbuffer control.
The expected value is approx 20-40% of ODbuffer control.

10.3. NEGATIVE CONTROL
As detailed in Section 9.1 (Sample Preparation), at least two Negative Control microwells must be included in each assay.
Calculate the mean OD value for the Negative Control control wells – ODnegative control
ODnegative control must be greater than 75% of ODbuffer control.
The expected value is approx 90-110% of ODbuffer control.

10.4. INTERPRETATION OF RESULTS
Specimens with a mean OD value less than 50% of the ODbuffer control should be interpreted as negative.
Specimens with a mean OD value ≥65% of the ODbuffer control should be interpreted as equivocal.
Specimens with a mean OD value between 50% and 65% of the ODbuffer control should be interpreted as positive.

11. PERFORMANCE LIMITATIONS
Contamination of the chromogenic Substrate may cause falsely high OD values and may cause quality control criteria to fail.
Washing the microwells too vigorously or incubating at a low temperature may cause falsely low OD values and may cause quality control criteria to fail.

The Oxoid Mycoplasma hyopneumoniae ELISA may be used for testing colostrum specimens for the presence of antibodies to M. hyopneumoniae using the test method and sample interpretation described in Sections 9 and 10.

12. REFERENCES
1. Feld NC, Qvist P, Ahrens P, Frisil NF, Meyling A
   A monoclonal blocking ELISA detecting serum antibodies to Mycoplasma hyopneumoniae.
2. Sorensen V, Barford K, Feld NC
   Evaluation of a monoclonal blocking ELISA and IHA for antibodies to Mycoplasma hyopneumoniae in SPF pig herds.
   Vet Rec 1992; 130: 488-90.
3. Sorensen V, Barford K, Feld NC
   Calculation of herd sensitivity and herd specificity for a monoclonal blocking ELISA detecting antibodies to Mycoplasma hyopneumoniae in pig serum and colostrum.