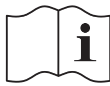

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PMT ELISA

REF K000911-9........................96 tests
K000921-9........................960 tests **EN**

A one-step enzyme immunoassay for the determination of *Pasteurella multocida* toxin (PMT).

1. INTENDED USE

The PMT ELISA is designed for the detection of *Pasteurella multocida* toxin: the causal factor of progressive atrophic rhinitis in pigs[1].

2. SUMMARY

Progressive atrophic rhinitis is a disease of pigs, caused by infection with toxigenic *P. multocida*[1]. The pathogenesis of the disease is linked to a single, well-characterised component: the *Pasteurella multocida* toxin (PMT). Other names for the PMT include dermonecrotic toxin (DNT); heat labile toxin (HLT); turbinate atrophy toxin (TAT) and osteolytic toxin. The clinical manifestations of the disease are shortening or distortion of the snout, sneezing, nasal discharge and epitaxis. Reduced growth rate is seen in severe cases. Subclinical forms of the disease also occur.

Host factors as well as other determinants – infectious or non-infectious – may influence the course of infection with toxigenic *P. multocida*. An important predisposing factor is infection with *Bordetella bronchiseptica*. Factors such as climate, housing and environment may influence the severity of infection as well as the persistence of toxigenic *P. multocida*, and thus, the clinical and economic burdens of the disease.

Health monitoring programmes should be based on laboratory tests for demonstration of toxigenic strains of *P. multocida* combined with clinical inspections of herds and snout sections at slaughter. The traditional laboratory tests are based on demonstration of the biological activitiy of toxin obtained from pure cultures of *P. multocida*. One of the main advantages of the PMT ELISA is that it assures a specific and sensitive demonstration of PMT in any type of sample, including extracts of primary mixed cultures obtained from e.g. nasal swabs or washings[2].

3. PRINCIPLE OF THE TEST

The kit contains microwells coated with mouse monoclonal antibody to PMT. Extracts of bacterial cultures and peroxidase-conjugated Fab’ fragment of rabbit antibody to PMT are incubated simultaneously in the microwells. After the incubation step, the microwells are washed and chromogenic subtrate is added. The colour reaction is stopped by the addition of acid. The presence of PMT in the extracts is indicated by a yellow colour in the microwells. The intensity of the colour is measured at 450nm and compared with the absorbance obtained for the positive and negative controls.

4. REAGENTS PROVIDED

Each kit contains sufficient materials for 96/960 determinations or 48/480 bacterial extracts assayed in duplicate. The shelf life of the kit is as indicated on the outer box label.

4.1. PMT ELISA CONTENTS

Instructions For Use.

Microtitration Plate 1/10 clear plastic 96 well Microtitration Plate of 12, 8 microwell strips coated with monoclonal antibody to PMT. A resealable plastic pouch is provided for storage of unused microwells.

White Strips 1/10 white plastic microplate of 12, 8 well strips – uncoated. The white wells are used for preparing extracts of bacterial specimens harvested from agar plates.

Sealing Tape 2/20 sheets of sealing tape.

One bottle of each of the following unless indicated otherwise:

Positive Control Antigen 1 x 1mL/2 x 1mL Affinity isolated, native, lyophilised PMT. Contains carrier protein.

Reconstitution Buffer 1 x 1mL/2 x 1mL buffered saline solution containing antimicrobial agent and detergent for reconstitution of Positive Control Antigen.

Conjugate 1 x 7mL/10 x 7mL of Peroxidase conjugated Fab’ fragment of rabbit polyclonal antibody to PMT in a buffer containing protein, coloured dye and an antimicrobial agent.

Wash Buffer Concentrate 1 x 50mL/10 x 50mL Wash Buffer concentrate (x25): Tris buffered solution containing antimicrobial agent and detergent.

Substrate 1 x 12mL/10 x 12mL Substrate stabilised peroxide and 3,3’-5,5’-tetramethylbenzidine in a dilute buffer solution. TMB has been reported to be non- carcinogenic. However, personal protective equipment is recommended to avoid direct exposure.

Stop Solution 1 x 25mL/5 x 25mL Stop Solution 0.46mol/L sulphuric acid

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

The PMT ELISA format allows for testing of up to 6 batches of specimens. 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 Monoclonal Antibody Coated Microwells

Open the plate pouch by cutting along the seal. Place unused microwells in the resealable plastic bag with the desiccant, carefully reseal the pouch and store at 2-8°C.

4.2.2 Positive Control Antigen

Add 800µL of Reconstitution Buffer to the lyophilized Positive Control Antigen. Mix the contents by gentle swirling. The Positive Control Antigen should be reconstituted at least 30 minutes prior to use. Reconstituted Positive Control Antigen should be stored at 2-8°C and used within three months. The Positive Control Antigen will be sufficient for at least 6 duplicate tests.

4.2.3 Conjugate

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

4.2.4 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).

4.2.5 Substrate

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

4.2.6 Stop Solution

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

5. ADDITIONAL REAGENTS

5.1. REAGENTS

Fresh deionised or distilled water for preparation of Wash Buffer.

5.2. ACCESSORIES

The following products are intended for use in conjunction with PMT ELISA. Contact your local distributor for further information.

Accessory Reagents for PMT serology (Code No. K003811-9)

6. EQUIPMENT

The following equipment is required:

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

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

Waste discard container with suitable fresh disinfectant

Microtitration plate shaker capable of a minimum speed of 500rpm with an orbital diameter of 1-3mm (optional). For information on suitability of plate shakers contact your local distributor

Automated plate washer (optional) or suitable equipment for washing 8 microwell strips (See Section 9.2.4)

Spectrophotometer or EIA plate reader able to read a 96 microwell plate of 8 microwell strips at an absorbance of 450nm with a reference at 620-650nm

37°C Incubator

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 Positive Control Antigen contains purified native PMT and should be handled and disposed of as a biohazard.

7.1.2 The Stop Solution contains sulphuric acid (0.46mol/L). Avoid eye and skin contact by wearing protective clothing and eye protection.

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

7.1.4 Do not pipette materials by mouth.

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

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

7.1.7 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.

7.2.3 Avoid contamination of reagents.

7.2.4 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.

7.2.5 Store deionised or distilled water for dilution of concentrated reagents in clean containers to prevent microbial contamination.

7.2.6 Microwells can not be re-used.

7.2.7 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.

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

7.2.9 Do not use Substrate showing a blue colour prior to its addition to the microwells.

7.2.10 Protect Substrate from light.

8. COLLECTION AND PREPARATION OF SPECIMENS

Suitable porcine microflora can be obtained from several sources, including nasal swabs, nasal washings, tonsillar swabs or from lung or tonsil tissues. Nasal swabs should preferably be obtained from the posterior part of the nasal cavity in live pigs by the use of commercially available swabs, such as cotton or alginate swabs. The swabs should immediately be transferred to a transport medium eg saline. The transport time to the laboratory should be minimized as much as practically possible, and the swabs should preferably be kept at low temperatures (approx 4°C) during transport[3].

Primary cultures of nasal swabs are prepared in Petri dishes (diameter 9cm) on selective blood agar medium containing neomycin sulphate 2µg/mL and bacitracin 3.5µg/mL[4]. In order to obtain an agar surface covered with numerous colonies, a few drops (50 to 100µL) of the transport medium should be distributed over the entire agar surface or by streaking the swab over the entire agar surface.

All colonies on the agar plate should be harvested after overnight incubation at 37°C. Add 2mL of deionised or distilled water to each plate and collect bacterial specimens using a Drigalski (“triangular”/”hockey stick”) spatula and a pipette. A smaller volume of water should not be used for the specimen preparation,

since this could cause antigen overloading.

9. TEST PROCEDURE

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

NOTE: Sample extraction must be carried out on the day prior to running the assay.

9.1. SAMPLE EXTRACTION

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.

Pipette 200µL of the harvested bacterial specimen into each of two white microwells. Locate specimens into microwells corresponding with the final position of the specimens in the coated microwells when the assay is run. Leave four microwells (eg microwells A1, A2, B1, B2) empty for the positioning of controls.

Cover microwells with a plate lid or sealing tape, and incubate overnight at 37°C for extraction to take place.

9.2. ASSAY PROCEDURE

NOTE: This procedure must not be followed for use with K003811-9 reagents.

NOTE: The assay procedure requires the use of a microtitration plate shaker. For information on suitability of shakers contact your local Oxoid subsidiary or distributor.

9.2.1 Conjugate Addition

Locate the required number of clear monoclonal antibody coated microwells into the microwell holder. Add 50µL of Conjugate to each microwell.

9.2.2 Specimen and Control Addition

Add 50µL of deionised or distilled water to each of the two negative control microwells (microwells A1 and A2).

Add 50µL of reconstituted Positive Control antigen to each of the two Positive Control microwells (microwells B1 and B2).

Transfer 50µL of the bacterial extract from the white microwells into the corresponding microwells of the clear microwells. It is recommended that a multichannel pipette is used for this operation.

9.2.3 First Incubation

Cover the microwells containing Conjugate, specimens and controls and incubate on a plate shaker at room temperature (**20-30°C**) with **shaking** for **60 minutes**.

9.2.4 Washing the Microwells

The microwells should be washed using freshly prepared working strength Wash Buffer (see Section 4.2.4).

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 a 2 minute soak period during the second wash or a total of a 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.

9.2.5 Substrate Addition

Add 100µL of Substrate to each microwell.

9.2.6 Second Incubation

Incubate the microwells on the plate shaker at room temperature (**20-30°C**) **without shaking** for **10 minutes, ± 2 minutes**.

9.2.7 Stopping the Reaction

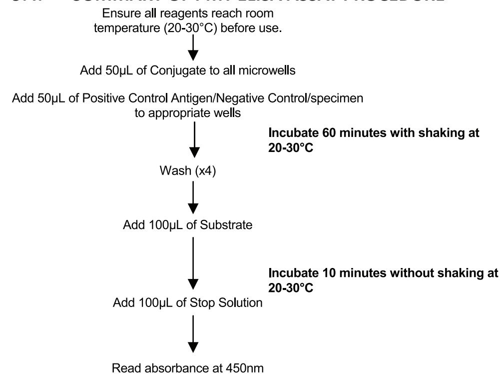
Add 100µL of Stop Solution to each microwell. Ensure thorough mixing in the microwells. The coloured product is stable for 30 minutes. **Do not expose to direct sunlight** as photobleaching of the coloured product may occur.

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 620-650nm), 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 PMT ELISA ASSAY PROCEDURE



10. QUALITY CONTROL AND INTERPRETATION OF TEST RESULTS

10.1. NEGATIVE CONTROL

As detailed in Section 9.2.2 (Specimen and Control Addition), two Negative Control microwells must be included in each assay.

Calculate the mean OD value for the two Negative Control wells - $OD_{Negative\ Control}$

$OD_{Negative\ Control}$ must be less than 0.150 absorbance units but greater than 0.000 absorbance units.

If $OD_{Negative\ Control}$ is <0.000 absorbance units then the reader should be reblanked on air and the wells re-read.

If $OD_{Negative\ Control}$ quality control requriements are not met, test results may be invalid and the assay procedure should be repeated.

10.2. POSITIVE CONTROL

As detailed in Section 9.2.2 (Specimen and Control Addition), at least 2 Positive Control microwells must be included in each assay.

Calculate the mean OD value for the two Positive Control control wells – $OD_{Positive\ Control}$

$OD_{Positive\ Control}$ must be greater than 0.500 absorbance units. If $OD_{Positive\ Control}$ is less than 0.500 absorbance units test results may be invalid and the assay procedure should be repeated.

10.3. SPECIMENS

Bacterial extracts should be considered as positive for the presence of PMT if both of the following criteria are met:

Mean specimen OD is ≥ $OD_{Negative\ Control}$ plus 0.100 absorbance units.

And

Mean specimen OD is ≥ 5 x $OD_{Negative\ Control}$

If only one of the above criteria is fulfilled, a retest of the remaining extract should be performed, or a repeat specimen requested.

11. PERFORMANCE LIMITATIONS

11.1. Contamination of the chromogenic Substrate may cause falsely high OD values and may cause quality control criteria to fail.

11.2. 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.

11.3. The PMT ELISA may also be used for the differentiation of toxigenic and non-toxigenic *P. multocida* in pure cultures. When testing pure cultures the extraction step used with primary mixed cultures can be omitted and the specimen can be tested immediately after being harvested from the agar plate.

11.4. The PMT ELISA may also be used in conjunction with PMT accessory reagents (Code No. K003811-9) for the detection of antibodies to PMT in serum or colostrum from animals suspected of having toxigenic *P. multocida* infection, or from animals that have been vaccinated with vaccines containing PMT or PMT analogues. For further details contact your local distributor.

12. BIBLIOGRAPHY

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13. PACKAGING

K000911-9........................96 tests

K000921-9........................960 tests

Symbol Legend

Symbol	Meaning
REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
i	Consult Instructions for Use
	Temperature Limitation
LOT	Batch Code
	Use By
	Manufacturer

IFU X7835A Revised March 2015

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For all enquiries please contact your local distributor.