Product information

SE/MR (#DEVS004)

SR/MR is an indirect enzyme immunoassay (EIA) for the detection antibodies to Erysipelothrix rhusiopathiae in porcine serum.

General Information

Swine Erysipelas is a widespread disease caused by Erysipelothrix rhusiopathiae bacterium.
The disease is manifested in several forms from acute and fatal outbreaks to mild infections (referred to as the urticarial form). Alternatively it may become established as a chronic infection. The latter includes cardiac and arthritic types as well as an abortive form, which may become more common.
Although it occurs in pigs of all ages, those between 3 months and 1 year are most susceptible.
The acute form of the disease in unweaned pigs has become increasingly common. The enzyme-linked immunoassay has proven to be a highly sensitive and specific tool for the diagnosis of all forms of the disease including the chronic and asymptomatic forms. It is, at the same time, of considerable interest in epidemiological studies. It may also be used to monitor herd response to vaccination.

Principle of the test

The Demeditec Diagnostics SE/MR test is an indirect enzyme-linked immunoassay (EIA).
The Erysipelothrix rhusiopathiae specific antigen is coated on 96 well plates.
Upon incubation of the diluted samples in the test well, antibody specific to Erysipelothrix rhusiopathiae binds with the coated antigen and remains in the well after washing off the unbound material, then a conjugate is added that binds any attached swine antibody. After that, unbound conjugate is washed away and chromogenic enzyme substrate is added. The color appearing in each well is proportional to the amount of swine antibody specific to Erysipelothrix rhusiopatiae present in the diluted sample.

Kit Composition

Product	Quantity
• 96-well microplate (in eight-well strips) coated with the specific Erysipelothrix rhusiopathiae antigen	5
• Vial 0 Washing Solution (10X)	2x100 ml
• Vial 1 Sample Diluent Solution (3X). Containing green dye	100 ml
• Vial 2 Conjugate Solution: Mab anti-porcine IgGs/HPRO solution. Ready to use. Containing red dye	30 ml
• Vial 3 Substrate Solution: ABTS solution. Ready to use.	30 ml
• Vial 4 Stop Solution : Oxalic acid solution. Ready to use	30 ml
• Vial 5 Positive Control: Positive Control Serum pre-diluted and ready to use.	2.2 ml
• Vial 6 Negative Control: Negative Control Serum pre-diluted and ready to use	2.2 ml
• Microplate adhesive cover	5
• Kit insert	1

Material needed but NOT provided

37°C incubator, precision single and/or multichannel pipettes with disposable pipette tips, tubes or dilution plate for diluting samples, 96-well plate reader, distilled or deionised water and plate-washing device.

Precautions for Users

Carefully read this kit insert. Store all reagents at 4ºC. Do not freeze. Unused strips must be resealed with silica gel as moisture can damage the plates. Do not expose substrate solution to strong light or any oxidising agents. The substrate solution is very sensitive even to trace levels of contamination and should therefore not be returned to the bottle once removed. An overage is supplied to allow removal of little quantites of remaining solution.Do not use components past expiry date and do not intermix components from kits with different lot numbers. Careful pipetting and washing throughout the procedure are necessary to maintain precision and accuracy. Do not pipette by mouth. Use gloves during the process. The stop solution is an organic acid, which is toxic and may be corrosive, handle with care. All wastes must be properly decontaminated prior to disposal. For veterinary use only.

Sample preparation

Positive and negative controls are ready to use and do not require dilution. The rest of the samples must be diluted 1: 200 in reconstituted Sample Diluents Solution.

It is recommended to perform a 2-step dilution protocol.
For example: First dilute in a tube 10 µl of sample in 390 µl of reconstituted Sample Diluents Solution, and then transfer 10 µl of this 1:40 diluted sample to the ELISA well into which has perviously been pipetted 40 µl of reconstituted Sample Diluents Solution. If it is allowed the use of round-bottomed tissue culture plates (dilution plates) and multi-channel pipettes able to accurately dispense a 5-µl volume, then the 2-step dilution protocol can be performed as follows:
First dilute 5 µl of sample in 95 µl of reconstituted Sample Diluents Solution pipeting each sample in the dilution plate, and then transfer 5 µl of this 1:20 diluted sample to the ELISA well into which has perviously been pipetted 45 µl of reconstituted Sample Diluents Solution.

Reagent Preparation.

All reagents must be allowed to come to room temperature before use.
Crystals may form in the washing and sample diluents solutions due to the high concentration of salts. To re-disolve this crystals the bottle should be repeatedly inverted prior to the following reconstitution steps. To obtain a faster resuspension of the crystals, resuspension for 10 – 15 minutes in a 28 - 37 ºC bath is allowed. Do not use this solutions upon it will be totally re-disolved. To avoid crystal formation in the concentrated 10x Washing solution, it can be stored at room temperature after the first precipitated resuspension.

- **Washing Solution (10x) (Vial Nº0): To reconstitute add 1 volume of Washing Solution concentrate to 9 volumes of distilled or deionised water (eg. to prepare 100 ml of reconstituted Washing Solution, mix 10 ml of the concentrate solution with 90 ml of distilled or deionised water). We recommend that this should be used within 7 days.**

- **Sample Diluent Solution (3x) (Vial Nº1): To reconstitute add 1 volume of Sample Diluent Solution concentrate to 2 volumes of distilled or deionised water (eg. to prepare 60 ml of reconstituted Sample Diluent Solution, mix 20 ml of the concentrate solution with 40 ml of distilled or deionised water). We recommend that this should be used within 2 days.**

Test Procedure.

1.Allow the reagents to come to room temperature and ensure adequate mixing by swirling or inversion.

2.Record sample and control locations on a 12x8 template sheet. The positive and negative controls must always be run in duplicate.

3.Remove the adhesive cover from the plate and add 50µl of the undiluted controls and 1:200 diluted samples to the appropriate wells.

4.Cover the plate with the adhesive cover and incubate 60 minutes at 37ºC.

5.Remove the adhesive cover and wash the plate 3 times with reconstituted Washing Solution (300 µl per well). Invert and firmly tap dry on absorbent paper.

6.Add 50 µl of Conjugate Solution (Vial Nº2) to each well.

7.Cover the plate with the adhesive cover and incubate 60 minutes at 37ºC.

8.Remove the adhesive cover and wash the plate 3 times with reconstituted Washing Solution (300 µl per well). Tap dry as above.

9.Add 50 µl of Substrate Solution (Vial Nº3) to each well. Shake gently the plate for 2 seconds.

10.Develop the chromogenic reaction for 15 minutes at room temperature (20-25 ºC) in the dark.

11.Add 50 µl of Stop Solution (Vial Nº4) to each well. Mix by gently tapping the side of the plate.

12.Wipe the under-surface of the plate free of dust etc. with a soft tissue. Read the plate using a Microtiter Plate Reader at 405 nm having first blanked on air. Record the results.

Test validation.

The test is valid if the mean OD_{405} of the Positive Control is superior to 0.9 and ratio (mean OD_{405} of the Positive Control /mean the negative control) is superior to 5,0.

Calculations.

For the interpretation of results, an IRPC value is required (Relative Index x100). The following formula is applied to obtain the IRPC value (using mean DO_{405} values for controls).

$$\text{IRPC} = \frac{OD^{405}\text{ Sample} - \text{Mean } OD^{405}\text{ Negative Control}}{\text{Mean } OD^{405}\text{ Positive Control} - \text{Mean } OD^{405}\text{ Negative Control}} \times 100$$

Interpretation of results.

SAMPLE	IRPC VALUE
POSITIVE	> 40
NEGATIVE	$\leq$ 40

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