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**Enzyme immunoassay for porcine reproductive and respiratory
syndrome**

猪繁殖与呼吸综合症免疫酶试验方法

(English Translation)

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Table of Contents

Foreword

1 Scope

2 Immunoenzyme test

2.1 Material preparation

2.2 Method of operation

2.3 Result determination

Foreword

Appendix A of this standard is a normative appendix.

This standard is proposed and managed by the Animal Husbandry and Veterinary Bureau of the Ministry of Agriculture.

This standard was drafted. Veterinary Diagnostic Center of the Ministry of Agriculture.

1 Scope

This standard specifies the Porcine Reproductive and Respiratory Syndrome (PRRS) immunoenzyme test and immunohistochemistry.

This standard applies to the detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) antibodies and PRRSV antigens in tissues in pig serum.

2.1.1 Reagents

2.1.1.1

Phosphate buffer (PBS). See section A for formulation. Chapter 1.

2.1.1.2

Preparation of antigen smears. PRRSV was inoculated into Marc-145 cells grown to 70% to 80% monolayer. 3d after inoculation~ 4d, when the lesion reached 50% to 75%, the monolayer of the dispersed infected cells was digested with trypsin, washed three times with PBS, and diluted to 1×10^6 fine Cell/mL. The chamber slides with 10 to 40 small holes were taken, and 10 μ L was added to each well. After drying at room temperature, fixed in cold acetone (4 ° C) 10min. Sealed and packaged at -20 ° C for use.

2.1.1.3

Standard positive serum. PRRSV test serum prepared from infected pigs.

2.1.1.4

Standard negative serum. no PRRSV-infected, unimmunized pig serum.

2.1.1.5

Enzyme conjugate. Horseradish peroxidase (HRP)-labeled Staphylococcal Protein A (SPA).

2.1.1.6

Substrate solution. See Appendix A for preparation. Chapter 2.

2.1.2 Equipment

- a) ordinary optical microscope;
- b) chamber slides with 10 to 40 small holes printed;
- c) micro-sampler, capacity 5 μ L ~ 50 μ L;
- d) 37 ° C constant temperature incubator or water bath.

2.1.3 Sample

The blood of the pig to be tested was collected and serum was separated. Serum should be fresh, transparent, non-hemolyzed, non-polluting, densely packed in sterile vials, 4 ° C or -30 ° C Save or check immediately. The test sera were serially numbered before the test and diluted 10 times with PBS.

2.2 Method of operation

2.2.1

Remove the antigen smear, dry at room temperature, add 10 times diluted serum to be tested and standard negative serum, standard positive serum, each blood Two viral cell wells and one normal cell well were added and placed in a humid chamber at 37 ° C for 30 min.

2.2.2

Rinse three times with PBS for 5 min each time and dry at room temperature.

2.2.3

Add the appropriately diluted enzyme conjugate and place in a humid chamber at 37 ° C for 30 min.

2.2.4

Rinse PBS three times for 5 min each time.

2.2.5

Place the chamber slide into the substrate solution and develop color for 5 min to 10 min at room temperature. Rinse twice with PBS and rinse with distilled water - times.

2.2.6

After drying, observe under ordinary light microscope and judge the result.

2.3 Result determination

2.3.1

In the case of a negative serum control and a positive serum control. the negative serum reacts with normal cells and virus-infected cells.

Colorless; positive serum reacts with normal cells colorless, reacts with virus-infected cells in a brownish-yellow to tan color, and the result can be determined; otherwise Retry.

2.3.2

The serum to be tested is colorless with normal cells and virus-infected cells, and can be judged as negative for PRRSV antibody.

2.3.3

The serum to be tested reacts with normal cells to be colorless, and the reaction with the virus-infected cells is brownish-yellow to tan, which can be judged as