UNIVERSIDAD COMPLUTENSE DE MADRID
Facultad de Veterinaria
Departamento de Sanidad Animal

REFERENCE LABORATORY

STANDARD OPERATING PROCEDURE FOR THE DETECTION OF AFRICAN SWINE FEVER VIRUS (ASFV) BY DIRECT INMUNOFLUORESCENCE (DIF)

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1. MATERIALS AND REAGENTS

- Organ impression smears from suspected ASF infected animals and no infected animals as a negative control.
- Anti-ASFV antibodies conjugated with fluorescein isothiocyanate (FITC)
- PBS pH7.2
- Slides
- Glycerine
- Fluorescence microscope
- Humid chamber

PBS (pH 7.2)

- ClNa [Merck 1.06404]---------- 8 gr
- ClK [Merck 1.04873]---------- 0.2 gr
- PO_4H_2K [Merck 1.06586] ---------- 0.2 gr
- PO_4HNa_2 [Merck 1.04936] ---------- 2.29gr
- H_2O destilada ---------- 1000 ml

2. METHODOLOGY

2.1 Prepare impression smears of test tissues on slides.
2.2 Fix them with heat during 15 minutes using a 60 vatios lamp located at 20 cm from the slide.
2.3 Add the immunoglobulin anti-ASFV conjugated with FITC at the recommended dilution.
2.4 Incubate it in a humid chamber at 37ºC for 30-40 minutes.
2.5 Wash three times with PBS pH 7.2
2.6 Add glycerine over the slide.
2.7 Examine under a fluorescence microscope.

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3. RESULTS

Positive tissue samples will show specific granular cytoplasmic fluorescence inside the infected cells.