



## **AFRICAN SWINE FEVER ANTIBODY DETECTION: INDIRECT IMMUNOFLUORESCENCE (IFI)**

### **• INTRODUCTION**

The IFI for ASF, is a common serological diagnostic method, where the sensitivity of the histological techniques and the specificity of immunological techniques are combined.

This technique was first adapted to the African swine fever (ASF) antibody detection by Bool et al. in 1961 and latter it was applied as a routine protocol by Sánchez-Botija et al. in 1970

This method uses fluorescein isothiocyanate conjugated against the immunoglobulins present in a sample serum, showing either immunological or fluorescent activity.

### **• MATERIALS AND REAGENTS REQUIRED**

- Monkey Stable (MS) cell culture on cover slips in "leighton" flasks or "Lab-Tek" chambers, infected with the ASF virus.
- ASF Positive and Negative sera
- Fluorescein conjugated anti-swine IGG (Nordic 4745)

#### **Carbonate/bicarbonate buffer 0,5M pH 9<sub>·</sub>**

Na HCO <sub>3</sub>	3.7 g
Na <sub>2</sub> CO <sub>3</sub>	0.6 g
Distilled water	100 ml

#### **Glycerine buffer pH 8**

Mix glycerine and carbonate/bicarbonate buffers in proportion 2:1.



### **PBS buffer pH 7.2\_**

Na Cl	8.0 g
KH <sub>2</sub> PO <sub>4</sub>	0.2 g
Na <sub>2</sub> HPO <sub>4</sub> 12H <sub>2</sub> O	2.9 g
KCl	0.2 g
Distilled water to	1000 ml

Check the pH before use. Store at + 4°C. For long term storage, keep at –20°C.

## • **METHODS**

1. Add test or control sera diluted 1/30 in PBS pH 7.2 to slides, in sufficient quantity. Incubate for 30 min. at 37°C in moist chamber.
2. Next, wash the cover slips exhaustively with PBS pH 7.2 , last time for 5 minutes.
3. Add fluorescein-conjugated protein A diluted in PBS pH 7.2, at recommended or pretitrated dilution. Incubate for 45 minutes at 37°C in moist chamber.
4. Wash them as previously described.
5. Then, dry the cover slips very carefully and mount them in glycerine buffer on a slide or in Fluoprep (Bio-Merieux 75521). Finally, examine the result using fluorescence microscopy.

## • **READING AND INTERPRETING THE RESULTS.**

Positive control shows a specific fluorescence near the nucleus. Negative control does not show fluorescence. Test sera will be classified as positive or negative in reference to the positive and negative controls.



## REFERENCES

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