A variant of the direct immunofluorescence technique used in the routine diagnosis of PRRS syndrome

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Abstract
Laboratory diagnosis of PRRS syndrome is based on virus detection, isolated strain characterization and antibody detection. Given the severity of the disease, rapid diagnostic methods are used to detect the nucleocapsid viral antigen present in the target organs (lymph nodes, lungs). From swine youth corpses from disease outbreaks, inguinal lymph nodes were taken, and from swine youth with characteristic respiratory symptoms, samples of oronasal fluid were taken. The nucleocapsid viral antigen was detected using the anti PRRSV monoclonal antibody kit labeled with fluorescein isothiocyanate (BIO 268). The smears made of lymph nodes and oronasal fluid to which they were identified, in the microscopic field, the described aspects were considered positive. Thus, 26 samples of lymph nodes (65%) and 9 oronasal fluid samples (45%) were positive, which were controlled to confirm PRRS virus presence by RT-PCR technique. All positive samples of lymph nodes and oronasal fluid positive to the IFD technique in the adapted working variant were confirmed as positive samples by the RT-PCR technique.

Key words: PRRS, lymphnode, IFD, RT-PCR

Introduction
Porcine Respiratory and Reproductive Syndrome (PRRS) was diagnosed in Romania in 1998 and is currently being spread in many swine farms (4).

The disease is produced by a RNA virus encompassed in family Arteriviridae, having two genotypes, respectively, type 1 (European) and type 2 (American). There are significant differences between these genotypes, represented by the variability of the gene sequences (5,6).

Laboratory diagnosis of PRRS syndrome is based on virus detection, isolated strain characterization and antibody detection. Given the severity of the disease, rapid diagnostic methods are used to detect the nucleocapsid viral antigen present in the target organs (lymph nodes, lungs) (1,4,5).

Since the immunofluorescence reaction performed on cryosections involves a complex endowment of the diagnostic laboratories, the research sought to develop a direct rapid technique for viral antigen detection, fingerprinting, lymph nodes and oronasal fluid.

Materials and methods
From swine youth corpses from disease outbreaks, inguinal lymph nodes were taken, and from swine youth with characteristic respiratory symptoms, samples of oronasal fluid were taken. The nucleocapsid viral antigen was detected using the anti PRRSV monoclonal antibody kit labeled with fluorescein isothiocyanate (BIO 268).

The used variant of the direct immunofluorescence reaction had the following steps depending on the pathological material used:
- the removal of glass blades with ethyl alcohol;
- calibration of samples from lymph nodes, on blades by fingerprint;
- centrifuging oronasal fluid samples and showing the sediment on the blades;
- blade welding and fixing in acetone;
- washing the blades with PBS-Blue Evans solution and drying the blades;
- addition of 0.1 microgram conjugated to fluorescein;
- examination of the fluorescent light with Optika microscope.

The confirmation of the obtained results was performed by the RT-PCR Operational Standard Procedure, the Real Time version, used in the Laboratory of Molecular Biology of the Pasteur SA Institute of Bucharest. For this purpose, four extraction kits (Qiagen and Roche, Germany) and two ORF 7-specific primers and the following primers were used.

- Primer PRRS -2 ORF 7: 5'-GCG AAT CAG GCGCAC WGT ATG-3';
- Primer PRRS-4 ORF 7: 5'- AGA AAA GTA CAG CTC CGA TGG -3';

A number of 40 samples of lymph nodes and a number of 20 oronasal fluid samples were examined by this technique.

**Results and discussion**

Inguinal lymph nodes were taken from fresh, suiting youth corpses from farms where PRRS syndrome has evolved as a primary disease. The lymph nodes were increased in volume, with firm consistency, and on the sectional area their color was red due to haemorrhagic inflammation.

Oronasal fluid samples were collected from suiting youth where PRRS syndrome clinically evolved in acute form.

A modified version of the immunofluorescence technique performed on cryosections was used in the research to be used as a method of diagnosing of PRRS syndrome because the cryosection technique requires adequate endowment.

On slides with ethyl alcohol, after drying, fingerprints were made on the lymphocyte section of lesions. Oronasal fluid samples were centrifuged and the sediment was uniformly exposed on the glass flaps. After drying, the smears made from the two types of pathogenic material samples were fixed in acetone solution for 15 minutes and then dried for 2 hours at room temperature. In the next step, the lamellae were rinsed with a mixture of saline phosphate buffer solution with Evans Blue and subsequently dried again. In the final step, the smears thus prepared were coated with the fluorescent conjugate, dried and covered with lamellae, and subsequently examined under a UV (20x and 40x) ultraviolet light microscope.

Microscopic lymph nodes have been screened for isolated cells, clustered cells, or large clusters of small, medium, large, plasma, and rare epithelial cell lymphocytes. In lymphocytes, the cellular contour was evident, the nuclei were well individualized, and the cytoplasm was bright fluorescent bright greenish appearance due to the presence of viral nucleocapsid antigens coupled to fluorescein-labeled monoclonal antibodies. Epithelial cells were rare, and the cytoplasmic fluorescence was very obvious.

In the microscopic field, smears of cells, predominantly epithelial with high fluorescence cytoplasm, were detected in oronasal fluid smears.

The smears made of lymph nodes and oronasal fluid to which they were identified, in the microscopic field, the described aspects were considered positive. Thus, 26 samples of lymph nodes (65%) and 9 oronasal fluid samples (45%) were positive, which were controlled to confirm PRRS virus presence by RT-PCR technique.

All positive samples of lymph nodes and oronasal fluid positive to the IFD technique in the adapted working variant were confirmed as positive samples by the RT-PCR technique.

The direct immunofluorescence reaction has been used so far only for the detection of the nucleocapsid antigen of the PRRS virus in cryosections performed from lymph nodes, pulmonary and other lymphoid organs. Our own research has been aimed at developing a simplified method as a routine routine method in the PRRS diagnosis (2).
The results obtained confirm that the IFD technique in the presented variant can be adapted, but more research is required to establish with certainly the degree of sensitivity and specificity of this method.

**Conclusions**
- The IFD variant used allowed lymphocyte and oronasal fluid smears to be detected with fluorescein-labeled monoclonal antibodies to detect different types of PRRS-infected cells.
- The IFD response following the described methodology detected the presence of viral antigens at 65% of the examined lymph nodes and oronasal fluid samples.
- For the use of the IFD technique as a rapid diagnosis method, it is necessary to continue the research on a much larger number of samples and in comparison to several diagnostic methods.

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