

ONE-STEP IMMUNOCROMATOGRAPHIC METHOD FOR THE DETECTION OF ELEVATED LEVELS OF PIG-MAP CONCENTRATION

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Introduction. Increases in basal pig APP concentration have been reported in response to experimental or natural infection, as well as under the effect of stressors like the change of facility, mixing of animals, or transport. Differences in APP serum levels have been found between high and low health status herds, and elevated levels of APP were associated with losses of productive performance. Several studies have demonstrated that APP serum concentration can also be used as a biomarker to assess the efficacy of therapeutic treatments or vaccination. Pig-MAP is one of the main APPs in pig. Its basal concentration is lower than 1 mg/mL, increasing up to 8-12 mg/mL during the acute phase response. In this study, a prototype qualitative membrane-based immunoassay for the detection of acute phase levels of Pig-MAP has been developed and evaluated in serum samples.

Material and Methods. The immunocromatographic test (ICT) device is formed by a semi-rigid plastic strip on which a chromatographic membrane and absorbent materials are attached. In the lower part of the strip there is a conjugate pad that contains colored microspheres coated with an anti Pig-MAP monoclonal antibody. The reaction zone contains two parallel lines in which a cooperant anti pig-MAP monoclonal antibody, and a control antibody are fixed. In the upper end is an absorbent that allows the chromatographic flow. The strip also contains a blood separator to be used with whole blood.

To perform the test, 10 ul of serum samples were added to microtiter plate wells, and incubated for 5 minutes with a dilution buffer containing a precise amount of Pig-MAP antibodies to block the Pig-MAP concentration present in normal state. A strip was introduced in each well, and the immunocromatography performed during 10 minutes. The excess of Pig-MAP present in the sample was captured by the antibody coated-colored microspheres that migrate upwards on the strip and were retained by the antibody fixed in the capture line. This leads to the apparition of a visible pink line (being its intensity dependant on protein concentration). The system also contains blue colored microparticules, that are retained by the control antibody to give a blue control line.

162 serum samples were analysed by the ICT. The concentration of Pig-MAP in the samples was determined by a sandwich ELISA (PigCHAMP Pro Europa S.A., Segovia, Spain), based on the same monoclonal antibodies that the ICT. The results obtained with the ICT were classified as positive (from slightly to clearly positive pink line for Pig-MAP and a blue control line), negative (only the blue control line).

Results. A cut off of 1.5 mg/ml was established for the normal serum. Serum samples were classified in two groups according to Pig-MAP concentration: lower than 1.5 mg/ml (“negative”) and higher than 1.5 mg/ml (“positive”). Of the 162 samples analysed 109 were lower than 1.5 mg/ml and 53 higher than 1.5 mg/ml. The sensibility of the ICT (% of ICT positive of the positive serum) was 90.5 %. The specificity (% of ICT negative of the negative serum) was 94.4 %. The positive predictive value (% of real positive detected by ICT of the total ICT positive) was 88.8% and the negative predictive value (% of real negative detected by ICT of the total ICT negative) was 95.3 %.

Discussion. Interest on animal APP and its use in animal production is increasing every year, and assays for the different species are currently under development. The ICT described here is an easy, fast and accurate method for the detection of animals suffering an acute phase response that results in an elevation of Pig-MAP concentration. The test showed a good sensibility (91%) and specificity (94%), and a very good negative predictive value (95.3%). The test can be applied to detection of “normal” or healthy animals and may be incorporated in the quality systems to evaluate the health status of pig herds at farms or slaughterhouses.