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Combination of laser microdissection with real-time PCR to determine the origin of isolated PAP particles up to their species level

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Abstract In the European Union, the reference method for the detection of Processed Animal Proteins (PAP) is light microscopy. Nevertheless, one of the drawbacks of the technique is its inability to determine the origin of detected animal particles up to species level. With the partial lifting of the EU total feed ban, additional analytical methods for enforcement of EU legislation are required to avoid 1 recycling of ruminant PAP and 2) intraspecies recycling of non-ruminant PAP. Up to now, the polymerase chain reaction (PCR) is the only method able to detect the presence of PAP and to determine their origin with sufficient sensitivity and specificity. Nevertheless, the possible presence of authorised milk or egg powder, fat and blood can interfere with PCR results. In 2010, Fumière et al. described an original protocol combining near infrared microscopy (NIRM) with real-time PCR to remove this limitation of PCR. Nevertheless, the manual isolation of particles at the microscopy step is still laborious and too tedious for routine analysis. An innovative and attractive alternative could be the use of a microscope coupled with a laser microdissection and catapulting system. The technique allows the isolation of single particles or parts of a particle without any physical contact reducing drastically the risks of contamination. AGES and CRA-W joined their forces to combine the two techniques and to analyse isolated particles with their respective DNA extraction and PCR protocols. Cleaning steps to eliminate DNA molecules sticking to PAP particles remain necessary in many cases as can be concluded from compared results obtained by both institutes but they confirm the interesting perspectives of this approach.

This communication is under the responsibility of the authors and does not reflect the view of the European Union Commission.

Keywords Real Time PCR;processed animal proteins;PAP;particles