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Implementation of the real-time PCR as official method of detection of processed animal proteins in the European Union reference laboratory network

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Abstract In the TSE Road map 2 describing the strategy on Transmissible Spongiform Encephalopathies for the period 2010-2015, the European Commission envisaged a possible gradual lifting of the feed ban but keeping the level of consumer protection unchanged. To achieve this goal, strict control rules are maintained: 1. Processed Animal Proteins (PAPs) coming from ruminant remain forbidden; 2. Ruminant cannot have PAPs in their diet unless few exceptions like fishmeals as milk replacers for young animals; 3. The intra-species recycling of PAPs is banned. In addition, PAPs will not be allowed in the feeds for herbivores (rabbit, horse,…) whatever their origin.

The implementation of this new legislation requires additional analytical methods for its enforcement. Besides a continual improvement of the light microscopy protocol, real-time PCR tests were or are assessed and validated for the purpose of the detection of PAPs and the determination of their species origin. Different PCR methods already proved their potential through previous inter-laboratory studies but always in the hands of their developers1. In 2009, a big step forward in the implementation of PCR methods in a network of labs was the development and the validation of a transfer protocol using plasmid calibrants through an international interlaboratory study conducted by the EURL-AP gathering 18 participants from Europe, Japan and Australia2. In March 2012, the ruminant PCR assays developed by TNO Triskelion used in combination with the CRA¬-W transfer protocol was officially declared as fit for the detection of PAPs in feed based on the results of an interlaboratory study involving 12 European participants3. The work to validate a pig and a poultry target is also in progress.

As the PCR is an indirect method targeting the detection of DNA, the presence of ingredients of animal origin authorised by the legislation (e.g. milk or egg powder, fats and blood powder) could interfere with the results. To solve this problem, different strategies are investigated (e.g. the PCR analysis of the sediment fraction or the combination of a laser microdissection and catapulting system with the real-time PCR).

Another aspect of the implementation of the PCR was the launching in December 2010 of a training program intended in a first step to beginners. Within 4 sessions, people from 19 NRLs attended courses alternating theoretical and practical aspects. Moreover, the EURL-AP produced a DVD explaining through video sequences the good laboratory practices to provide reliable PCR results.

A final evaluation of the successful implementation of the PCR in the EURL-AP network is under progress with the interlaboratory study aiming to evaluate the ability of the NRLs to detect the presence of ruminant in DNA samples as well as in feed samples.

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Keywords Real Time PCR;calibrant;plasmid;cut-off, PAP;processed animal protein