P15

Comparison by two complementary assays of the absolute copy numbers of plasmid calibrants used for the determination of a PCR cut-off

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Abstract In the framework of a European relaxing of the feed ban, PCR assays to determine the species of origin of processed animal proteins (PAP) are validated and implemented in a European network of 26 National Reference Laboratories. A ruminant PCR assay developed by TNO Triskelion bv was recently validated by the European Union Reference laboratory for Animal Proteins in feedingstuffs (EURL-AP)[1]. However, as for other natural PCR targets, late amplification signals are sometimes observed even with feedingstuffs free of ruminant material. The determination of an accurate cut-off is therefore needed to interpret reliably the PCR results qualitatively. In 2009, the EURL-AP validated a transfer protocol to determine the cut-off of a PCR platform (combination of a thermocycler and a master mix) for a PCR assay[2]. This protocol uses plasmid solutions as calibrants containing the target cloned in pUC18. Formerly, the copy number of the target was estimated by spectrophotometry on a highly concentrated solution of linearized plasmid. Solutions at nominal concentrations of 128, 32 and 8 copies/µl were obtained by successive volumetric dilutions of the highly concentrated solution into a maize background DNA. At such low final DNA concentrations (~fM), the precise number of copies cannot be determined by classical spectrophotometry methods and may fluctuate from batch to batch depending on the repeatability of the dilution process.

Consequently, the EURL-AP and the EC-JRC-IRMM joined their forces to improve the accuracy on the determination of the calibrant copy number using a digital PCR (dPCR) apparatus "BioMark™ HD System" (Fluidigm Corporation, South San Francisco, CA, USA). The nature of the target (a highly repeated ruminant target) complicated this task due to the presence of contaminant ruminant DNA in some commercial reagents used for the dPCR. Two different strategies were tested: the first one kept the original primers and probe targeting the cloned ruminant sequence in pUC18 and a loading reagent free of ruminant DNA but not originally dedicated to dPCR; the second one consisted of the normal loading reagent associated to a newly designed PCR assay targeting a border region between the cloned ruminant target and pUC18. Advantages and drawbacks of each strategy are explained and their results exposed and compared.

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Keywords digital PCR;dPCR;calibrants;plasmid;cut-off;PAP;processed animal proteins

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