EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
**Food & Feed Compliance**

# Mandate for an ENGL Working Group on multiplex PCR Methods (WG-mpPCR)

## Background

Multiplex PCR is a valuable approach to simultaneously detect two or more DNA target sequences in one reaction. It is increasingly applied because of its cost-benefits and possibility to increase the GMO screening coverage with similar or less number of reactions in comparison to singleplex PCR.

Multiplex PCR requires specific instrumentation, reagents as well as expertise for accurate application. Compared to singleplex PCR methods, development and validation of multiplex PCR methods are more sophisticated. Specific considerations and adaptions for their application are required (primer/probe design and concentration, fluorophores and quenching, instruments, mastermixes etc.).

Some aspects concerning multiplex PCR are already addressed in ENGL guidance documents (MPR, MVfr, dPCR). However, information and guidance are scattered and relate only to specific performance parameters and PCR applications (e.g. asymmetric LOD; dPCR application; method verification). Additional information and guidance for ENGL laboratories is required for further developments, applications and routine use. The dispersed information should be compiled, reviewed and consolidated into one guidance document for multiplex PCR.

## Tasks

The WG should review the following aspects, identify needs for provision of guidance to ENGL laboratories and propose approaches to address them:

- Review existing (validated) multiplex PCR methods (qualitative, quantitative, element-/construct-/event-specific, species-specific)
- Review of multiplex PCR instruments and reagents; fluorophore/ quencher choice and combination
- Validation parameters (additional bioinformatics; *in silico* specificity and sensitivity tests etc.); relevant performance requirements and criteria
- Combination of existing singleplex PCR modules
- Modularity of multiplex PCR assays (exchange of modules)
- Transferability/exchange of PCR platforms/instruments
- Multiplex PCR and quantification
- Multiplex digital PCR
- In-house validation; method verification

Other relevant aspects may be added to this list.

## Timeline

The first meeting should take place within the the end of 2017. At this meeting a work plan will be established. A total of 4 meetings (virtual or physical) should be sufficient to finalise the work.