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BMT/11/26
ORIGINAL: English
DATE: September 18, 2008

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

CODEX WORK ON DNA-BASED METHODOLOGY

Document presented by an expert from the
Food and Agriculture Organization of the United Nations (FAO)

UPOV Working Group on Biochemical and Molecular Techniques

Madrid, 16-18 September 2008

Codex work on DNA-based methodology

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Joint FAO/WHO Food Standards Programme

Joint FAO/WHO Food Standards Programme - Codex Alimentarius

- Intergovernmental Organisation
- The Commission meets every year in FAO or WHO
- Subsidiary bodies/ Codex Committees and Task Forces
- Scope : All aspects of food safety (including chemical and microbiological contamination, safety of foods derived from biotechnology) and food quality

Background

- Codex work on foods derived from biotechnology/ genetically modified foods
  - risk analysis and safety assessment : Ad hoc Task Force on Foods Derived from Biotechnology (work completed)
  - methodology: detection and identification of GM material (Committee on Methods of Analysis and Sampling)
  - labelling (Committee on Food Labelling - ongoing)
- Criteria for the Methods for the Detection and Identification of Foods Derived from Biotechnology: General Approach and Criteria for the Method (under development)

Guidelines for the Validation and Quality Control Requirements for the Analysis of Foods Derived from Biotechnology

- Method criteria
- Definitions (specific definitions in addition to current definitions of general application)
- Method development to formal validation: applicability of the method, validation process, modular approach to method validation
- Method acceptance criteria
- Collaborative Trial Requirements
- Validation of PCR Methods (Quantitative / Qualitative)

Guidelines for the Validation and Quality Control Requirements for the Analysis of Foods Derived from Biotechnology

- Validation of a Protein based method
  - o Quantitative / Qualitative
- Units of Measurement
- Measurement Uncertainty
- Guidance on Laboratory set up and operation Reference Materials
- Sampling
- Concentration Distributions
- Reference Materials

Applicability of the Method

- the method should be applicable to the matrix concerned. in the case of « general purpose » methods to identify and quantify GM material, at least one generally applicable extraction method should be available
- the extraction should yield DNA in sufficient quantity, structural integrity and purity to allow adequate amplification of DNA during the PCR step
  - can be tested by setting up series of the template DNA and determining the Ct-value (threshold number of cycles at which the measured fluorescence signal crosses the defined threshold value betwen dilutions) for each dilution – used to estimate the efficiency of PCR in real time PCR analysis
  - due to deterioration of protein during processing, protein based methods are generally applied to minimally processed foods

Acceptance Criteria

- conditions: the method should detect and quantify the specific target and taxon-specific DNA sequence or the protein: use of DNA-based or protein-based methods
- the method should fulfil the following requirements
  - DNA based transformation event-specific methods should allow for unequivocal detection /identification / quantification of a transformation event: target a transformation event –specific genomic region
  - qualitative tests performed so that the number of positive and negative subsamples (pools) from a sample can lead to an estimate of the biotechnology derived grain content of the material
  - all methods should be applicable to the material specified in their scope, and to appropriate quality control and reference materials when availalble

Information about the method

- Primer pairs: sufficient justification for the selection of primer pairs /fluorescent oligonucleotide probe for the target gene and the reference gene should be provided
- Selectivity Testing
- Stability Testing
- Sensitivity Testing
- Robustness Testing
- Cross-reactivity

Practical Application of the Method

- Applicability
  - indication of the matrix (processed foods, raw materials), type of sample, range to which the method can be applied should be given. Relevant limitation of the method should also be addressed
- Operational characteristics and practicability of the method
- Operator skills requirements

Method Validation Performance

- whether the method is instrument or chemistry specific (such as stability of reagents, heating and cooling characteristics, recording of fluorescence, qualitative or quantitative
- Amplicon length: may influence PCR performance. selection of shorter amplicon sizes will increase the possibility of a positive signal in the analysis of processed foods
- whether single- or multi-plex PCR amplifications are undertaken
- differences between PCR-based and immunological methods concerning validation criteria

Validation of a Quantitative PCR Method

- Accuracy
- Applicability
- Dynamic range – range of quantification
- LOD and LOQ: if the method is used at concentrations close to the LOD and LOQ (typically 0.01-0.05%), their assessment will be part of the validation procedure
- Practicability
- Repeatability Standard Deviation: should be ≤ 25% or as close as practicable over the whole dynamic range of the method

Validation of a Quantitative PCR Method

- Reproducibility Standard Deviation: should be ≤ 25% or as close as practicable at the target concentration and over the majority of the dynamic range.
- Ruggedness (Robustness)
- Sensitivity
- Target Specificity of the detection and reference genes should be demonstrated by providing experimental evidences, testing the method with non-target r-DNA transformation events and non-r-DNA plants

Validation of a Qualitative PCR Method

- False positive rates
- False negative rates
- Ruggedness
- Acceptance Criteria Value and Interpretation of Results

Validation of a Qualitative PCR Method:
Table 1 :criteria for scoring qualitative PCR analyses

PCR result (GM analyte)	**PCR result (endogenous)**	**Scoring of test**
Positive	Positive	Positive
Negative	Positive	Negative
Positive	Negative	Repeat
Negative	Negative	Repeat

Table 2: Criteria for scoring duplicate qualitative PCR analyses scored as per Table 1

Result 1	**Result 2**	**Result of the analysis**
Positive	Positive	Positive
Negative	Positive	Repeat/indeterminate
Positive	Negative	Repeat/indeterminate
Negative	Negative	Below LOD

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