Techniques for detection and identification

5-1 Choice methods

Experimental design

- The scope is limited to grains and seeds

- Separation of activities in different areas (the sample preparation should be in the different room to prevent cross contamination due to dust)

- Forward flow for DNA

  - Sample preparation
  - Extraction

- Depending on functionality (equipments) and expertise of the lab and regulatory purposes and requirements

- Screening, identification or quantification

Sample handling and preparation

- EU, ISTA and ISO guidelines

- Sample size, integrity of the sample and clear identification of the sample with a company documentation

- The storage of the laboratory sample received and/or the retention sample

- The storage conditions should maintain the integrity of the test portion and retention sample

- A procedure for the disposal of the samples

- The laboratory sample must be a subsample and homogenized

- The subsampling must maintain the representative nature of the laboratory sample

- The smaller the particle size the better the sensitivity of the PCR

- The larger the test portion the better the representation of the hole sample

- The test portion must be conducted ideally in minimum duplicate

- Need of a system for the unique identification of the test portion throughout the process

As well as the retention sample

- Labo personel should only be part of sampling at lab level

Applicability of matrices

- Grains and seeds

5-2 protein methods

- Commercial produced and specific for a particular transgenic proteins (S+E)

- LOD varies between different strips and different suppliers (S+E)

- Identifying protein is not equivalent to event detection (S+E)

- Multiple strips or plates are required for screening (S+E)

- The expression levels of the transgène protein in the LMO may vary depending on environment conditions (S+E)

- Rapid point of entry and field monitoring(S)

- Strip readers may a offer a semi quantitative approach but the level of uncertainty is high(S)

- The sensitivity of the strips or ELISA may not be sufficient to detect adventitious presents of an LMO(S+E)

- The cost is relatively low and easy to implement

- **Laterol flow strip (Theoroy is in technical tools and guidances)**

- Sample preparation is the same for DNA (except the partical size may be bigger)

- The proteins are extracted crudely according to the manufacturer instructions

- The storage of the strips is important in maintaining the ability of the strip to function correctly

- Centrifugation can be used to remove particles from the crude protein extracts

- It is important that the control line be visible and affiant target line and to consider that a faint target line does not necessarily indicate low levels of LMOs

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- **ELISA (Theoroy is in technical tools and guidances)**

- Need specialized equipments for quantitative use

- Follow manufacturer instructions

- The kits must be keept at the correct temperature to maintain their functionality

- Sample preparation is the same for DNA (except the particle size may be bigger)

- The proteins are extracted crudely according to the manufacturer instructions

- Centrifugation can be used to remove particles from the crude protein extracts

- **Limitations (Theoroy is in technical tools and guidances)**

- Limited availability of kits for different transgène proteins and there is a high probability for false positive
- Negative results on either ELISA or strip does not exclude the present of untested LMOs

5-3 DNA methods

All methods used must be verified in the lab conditions

- **DNA extraction and handling**

- Can use an commercial kits or other validated methods (references)
- Have to be careful for the cross contaminations
- Different controls can be used
    - Positive extraction controls
    - Extraction blank controls
    - Environmental control
- The integrity and yield of the DNA should be checked either through spectro, fluoremetry or gel electrophoresis (references)
- The extracted DNA must be stored appropriately

- **Qualitative PCR**

- Dilutions of the DNA must be made to test for sample inhibitions to avoid false negatives
- The amount of DNA should ideally be between 20 and 200ng/µl of DNA
- The following controls are required:
    → Positive control
    → Negative /blank control
- Cross contamination is an important consideration when gel electrophoresis of amplified products is performed. The forward flow must be maintained
- Dedicated equipment for PCR set up, for PCR and gel electrophoresis
- The preparation of the PCR set up should be separated to the edition of the DNA
- Use filter tips and gloves without powder (because powder derived from maize may be used)
- The reagents should be aliquoted

- DNA and RNA free plastic ware and disposable tubes
- Regular cleaning and decontamination of the laboratory is necessary
- Reagent set up and the addition of DNA or DNA controls can be performed in a dedicated dead air box/Biosafety cabinet/PCR hood
- Qualitative gel based methods are less sensitive than real time methods
- The advantage with the real time PCR is that contamination is minimized since post-PCR handling is not required
- Qualitative gel electrophoresis methods require post PCR verification
- PCR performed in minimum of duplicate
- Each PCR assay requires separate optimization and verification
- The results must be consistent between replicates
- Sample inhibition can be checked by setting up additional sample reactions in duplicate that are spiked with known quantities of positive DNA to determine the ability of the assay to amplify the spiked DNA

- **Quantitative real time PCR**

- Dilutions of the DNA must be made to test for sample inhibitions to avoid false negatives
- The amount of DNA should ideally be between 20 and 200ng/µl of DNA
- The following controls are required:
    - → Positive control
    - → Negative /blank control
    - → Standards with three data points ideally in duplicate (CRM or plasmids)
- The relative amount of the transgene target gene is expressed as a percentage relatively to an endogenous reference gene
- If using CRM material as standards is better to express the results in percentage m/m While compared to plasmid percentage CP/CP
- Dedicated equipment for real-time PCR is required
- The preparation of the PCR set up should be separated to the edition of the DNA
- Use filter tips and gloves without powder (because powder derived from maize may be used)
- The reagents should be aliquoted
- DNA and RNA free plastic ware and disposable tubes
- Regular cleaning and decontamination of the laboratory is necessary
- Reagent set up and the addition of DNA or DNA controls can be performed in a dedicated dead air box/Biosafety cabinet/PCR hood
- The advantage with the real time PCR is that contamination is minimized since post-PCR handling is not required
- Real-time PCR performed in minimum of two dilutions in duplicate
- Each real-time PCR assay requires separate optimization and verification
- For real-time PCR quantification a positive control of known percentage can be used as internal control
- Requirements for the standard curves must be meet (references)

- The LOQ is sufficient for the scope of analysis

- **PCR-BASED SCREENING STAGTEGIES (Matrix approach)**

- The screening should take into consideration the regulatory scope and approvals of LMOs in a country
- The matrix approach can be used for screening
- The results of the analysis are only applicable to the elements tested
- Common elements in LMOs can be used for LMO detection
- It is preferable to follow a screening approach with event detection

- **Interpretation of results**

- Results have to be analyzed in terms of LOD and LOQ of the assay

- The result of the duplicate sample must be consistent

- Acceptable analytical practice must be used in analysis (references)

- The measurement of uncertainty must be indicated for quantitative analysis (references)

- Prior to sample analysis, controls must meet the necessary quality control criteria (references)

- **Limitations of DNA based methods**

- (see above)

- Lack of DNA sequence information for a particular event make it impossible to design appropriate assays

- The laboratory can only detect the LMOs that are been tested before

- high cost and specialized equipment and expertise are required

- illegal events are likely to be difficult to detect

- Availability of controls

- Since DNA based testing is more complex it is important to verify the assay

5-4 other novel technologies/strategies for LMO detection

- (See technical tools and guidance)