SAMPLING AND LABORATORY SAMPLE PREPARATION

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Outline

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- Sampling and sample preparation equipment
- Environmental and safety issues
- Generic sampling process
- Sample preparation
- Sample storage and disposal
- Quality control during sampling and sample preparation
- Conclusion
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Introduction

- Sampling - procedure through which a portion of a substance, a material or a product is taken in order to provide a representative sample of the total, for testing.
- Sampling and testing for the presence of GM material may be carried out at several points "from farm to fork".
- e.g. samples of the seed before sowing
- plants in the field
- harvested crops
- various points during further processing

Introduction cntd

- How to take samples and test for GM content will be a balance between:

- which analysis will be most relevant
- costs of this analysis

Introduction cntd

- Testing results only as good as the sample which has been tested – limit of detection determined by least sensitive part of procedure.
- Sample preparation steps shown to contribute to the largest error component in an analytical determination.
- Ensuring proper sampling and sample handling supports the validity and applicability of any laboratory results

Introduction cntd

- Lab must "have a sampling plan and method when it carries out sampling of substances, materials or products for subsequent testing…" (ISO 17025:2017 Clause 7.3)
- Sampling plan should identify the selection, withdrawal, preservation, transportation and preparation of the primary sample in a statistically representative manner.
- Sample must be sufficiently large to allow reliable detection at desired sensitivity.

Some Definitions

Lot

Distinct and specified quantity of material dispatched or received at one time and covered by a particular contract or shipping document

Laboratory sample or Bulk

Sample as prepared (from the lot) for sending to the laboratory and intended for inspection or testing

Reserve sample

Left-over material from the test samples which is representative of each Lot usually adequate for two more full tests.

Some Definitions

Homogeneity

Degree to which property or constituent is uniformly distributed throughout a quantity of material – the sameness of things.

Increment

A portion of material extracted from the laboratory sample when a sub-sampling process is required

Incrementing (composite sampling)

Collection of many random increments from lab sample to make up the subsample - all increments must have the same probability of ending up in the sample.

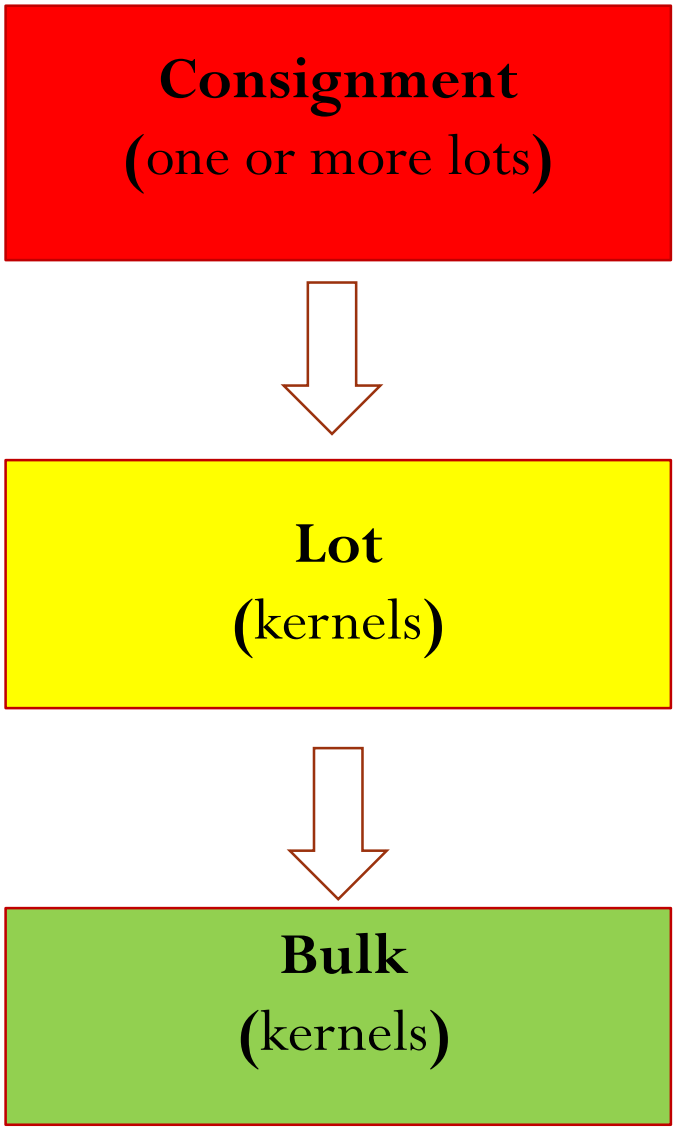
Sampling plan

The following elements should be considered in the sampling plan:

- purpose for which the sample is taken
- client's requirements
- selection of sampling sites
- sampling frequency and timing
- type of sampling containers
- on-site measurements, environmental conditions

The Effect of Sample Preparation

PREPARATORY STEPS **SAMPLE TYPES** **PROBLEMS**

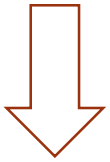


Sampling

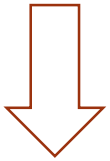
Distribution of GM – kernels unknown

PREPARATORY STEPS **SAMPLE TYPES** **PROBLEMS**

Sub -sampling



Laboratory
(kernels**)**



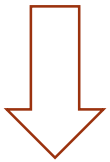
Grinding

Laboratory
(particles**)**

Is the distribution and proportion of GM and non-GM particles the same as that of GM and non-GM – kernels after grinding?

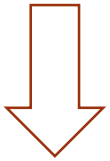
PREPARATORY STEPS **SAMPLE TYPES** **PROBLEMS**

Sub -sampling



Test
(particles)

DNA extraction



Test/Analytical
(molecules)

Different contributions of GM and non-GM – copies of DNA from different tissues after extraction?

Sampling plan cntd

The following elements should be considered in the sampling plan:

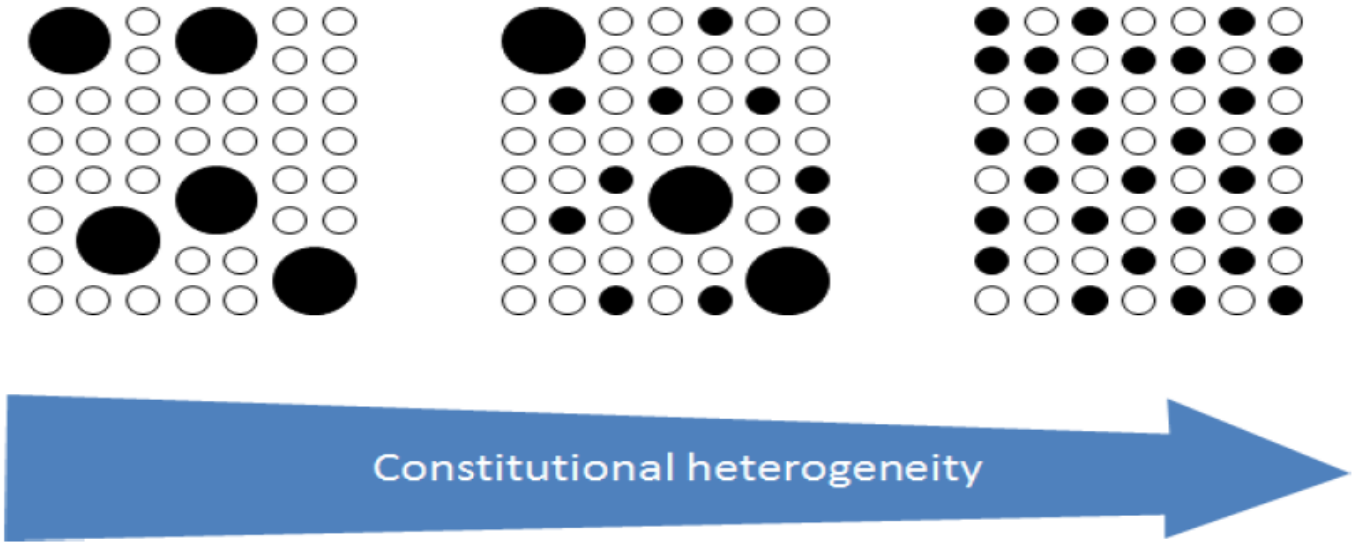
- sample size
- holding conditions
- preservatives
- homogeneity
- appropriateness of the sample

General considerations related to sampling

- Sampling and sample reduction must be performed using appropriate techniques and clean equipment in good condition
- The lot to be sampled from must be as uniform as practicable.
- Distributional and constitutional heterogeneity should be avoided

General considerations related to sampling

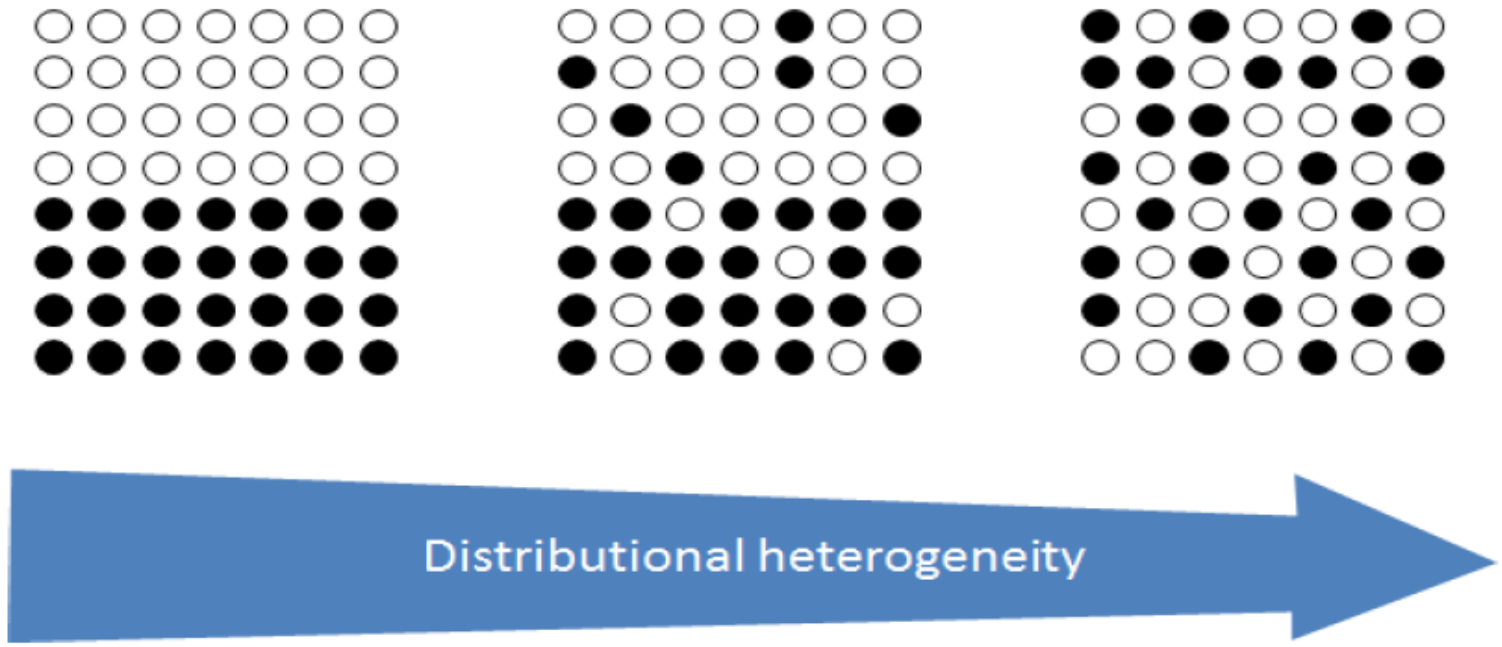
Constitutional heterogeneity is the existence of large overall composition based differences.



This can be reduced by reducing the average particle size through crushing/cutting

General considerations related to sampling

Distributional heterogeneity is the non-random distribution of particles in the sample and is caused by different densities, sizes or shapes of particles .



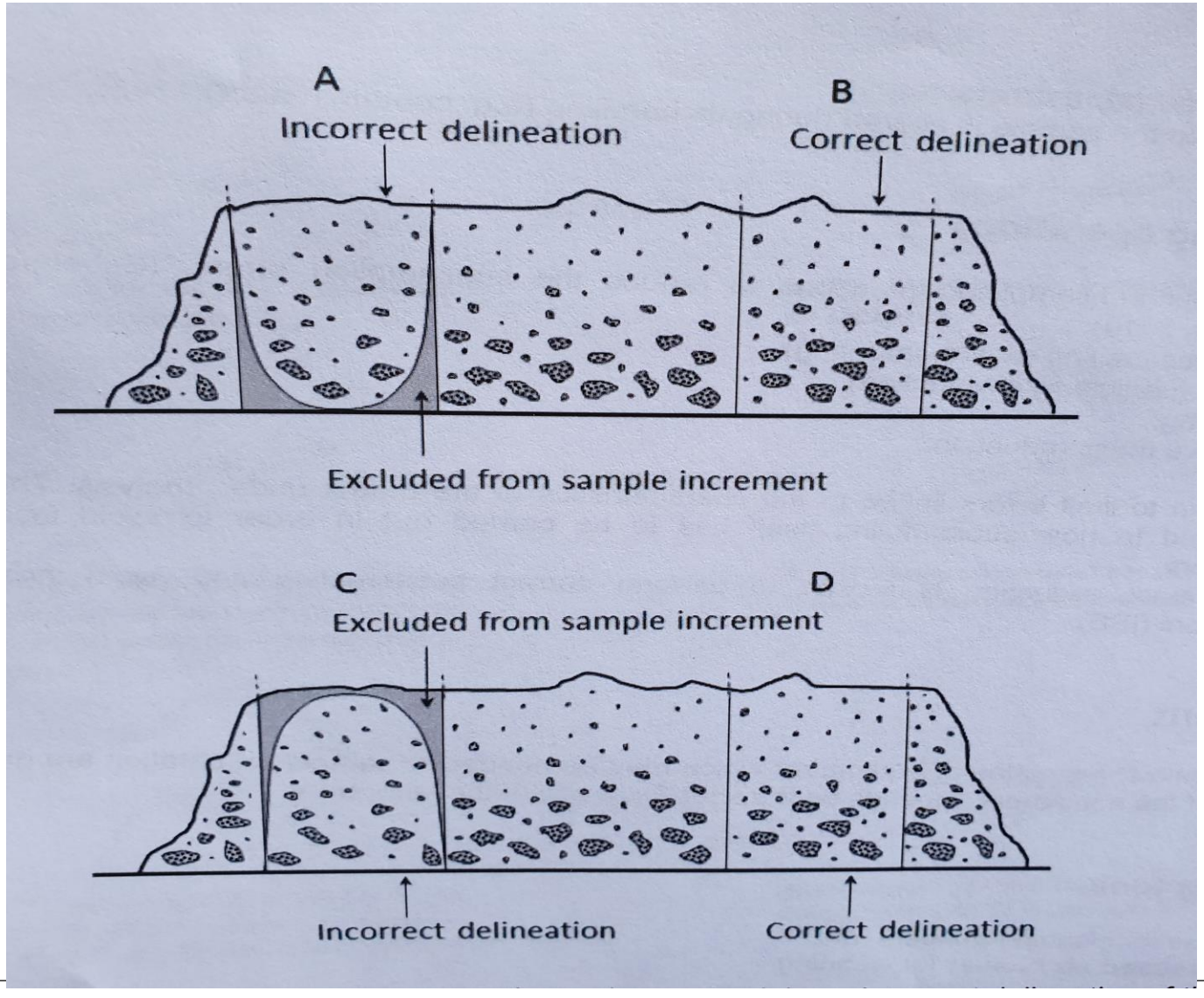
It can be eliminated by mixing, blending or incrementing.

General considerations related to sampling

Sampling and sub sampling process associated with three main errors;

- Increment delineation errors – This is mainly caused by the sampling tool
- Increment extraction errors – Fragments with their centre of gravity inside the delineated increments should become part of the composite subsample
- Increment preparation errors – mainly as a result of contamination

Inappropriate extraction tools - incorrect delineation of the increment



Sampling and sample preparation equipment

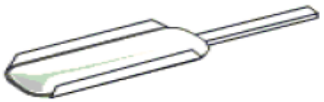
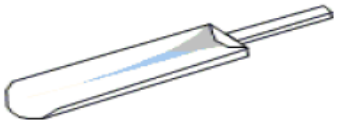
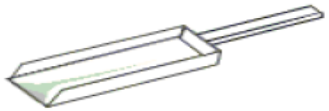
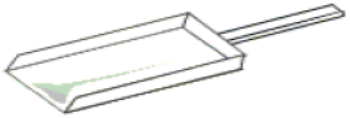
The design of sampling equipment is critical in order to avoid sampling errors.

- Cleaning tools - brushes, compressed air blower, vacuum cleaner and ultrasound bath.
- Sampling tools - spoons, spatulas and shovels
- Sample preparation – balance
- Drying systems – Lyophilisation system, drying ovens and incubators

Sampling and sample preparation equipment

- Particle size reduction – grinders, dust extractors, riffle splitters, motor and pestle, overhead mixer and plastic bags.
- Storage equipment – wide mouthed plastic bottles, plastic bags, refrigerator and freezer
- To avoid increment delineation errors, spoons, spatulas and shovels should be square-edged.

Sampling and sample preparation equipment

Incorrect design		Correct design
	Spatula	
Flat without edges: material segregates when falling off each side		Square edges: prevent material from falling off each side
	Scoop	
Round shape: material at the top of the flattended sample has more chance to be part of an increment than the material at the bottom		Square shape: all material has the same chance to be part of an increment
	Shovel	
Round shape: material at the top of the flattended sample has more chance to be part of an increment than the material at the bottom		Square shape: all material has the same chance to be part of an increment

Environmental and safety issues

- Sample preparation work should be dedicated to a special room or working area
- All equipment should be cleaned during sample preparation and after. Wash solutions like bleach, HCl and H_2O_2 are recommended
- Appropriate PPPs should be worn. Dust ventilation system has to be operated.

Generic sampling procedure

- Lot preparation and conditions for sampling
- Establishing sampling intensity
- Taking primary sample
- Obtaining the composite sample
- Obtaining laboratory-bound sample
- Transportation of laboratory sample

Lot preparation and sampling intensity

- The lot must be uniform otherwise establish a statistically verifiable way of ensuring uniformity
- It should also be arranged in such a way that every part of the lot is conveniently accessible
- Sampling intensity depends on the type of material and population size

Lot preparation and sampling intensity

- For example, for ISTA - sampling seed lots in containers holding up to 100 kg seed, the following sample intensity can be considered;

Number of containers	Minimum number of primary samples to be taken from each container
1-4	3
5-8	2
9-15	1
16-30	15 primary samples, one each from 15 different containers
31-59	20 primary samples, one each from 20 different containers
60 or more	30 primary samples, one each from 30 different containers

Taking primary sample

- Primary samples of approximately equal size must be taken from the lot
- When the lot is in containers, the containers to be sampled must be selected at random or according to a systematic plan throughout the lot.
- Primary samples must be drawn from the top, middle and bottom.
- When the lot is bulky, the primary samples must be drawn from random positions.

Obtaining composite and lab sample

- Primary samples are physically compared with each other during sampling
- If they are uniform, they are combined to form a composite sample.
- The laboratory submitted sample is then obtained by reducing the composite sample to an appropriate size (mass reduction)

Obtaining composite and lab sample

- Number of mass reduction can be achieved through different ways;
- Fractional shovelling – Lab sample is split into different samples by collecting increments from the same sample. Increments are then combined and homogenised.

Obtaining composite and lab sample

- Spoon method – Sample is spread out in an even layer and random increments are taken and combined into a subsample.
- Long pile method – Sample is poured out in a long pile. Several cross sections are then taken out of the pile and all cross sections put together lead to a subsample.

Transportation

- "The laboratory shall have a procedure for the transportation, receipt, handling, protection, storage, retention and disposal or return of test items……Precautions shall be taken to avoid deterioration, contamination, loss or damage of the item…" (ISO 17025:2017 Clause 7.4.1)
- The sample should be transported to the laboratory in a manner that makes sure that its integrity is protected.

Sample preparation

On arrival at the lab, sample goes through the following processes before analysis;

- Laboratory sample check
- Mass reduction
- Particle size reduction
- Partial drying
- Special sample prep procedures
- Test portion
- Storage and disposal

Laboratory sample check

- Laboratory sample is registered and identified using a unique number
- The sample should be free from any sign of damage and should be cooled or frozen if prescribed by the shipment conditions
- Deficiencies should be documented and eliminated whenever possible

Laboratory sample check

- In case of cross contamination, the sample is not fit for further analysis
- Also check on the sample size. It should be stated if it is below the minimum sample intake.
- It is also advisable to fix a maximum size to avoid the mass reduction step

Mass reduction

- If grinding is required - recommended to use the whole sample.
- Mass reduction can only be done if a part of the sample is to be used.
- When a dry sample has lumps and is of particle sizes above 6 mm, the whole laboratory sample should be pre-ground before mass reduction.

Mass reduction

- Lab mass reduction can be achieved through any of the following;
  - Fractional shovelling
  - Spoon method
  - Long pile method

Particle size reduction

Particle size reduction can be achieved through the following processes;

- Chopping – mechanically cutting into smaller pieces
- Crushing – for samples with large and hard lumps
- Cutting – can be done using rotating or stationary knives
- Blending – employed for semi-solid material
- Milling/grinding – using a combination of cutting, shearing and pressure
- Pulverizing – action of various mills to reduce particle size

Particle size reduction

Size reduction techniques should take into consideration the following aspects;

- Physical and chemical properties of the material
- Maximum allowable initial particle size
- Final desirable particle size range
- Quantity of material and expected sample throughput
- Required grinding efficiency
- Sample loss due to adherence to grinding surface
- Decontamination requirements for the equipment

Lab samples size

Threshold Value %	Laboratory sample size (number of particles)	
	Homogenous distribution of GM particles	Heterogeneous distribution of GM particles
0.1	35 000	100 000
0.5	7 000	20 000
1	3 500	10 000
2	1 750	5 000
5	700	2 000

Particle size reduction

- To minimize the loss of integrity of DNA molecules, heating during grinding must be limited.
- Mixing is required after grinding to homogenize the sample.
- The best recommended mixing technique is the plastic bag technique.
  - Ground material is put in a plastic bag that is closed
  - Approximately one half of open space is left in the bag
  - Homogenization is then achieved through 20 successive reversals of the bag

Partial drying

- Some semi-solid matrices e.g. forages, it is important to partially dry before fine grinding
- Partial drying can be done using either a forced-air oven or microwave heat
- Drying should be done at temperatures below 60^0C so that the DNA content of the sample is not affected.

Special sample Preparation

Some special sample treatments are done for different sample matrices;

- Samples with high fat – process the sample in a frozen or chilled state. Use dry ice during grinding to avoid melting
- Viscous sample – e.g. honey, heating to about 60^0C improves homogeneity
- Liquid samples – centrifuge and extract DNA from the pellet
- Plant tissue – freeze dry and grind or freeze in liquid nitrogen and then grind to a homogenous sample.

Test portion

- Mixing of sample before taking test portion for extraction, is important for homogenisation
- Test portion must contain an adequate mass in accordance with the fundamental subsampling error and maximum particle size.
- The maximum test portion size for DNA extraction is usually 5 g.

Storage and disposal

- The integrity of the laboratory sample should be maintained throughout the analytical and post analytical phases until disposal.
- Proper storage includes refrigeration, controlled humidity and UV light
- Storage will obviously depend on the sample matrix.
- The laboratory should have a clearly documented storage and disposal policy

Quality Control during sampling and sample preparation

- The laboratory is required to have a procedure for the monitoring of results validity.
- The sampling and sample preparation phases should therefore be guided by specific quality control procedures
- Quality control should mostly be ensured during particle size reduction, homogenization, and taking of the test portion for further analysis

QC during particle size reduction

- Particle size reduction has to be done using calibrated equipment.
- A typical example is for grinding;
  - Ground product should usually contain 80% of particles below half the sieve mesh size
  - 95% of the material should be below the sieve nominal mesh size.
- In-between samples cleaning process effectiveness can be tested through doing a negative sample test. A negative sample is run after a positive sample (>5%) with the cleaning exercise having been done between samples.

QC during sample homogenizing

- Particle size reduction usually promotes segregation and improper mixing only promotes this problem
- Quality control is therefore crucial during the mixing stage.
- The unmixed layers method can be used to control the laboratory mixing method

QC during sample homogenizing

- A quarter of the storage container is filled with a ground light coloured material with a further quarter filled with a ground coloured material. The time needed to mix the contents until they are visually blended is noted.
- The realistic densities of usual test material has to be taken into account.

Test portion representativeness

- It is essential to ensure that the test portion is sufficiently representative of the sample from which it is taken
- This can be ensured through replicate analysis (six) of a level just above the method LOQ/LOD.
- The RSD should not exceed 25% otherwise the test portion should be increased or the mean particle size of the yielded ground material should be reduced.

Conclusion

- In each step during sampling, an error is introduced.
- Challenge is to minimize the unavoidable sampling error.
- The first sampling stage in this process normally regarded as the most critical one
- The actual distribution of the genetically modified material in the lot is not known beforehand.

Conclusion

- Sampling and sample preparation undoubtedly the major contributors to measurement uncertainty
- It is crucial for the laboratory to have a clear sampling plan as well as a detailed sample preparation procedure.
- Also important to ensure that there is adequate quality control at every stage of the sampling and sample preparation process

References

- ISTA (2018), *International Rules for seed Testing*, International Seed Testing Association, Bassersdorf, Switzerland.
- European Commission (2014), *JRC Technical Report,* Guidelines for sample preparation procedures in GMO analysis, Luxembourg: Publications office of the European Union.
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- ISO 7002:1986, Agricultural food products – layout for a standard method of sampling from a lot
- ISO/IEC 17025:2017, General requirements for the competence of testing and calibration laboratories

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Thank you!