

Asia Sub Regional Training-on-Trainers Workshop on the Identification
and Documentation of Living Modified Organisms

Sampling Methodology



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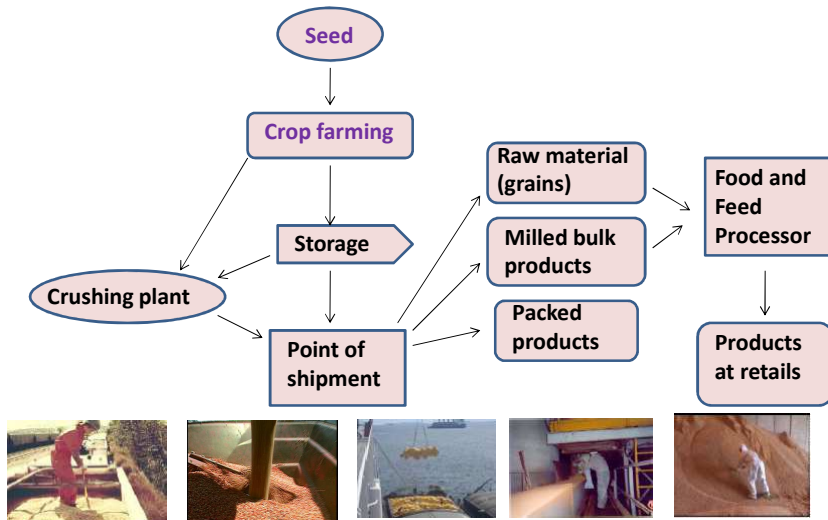
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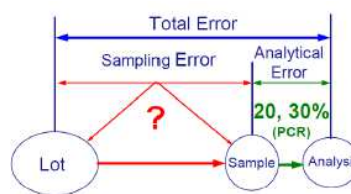
Outline

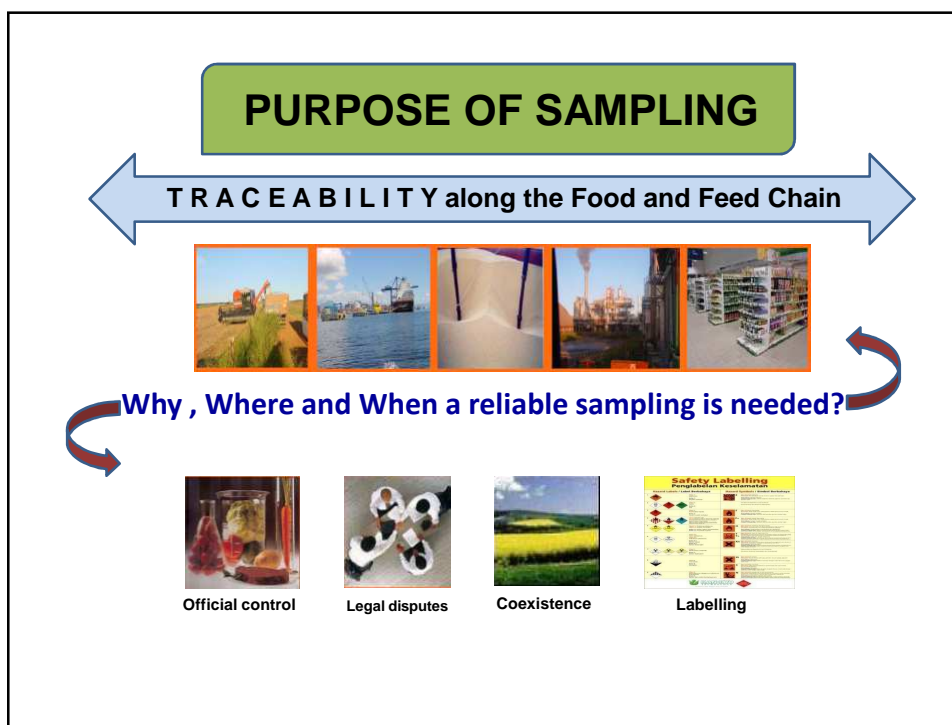
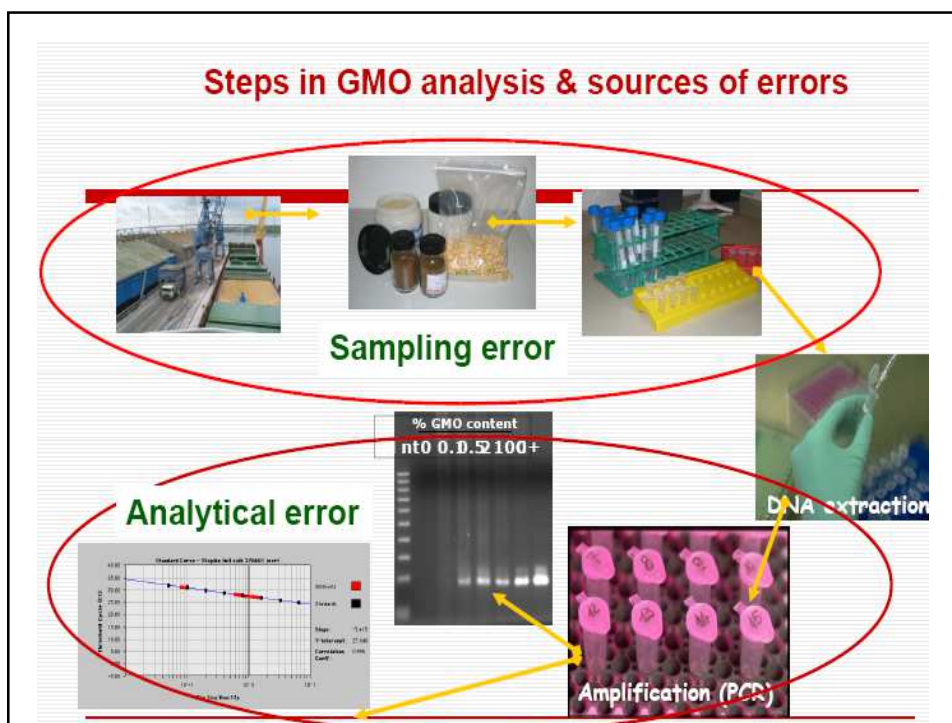
- **Introduction to sampling**
- Sampling of seeds
- Sampling along the food and feed supply chain

Complexity of Sampling in the Supply Chain



- Sampling: **most crucial first step in any analytical process**
- Objective of a good sampling plan: To provide a representative sample for the analysis and to minimize the error during detection of GMOs
- Sampling should be accurate
- Wrong sampling plan can greatly affect the reliability of the measured GMO levels





- Sampling depends on **content of the analyte - the lower the GMO concentration is the higher is uncertainty**
- The reliability of the analytical result is affected not only by sampling, but also by **sub-sampling uncertainty**, defined as a secondary sampling stage aiming at homogenizing the laboratory sample and preparing the test portion
- An important issue: **statistical distribution of the population from where the sample is taken** (homogeneous versus heterogeneous distribution – the latest being characteristic for GMOs).

Sampling Equipment

Type of Equipment	Equipment	Used for:
Triers/Probes	Partitioned Sleeve type trier	Horizontal or vertical probing
	Non -partitioned sleeve type trier	Horizontal probing only
	Nobbe trier	Horizontal probing only
Containers	Buckets Primary bucket Clean paper Composite bucket	Collection of primary samples
	Submitted sample container	Seal and submit sample to laboratory
Dividers	Boerner type divider Riffle-Soil divider Centrifugal divider Rotary divider	Divide primary samples to submitted sample size
Sealing equipment	Adhesive stickers Snap-on metal clips Plastic zip ties	Sealing lot and samples

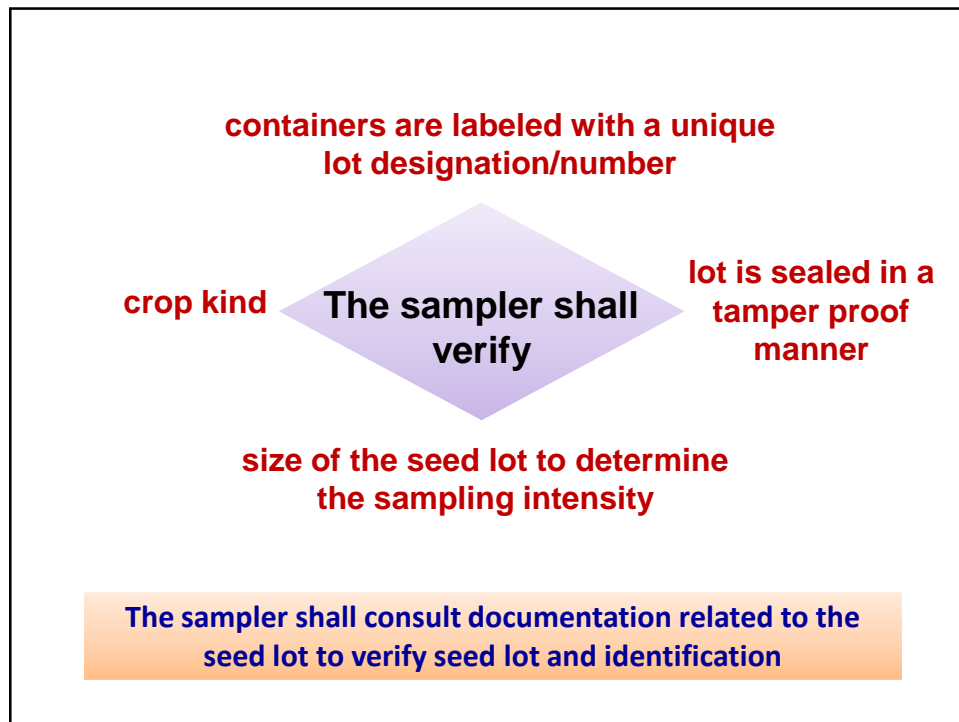
Preparation For Sampling the Seed Lot

The sampler shall:

- a) **communicate with the operator, grower, owner/manager** of the establishment regarding the **number of seed lots for sampling and their location** in the warehouse.
- b) Ensure that the seed lot is **accessible for sampling**.
The sampler must exercise **due diligence with respect to safety**

The sampler shall:

- c) **refuse to sample when a seed lot is:**
 - **not labeled**
 - **labeled for export only** (unless an ISTA International Seed Lot Certificate is requested or a certificate of analysis from a government laboratory is requested)
 - **labeled for further conditioning**
 - **being sampled for an ISTA International Certificate and exceeds the maximum lot size.**



A 'good' sampling practice should:

- minimize the unavoidable sampling error,
- ensure that the sample is representative of the entire population (statistical background, resources, facilities, skilled people)

"Primary" Sampling Programs

- Sites (Where?)
- Types (Which?)
- Times (When)
- How Many ?

(control plans, monitoring, surveillance, HACCP, exposure assessment)

"Secondary" Sampling

Actual drawing of the sample for the analysis

Accuracy of Sampling

Accuracy of sampling depends on

- The **thoroughness** of the blending of the seed lot from which the sample is drawn
- The sampling **equipment**
- The care used in drawing the primary samples
- The care with which a **number of primary samples** drawn from several containers are mixed to form a composite sample representing a seed lot
- The care used in **mixing and reducing the composite sample** to obtain the required submitted sample for submission to the laboratory

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- **Sampling of seeds**
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Sampling of seeds



➤ The purpose of **seed sampling** is to obtain a **representative sample** of a seed lot.

➤ Two organizations are aimed at ensuring uniformity in seed testing on the international level,

1) **Association of Official Seed Analysts (AOSA)**

2) **International Seed Testing Association (ISTA).**

✓ **AOSA** is an organization of member laboratories across the **United States and Canada.**

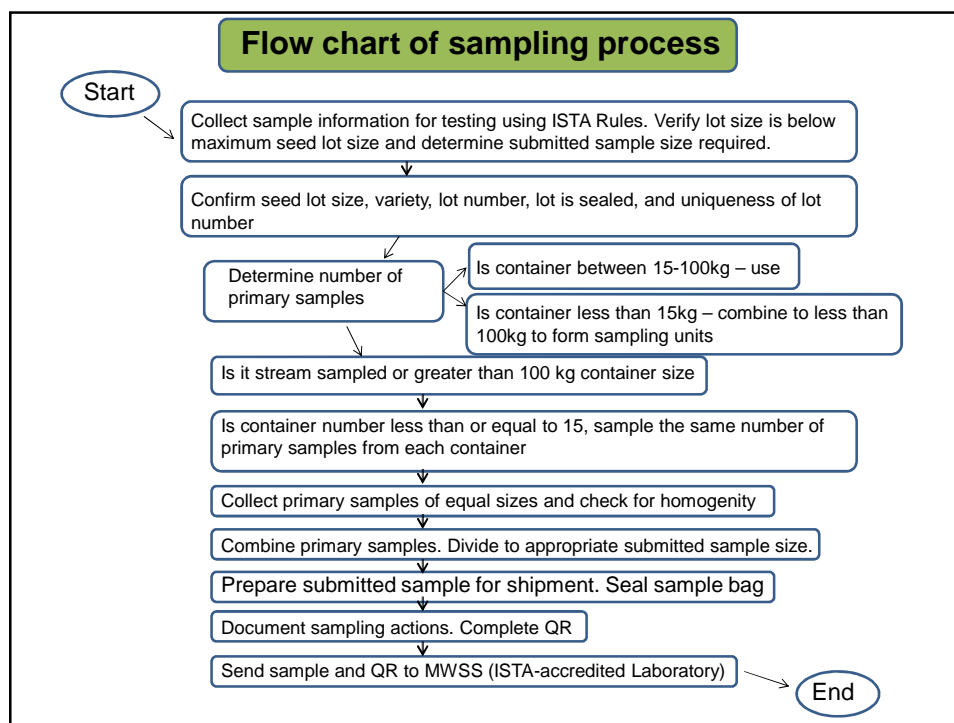
✓ **ISTA** is spread in **79 countries worldwide** and has **around 120 accredited member** laboratories.

Both organizations develop, adopt and publish standard procedures for sampling and testing seeds and issue certificates of seed quality

Sampling of seeds – seed lots

Minimum sampling intensity for seed lots in containers

Weight of individual container in the seed lot	Weight of lot (Kg or number of container)	Number of primary sample
>100 Kg	Up to 500 Kg	at least 5
	501 - 3000 Kg	1 for each 300 Kg , but not less than 5
	3,001 - 20,000 Kg	1 for each 500 Kg , but not less than 10
	20,001 kg and more	1 for each 700 Kg , but not less than 40
15 – 100 Kg Inclusive	1 – 4 containers	3 from each container
	5 – 8 containers	2 from each container
	9 – 15 containers	1 from each container
	16 –30 containers	15 from the seed lot
	31 –59 containers	20 from the seed lot
	60 or more containers	30 from the seed lot
Containers <15 Kg	<ul style="list-style-type: none"> Containers shall be combined into smaller units not exceeding 100Kg (20 Containers of 5 Kg, 33 containers of 3 Kg or 100 containers of 1 Kg) For seed mats and tapes, small packets and reels may be combined to sample units that not exceeding 20,00,000 seeds The sampling units shall be regarded as a containers Intensity is performed as defined for containers of 15 – 100 Kg 	



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Bulk Lots Sampling - Adopted Protocols

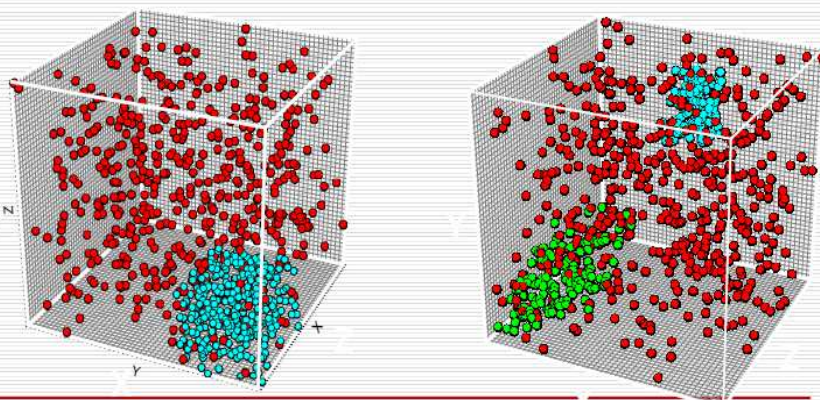
- International Seed Testing Association (ISTA)
- US Dept. of Agric. Grain Inspection (USDA/GIPSA)
 - EU Aflatoxin Directive 98/53
 - WHO/FAO *Codex Alimentarius*
- Int. Organization for Standardization (ISO)

Binomial or
Poisson
distribution

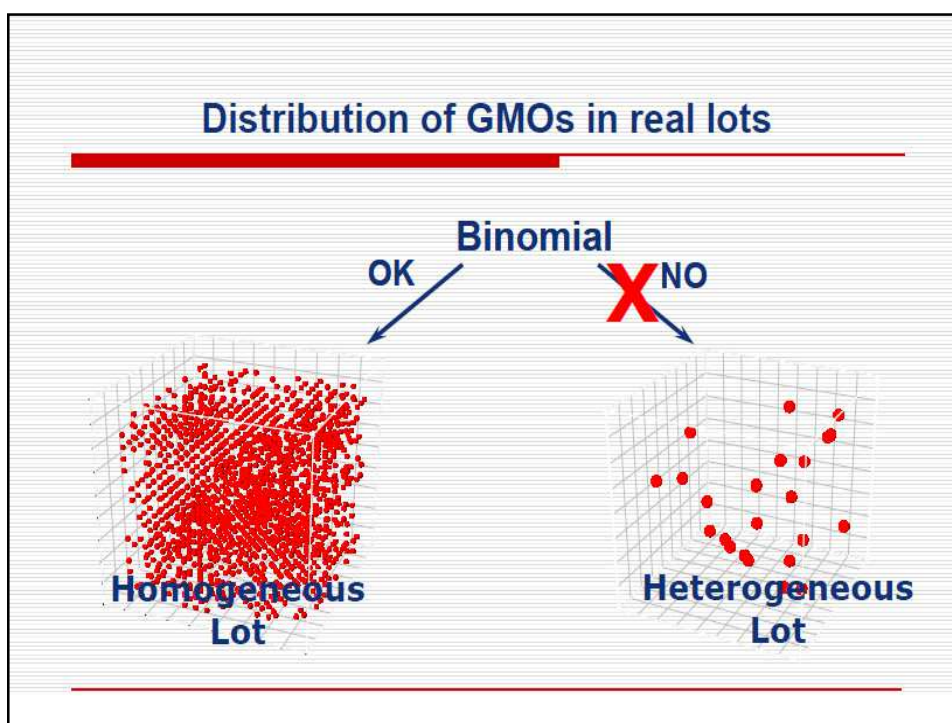
- GMO distribution in the lot must be known before their application to GMO survey
- Recommendation 2004/787/CE - Based on a general model that allows estimating GMO content of a lot without imposing any distribution

Distribution of GMOs in real lots (cont.)

Other possible scenarios: different GMOs with different distributions



Distribution of GMOs in real lots



Sampling Trier Selection for Specific Seed Types

Seed Type	Example Crop Kinds	Minimum Slot Width	Containers 100 kg (220 lbs.) or less	Containers Greater Than 100 kg (220 lbs.)	
				Horizontal Probing ¹	Vertical Probing
Small, maximum seed length 4 mm (~ 1/8")	Clovers, timothy, Brassicas, fine grasses (e.g. bluegrass), small vegetables (e.g. carrot, alfalfa)	7 mm ~ 1/4"	Sleeve Nobbe	Sleeve Nobbe	Partitioned sleeve
Medium, maximum seed length 8 mm (~ 5/16")	Cereals, flax, medium grasses (e.g. orchardgrass), medium vegetables (e.g. asparagus)	14 mm ~ 1/2"	Sleeve Nobbe	Sleeve Nobbe	Partitioned sleeve
Large, maximum seed length 15 mm (~ 5/8")	Pea, bean, soybean, corn, large grasses (e.g. brome, fescue, ryegrass), large vegetables (e.g. cucumber)	19 mm ~ 3/4"	Sleeve Nobbe	Sleeve Nobbe	Partitioned sleeve
Very chaffy grasses	Chaffy bromegrass, fescue	N/A	Hand	See note ²	See note ²
Mixtures	Lawn, forage, ground cover	Based on the size of the largest component of the mixture and flowability of mixture	Sleeve Nobbe	Sleeve Nobbe	Partitioned sleeve

Types of Trier

1) Sleeve Trier

- a) multi-openings without partitions
- b) multi-openings with partitions



Figure 12d

Sleeve Trier

2) Bin Trier

3) Nobbe Trier



Nobbe Trier



Bin Trier

Primary Samples

- i. A primary sample is a small portion taken from the seed lot during **one sampling action**
- ii. Before sampling the lot, the appropriate method of sampling based on the crop kind and the **packaging of the seed must be selected**
- iii. The instruments being used must neither damage the seed nor select according to **seed size, shape, density, chaffiness or any other quality trait**
- iv. Each **primary sample** is obtained by passing the sampling equipment through the **seed once**
- v. Each trier or pass of the sampling equipment is one primary sample



The trier opening size must be two times (2x) greater than the longest part of the seed to be sampled

Types of Sampling

1) Automatic Sampling



Automatic sampling: A stream of seed requires a device that samples across entire seed stream and which no seed bounces out



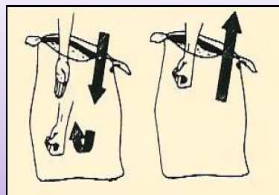
Containers to collect seed from automatic sampling devices shall be sealable to restrict access to only the authorized sampler

2) Manual Stream Sampling



Manual Stream Sampling

3) Hand Sampling



Types and Methods of Lot Sealing

- a) Adhesive patches and tapes
- b) Metal snap-on seals
- c) Sealing by Stitched Label
- d) Sealing by stitching
- e) Self sealing containers
- f) Hermetically Sealed



Allowed



Not Allowed

Bagged seed is considered sealed in valve-pack bags or sewn bags (must be no evidence of restitching).

Marking and Tagging the Seed Lot

The containers must be labeled with:

- crop kind
- variety name
- lot number
- weight of the seed container



Example of a tag used to “mark” all the bags of a seed lot

Types of Labels

a) Tear -resistant labels

b) Adhesive labels

c) Pre-printed labels

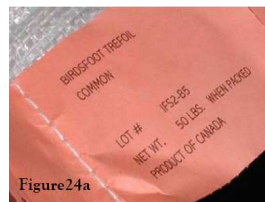


Figure 24a



Figure 24b

Source	Lot Size	Bulk Sample	Laboratory Sample
ISTA	Varies according to species 10t to 40t max	1 Kg	1 Kg = 3000 kernels
USDA/ GIPSA	Up to 10 bushels (=254.000 Kg) or 10.000 sacks if the lot is not loose	Equivalent to laboratory samples	2.5 Kg, but not less than 2 Kg
ISO 13690	Up to 500 t	Not indicated	>1 Kg (for Kernels)
USDA/ GIPSA Starlink	Follow general USDA/ GIPSA guidelines	2.5 Kg	2.400 Kernels
EU Dir. 401/2006	No limits if not separable, otherwise Up to 500t	1-10 Kg, Lot size <50Kg	1-10 Kg
CFN	Up to 500t	60 Kg	10. 000 Kernels
ISS	No limit	24 Kg	24 Kg
WHO/FAO (CX/MAS 01 /3)	Discussed, but not specified	Not indicated	Not discussed

Acknowledgement

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Further Reading

- 1. International Rules for Seed Testing (ISTA), 2010**
- 2. ISTA Handbook of Seed Sampling**

THANK YOU

