ISTA Rules for Seed Sampling

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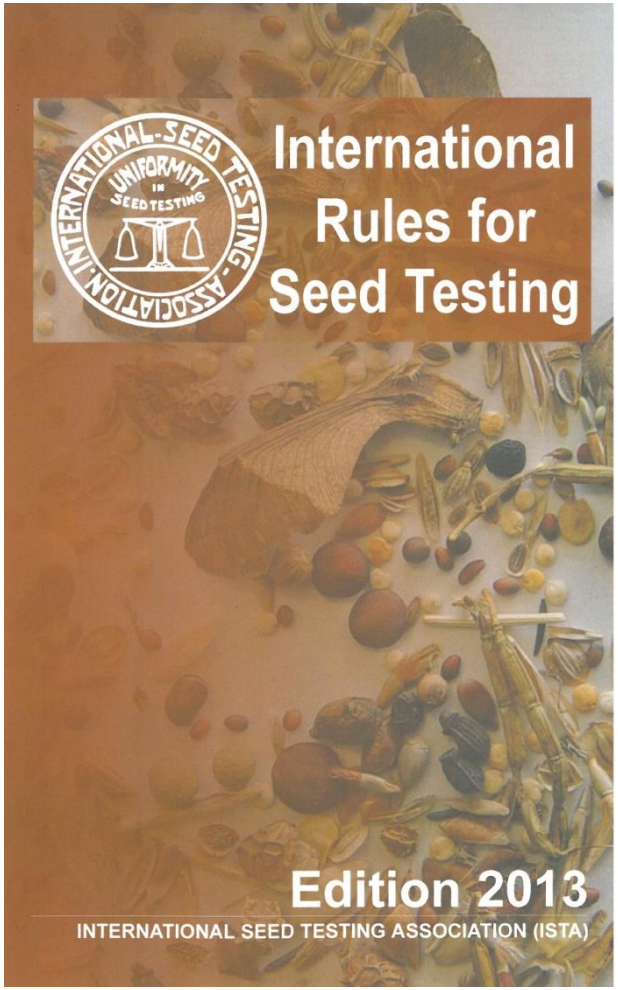
Chapter 2: Sampling



Chapter 2: Sampling

2.1 Object

The object of sampling is to obtain a sample of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot.

2.2 Definitions

2.2.1 Seed lot

A seed lot is a specified quantity of seed that is physically and uniquely identifiable.

2.2.2 Primary sample

A primary sample is a portion taken from the seed lot during one single sampling action.

2.2.3 Composite sample

The composite sample is formed by combining and mixing all the primary samples taken from the seed lot.

2.2.4 Subsample

A subsample is a portion of a sample obtained by reducing a sample.

2.2.5 Submitted sample

A submitted sample is a sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a subsample thereof. The submitted sample may be divided into subsamples packed in different material meeting conditions for specific tests (e.g. moisture or health).

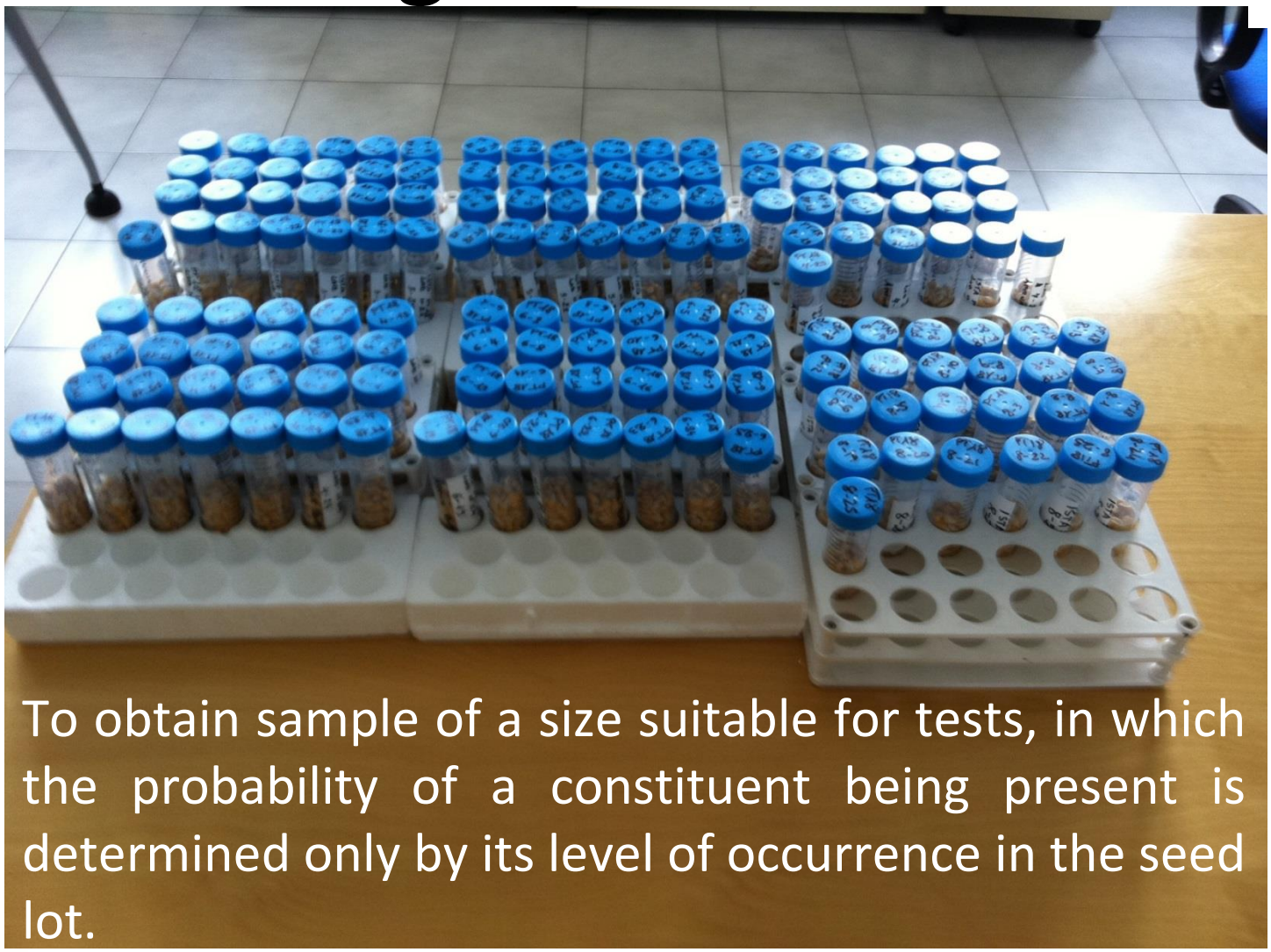
2.2.6 Duplicate sample

A duplicate sample is another sample obtained for submission from the same composite sample and marked "Duplicate sample".

2.2.7 Working sample

The working sample is the whole of the submitted sample or a subsample thereof, on which one of the quality tests described in these ISTA Rules is made and must be at least the weight prescribed by the ISTA Rules for the particular test.

Object of sampling according to the ISTA Rules



To obtain sample of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot.

Seed samples are addressed to quality evaluation

- technical purity and other seeds by number
- germination, viability
- moisture content
- variety testing
- GMO testing
- field plots
- SEED HEALTH TESTING
- ……

ISTA sampling: the principle

ONE SEED LOT

ONE SUBMITTED SAMPLE

ONE CERTIFICATE

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The seed lot

A seed lot is a specified quantity of seed that is physically and uniquely identifiable (labelled/marked).

The seed lot:

- shall not exceed a maximum lot size, e.g. 40 t in maize, 30 t in cereals and pulses, 10 t in rape seed (+ 5% tolerance);
- when sampled, must be packed and sealed (or sampled when entering the containers, under the control of the sampler);
- shall be as uniform as practicable (in cases of doubt: heterogeneity test);
- each part of the seed lot should be accessible.

The seed lot

A VERY IMPORTANT PRE-REQUISITE

The seed lot:

- shall be as uniform as <u>practicable</u>

It is practically impossible to produce a perfectly uniform seed lot. Representative samples may be obtained only by appropriate sampling techniques.

Uniformity is a dynamic characteristic.

After harvesting: the proportion of impurities is at its maximum and the uniformity state minimum.

The objects of blending and processing are cleaning and random distribution of constituents.

Heterogeneity in a seed lot

Differences in seed weight, shape and flowing properties between the constituents (seeds, impurities) can cause separation -> heterogeneity in a seed lot.

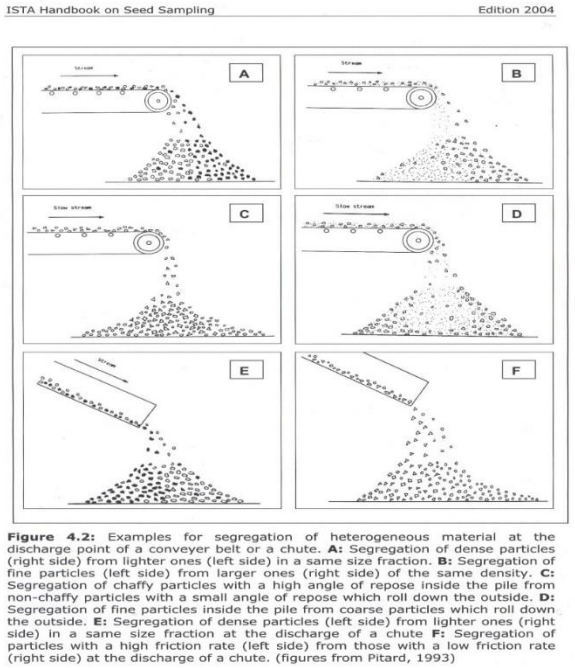

Figure 4.2: Examples for segregation of heterogeneous material at the discharge point of a conveyer belt or a chute. **A:** Segregation of dense particles (right side) from lighter ones (left side) in a same size fraction. **B:** Segregation of fine particles (left side) from larger ones (right side) of the same density. **C:** Segregation of chaffy particles with a high angle of repose inside the pile from non-chaffy particles with a small angle of repose which roll down the outside. **D:** Segregation of fine particles inside the pile from coarse particles which roll down the outside. **E:** Segregation of dense particles (left side) from lighter ones (right side) in a same size fraction at the discharge of a chute **F:** Segregation of particles with a high friction rate (left side) from those with a low friction rate (right side) at the discharge of a chute. (figures from Pitard, 1993)

Source: ISTA Handbook on Seed Sampling

In case of obvious evidence of heterogeneity, sampling has to be refused.

In cases of doubt, a heterogeneity test can be made.

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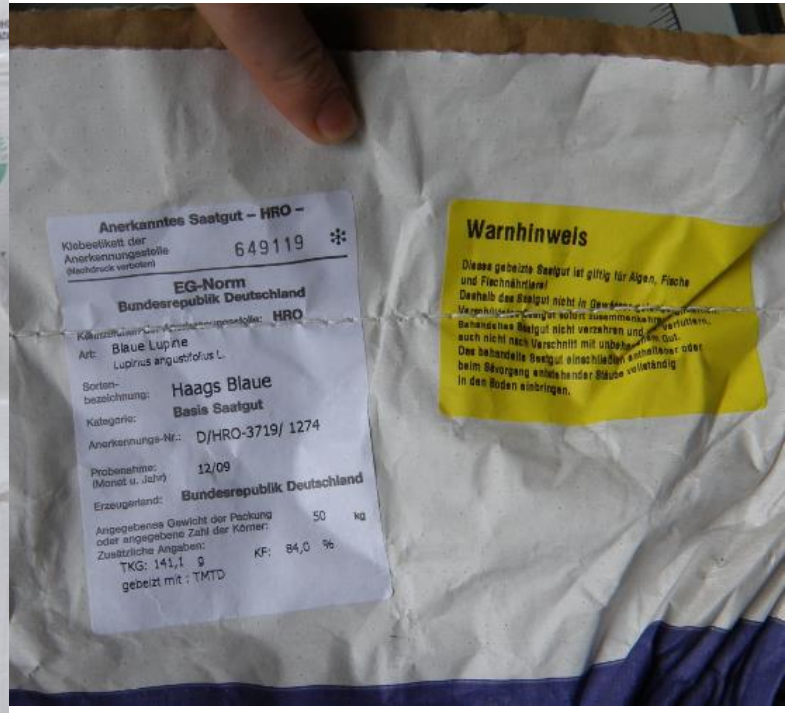
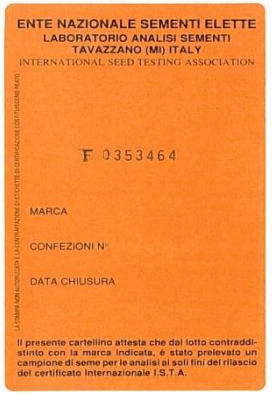
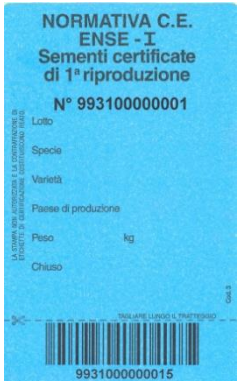
Identification of seed lot







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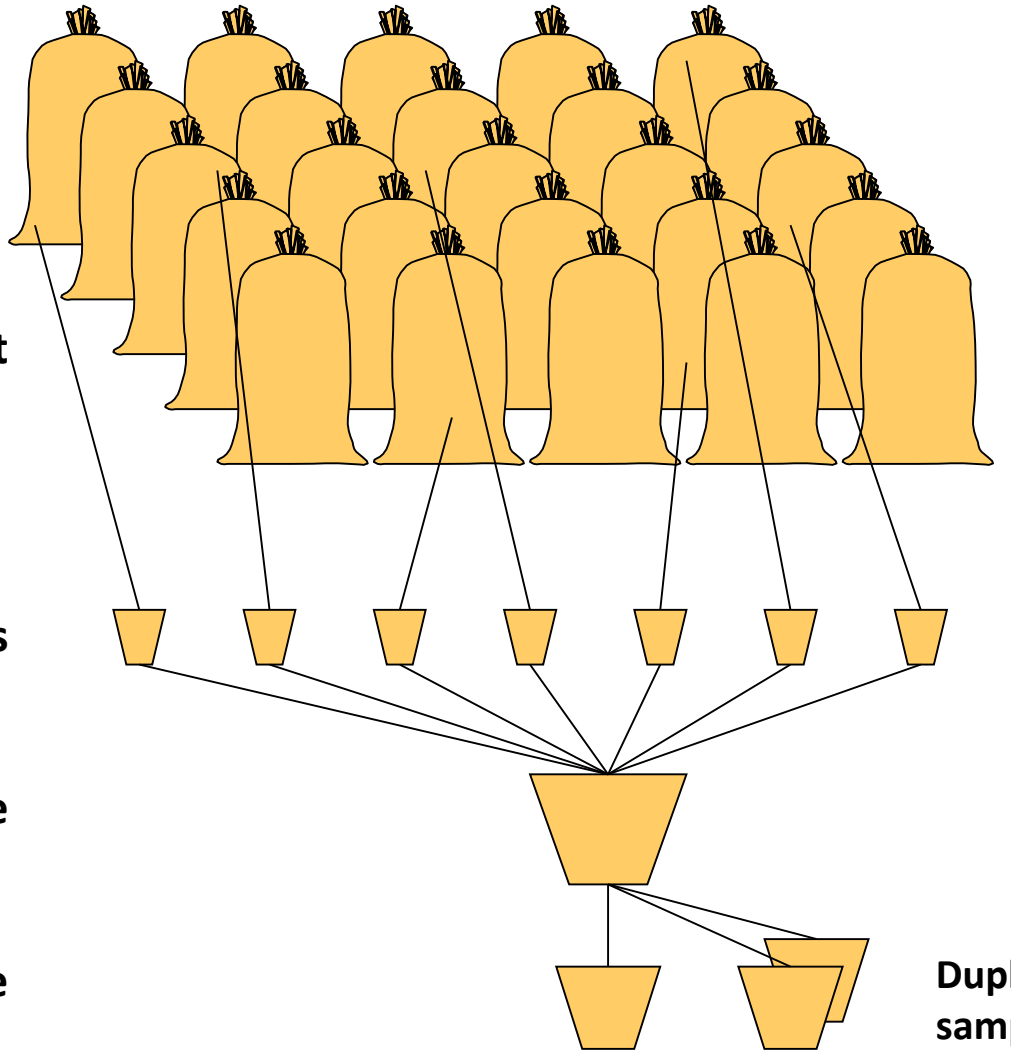
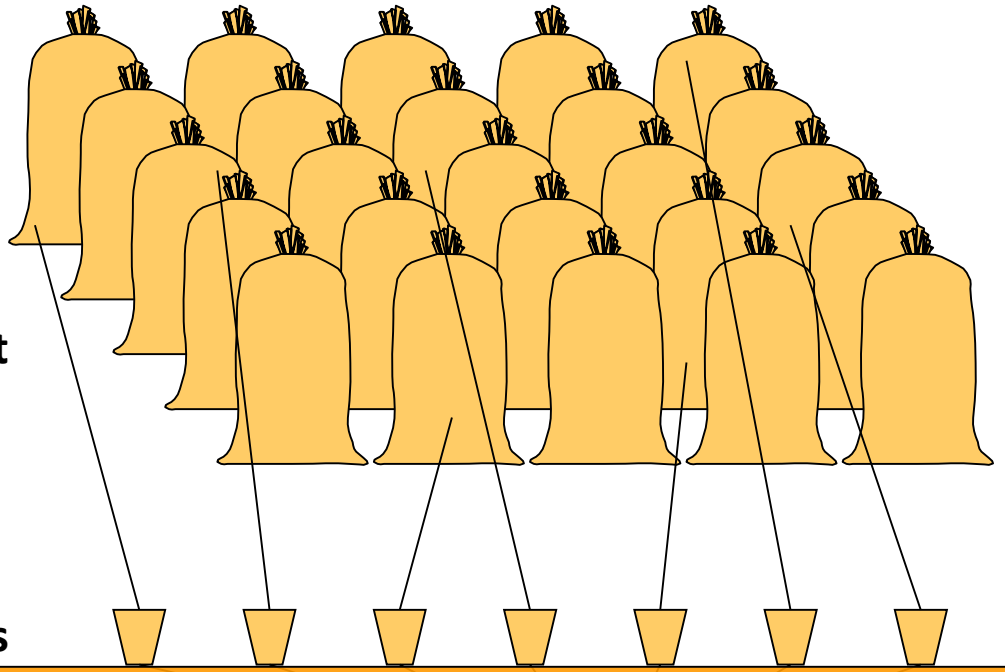
ISTA´s sampling scheme



Taking primary samples



seed lot

primary samples

composite sample

submitted sample

Duplicate samples

Sampling intensity

- Sampling intensity in the ISTA Rules has been established to meet statistical requirements.
- Minimum numbers of primary samples have been defined for three different situations:
  - containers between 15 and 100 kg
  - containers smaller than 15 kg
  - containers larger than 100 kg (or streams of seed entering containers).

Primary sample: a sample from one single sampling action

Sampling seed lots in containers between 15 and 100 kg

Containers in the seed lot	*Minimum number of primary samples*
1 – 4 containers	3 primary samples from each container
5 – 8 containers	2 primary samples from each container
9 – 15 containers	1 primary samples from each container
16 – 30 containers	15 primary samples from the seed lot
31 – 59 containers	20 primary samples from the seed lot
60 or more containers	30 primary samples from the seed lot

Sampling seed lots in containe smaller than 15 kg

- Containers are combined to sampling units of 100 kg
- Sampling scheme for containers between 15 kg and 100 kg is followed by taking the sampling units as containers

Sampling seed lots in containers greater than 100 kg and from the seed stream

Lot size	***Minimum number of primary samples to be taken***
Up to 500 kg	At least five primary samples
501 - 3 000 kg	One primary sample for each 300 kg, but not less than five
3001 - 20 000 kg	One primary sample for each 500 kg, but not less than 10
20 001 kg and above	One primary sample for each 700 kg, but not less than 40

Special case: up to 15 containers – regardless the size – the same number of primary samples shall be taken from each container.

Instruments for taking samples from seed lots

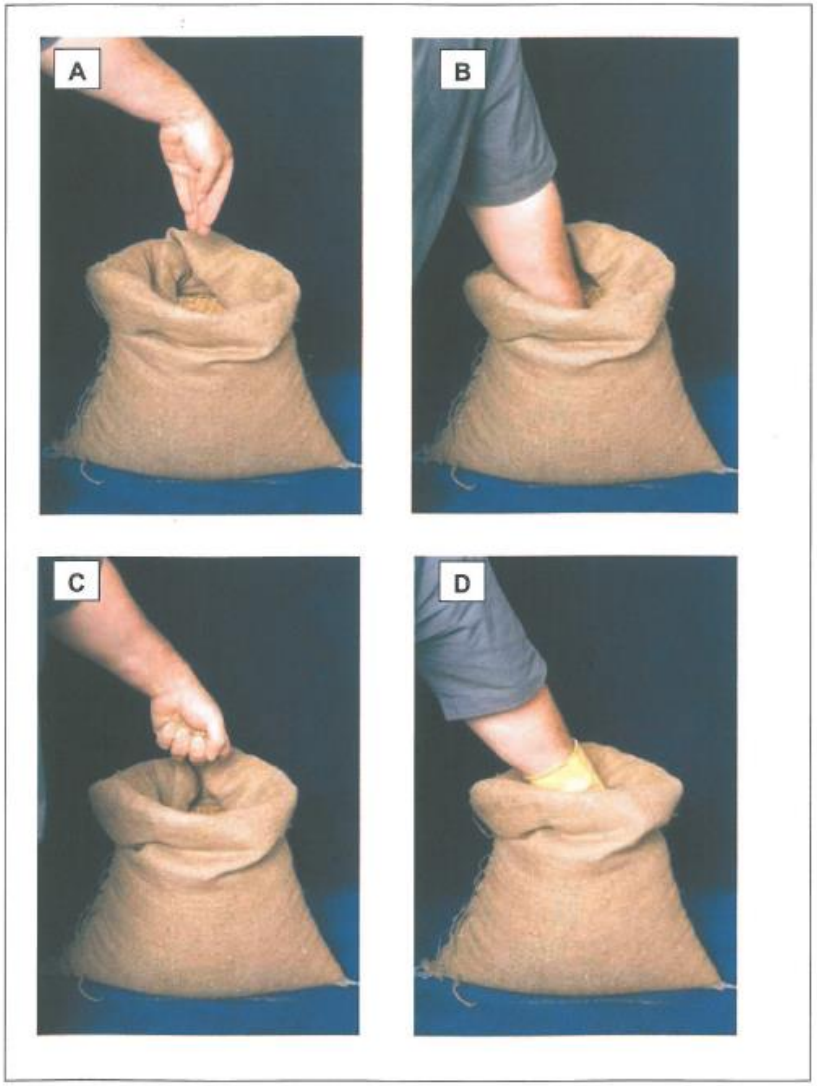
- By hand

A. Push the open hand into the container
B. Close the hand with seeds inside
C. Withdraw the hand by taking great care that fingers remain tightly closed around the seeds so none may escape
D. In case of treated seeds, use appropriate gloves

Recommended in case of:

- ***Risk of contamination with pathogen*** ***(for SHT: the sampling tools must be cleaned and disinfected!)***
- ***Risk of damage to the seeds***

After sampling by hand the containers must be sealed under the supervision of the seed sampler.



Source: ISTA Handbook on Seed Sampling

Instruments for taking samples from seed lots

- Triers approved ISTA

THE NOBBE TRIER (DYNAMIC SPEAR)
The width of the hole should be at least two times the maximum diameter of the seed.
The length of the hole should be between two and five times the width of the hole.

In the photo:
MI-1: to be used for maize, soybean
MI-2: to be used for wheat
MI-3: to be used for rape

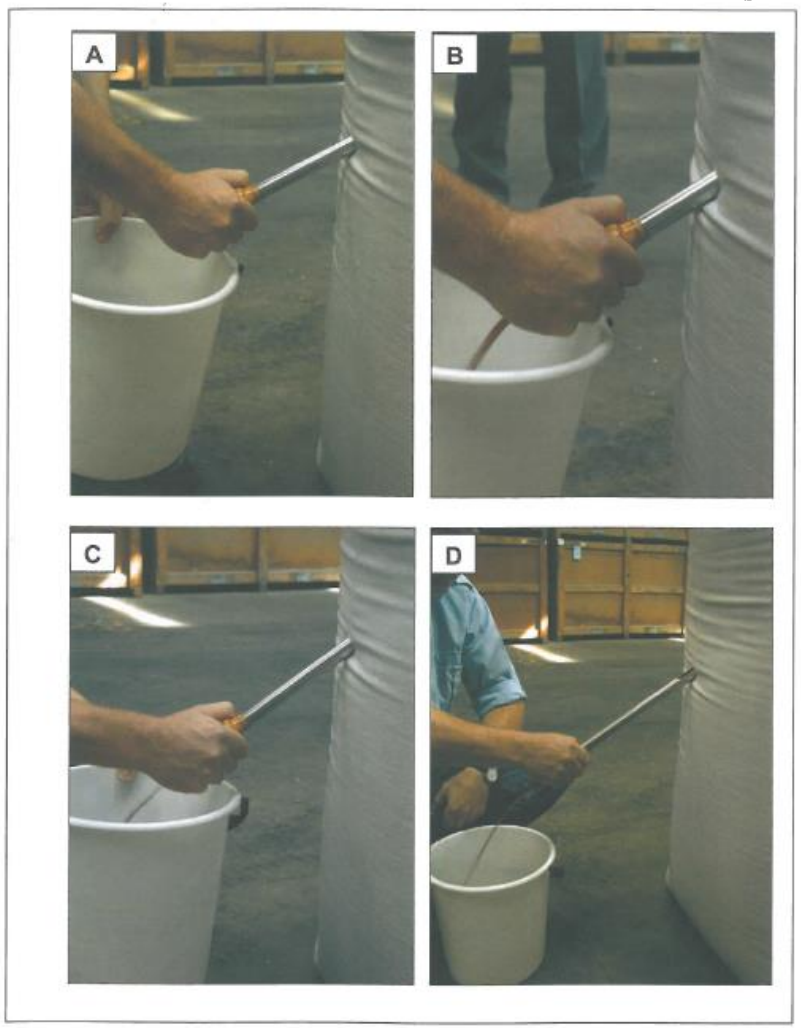


Figure 5.3: How to use a Nobbe trier. **A**: Pushing the trier into the bag with the opening upside down. **B, C** and **D**: Turning around the trier and withdrawal from the bag.

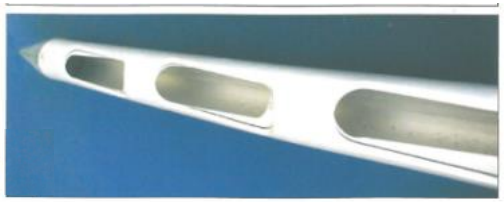
Source: ISTA Handbook on Seed Sampling

Instruments for taking samples from seed lots

- Triers approved ISTA

THE SLEEVE TYPE (SAMPLING STICK)
It consists of a inner and an outer tube.
Some model have partitions, other have only one cavity.
Basing on the ISTA Rules, a stick without partitions can be used only horizontally.

THE SPIRAL TRIER
It consists of a inner and an outer tube.
It doesn't have partitions, but in the inner tube the slots are in a spiral arrangement.
Basing on the ISTA Rules, it can be used vertically.



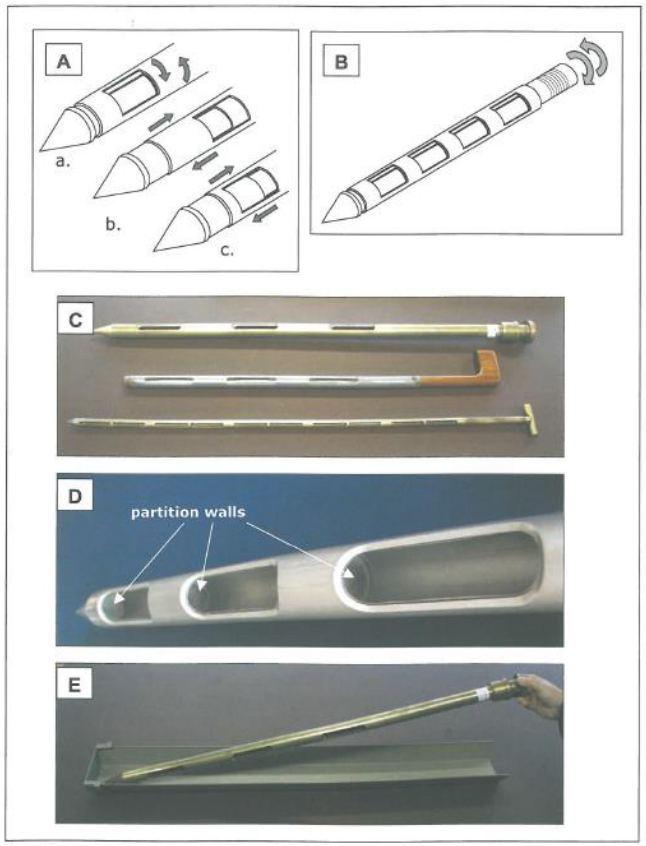


Figure 5.4: The stick trier. **A**: Schematic drawing of different types for opening and closing the chamber (a: twisting the tubes. b: longitudinal pushing the outer tube c: longitudinal pulling the inner tube). **B**: Schematic drawing of a whole stick that is opened and closed by twisting the tubes. **C**: Different types of stick triers. **D**: A stick trier with compartments for vertical use **E**: Special receiving pan for emptying a stick trier.

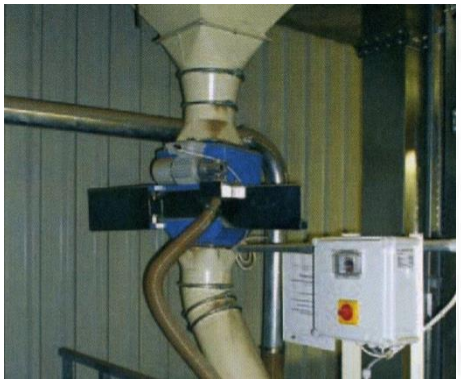
Source: ISTA Handbook on Seed Sampling

Source: ISTA Method validation report 2009

Instruments for taking samples from seed lots

- Automatic sampler

It must fulfill specific conditions (e.g. it uniformly samples the cross section of the seed stream; the material entering the instrument does not bounce out again).



INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)

Technical Guideline

Protocol for the approval of automatic seed samplers

Note: Any copies of this document are not subject to change service

	Created by	Reviewed by	Approved by
Date:	January 2012	May 2012	November 2012
Name:	Bulking and Sampling Committee (BSC)	Executive Committee (ECOM)	ECOM / BSC
Signature:			
Valid from: 28.11.2012			

Source: ISTA Handbook on Seed Sampling and personal files

http://seedtest.org/upload/cms/user/TCOM-TG-01-Protocolautomaticseedsamplers-V1.0.pdf

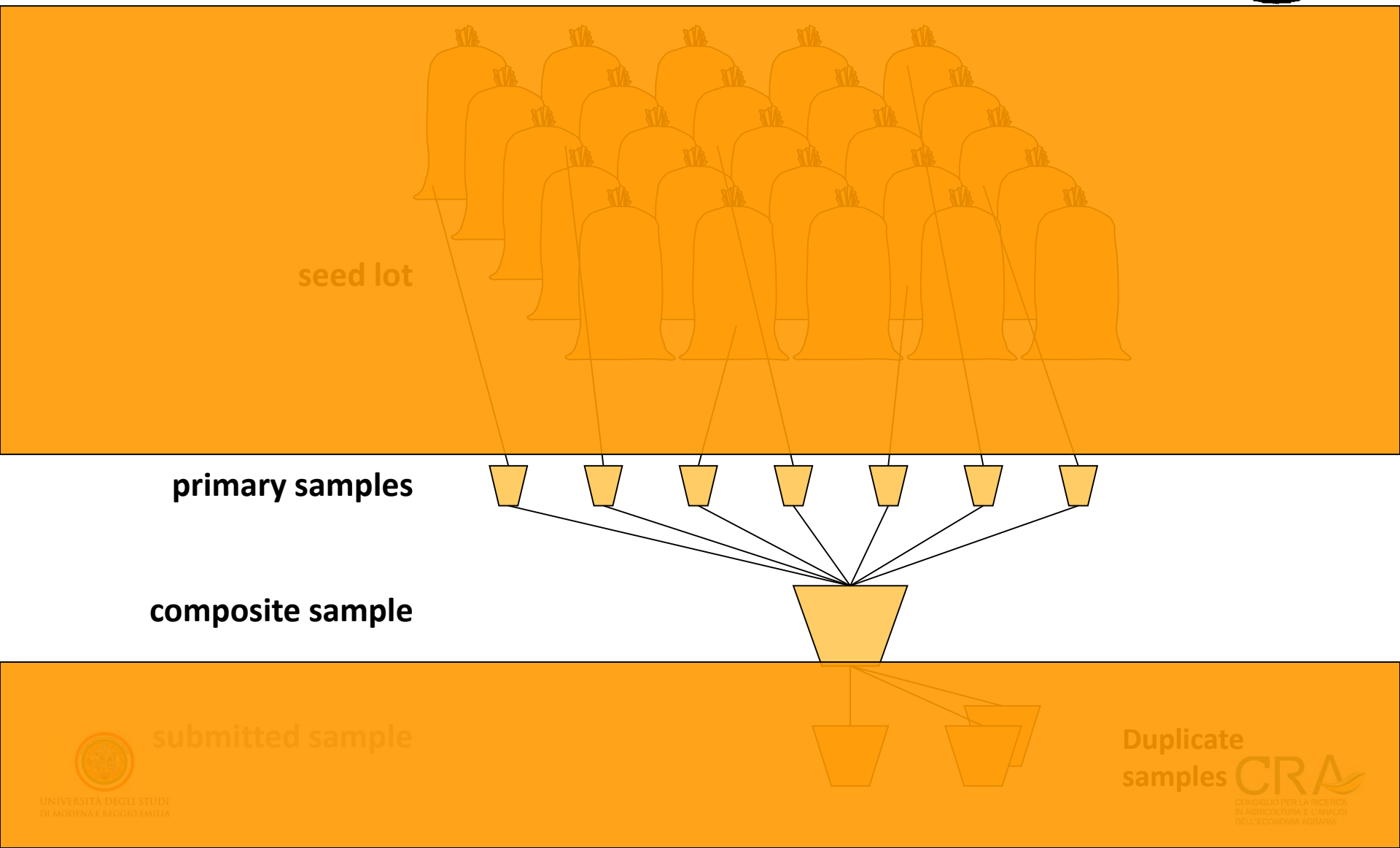
Instruments for taking samples from seed lots

- Triers **NOT** approved ISTA

The “thief trier” is not validated. It significantly over-represents the outer part of the container.

It cannot be used for ISTA purposes. It is recommended **not to use it at all**!

Obtaining the composite sample



Sampling under control of seed sampler

- Uniformity of composite sample is checked

Where possible, primary samples are compared with each other during sampling.

Where primary samples are collected directly into one containers, the composite sample must appears uniform.

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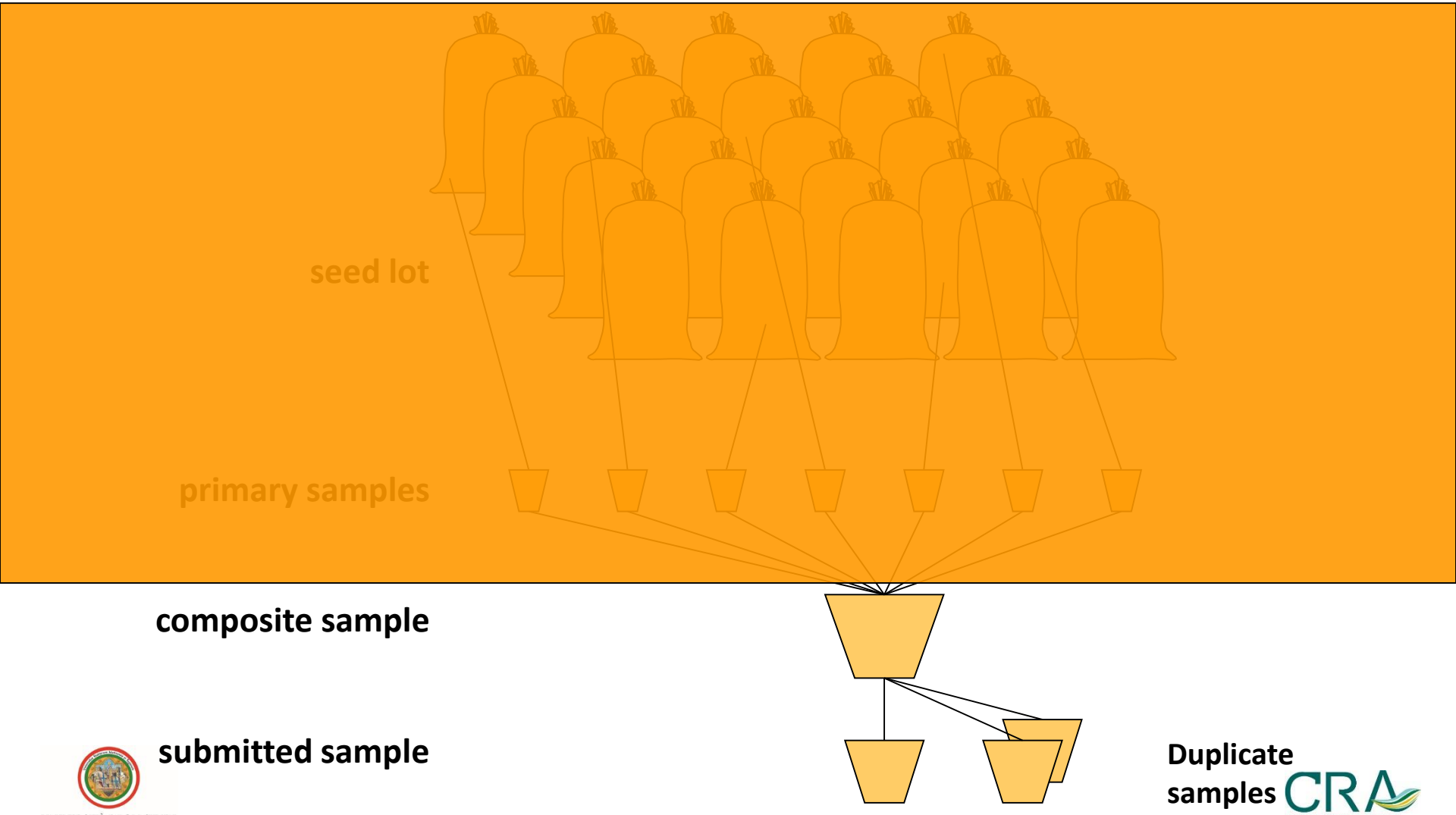
Obtaining the submitted sample



Submitted sample

- shall be obtained by reducing the composite sample by one or more ISTA reduction methods; if it not possible, the composite sample is submitted to the laboratory;
- the submitted sample must be packed in appropriate containers, identified and sealed;
- the sample size is listed in Table 2A; in general 25000 seeds, at maximum 1 kg; for specific tests (seed health testing, GMO testing, variety testing) greater sample sizes may be required;
- special instructions are provided for specific purposes (e.g. sample addressed to seed health test, to moisture determination, samples of pelleted seeds).

Submitted sample for SHT

- The submitted sample must be packed in appropriate containers, identified and sealed: for SHT moisture proof containers are not recommended (to be used only if suitable storage conditions maybe assured).

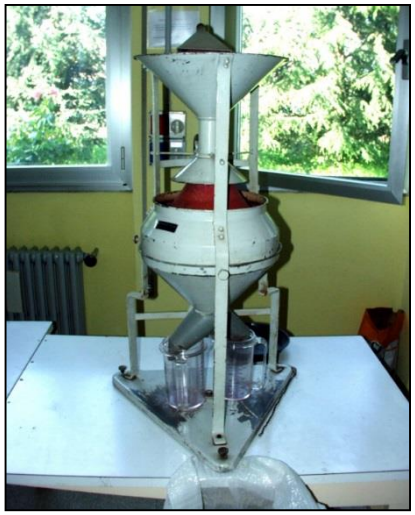
- the sample size listed in Table 2A may not fit the purpose of SHT: it is up to the laboratory to provide the samplers with specific instructions.

Sample reduction (at the sampling site, at the laboratory)

Sample reduction methods (can be used also in combination):

- at first, the sample must be thoroughly mixed
- reduction by sample dividers (not for SHT!)
- reduction by hand (for SHT!)

SOIL/RIFFLE DIVIDER



CONICAL DIVIDER

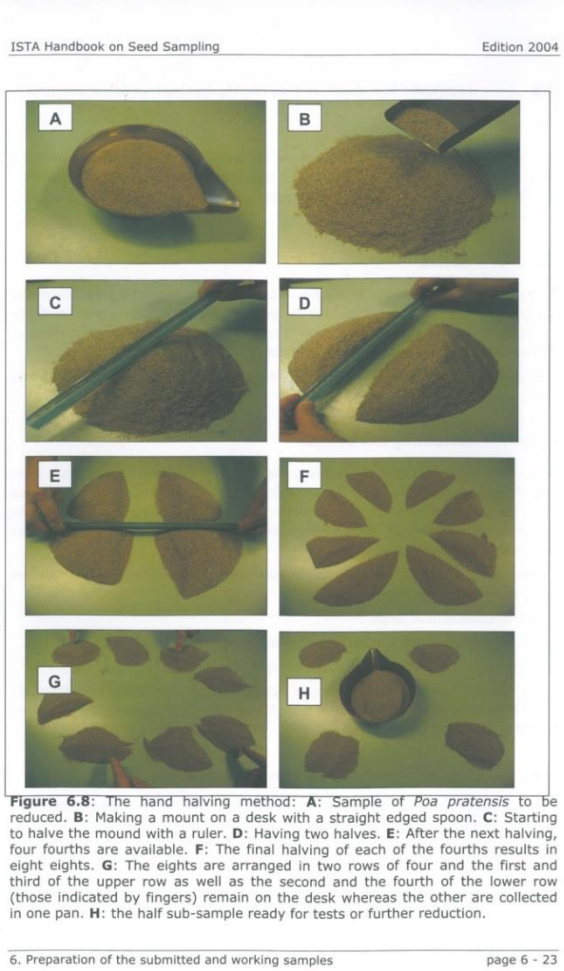
ROTARY DIVIDER

Sample reduction
(at the sampling site, at the laboratory)

Whenever possible, ISTA recommends to use mechanical reduction methods, more independent of the person, more robust. In specific situations (very chaffy seed, unprocessed seed, seed health testing) hand reduction methods are available:

- Hand halving method
- Spoon method
- Modified halving method

} ➔ SHT



Source: ISTA Handbook on Seed Sampling

Thank you for your attention!

Any questions?