




Sampling methodology for detection of living modified organisms

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Sampling Procedure

- The Detection of LMO is usually carried out with a small portion of seed drawn from the seed lots. The objective of sampling is to ensure that the portion of the seed taken for testing is a true representative of the entire lot.
- A good sampling procedure is essential while dealing with bulk consignments to get a uniform and representative sample for testing from plant quarantine viewpoint.



Sampling Procedure

- International Seed Testing Association (ISTA Rule, 1999)
- Bureau of Indian Standards (IS : 2814-1964)





ISTA guidelines for sampling

- The International Seed Testing Association (ISTA) has laid down the rules and defined units for the sampling purpose.



Seed lot

- 1) A seed lot is a specific, identified quantity of seed whose purity and quality is homogenous throughout entire lot.
- 2) The maximum size of seed lot prescribed for agricultural and horticultural seed is 20,000 kg.

Seed sampling

- Under ISTA method several individual samples drawn from a lot, constitute **primary sample**. These are combined to make **composite sample**, which is usually much larger than the required and must be reduced before submitting to the laboratory. These samples are known as **submitted sample**. From the submitted sample, a further reduced sample is obtained for actual testing, which is called as **working sample**

Sampling intensity


Lot size	No. of primary samples to be drawn
Up to 500 kg	At least five primary samples except for lots less than 50 kg but not less than three need to be taken.
501 to 3000 kg	One primary sample for each 300 kg but not less than five
3001 to 20,000 kg	One primary sample for each 500 kg but not less than 10 samples

Sampling intensity	
No. of container (s)	No. of primary samples to be drawn
Up to 5 containers	Sample each container and at least five primary samples
6 – 30 containers	Sample at least one in every three containers but not less than five primary samples
31 containers or more	Sample at least one in every five containers but not less than 10 primary samples.

Minimum weight of submitted samples for various crop species	
Minimum weight of submitted samples	Crop species
1,000 g	<i>Avena sativa</i> , <i>Triticum aestivum</i> , <i>Hordeum vulgare</i> , <i>Zea mays</i> , <i>Phaseolus</i> spp., <i>Pisum sativum</i> , <i>Cicer arietinum</i> , <i>Secale</i> , cereal, <i>Leucaena leucocephala</i> , <i>Glycine max</i> , <i>Gossypium</i> spp., <i>Helianthus annuus</i> , <i>Arachis hypogea</i> , <i>Lupinus</i> spp., <i>Vigna unguiculata</i> , <i>Cucurbita</i> spp., <i>Cajanus cajan</i> and <i>Dolichos lablab</i> .
900 g	<i>Prunus avium</i> , <i>Sorghum vulgare</i>
500 g	<i>Beta vulgaris</i> , <i>Prunus serotina</i>
400 g	<i>Oryza sativa</i> , <i>Calopogonium mucunoides</i>
250 g	<i>Sorghum sudanense</i> , <i>Trifolium subterraneum</i> , <i>Spinacia oleracea</i>
200 g	<i>Sinapis alba</i>



Minimum weight of submitted samples	
150 g	<i>Capsicum</i> spp, <i>Cucumis melo</i> , <i>Cucumis sativus</i> , <i>Pennisetum typhoides</i> , <i>Solanum melongena</i> , <i>Linum usitatissimum</i> , <i>Lycopersicon esculentum</i>
100 g	<i>Brassica napus</i> , <i>B. oleracea</i> , <i>B. rapa</i> , <i>Pinus caribaea</i>
80 g	<i>Allium cepa</i> , <i>Stylosanthes</i> spp.
70 g	<i>Sesamum indicum</i>
60 g	<i>Cuminum cyminum</i> , <i>Trifolium alexandrinum</i>
50 g	<i>Malus</i> spp., <i>Medicago lupulina</i> , <i>M. sativa</i> , <i>Melilotus</i> spp., <i>Rosa</i> spp., <i>Trifolium pratense</i> , <i>Cichorium intybus</i> , <i>Allium fistulosum</i>
40 g	<i>Brassica chinensis</i> , <i>Cichorium endivia</i> , <i>Picea abies</i> , <i>Brassica nigra</i>
30 g	<i>Lactuca sativa</i> , <i>Daucus carota</i> , <i>Ulmus</i> spp.
25 g	<i>Nicotiana tabacum</i> , <i>Apium graveolens</i>

Sampling equipment and their use



The stick or sleeve-type trier



- It consists of hollow brass tube inside a closely fitting outer sleeve which has a solid pointed end. Both the sleeve and the inner tube have open slots in their walls so that when tube is turned until the open slots on the sleeve are in line with that of tube, seeds can flow into the cavity of tube. When the tube is given half turn the slots are closed. The tube length and diameter vary for use with different kinds of seed and various sizes of containers.

Sampling as per Bureau of Indian Standard (IS : 2814 – 1964)

Sampling grains in bags

No. of bags in lot	No. of bags to be sampled
Up to 30 bags	All bags
31 to 300 bags	30 bags
301 to 1000 bags	50 bags
1001 to 2000 bags	100 bags
2001 and above	5% of bags to be sampled





Sampling grains in bags

- The primary samples are drawn by inserting slotted tube sampler (IS : 2815-1964) diagonally in about one third of the bag to be sampled. The sampler shall be inserted into the bag in the 'closed position' with opening slots facing downward position. When the sampler inserted to the desired position, it may be turned to 'open position' such that opening slot face up wards to allow grains filling into the cavities of inner tube. The sampler shall be turned back to close position before taking out of bag.



Sampling grains in bags

- When a *Parkhi* (IS : 2816-1964) is used for sampling, it shall be inserted into the bag diagonally and samples are drawn by gently turning in such a way that the grain fall into the collecting tray.




Sampling grain in bulk



Quantity of consignment in bulk	No. of spots from which sample are drawn
Up to 300 tonnes	30 spots
301 to 1,000 tonnes	50 spots
1,001 tonnes and above	100 spots



Sampling grain in bulk





- Sampling may be done by selecting the spots at random and shall be done with bin sampler.
- The bin sampler can be used only to a depth of 1.5 meters. The sampler shall be inserted at an angle in closed position till desired depth is reached and then opened to collect the grains and pulled out after being closed.
- Sampling of the grains while in motion or from the conveyor belt shall be drawn with either scoop or a pelican type of sampler at timely intervals.



Sampling by hand

- According to ISTA rules, one of the following methods may be used in obtaining working sample:
- Mechanical divider method  
- Random cup method
- Modified halving method and
- Spoon method



Sampling for germplasm

- Where it is not possible to draw the working sample such as high quality germplasm material, it is prescribed that at least one half of the seeds imported may be examined. Even if it is not possible to examine one half of the seeds received, it is suggested that the entire quantity of the seed may be examined for quarantine purpose.



Thanks

