Workshop and Training Program on Sampling and Detection Methods Applied to Transgenic Crops

November 17 – 19, 2011, NIN, Hyderabad, India

It Starts and Ends with Sampling

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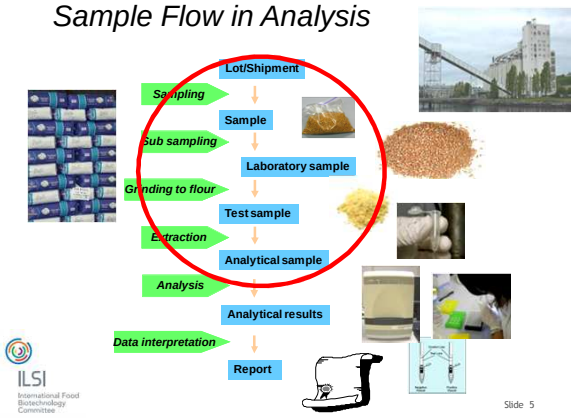
Sampling

- Why sampling is necessary
- How do we Sample?
- Use of sampling approaches
- Conclusions

Why Sample?

- Much more cost effective than inspecting the entire lot
- 100% certainty requires inspection of every particle
- Testing is destructive
- Sampling precludes 100% certainty – no guarantees of "non-transgenic" or "transgenic-free"
- Design sampling to provide high levels of confidence that sample 'represents' bulk lot

Sample Flow in Analysis



Processing a sample

1. Take a sample of the seed, grain or food and send to the laboratory
2. Inspect the sample and record characteristics
3. Obtain a subsample (Laboratory sample)
4. Grind the sample (if seed/grain, or particulate food)
5. Obtain a subsample (Test sample)
   - Possibly re-grind the sample
6. Extract the analyte from the subsample
7. Measure the efficiency of extraction, and quality of extract
8. Obtain a subsample (Analytical sample)
9. Perform the analysis

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Step 1: Sampling the Lot

Rules
ISO 6644:2002
ISO 13690:1999
Codex GAC/GL 50-2004

Taking a Grain Sample

USDA

ISO 24333/2009
Codex GAC/GL 50-2004
IWA on sampling





Step 2: Arrival at the laboratory

- The handling of a sample once it arrives at the laboratory is a critical step.
- Samples must be followed in a record system to ensure they are handled appropriately.
  - High throughput laboratories typically have a computer system with database that tracks samples through the system
  - Low throughput laboratories typically do this by hand

The Laboratory Sample

Step 3: Inspecting the Sample

How much sample have you received ?

What is in the sample ?

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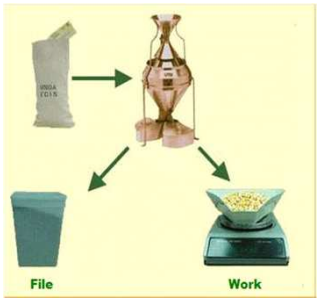
What's in your sample?

Step 4: Subsampling for Multiple Uses

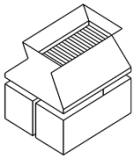


A sample may be tested for multiple analytes, and a file (retain) sample may be required

Subsampling

Boerner divider

Riffle multiple-slot divider

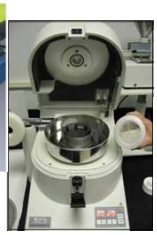


Step 5: Reducing the Laboratory Sample (by Grinding)

How much sample will you analyze ?

How does it affect your LOD ?

Grinding the Sample



Effect of Sample and Particle Size

- All samples consist of particles
  - Grain
  - Flour, meal
  - Molecules in solution (protein, DNA)
  - Containers
- A sample is a subset of particles from the bulk lot
- Sample size and particle size determine the number of particles in sample
- More particles are present in a sample of finer particles

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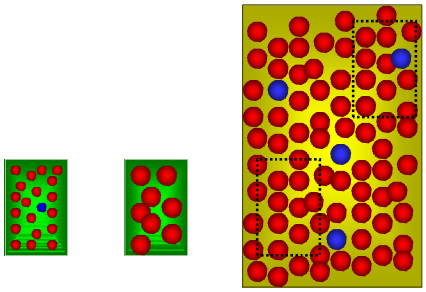
Sample Size and Particle Size



~~Zero~~ or Not Detected

Zero can only be tested by analyzing every seed !

Can be "not detected" by qualitative analysis;
- confidence depends on number and size of pools, and error rates

Can be "not detected" by quantitative analysis;
- no signal, but can be analytical method failure
- by false positive results depending on interpretation

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Enough seeds need to be tested to obtain a meaningful result.

If one transgenic seed is contained in a total of:	Testing a sample of 200 seeds yields a positive result:	Testing a sample of 1,000 seeds yields a positive result:	Testing a sample of 3,000 seeds yields a positive result:	Testing a sample of 10,000 seeds yields a positive result:
1,000 seeds	18% of the time	63% of the time	95% of the time	99.99% of the time
10,000 seeds	2% of the time	9.5% of the time	26% of the time	63% of the time
80,000 seeds	0.2% of the time	1.2% of the time	3.7% of the time	11.8% of the time
1,000,000 seeds	<0.1% of the time	<0.1% of the time	<0.1% of the time	3% of the time

LOD of the method must be taken into account
LOQ is important if quantification is required

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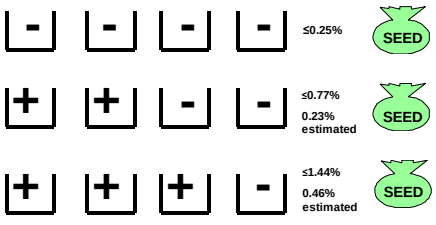
Threshold Testing

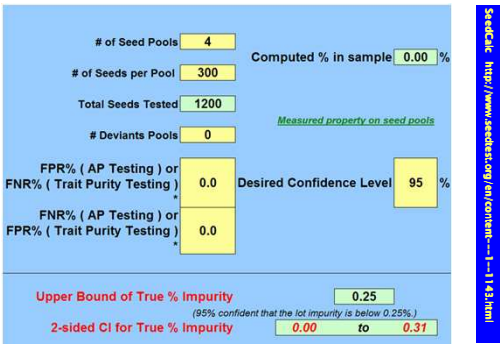
- Test to control distribution of grain
- Determine statistical probability that load is above or below threshold
- Use existing equipment and infrastructure
- Well established sampling protocols (e.g. USDA) to obtain a representative sample
- Rapid, protein strip tests
- Knowing the number of particles (seeds) in the sample allows estimation of maximum % transgenic

The Seed Pooling Test Strategy

(4 pools of 300 seeds, 95% LOC)



0 of 4 pools positive - Seedcalc.xls



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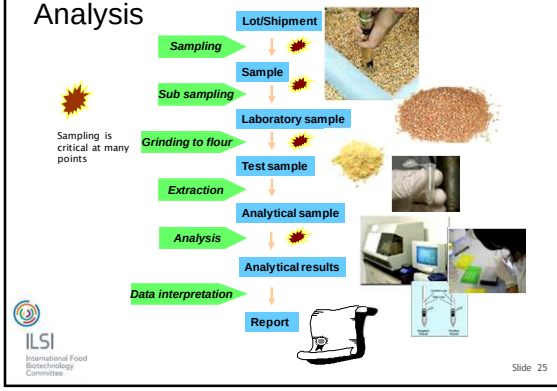
Sampling is Involved at Every Step in Analysis



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Sampling of DNA for analysis

- As the amount of DNA extracted from the sample becomes lower, sampling error becomes proportionally larger.
- 100 ng of DNA samples at a level of 0.1% (wt/wt) would produce GMO DNA estimates no better than 30% of the mean value, 95% of the time – a poor level of accuracy……..

The limits of GMO Detection.
Kay, S., & Van den Eede, G.
(2001) *Nature Biotech.*, 19, 405

Summary

- Sampling is the best way to get an estimate of the % transgenic in a lot
  - Improper sampling leads to false, imprecise and variable results
  - Representative samples are used
- Sample size is a balance between
  - Sensitivity
  - Cost
  - Probability of false negative results
- Sampling occurs throughout the analytical process

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Thank you

Discussion