PHL-IL and Mycotoxins

Andreia Bianchini, PhD
University of Nebraska - Lincoln

# Concerns About Mycotoxins

- Where populations have a single dietary staple
  - May be exposed to great amounts
  - Acute and chronic toxicity possible
  - Less developed countries – more direct exposure
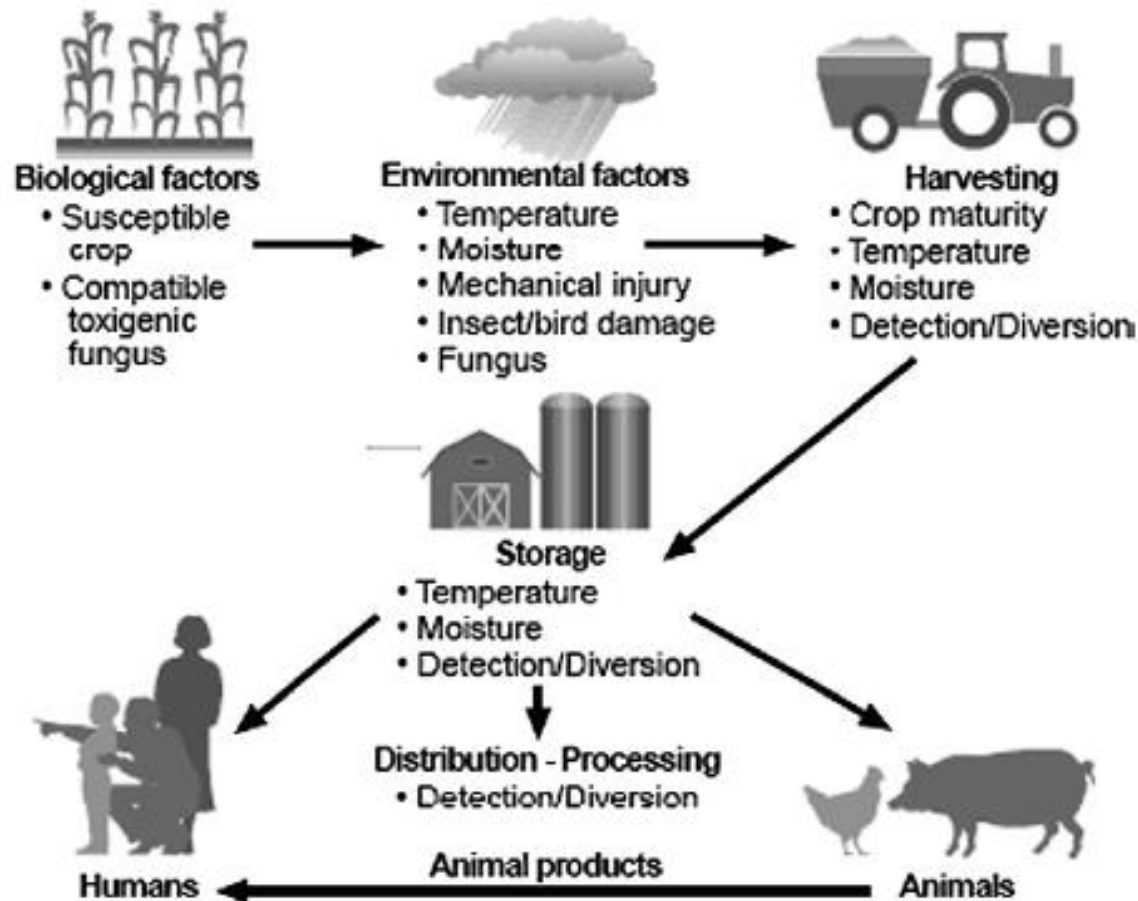
- Where diets are diverse
  - Low levels of exposure
  - Foods of better quality – lower amounts
  - More developed countries – direct and indirect exposure
    - ❖ Food Ingredients
    - ❖ Residues in animal products – milk, eggs, edible organ tissues
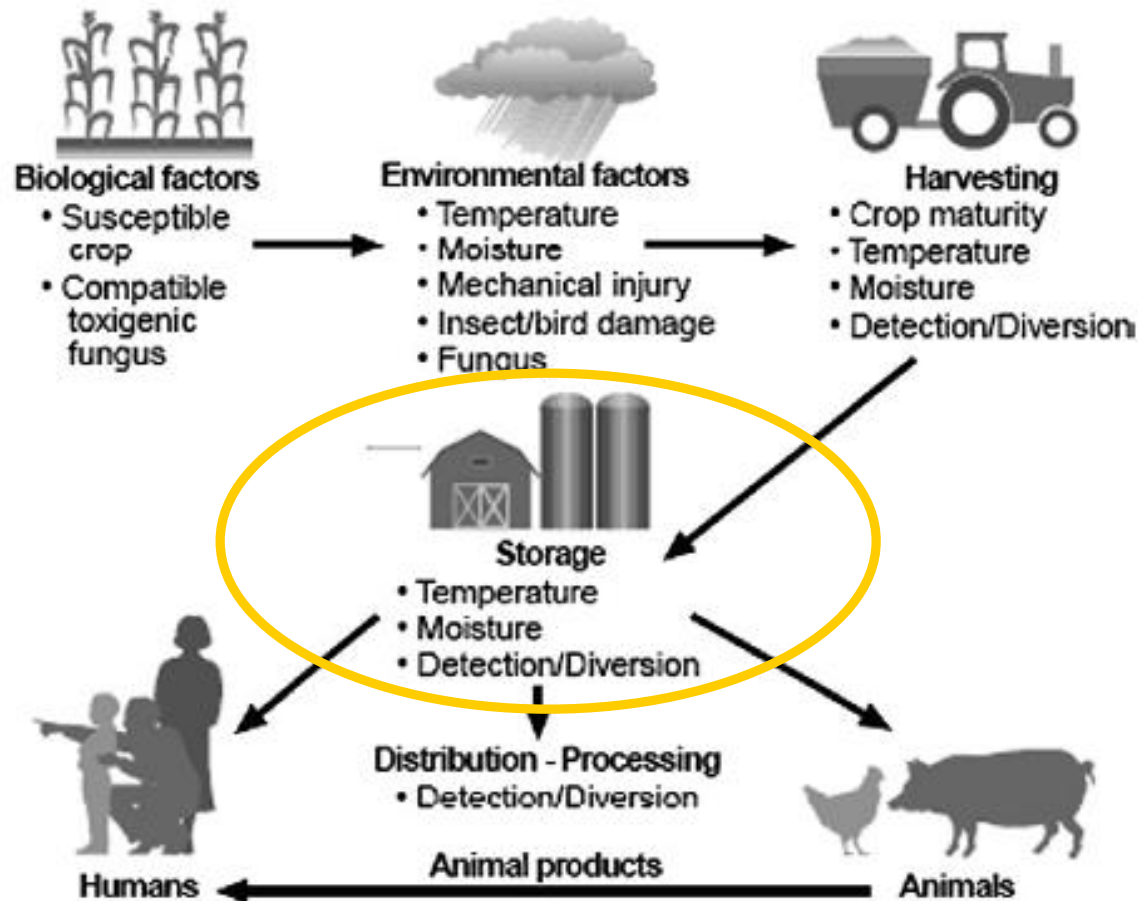
Mycotoxins: a multi-faceted issue
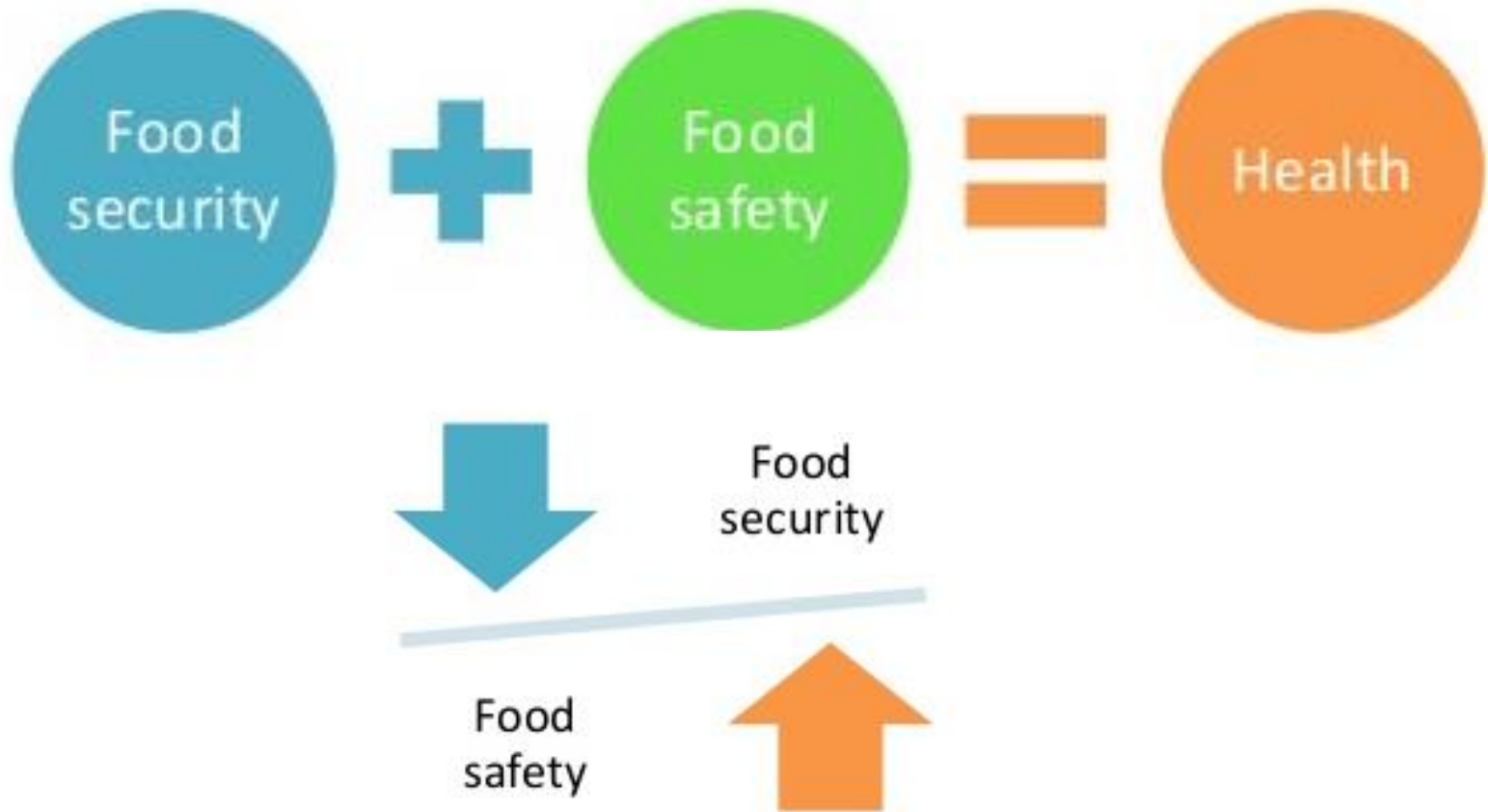
Mycotoxin Occurrence in the Food Chain

Factors affecting mycotoxin occurrence in the food chain (CAST, 2003).

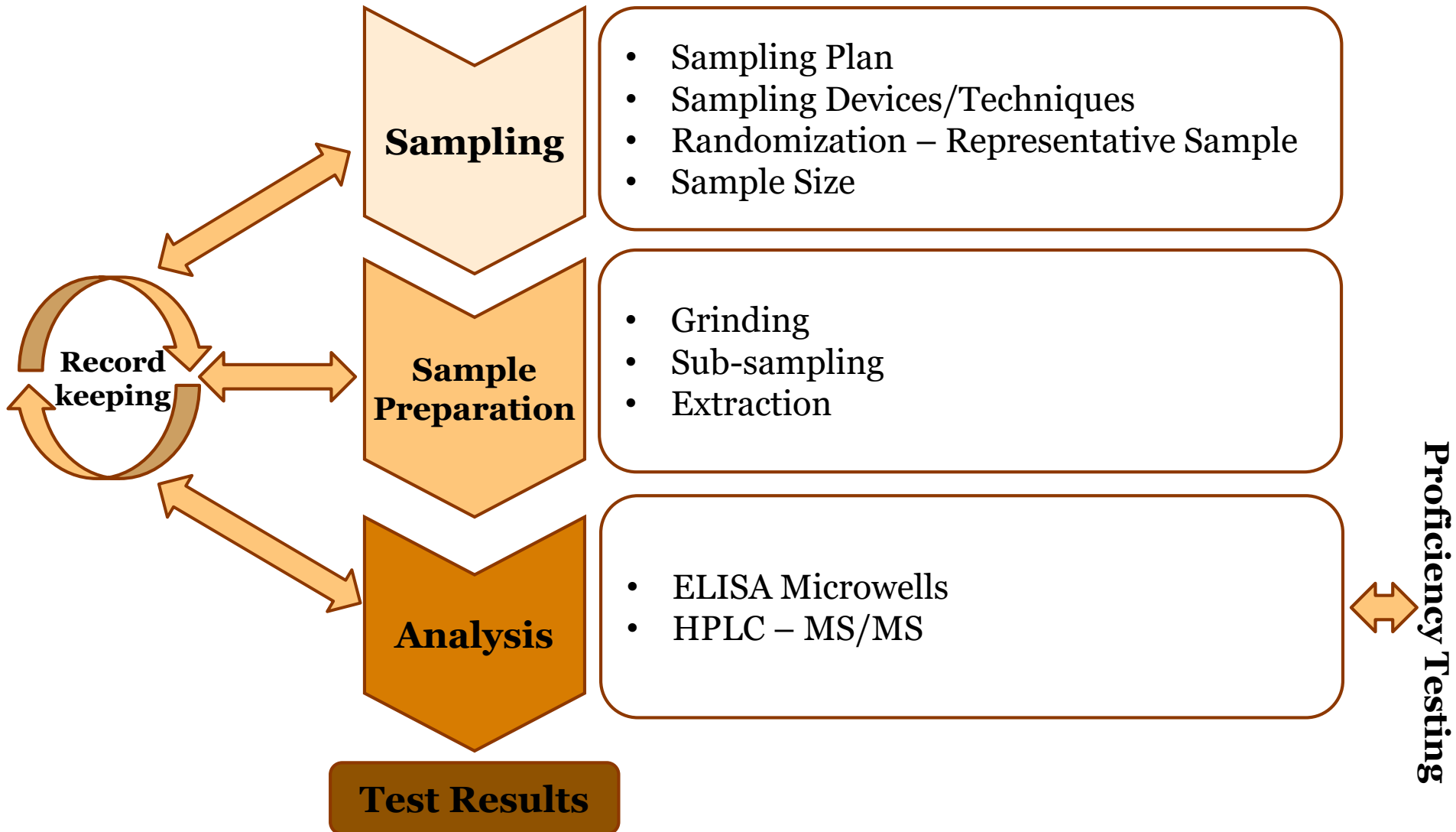# Mycotoxin Occurrence in the Food Chain

Factors affecting mycotoxin occurrence in the food chain (CAST, 2003).

PHL-IL Efforts in Target Countries

- Bangladesh:
  - Aflatoxin and Fumonisin in rice
- Ghana:
  - Mycotoxin baseline in corn
- Ethiopia:
  - Aflatoxin, Fumonisin, DON and Ochratoxin in maize, wheat, chickpea and sesame
- Guatemala:
  - Aflatoxin and Fumonisin in corn
- Afghanistan:
  - Aflatoxin and Ochratoxin in nuts and raisins
  - Ochratoxin, T-2 and Deoxynivalenol in wheat and flour

Mycotoxin Analysis – Main Steps

Sampling

- Sampling Plan
- Sampling Devices/Techniques
- Randomization – Representative Sample
- Sample Size

Sample Preparation

- Grinding
- Sub-sampling
- Extraction

Analysis

- ELISA Microwells
- HPLC – MS/MS

Test Results

Record keeping

Proficiency Testing

Sampling

- Major source of error and variation
- Mycotoxins are not evenly distributed in a lot
- Not every kernel or nut is contaminated
- A few kernels can contaminate large lots
- **"Cherry-picking"**

Sampling – Sample Size

Table 1. Effect of uneven distribution of mycotoxin on test results

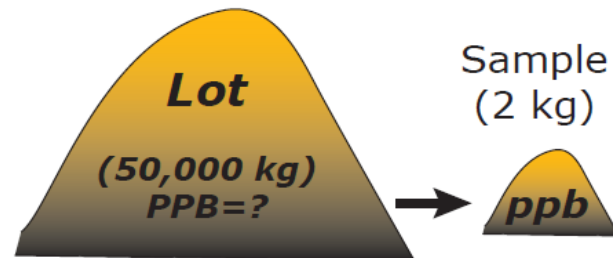| Mycotoxin	Commodity	Laboratory		
		Afghanistan	**Austria**	**USA (UNL)**
Aflatoxin (µg/kg)	Walnut-551	25	< LOD	< LOD
	Walnut-554	22	< LOD	< LOD
	Pistachio-612	< LOD	142	46
	Pistachio-624	< LOD	14	100
	Almond-504	14	< LOD	< LOD
	Raisin-269	18	< LOD	

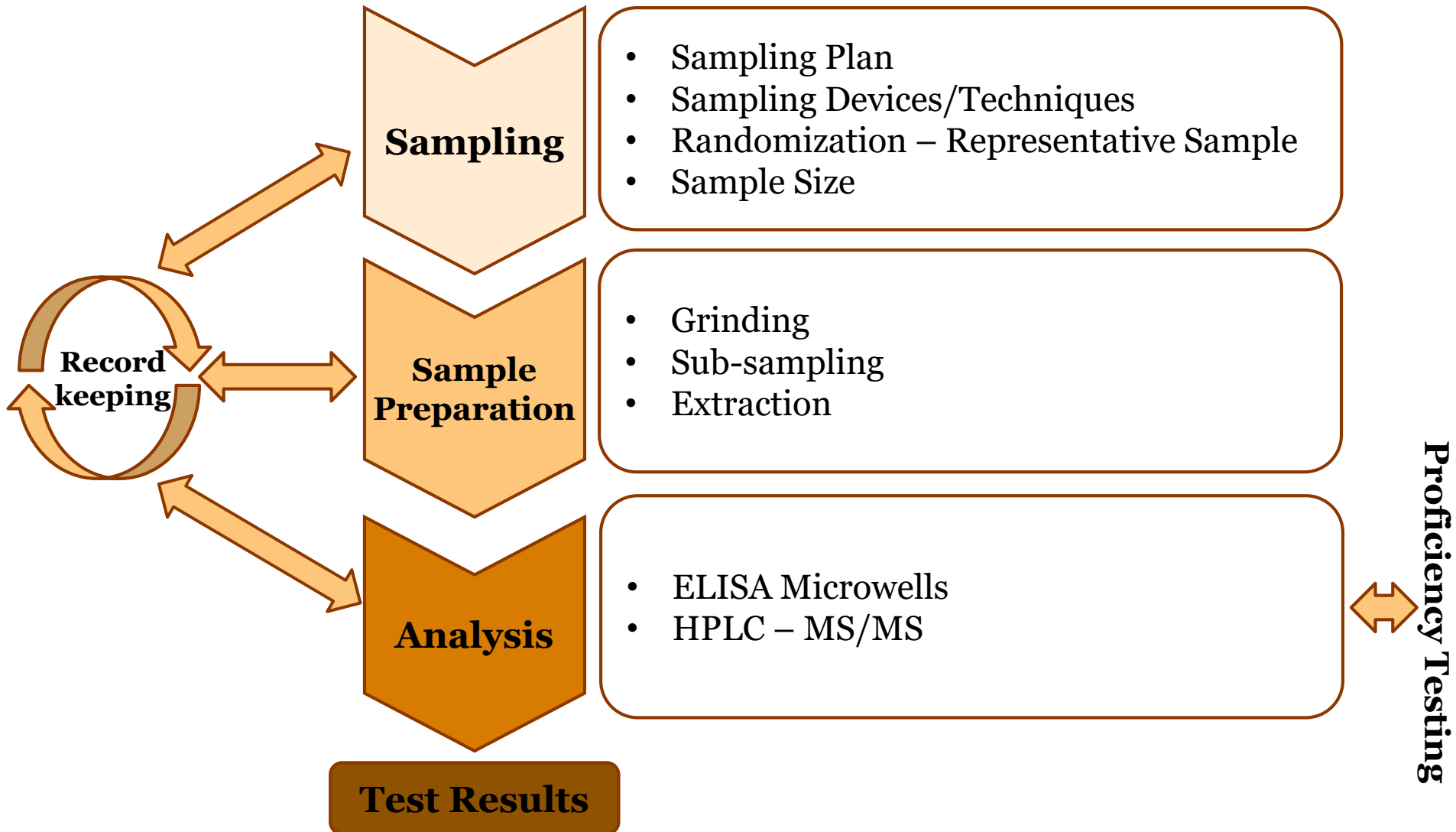# Solutions

Representative Sample

What is PHL-IL doing to ensure sample representativeness?
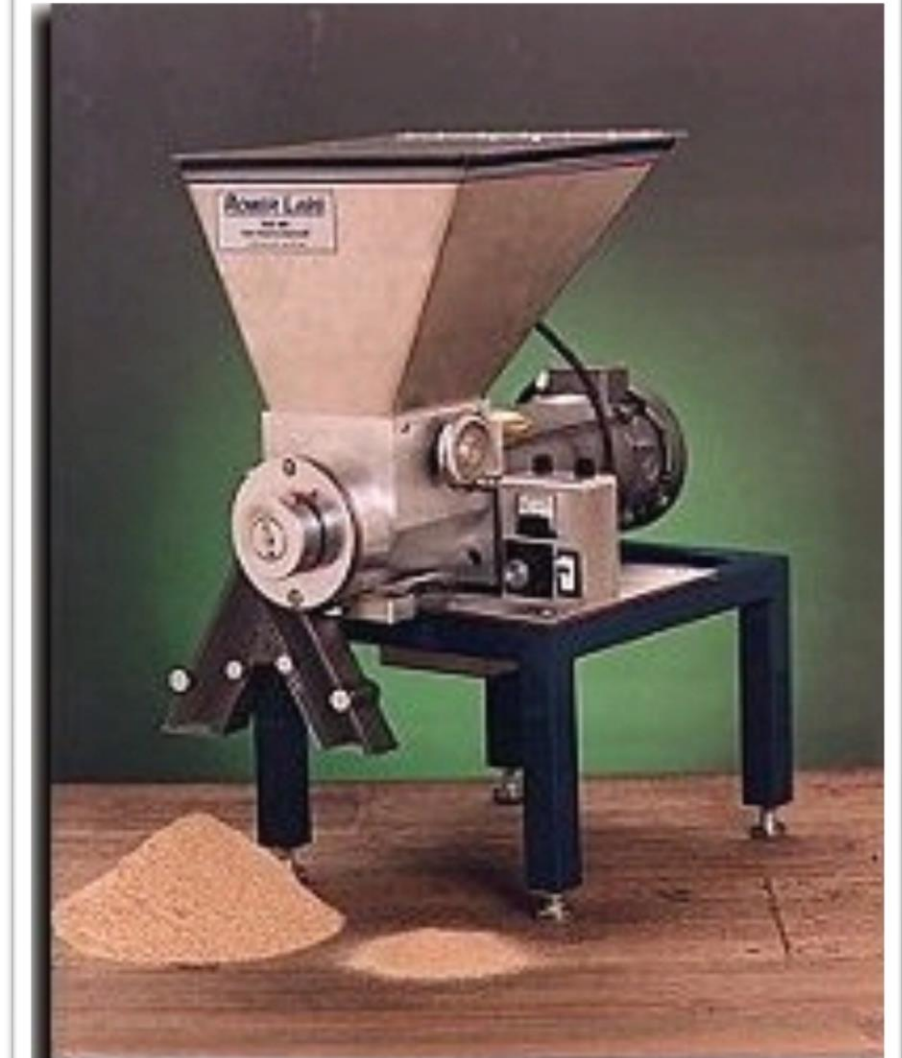- Use of probes?
- Mixing of subsamples?

Any help needed in this area?

Mycotoxin Analysis – Main Steps

Sampling
- Sampling Plan
- Sampling Devices/Techniques
- Randomization – Representative Sample
- Sample Size

Record keeping

Sample Preparation
- Grinding
- Sub-sampling
- Extraction

Analysis
- ELISA Microwells
- HPLC – MS/MS

Test Results

Proficiency Testing

Sample Preparation – Size Reduction

Mycotoxin Analysis – Splitting

Table 2. Effect of "splitting before grinding" on mycotoxin test results

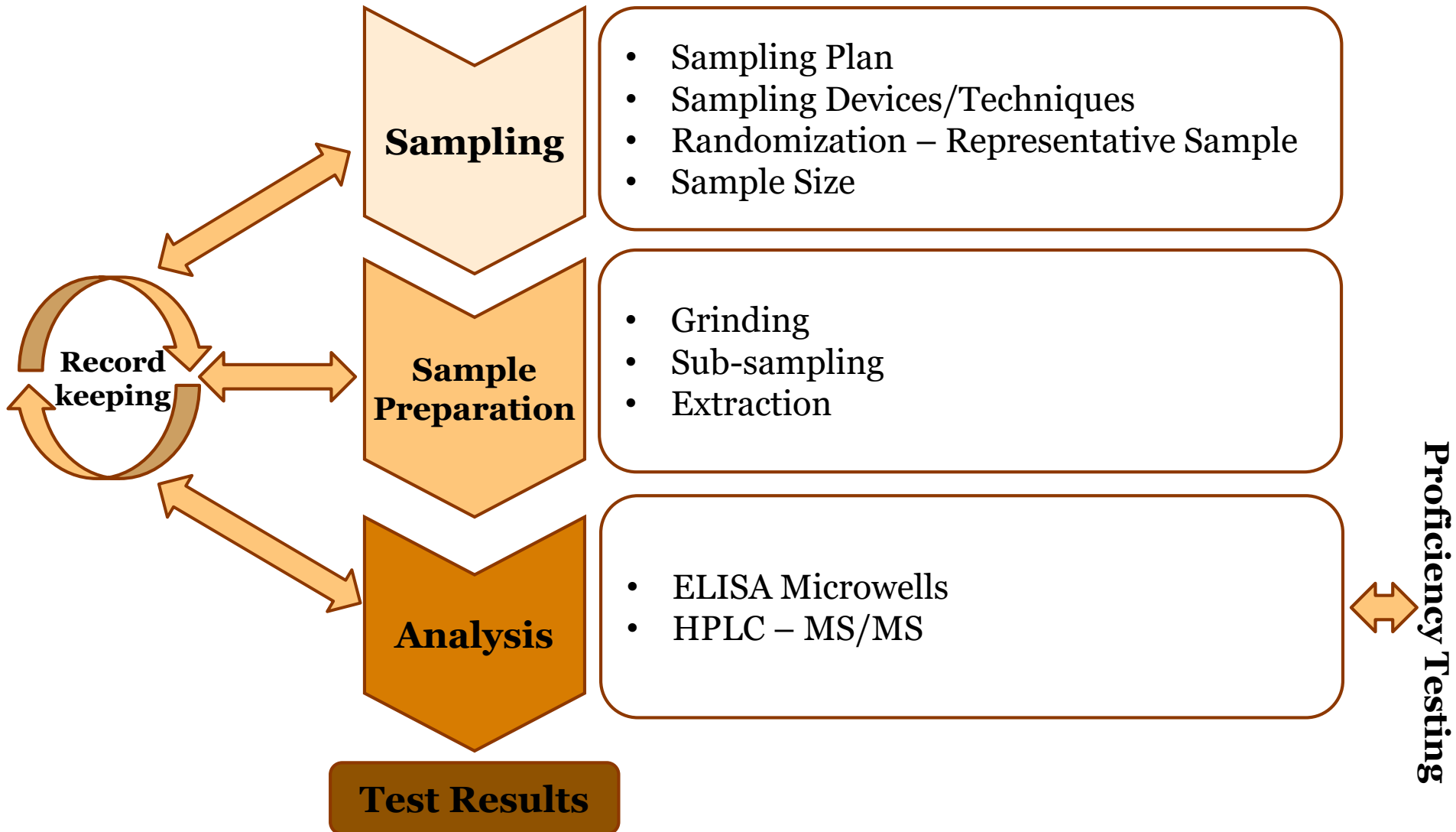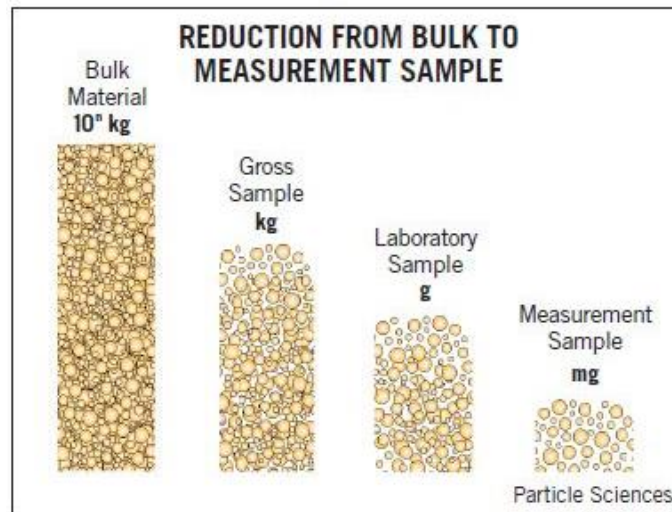| Mycotoxin (µg/kg)	Commodity	Batch 1	Batch 2		
		Afghanistan	Austria	USA (UNL)	USA (KSU)
Aflatoxin	Wheat-68	5	< LOD	< LOD	< LOD
	Wheat-110	10	< LOD	< LOD	< LOD
	Walnut-551	25	< LOD	< LOD	
	Pistachio-624	< LOD	15	95	
Deoxynivalenol	Wheat-3	3500	< LOD	< LOD	< LOD
	Wheat-14	1290	< LOD	< LOD	< LOD
Ochratoxin	Raisin-296	13	< LOD		
	Raisin-302	< LOD	10		

Solutions

Ideal Sample Preparation

What is PHL-IL doing to ensure sample representativeness?

- Use of mills?
- Grinding all sample and sub dividing at later steps?

Any help needed in this area?

Mycotoxin Analysis – Main Steps

Sampling

- Sampling Plan
- Sampling Devices/Techniques
- Randomization – Representative Sample
- Sample Size

Sample Preparation

- Grinding
- Sub-sampling
- Extraction

Record keeping

Analysis

- ELISA Microwells
- HPLC – MS/MS

Proficiency Testing

Test Results

Analytical Procedures

Sources of bias:

- Less than 100% of the mycotoxin may be extracted
- Other compounds may be extracted
- Mycotoxin standards may not be exact
- Instruments may not be correctly calibrated
- Technician errors
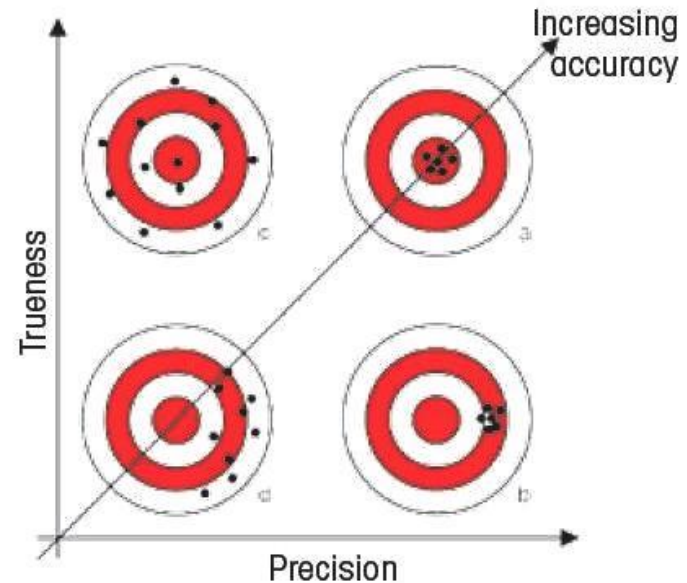
Solutions

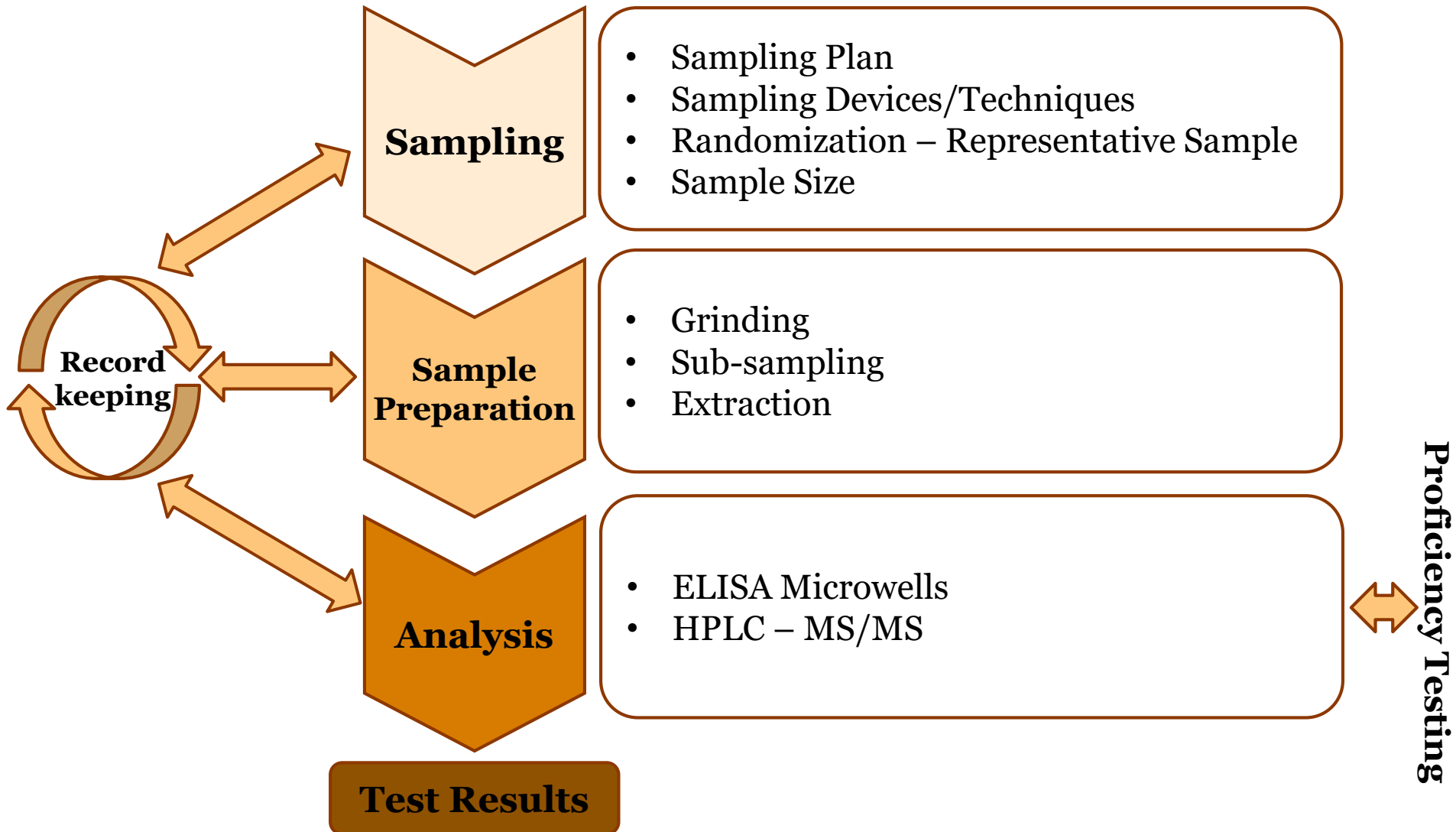**Monitoring/Verification and Training**

What is PHL-IL doing to ensure sample representativeness?
- Training of technicians?
- Verification of tests results?
  - Parallel testing of a few samples
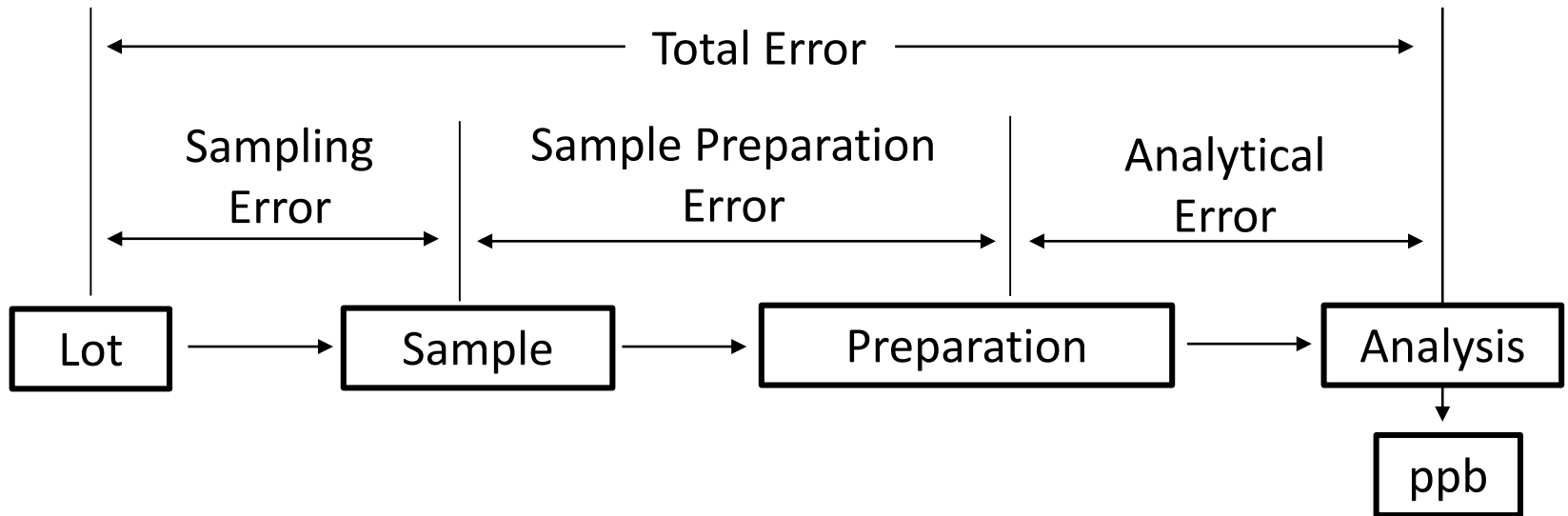 - Proficiency testing

Any help needed in this area?

PHL Innovation Lab
Guatemala

TITTLE: Validation test for total mycotoxin quantification, Agravision®

Written by: J. Rodrigo Mendoza   Edited by: Andréia Bianchini
Valid since: 02/22/2016   Version: 1

PURPOSE
1. To utilize reference material and/or food samples (i.e. corn samples with levels of mycotoxin determined by a laboratory of reference) to validate proper functioning of Agravision® mycotoxin reader, kits and proficiency of technicians running the test.

PROCEDURE

Mycotoxin Analysis – Main Steps

Sampling
- Sampling Plan
- Sampling Devices/Techniques
- Randomization – Representative Sample
- Sample Size

Sample Preparation
- Grinding
- Sub-sampling
- Extraction

Analysis
- ELISA Microwells
- HPLC – MS/MS

Record keeping

Proficiency Testing

Test Results

Mycotoxin Testing: Total Error