



# Mycotoxin analysis and the role of sampling

# Romer Labs...

## a leading global supplier of diagnostic solutions

- mycotoxins
- food allergens & gluten
- microorganisms
- genetically modified organisms (GMO)
- other food contaminants

## operate 6 fully accredited service labs

- United States
- Austria
- UK (2)
- Singapore
- China

# Why Are We Testing?

Mycotoxin legislation – i.e., European Union

- Prohibits the use of Products not complying with **maximum levels**
- For **Aflatoxins (B<sub>1</sub>+B<sub>2</sub>+G<sub>1</sub>+G<sub>2</sub>), Aflatoxin M<sub>1</sub>, Ochratoxin A, Patulin, DON, Fumonisin (FB1 + FB2) and Zearalenone maximum levels** have been established
- For **T-2 and HT-2 Toxin new recommendation since March 2013**
- **Regulation for Ergot Alkaloids since January 2022**
- Detailed list of regulated products for each mycotoxin
- Legislation contains **regulations for sampling** and **method performance criteria**

# 1881/2006/EC – Aflatoxins

| Élelmiszerek (*) |   | Felső határértékek (µg/kg) |  |                |
|------------------|---|----------------------------|--|----------------|
|                  |   | B <sub>1</sub>             | A B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> és G <sub>2</sub> összege | M <sub>1</sub> |
| 2.1.             | <b>Aflatoxinok</b>  |                            |  |                |
| 2.1.1.           | Az emberi fogyasztás vagy élelmiszer-összetevőként történő felhasználás előtt válogatásnak vagy egyéb fizikai kezelésnek alávetett földimogyoró   | 8,0 (*)                    | 15,0 (*)   | —              |
| 2.1.2.           | Az emberi fogyasztás vagy élelmiszer-összetevőként történő felhasználás előtt válogatásnak vagy egyéb fizikai kezelésnek alávetett diófélék   | 5,0 (*)                    | 10,0 (*)   | —              |
| 2.1.3.           | Közvetlen emberi fogyasztásra vagy összetett élelmiszerek összetevőjeként történő felhasználásra szánt földimogyoró, diófélék és ezek feldolgozott termékei                                 | 2,0 (*)                    | 4,0 (*)  | —              |
| 2.1.4.           | Az emberi fogyasztás vagy élelmiszer-összetevőként történő felhasználás előtt válogatásnak vagy egyéb fizikai kezelésnek alávetett szárított gyümölcs                                       | 5,0                        | 10,0   | —              |
| 2.1.5.           | Közvetlen emberi fogyasztásra vagy összetett élelmiszerek összetevőjeként történő felhasználásra szánt szárított gyümölcs és annak feldolgozott termékei                                    | 2,0                        | 4,0  | —              |
| 2.1.6.           | Valamennyi gabonaféle és a gabonafélékből származó valamennyi termék, beleértve a feldolgozott gabonatermékeket is, a 2.1.7., 2.1.10. és 2.1.12. pontban felsorolt élelmiszerek kivételével | 2,0                        | 4,0  | —              |
| 2.1.7.           | Az emberi fogyasztás vagy élelmiszer-összetevőként történő felhasználás előtt válogatásnak vagy egyéb fizikai kezelésnek alávetett kukorica   | 5,0                        | 10,0   | —              |
| 2.1.8.           | Nyerstej (*), tejalapú termékek előállításához használt tej és hőkezelt tej   | —                          | —  | 0,050          |

# 401/2006/EK - Sampling

| Árucikk                           | A tétel tömege (tonna)  | Az altételek tömege vagy száma | A részminták száma | Az egyesített minta tömege (kg) |
|-----------------------------------|-------------------------|--------------------------------|--------------------|---------------------------------|
| Gabonafélék és gabonakészítmények | $\geq 1\ 500$           | 500 tonna                      | 100                | 10                              |
|                                   | $> 300$ és $< 1\ 500$   | 3 altétel                      | 100                | 10                              |
|                                   | $\geq 50$ és $\leq 300$ | 100 tonna                      | 100                | 10                              |
|                                   | $< 50$                  | —                              | 3–100 (*)          | 1–10                            |

(\*) A tétel tömegétől függően – lásd 2. táblázat.

# 152/2009/EK – 691/2013/EK sampling for feed

| 5.A.2.     | Elemi minták                                  |  |
|------------|---|--|
| 5.A.2.1.   | Ömlesztett takarmány:                         | Az elemi minták minimális száma:   |
| 5.A.2.1.1. | 2,5 tonnát meg nem haladó mintavételi tételek | hét  |
| 5.A.2.1.2. | 2,5 tonnát meghaladó mintavételi tételek      | $\sqrt{a}$ mintavételi tételt (*) kitevő tonnák számának húszszorososa, de legfeljebb 40 elemi minta |
| 5.A.2.2.   | Kiszerelt takarmány:                          | Mintavételre kerülő töltött göngyölegek minimális száma (**):  |

5.3. Az igen nagyméretű tételek esetében az elemi mintákra vonatkozó mennyiségi követelmények A nagyméretű mintavételi tételek (> 500 tonna) esetében az elemi minták szükséges száma  $c = 40$  elemi minta +  $\sqrt{\text{tonna}}$  a takarmányban egyenletesen eloszló anyagok és termékek ellenőrzése esetén, vagy 100 elemi minta +  $\sqrt{\text{tonna}}$  a takarmányban feltételezhetően egyenetlenül eloszló anyagok és termékek ellenőrzése esetén.

# Sampling - definition

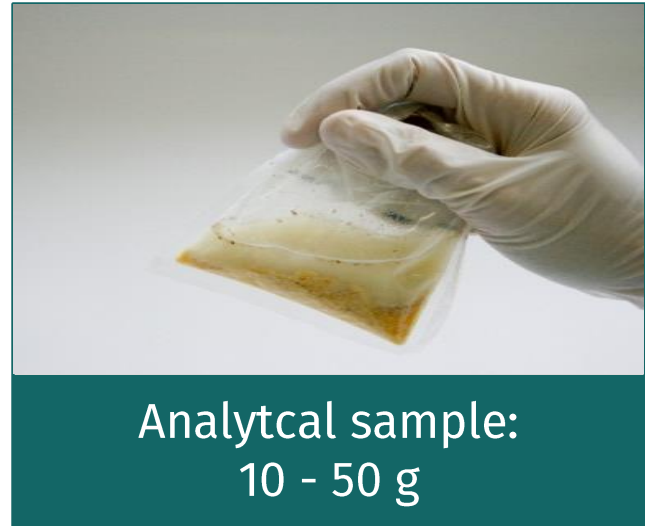
Statistical procedure, to take a sample from a population. Then based on the sample testing results we draw conclusions to the population.

# Sampling in practice

Average mycotoxin concentration should be the same



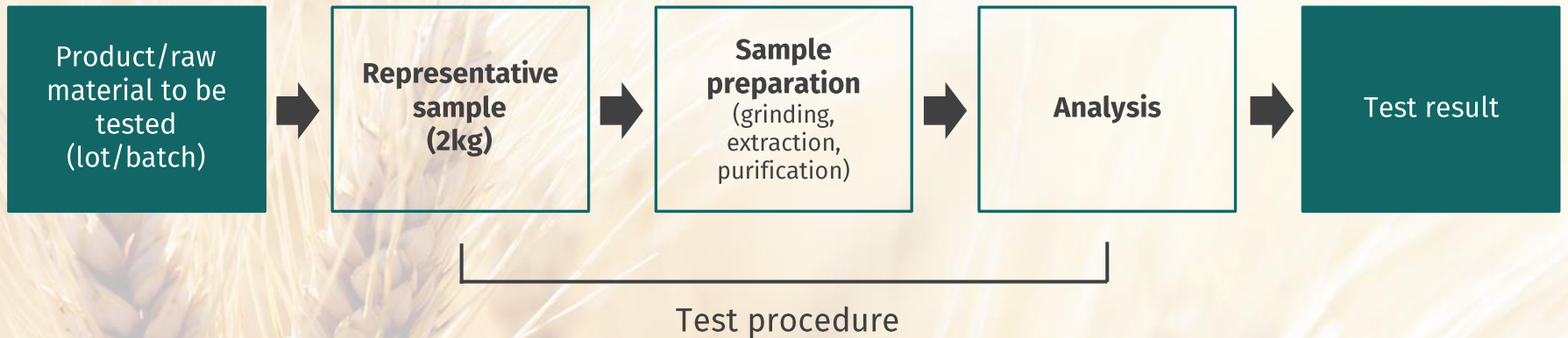
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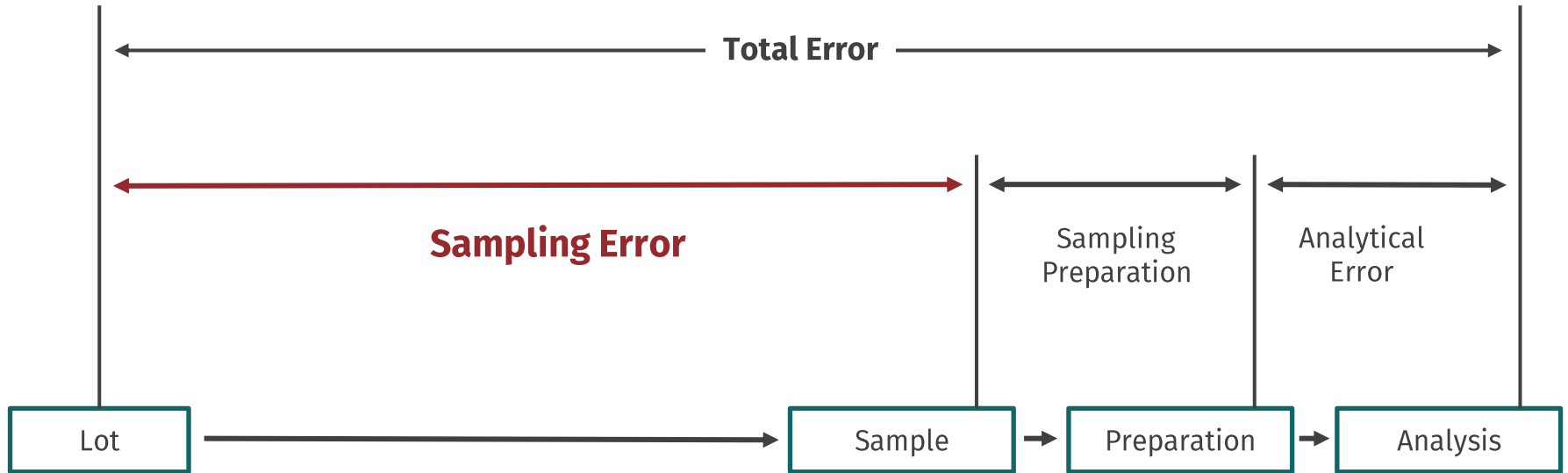
# Mycotoxin Test Procedure

## Overview



# Testing Variability

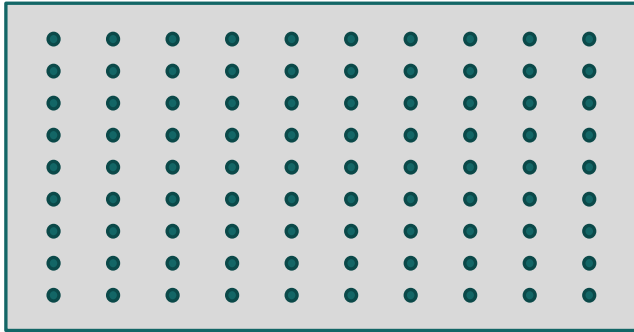
*Sampling contributes to up to 88% of the total uncertainty\**



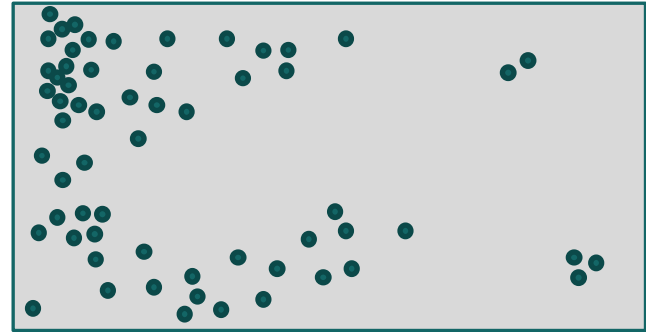
Source: Whitaker & Dicken, 1974

# The „Distribution-Problem“

Mycotoxins are not uniformly spread throughout the entire batch, but rather vary in their levels of contamination across different areas



**Even** distribution  
(example: proteins)



**Uneven** distribution  
(mycotoxins)

# The „ppb – Problem“

Mycotoxins are very potent compounds causing human and animal health problems at concentrations as low as parts per billion (ppb)

- 1 Part in 1,000,000,000
- 1 second in 32 years
- A grain of sand in 22 kg
- 1 kernel of corn in 3.5 rail cars

$$1\text{ppb} = 1\mu\text{g}/\text{kg}$$

# Sampling risks

Overestimation



Good lot accepted



Good lot rejected



Underestimation



Bad lot accepted



Bad lot rejected



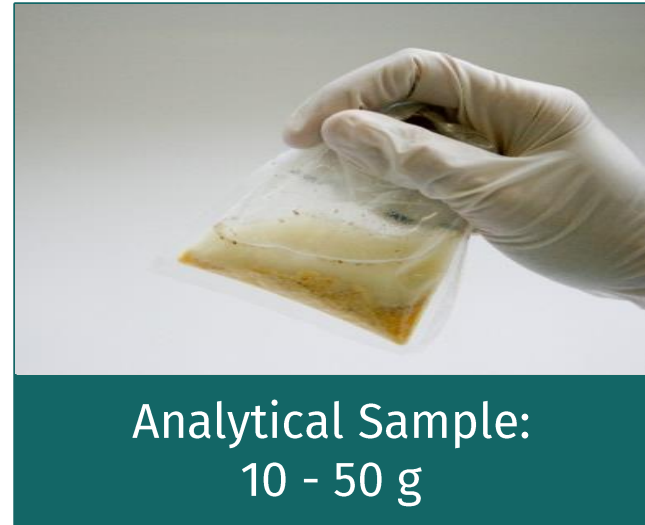
0

# The Quest for a Representative Sample

Same average mycotoxin concentration



=



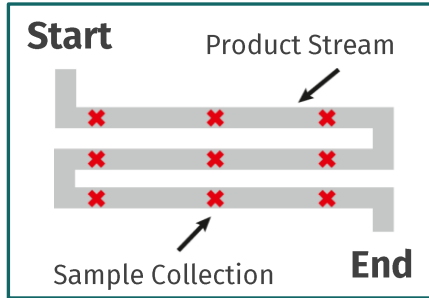
# Selecting a representative sample

- Consider number of samples required for proper analysis.
- Collect small incremental samples from several areas of the lot.
- Combine these samples to a single aggregate sample.
- Reduce to the final sample to be used for analysis.
  
- **Random selection**
- Each grain kernels or pellet particles should have **equal opportunities** to make it to the sample
- The sampling process should be unbiased

# Basic Principles to Obtain a Representative Sample - I

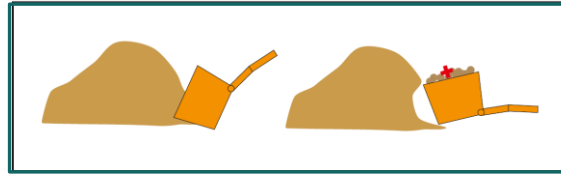
- Consider number of samples required for proper analysis.
- Collect small incremental samples from several areas of the lot.
- Combine these samples to a single aggregate sample.
- Reduce to the final sample to be used for analysis.

## Sampling during transfer (i.e. loading bucket, loading spout)



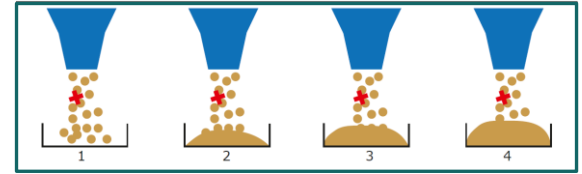
### Sampling from a moving stream:

The **red x** represents the place where the incremental sample should be collected.



### Sampling during transfer with loading bucket:

The **red x** represents the place where the sample should be collected.



### Sampling from the moving stream of a loading spout:

The **red x** represents the place where the sample should be collected.



# Basic Principles to Obtain a Representative Sample - II

## Dynamic sampling

### Sampling from moving grain flow

#### a. Manual sampling



Pelican sampler

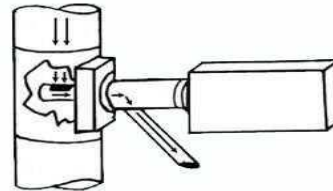


Ellis sampler

#### b. Automated sampling



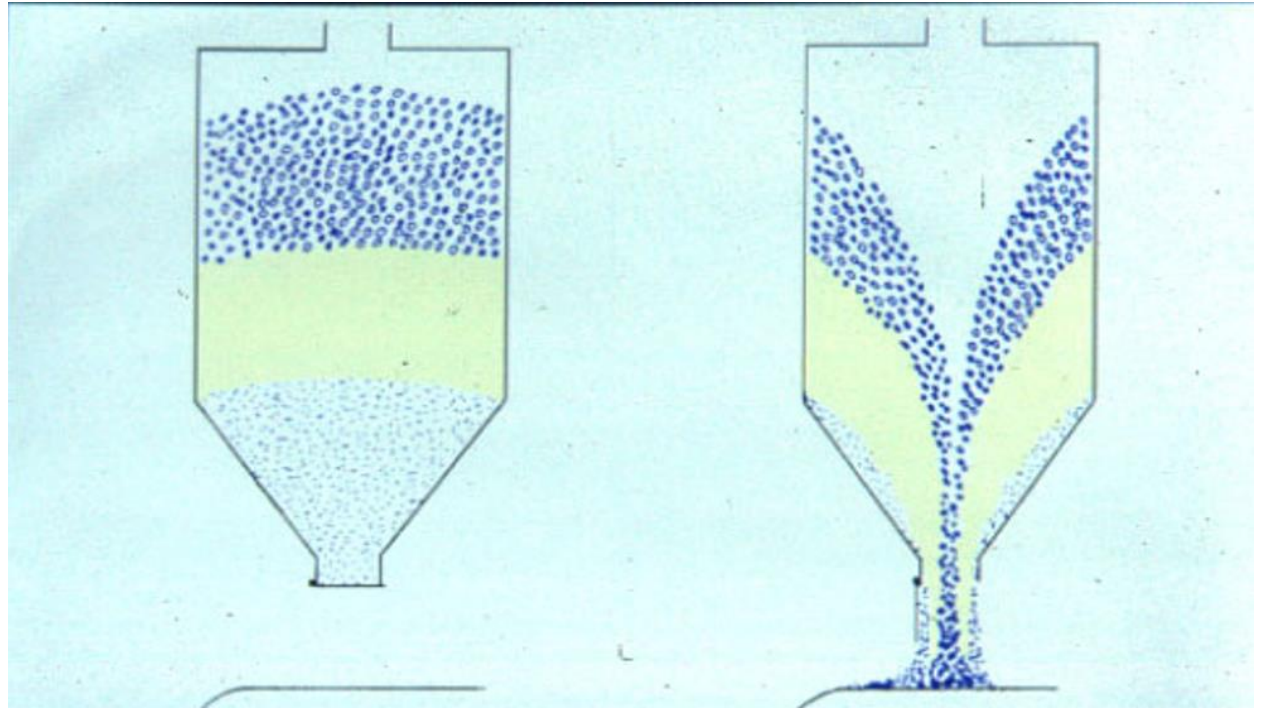
Diverter-type sampler for grain particles



Point-type mechanicals sampler for powders

# Segmentation

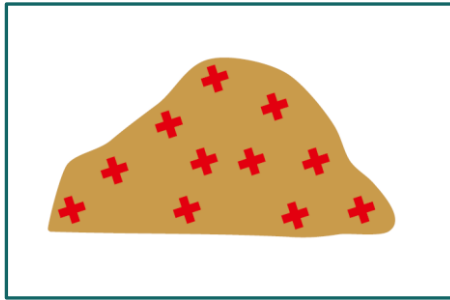
- Size
- Shape
- Weight



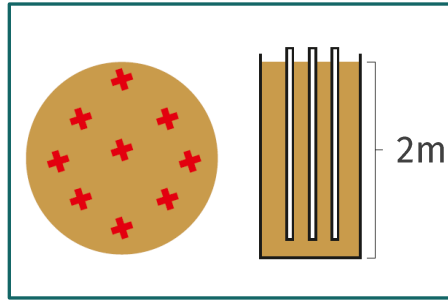
# Basic Principles to Obtain a Representative Sample - III

- Consider number of samples required for proper analysis.
- Collect small incremental samples from several areas of the lot.
- Combine these samples to a single aggregate sample.
- Reduce to the final sample to be used for analysis.

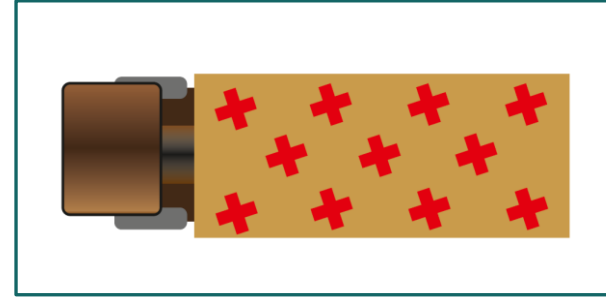
## Sampling in place of storage (grain heap, storage silos) and bulk samples in trucks



Sampling of **grain heaps**.  
The **red x** represents the place where the sample should be collected.



Sampling of grains in a **tower silo**.  
The **red x** represents the place where the sample should be collected.

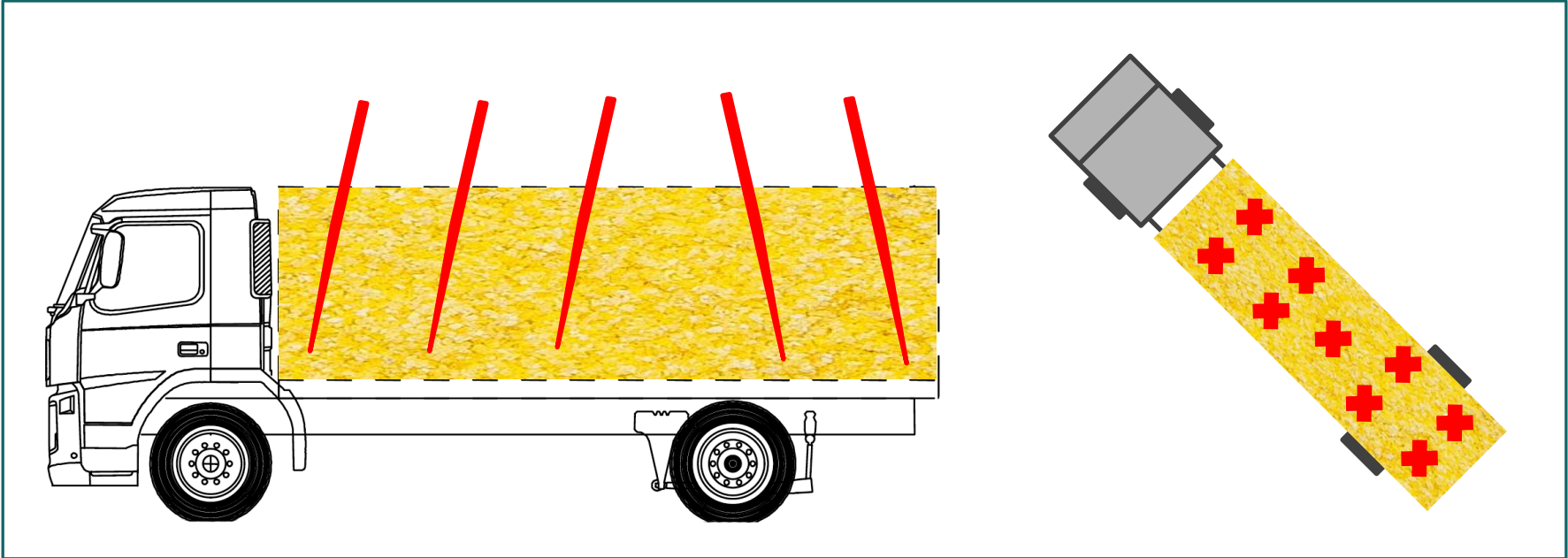


Sampling of grains in a **truck**.  
The **red x** represents the place where the sample should be collected.

# Sampling devices



# Sampling from trucks





**Lot**

**Pooling**

**Aggregate sample**

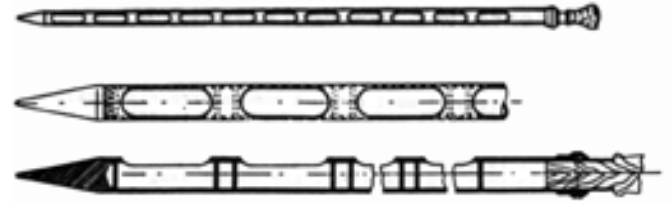
**Grinding**

**Subsampling**

**Analytical sample**

# EU Sampling Recommendation

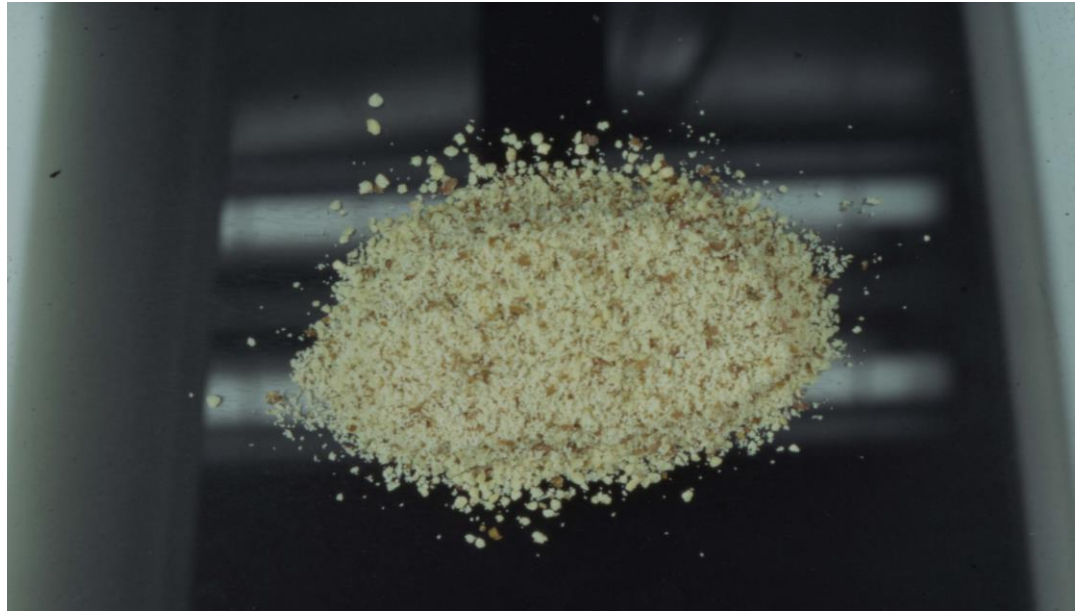
“< 50 tones cereals, take  
10+ incremental samples  
of 100 g each”



Use a grain probe or trier

# Sample preparation

- Grinding
- Subsampling
- Extraction
- Sample clean-up
- Concentration





# Subsampling

## **The aim?**

To select a small amount of material (e.g. grain or animal feed) from a sample that has the same concentration of the analyte of interest (e.g. mycotoxins).

## **How can it be achieved?**

We have to ensure that the subsample contains a small particle of each grain kernels or feed pellet from the sample.

# Specific mill



# Mikotoxin analysis



## Rapid testing

Antibody based methods

On-site testing



## Reference testing

Chromatography

Laboratory testing



# Reference testing

Confirmatory analysis (HPLC, LC-MS/MS)

- Complex matrices
- Multi-mycotoxin analysis
- High accuracy and sensitivity
- When official methods are requested



# Reference testing

Test report example

**Probennr.: AT-32203-4**

Beschreibung: Corn 4,16.08.2023

Matrix: Mais

Zustand: gemahlen, ungekühlt

Gewicht: 24 g

Verpackung: Kunststoffröhrchen

## Prüfverfahren und Ergebnisse

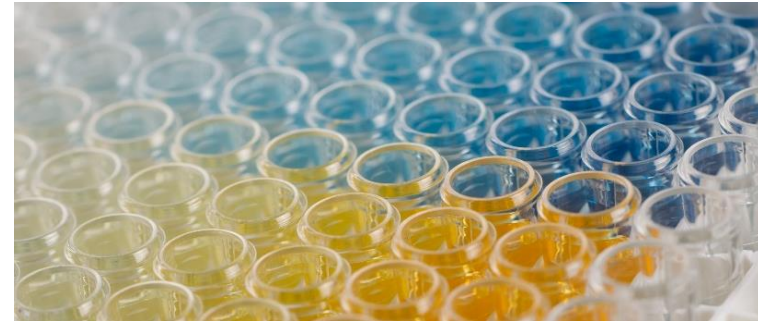
Test Ausführung: 22.08.2023

| Parameter    | Wert ± MU  | Einheit | NWG | Methode  | Artikelnr. |
|--------------|------------|---------|-----|----------|------------|
| Fumonisin B1 | 3290 ± 990 | µg/kg   | 10  | AT-SOP31 | 30000902   |
| Fumonisin B2 | 1210 ± 360 | µg/kg   | 10  | AT-SOP31 | 30000902   |

# Rapid testing - ELISA

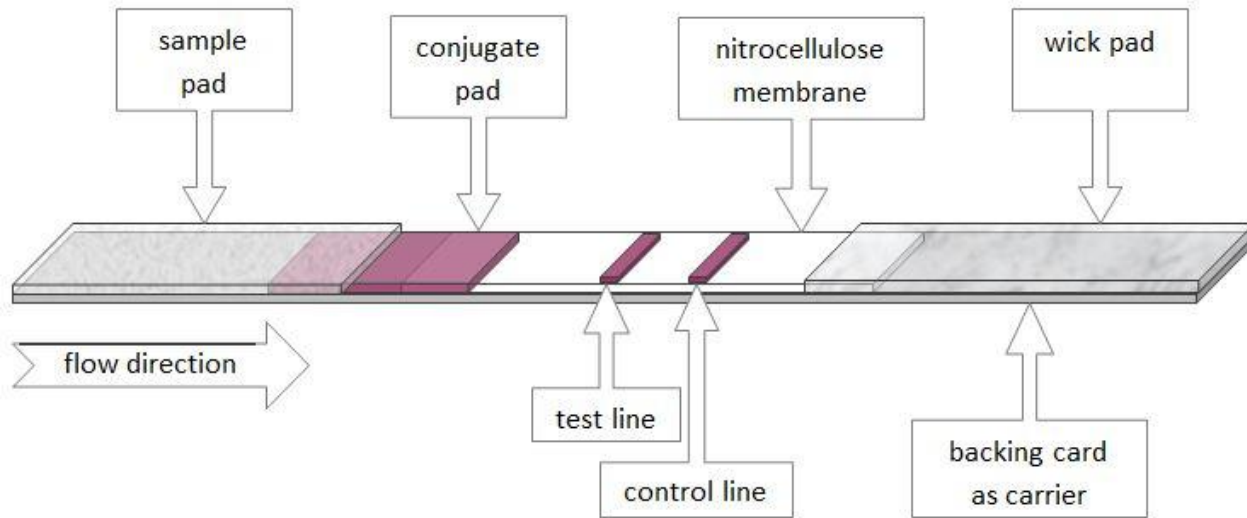
Laboratory conditions, screening assay

- Beneficial for high sample number
- When time is important – fast results
- Laboratory background, low/medium volume investment
- Simple protocol, ease-of-use



# Rapid testing - LFD

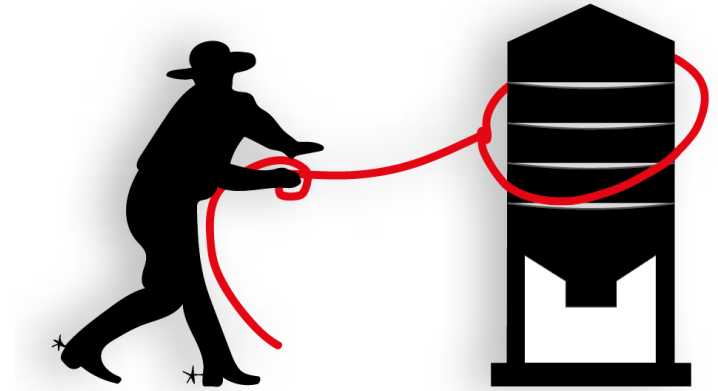
## Competitive LFD



# The “Grain Wrangler”

## Profile:

- Usually found at raw material reception points
- decides whether to accept, reject or otherwise segregate ingredients/ grains, based on their levels of mycotoxin contamination
- Interested in time to result, simple test procedure, robust product design, connectivity





# The “Artisan Tester”

## Profile:

- QC for highly refined products such as citric acid, starch, high fructose corn syrup
- lab environment and technical staff
- Look to free up more complex equipment and labor time for other necessary lab work
- Interested in precision and flexibility



# The “Mycotoxin Cop”

## Profile:

- Usually, part of certification companies or regulatory agencies
- Assures compliance with regulations and thresholds
- Mainly test on-site
- Interested accuracy and usability
- Test strips and readers must be able to
- Maintain quality performance despite always being on the move



# The “Animal Guardian”

## Profile:

- Usually found around a farm
- Tasked to manage the mycotoxin risk while keeping an eye on animal health and performance
- Interested in a fast, sensitive and easy-to-use test kits which enables him to choose the right mycotoxin risk management strategy.



# AgraStrip® Pro WATEX® & AgraVision™ Pro

**AgraStrip® Pro WATEX®** test system includes the AgraVision™ Pro reader and the

**AgraStrip® Pro WATEX®** test kits:

- Total aflatoxins
- Deoxynivalenol (DON)
- Total fumonisins
- Zearalenons (F-2)
- Ochratoxin A
- T-2/HT-2



# AgraStrip® Pro WATEX®

| item                                  | Quantitation range | LOD        | LOQ       |
|---------------------------------------|--------------------|------------|-----------|
| AgraStrip® Pro Total Aflatoxin WATEX® | 0 – 460 ppb        | 2/1 ppb*   | 3/1.5 ppb |
| AgraStrip® Pro Deoxynivalenol WATEX®  | 0 – 44 ppm         | 0.1 ppm    | 0.2 ppm   |
| AgraStrip® Pro Total Fumonisin WATEX® | 0 – 44 ppm         | 0.1 ppm    | 0.2 ppm   |
| AgraStrip® Pro Zearalenone WATEX®     | 0 – 1650 ppb       | 25 ppb     | 40 ppb    |
| AgraStrip® Pro Ochratoxin A WATEX®    | 0 – 100 ppb        | 2/0.5 ppb* | 3/1 ppb*  |
| AgraStrip® Pro T-2/HT-2 WATEX®        | 0 – 1000 ppb       | 15 ppb     | 20 ppb    |

\* High sensitive methods available for aflatoxins and ochratoxin A

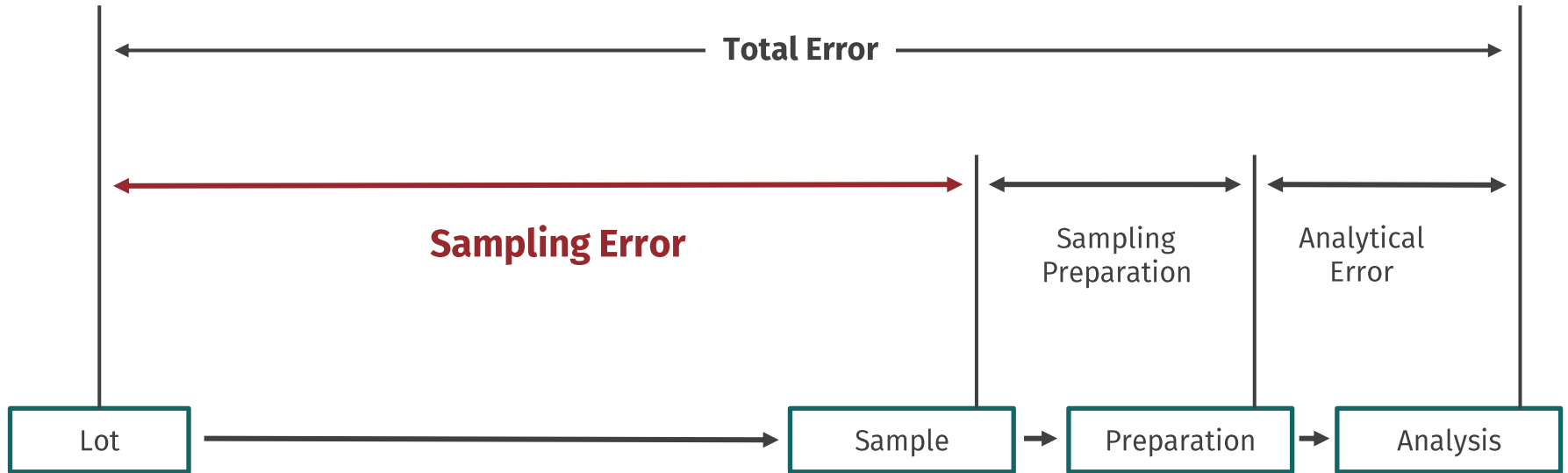
# Testing methods

## Summary

- Different methods available
- Decision factors: fitness for purpose
  - Testing time
  - Laboratory background, budget
  - Skilled personnel
  - Commodities to be tested (matrices)

# Testing Variability

*Sampling contributes to up to 88% of the total uncertainty\**



Source: Whitaker & Dicken, 1974

# Thanks for your attention!

For more information visit  
[www.romerlabs.com](http://www.romerlabs.com)