



# An integrated approach to mycotoxin testing in the poultry feed chain

Bankok, March 2010

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# OUTLINE

- 🌿 **Mycotoxins in poultry feed**
- 🌿 **Integrated testing plan**
- 🌿 **Sampling**
- 🌿 **On-site testing**
- 🌿 **Laboratory testing**



# Mycotoxin contaminated feed materials

- **Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub> – nuts, seeds, maize**
- **Fumonisin B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> – maize (corn)**
- **Zearalenone in wheat and maize**
- **Deoxynivalenol (DON) – wheat & grain**
- **Ochratoxin A – all cereals**



# Feed risk components for aflatoxin B<sub>1</sub> contamination

Groundnut

Copra

Palm kernel

Sunflower seeds

Maize

Babassu

Cotton seed

Rice & rice bran



# Feed risk components – DON & T-2 toxin

**Wheat**

**Barley**

**Oats**

**Maize**



**Dried Distillers Grains with Solubles (DDGS)**



# Feed risk components – Fumonisin

**Maize**

**Maize germ meal**

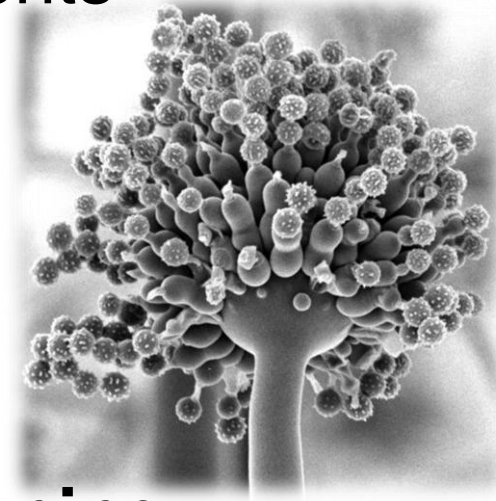
**Corn steep liquor**



# Integrated approach

Identification of high-risk feed components

- Screening of feed components
- Screening of compound feed



**Testing for multiple toxins and screening at relevant levels**

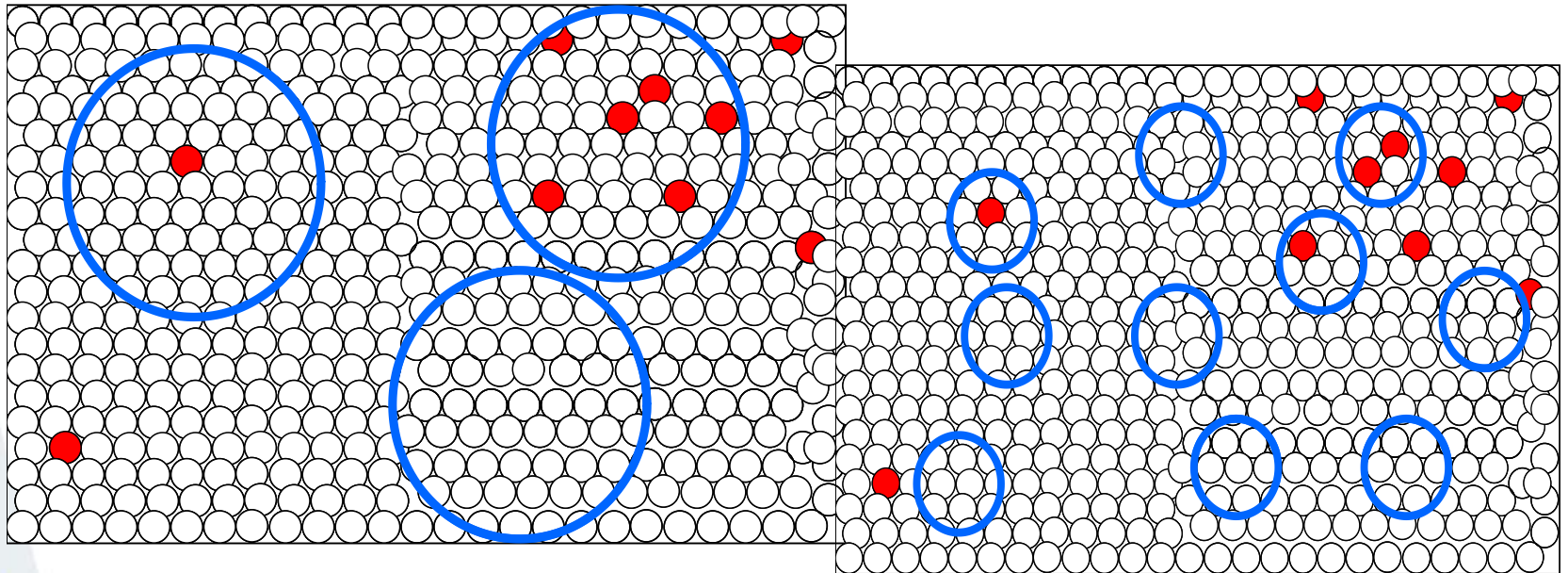


# Heterogeneous contamination problem

Infection is a random event – uneven contamination

Taking a representative sample presents a major difficulty

Need to follow systematic sampling plan





# Adoption of adequate sampling strategy

- ❧ Mycotoxins in feed are not uniformly distributed
- ❧ Heterogeneous contamination – hot spots
- ❧ Large sample sizes need to be taken



# EU Sampling requirements for aflatoxins in poultry feed

Commission Regulation (EC) No 401/2006 of Feb 26<sup>th</sup> 2006

<b>Lot weight in tonnes</b>	<b>Sub-lot</b>	<b>No of incremental samples</b>	<b>Aggregate sample size</b>
>1500	500 tonnes	100	10 kg
300- 1500	3 sub-lots	100	10 kg
50-300	100 tonnes	100	10 kg
<50	None	3-100	1-10 kg



# Testing for aflatoxins in poultry feed

Sampling & sub-sampling



Grinding and homogenization (10 kg)



Extraction into solvent



Screening

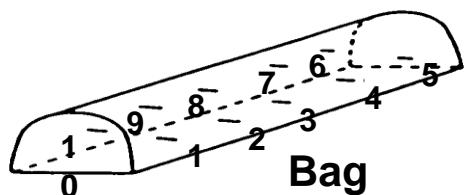


Instrumental analysis

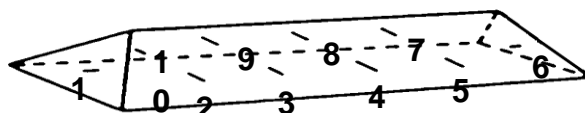


# Homogenization of sample prior to analysis

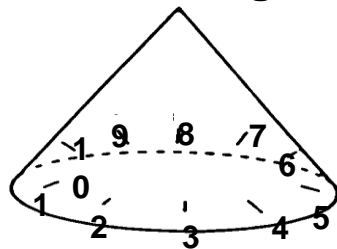
- Feed samples must be milled
- Milled samples thoroughly mixed
- Sub-sampled
- Slurry-extraction



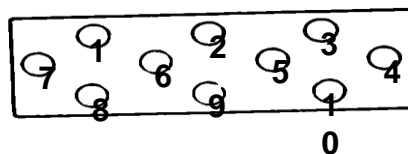
Bag



Wedge Shaped Pile



Conical Pile



Truck



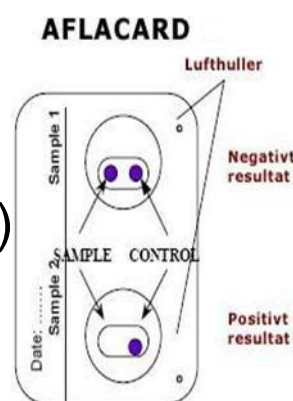
# Antibody technology

- Antibodies can be obtained specific to aflatoxin B<sub>1</sub>
- Antibodies have high specificity
- Antibodies can be used in various formats to provide sensitive and specific test kits
- End-points are usually colour changes
- Cut-off point for test can relate to regulatory limit
- Intensity of colour can be quantified



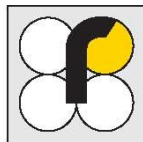
# Typical rapid screening – R Biopharm AFLACARD B1

Extract 50 g sample in blender  
↓ 100 mL methanol/water  
Filter minimum of 10 mL  
↓ using syringe  
Apply 500 uL to card membrane  
↓ allow to pass 5 min  
Apply 100 uL of conjugate  
Apply 100 uL buffer  
Apply 100 uL substrate wait 5 min  
Apply 100uL Stop solution  
↓  
Read result visually (coloured spot)



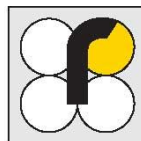
# Comparison of screening options for aflatoxin testing in feed

Product	Brand name	No tests	Analysis time	Reading result	LOD ppb
Test card	AFLACARD B1	2/card	5 min	Visual	2
LFD	RIDA®QUICK Aflatoxin	1/LFD	4-16 min	Visual/ Reader	4,10,20
ELISA	RIDASCREEN ® FAST SC	48 or 6 strips	15 min	Reader	2
IAC	AFLASCAN®	1 per column	20 min	Visual UV	1



# Comparison of screening options for OTA testing in feed

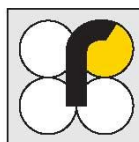
<b>Product</b>	<b>Brand name</b>	<b>No tests</b>	<b>Analysis time</b>	<b>Reading result</b>	<b>LOD ppb</b>
<b>Test card</b>	<b>OCHRACARD</b>	<b>2/card</b>	<b>30 min</b>	<b>Visual</b>	<b>2</b>
<b>ELISA</b>	<b>RIDASCREEN® Ochratoxin A</b>	<b>96 or 12 strips</b>	<b>45 min</b>	<b>reader</b>	<b>2.5</b>
<b>IAC</b>	<b>AFLASCAN®</b>	<b>1 per column</b>	<b>20 min</b>	<b>Visual UV</b>	<b>1</b>





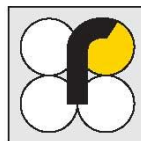
# Comparison of screening options for DON testing in feed

<b>Product</b>	<b>Brand name</b>	<b>No tests</b>	<b>Analysis time</b>	<b>Reading result</b>	<b>LOD ppm</b>
<b>LFD</b>	<b>RIDA®QUICK DON</b>	<b>1/LFD</b>	<b>5 min</b>	<b>Visual</b>	<b>0.5 or 1.25</b>
<b>ELISA</b>	<b>RIDASCREEN® FAST DON SC</b>	<b>48 or 6 strips</b>	<b>8 min</b>	<b>reader</b>	<b>0.07</b>
<b>IAC</b>	<b>DONPREP®</b>	<b>1 per column</b>	<b>20 min</b>	<b>HPLC</b>	<b>1</b>



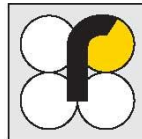
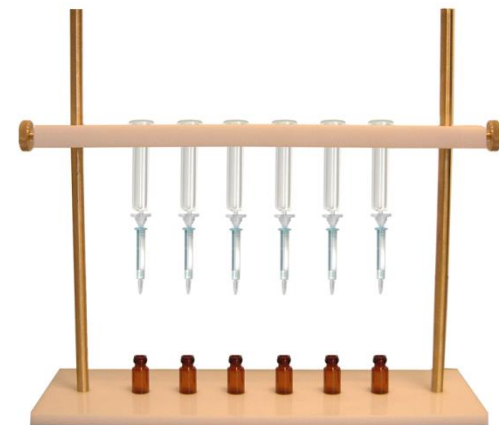
# Comparison of screening options for fumonisin testing in feed

<b>Product</b>	<b>Brand name</b>	<b>No tests</b>	<b>Analysis time</b>	<b>Reading result</b>	<b>LOD ppm</b>
<b>LFD</b>	<b>RIDA®QUICK Fumonisin</b>	<b>1/LFD</b>	<b>5 min</b>	<b>Visual/reader</b>	<b>0.8-4.0</b>
<b>ELISA</b>	<b>RIDASCREEN FAST Fumonisin</b>	<b>48 or 6 strips</b>	<b>15 min</b>	<b>reader</b>	<b>0.2-6.0</b>
<b>IAC</b>	<b>FUMONIPREP</b>	<b>1 per column</b>	<b>20 min</b>	<b>HPLC</b>	<b>-</b>



# Instrumental analysis – R-Biopharm Aflatest®

- Extract 50 g sample in blender
- Filter or centrifuge
- Apply to immunoaffinity column
- Wash column
- Elute from column
- Carry out HPLC analysis
- Separation of aflatoxins and quantification by fluorescence



# Conclusions

- Aflatoxin contamination - major concern for poultry
- Some feed components are 'high risk' and **MUST** be controlled
- Simple screening tests available
- Sampling critical to achieve meaningful result

# Thank you



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