Rapid methods for chemical contaminants in feed: industrial needs and scientific perspectives

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Industrial needs; Nutreco / BU Hendrix

- Headoffice in The Netherlands
- Listed at NYSE Euronext stock exchange A'dam
- Animal-nutrition with 9 Business-units
- Aqua-culture with 4 Business-units
- Over 120 production and processing plants in 30 countries
- Multinational workforce of approximately 10,000 employees
- Revenue in 2010 of EUR 4.9 billion

- Animal nutrition ➔ Business-unit Hendrix
  - Total annual volume 2.5 million tonnes compound feed
  - Top player on the Benelux, market share of 12%
  - Ten compound feed plants in the Benelux and Germany
  - Workforce of approximately 650 employees

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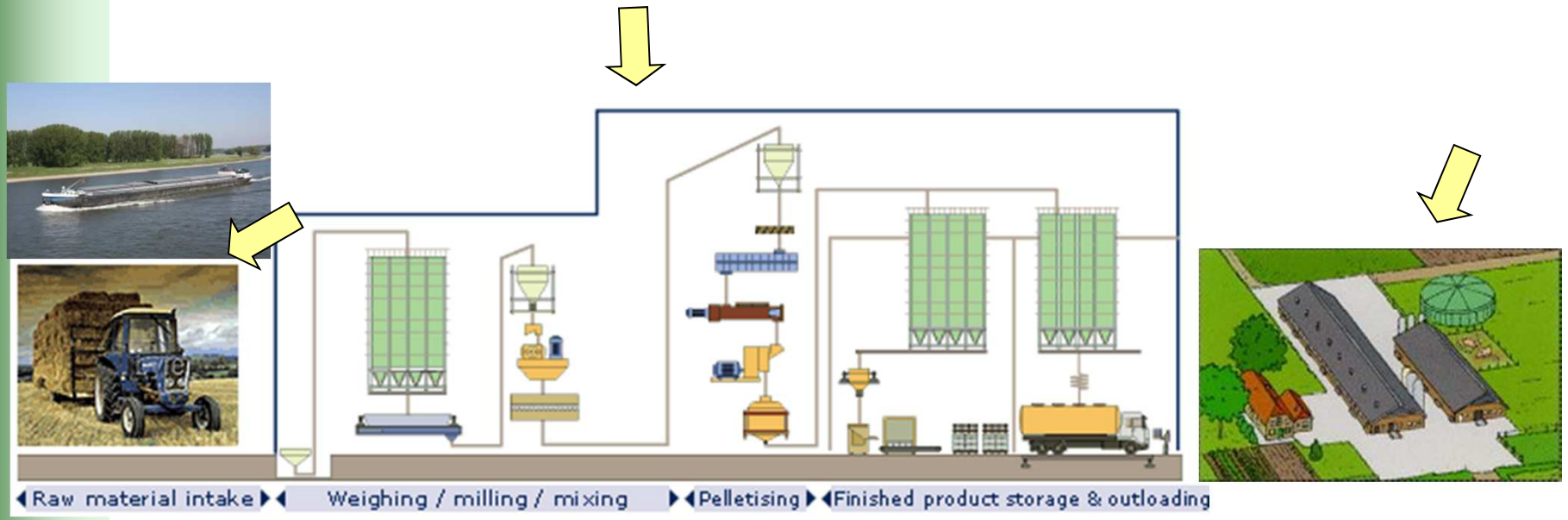
Compound feed production



Examples of industrial needs:

- Mycotoxins
- Coccidiostats

Mycotoxins (supply)

- Riskproducts; grains, processed grainproducts
  - Risk management on supplier/orgin
  - Dedicated feed-mills; can we steer supply?
  - Status depending on harvest and storage conditions
  - In product different mycotoxins can be present with different effects on different animals

1. Supply; from farmer/supplier to feed mill
   - Time of transport (ig North France)
   - Lot's of trucks and ships coming at feed-mills
   - Limited lab-facility
   - Education operators
   - Limited storage

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Mycotoxins (Farmer)

2. Farmers

- Professional partners
- Development: concentrated feeds
- Own crop (dry)
- Purchase wet products (ig Wheatstarch)
- Mixing feed at the farm
- Advise by Feed-producer
- No lab-facility

➢ Need for reliable simple fast multi methods

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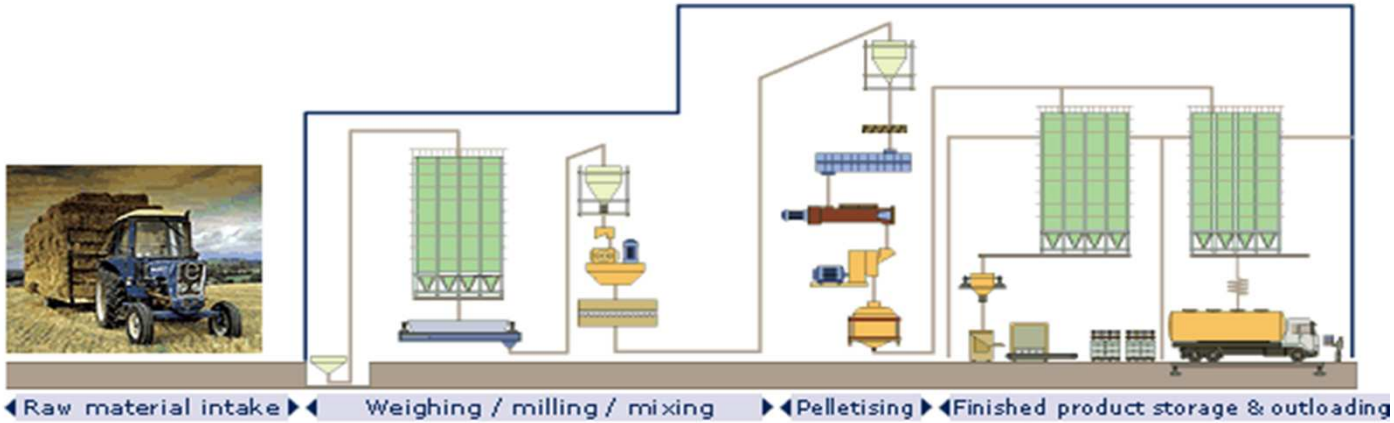
Coccidiostats



- Legislation coc's for non-target-animals
- Part of feed with and part without coc's
- Cross-contamination during feed-production
- Cross-contamination due to feed from farmers
  - Unknown levels of coc's; present or not; concentrationlevel
  - Adjustment recipy might be necessary

- Need for fast, reliable screening methods

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CONffIDENCE in a nutshell

CONtaminants in *f*ood and *f*eed:

Inexpensive DEtectioN

for Control of Exposure

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CONffIDENCE passport

- Collaborative Project: FP7 (European Commission)
- Duration: May 2008 – April 2012
- 16 partners from 10 countries, representing universities, research institutes, industry and SMEs
- Budget: 7.5 Mio €
- Coordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)
- Industrial feed partner: Nutreco

The target contaminants for feed

- Mycotoxins
- Coccidiostats
- Antibiotics: tetracyclines
- Alkaloids:
  - Ergot alkaloids
  - Pyrrolizidine alkaloids
  - Tropane alkaloids
- Heavy metals speciation: inorganic arsenic, methyl mercury in *fish feed*
- POPs in *fish feed*
  - Dioxin-like PCBs
  - Brominated flame retardants

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Results - Example 1: Mycotoxins

- Work coordinated by ISPA, Bari (IT):
  Angelo Visconti / Veronica Lattanzio

Mycotoxins: products and compounds

WHEAT

OAT

Target toxins: DON, ZEA, T-2 and HT-2 toxins

MAIZE

CORN GLUTEN FEED

Target toxins: DON, ZEA, FB_1, FB_2, T-2 and HT-2 toxins

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Mycotoxins: extraction procedures

wheat, oat

(DON, ZEA, T2/HT2)

Ground sample (10g)

↓

Methanol /water 80/20, 50 mL

High speed blending, 2min

↓

Extract dilution

↓

Dipstick analysis

3 min

maize, maize-feed

(DON, ZEA, T2/HT2, FB_1/FB_2)

Ground sample (10g)

↓

Water (40 mL)

High speed blending, 2min

Add Methanol (60 mL)

High speed blending, 2min

↓

Extract dilution

↓

Dipstick analysis

6 min

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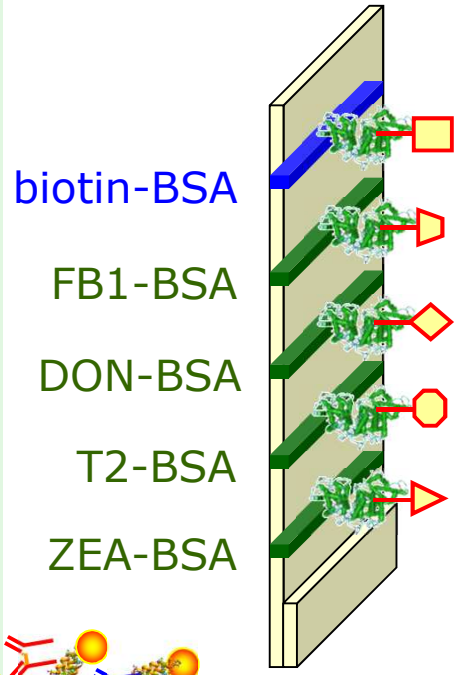
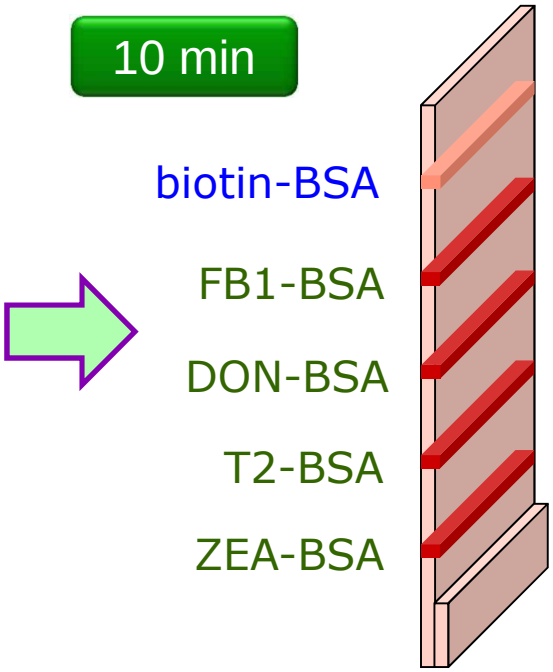
Mycotoxins: prototype multi-dipstick

- *Indirect competitive immunoassay; 10 min incubation at 40 ºC*

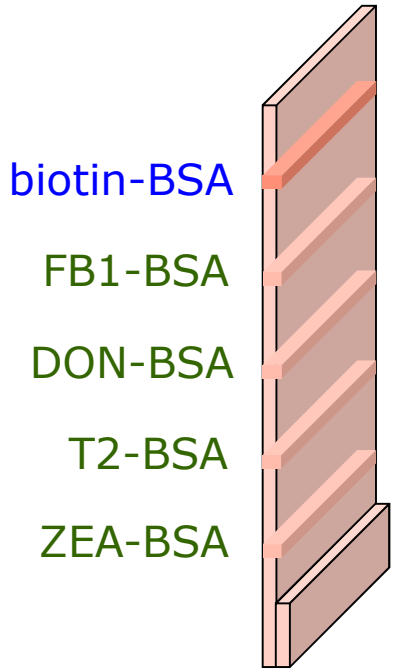


10 min



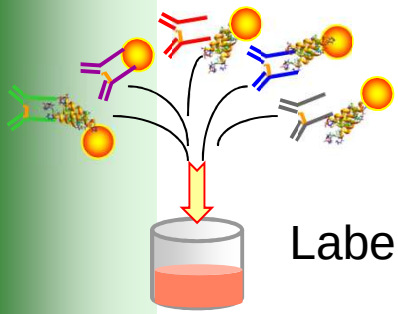
NEGATIVE sample

Test Lines darker than CTRL line



POSITIVE sample

Test Lines lighter than CTRL line



Labelled antibodies

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Mycotoxins: procedure for maize feed

Total analysis time: 30 min

- ✓ Add water; 2 min blending
- ✓ Add methanol; 2 min blending

Dilution and analysis

Incubation at 40 C, 10 min
Migration, 10 min

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Mycotoxins detection: dipstick reader

- Multiplex dipstick reader instrument in development to give a numerical result
- This allows the use of a “cut-off value”
- This will probably allow to use the method for semi-quantitative purposes

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Mycotoxins: summary

- Screening of the main *Fusarium* toxins at action levels in feed is possible
- Total test time: 30 minutes
- Easy-to use and inexpensive equipment: blender, dipsticks, incubator, reader
- Test can be applied in a small feed mill laboratory (and on-farm after training ?)
- Test kit is under development (Unisensor)

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Results - Example 2: Tetracyclines

- Work coordinated by Fera, York (UK): Sara Stead

Dipstick for tetracyclines in feed

Receptor (TetR) based dipstick for:

oxytetracycline, tetracycline, chlortetracycline, doxycycline

Assay protocol

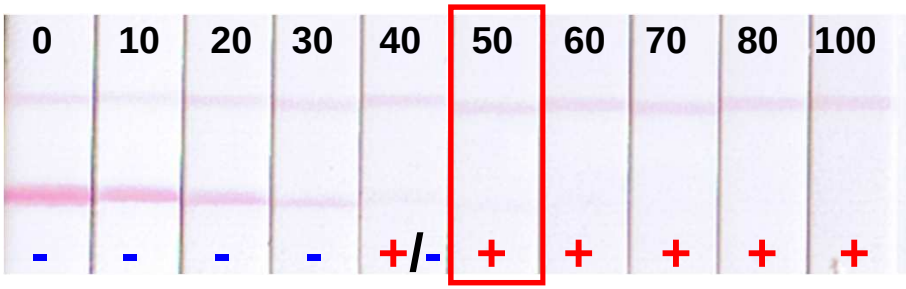
Sample **-** dilution (**10x)** in buffer

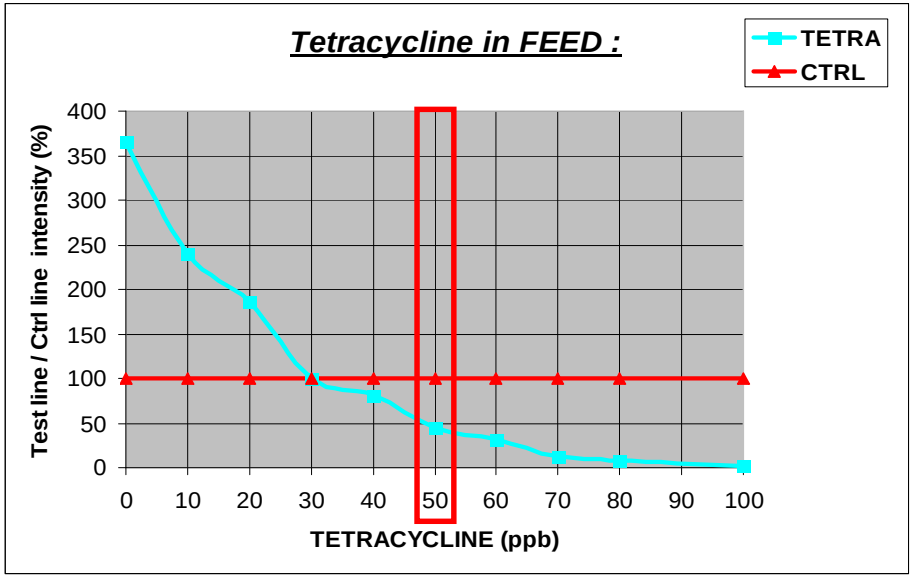
Homogenise (2 min)

Centrifugation (2 min)

200 µl of sample + reagents

Test : **10 min** at **RT**





Tetrasensor

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fera The Food and Environment Research Agency

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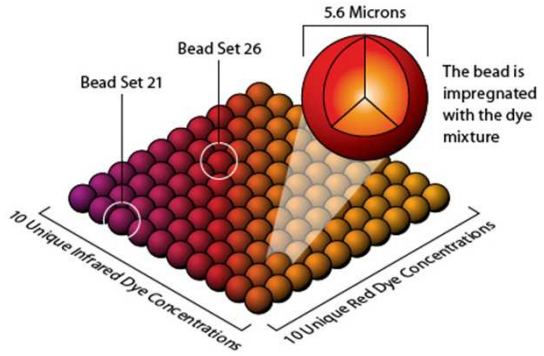
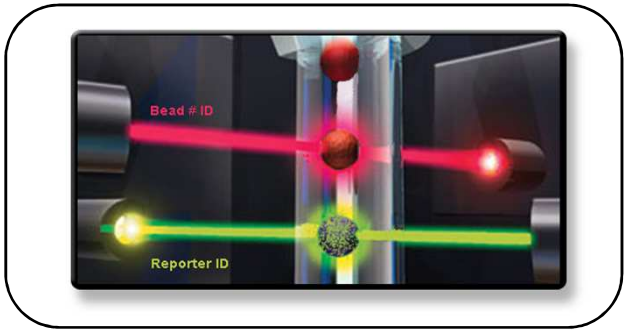
Results - Example 3: Coccidiostats

- Work coordinated by JRC-IRMM, Geel (BE), Ursula Vincent

Coccidiostats: cross-contamination

- Lasalocid A, monensin, salinomycin, narasin, nicarbazin and diclazuril in feed
- Multiplex immunoassay: Flow cytometry
- Very promising results at 1 % and 3 % cross-contamination levels





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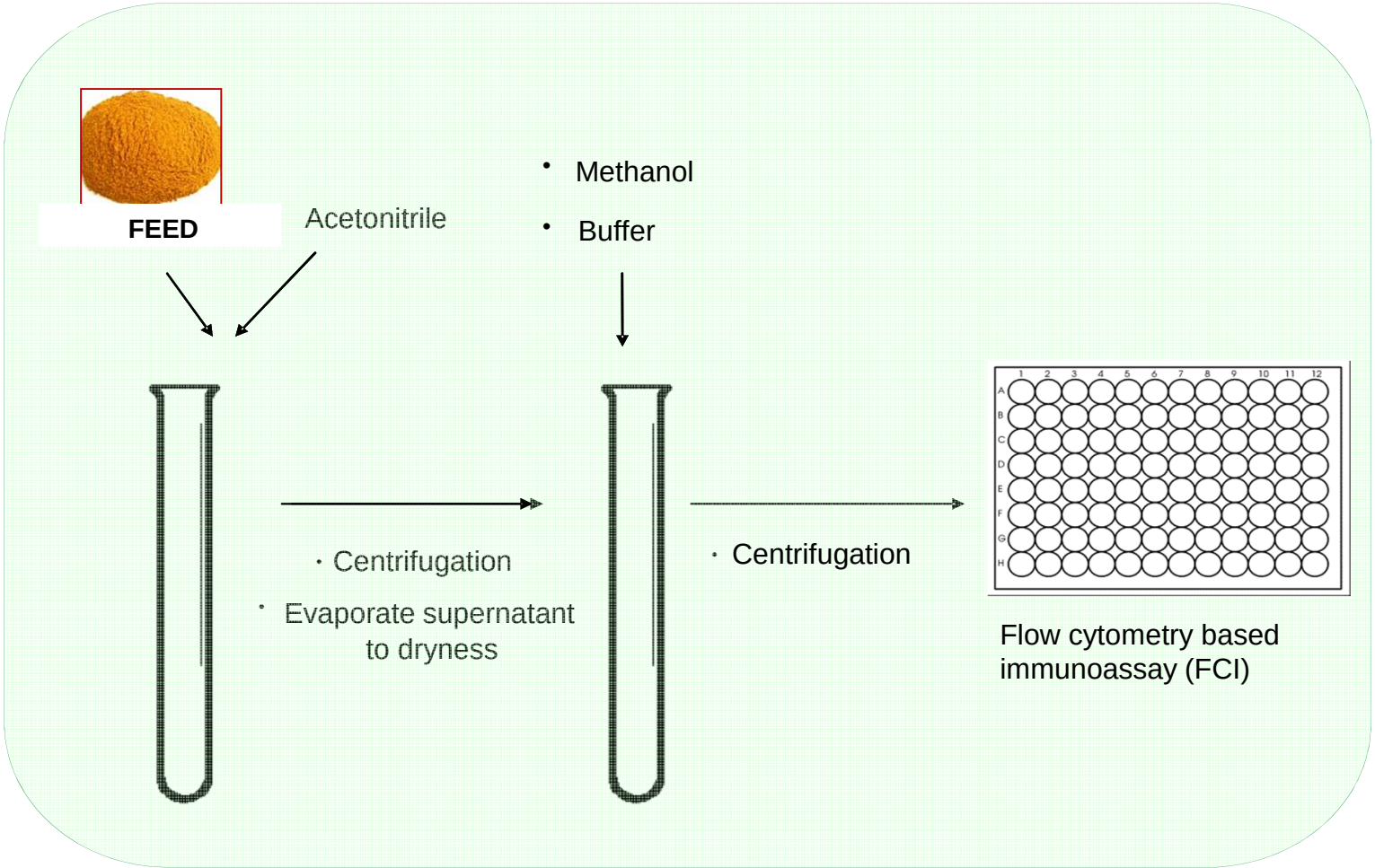
6-Plex assay for coccidiostats in feed



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JRC

irmm Institute for Reference Materials and Measurements

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Coccidiostats: summary

- Screening of 6 main coccidiostats at 1 and 3 % cross-contamination level seems possible
- Multi-screening of 40 samples per day per analyst
- Probably suitable for semi-quantitative purposes
- Equipment is commercially available
- Test could be applied in medium-size feed mill laboratories after training of the technicians
- Test kit not (yet) under preparation

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Other tests

- Alkaloids in cereals, feed and honey: multiplex dipsticks for ergot, tropane and pyrrolizidine alkaloids; work coordinated by RIKILT (NL), Hans van Egmond
- Promising results for major representatives of these emerging toxin classes

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More information

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