



**Experiences, good and bad, in the use of rapid methods:**

**- allergens, mycotoxins & microorganisms -**

**Charlotta Engdahl Axelsson, Eurofins Food & Agro AB**



# Eurofins all over the world



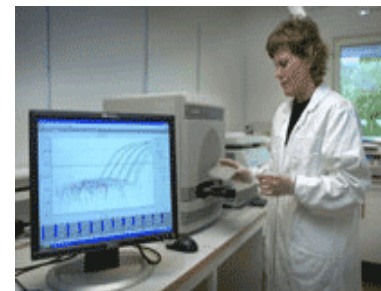
## Introduction

- Example of rapid methods
- Acceptance



## Experiences

- DNA based methods
- Immunobased methods
- NIT



# What is a rapid method?

Depends on the perspective and is different for different areas

**Microbiology:** Alternative to traditional standard methods which enables a more rapid result

**Chemistry:** ≈Alternative - usually meaning screening methods that require little investments



---

## Examples of rapid technologies used for allergens, mycotoxins and microorganisms:

- Immuno based methods
- DNA based methods
- Cultural methods alternative to standard methods
- NIT
- LC MS MS

## Food analysis - differences between areas



Area	“Official” standard	Acceptance of alternative methods
Microorganisms	ISO/NMKL	OK for official control and according to 2073/2005/EC if validated (ISO 16140 or similar)
Allergens	No EN methods standard exist. There are examples of standards in eg. Germany and Japan.	Proprietary ELISA methods are commonly used.
Mycotoxins (Fusarium-, ochra- and aflatoxins)	EN standards exist	LC MC MC can be used for official control (performance criteria is used). ELISA is widely used for screening of grains etc.

PCR as a tool for measuring microorganisms in food instead of standard methods: Discussions started in mid 90:es.....

Now PCR is commonly used for detection of pathogens, eg:

- Salmonella
- Listeria
- Yersinia
- Vibrio
- Campylobacter
- STEC/EHEC

More methods are coming or already existing.....

## Microorganisms:

- Benefits during outbreaks
- Good selectivity
- Very rapid results compared to traditional methods
- Other benefits than speed



## Benefits during outbreak (1)

An outbreak of EHEC (E. coli O157:H7) in Sweden 2002 involving 40 cases (12 received HUS)

A lot of food samples were analysed in order to trace the outbreak



Almost impossible to use the standard culture-IMS based method due to the extensive work with all confirmations of more or less suspected colonies growing on the plates

PCR rapidly picked up the infected sample and the source was successfully traced

## Benefits during outbreak (2)

Outbreak of *Salmonella cubana* 2003

49 swine herds were infected

Another 28 herds had been feed  
contaminated feed

At the same time as:

- new legislation
- an AFNOR certificate was issued  
(validated according to ISO 16140)
- accreditation of the method

A lots of pig carcasses were saved  
from destruction



---

## Evaluation 2002:

Fifty-seven samples representing 5 food matrices and environmental swabs were inoculated with 1-10 cfu *L. monocytogenes* per gram in combination with 600-2000 cfu of *L.innocua* per 25 g

NMKL 136 (3-5 d): Detection of *L. monocytogens* was not possible

PCR (2d): No false false negative results

## Evaluation 2009 new more rapid version of PCR system

Fifty food samples representing 8 matrices were spiked with 3 cfu of *Listeria monocytogenes* per gram in combination with 400 of *Listeria innocua* per 25 g

Number of false negatives:

NMKL 136 (3-5 d)

Rapid culture based alt. (2d): 4 samples

PCR (1d) : 1 sample

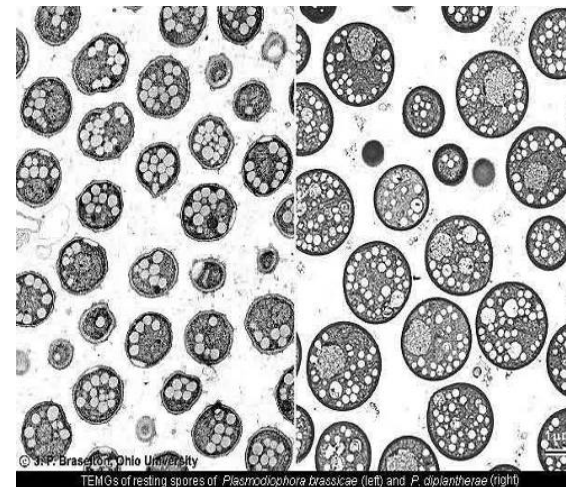
No false positives



## PCR – Rapid compared to traditional methods (*Plasmodiophora brassicae*)

Plasmodiophora is an Oomycetes

- *Plasmodiophora brassicae* is an obligate endoparasite having resting spores
- Taxonomically they are included in the Protists (eucaryote).



PCR – Rapid compared to traditional methods  
Example: Analysis of *Plasmodiophora brassicae*

If low numbers of resting spores are present in soil Brassica crops can be infected.

Risk for infection is related to the quantitative number



Much more rapid than traditional bio-assays !

Traditional analysis takes 6-8 weeks !!

By using a quantitative real-time PCR assay a result be completed within 4h!

This means that the soil can be controlled prior sowing in order to culture a non-susceptible crop if the soil is infested



- Specificity
- Repeatability
- Linearity
- Limit of detection
- Accuracy

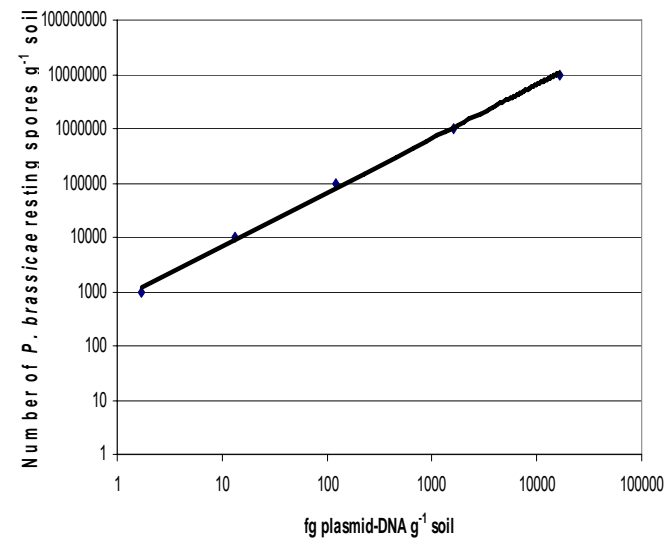


Fig.: Correlation between the concentration of target DNA and nr of spores

Constructed and validated in cooperation with Swedish University of Agricultural Sciences

### **Microorganisms:**

- Matrix effects and construction of unsuitable controls
- Example of not well adapted software for PCR kits
- Lack of correlation to standard methods – a challenge

### **Matrix effects and construction of appropriate controls**

An early version of a PCR method developed for feed and feed ingredients were not performing sufficiently well:

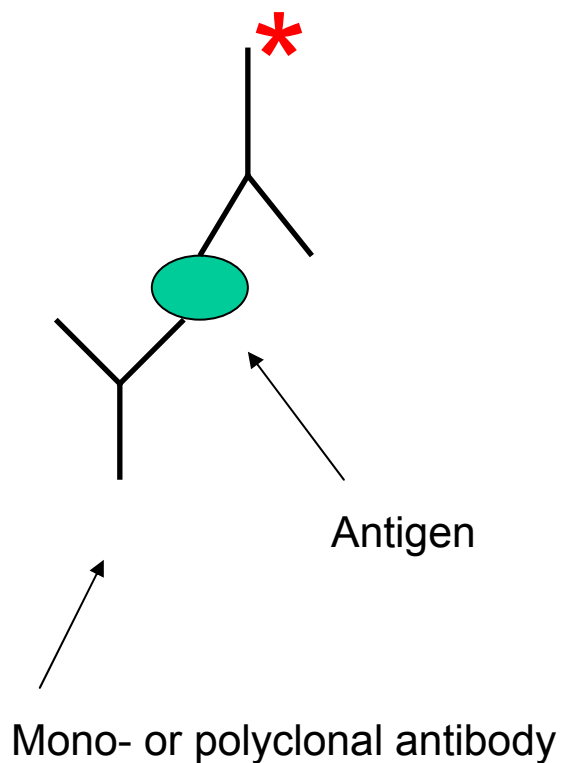
- Sensitivity and specificity of the PCR enzyme (Tth)
- The probability of detecting Salmonella in rape seed when spiking with low levels of Salmonella was only half of the probability when the NMKL method was used (n=50). (Other matrices OK or better.)
- The internal control for the PCR system was too high to track minor inhibitions

- Sometimes lack of correlation to standard methods:  
Difficult for customer to know if the result require an action
- Legal aspects versus riskassesment?
- How validation data looks like is essential (and the performance at the lab of course)
- The formal approval of the method – with or without confirmation



### Kit based systems for pathogen detection

- Software for automatic interpretation of PCR/DNA curves from manufactures have not always interpreted data correctly
- Careful evaluation of positive results



## Principle:

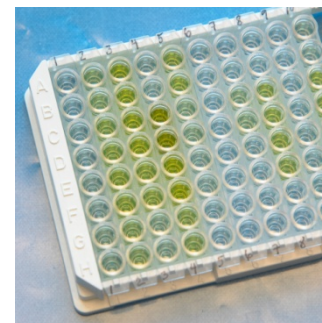
The antigen (allergen) is recognised by an antibody linked with an enzyme.

Thereafter the enzyme converts a substrate to a chromogenic/fluorogenic substance

## Main variants of ELISA:

Sandwich ELISA

Competitive ELISA



## **Good:**

- Rapid and "simple" to perform
- Sensitive, measure proteins
- Potentially dangerous foods can be traced

## **Bad experiences using kit**

- Cross-reactions (specificity)
- Relation to the "real value"
- Different kits use different kit calibrants
- A high variation between subsamples - small part of samples is extracted
- The degree of signal is affected by processing (and food matrix)
- Variation between batches



## Analysis of caseine

Wheat- and ryeflour	3-4 ppm
Eggyolkpowder	50
Minced meat (raw)	3-4
Chicken (raw)	7
Fish, cod (raw)	7
Fish, tunafish (raw)	13
-	
Milk	ca 10000



### Relation between extracted value and the value in the sample

#### **Validation of Tropomyosin in 2004:**

Different species within Crustacean (shrimps etc) were evaluated.

Results refers to Tropomyosin/total protein in extracted solution

Only 3-7% of all proteins are extracted !

#### **Reporting??**

LoD solution corresponds to 100-200  $\mu\text{g}$  Crustacean /g of sample



## The result depends on choice of calibrant

Kit A: soja beans, kit B: soja meal

Product	Dilution	Soyproteins (ppm)	
		Kit A (ppm)	Kit B (ppm)
Soja protein isolate	10000	<1	2,3
Chocolate milk (UHT)	1000	<1	4,4
Soja concentrate	1000	<1	4,5
Soja milk	47000	1,8	4,9
Soja beans	50000	2,5	7,2

## Analysis of gluten in cereal based baby food: FAPAS round 2737 (ca 130 lab)

Sample	Kit	Resultats ppm (mean)
A	R-Biopharm	60
	Tepnel	34
	Neogen	90
B	R-Biopharm	261
	Tepnel	146
	Neogen	316

Due to:

- different antibodies
- extraction solutions
- reference materials

Results depends on the kit used

Limits are settled in regulation 41/2009/EC without specifying method or sampling procedure (only indirectly recommending antibody)

High variation between subsamples may occur despite through mixing – only a small amount of sample is extracted



Kits are better validated prior to release

There exists a new harmonised validation protocol from AOAC giving guidance on how to conduct a validation study (Abbott et al. 2009)

Reference materials are agreed upon/developed

Confirmatory methods are necessary eg. LC MS  
MS

## Evaluation in 1991 (Fusarium toxins):

- T2: Cross-reactions to HT-2, potential as a screening tool
- Zearalenone: No false positives
- DON: LoD to high (500 ppb)



ELISA methods are used today as a screening tool for grain (T2, DON, zearalenone)

Generally good agreement when results are checked against LC MS MS

Validation data from kitmanufacturers? Specificity?

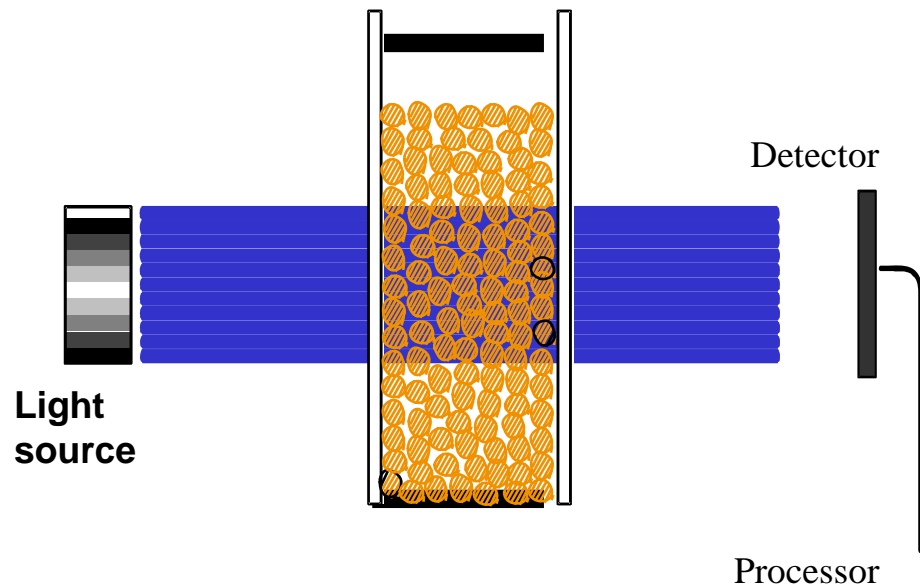
Performance around limits is important

# NIT – Near Infrared Transmittance

Has been developed for analysis of ergosterol and screening of mycotoxins in grain by Thomas Börjesson, Svenska lantmännen.

The objective is to have a very rapid "at site" screening tool for evaluation of the hygienic quality of grain as well as an analysis with a good precision





Transmittance - pathlength  
18 mm for small grains

Wavelength range - normally 850 -  
1050 nm

No sample preparation

Analysis time 1.5 minutes

## Transmittance (NIT), Foss

NIT analysis can be used for screening grains for containing potentially high levels of mycotoxins

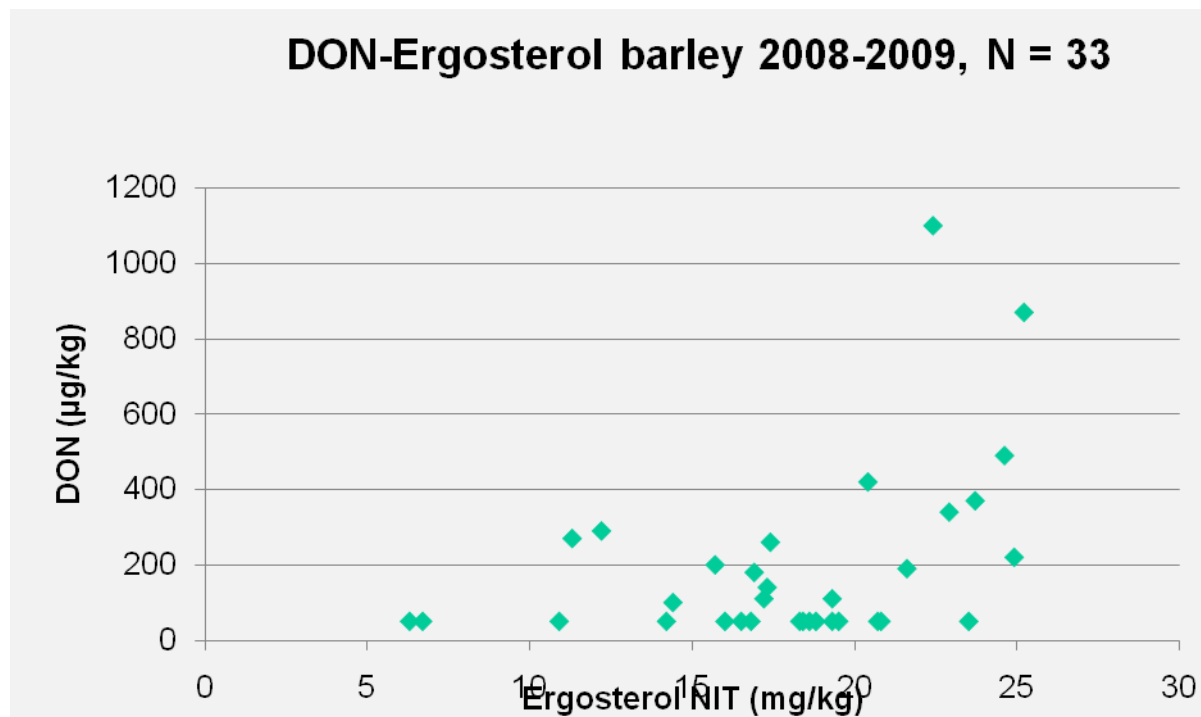


Fig: Thomas Börjesson

Extensive calibrations between ergosterol as measured by HPLC and between NIT instruments have been performed

Once calibration has been established a good precision in the analysis is obtained

Repeatability: CV <5%

Reproducibility: CV <10%



Special thanks too:



**Prof. Anders Jonsson, Swedish University of  
Agricultural Sciences**

**Dr. Ann-Charlotte Wallenhammar, Agricultural  
Society, Sweden**

**Dr. Thomas Börjesson, Lantmännen Sweden**

**thomas.borjesson@lantmannen.com**

**MSc Charlotta Almqvist, Eurofins Sweden**

**Dr. Rikard Westbom, Eurofins Sweden**





**Thank you for your attention!**

Charlotta Engdahl Axelsson

[charlottaengdahlaxelsson@eurofins.se](mailto:charlottaengdahlaxelsson@eurofins.se)

