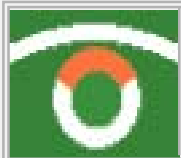


## Finding the traces:

# Qualitative and quantitative detection of animal DNA in food and feed

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## Control compliance of legal regulations

- ban of MBM from feedstuff
- codex regulations for food composition

### Universal

Mammals  
Poultry

### Species-specific

Cattle  
Horse  
Swine  
Chicken  
Turkey  
  
Mouse



## **Sensitivity**

mitochondrial DNA target sequences  
high copy number

## **Specificity**

Race-spanning !

different degrees of conservation

## **Degraded material**

short target sequences (61 - 81bp)  
competitive IPC

## **Standardized DNA-extraction**

weighed amount tissue extracted  
as reference magnitude

# Specificity

between species

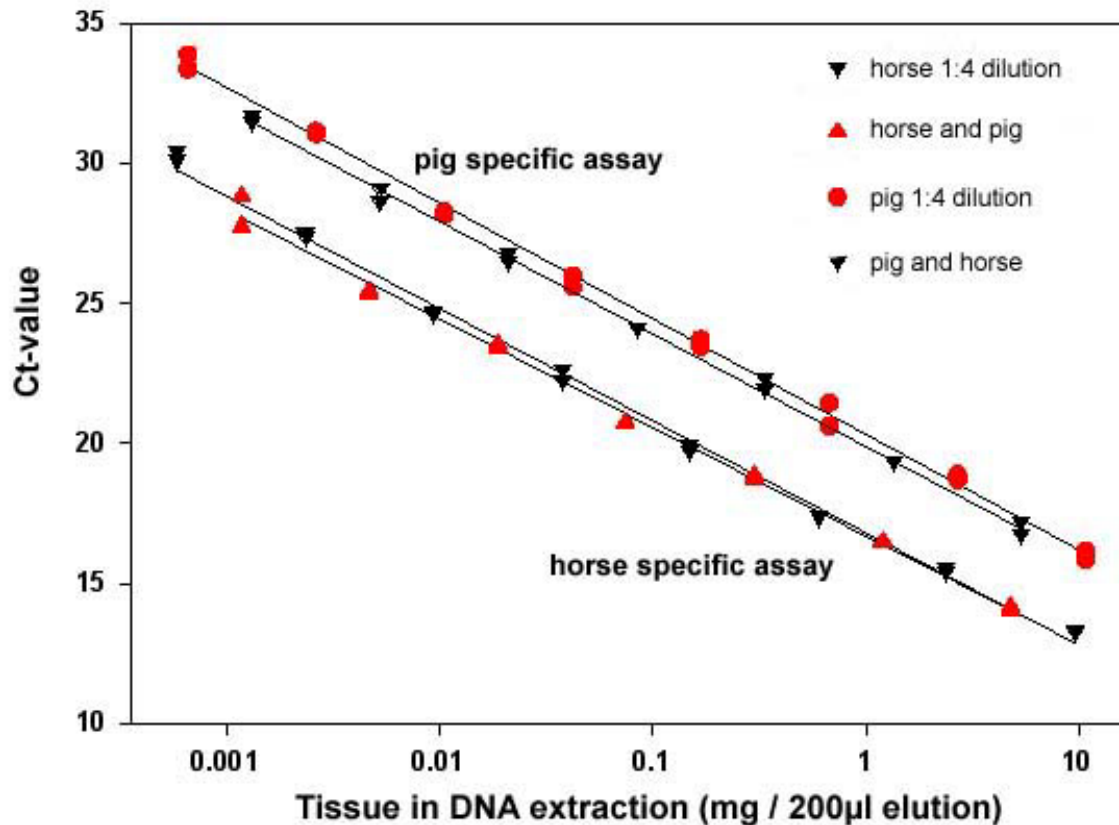
within species

- ✓ Alignments of sequence data
- ✓ Tests / confirmation on a variety of species / individuals
- ✓ Precision of quantification in presence of non-target DNA

# Specificity

between species

within species



statistical verification  
of regression interaction  
using GLM procedures

all experiments with  
non-significant results  
and hence no effect of  
non-target DNA on  
quantification.

# Specificity

between species

within species

## 60 cattle

(Simmental, Murbodner, Braunvieh cattle  
Tuxer, Limousin, Ennstaler Bergscheck)



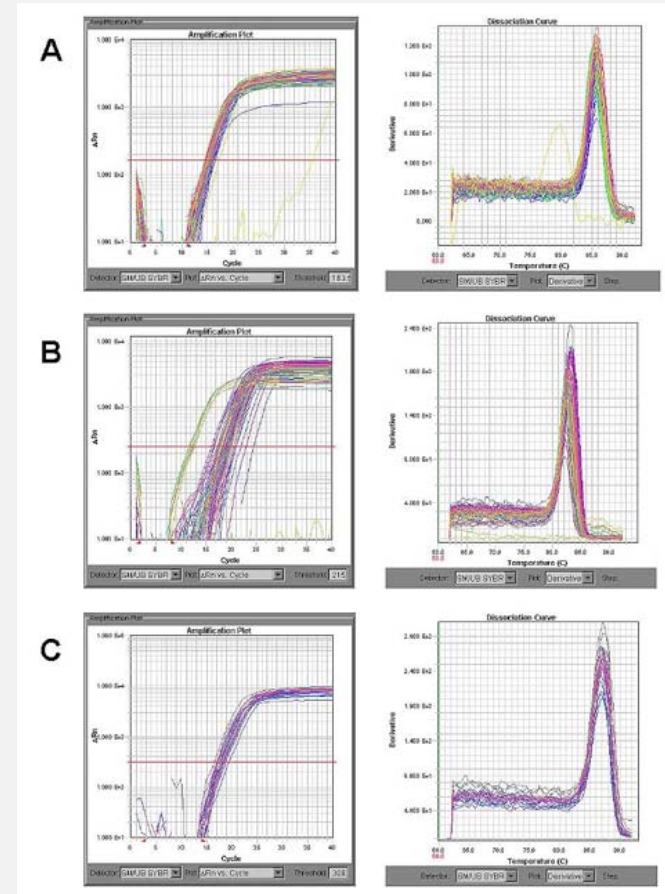
## 60 pigs

(Edelswine, Landrace, Duroc,  
Mangaliza, Pietrain, Hampshire, Turopolje)



## 24 horses

(Trakhener, Austrian Warmblood, Quarter Horse,  
Andalusian, Holsteiner, Welsh Pony, Paint, Appalosa,  
Shetlandpony, Connemara, Oldenburger, Pinto  
Wurttemberger, New Forest Pony, Shire Horse,  
Missouri Foxtrotter, Saddlebred, Berber, Tinker,  
Traber, Shagya Araber, Fjordpony)



# Sensitivity

fresh tissue DNA

degraded DNA:  
heat and pressure treated tissue

- Repeated DNA extractions from various amounts of tissue
- Repeated serial dilutions
- Repeated amplifications

## Sensitivity

fresh tissue DNA

0.1  $\mu\text{g}$  material  
in 200  $\mu\text{l}$  elution buffer

equiv. to 0.5  $\text{ng}/\mu\text{l}$

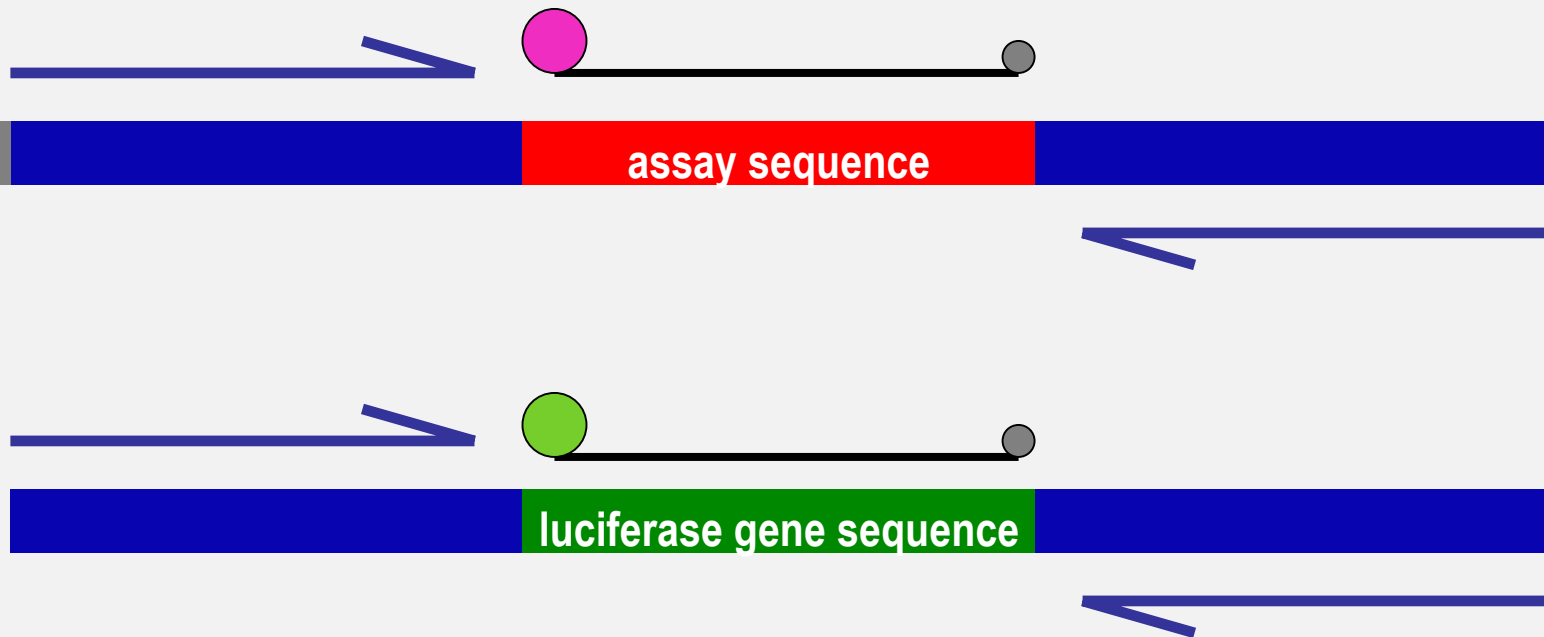
degraded DNA:  
heat and pressure treated tissue

0.1  $\text{mg}$  material  
in 200  $\mu\text{l}$  elution buffer

equiv. to 0.5  $\mu\text{g}/\mu\text{l}$

# Quantification of MBM in feedstuff

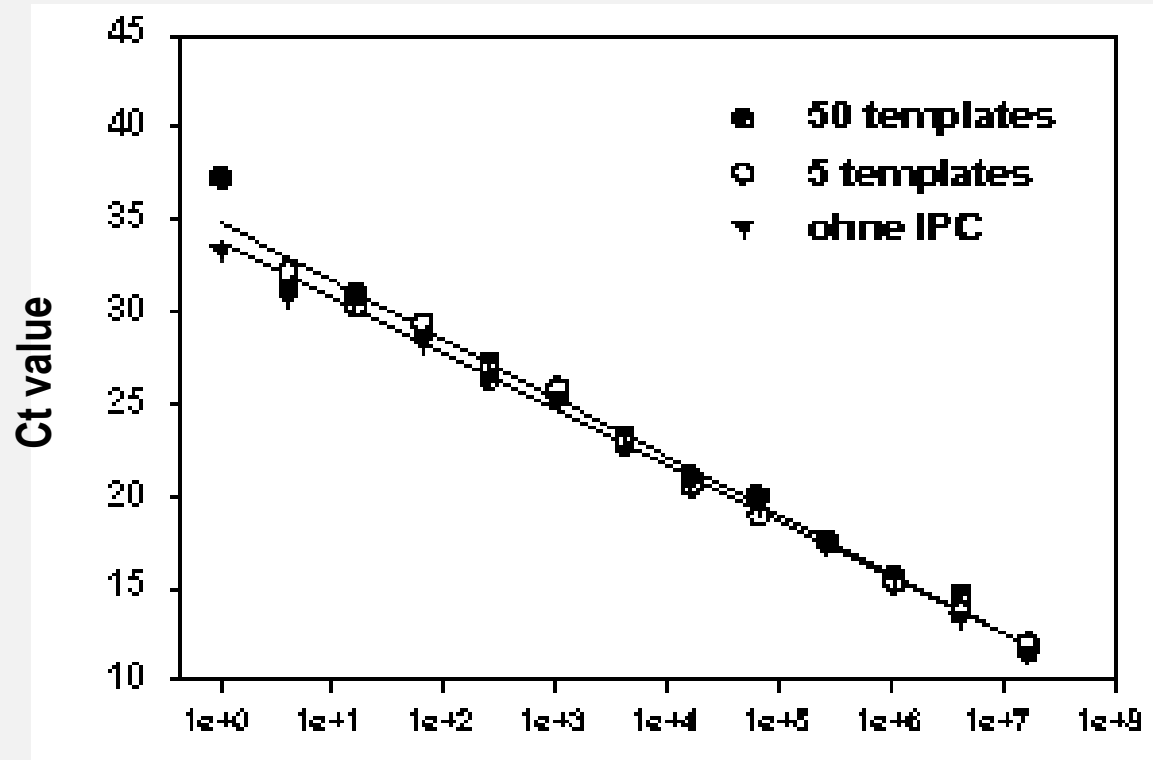
## Internal positive amplification control : IPC



synthetic template with sequence of assay primers and luciferase gene probe

# Internal positive amplification control : IPC

## Effects on target assay performance



Standards: comparison of material for standard DNA preparation

**Autoclaved fresh muscle  
tissue from species**

**MBM**

### **Advantages**

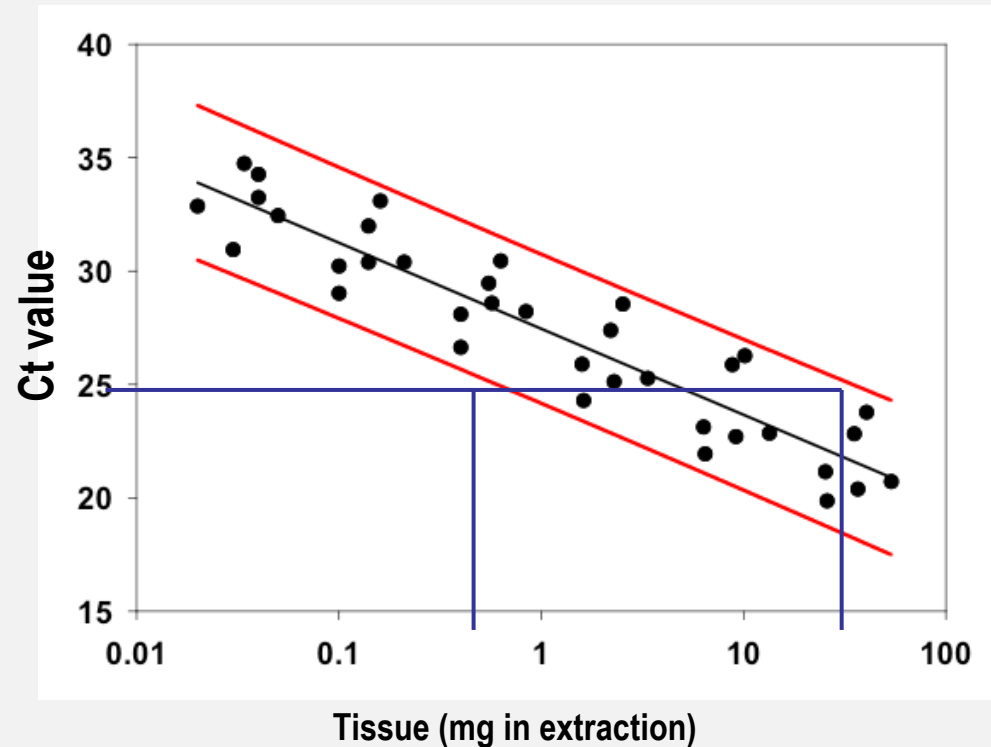
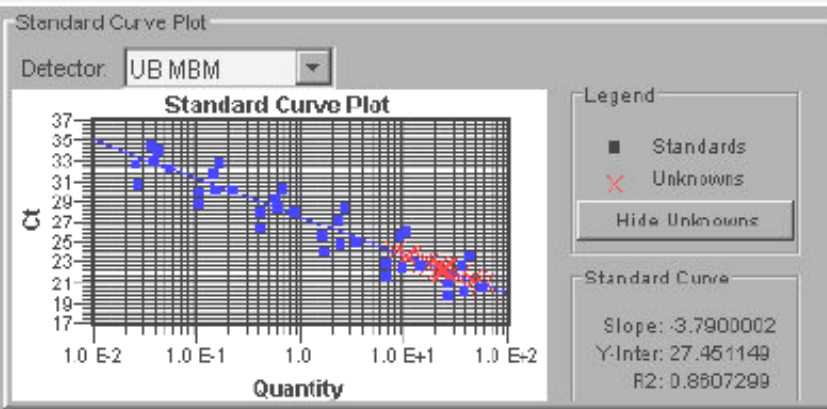
- defined portion of species-specific material
- intermediate mitochondria density
- controlled material processing

### **Advantages**

- "genuine" material

## Standards: comparison of sources I

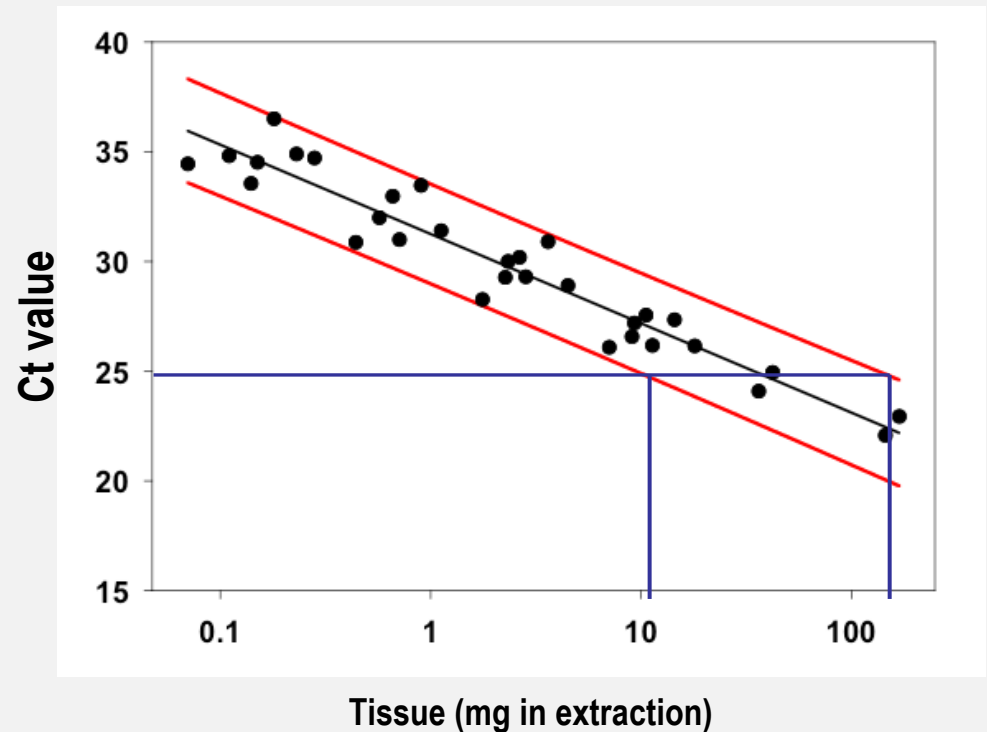
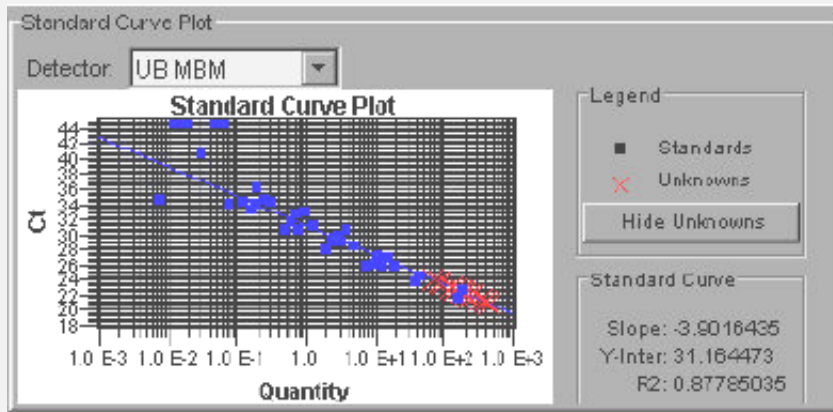
# MBM charge quantification using autoclaved fresh muscle tissue



2 independent DNA extractions of each, cattle, horse and pig

## Standards: comparison of sources II

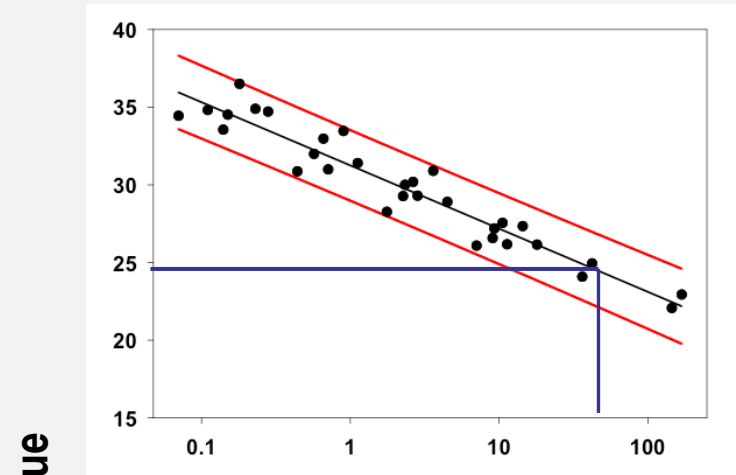
# MBM charges quantification using MBM standards



7 independent DNA extractions of 2 MBM lots

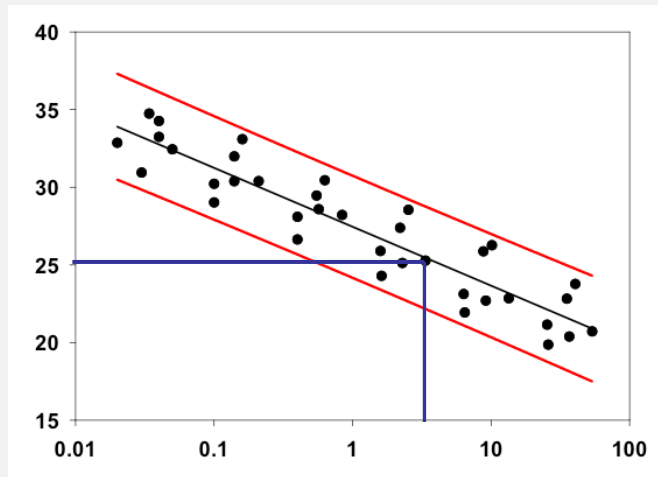
# Standards: comparison of sources III

## MBM recovery rates in 0.1% feedstuff admixture using different standards



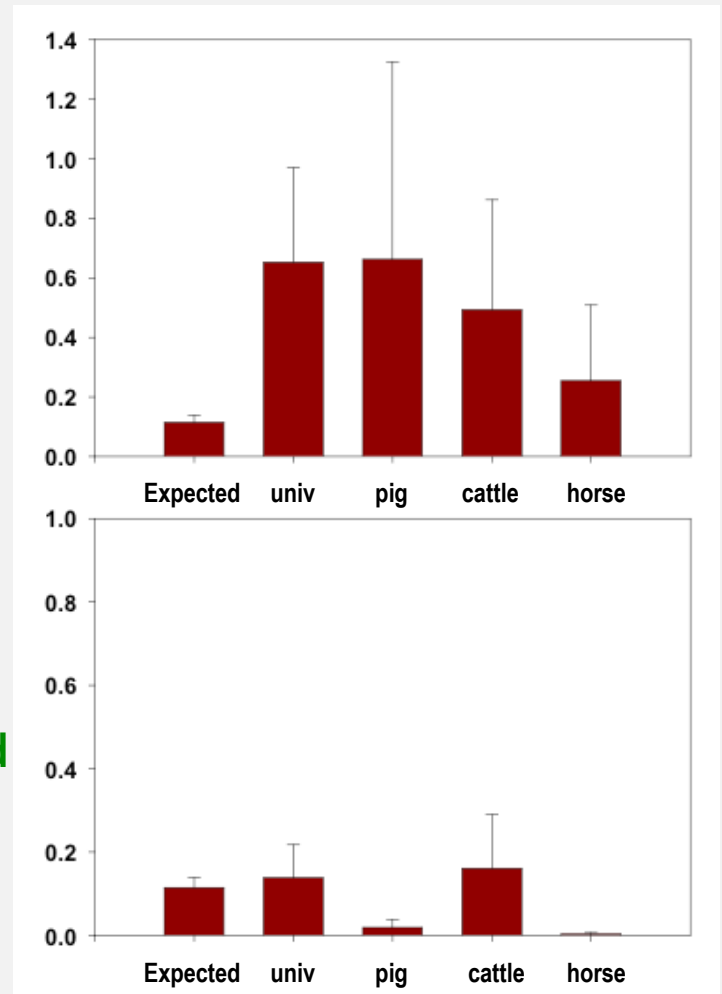
Ct value

MBM



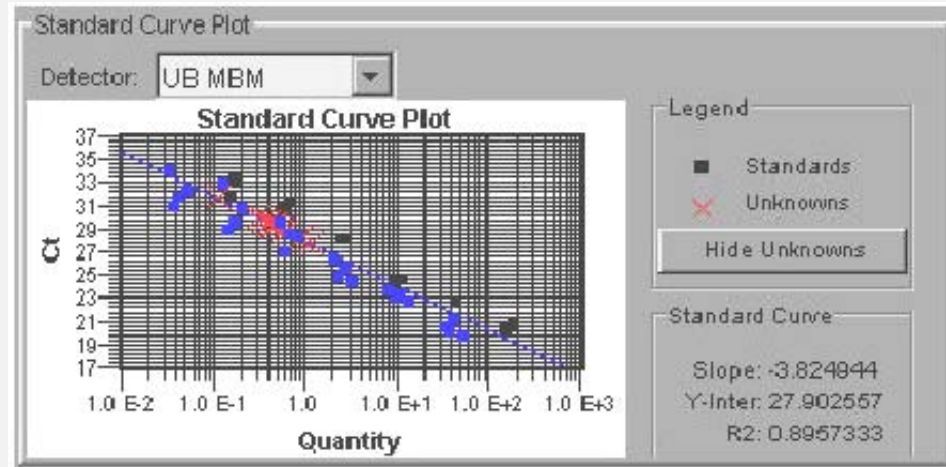
Tissue (mg in extraction)

autoclaved

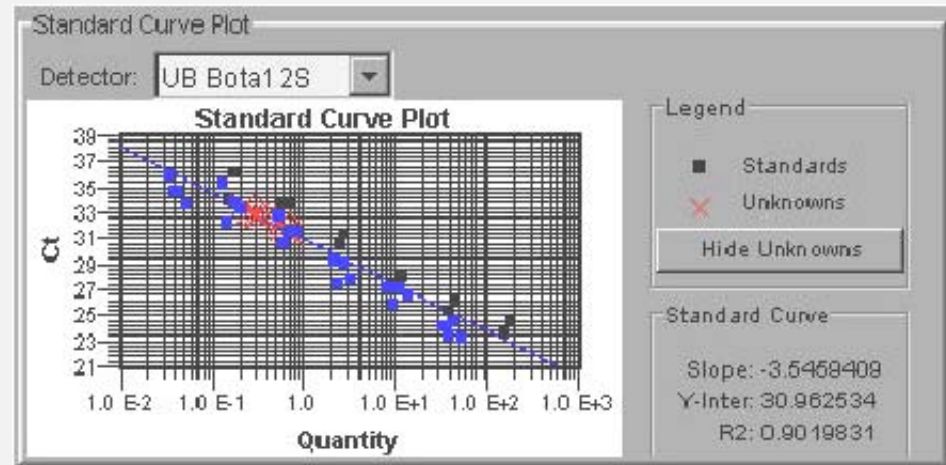


# Quantification of 2% MBM content in feedstuff admixture

Universal mammalian assay



Cattle specific assay



**Constraints and assumptions -**  
**good reasons for a confidence interval approach for judgement**

**DNA extraction yield variation**

**minor variation in PCR efficiency not recognized in IPC amplification**

**inhomogeneity of MBM in feedstuff samples**

**inhomogeneity of MBM composition itself**

**inhomogeneity of mitochondrial DNA density in tissues included to MBM**

**degree of target DNA decay, differences due to treatment**

**“carrier-DNA” effects of nontarget DNA in reactions**



## **Summary and conclusions**

**Set of assays for universal and species-specific quantitative detection of DNA**

**Competitive internal positive control**

**Quantification sensitivity of assays at 0.1% MBM in feedstuff**

**Evaluation of sources for standard-DNA preparation**

**Application of confidence intervals to quantification and reasoning thereof**

**Reliable, fast, cheap**

**More objective than other methods (e.g. microscopy)**

**Potential for automation and routine use**





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