



# Identification of Animal Proteins by Alternative Techniques

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## Looking for a needle in a haystack!

### **Prohibited processed animal protein:**

includes mammalian meat and bonemeal, meat meal, bone meal, hoof meal, horn meal, greaves, poultry meal, poultry offal meal, feather meal

### **Restricted proteins:**

Fishmeal, Blood products, Blood meal (only to be fed to farmed fish), Di-calcium phosphate and tri-calcium phosphate (of animal origin only – not mineral)



## Looking for a needle in a haystack!

Acorn, Alfalfa, Barley, Barley distillers, Barley molasses, Beet (Ext + pulp), Bean sprouts, Biscuit meal, Biscuit waste, Black syrup, Blood meal, Butter milk, Cadenza, Cake mix, Coffee, Citrus, Cocoa bean (kernel and shell), Concentrated grass, Copra, Corn cobs, Cooked pulse mix, Corn gluten, Cotton seed, Crisps, Denatured milk, Fat filled whey, Feather meal, Feather meal (hydrolysed), Fish meal (white and oily), Field beans Gelatine, Gluten, Indian rice bran, Lignin, Limestone, Linseed, Linseed (Ext + flake), Liquid lysine, Locust beans, Lupin, Maize distillers, Maize gluten, Malt (culms + offal), Mango, Milk powder (skimmed + spray dried), Minerals Molasses, Mono - calcium phosphate, Nigerian palm kernel, NI straw, Noodles, Oats, Olive pulp, Palm kernel, Peanut, Peas, Prairie gluten, Prairie meal, Rape (meal and seed), Rice, Salt, Sesame seed, Shea-nut, Shrimp meal, Sodium bicarbonate, Soda seed, Sopralin, Soya, Soya oil, Soya hulls, Spice, Starch, Straw products, Sugar beet, Sunflower, Tallow, Wheat



**Denatured product**

**Wide range of contaminants**

**Some permitted materials**

**Variable vegetable matrix**

**Low concentration**

**Species detection?**



## Hunts Needle in a Haystack

HOW LONG does it take to find a needle in a haystack? Jim Moran, Washington, D. C., publicity man, recently dropped a needle into a convenient pile of hay, hopped in after it, and began an intensive search for (a) some publicity and (b) the needle. Having found the former, Moran abandoned the needle hunt.

## Approaches

- Classical Microscopy
- Immunoassays
- Near infra-red
- Polymerase Chain Reaction (PCR)
- Mass-spectroscopy

## **Classical Microscopy**

- Only official method within the EU
- Two levels of detection
  - Detection of animal structures
  - Differentiation between terrestrial and fish structures
- EU validated for the detection of 0.1% MBM within a feed matrix
- Excellent screening tool for detection of animal materials



## Classical Microscopy - Limitations

- Limited to detection of materials showing solid structures
- No soft tissue detection (brain, spinal chord etc)
- Limited speciation capabilities
- Operator dependent
- Quantification ????





## Immunoassays – protein detection

- Target species specific heat stable proteins
- Species level detection
- Documented protein targets – Troponin
- Wide range of testing platforms
  - Lateral flow devices
  - ELISA
  - Electrophoresis methods
  - DELFIA

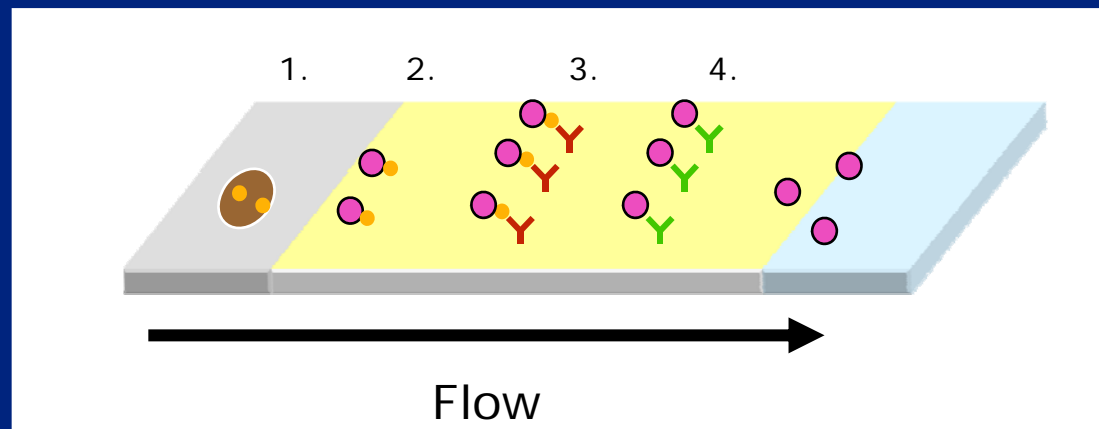




## Immunoassays – protein detection

### Lateral Flow Devices - Dipsticks

- Proteins flow through the absorbent wick
- If positive, a protein-antibody-coloured particle complex is formed
- Complex is fixed and concentrated using a second antibody



## Immunoassays – protein detection

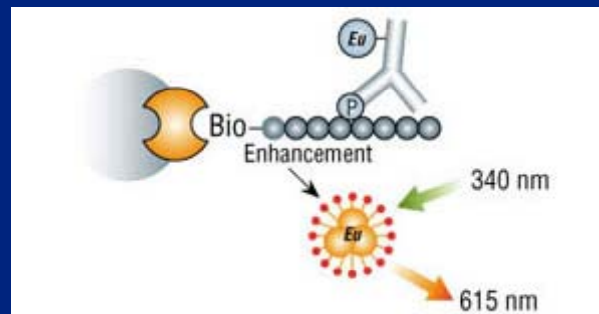
### Lateral Flow Devices - Dipsticks

- Easy to use
  - Very mobile
  - High throughput
  - Cost effective screening
- 
- Limited sensitivity ?
  - Cross reactivity issues
  - Sample matrix issues



## Immunoassays – protein detection

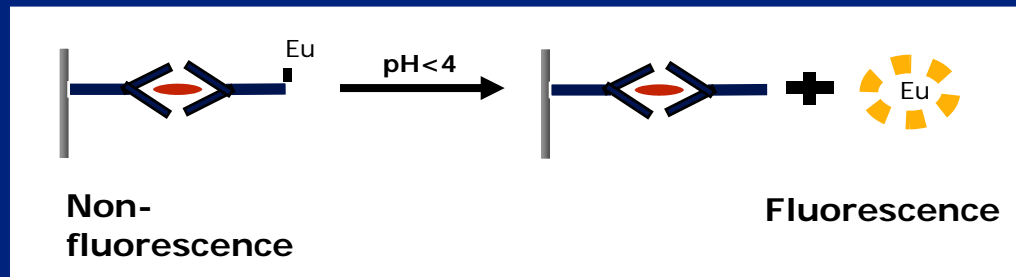
### DELFLIA - Dissociation Enhanced Lanthanide Fluorescent Immuno Assay



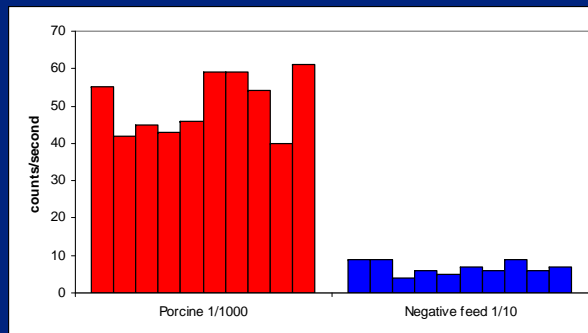
- Variation on the common ELISA
- Potentially greater sensitivity than ELISA
- Lanthanide chelate labels ( $\text{Eu}^{3+}$ ) with unique fluorescent properties
- Dissociation-enhancement

## Immunoassays – protein detection

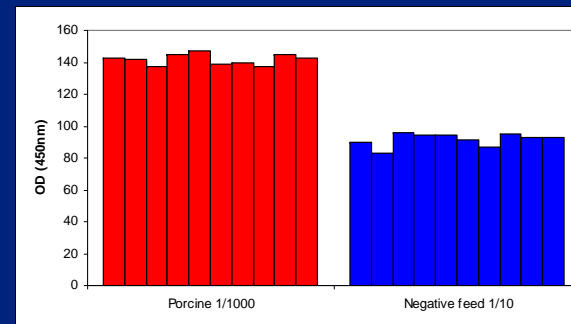
### DELFA - Dissociation Enhanced Lanthanide Fluorescent Immuno Assay



#### DELFA

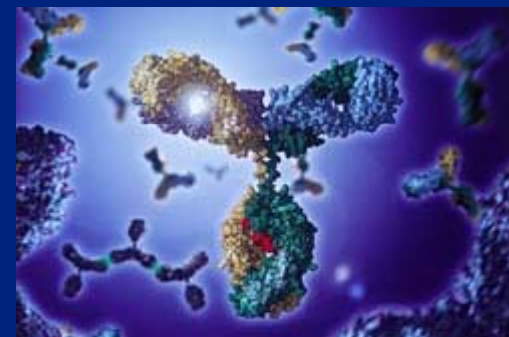


#### ELISA



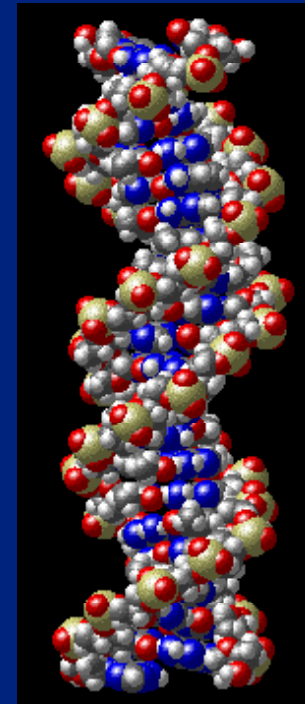
## Immunoassays – protein detection -Limitations

- Performance is processing temperature dependent
- Cross reactions
- Interference by vegetable components
- Dependent on antibody quality and supply
- No quantification



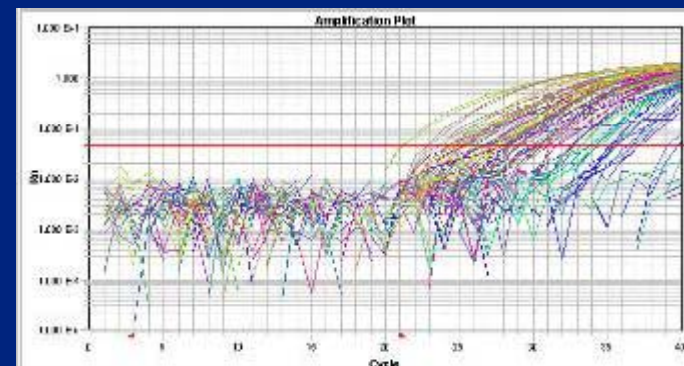
## Polymerase Chain Reaction

- Targets heat stable DNA
- Species level detection (Bovine, Ovine, Porcine, Avian etc)
- Good result in pre-validation
- Standard laboratory technique
- Rapid, sensitive and reproducible results



## Polymerase Chain Reaction - Limitations

- Not EU validated - Not yet
- Detects animal DNA rather than banned material
  - Permitted material – milk powders
  - Contamination at feeding troughs
- Equipment and set up costs?
- No quantification

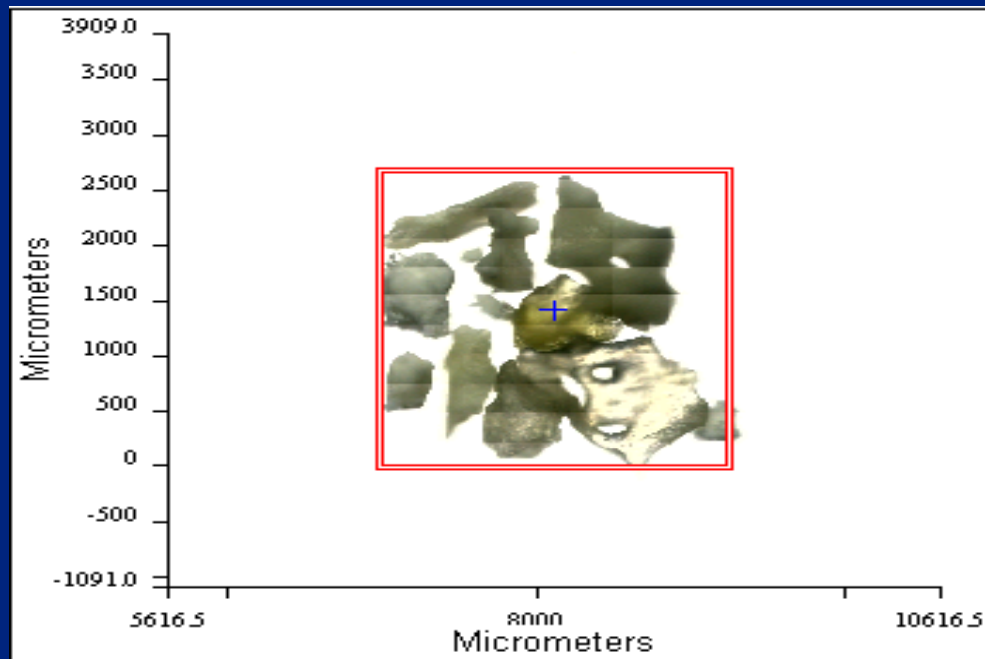




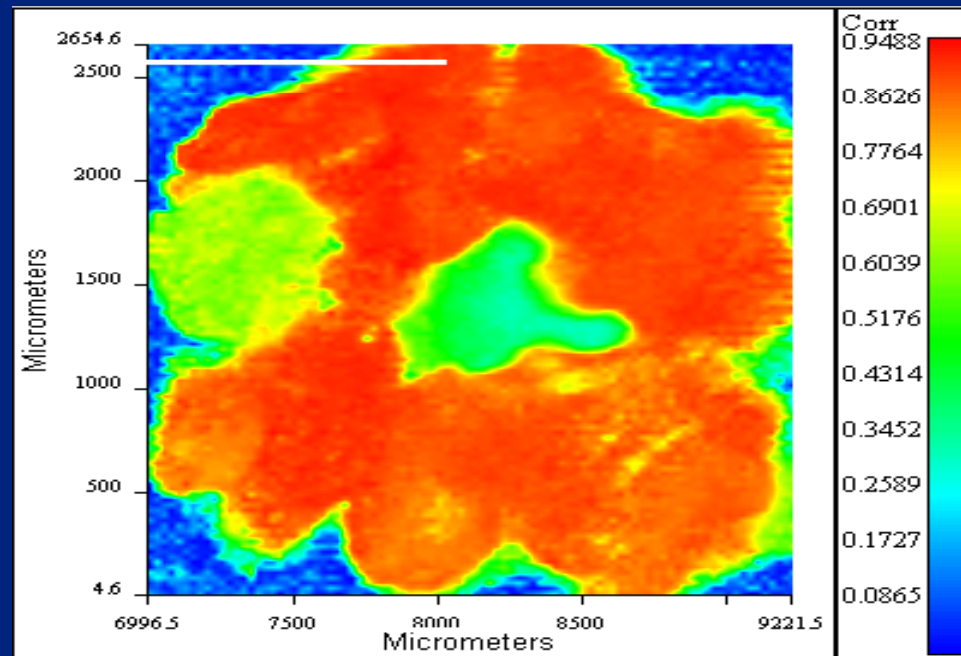
## **Near Infrared Microscopy**

- Reduces the operator dependence from classical microscopy
- Possibly will reduce the operator limitations in CM quantification

## Near Infrared Microscopy



## Near Infrared Microscopy



## Near Infrared Microscopy - Limitations

- Limited to detection of materials showing solid structures
- No soft tissue detection (brain, spinal chord etc)
- Limited speciation capabilities
- ~~Operator dependent~~

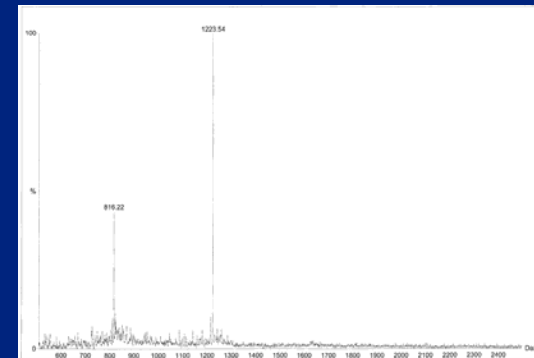
## Mass spectroscopy

- Target species specific heat stable proteins
- Specific extraction of selected proteins
- Detection of selected protein using mass spectroscopy
- Could give:
  - good level of detection
  - low cross reactivity
  - good degree of confidence



## Mass spectrometry - Limitations

- Novel work ?
- Efficient protein extraction is vital
- Requires consistently present heat stable target proteins
- No quantification



## Importance of a multiple test strategy

- Complexity of the task
  - Detection difficulties
  - Qualitative/ quantitative
  - Animal screen / species detection





## Importance of a multiple test strategy

- Match complementary elements of the tests to meet the requirements of an effective feed surveillance programme

Screening tests : Microscopy / PCR / Dipsticks / NIRM?

Animal Structure detection : Microscopy / NIRM

Soft tissue detection : Immunoassay / PCR

Species detection : Immunoassay / PCR / NIRM + PCR

Quantification : Microscopy ??? / NIRM ???



**Thank you**

