Veterinary Laboratories Agency

SAFEEDPAP

Perspectives of Immunological Methods

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Considerations

- Targets
  - Processed to at least 133°C, 3bar pressure
  - Derived from bovine, ovine, porcine, avian
  - Derived from waste carcase material (bone/ muscle/ connective tissues)
  - Diverse range of materials

- Test sample
  - Detection at low concentrations
  - Complex background of vegetable ingredients

What do we need from our immunological test?

- Good test sensitivity across a wide range of test samples
- No false positives
- Good reproducibility
- Easy to apply
- Cost effective

What do we need, in order to come up with an immunological test?

- Antibodies
- Sample handling
- Protein extraction
- Testing platforms
- Validation

Antibodies

- Monoclonal or Polyclonal
- Raised against species specific thermally stable proteins
- Good specificity
- Robust

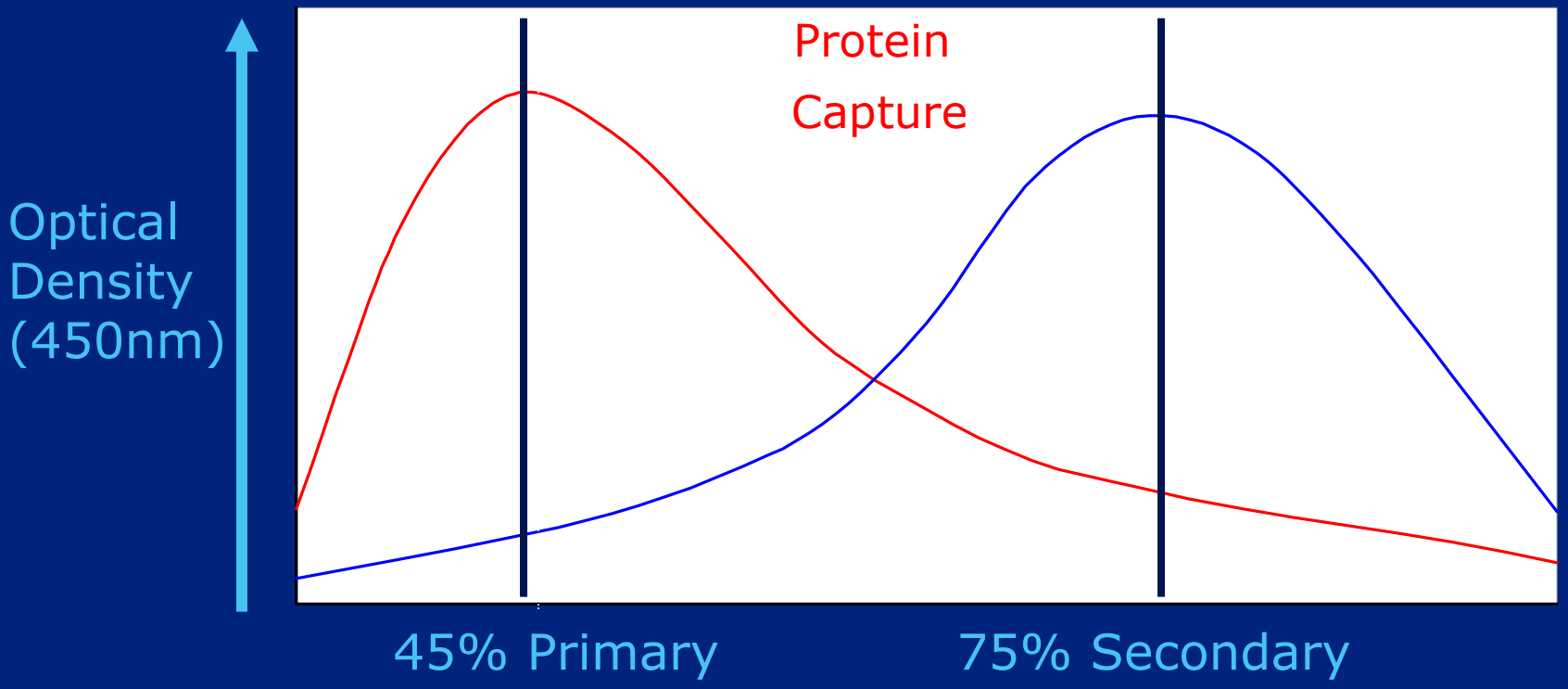
Sample handling

- Pelleted samples / meals
- Large sample sizes
- Liberating proteins from fats

Protein extraction

- Specific extraction of target protein
- Minimising the effect of vegetable components

Protein extraction



Protein extraction

- Specific extraction of target protein
- Minimising the effect of vegetable components
- Minimising the effect of non-specific binding agents / False positives

Protein extraction

- Specific extraction of target protein
- Minimising the effect of vegetable components
- Minimising the effect of non-specific binding agents / False positives
- Overcoming problems with fats and oils

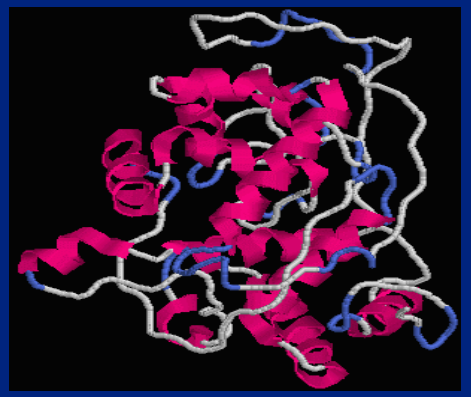
Testing Platforms: ELISA

- Antibodies bind to the extracted/ concentrated protein target protein to a plastic well
- Second antibody bound to an enzyme
- Colour change to indicate positives read on an automated reader

Testing Platforms: ELISA

Benefits of ELISA

- Simple to carry out
- Standard technique
- High throughput
- Reasonably cost effective
- Good supply of standard reagents

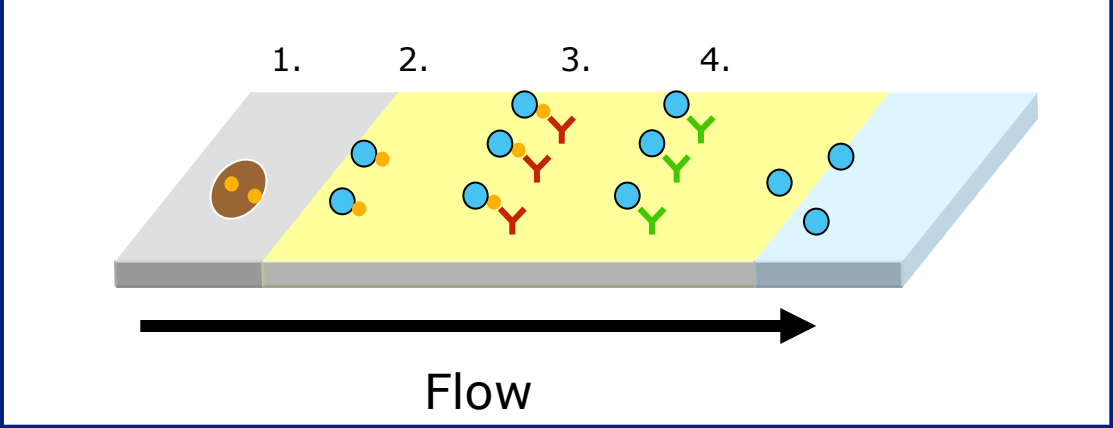


Testing Platforms: ELISA

Drawbacks of ELISA

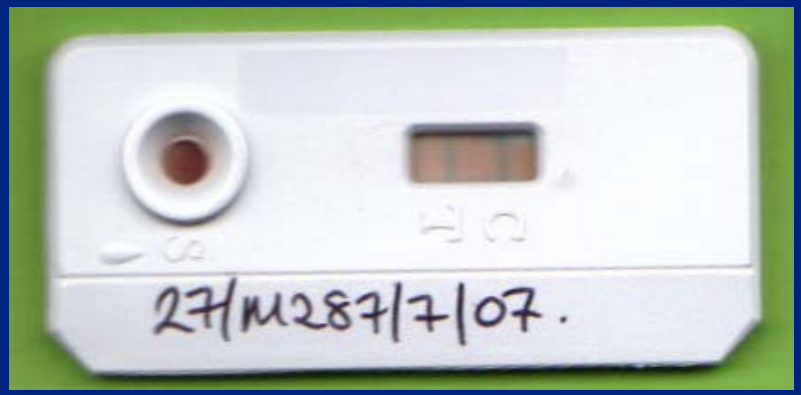
- Temperamental !!!!
  - Standardisation, specificity, matrix effects, abnormal forms, interferences, needs experience
- Requires plate washers and microplate readers.

Testing Platforms: Lateral Flow Devices



1. Protein extract added to absorbent pad and wicked through a reagent zone on the test strip
2. If positive, a protein-antibody–coloured particle complex forms (conjugation pad).
3. The complex is captured by a second antibody fixed to the membrane.
4. Unbound coloured particles continue and are fixed to form a control line.

Testing Platforms: Lateral Flow device



Benefits of LFDs

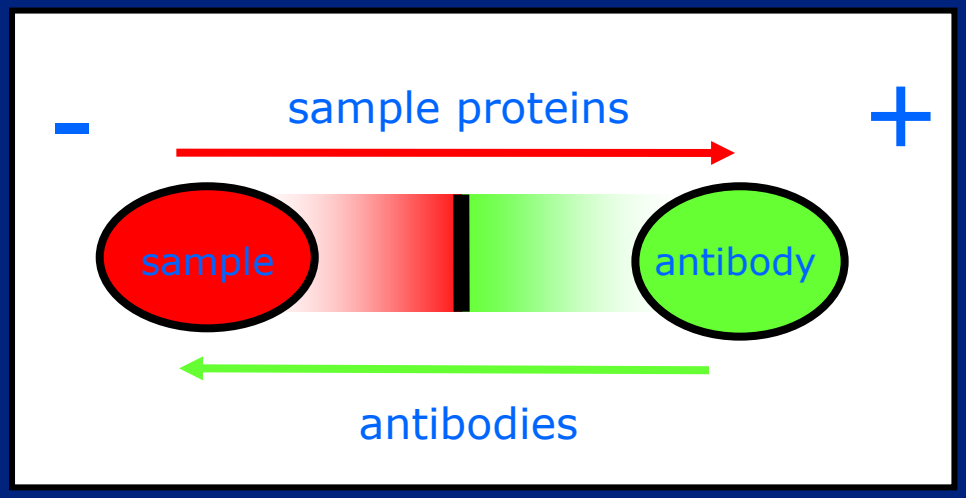
- Simple to carry out
- Quick
- Very mobile – can be used anywhere?
- High throughput
- Reasonably cost effective
- Easily distributed as kits

Testing Platforms: Lateral Flow Devices

Drawbacks of LFDs

- Sensitivity ?
- False positives ?
- Visual interpretation

Testing Platforms: Counter immuno electrophoresis



- Test proteins and antibodies brought together in a gel slide under the influence of an electric current
- If positive material is present the a complex is formed between the two wells

Testing Platforms: CIE

Benefits of CIE

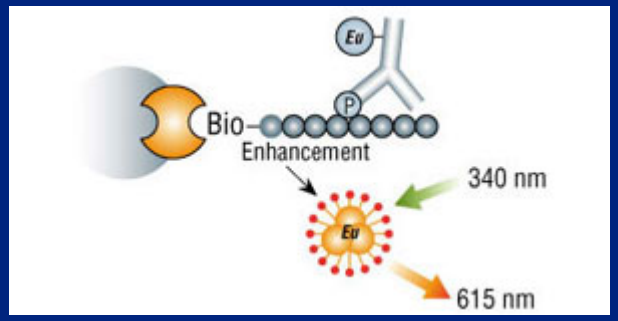
- Reasonably high throughput
- Reasonably cost effective

Drawbacks of CIE

- Slow
- Visible interpretation

Detection Techniques: DELFIA

Dissociation Enhanced Lanthanide Fluorescent Immuno Assay



- Variation on the common ELISA
- Potentially greater sensitivity than ELISA
- Includes:
  - Binding to the surface of a microtitre plate
  - Washing
  - Enhancement

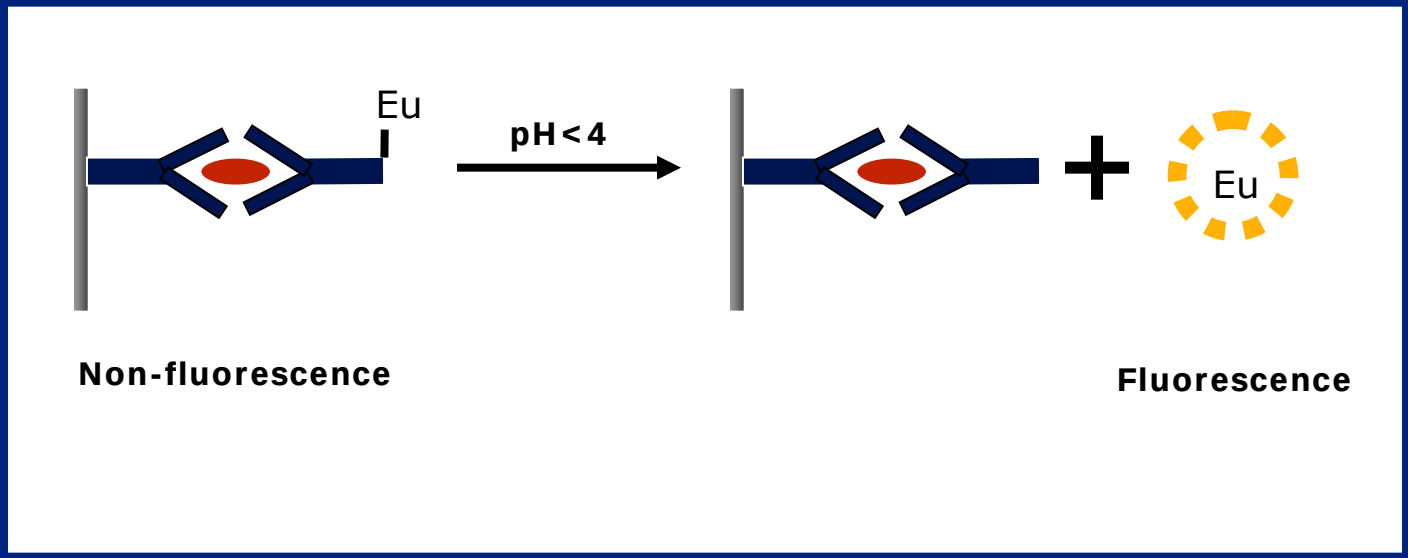
Detection Techniques: DELFIA

Enhancement

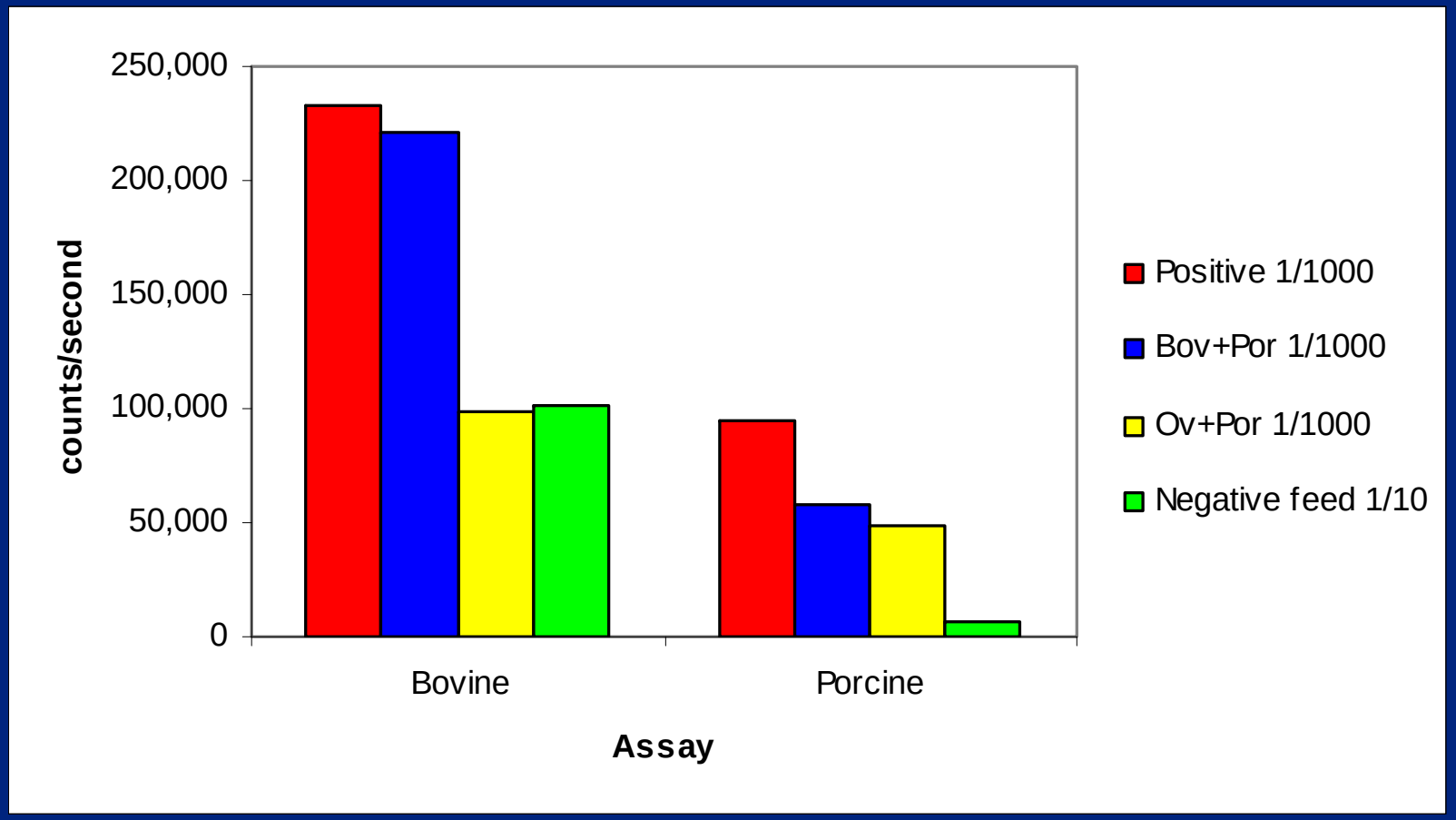
- Lanthanide chelate labels (Eu^{3+}) with unique fluorescent properties.
- Fluorescent lifetime of specific signal is several orders of magnitude longer than non-specific background.
- Large stokes shift ie. difference between excitation and emission wavelengths.
- Narrow emission peak.

Detection Techniques: DELFIA

Dissociation - Enhancement

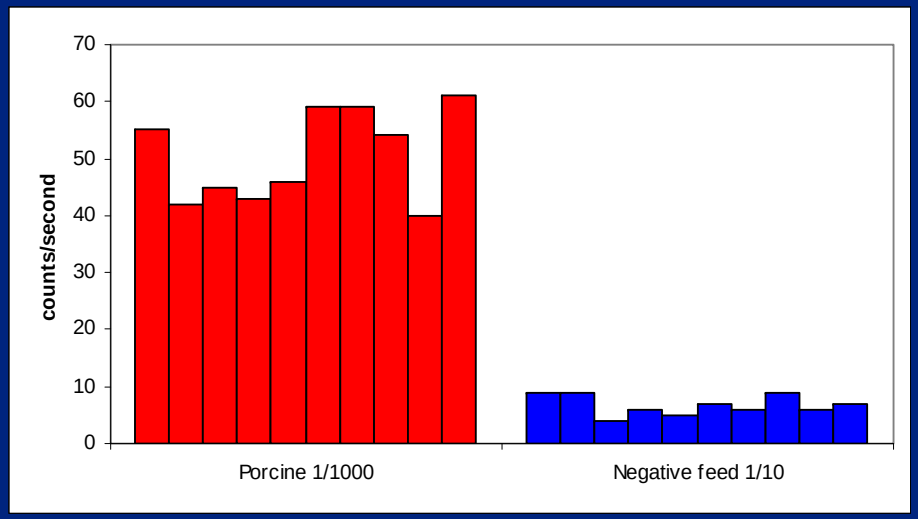


Detection Techniques: DELFIA

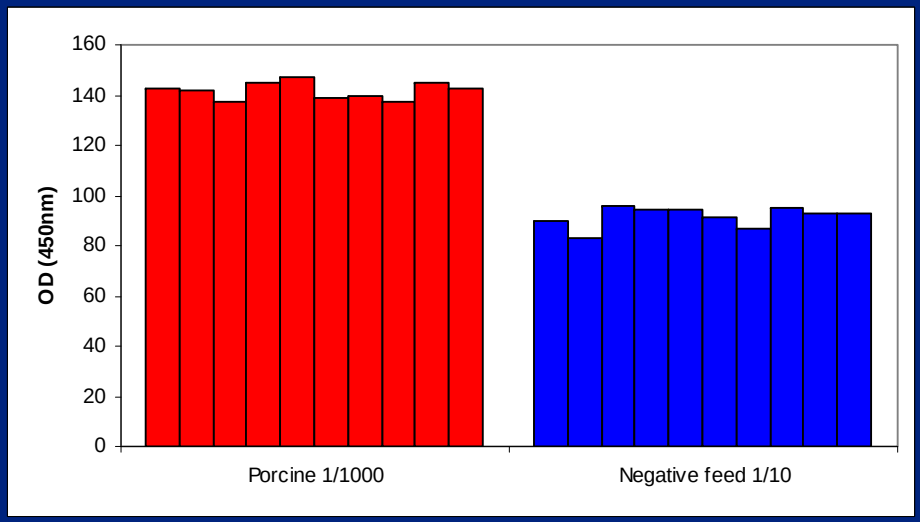


Detection Techniques: DELFIA

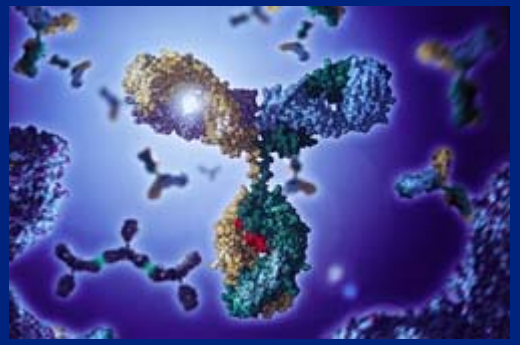
DELFIA



ELISA



Your Immunological Method Is Only As Good As Your Antibodies



Thank you