Enzyme Linked Immunosorbent Assay (ELISA)

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Outline

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• Conclusion
Background

- Plate technique used to detect and quantify proteins, antibodies, hormones, peptides, etc.
- Passive adsorption of antibodies to antigens
- Substrate color change; spectrophotometer or fluorometer

**COATING**
Polystyrene plate is treated with a solution of either antigen or antibody.

**BLOCKING**
An unrelated protein-based solution is used to cover all unbound sites on the plates.

**DETECTION**
Enzyme-conjugated antibody or antigen binds specifically to the target antigen or antibody.

**READ RESULTS**
Substrate is added and the signal produced by the enzyme-substrate reaction is measured.

Laboratory Info:
http://laboratoryinfo.com/elisa/
Types

• 4 different types
  – Direct
  – Indirect
  – Sandwich
  – Competitive
Direct Assay

- Pure antigen coated on plate walls
- Enzyme-linked antibody added to wells; binds to antigen
- Substrate added for color change
- Fast and simple

BosterBio
Indirect Assay

- Pure antigen coated on plate walls
- Sample containing 1° antibody added
- 2° antibody bound to enzyme is added to bind to 1° antibody
- Increased sensitivity


BosterBio
Sandwich Assay

• Capture antibody immobilized on plate walls
• Sample containing antigen binds to capture antibody
• 1° antibody added, binds to antigen, creates a “sandwich”
• 2° antibody linked with enzyme binds to primary antibody
• High sensitivity; two antibodies bind to antigen
• Suitable for complex samples; antigen doesn’t require purification
Competitive Assay

• Purified antigen coated on plate walls
• Sample containing unknown antigen added
• If both bound and free antigens are identical, will compete with each other to bind to the antibody
• Decrease in signal = high antigen concentration

BosterBio
Competitive Assay

![Graph showing absorbance (450 nm) vs. [MT] (μg/ml) with data points and a trend line. The x-axis ranges from 0.0001 to 10, and the y-axis ranges from 0 to 0.3.](https://openi.nlm.nih.gov/detailedresult.php?img=PMC3281953_pone.0031185.g001&req=4)
Applications

• Medical
  – HIV; indirect assay
  – Pregnancy tests; sandwich assay

• Quality control
  – Food industry; sandwich assay
Pregnancy test strips

http://www.macmillanlearning.com/catalog/static/whf/kuby/content/anm/kb07an01.htm
Qualification and application of an ELISA for the determination of Tamm Horsfall Protein (THP) in human urine and its use for screening of Kidney Stone Disease

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Figure 1: Determination of the optimal concentration of WGA for coating onto microtiter plate. A series of diluted WGA from 1 - 100 μg/mL were investigated at a serial diluted urine (1:5, 1:10, 1:20, 1:40, 1:80, 1:100) and non-diluted urine. The anti-THP dilution and secondary HRP conjugate dilution were 1:10,000 and 1:3,000, respectively. The optimum WGA coating concentration was 10 μg/mL for urine diluted at 1:10.
Figure 4: The correlation between ELISA and SDS-PAGE methods in measurement of urinary THP concentrations. The □ symbols represent an individual urine sample with its corresponding THP concentrations as measured by the methods. The THP concentrations assayed by ELISA method was correlated positively to SDS-PAGE ($r = 0.648$, $P = 0.0004$). The level of significance according to Pearson correlation of coefficient was set to alpha $= 0.01$. 
Advantages and Disadvantages

- Inexpensive
- High sensitivity
- Simple, quick
- Little to no health hazards; no radioactivity

- Tedious
- Cross-reaction may occur
- May give false results for HIV; use Western Blotting to verify

Conclusions

• Useful for simple detection
• 4 different types used depending on specificity and cost
• Highly sensitive
References

2. Laboratory Info: http://laboratoryinfo.com/elisa/
Thanks!