ELISA
Enzyme Linked Immune Sorbent Assay
Test are Specific and Sensitive
It is the most common, widely used serological test for Ab or Ag detection.
Ag or Ag are labelled by linking of enzyme.
These test can be automated.
It is method to determine the concentration of material.
Basic Principle of ELISA

1. Add antigen to the solid surface.
2. Add antibody-enzyme conjugate.
3. Add substrate (colorless).
4. The substrate is converted into a product, which is detected.
ELISA Types

- Direct (Sandwich)
- Indirect
- Competitive
Direct method (Sandwich)  
( Detection of Ag )

1. Antibody-coated well
2. Add antigen to be measured
3. Add enzyme-conjugated secondary antibody
4. Add substrate and measure color
5. Wash
Peroxidase  \[ \text{H}_2\text{O}_2 \]
Alkaline phosphatase  \[ \text{P-Nitro phenyl Phosphate} \]

- Colour proportional to Antigen in patient sample.
Indirect method
(Detection of Ab)

1. Antigen-coated well
2. Add specific antibody to be measured
3. Add enzyme-conjugated secondary antibody
4. Add substrate (S) and measure color

Wash
Colour proportional to Ab in patient sample.
Competitive method

Working Principle of the competitive ELISA (ELISA) enzyme linked immunosorbent assay

- Antibody
- Tracer (Antigen + Enzyme)
- Antigen (Hormone)
- Substrate
- Modified Substrate (coloured)

1. Add 100 µl unknown solution & 100 µl tracer solution
2. Remove excess antigen
3. Reaction of the substrate
4. Photometric detection
- Colour inversely related to Ag in patient serum.
Application Of ELISA

- Hormones in the serum like Thyroid hormones, Insuline....
- Tumor marker like AF1, PSA
- Infectious Disease like Bacterial toxin, Viruses, Hepatitis – B Surface antigen
- Assay of the Ab in serum infectious disease like Rubella Viruses, HIV etc...
- Assay of auto Ab or Anti DNA, anti-nuclear Abs etc...
Advantage:

- No Radiation hazard
- High sensitive
- Obtain quick and accurate result
- Minimal discomfort
- Used in wide variety of test
- It doesn’t need costly instrumentation.

- Antigens of very low or unknown concentration can be detected since capture antibody only grabs specific antigen.
Disadvantage:

- Monoclonal antibodies more difficult to separate.
- Enzyme/substrate reaction is short term so microwells must be read as soon as possible.
- Good time management required.
- Monoclonal antibodies can be costly.
- Require good skill.
- Require good quality of ELISA kit.
<table>
<thead>
<tr>
<th><strong>RIA</strong></th>
<th><strong>ELISA</strong></th>
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<tbody>
<tr>
<td><strong>Radioactive Hazard.</strong></td>
<td>It is not Hazard.</td>
</tr>
<tr>
<td>Used estimation of <strong>very small concentration.</strong></td>
<td>Used estimation of <strong>small concentration.</strong></td>
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<tr>
<td><strong>Very high cost</strong> equipment</td>
<td><strong>Low cost</strong> equipment</td>
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<tr>
<td>Cheap reagent.</td>
<td>Costly reagent.</td>
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<tr>
<td>Value measured in <strong>curie &amp; microcurie</strong></td>
<td>Value is measured in <strong>micro.</strong></td>
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<tr>
<td><strong>Require certificate &amp; training from RIA centre.</strong></td>
<td>No training nor certificate require.</td>
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