Measuring Biological Material: Immunoassays

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Lecture Objectives

1. Define what an immunoassay is
2. Explain how antibodies bind specific antigens
3. Understand different labelling strategies
4. Describe the difference between an ELISA and Luminex assay
5. Describe the principles of a whole blood assay
6. Understand how a lateral flow assay works
7. Recommend sample types for an immunoassay

Immunoassays rely on antibody-analyte binding

- Biochemical test that identifies and measures specific molecule (analyte) in solution (serum, urine, plasma…)
- Broad range of analytes (lipids, hormones, peptides, proteins)
- Popular and frequent approach
- Qualitative and quantitative
- High throughput

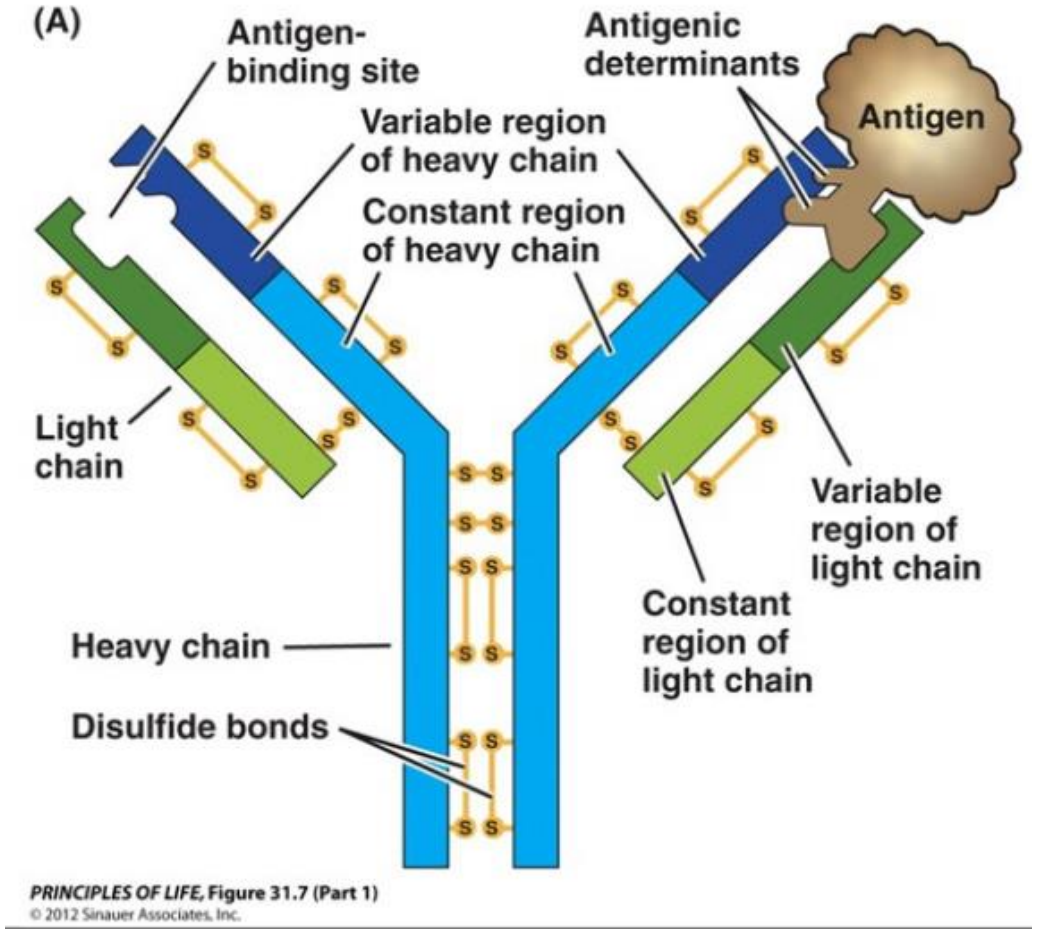
Image source: www.shutterstock.com

The immune response is how the body recognizes and protects itself

- Encounter multiple different “attacks”
- Antigen-antibody binding
- Antigens/immunogens:
  - Molecule capable of being recognized and bound by immune system
  - **Anti**body **Gen**erating
  - Immunogens are antigens capable of stimulating immune response (humoral or cell mediated)
  - Haptens (to fasten) are small molecules that elicit an immune response **only** when bound to carriers (proteins)

Antibodies

- Immunoglobulin
- Large, Y-shaped glycoprotein
- Able to recognize antigens via Fab's variable region (antigen binding fragment)
  - Region that binds to antigens
  - Paratope at tip of "Y"
  - "Lock" specific for epitope on antigen "key"
- Allows precise binding



Immunoassays

- Usually use antibodies (can also use antigens)
- Inherent properties of antibodies
  - Ability to recognize and bind specific molecule in complex mixture
  - Accurate and sensitive
- Polyclonal vs monoclonal?
  - Poly: collection of antibodies from different B cells that recognize multiple epitopes on the same antigen
  - Mono: single antibody producing B cell and therefore only binds with one unique epitope
- Key feature is measurable signal in response to binding = intensity = quantity

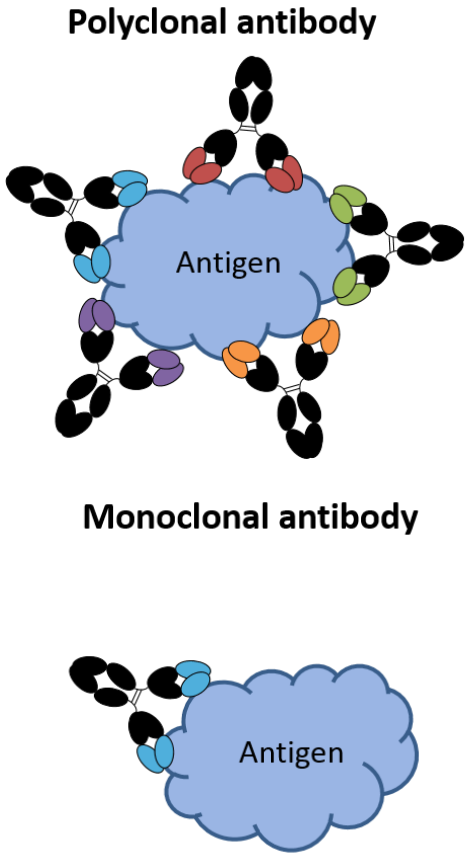
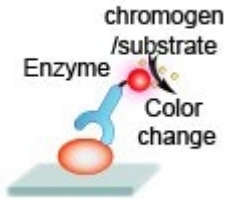


Image source: www.absoluteantibody.com

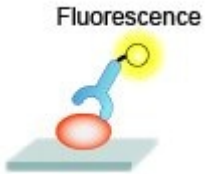
Immunoassays use different labels to measure amount of analyte

1. Radio Immunoassays

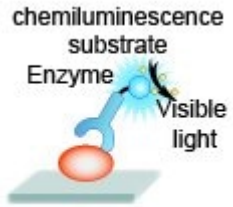
2. Enzyme Immunoassays



3. Fluoro Immunoassay



4. Chemiluminescence Immunoassays



5. Immunochromatographic Assay

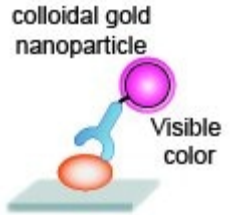


Image source: www.absoluteantibody.com
www.creativediagnostics.com

ELISA: Enzyme-Linked Immunosorbent Assay

- Capture of analyte either DIRECT OR INDIRECT
- Direct: absorption of analyte onto surface
- Indirect: pre-coated capture antibody (sandwich)

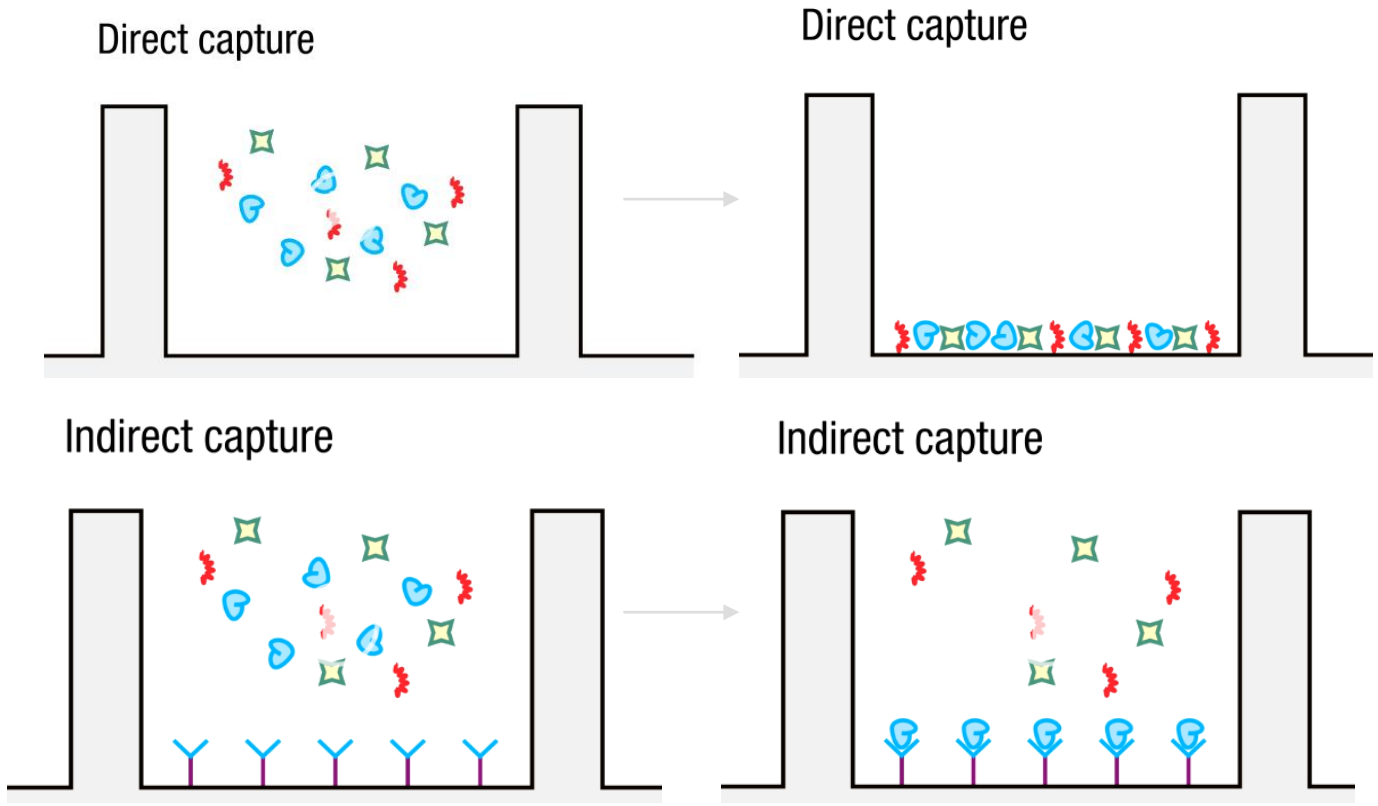
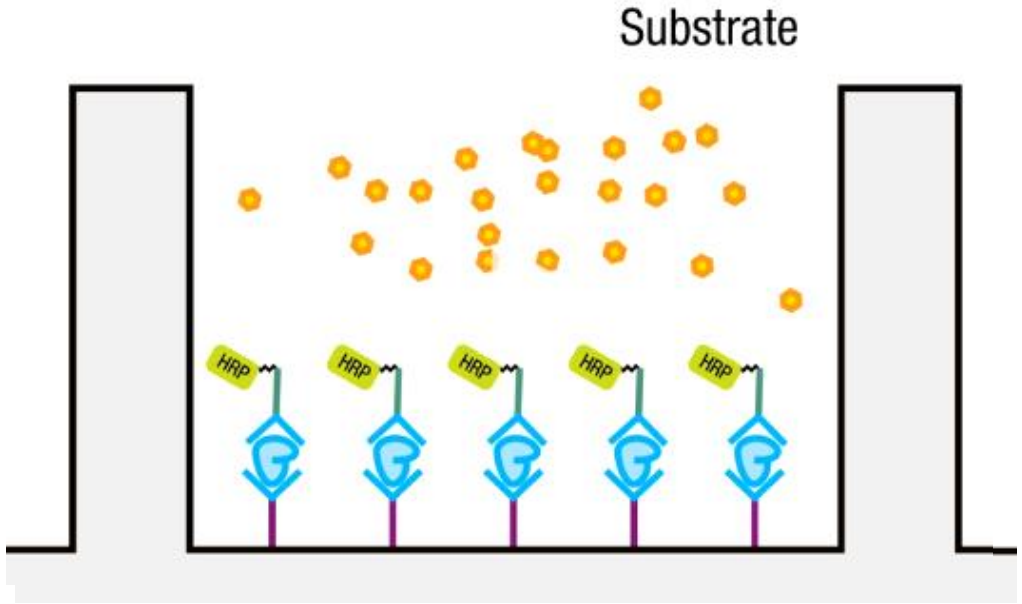


Image source: www.thermofisher.com

ELISA: Enzyme-Linked Immunosorbent Assay

DIRECT DETECTION



INDIRECT DETECTION

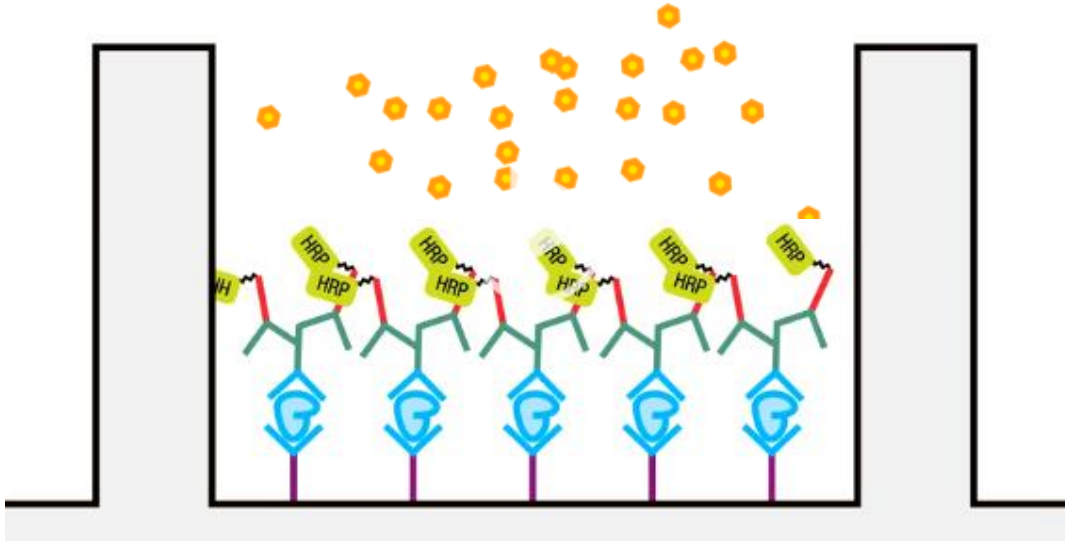
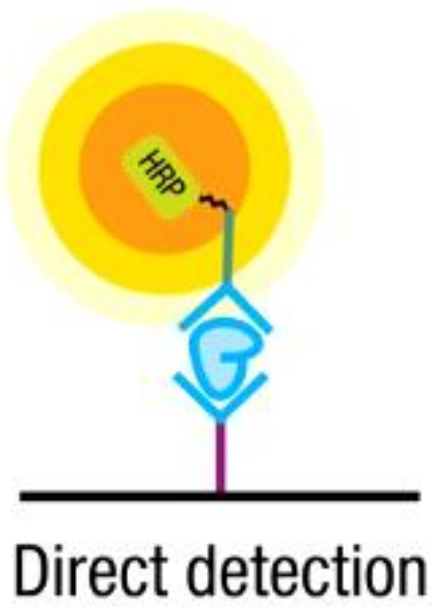


Image source: www.thermofisher.com

ELISA: Enzyme-Linked Immunosorbent Assay

DIRECT DETECTION

- Fewer steps
- Minimal signal amplification



INDIRECT DETECTION

- Versatile
- More sensitive
- Requires more steps
- Cross reactivity of 2°

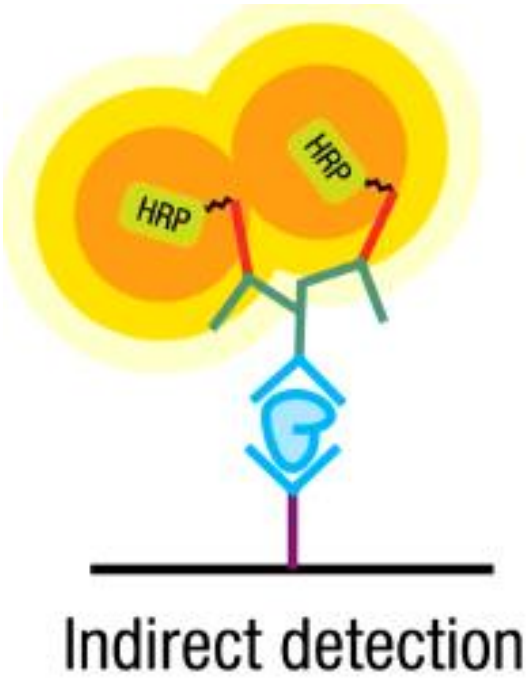


Image source: www.thermofisher.com

To summarize

1. Direct ELISA
   - Fewer steps, faster, less reagents
   - Non-specific binding of antigen, high background, no signal amplification

2. Indirect ELISA
   - Longer procedure, cross reactivity of secondary AB
   - Highly sensitive, more flexible (multiple 1° AB, single 2° AB)

3. Sandwich ELISA
   - Longer procedure, optimization required
   - Matched antibody pairs (capture and detection) = Highly specific, high sensitivity (2-5 times >)

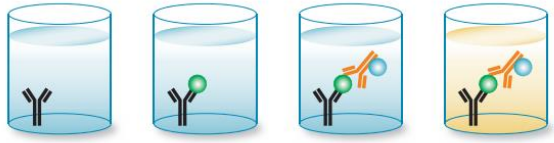


Image source: www.biorad.com

HIV ELISA

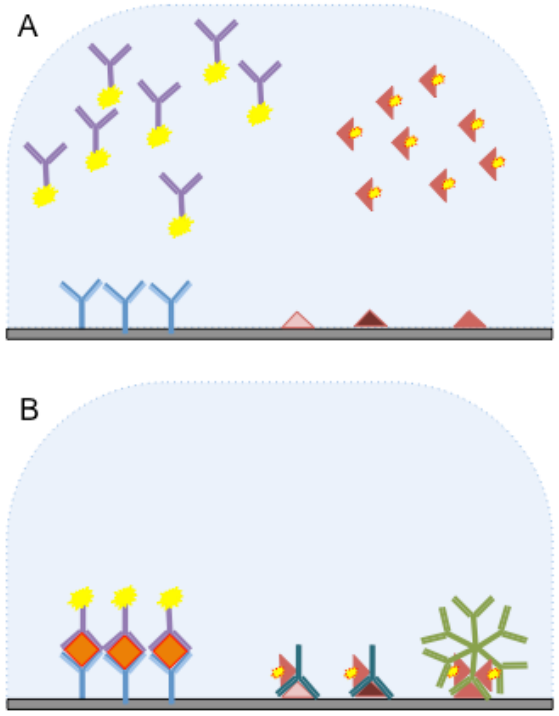
- Combined HIV antibodies and HIV P24 antigen in blood

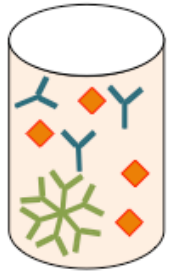
Anti-P24 Polyclonal antibody

Anti-P24 capture monoclonal antibody

Labelled antigen

Synthetic peptide capture antigens for HIV 1 and HIV 2





Human sample to be tested

Patient antibody IgG

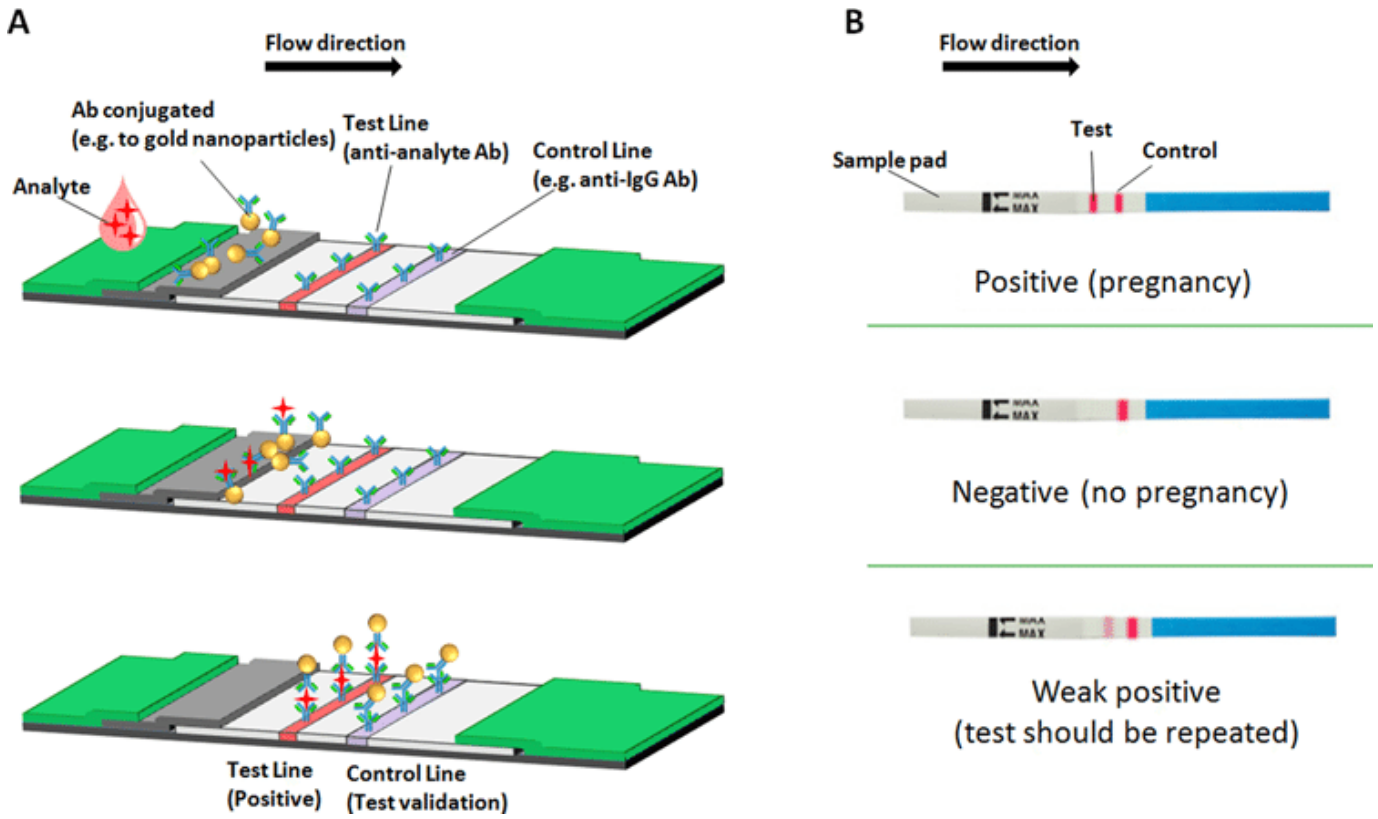
Patient antibody IgM

P24 antigen in patient blood

Image source: www.wordpress.com

Lateral Flow Tests

- Home pregnancy test
- Fast, simple
- No specialized equipment
- POC
- Mostly qualitative
- Time vs sensitivity



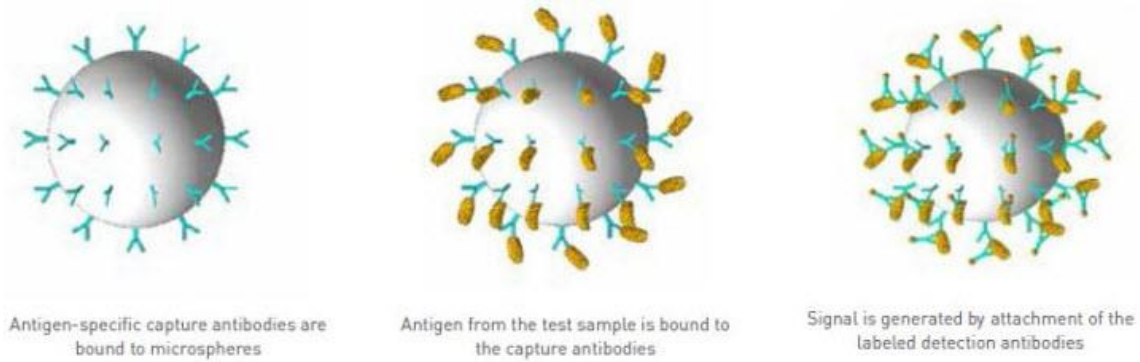
Luminex

- Immunoassay with multi-analyte profiling
- Up to 500 analytes in single drop
- Detection and quantification of secreted proteins (cytokine, chemokines, growth factors…)
- High throughput compared to ELISA

Image source: www.luminexcorp.com

Luminex

- Colour coded magnetic beads
- Pre-coated with capture antibodies
- Each bead is a “mini-ELISA”



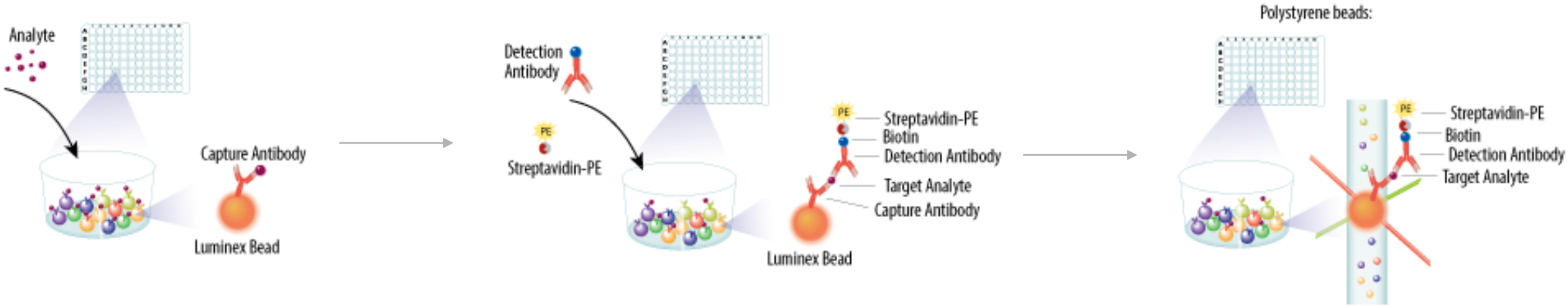
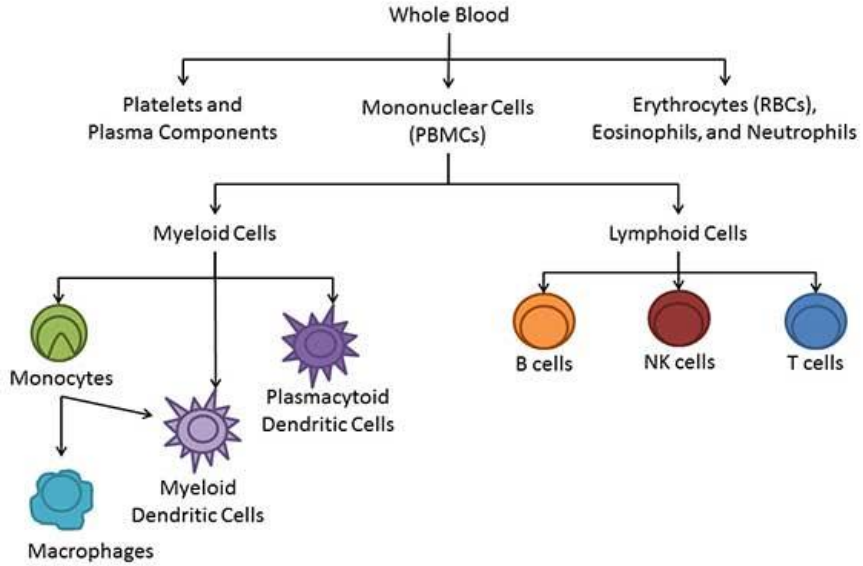


Image source: www.rndsystems.com

Whole blood Assays

- *In vitro* stimulation with specific antigens
- Why?
  - Approximates the state of circulating cells *in vivo*
  - Measure cellular immune response
  - Cytokines, chemokines, T-cell responses
- IGRA for *M tb*
  - Antigens: ESAT-6, CFP 10, TB 7.7
  - Measure: Interferon gamma



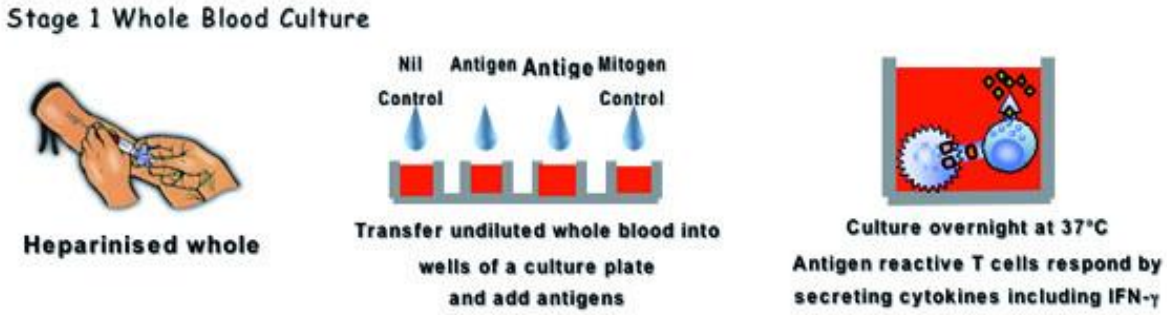


Image source: www.lalpathlabs.com

Specimen Type

- Serum and plasma most common
- Whole blood
- Urine: drug testing
- Cerebrospinal fluid for blood-brain barrier integrity
- Saliva, sweat, tears, stomach fluids and bronchoalveolar lavage fluid
- Amniotic fluid, cord blood: fetal exposure to drugs

Serum		Plasma	
Advantage	**Disadvantage**	**Advantage**	**Disadvantage**
Cleaner sample	Latent clotting can lead to fibrin formation	Faster turn-around time	Platelets and cells (typically white cells) often trapped above gel or found at plasma red cell interface in non-gel tubes
Considered the gold standard for some analyses	30 minute delay for clot formation	Representative of circulating blood	Metabolism and lysis of cells can alter test results
More stable once separated	Clot retraction elevates potassium levels relative to plasma values	Increased sample volume	Considered less stable especially when stored

CANCER GENOMICS & PROTEOMICS 3: 227-230 (2006)

Review

Considerations Regarding the Use of Blood Samples in the Proteomic Identification of Biomarkers for Cancer Diagnosis

CHRISTINE V. SAPAN and ROGER L. LUNDBLAD

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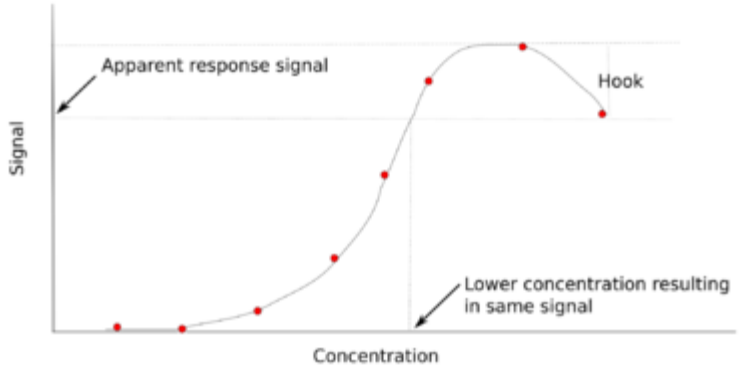
Image source: www.austincc.edu

Pitfalls

Interferences: false positives and negatives

1. Endogenous interference (matrix effect): e.g. haemoglobin
2. Cross reaction of antibodies
3. System related errors: probe contamination, carry over, pipetting
4. Hook effect: false negatives/inaccurately low results



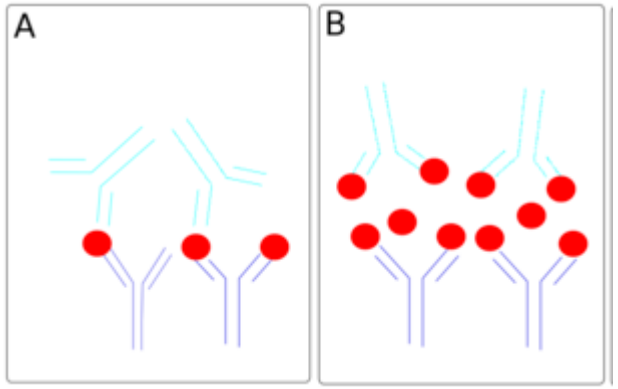


Image source: adapted from Schiettecatte et al.

Takeaway messages

1. Immunoassays are analytical methods dependent on antibody-antigen binding
2. Immunoassays measure the presence and concentration of analytes in fluid
3. Immunoassays are highly sensitive and specific
4. The enzyme-linked immunosorbent assay is a test that uses antibodies and color change to identify an analyte
5. Luminex technology allows multiplexing up to 500 analytes
6. Whole-blood assays are well-suited for measuring cytokine production while keeping the physiological environment
7. Consider the sample type!