Immunoassay techniques

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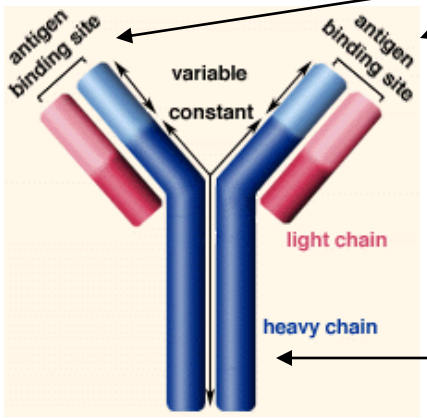
Immunoassays - definition

An immunoassay is any test that **uses an antibody to detect** a given analyte.



The Fab regions provide specific recognition of the analyte, and will vary from assay to assay

The Fc region is constant for a given antibody type (IgG in most assays)

- All immunoassays have 3 fundamental parts
  - The antigen being measured
  - A specific antibody
  - A method to detect antigen/antibody binding

Immunoassays - types

Although all immunoassays rely on antigen/antibody binding, there are many technical variations on how this can be performed.

- Method of detection used to measure analyte
  - Radioactive, enzymatic, chemiluminescent, fluorescent, precipitation
- Competitive vs non-competitive assays
  - Does the analyte in the patient sample need to compete with a labelled analyte for binding to the antibody?
  - Noncompetitive assays tend to be more sensitive than competitive
- Homogenous vs heterogenous
  - Does unbound antibody need to be separated in order to read result?
  - Homogenous assay tend to be faster – heterogenous assays tend to have reduced background

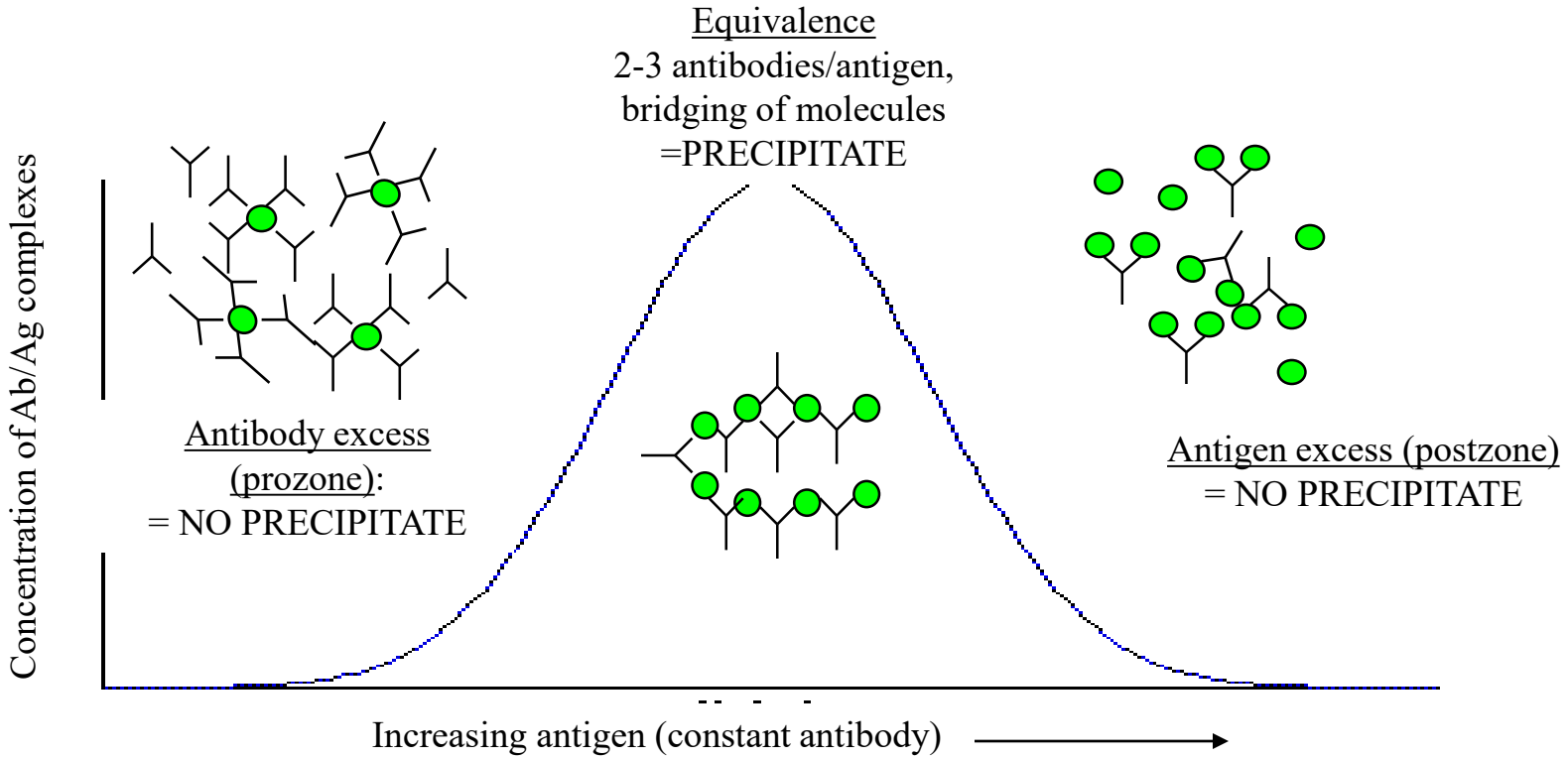
Definitions of different immunoassay types

Abbreviation	Name	Detection	Competitive	Separation	Comment
RIA	Radioimmunoassay	Radioactive	Competitive	Heterogenous	
IRMA	Immunoradiometric assay	Radioactive	Noncompetitive	Heterogenous	
EIA	Enzyme immunoassay				(general term referring to any assay utilizing enzymatic detection)
ELISA	Enzyme-linked immunosorbent assays	Enzymatic	Either (most noncompetitive)	Heterogenous	
Sandwich ELISA		Enzymatic	Noncompetitive	Heterogenous	ELISA using 2 Abs (one to capture, 2nd to detect)
EMIT	Enzyme-multiplied immunoassay technique	Enzymatic	Competitive	Homogenous	
CEDIA	Cloned enzyme donor immunoassay	Enzymatic	Competitive	Homogenous	
IFA	Immunofluorescent assay	Fluorescent	Noncompetitive	Heterogenous	Primarily used to refer to microscopic evaluations of tissue or cell culture
FIA	Flourescent immunoassay	Fluorescent	Either	Either	
FPIA	Flourescentce polarization immunoassay	Fluorescent	Competitive	Homogenous	
CLIA	Chemiluminescent immunoassay	Chemiluminescence	Either	Either	
ICMA	Immunochemiluminometric assay	Chemiluminescence	Usu noncompetitive	Usu heterogenous	

- Similar terms are often used interchangeably in the literature (eg, RIA and IRMA)

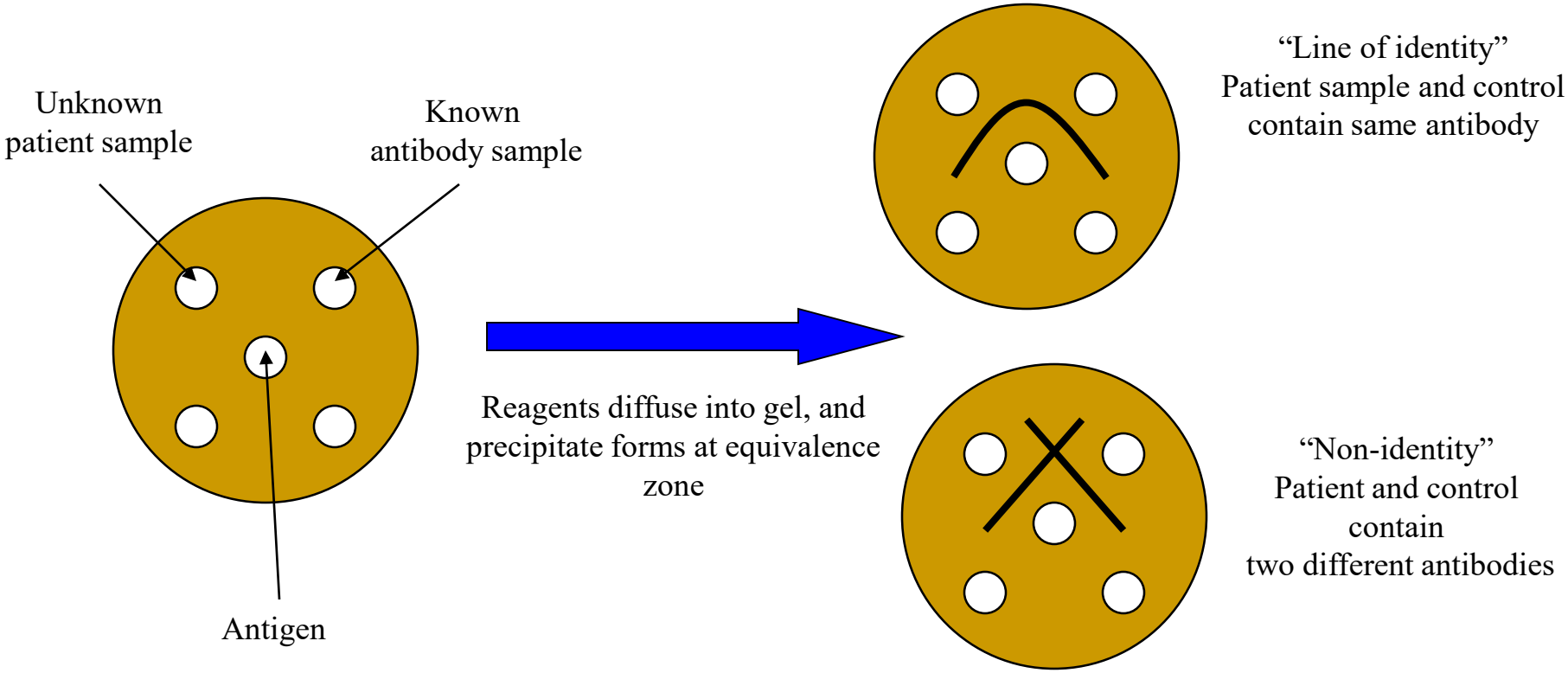
Qualitative immunoassays - the preciptin reaction

- Binding of antibody to antigen takes place in three phases, depending upon the relative amounts of antibody to antigen



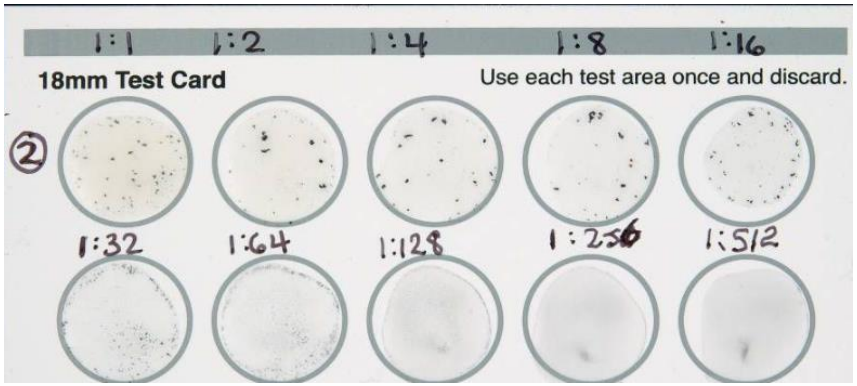
Ouchterlony dish diffusion (aka double diffusion)

- Qualitative gel-based assay to assess similarity of different antibodies
  - Historical assay – rarely used now (but shows up on boards)



Agglutination

RPR



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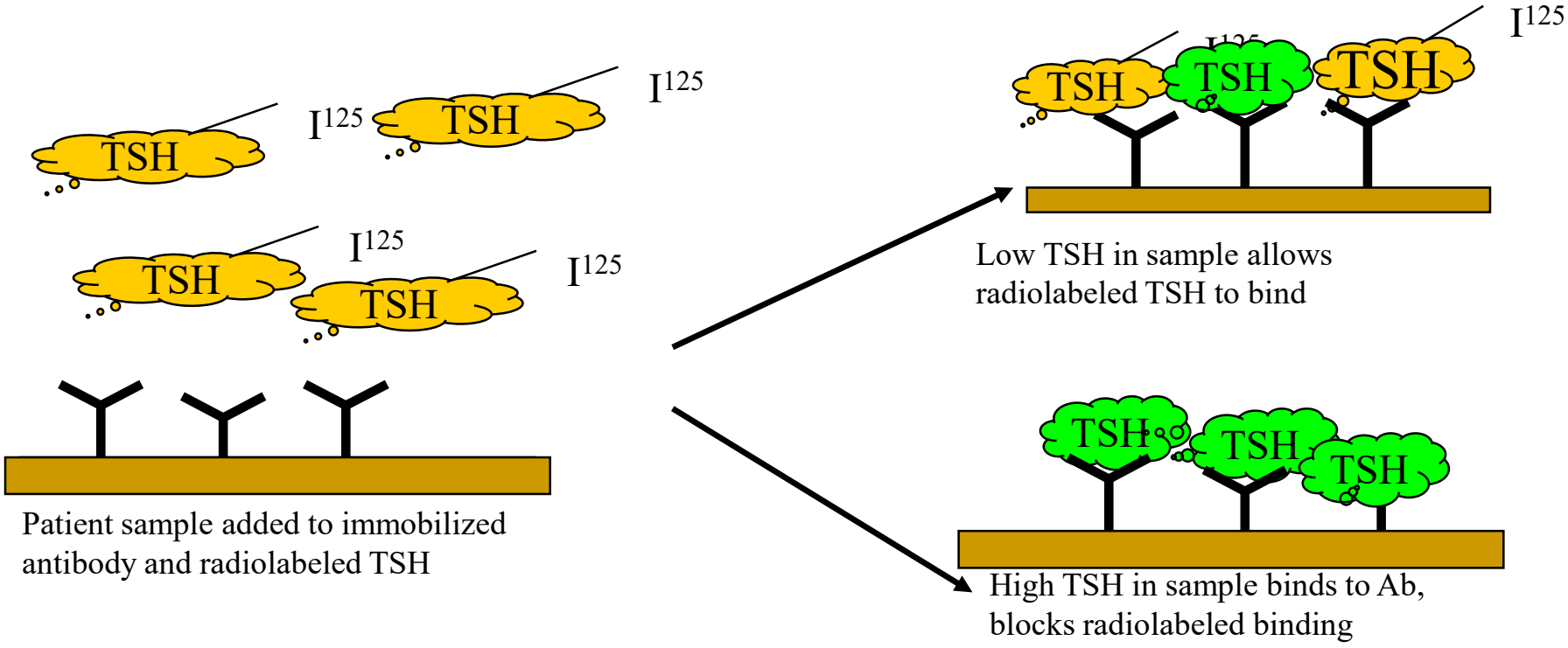
Quantitative immunoassays - labelled immunoassays

- Labelled immunoassays are used to increase low-end sensitivity by a variety of methods
  - Enzymatic labelling: amplifies signal
  - Chemi- or bio-luminescence: lowers detection limits

- Different types of immunoassays exist
  - Competitive (Ag* + Ag (px) + limiting Ab)
  - Non-competitive (Ab + Ag (px) + 2nd Ab)
  - Heterogenous assay (free and bound Ab give similar signal and must be separated, i.e. ELISA)
  - Homogenous assay (only bound Ab gives signal and therefore does not need to be separated before assay, i.e., FPIA & EMIT)

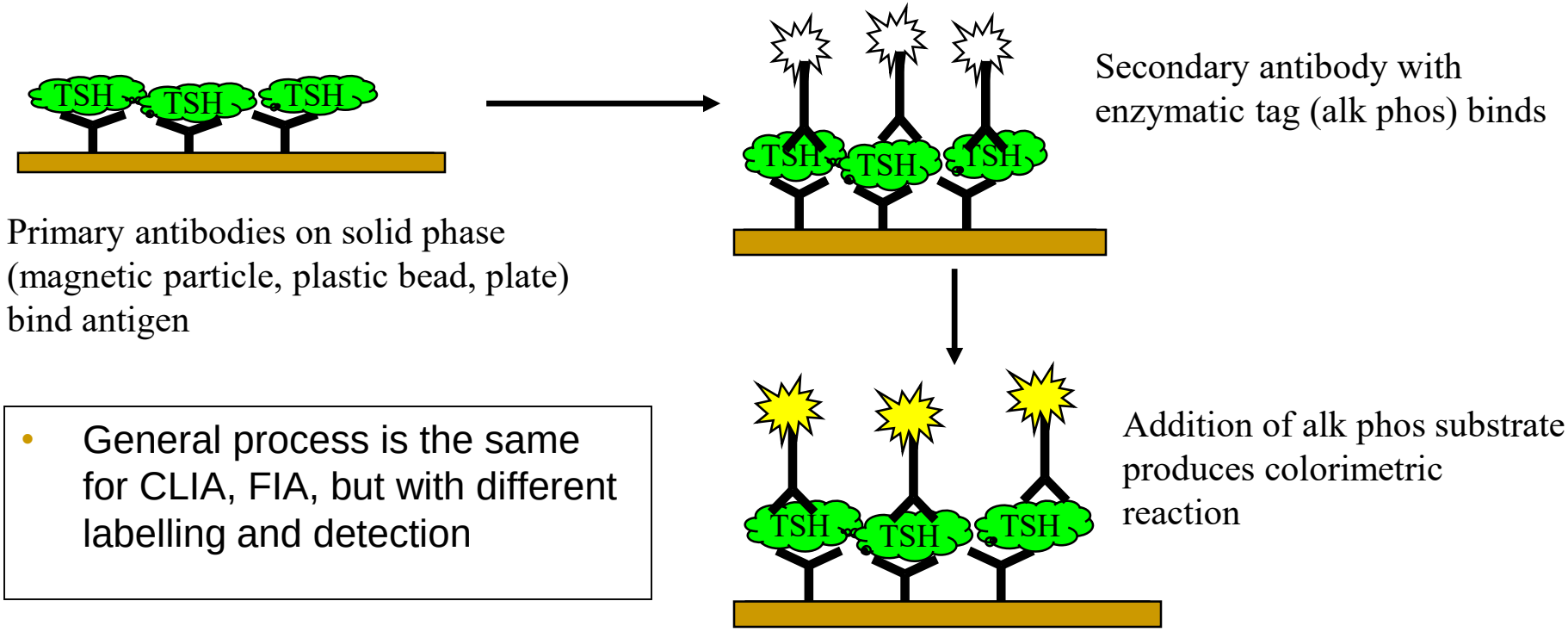
Quantitative immunoassays - radioimmunoassay (RIA)



Pros: Very sensitive (particularly for small molecules such as hormones), minimal background

Cons: Use of radioactivity, requires labeling of antigen or antibody without altering binding, manual and labor intensive assays

Quantitative immunoassays - enzyme-linked immunosorbant assay (ELISA)



Primary antibodies on solid phase (magnetic particle, plastic bead, plate) bind antigen

Secondary antibody with enzymatic tag (alk phos) binds

Addition of alk phos substrate produces colorimetric reaction

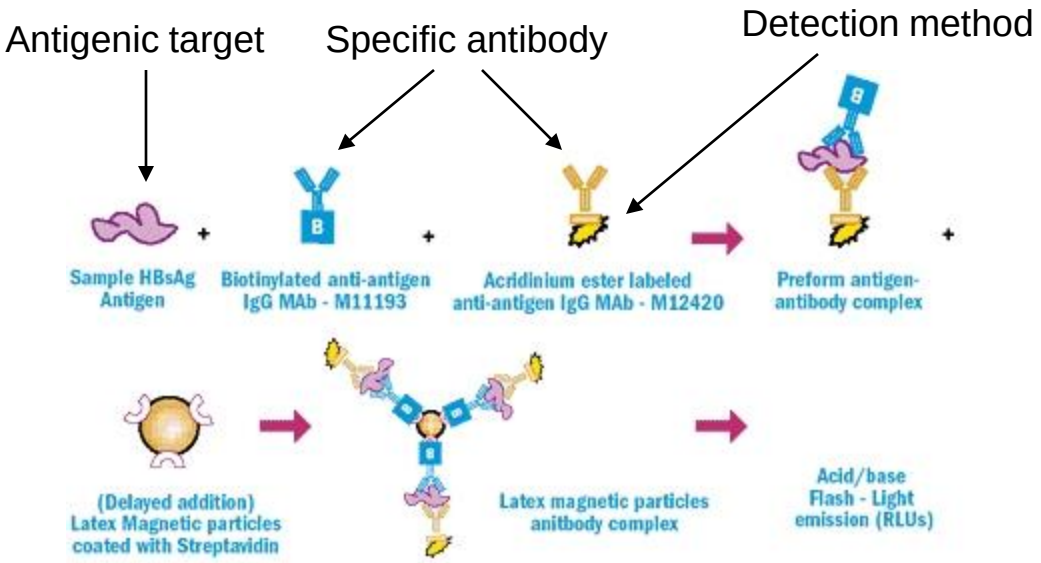
- General process is the same for CLIA, FIA, but with different labelling and detection

Pros: Enzymatic amplification increases sensitivity (low ng/ml), easily automated, rinsing reduces background

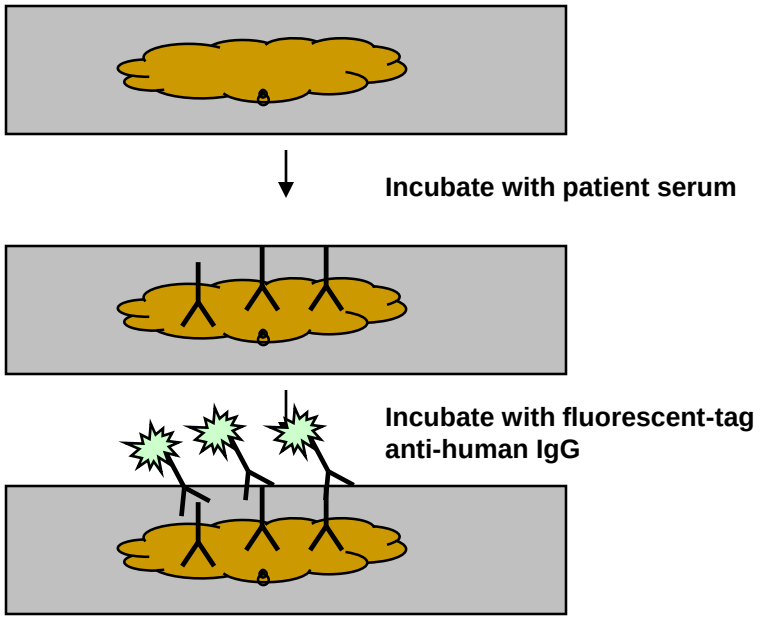
Cons: Possibility for specific inhibitors (HAMA), hook effect for sandwich ELISAs

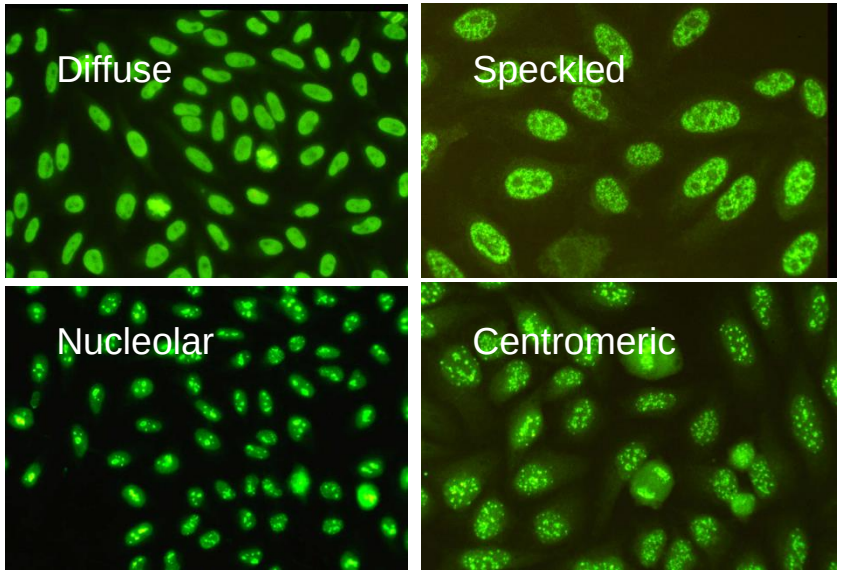
Example: CLIA – HBsAg on Advia Centaur

Anti-Hepatitis A, IgM	Prolactin
Total Hepatitis A Virus	Alpha Fetoprotein in Maternal Serum
Hepatitis B Core IgM	Human Chorionic Gonadotropin in Maternal Serum
Anti- Hepatitis B Core Total	Red Blood Cell Folate
Hepatitis B Surface Antibody	Cortisol
Hepatitis B Surface Antibody Quantitative	Parathyroid Hormone
Hepatitis C Virus IgG Anitbodies	Rapid Parathyroid Hormone
Hepatitis B Surface Antigen	Testosterone
Hepatitis B Surface Antigen Confirmatory	BNP Centaur
Homocysteine	C-Peptide
Alpha Fetoprotein	
Insulin	



Fluorescent immunoassays - IFA

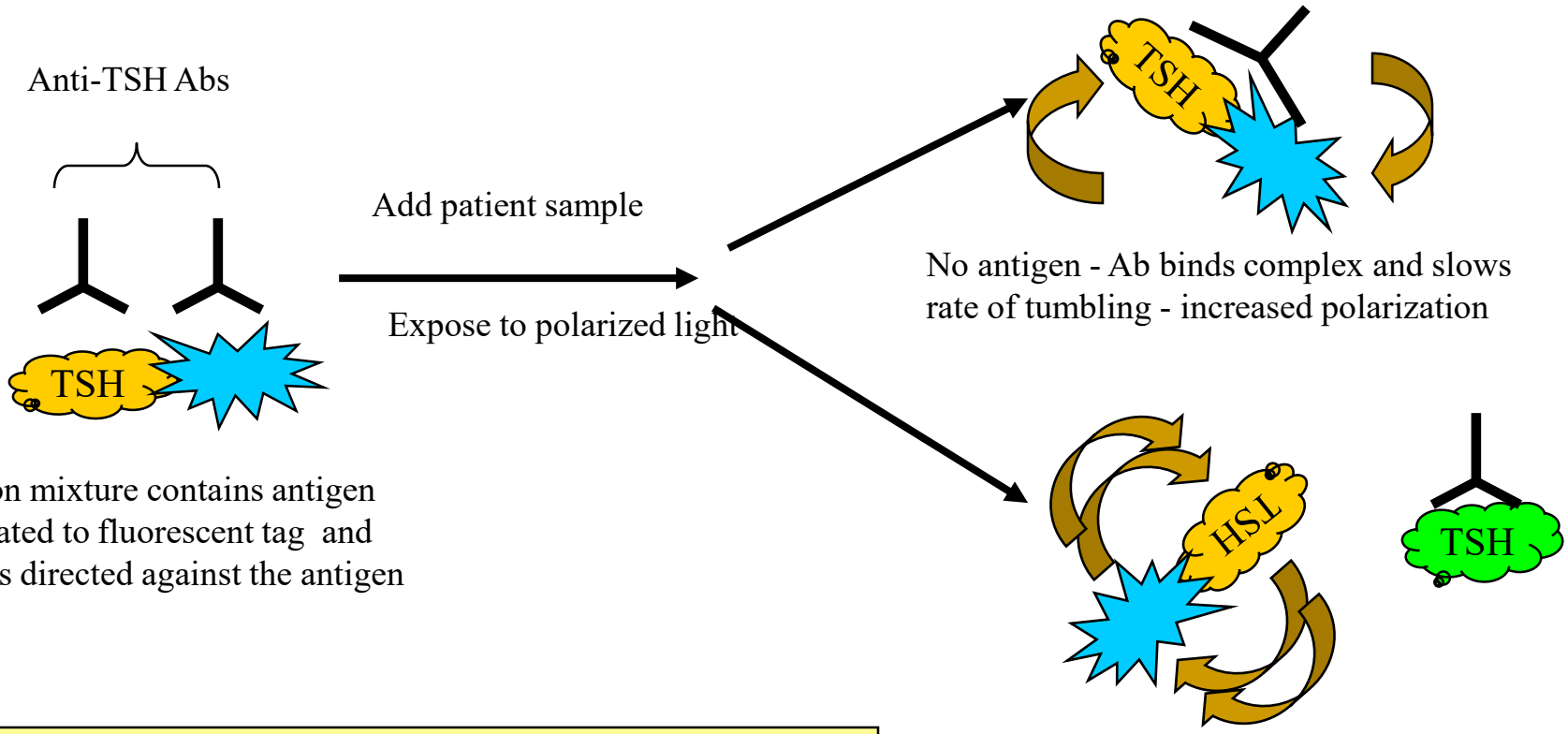




Pros: General screen w/o knowing specific antigens

Cons: Manual assay, requires interpretation

Quantitative immunoassays - fluorescence polarization



Pros: Very sensitive, rapid

Cons: Background fluorescence in human samples, requires specific instrumentation.

Conclusions

- Immunoassays are one of the most commonly-used types of tests in the clinical laboratory today
- Although many variations exist, they all share common components of antigen, antibody, and a detection method.
- The primary differences between immunoassays relate to the type of signal generated, competitive vs non-competitive measurements, and homogenous vs heterogeneous assays.