Cihan University/ Sulaimaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab.5: Labeled immunoassays

Radioimmunoassay (RIA)

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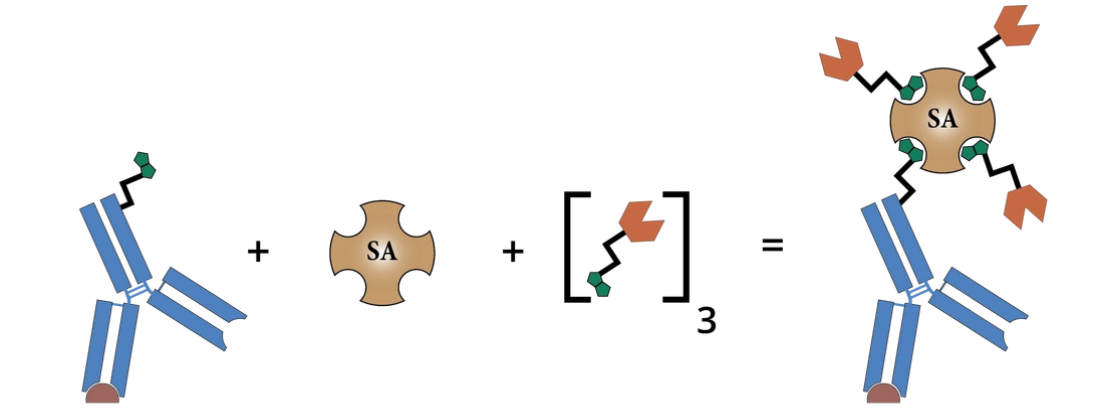
Labeled Immunoassays

- Differ from unlabeled techniques by including a detection molecule (label) in the test system.
- Most current techniques utilize **non-isotopic labels** to generate a light signal. Depending on manufacturer design, labels may include a **colorimetric substrate**, **fluorescent compound (fluorophore),** or **luminescent molecule**.
- Earlier generation immunoassays used radioactive isotope elements as the label, but these techniques are less commonly used in clinical laboratories today.
- Labels are sometimes referred to as “tracer” molecules because they allow for tracing of the detection signal.

Antibody can be labeled by:

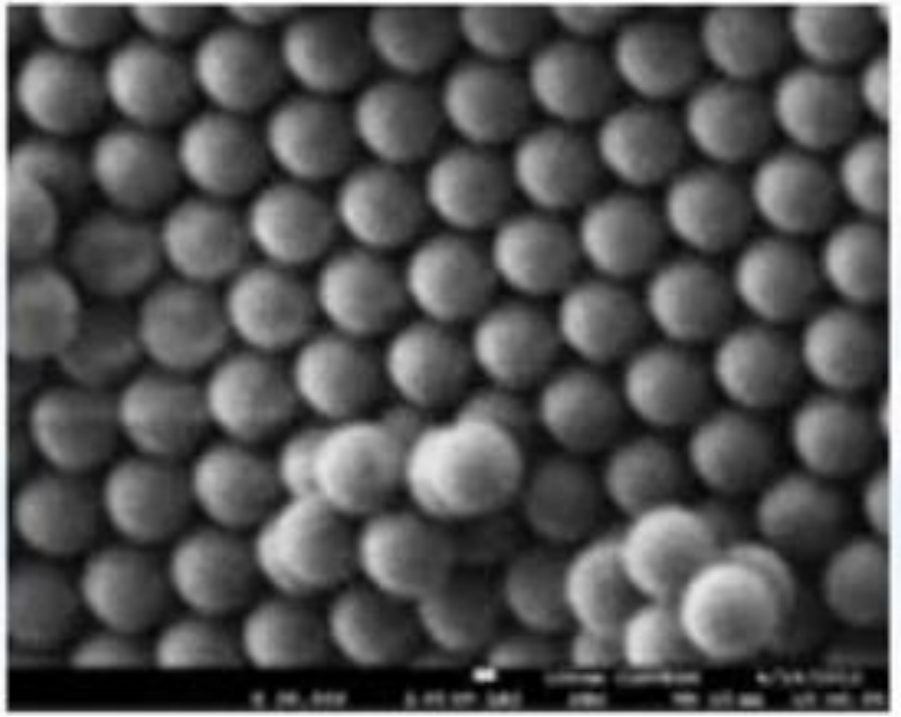
1- Small Particles (Nanoparticles)

- The process of biotin labeling is known as biotinylation. Protein biotinylation involves covalently attaching biotin molecules to other biomolecules, such as antibodies, peptides, proteins, nucleic acids (DNA and RNA), oligonucleotides, and others.
- Biotin- Used in Lateral Flow Immunoassay, Western Blot, ELISAs, Flow Cytometry.



2- Particles

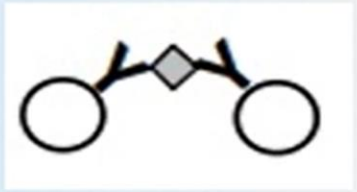
- Microspheres- Polystyrene latex- based microsphere, magnetic beads.
- Use in Lateral Flaw Immunoassay, Latex Agglutination Test, Fluorescence microsphere immunoassays (Luminex), Magnetic ELISA, Magnetic Immunoassays.



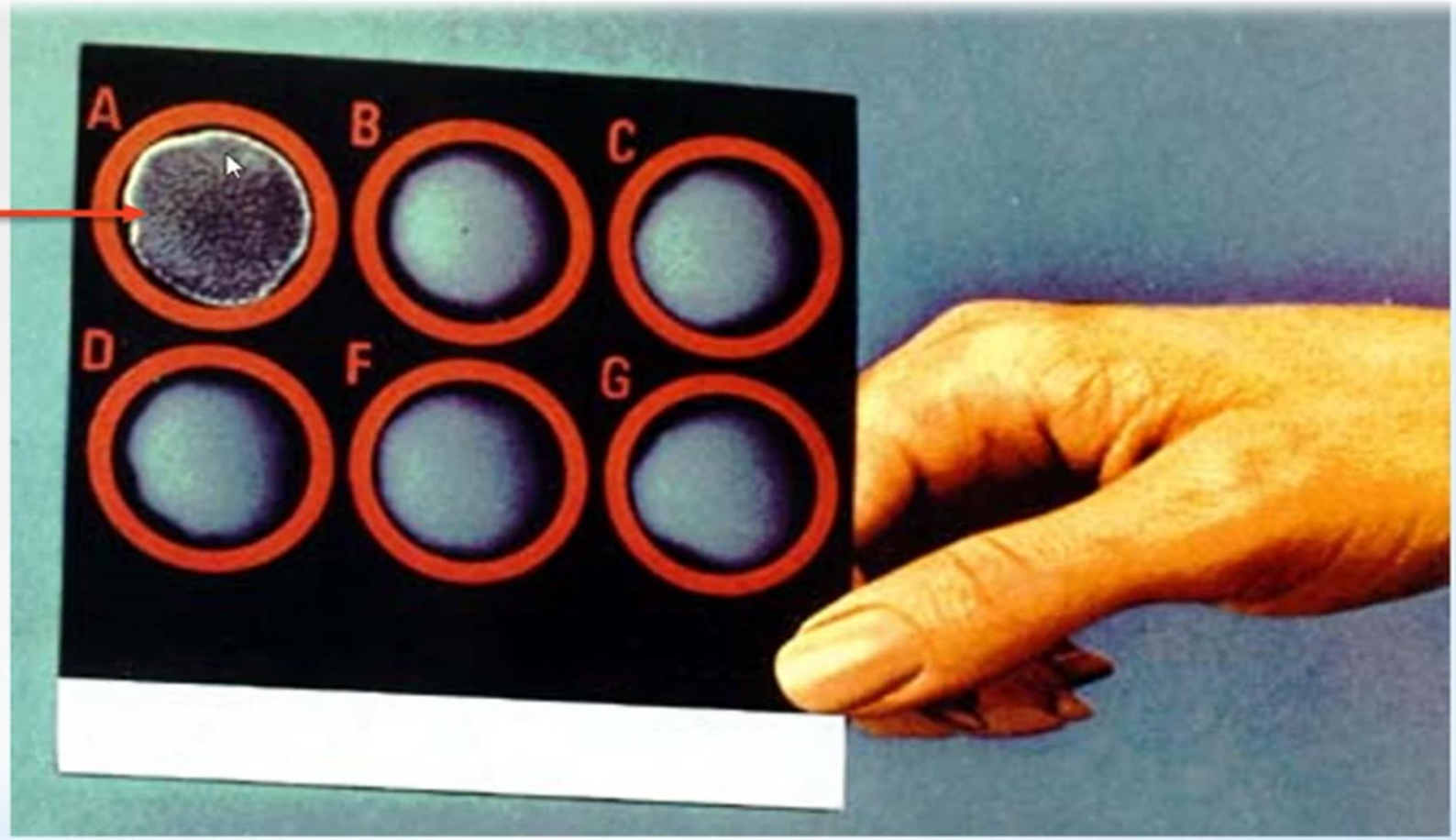
Microsphere. SEM image.

Example of Latex Agglutination Test

Agglutination

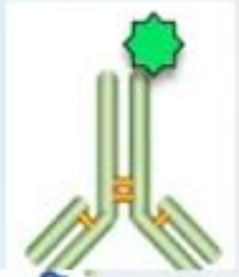


Particle agglutination
(clumping of microspheres,
to look like curdled milk)



Circle A shows a positive test (agglutination of the latex particles.) The other circles B-E show negative tests (no agglutination of the latex particles).

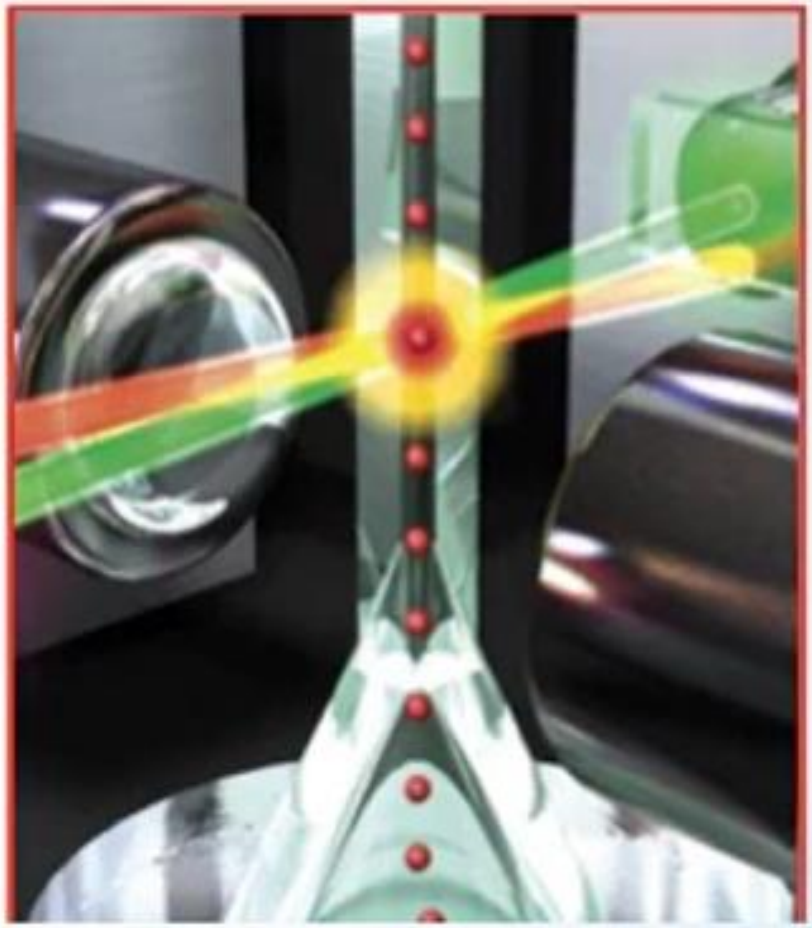
Florescent Microsphere Immunoassays



Detection antibody
(specific antibody labelled
with fluorescent reporter)

Target antigen

Microsphere with specific antibody

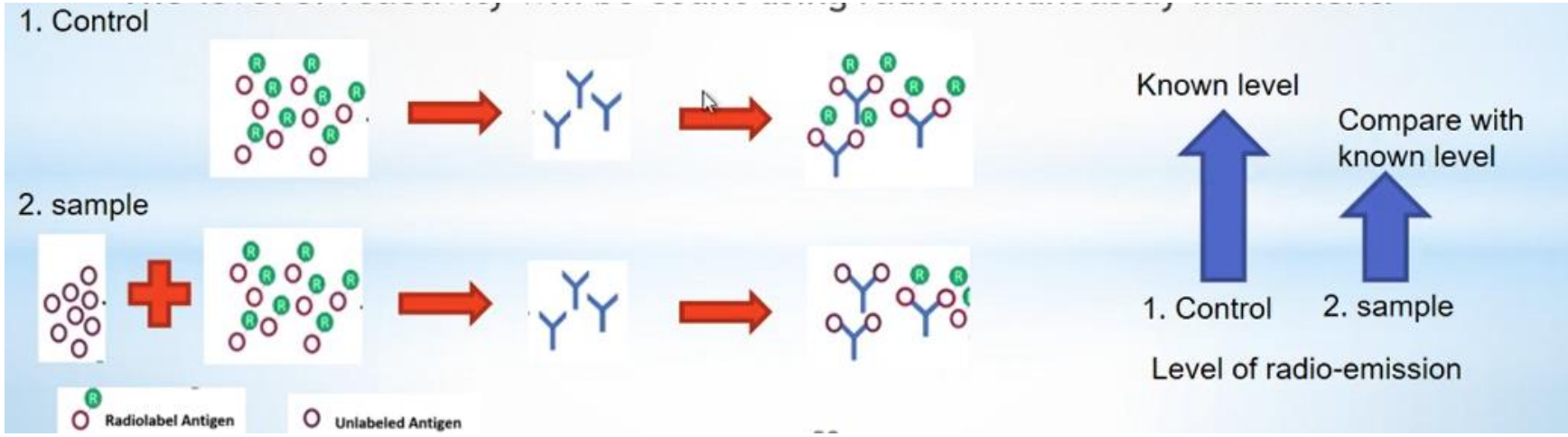


Green laser to count
positive / negative

Red laser to
identify bead
(specific for
target antigen)

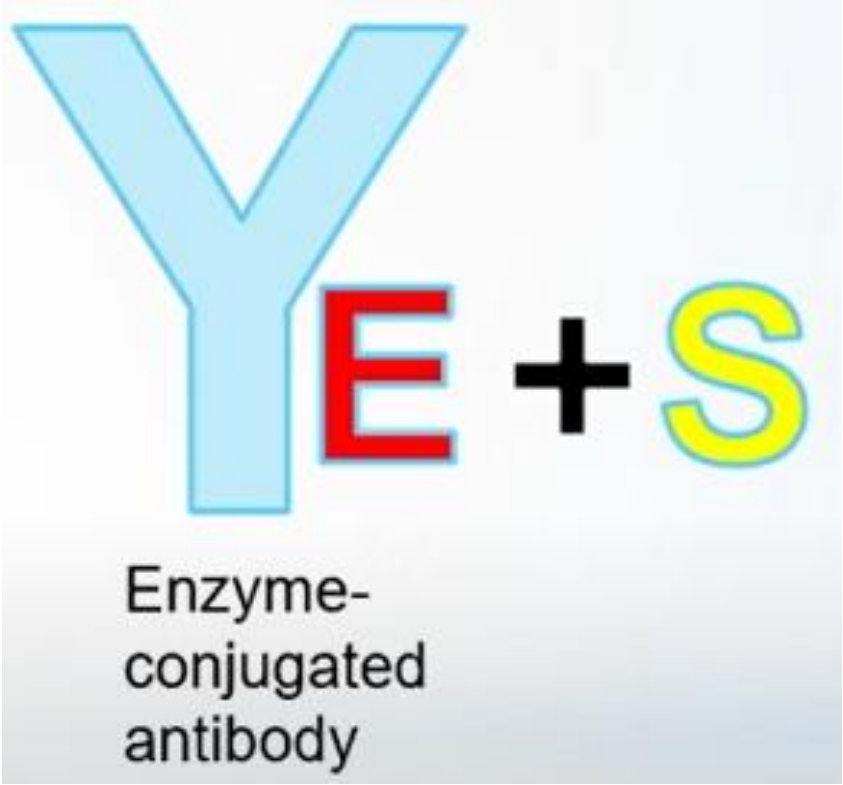
3- Isotope

- Radioisotopes- labelled antigen/ Antibody,
- Use in **Radioimmunoassay.**
- Is the **most sensitive** and **specific methods** of *invitro* assay techniques used to measure concentrations of antigen.
- Example: Radioisotopes- labelled target antigen+ sample (unknown specific antibody).
- The level of reactivity will be count using radioimmunoassay instrument.



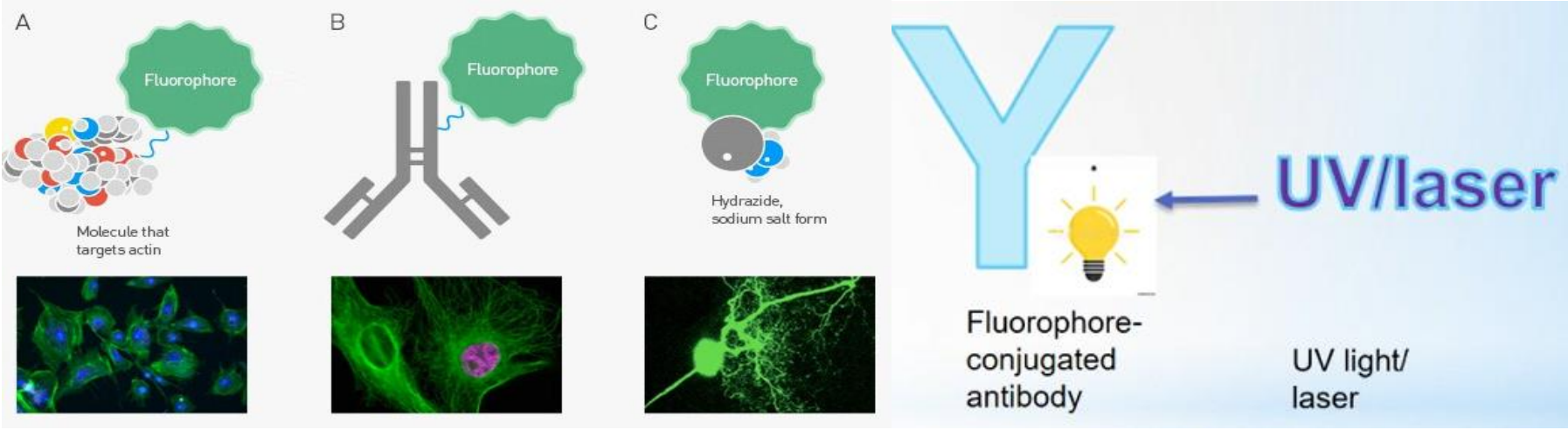
4- Enzymatic Protein

- Uses an enzyme linked to an antibody to detect antibody/ antigen.
- To use enzyme- labelled antibody, samples are incubated with a substrate that is catalyzed by the enzyme to produce a colored product (chromogenic assay) or light (chemiluminescent assay).
- Color producing assays are useful for ELISA, Lateral Flow Immunoassay, Western blots, and immunohistochemistry.
- While light- producing reactions are most frequently used in Western blot and chemiluminescence Immunoassay (CLIA).



5- Florescent dyes/ Fluorophore

- The use of fluorophore- labelled antibodies,
- Since fluorescent dyes are directly conjugated to the antibody, no enzyme/ substrate or binding interactions are required for detection.
- Therefore, the amount of fluorescent signal detected is directly proportional to the amount of target protein in the sample.
- Fluorescent labels are used in highly quantitate assays like, flow cytometry, western assays.



Immunoassay Techniques

Perkin Elmer Wizard2 2470 Automatic Gamma Counter

Radioimmunoassay

- Radioimmunoassay (RIA) is an elegant technique in clinical immunology and analytical chemistry.
- If substance to be analyzed is in very low quantities in the order of microgram, nanogram, conventional methods like gravimetric or colorimetric method fail.
- RIA finds extensive application in the assay of many substances which are present in trace amount in blood.
- So, RIA very sensitive in vitro assay technique used to measure concentrations of antigens (for example, hormone levels in the blood) by use of antibodies.

History of RIA

- The technique was introduced in 1959 by *Rosalyn Yalow* and *Solomon Berson* as an assay for the concentration of insulin in plasma.
- It represented the first time that hormone levels in the blood could be detected by an *invitro* assay.
- In 1977, Dr. Rosalyn Yalow became the first female to win a Nobel Prize with her work on the radioimmunoassay.

Advantages

1. Radioimmunoassay is widely-used because of its great sensitivity.
2. By using high affinity antibodies, possible to detect a few picograms (10−12 g) of antigen in the tube.
3. The greater the specificity of the antiserum, the greater the specificity of the assay.

Disadvantages

1. The main drawbacks to radioimmunoassay are the expense and hazards if preparing and handling the radioactive antigen.
2. Both ^{125}I or ^{131}I emit gamma radiation that requires special counting equipment;

References

- Kim, J. H., Lee, S. Y., & Lee, S. K. (2021). Development of novel lab-on-a-chip platform for high-throughput radioimmunoassay. Applied Radiation and Isotopes, 168, 109526.
- Alhabbab, R.Y. (2018). Radioimmunoassay (RIA). In: Basic Serological Testing. Techniques in Life Science and Biomedicine for the Non-Expert. Springer, Cham.
- https://microbenotes.com/radioimmunoassay-principle-uses-and limitations/#radioimmunoassay-ria-requirements