SAWA UNIVERSITY

VIROLOGY

radioimmunoassay (RIA)

Sawa University — جامعـــــة ســـــــاوة الاهلية

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Three :Stage . — المرحلـــــــــــة الثالثة

الجانب العملي — محاضرة رقم 8...

Practical — Lecture No.8..

تدريسي المادة : م. د. سيف علي

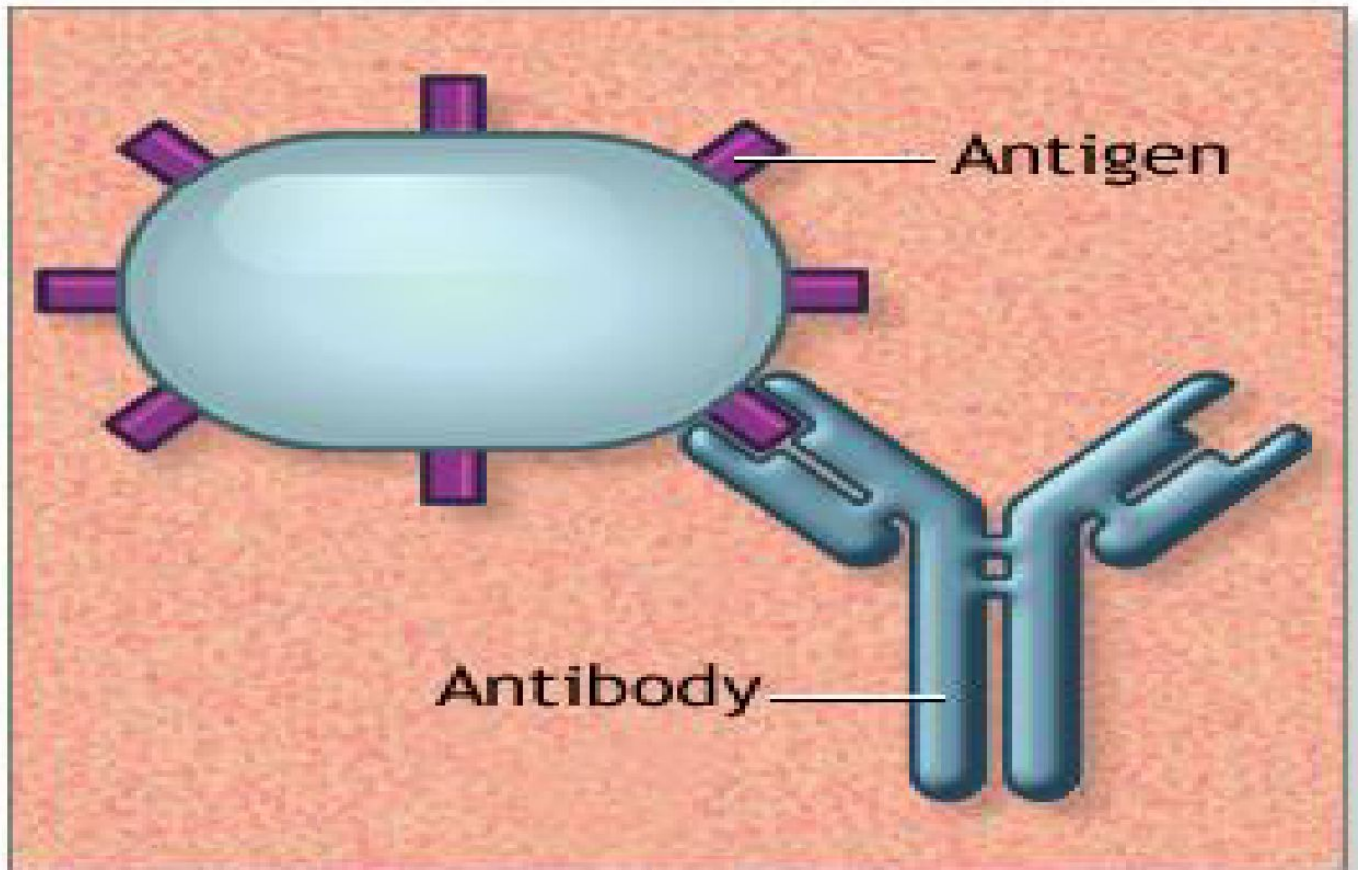
Radioimmunoassay (RIA)

is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies.

Radioimmunoassay (RIA)

Principle of Radioimmunoassay

1) An immune reaction (i.e. antigen, antibody binding)



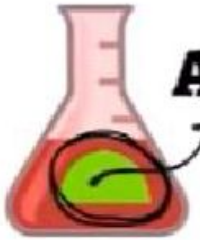
2. A competitive binding or competitive displacement reaction. (It gives specificity)

This is a phenomenon wherein when there are two antigens which can bind to same antibody, the antigen with more concentration binds extensively with the limited antibody displacing other. So here in the experiment, radiolabelled antigen is allowed to bind to high affinity antibody. Then when patient serum is added unlabeled antigens in it start binding to the antibody displacing the labeled antigen.

3. Measurement of radio emission. (It gives sensitivity)

Once the incubation is over, then washings are done to remove any unbound antigens. Then radio emission of the antigen antibody complex is taken, the gamma rays from radio labeled antigen are measured

Serum



A- antigen

1.

Anti A

Antibodies

2.

Radiolabeled

A antigen

radioisotopes

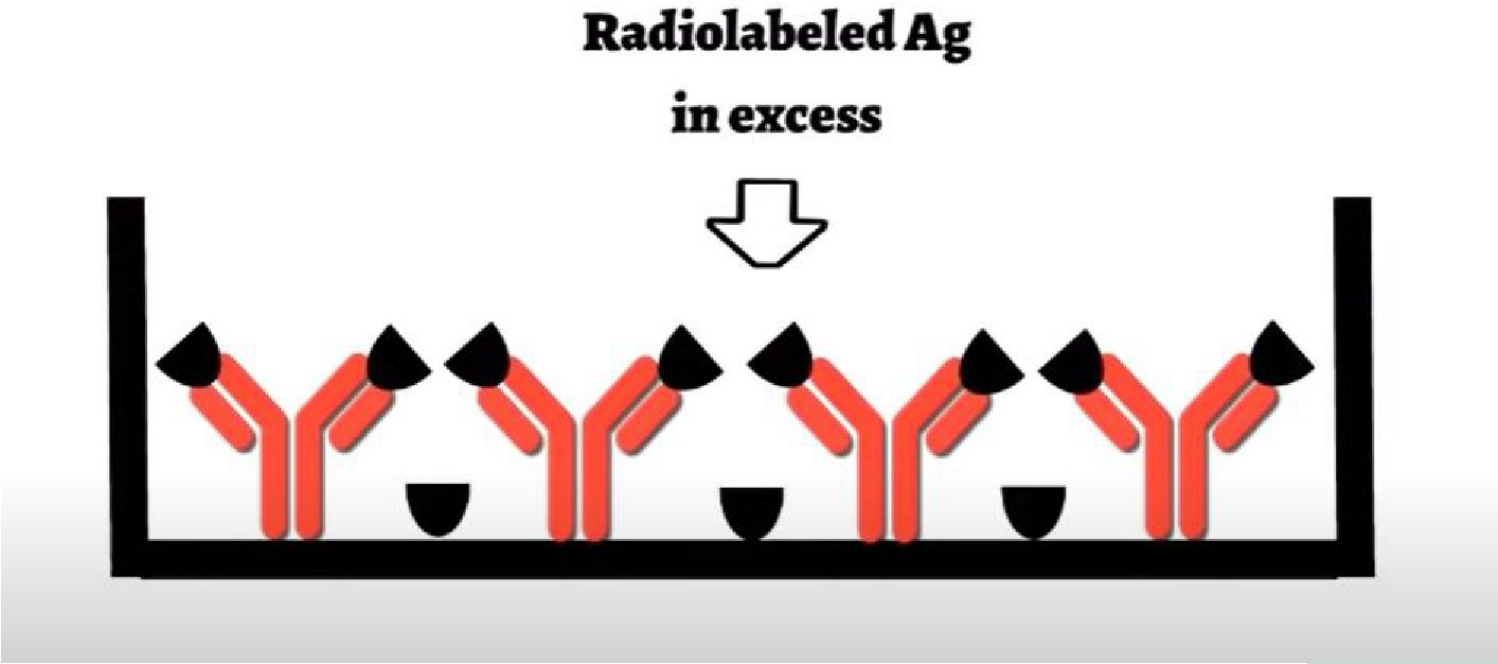
Tritium ^{125}I

3.

Unlabeled

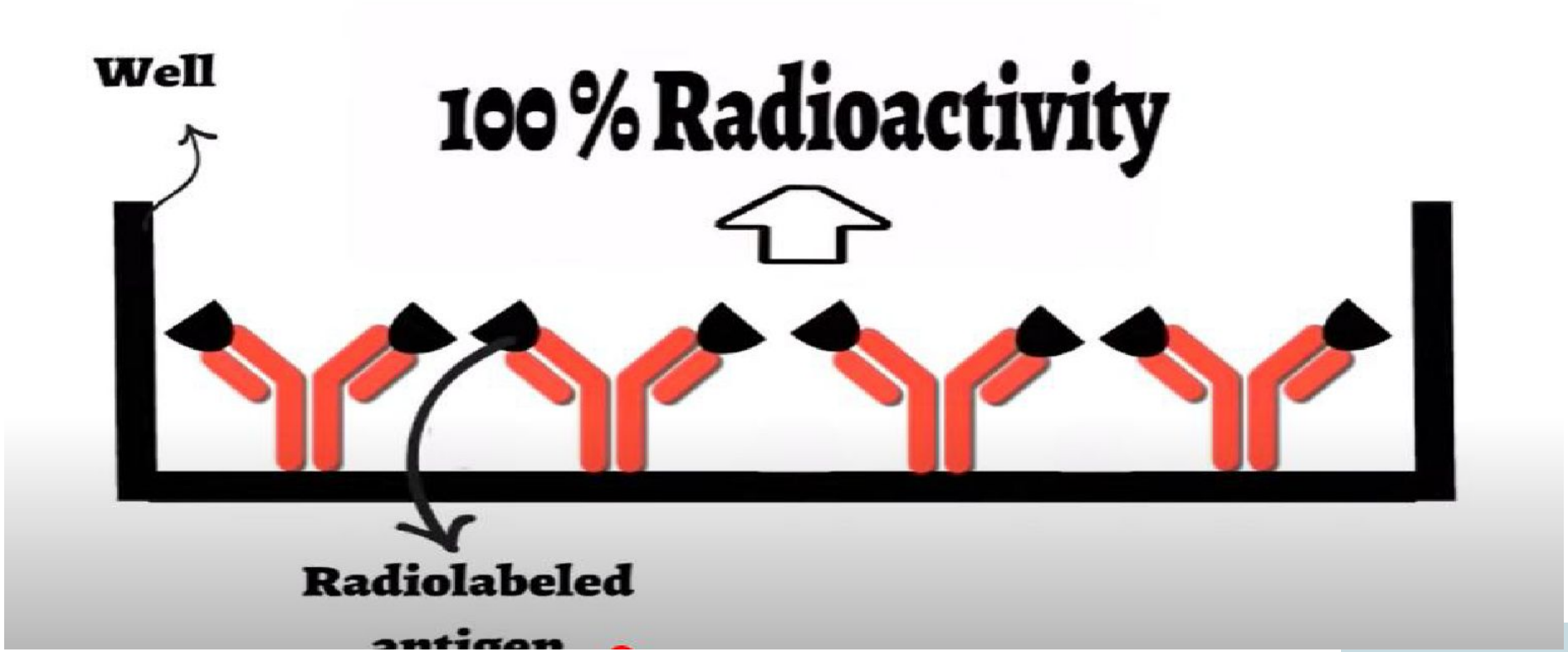
A antigen

1.The labeled antigen (The antigen is generally labeled with I125) is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody.

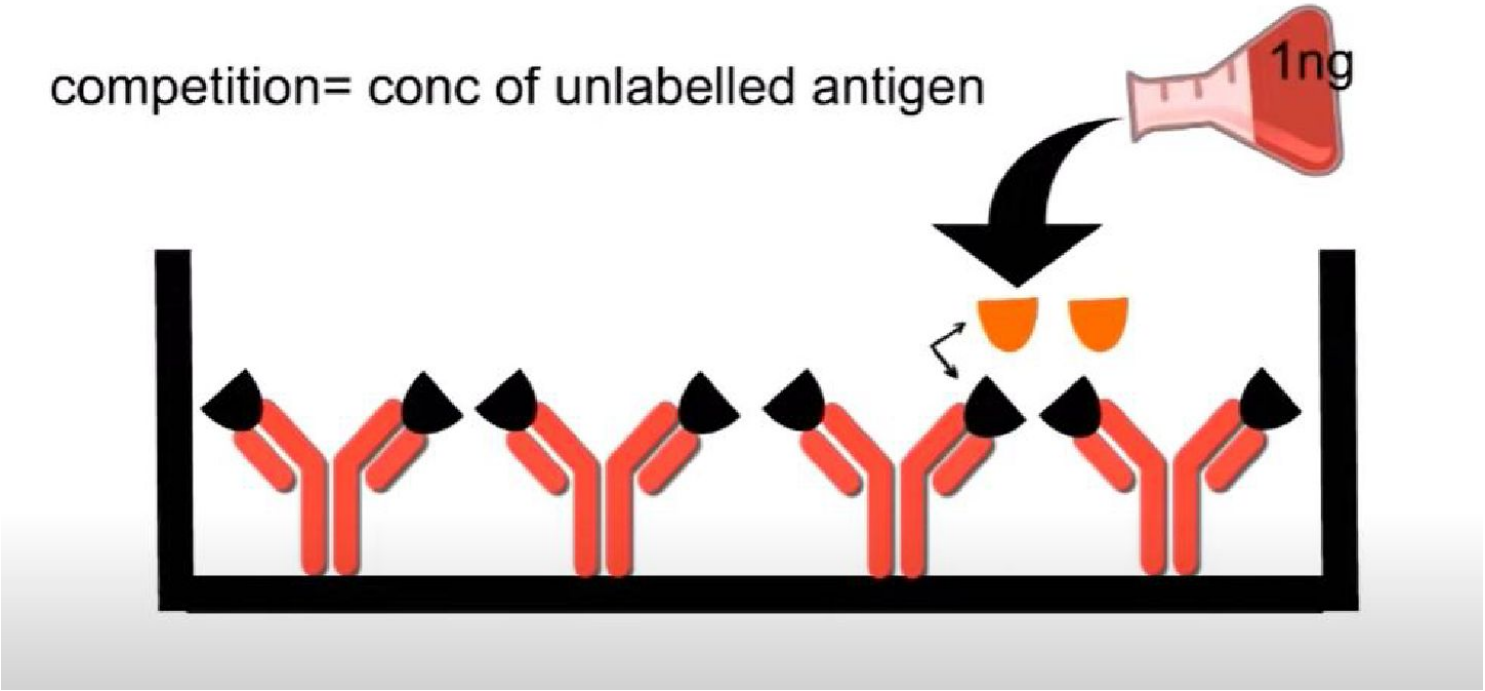


2. Wash to remove unbounded antigens

3. Measurement of radio emission.

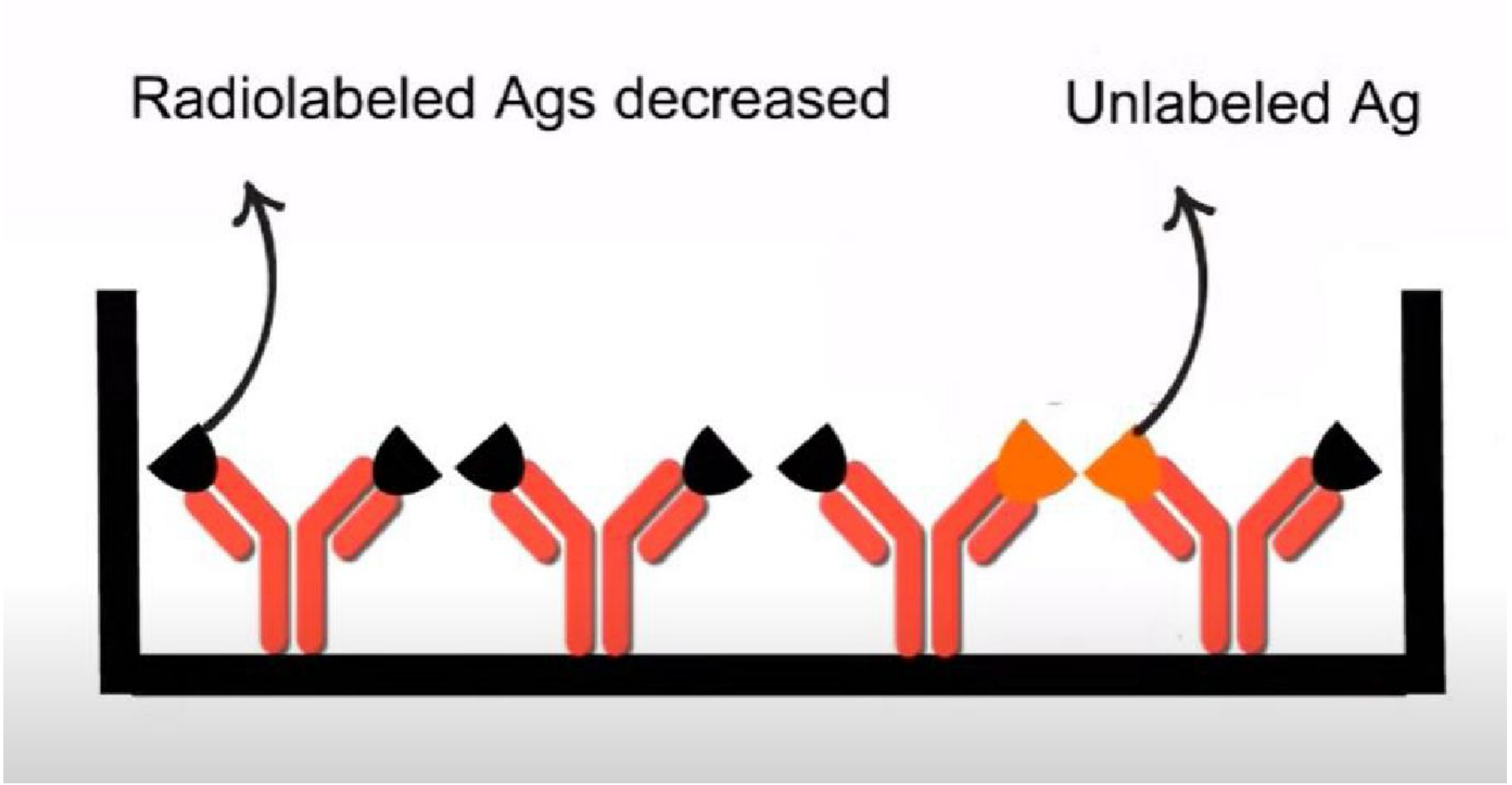


4.Then test samples of an unlabeled antigen of unknown concentration are added in progressively more substantial amounts. so the two kinds of antigen compete for available binding sites on the antibody.



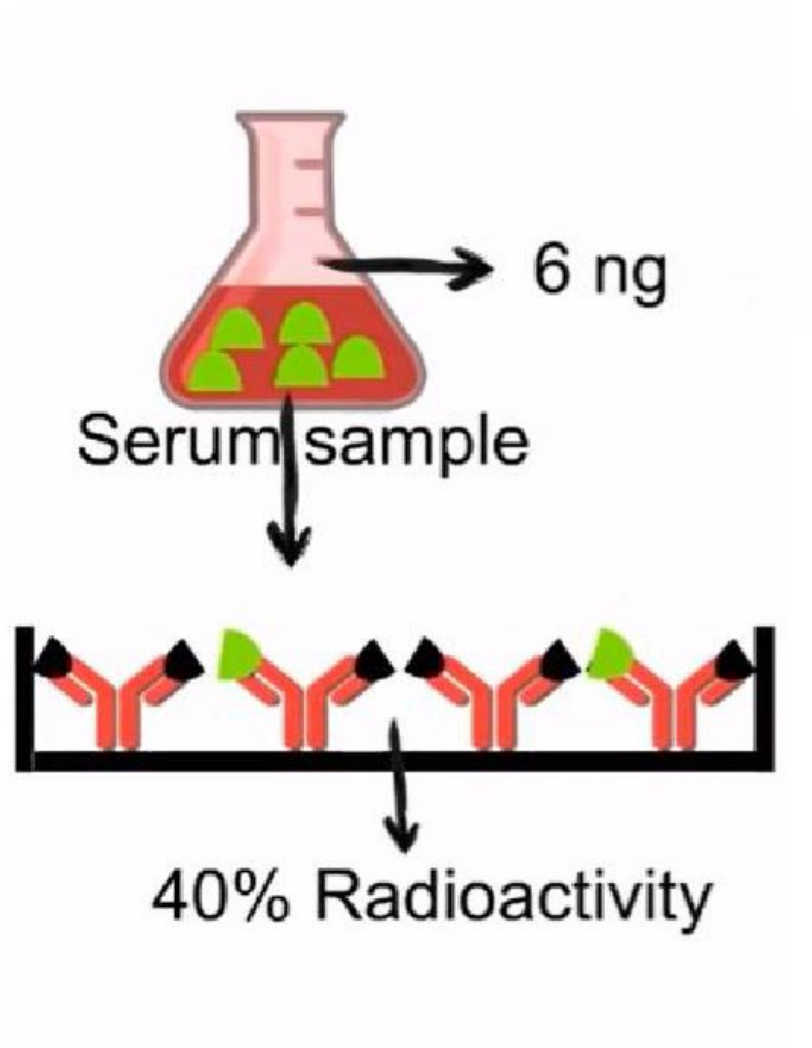
5. Washing

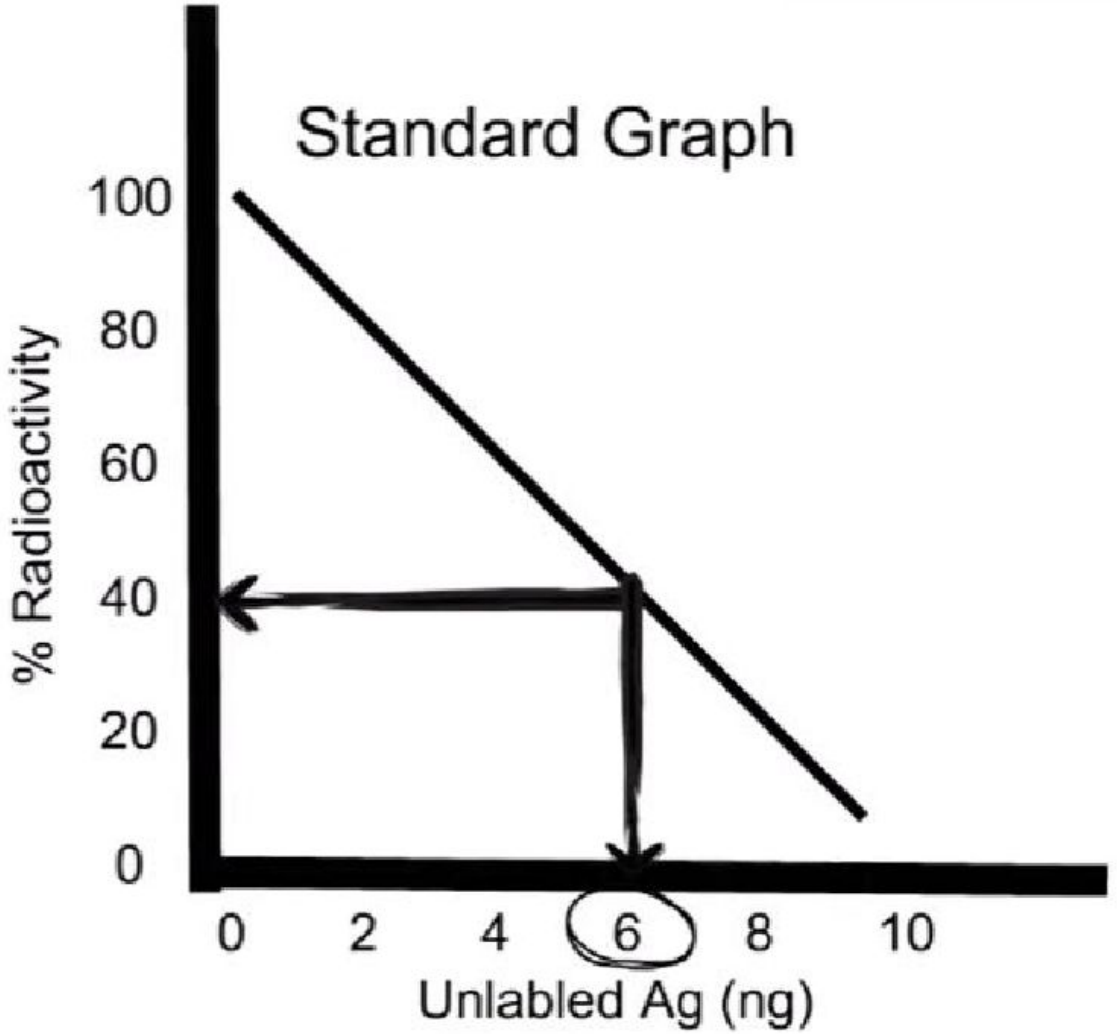
6. Measurements of radio emission for the mixture


Radiolabeled Ags decreased
Unlabeled Ag

7. A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen, and the concentrations of antigen in patient samples are extrapolated from that curve.

Unlabeled Ag (ng)	Radioactivity %
0	100
1	90
2	80
3	70
4	60
5	50
6	40
7	30


6 ng
Serum sample
40% Radioactivity


Standard Graph
% Radioactivity
100
80
60
40
20
0
0
2
4
6
8
10
Unlabled Ag (ng)

Uses of RIA:

1)The test can be used to determine very small quantities (e.g. nanogram) of antigens and antibodies in the serum.

2)The test is used for quantitation of hormones, drugs, HBsAg, and other viral antigens.

3)Analyze nanomolar and picomolar concentrations of hormones in biological fluids.

The main disadvantages of RIA

A) The expense and hazards of preparing and handling the radioactive antigen.

B) Both 125I or 131I emit gamma radiation that requires special counting equipment.

C) The body concentrates iodine atoms- radioactive or not in the thyroid gland where they are incorporated in thyroxin (T4).

Any questions?