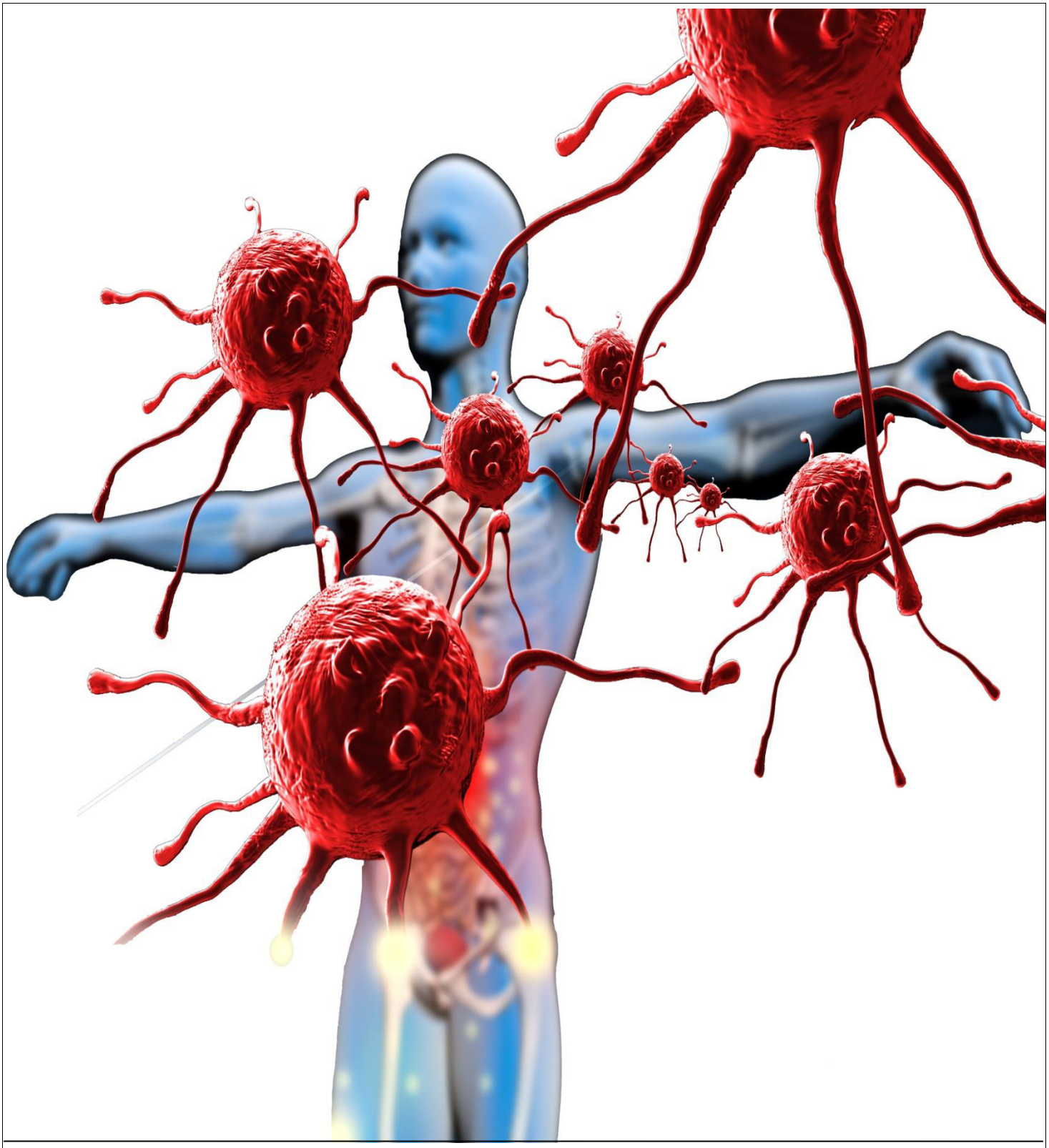


IMMUNOLOGY

Done By: Amani AlHalabi

#7

Dr. Hassan Abu Al-Ragheb

By Mohammed Nawaiseh

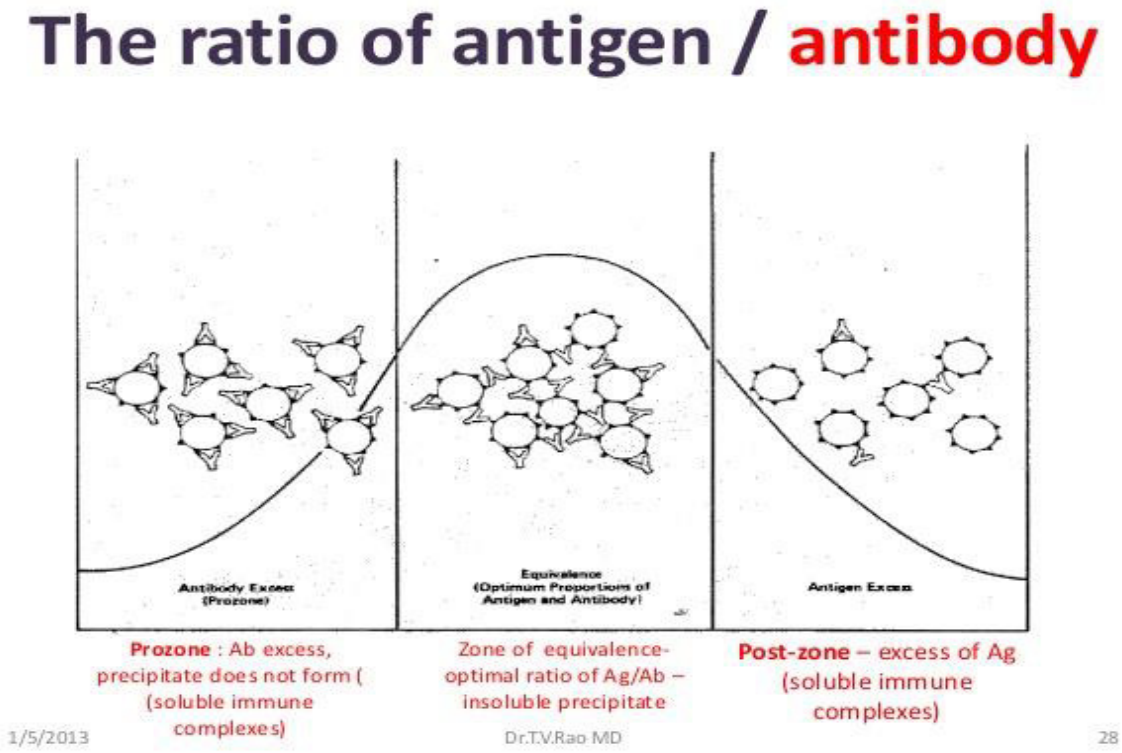
Serology

Ag-Ab interactions occur frequently in our bodies, but here, by saying "Serological reactions", we're more concerned with detection of Ags / Abs/ and their reactions in "serum" in labs.

- One important unique feature of Ag-Ab reactions is their **SPECIFICITY**. *[once you have an Ag, you must have its specific Ab. Always: 1+2=3!]*
- The normal result of Ag-Ab interaction is forming a **precipitate** of these complexes or an **agglutination** reaction.

A. *Precipitations*

- A precipitation precipitates as a result of an Ab *meeting* its soluble specific Ag.
- A precipitation doesn't form at any concentrations of Ags or Abs; there must be *certain concentrations* of these molecules to interact and precipitate.
- If we have excess Abs "Pre-zone"/"Pro-zone" → no precipitation
- If we have excess Ags "Post-zone" → no precipitation
- At the *"zone of equivalence"* (latex formation) , where [Ag] almost = [Ab] → Precipitation occurs (maximally).



- Sometimes you need to dilute either the Ags or Abs to reach the concentrations of the zone of equivalence. **"Serial Dilutions"**
  [Imagine you have many test tubes, each of which contains the same amounts of Ag "X" with the same concentrations. You start adding a serum with Abs at certain concentration to the first tube, then dilute it (1:2) and add it to the 2nd tube, then dilute it (1:4) in the 3rd , etc.
  You keep diluting the serum and Abs by known percentages, until a *precipitation* is formed in a tube; you conclude here that these are the concentrations of the zone of equivalence for both Ag and Ab; **"The titter of the serum":** the Ab dilution at which the Ag is detected. Before the precipitation, you were in the "pre-zone" where the Ab is found in excess. Also after the occurrence of the precipitation expect to have again some negative results later on when the Ags become more than the Abs; "post-zone".]

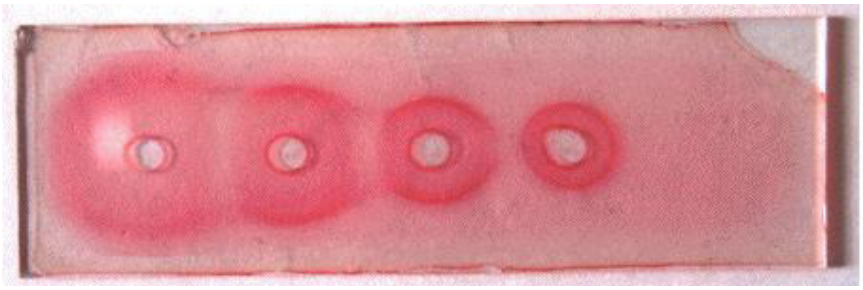
- Precipitations are very difficult to be seen by naked eye in a liquid solution, that's why we resort to special instruments (quick+expensive) to detect these precipitations formed in the test tubes; we actually expose the tube to light, some of the light will be transmitted, and some will hit the immune complexes in the solution and be scattered (deviated). We either measure the transmitted or the scattered light and count their ratio to the original light to see if there are many immune complexes (more scattered) or not, by plotting all results to a chart with certain standards.
  - If we measure the transmitted light → **Turbidimetry**
  - If we measure the scattered light → **Nephelometry** (the dr says nephalo)

  ➔ These 2 tests are quantitative & qualitative; they measure the light and the amount of Ag or Ab we're looking for.

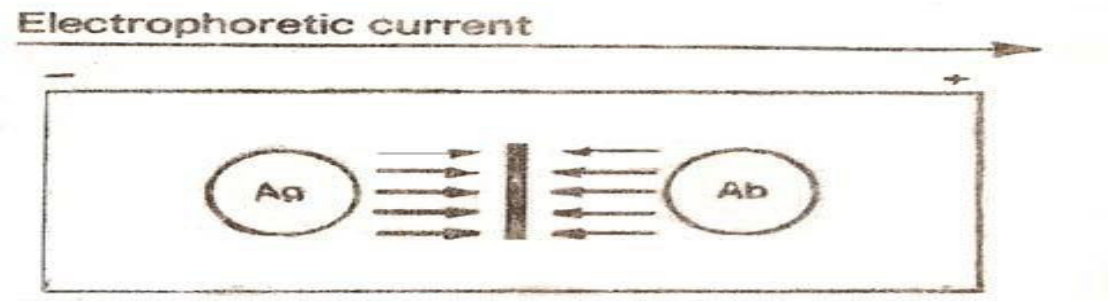
- Precipitations can be detected manually and by the naked eye only if they are formed in semi-solid mediums. Using a slide of glass covered with *agarose*, put the Abs and Ags in different holes in the agarose layer, leave it for about 24 hours, the Ags and Abs will diffuse in the medium to a point where their concentration are in the *zone of equivalence* and

they will precipitate there. You don't need to dilute the solutions here, they are diluted by themselves by diffusion, **"Double Diffusion"**.

- Ex: [If you put the Ags and Abs in certain concentrations and get the line of precipitation in the middle between them, then you decreased the concentration of Abs and did the experiment once again, you'll notice the line of equivalence shifted more close to the Abs hole; the Ags had to be diluted and diffused more toward the new decreased concentration of Abs.]



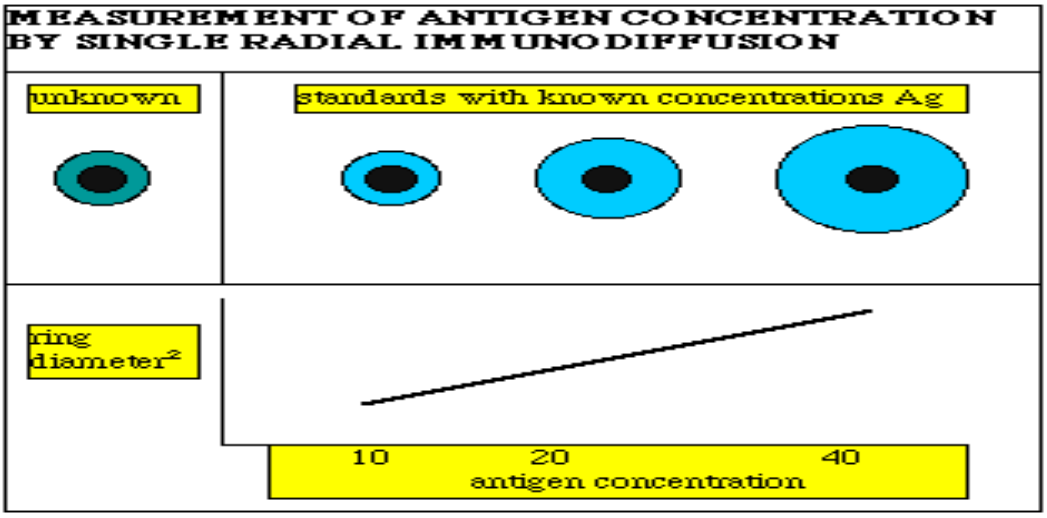
- In some cases, like meningitis, you cannot wait for 24 hours to get the results from lab, you need them within a couple of hours. You use the **"Counter-current immuno-electrophoresis"**; You examine the CSF to detect the presence of bacterial Ags by adding Abs against them in the media with electrical current and balancing pH to control them to move in the proper direction.



➔ The previous 2 tests are considered qualitative, not quantitative; they indicate the presence of the relevant Ag or Ab. *But we can modulate the experiment and use the electrophoresis to measure the quantity, and it will become quantitative too,* By bringing the agarose layer mixed uniformly with the Abs (now concentrations of Abs are constant in any side of the layer), then applying the Ags which will diffuse within 24 hours and precipitate in a circular manner along the suitable concentrations (zone of equivalence).

[The greater the [Ag], the bigger the circle; needs more area to be diluted enough.
REMEMBER: Area of circle = 14.3* r^2 , and we can measure the diameter/2 instead

of measuring the r with is difficult sometimes. When you plot the different concentrations with their corresponding "r"s, you will get a straight line →

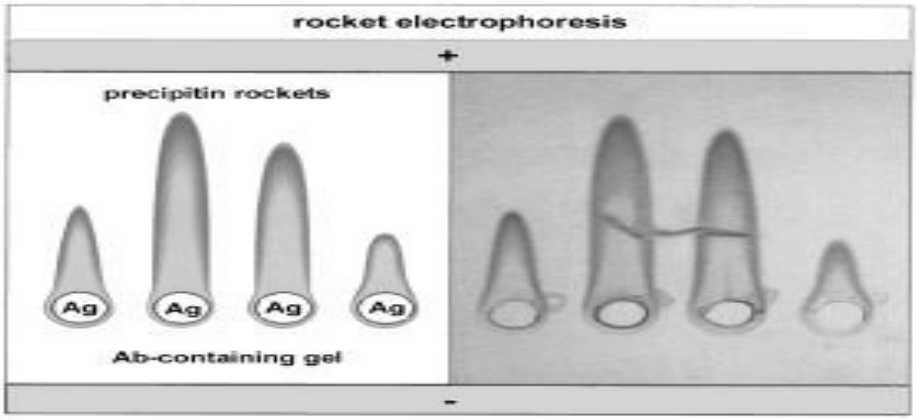


This is the: **"Single radial immunodiffusion"** [single: one element is moving / radial: radially producing a circle]

➔ This test is qualitative (the appearance of circles) and quantitative too.

- If you need the result within few hours: use **"Rocket electrophoreisis"**; you need to expose the reactants to an electrical current, and start at neutral pH to assure that Abs are not moving initially, then you assign standards, and apply the current, the current will affect the Ags and make them migrate toward the cathode for example. You will finally measure the distance travelled by Ags before their stopping at certain points, and relate these distances to their corresponding concentrations to know those of the zone of equivalence. When plotting the distance vs. concentrations → straight line!

➔ Quantitative method.



B. Agglutinations

- A particulate Ag (epitope) which agglutinate with an Ab.
- More sensitive than precipitations, and can be seen by naked eye. (no special instruments)
- Easily done, like in blood group detection; when you mix the RBCs of "A" Ags, with a serum containing Anti-A antibodies → the cells clot together, are cross linked, agglutinate, and you can see that agglutination.
- You need to mix them in concentrations of the zone of equivalence.
- You can use the "Serial Dilution" to identify the titter of the serum.

- Another example:
  - ➔ Widal test: looking for Abs against **Salmonella Typhi** Ags in the serum of the patient.
    [We dilute the serum, then add some Ags; if we find the titter of the serum significantly elevated (eg: 1/320), this indicates the presence of the infection. But if it was, for example, = 1/8, this is not always significant, because in areas with endemic salmonella typhi, such results may be normal due to past infections or other factors, so we wait for few days-week, then do another test with the same amount of Ags, if the titter were found to be = 1/128, this indicates that the concentration of Abs has increased as a result of true ongoing salmonella infection and the body is producing more Abs.]
  - ➔ This is known as "Rising Titter"; a titter should be increased by 4 folds (2 tubes) or more to be considered significant.
    (less than 4 folds may be a result of experimental error)
- Other infections like **Brucellosis** can be measured by the same method.
- Direct Agglutination: the epitopes involved are actual parts of the particles; like "A" or "B" Ags on RBCs, they're actually parts of the cellular membranes, or like an epitope on the surface of bacteria.
- Passive Agglutination: the epitopes are introduced to the cells or particles, e.g: Latex particles (can be saved for long time (stable) and used later on, unlike RBCs which die in a short time.), not parts of the particles themselves.

➔ One example is the pregnancy test; when you buy a strip from the pharmacy and expose it to the urine of a lady, the Ags (hCG hormone in the urine) if present, will agglutinate with the Abs introduced on the strip on Latex particles (Anti-hCG antibodies) → a pregnant lady ! ☺

[Pay attention to that you can either introduce the epitope or the Ab on the latex particles.]

Introduction to Coomb's test (Anti-Globin test)

- Used widely in blood banking, and in detection of haemolytic diseases of newborns.
- IgM, "Complete Ab", is a big molecule with many Ag-binding sites; easily cross links Ags.
- Agglutination of RBCs (negatively charged = repulsion, gap) with IgM molecules is easier than doing so with IgG molecules; IgG cannot cross link Ags on cells.
- IgG is considered an "Incomplete Ab"; it sensitizes the RBC (adheres to it) but doesn't induce agglutination due to the presence of large gaps.

➔ Remember: 3 main steps:

1. Sensitization of RBCs (to have the Abs on their surfaces)
2. Cross linking
3. Agglutination

What to do in order to cross link Ags using IgGs?

- We can produce Abs against the Fc fragment of IgGs, these Abs when introduced to the IgGs within Ags, they will cross different IgGs together allowing them to pull their Ags closer → Agglutination

That’s why it's called: Anti-Globin test; we're using Abs against Globins (Igs)

➔ One last example: Rhesus (D) Ag on RBCs;
The problem arises when a –ve D mother gets pregnant of a +ve D baby, after childbirth, some +ve Ags may pass to her blood from the baby, inducing an immunization (Abs) against +ve D Ags in her body of the type IgG → Can cross the placenta and affect the 2nd baby, causing phagocytosis of fetal cells by splenic macrophages, haemolytic anaemia of excess degradation!

- To test for Rhesus incompatibility diseases, you either test the:
  1. Sensitization of Fetal cells [Direct]
  2. Serum of the mother [Indirect]

& these will be discussed in lecture 8 إن شاء الله (:
