ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

- Description: It is a plate-based assay technique used to investigate the presence of antigens (Ag) in the suspicious material or antibodies (Abs)against them, using enzymes labeled antibodies (conjugate) and substrate.

Why do we use ELISA?

- According to the purpose, to detect Antibody (Ab) or Antigen (Ag)

- It is Very Sensitive test

What we need to perform ELISA?

- ELISA plate (polystyrene, polyvinyl vb)
- Conjugate
- Substrate
- Virus (known or suspicious)
- Sera (known or suspicious)
- Test solutions
  - Positive and negative controls
  - Detection solutions
  - Stop solutions
  - Wash buffer
- ELISA reader

https://www.gbo.com/en-es/spotlight/article/an-introduction-to-elisa-protocol

ELISA plate reader
Definition, Principle, Procedure, Application, Advantages

Science Info

https://scienceinfo.com/elisa-plate-reader-definition-principle-procedure-application-advantages/

Conjugate

- Definition: Enzyme labeled antibody.

  (Marker + Antibody)

- The marker enzymes used in the ELISA test are peroxidase, alkaline phosphatase, glucose oxidase, B-galactosidase, etc.
- The most frequently used markers are peroxidase and alkaline phosphatase.

ANTIBODY IN CONJUGATE;

- It is specific for the virus.

  E.g., if the ELISA test is performed to investigate the presence of the "A" antigen, the antibody is against the "A" antigen

- It is specific to the primary antibody.

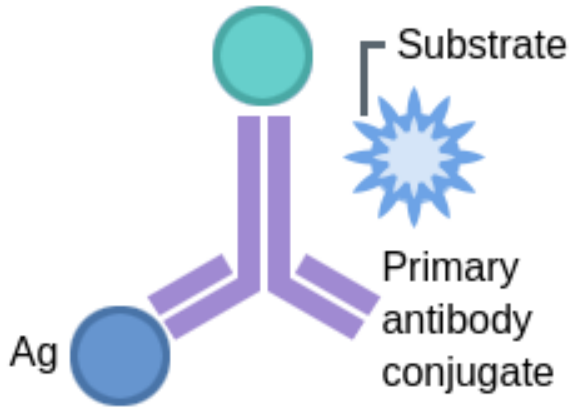
  E.g., if the presence of an antibody is sought in bovine serum, anti-bovine IgGs are in the structure of the conjugate.

➢ The conjugate was prepared against the antigen in the direct ELISA test and against the primary antibody in the indirect ELISA test.

Substrate

- It reacts with the enzyme of the conjugate structure to reveal the formation of antigen-antibody complexes.

- As a result of the reaction, blue, green and yellow products are formed according to the substance in the structure of the substrate.



Direct ELISA

https://theory.labster.com/types-elisa/

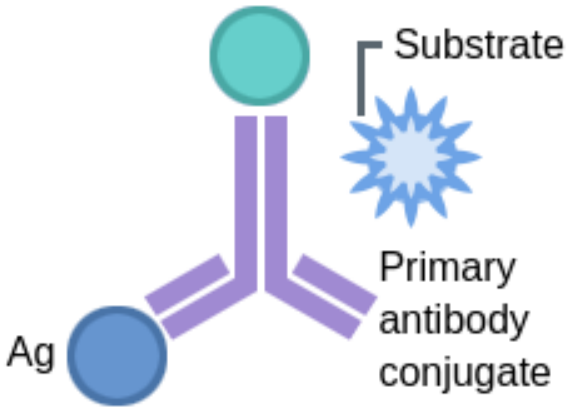
Conjugate-Substrate Correlation

- **Conjugate+Substrate = COLOR**
- **Peroksidase-** OPD - Orange

  5-Aminosalicylic acid - brown

  Orthotolidine- blue

  TMB- blue
- **Alkaline Phosphatase -** nitrophenil fosfat- yellow



Direct ELISA

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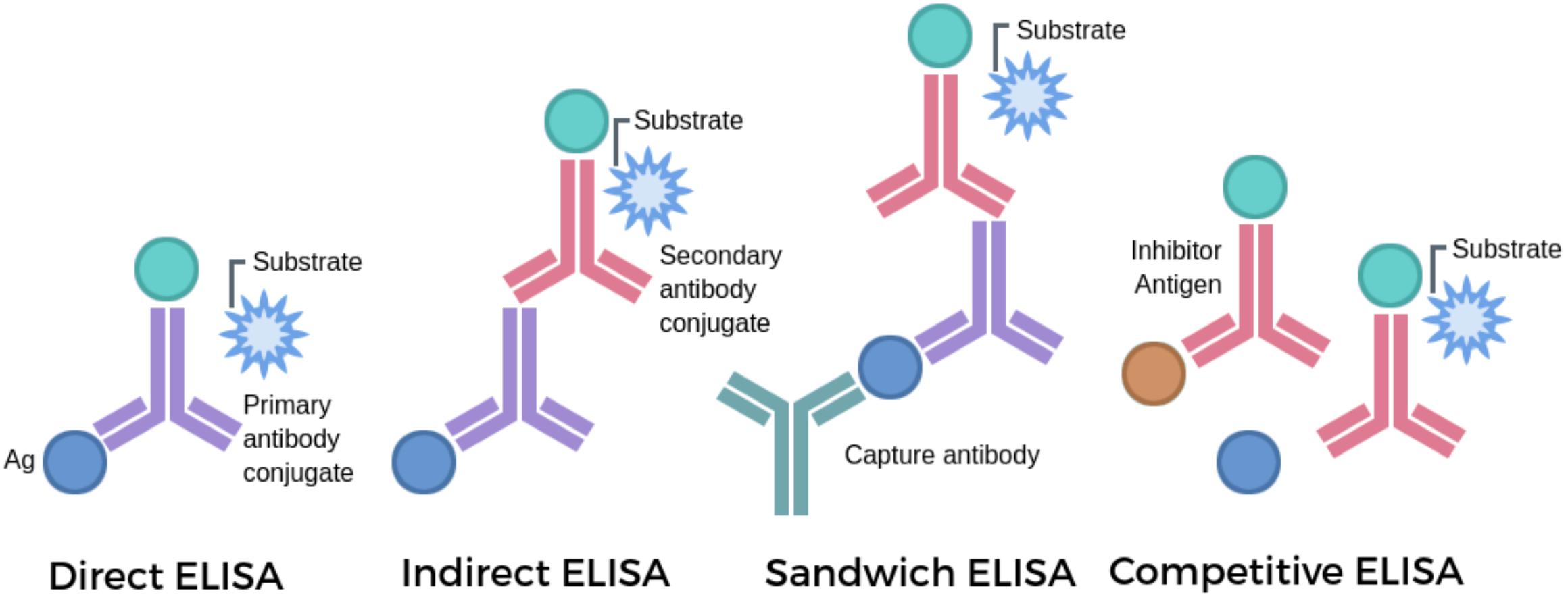
Types of ELİSA



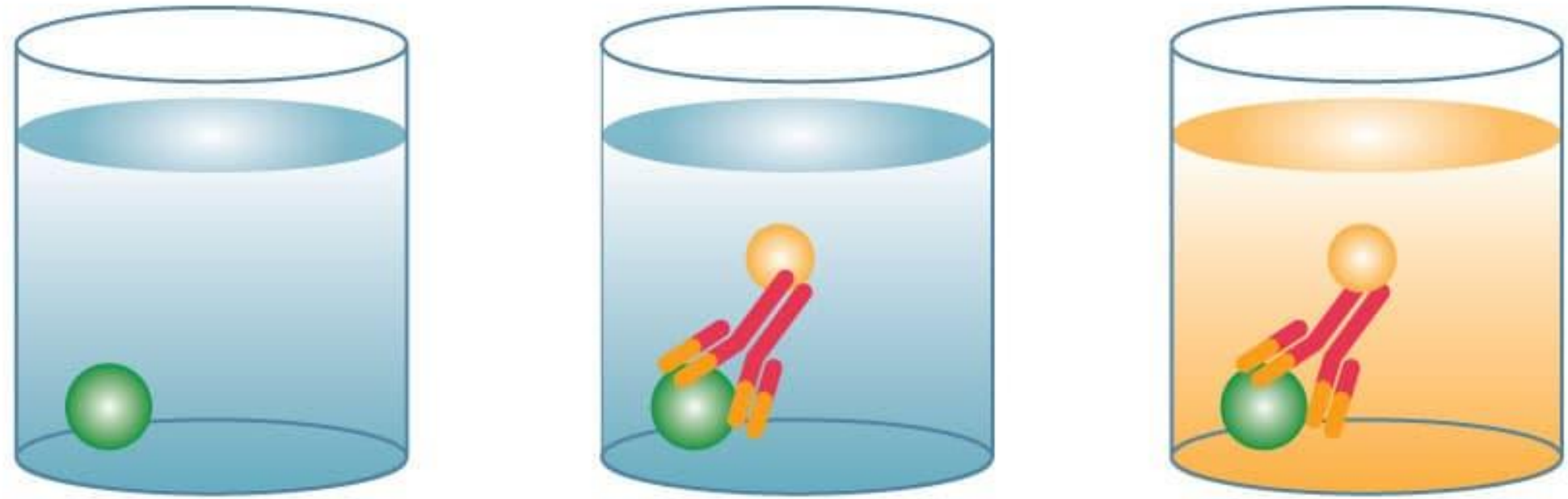
https://theory.labster.com/types-elisa/

Direct ELISA

- Antibody-coated 96-well microplate which aims to detect antigen
- The essence of the method is to examine the presence of the antigen using antibodies specific for the suspected antigen coated on an ELISA tablet.
- In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein.
- Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample.
- Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA.

https://www.rndsystems.com/resources/what-is-an-elisa-and-elisa-types#:~:text=In%20a%20direct%20ELISA%2C%20an,of%20analyte%20in%20the%20sample.

1. Add samples to wells.
2. Cover plate and incubate at room temperature for 1-2 hours (according to test).
3. Discard the liquid.
4. Wash the wells with wash solution 3-5 times.
5. Add conjugate to wells.
6. Cover plate and incubate at room temperature
7. Discard the liquid.
8. Wash the wells with wash solution 3-5 times.
9. Add substrate to each well.
10. Cover plate and incubate at room temperature (in a dark place)
11. Add the stop solution to each well. The solution in the wells should change from blue to yellow.
12. Read the absorbance of each well at 450 nm or 550 nm. (according to test)



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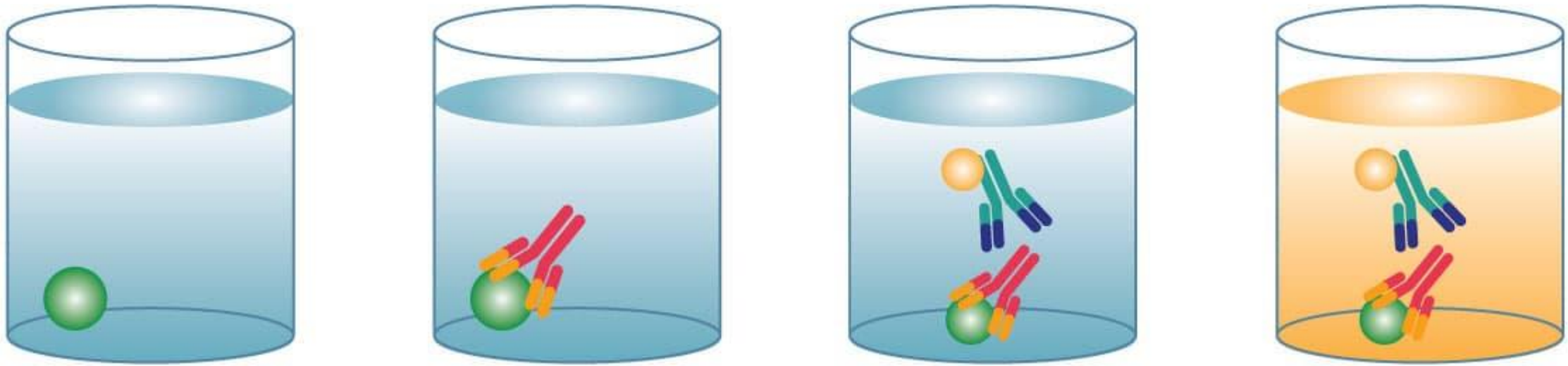
Indirect ELISA

The method is used to measure the detection of antibodies in the suspected serum.

The tablet is coated with known antigen.

1. Add suspected blood serum.
2. Cover plate and incubate at room temperature for 1-2 hours (according to test).
3. Discard the liquid. Washing step
4. Add the conjugate. (Anti-Antibody)
5. Cover plate and incubate at room temperature for 1-2 hours (according to test).
6. Discard the liquid washing step
7. Add the substrate.
8. Cover plate and incubate at room temperature
9. Add the stop solution to each well
10. Read the absorbance

NOTE: Washing is carried out after every hour, after the appropriate period of incubation.



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Indirect ELISA

1. Antigen/sample is added to plate.
2. Blocking buffer is added to block remaining protein-binding sites.
3. Next a suitable ***primary antibody*** is added.
4. A suitable ***secondary antibody – HRPO conjugate*** is then added which recognizes and binds to the primary antibody.
5. TMB substrate *(Leinco Prod. No. T118)* is added and is converted by HRPO to detectable form.

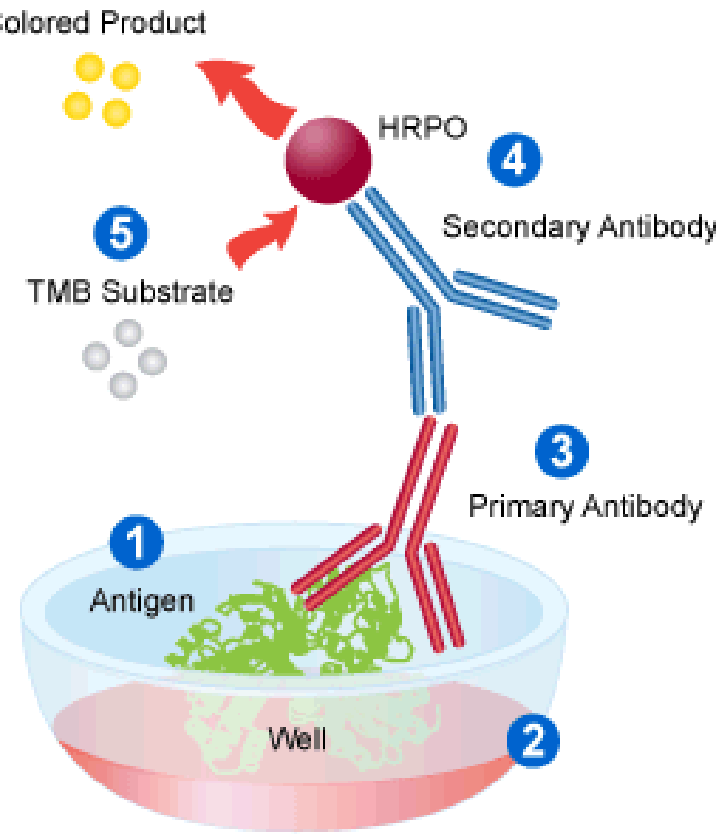

Diagram 1: *Illustration of Indirect ELISA method.*

https://www.leinco.com/indirect-elisa-protocol/