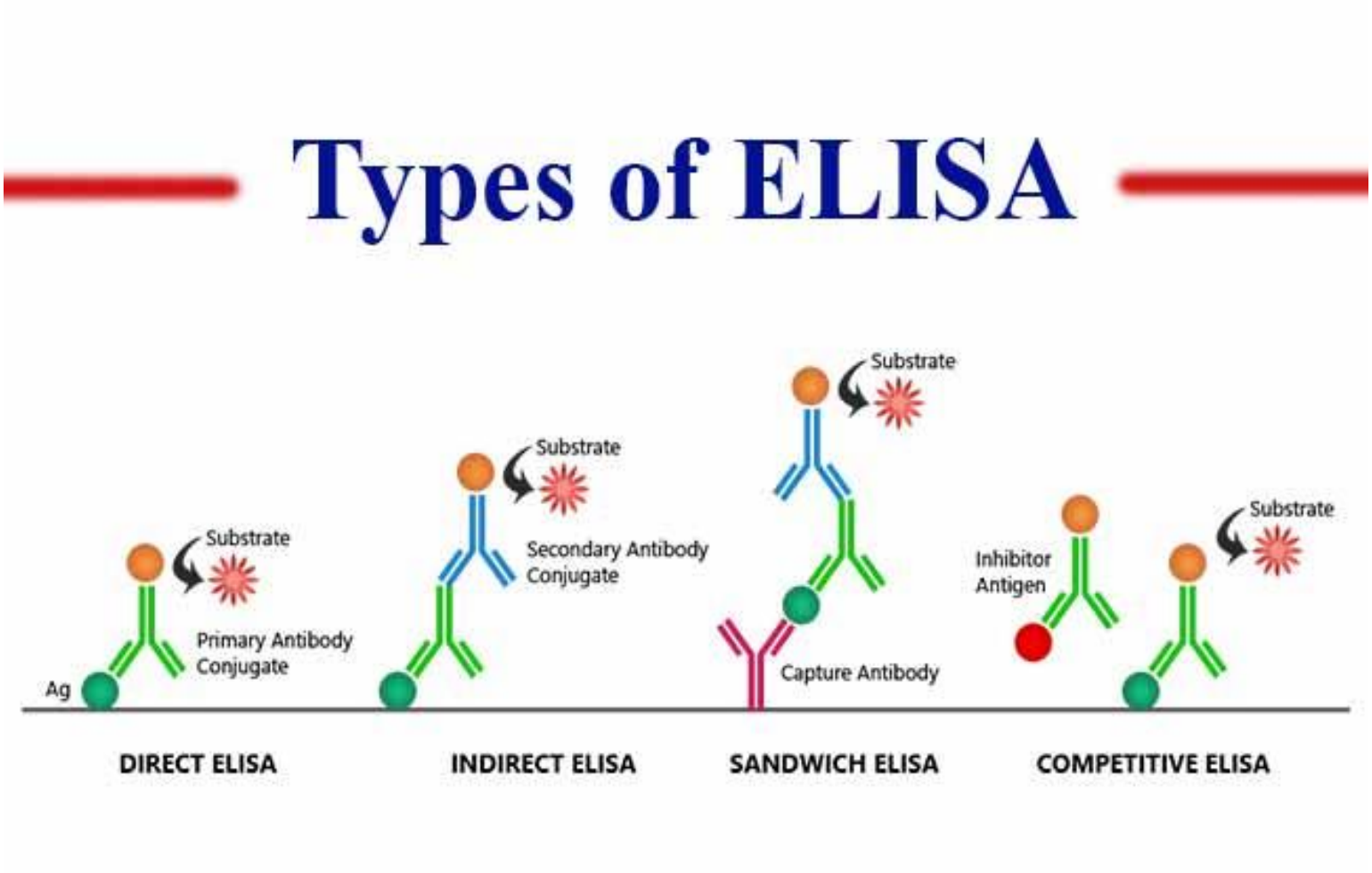
ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

- Can Be Used To Detect Both Antibody and Antigen
- Very Sensitive, pg/mL

- Different Types
  - Sandwich
  - Direct
  - Indirect
  - Competitive

http://immunologynotes.com/enzyme-linked-immunosorbent-assay-elisa/



Conjugate

- **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

- Detection is specific for the agent (eg if the ELISA test is performed to investigate the presence of the "A" antigen, the antibody is against the "A" antigen)
- The primer is antibody-specific. (For example, if there is an antibody present in bovine serum, the anti-bovine IgGs are found in the conjugate structure)
- Direct ELISA test against conjugate antigen, indirect ELISA test against primer antiserum.

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, yellow, etc. according to the structure of the chromogen material in the structure of the substrate. Colors occur in products.

Methods

- Direct ELISA (for Ag detection)
- Indirect ELISA (for Ag or Ab detection)

MATERIAL

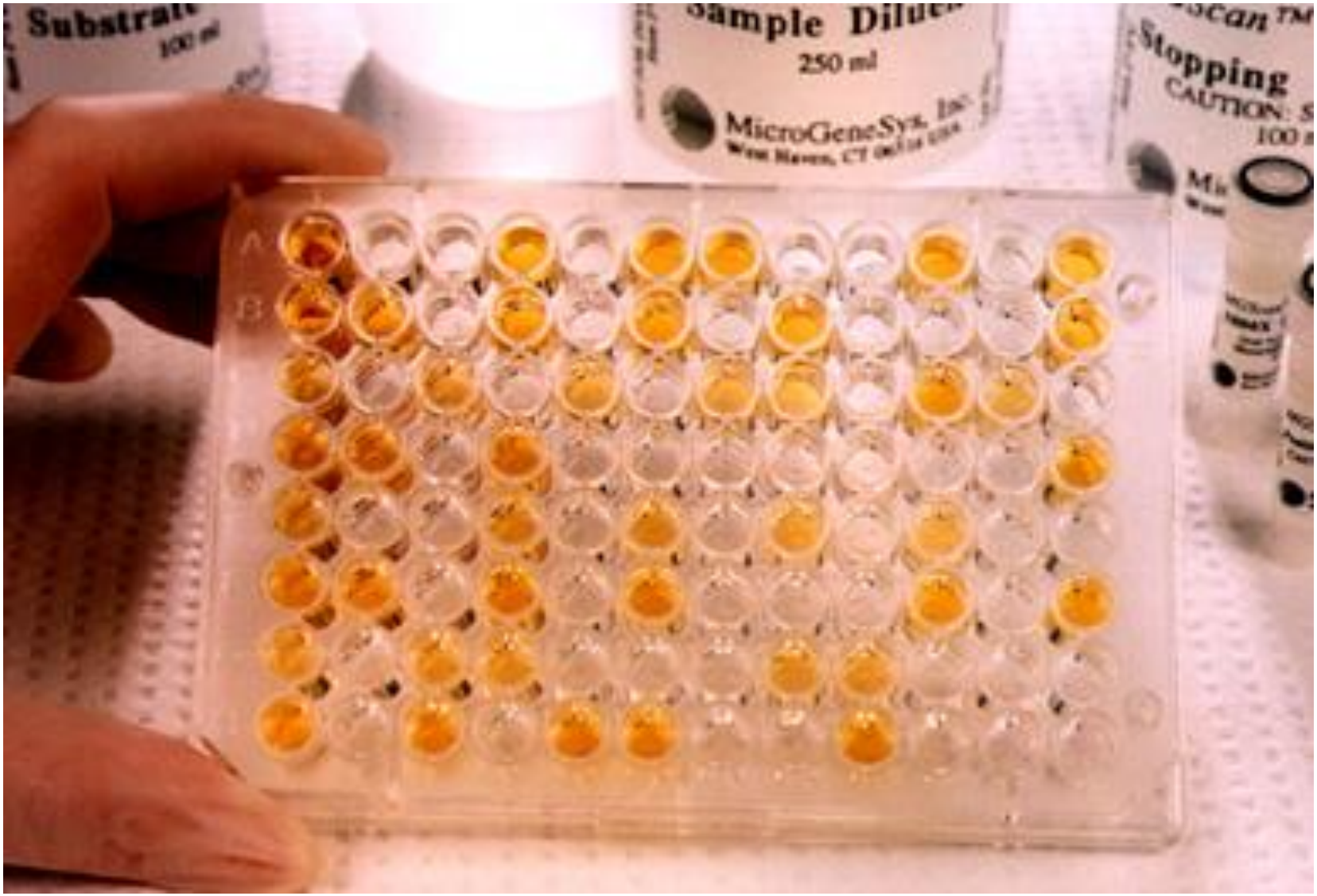
- 96-well microplate
- Detection antibody (usually biotinylated)
- Standard
- HRP conjugate (antibody or streptavidin)
- Diluent buffers
- Wash buffer
- Chromogenic substrate (usually TMB)
- Stop solution
- Plate covers

DIRECT ELISA

- Antibody-coated 96-well microplate which aims to detect antigen

Method

- Add sample to wells. Cover plate and incubate at room temperature for 2 hours.
- discard the liquid.
- Wash wells.
- Add conjugate to wells. Cover plate and incubate at room temperature
- discard the liquid.
- Wash wells 4 times.
- Add substrate to each well.
- Develop plate at room temperature in the dark
- Add 100 µL of stop solution to each well. The solution in the wells should change from blue to yellow.
- Read the absorbance of each well at 450 nm and 550 nm.


Substrat
100 ml
ample Dilue
250 ml
MicroGeneSys, Inc.
Stopping
CAUTION:

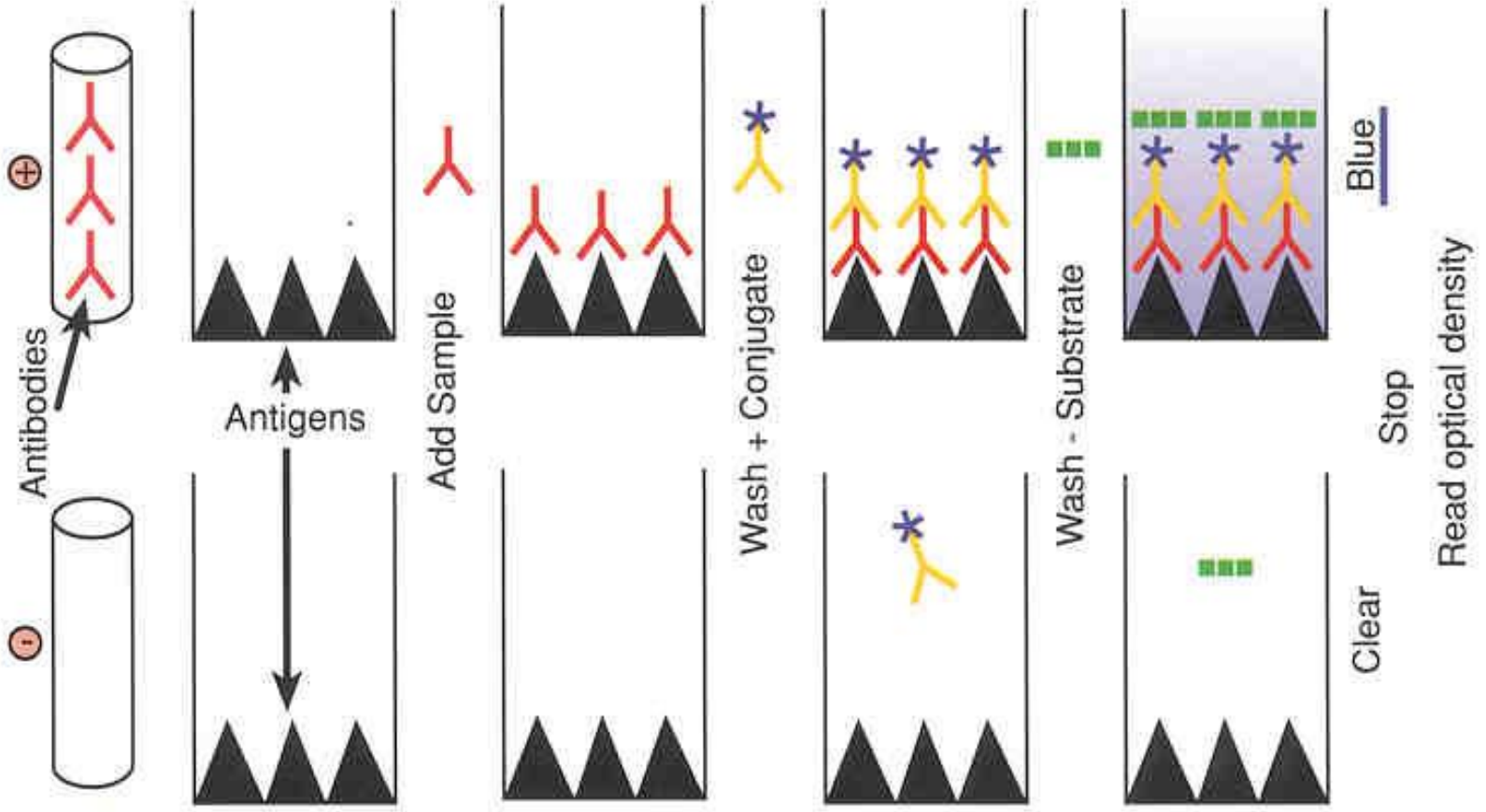
Indirect ELISA

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

method

- 1. The tablet is coated with known antigen.
- 2. Add suspected blood serum.
- 3. Add the conjugate. (Anti-Anticor)
- 4. Place the substrate.
- NOTE: Washing is carried out after every hour, after the appropriate period of incubation.

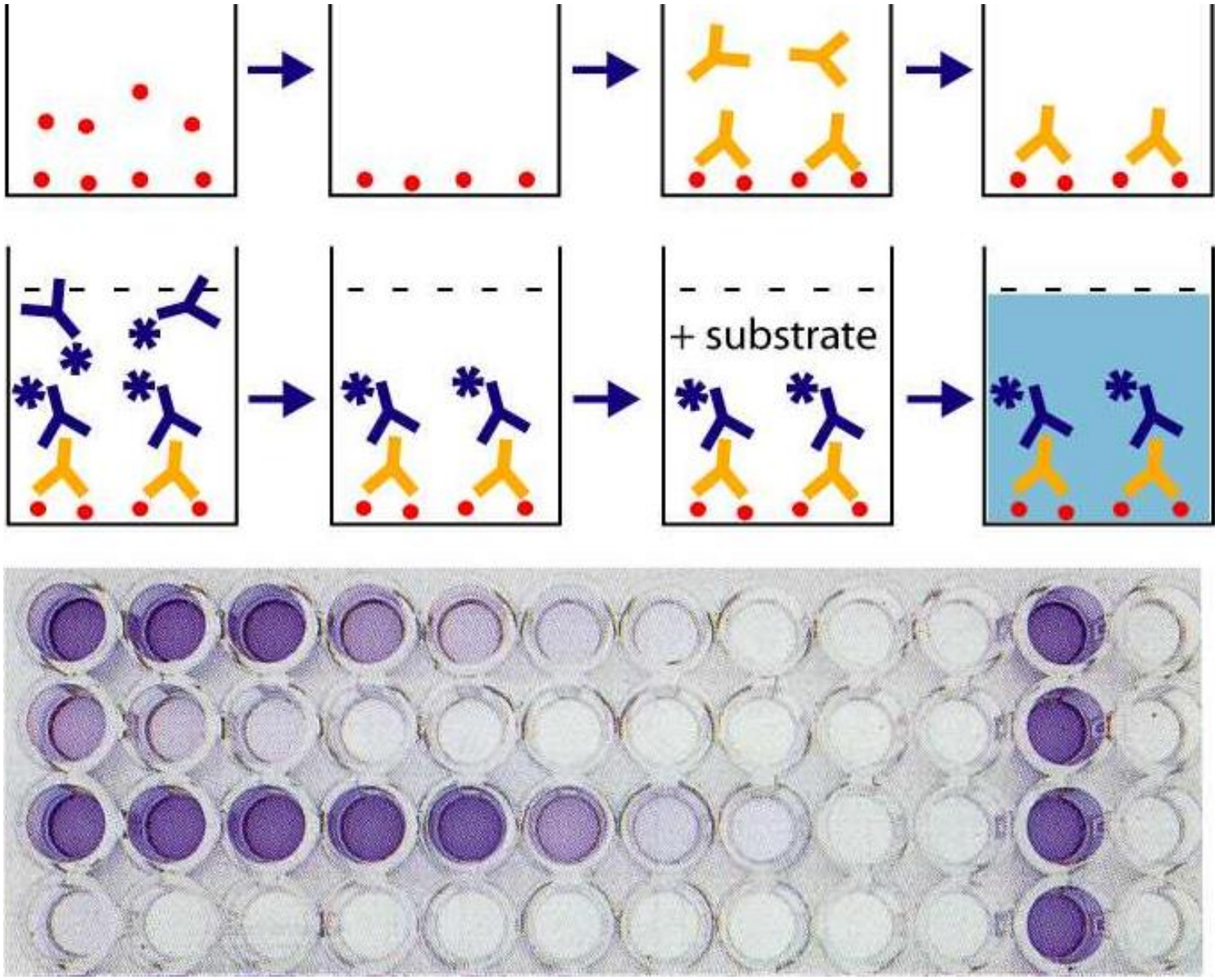
Diagram of steps in an Indirect ELISA



IDEXX PRODUCTION ANIMAL SERVICES

1-800-548-9997 (Livestock)
1-800-943-3999 (Poultry)

09-62241-00


+ substrate