Molecular Biology Research Unit

Principals of ELISA

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SEROLOGY:

SCIENTIFIC STUDY OF SERUM AND OTHER BODY FLUIDS.

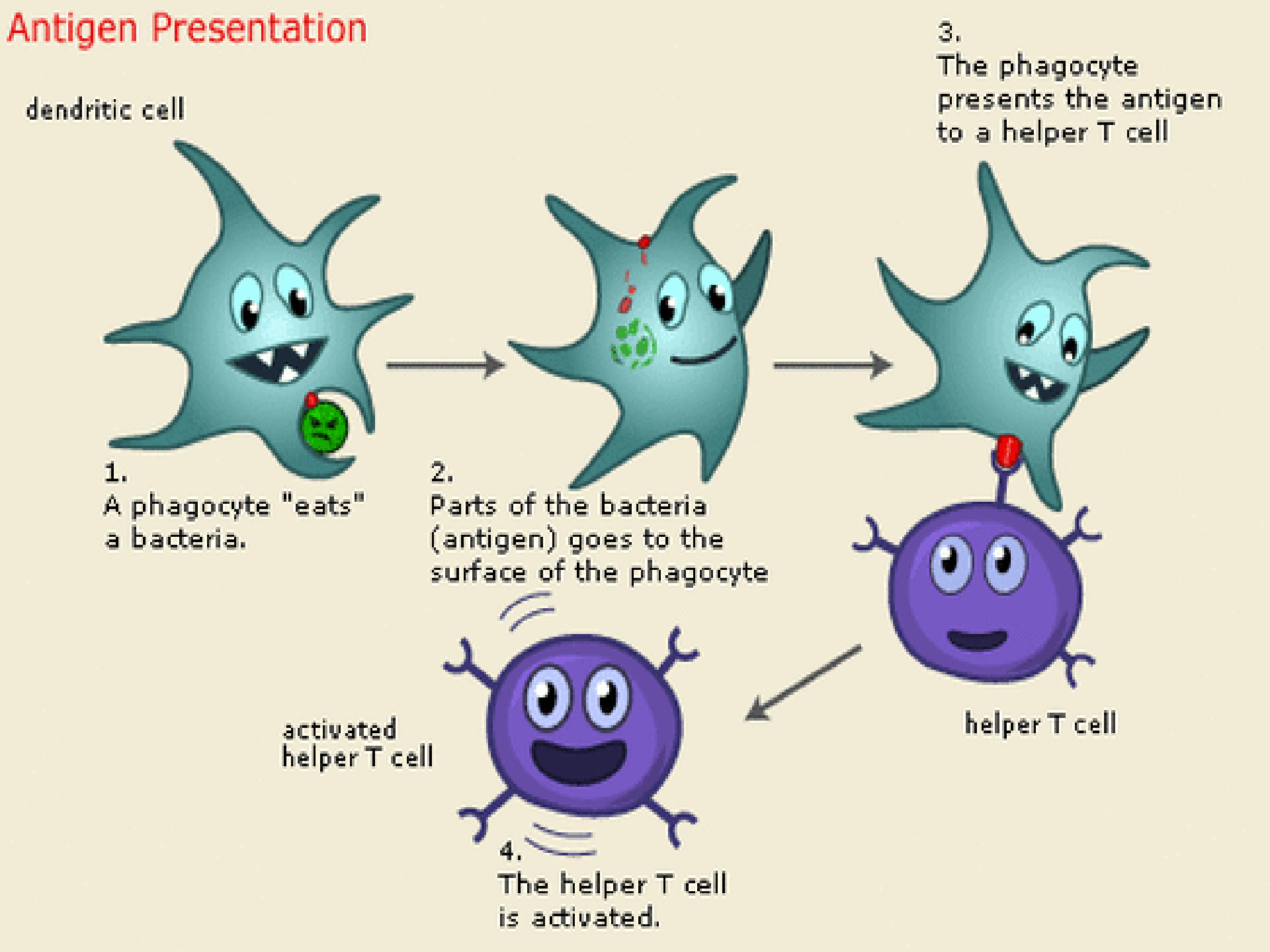
IN PRACTICE:

SEROLOGICAL TEST USUALLY REFER TO
THE DIAGNOSTIC IDENTIFICATION OF ANTIBODIES IN THE SERUM.

Serological test is a reaction between

Ag + Ab

Antigen Presentation



DIRECT SEROLOGICAL TEST
→ DETECT ANTIGENS

INDIRECT SEROLOGICAL TEST
→ DETECT ANTIBODIES

Classified into Three main categories:

A- Tests that directly measure the binding between Ag and Abs→ ELISA, Radio immune assay, FA, IFA called *Primary tests*.

B- Tests that detect the results of Ag and Abs binding (precipitation test, agglutination test, complement fixation test, neutralization test) called *Secondary binding test.*

C-Test that detect the quantity of Abs → e.g. Tube agglutination test (primary test) or (secondary tests).

ELISA test

Enzyme Linked Immuno-Sorbent Assay

History

Before the development of the ELISA, the only option for conducting an immunoassay was radioimmunoassay, using radioactively labeled antigens or antibodies, because potential health hazards of these materials, a safer alternative was sought.

In 1971, Peter Perlmann and Eva Engvall at Stockholm University in Sweden, and Anton Schuurs and Bauke van Weemen in the Netherlands independently published papers that synthesized this knowledge into methods to perform EIA/ELISA.

History

Nowadays

In 2012 an ultrasensitive, enzyme-based ELISA test using nanoparticles as a chromogenic reporter was able to give a naked-eye color signal.

A blue color appears for positive results and red color for negative.

This detection only can confirm the presence or the absence of Ag/Ab not the actual concentration.

Materials needed in ELISA Testing

1. **ELISA Readers:** Readers need to have appropriate filter (650 nm and 450 nm).
2. **Pipette:** Are available as fixed as well as adjustable volume as well as single channel and multi-channel.
3. **Washing system:** It can be manual system that washes one row or column at a time or semi automated systems that wash one strip or plate at a time or fully automated systems that can process multiple plates.

Reagents needed for the testing:

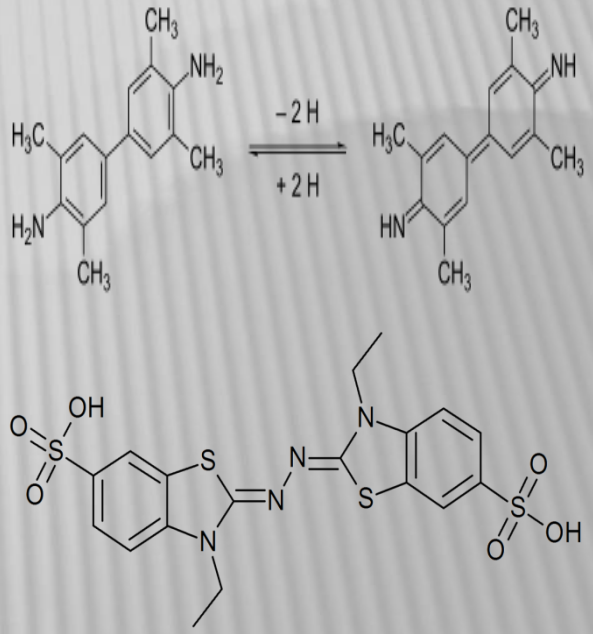
1. **Coated plates:** The 96-well plates are made of polystyrene and coated with either inactivated antigen or antibody. The function of the plate has to hold the immobilized either antigen or antibody.
2. **Controls:** Negative and positive controls are provided in each kit. The controls help to normalize or standardize each plate. Controls are also used to validate the assay and to calculate sample results.
3. **Conjugates:** ELISA conjugates are enzyme labeled antibodies that react specifically to plate bound sample analytes.

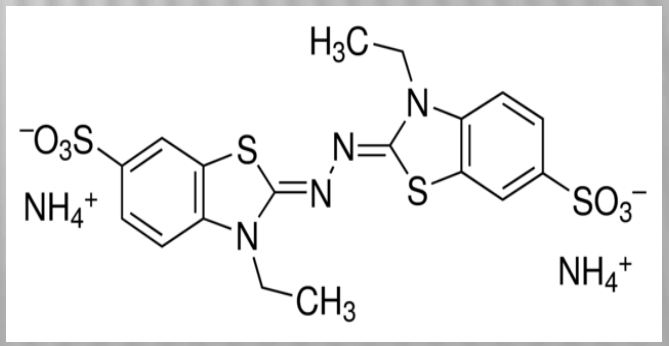
Reagents needed for the testing:

4. **Washing buffer:** It acts as a buffered solution containing detergent to wash unbound material from the plate.
5. **Substrate:** 3,3',5,5'-Tetramethylbenzidine or TMB is a chromogenic substrate used as a visualizing reagent in ELISA. TMB is a white crystal powder that forms a pale blue-green liquid in solution with ethyl acetate. TMB is degraded by sunlight and by fluorescent lights.
6. **Stop solution:** It stops the enzyme substrate reaction and color development (0.16M sulfuric acid/TMB).

Types of substrates:

- **TMB: 3,3’,5,5’-Tetramethylbenzidine**
- **ABTS: 2,2′-Azino-bis (3,ethylbenzothiazoline-6-sulfonic acid).**
- **OPD: (O,phenylenediaminedihydrochloride).**





KPL

Comparison of ABTS, TMB, and OPD Peroxidase Substrate Systems

Figure 1: Substrate Performance Before Reaction is Stopped

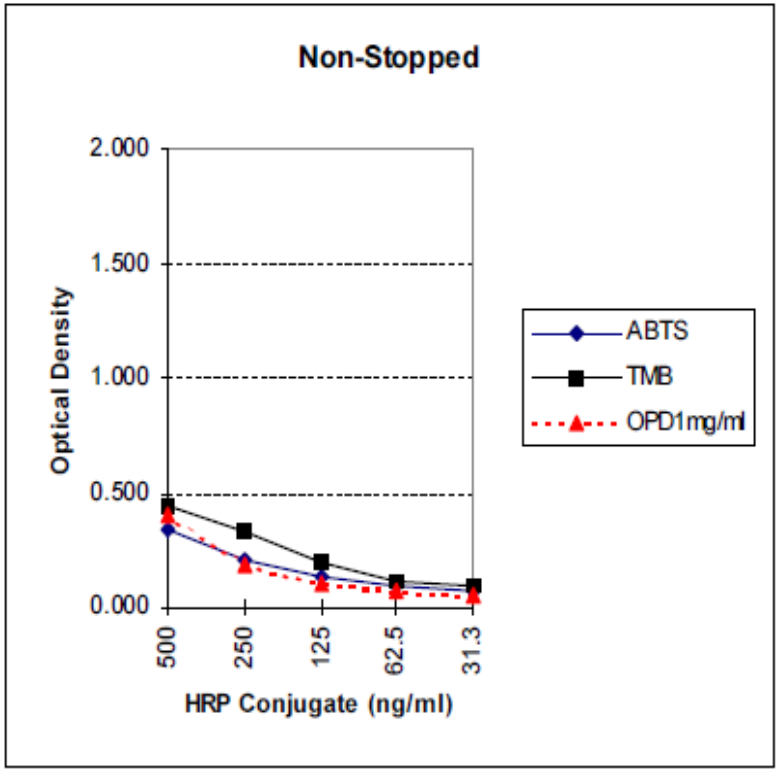
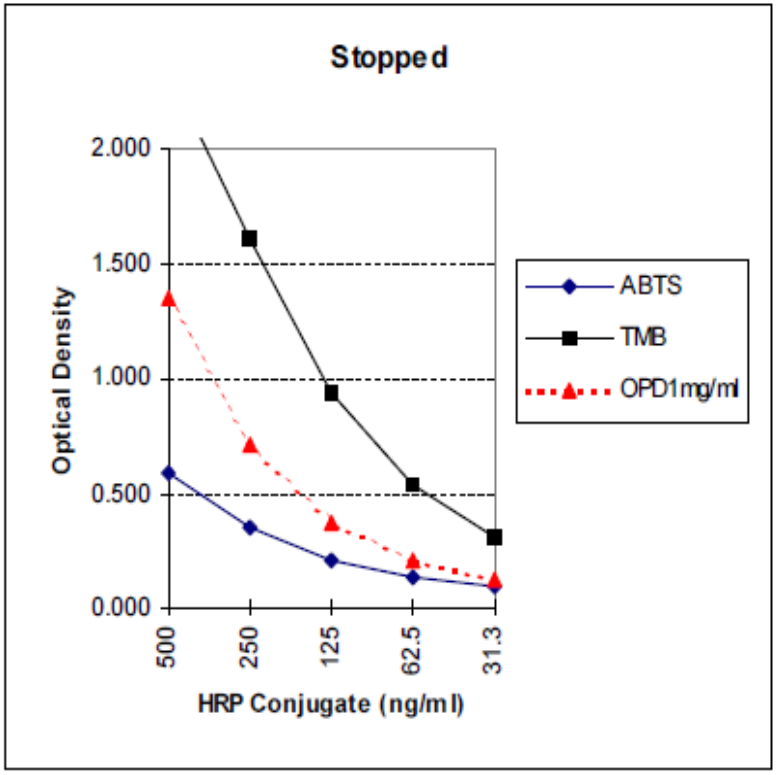


Figure 2: Substrate Performance After Reaction is Stopped



ELISA KIT

ELISA TEST (STEP BY STEP):

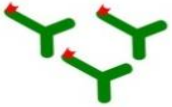
Reaction steps

1. Coat microtitre plate wells with Ag

2. Block unoccupied sites with non-specific protein

3. Incubate with serum, containing primary Ab against specific Ag

4. Incubate with enzyme-labeled second Ab, which binds primary Ab

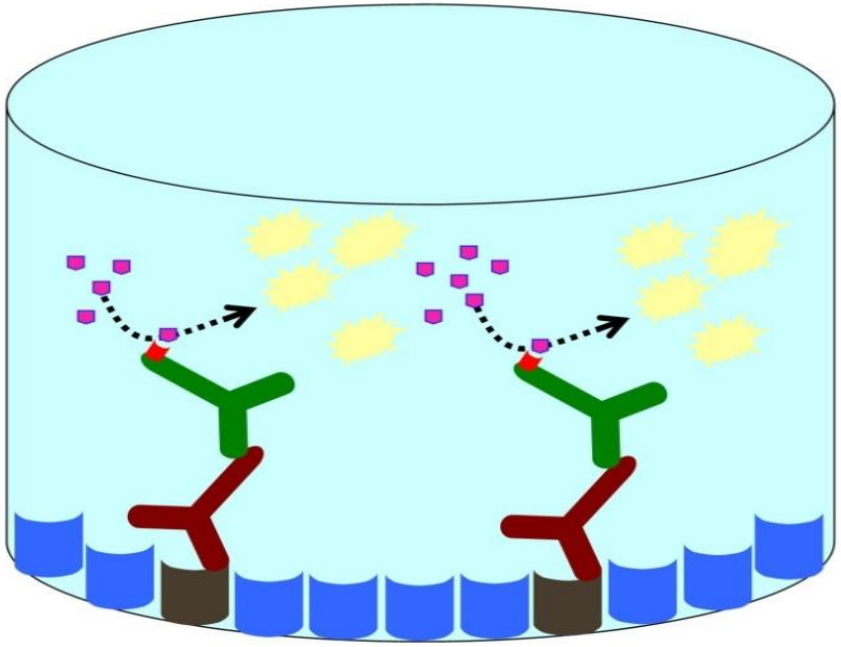


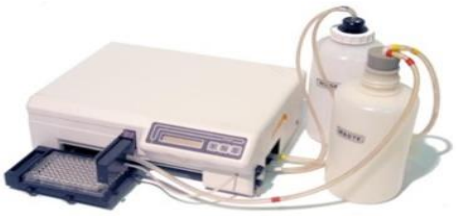
Wash after each of above steps

5. Add substrate

Formation of coloured product is an indication of presence of specific Ag

6. Stop enzyme-substrate reaction (Optional).
Read OD value using plate reader





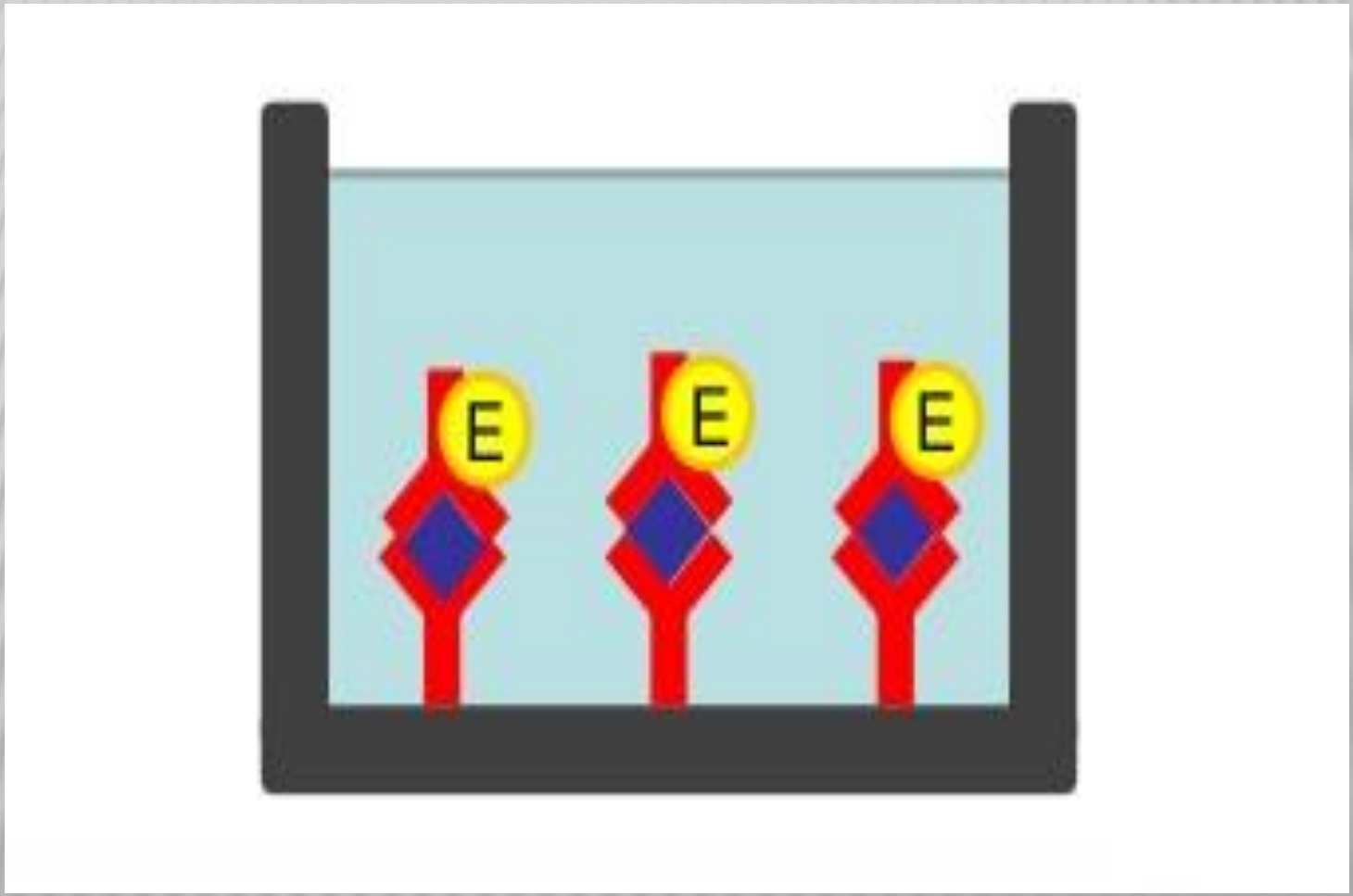
Microtiter plate washer

Microtiter plate reader

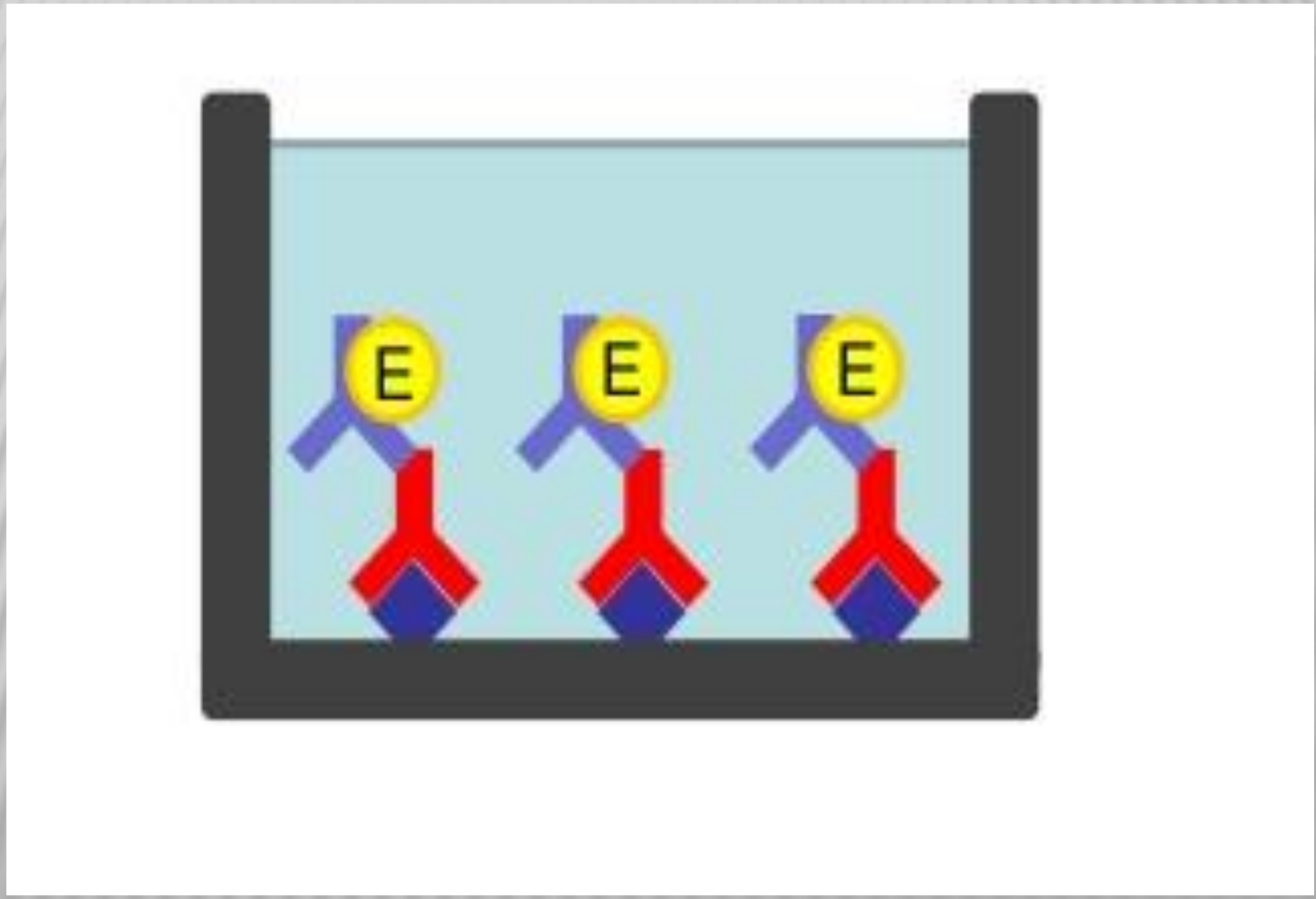
Types Of ELISA

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA

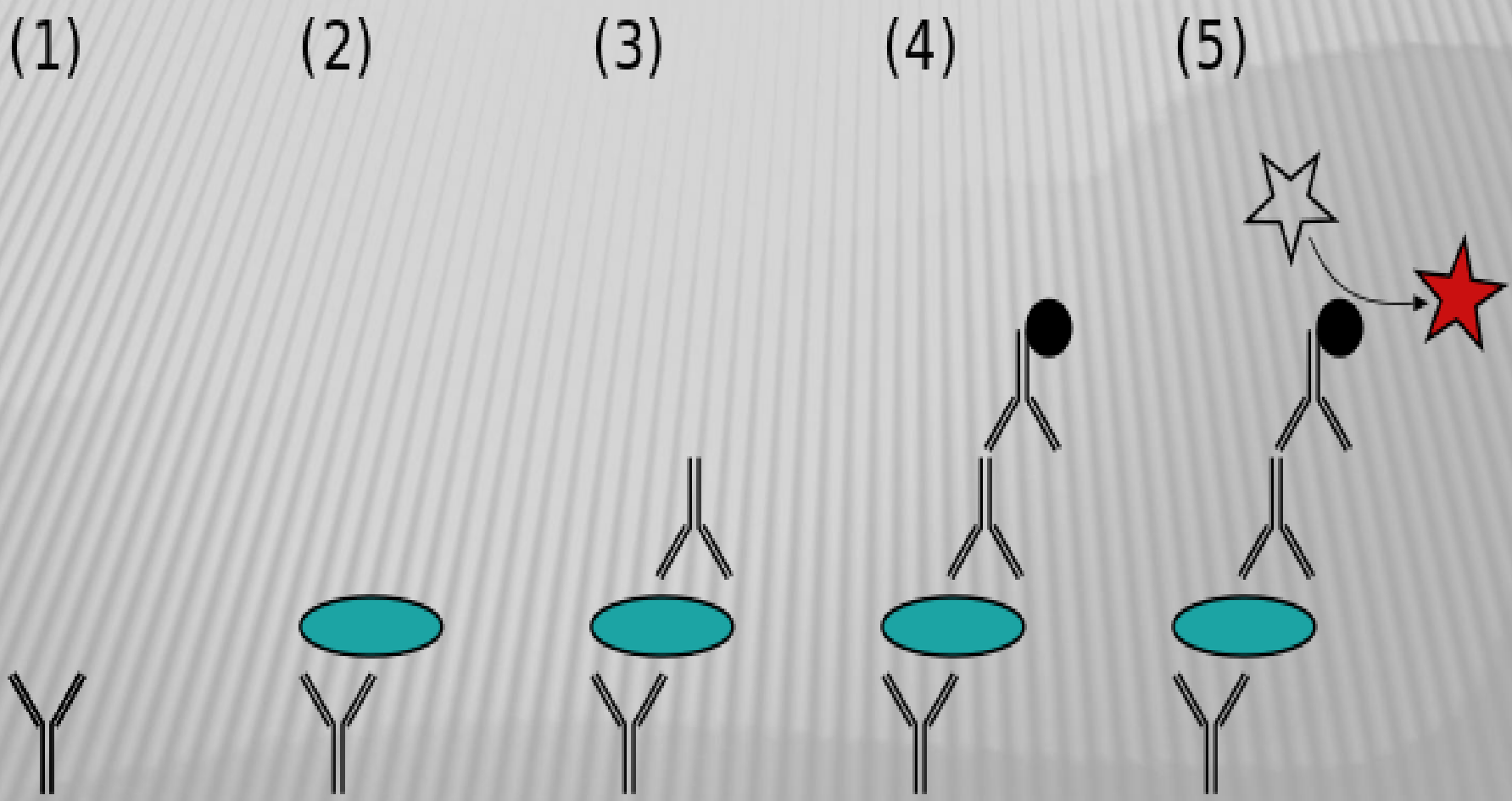
Direct ELISA



Indirect ELISA



Sandwich ELISA



Competitive ELISA

In this test, antibody is first incubated in solution with a sample containing antigen.

The antigen-antibody mixture is then added to the plate which is coated with antigen.

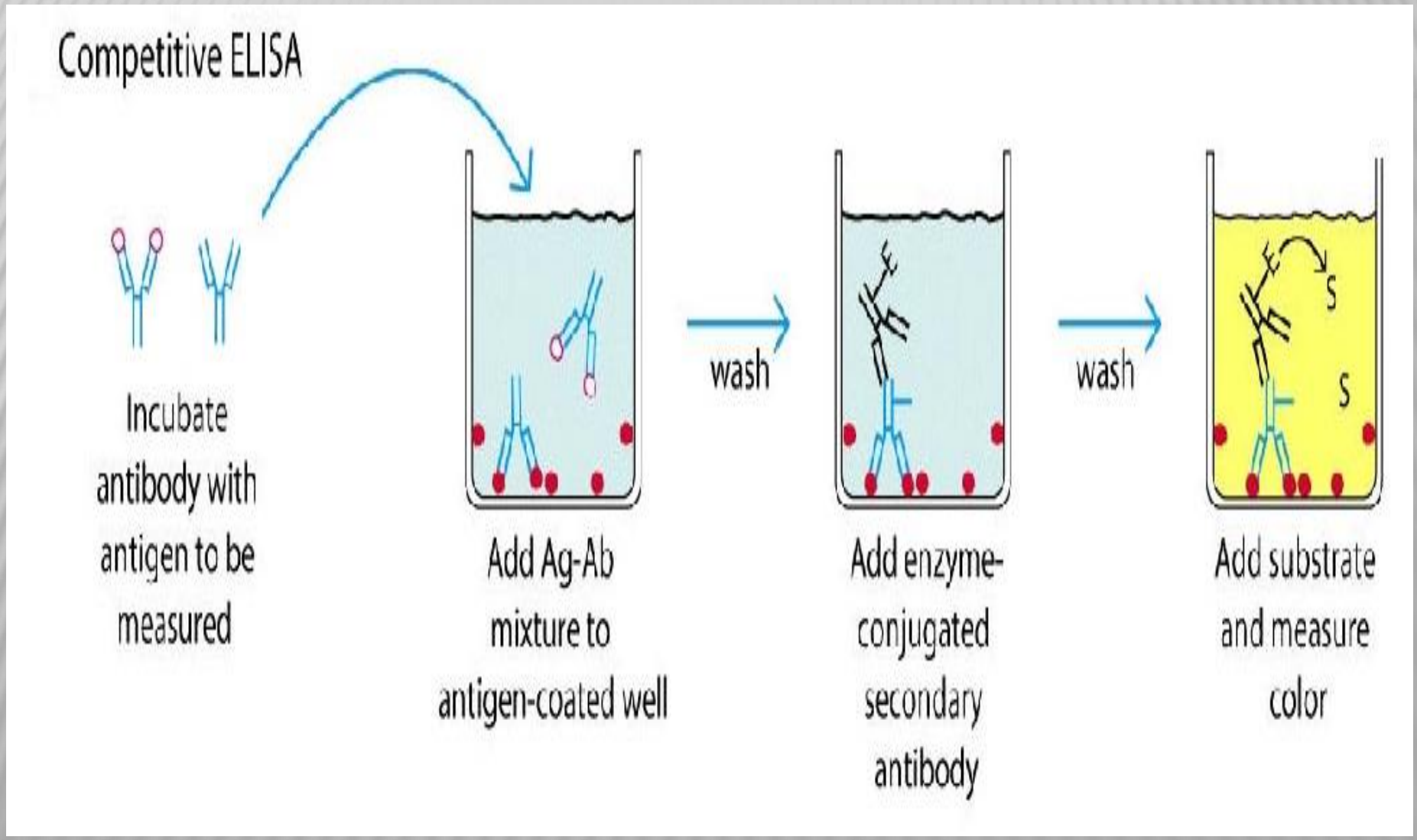
The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.

Competitive ELISA

After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well.

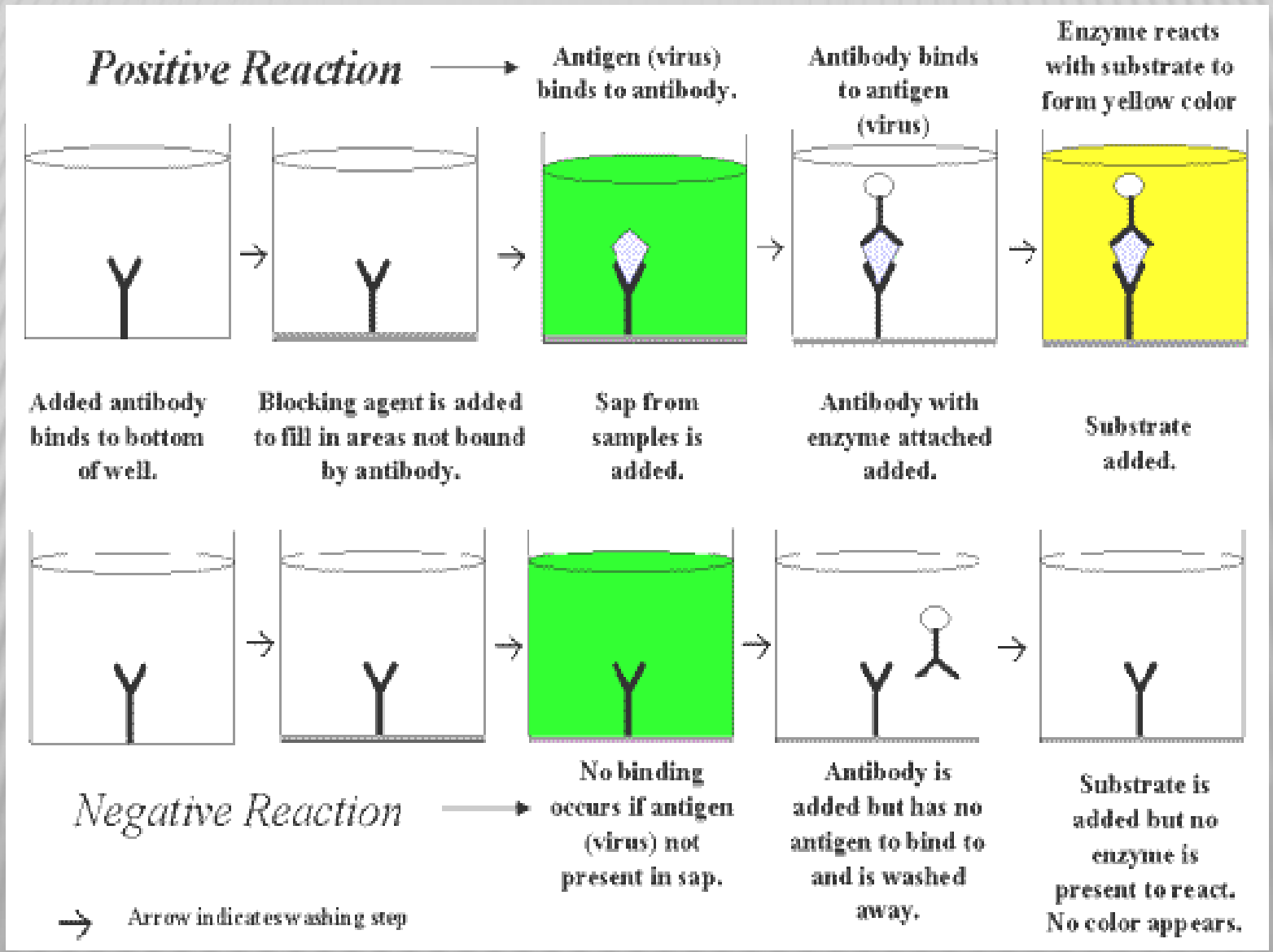
The higher the concentration of antigen in the sample, the lower the absorbance.

Competitive ELISA

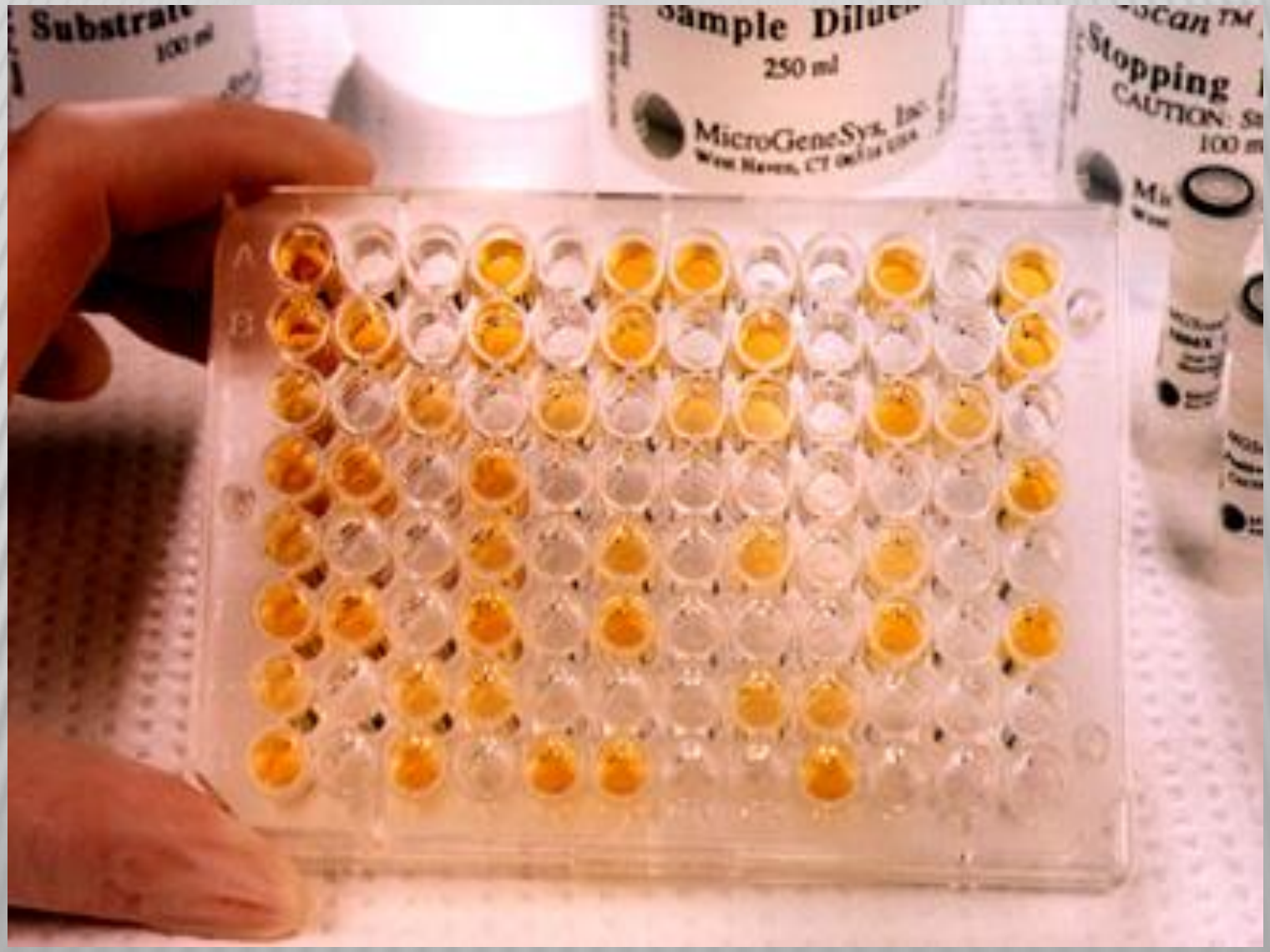


ELISA
(+) wells
And
(-) wells

ELISA (+) and (-) wells

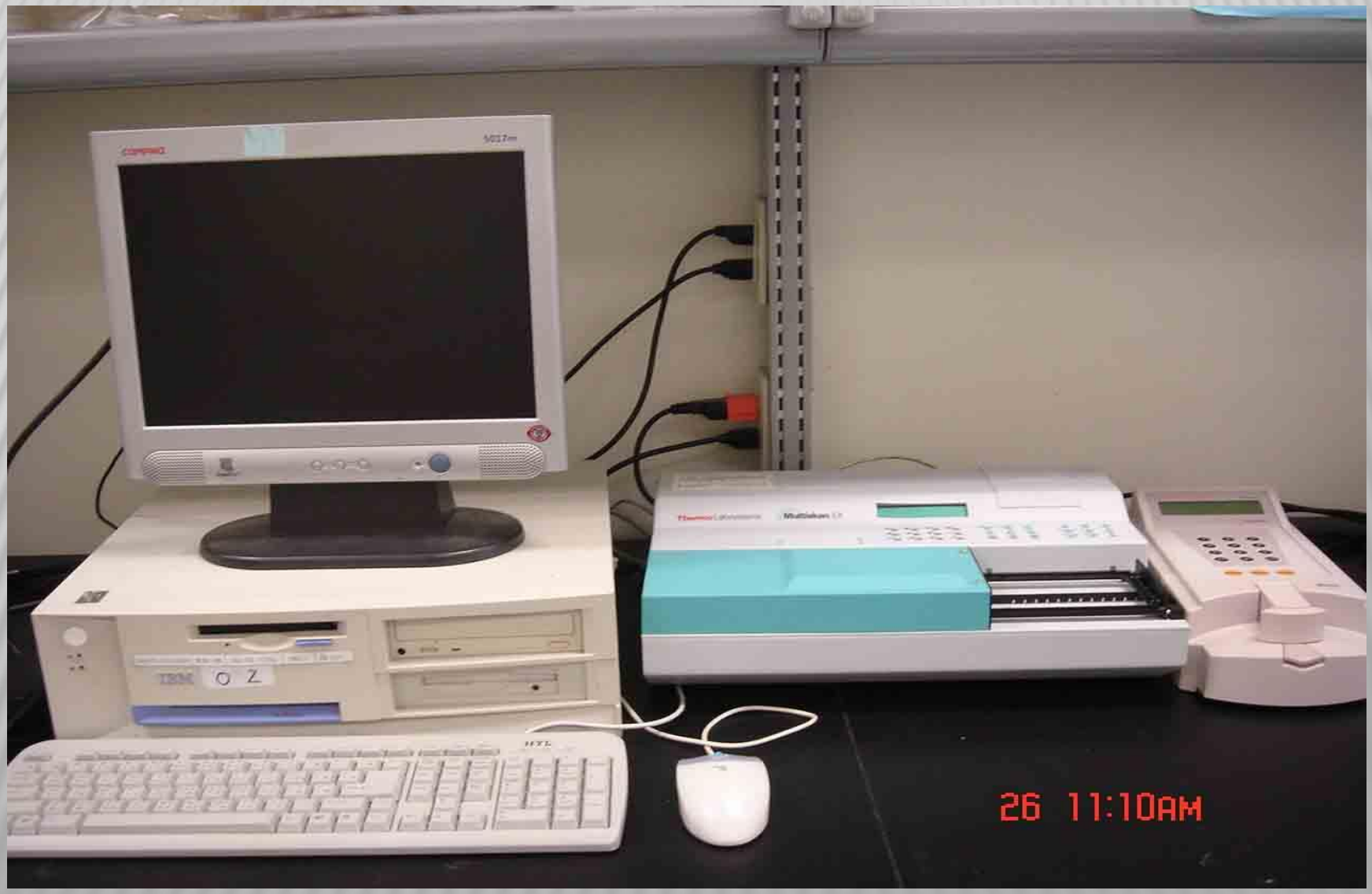


ELISA Plat


Substrate
100 ml
Sample Dilue
250 ml
MicroGeneSys, Inc.
Stopping
CAUTION:

ELISA Reader

ELISA Reader



ELISA Reader


heights
let's
new
reach
together


THANK
YOU