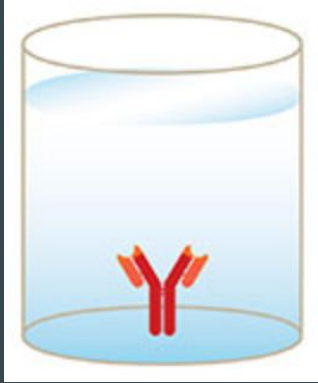


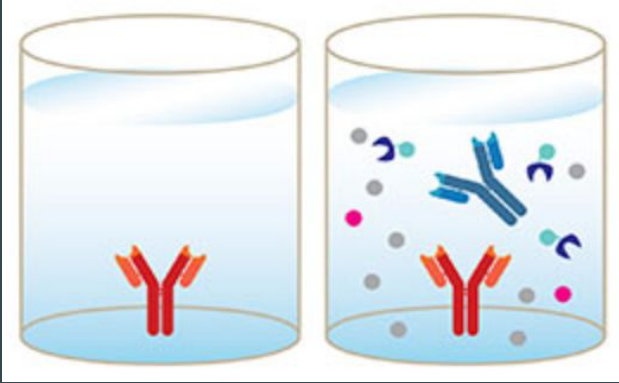
# An ELISA example...



We coat wells with a secondary capture antibody.

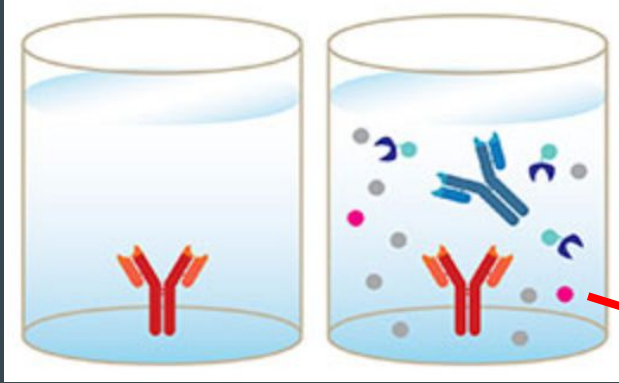
(Remember... the goal of an ELISA is to quantify the unknown concentration of an analyte in a given sample.)

# An ELISA example...



Then, roughly at the same time we add...

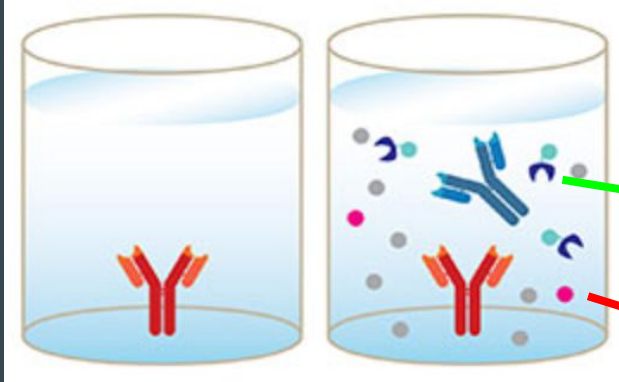
# An ELISA example...



Then, roughly at the same  
time we add...

A solution with antigen

# An ELISA example...

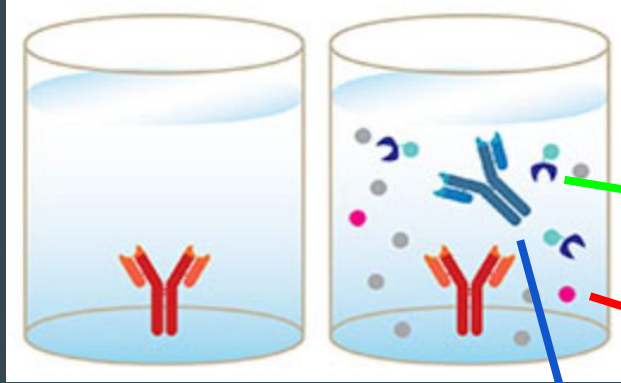


Then, roughly at the same  
time we add...

A solution with enzyme-conjugated antigen

A solution with antigen

# An ELISA example...



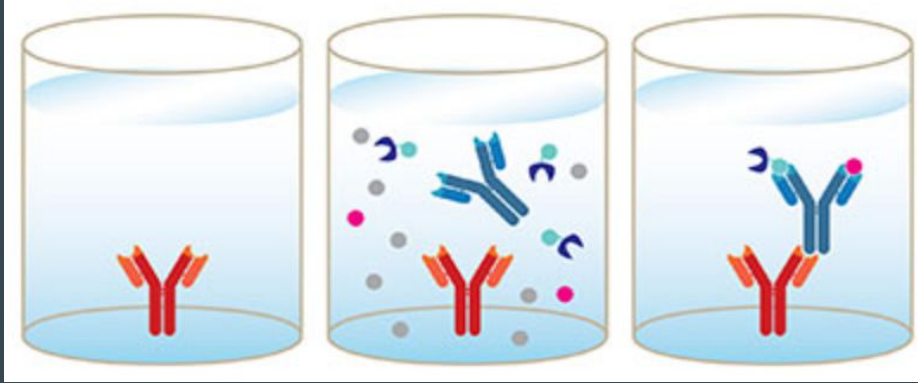
Then, roughly at the same time we add...

A solution with enzyme-conjugated antigen

A solution with antigen

A solution with primary detection antibody

# An ELISA example...



The primary antibody  
binds with...

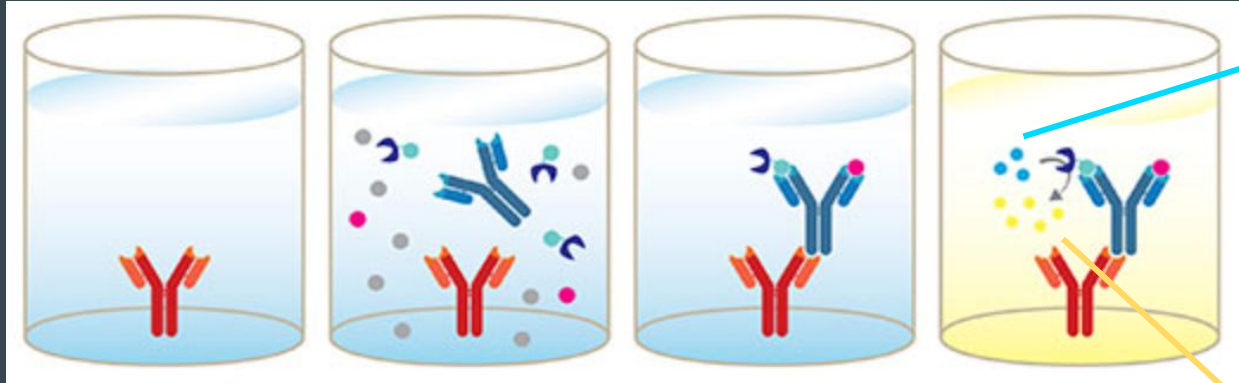
the sample antigen

the conjugated antigen

& secondary antibody  
coated to the plate

We then wash away any  
excess antigen and  
enzyme-conjugated  
antigen from the well.

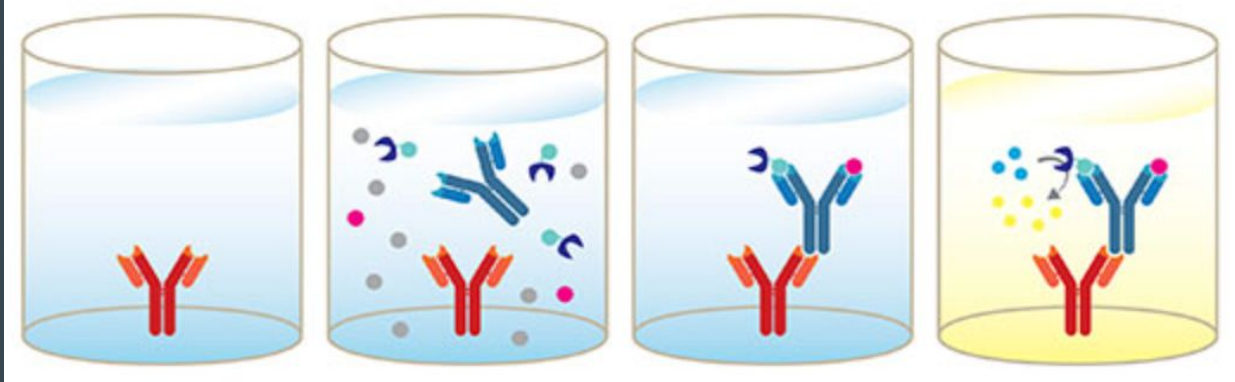
# An ELISA example...



Then we add substrate solution. It reacts with the enzyme attached to the conjugated antigen.

The reaction produces a yellow product when stopped with an acid solution.

# What type of ELISA is this?

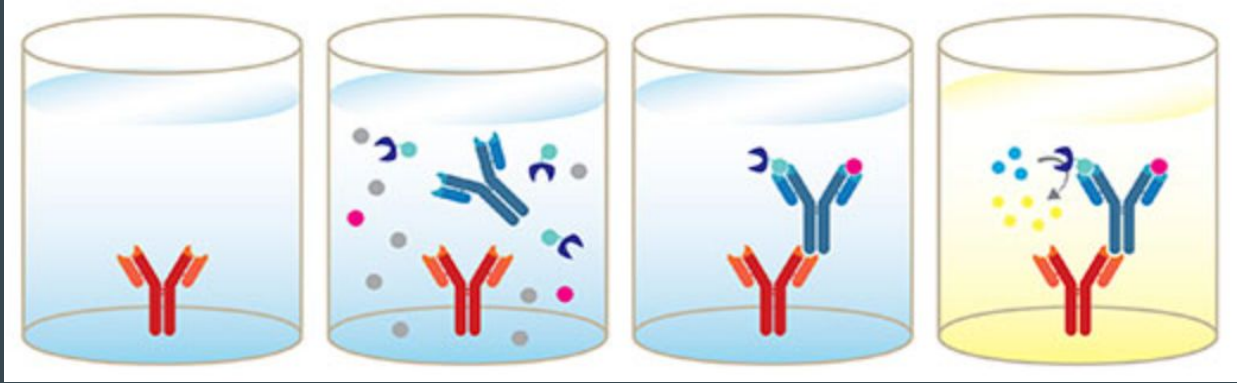


- A. Sandwich
- B. Competitive
- C. Direct

Write down your answers on a scrap of paper and pass to me.

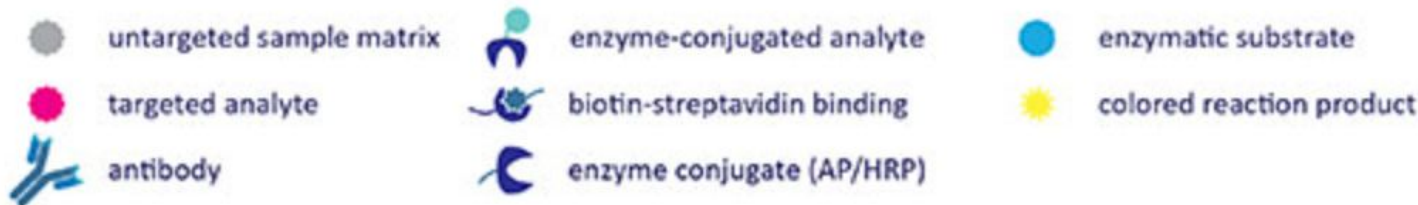
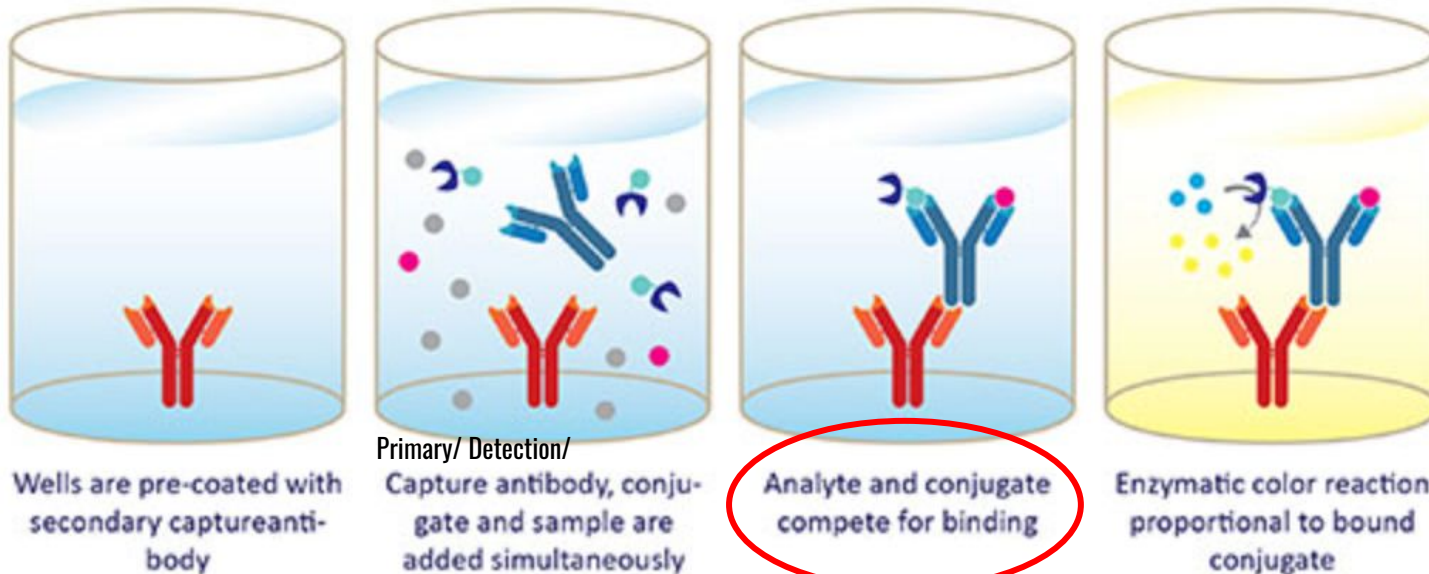


# What type of ELISA is this?

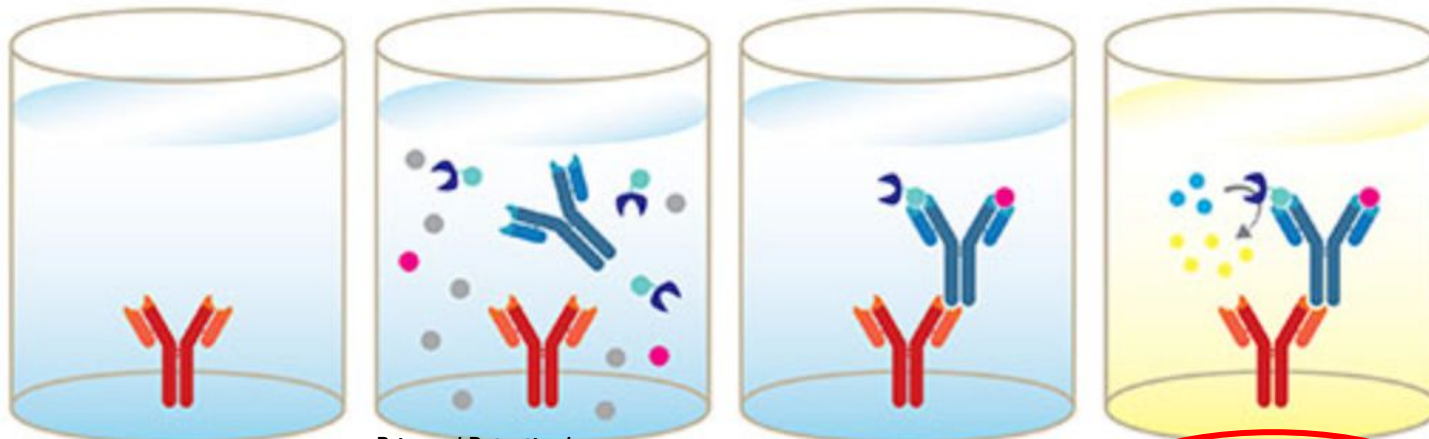


- A. Sandwich
- B. Competitive
- C. Direct

# Competitive ELISA



# Competitive ELISA

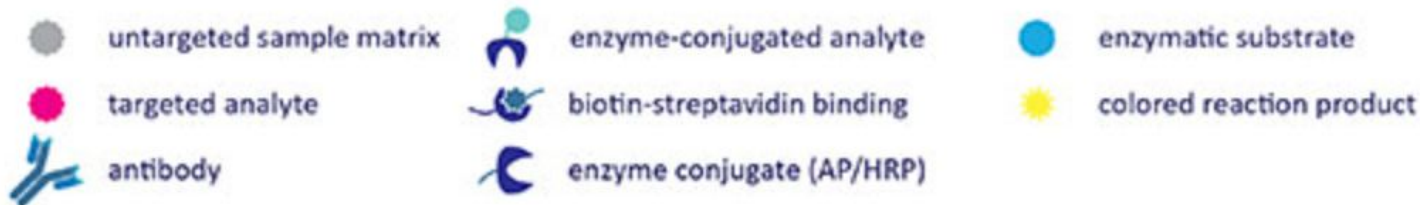


Wells are pre-coated with secondary capture antibody

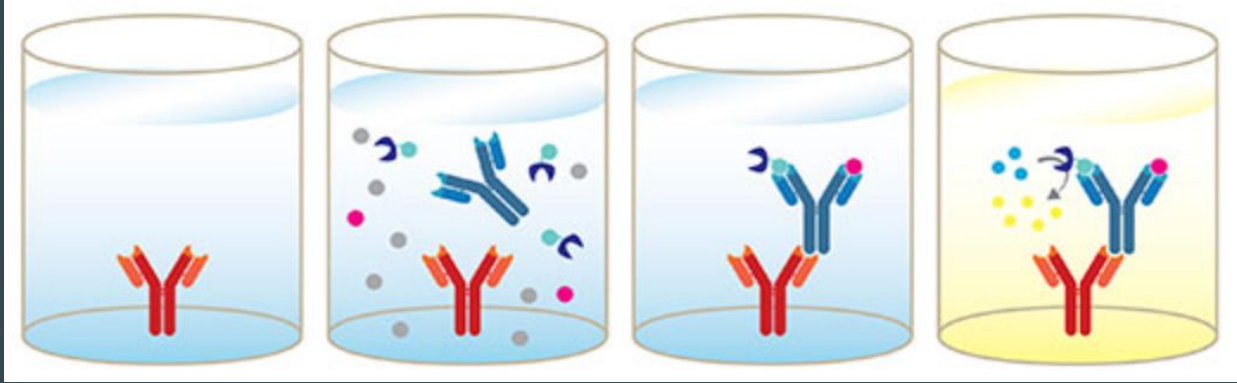
Primary/ Detection/  
Capture antibody, conjugate and sample are added simultaneously

Analyte and conjugate compete for binding

Enzymatic color reaction proportional to bound conjugate



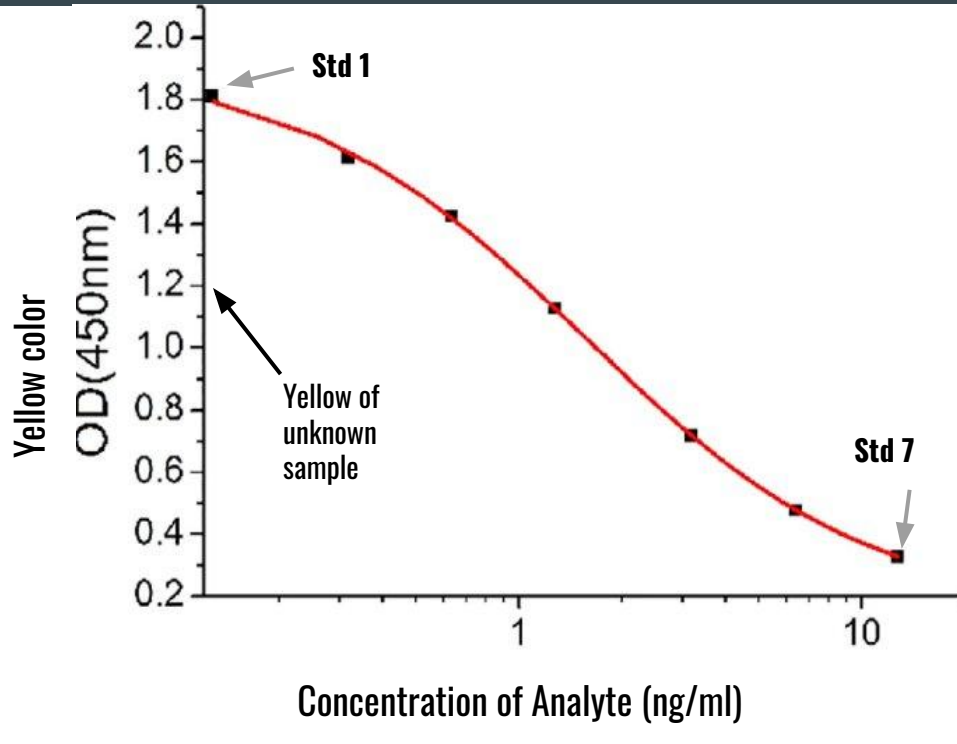
# Competitive ELISA



How does the color reaction help us figure out the unknown concentration of the analyte?



# The Standard Curve



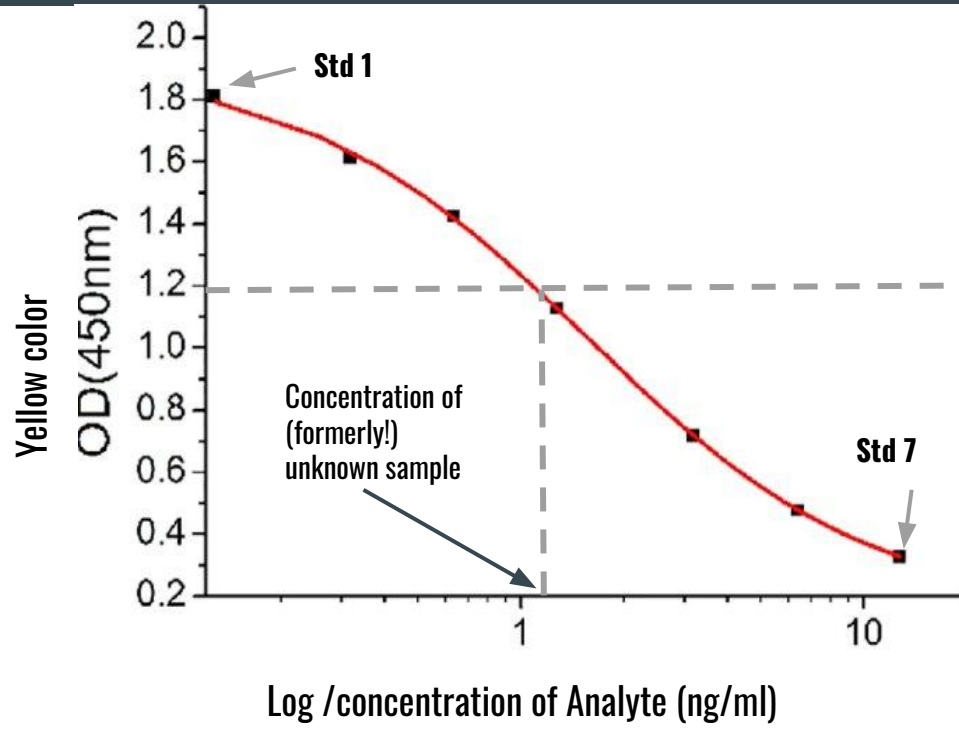
After a plate reader quantifies the yellow color (OD) for each well in the plate...

We use samples of KNOWN concentration to show the relationship of OD's (y-axis) with concentration.

Then when we have an UNKNOWN concentration, we take its OD...



# The Standard Curve



... and infer its corresponding concentration from the curve.