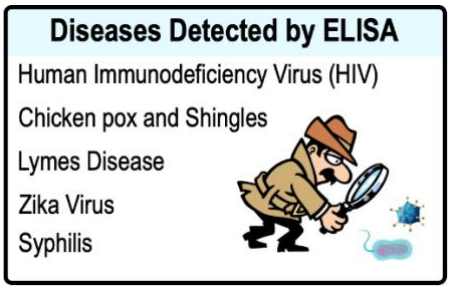
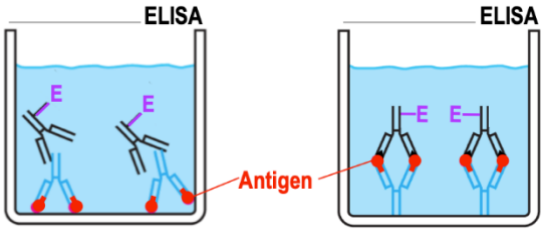
CONCEPT: ELISA

- _____ (Enzyme-Linked Immunosorbent Assay): technique using *antibodies* to *detect* & *quantify* proteins in a sample.
  - □ Samples could be blood or urine from a patient or a solution from cells grown in a lab.
  - □ ELISA is appropriate for diagnosing many diseases and screening _____ samples *at once*.
- Several types of ELISA exist including _____ ELISA & _____ ELISA.
  - □ *Indirect ELISA*: the _____ is coated on a surface & detected with antibodies.
  - □ *Sandwich ELISA*: the _____ is coated on a surface & antigen is "*sandwiched*" between antibodies.

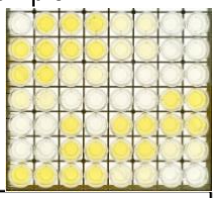


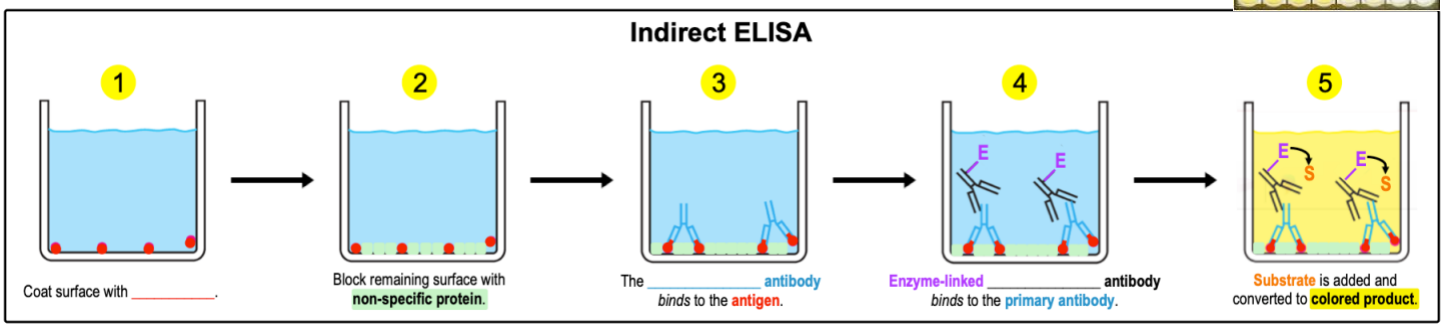


Indirect ELISA Set-Up

- Indirect ELISA can be set-up & performed in _____ general steps:
  1. Adhere _____ of interest in a sample to an inert surface in the wells of a microplate.
     - □ Separate different samples into different wells of the microplate.
  2. _____ any unoccupied sites on the surface by washing with a nonspecific protein (ex. casein).
  3. Treat surface with a _____ *antibody* specific to antigen of interest & *wash away* any unbound antibody.
  4. Treat surface with an *enzyme-linked-*_____*-antibody* specific to primary antibody.
     - □ Enzyme linked to the secondary antibody *catalyzes* a reaction forming a _____ product.
  5. Add _____ for the enzyme-linked-antibody & *monitor* color intensity.
     - □ Color intensity is *directly* _____ to the amount of antigen present in the sample.

EXAMPLE: Indirect ELISA.

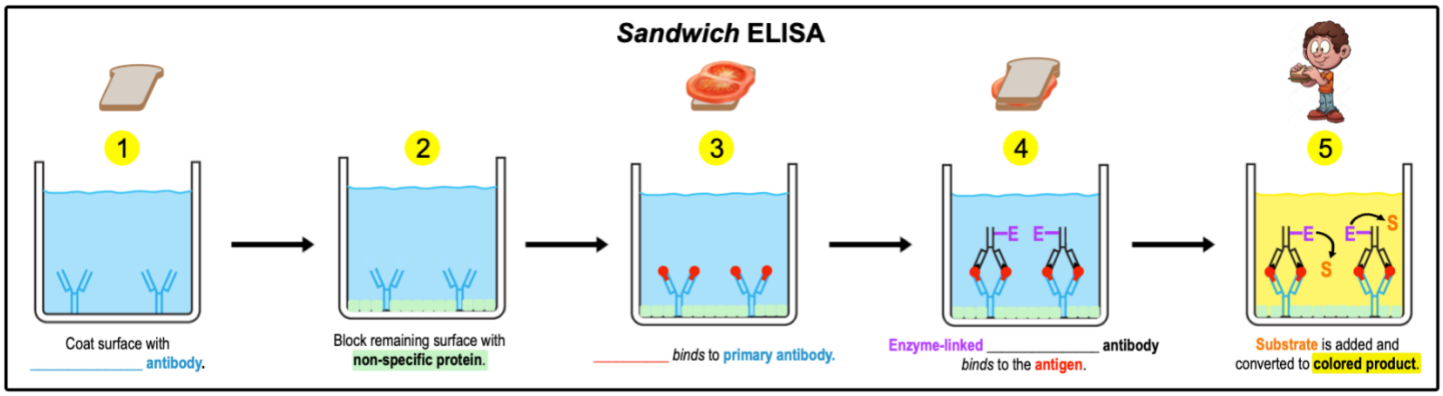




CONCEPT: ELISA

●Sandwich ELISA is performed with *similar, parallel-like* steps.

□ Provides higher sensitivity and specificity than indirect ELISA but can present more challenges to perform.



PRACTICE: An ELISA can be used for:

a) Quantitative analysis.
b) Size analysis.
c) Sequence analysis.
d) Structure analysis.

PRACTICE: In an Indirect ELISA, the enzyme-linked antibody will attach to:

a) The patient's antigen.
b) The antigen binding region (F_{ab}) of the primary antibody.
c) The constant region (F_c) of the primary antibody.
d) The wall of the microtiter well.
e) The constant region (F_c) of the secondary antibody.

PRACTICE: ELISA is a common application of fluorescence used for its ability to detect faint biochemical signals. How does ELISA detect its analytes of interest?

a) Antibodies that bind specifically to the analyte of interest fluoresce once bound to the analyte, creating a quantifiable signal to record.
b) As analytes bind the antibodies on the polymeric support of the assay, a fluorescent signal is released as each analyte gets bound.
c) Different amounts of a fluorescent standard are added to the ELISA assay and a calibration curve is made in order to make an estimate as to how strong the signal is.
d) An enzyme, attached to an antibody bound molecule of analyte, catalyzes multiple cycles of a reaction that generates fluorescent product.