Universitas Esa Unggul

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INTRUMENTASI BIOTEKNOLOGI

Program Studi Bioteknologi

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Meeting 8

Universitas Esa Unggul

Serology Equipment

Elisa (Mikroplate reader, mikroplate washer)

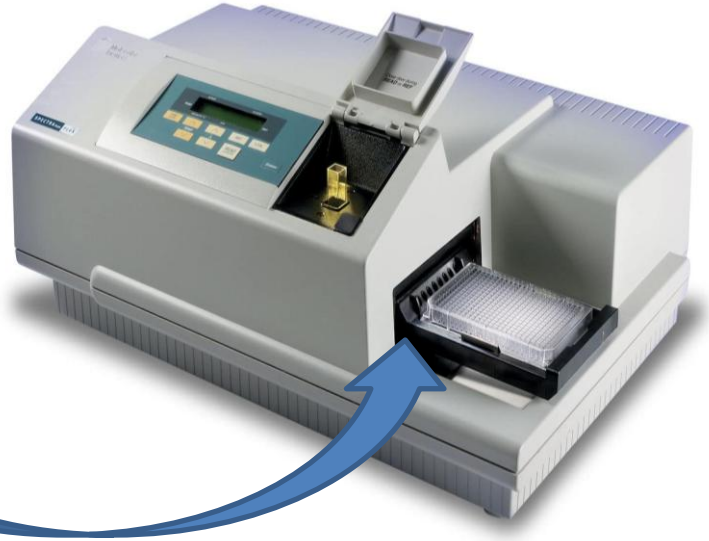
Elipsot Reader

Multichannel pipet

Western Blot transfer

Tujuan Perkuliahan

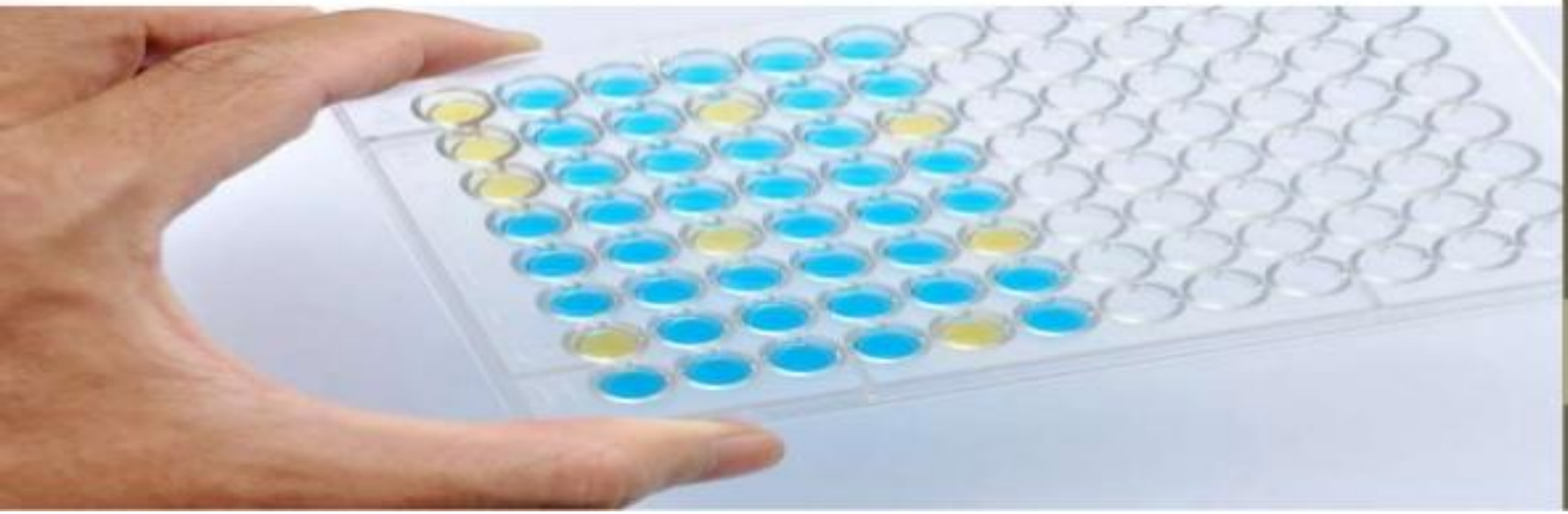
- Mengidentfikasi alat serologi: mikroplate reader, mikroplate washer, Elispot reader dan multichannel pipet
- Mengetahui prinsip bekerjanya alat-alat tersebut Mengidentfikasi alat serologi: western blot



Elisa (Enzyme-linked immunosorbent assay)

Definition:

- The enzyme-linked immunosorbent assay (**ELISA**) is a common laboratory technique which is used to measure the concentration of an analyte (usually antibodies or antigens) in solution.



History of Elisa

Rosalyn Sussman 1960

Eva Engvall 1971

Introduction of Elisa

- Enzyme Linked Immunosorbent Assay (ELISA)
- Term Was Coined By Engvall and Pearlmann in 1971
- Similar To RIA, Except No Radiolabel
- Can Be Used To Detect Both Antibody and Antigen
- Very Sensitive, pg/mL
- Relies on Monoclonal Abs
- ELISA may be run in a qualitative or quantitative format.

Principle ELISA

- Antigen bound to ELISA plate
- Specific antibodies bind to antigen
- Antibodies conjugated with an enzyme
- Addition of substrate results in a colored product
- Intensity of color detected by ELISA reader or spectrophotometer
- Both antigen & antibody can be coated onto polystyrene plates
- Both antigen & antibodies can be conjugated with enzyme

Basic Terms:

- **Adsorption:**

The process of adding an antigen/antibody, diluted in buffer, so it attaches to the solid phase on incubation.

- **Washing:**

The simple flooding & emptying of wells with a buffered solution to separate bound from un-bound reagents in ELISA

Basic Terms:

- **Antigen:**

Any molecule that elicits the production of antibodies when introduced into body.

- **Antibodies:**

Proteins produced in response to antigenic stimuli.

- **Enzyme conjugate:**

An enzyme that is attached irreversibly to an antibody. e.g: Horse-redish peroxidase (HRPO).

Basic Terms:

- **Chromogen:**

A chemical alters color as a result of an enzyme interaction with substrate (color reaction used as signal) e.g Trimethyl benzidine (TMB).

- **Stopping:**

The process of stopping the action of an enzyme on a substrate.

- **Reading:**

Spectrophotometric measurement of color developed in ELISA

Equipments:

1) **Microwell Plate:**
Flat bottom polystyrene plate, contains 8 x 12 wells holding 350 µL each.

Equipments:

2) Multipipette :

An 8-channel 100 µL pipette is a good help for even small-scale work.

Equipments:

3) Washing Device:

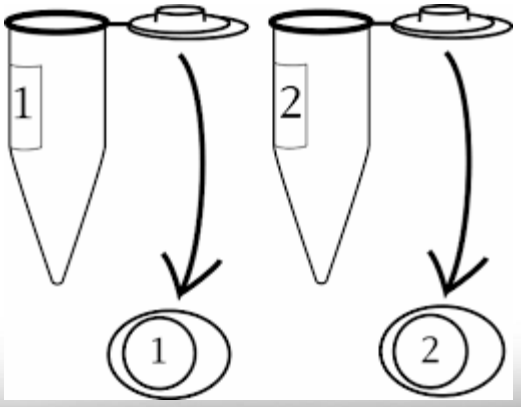
- manually operated washing devices.
- may be of use particularly when there is a risk that the samples tested in ELISA contain infectious material, so must be collected for subsequent disinfection.

Equipments:

4) Microplate washer:

- These are very efficient with unusually low carry-over contamination.

Equipments: ELISA



Equipments: ELISA

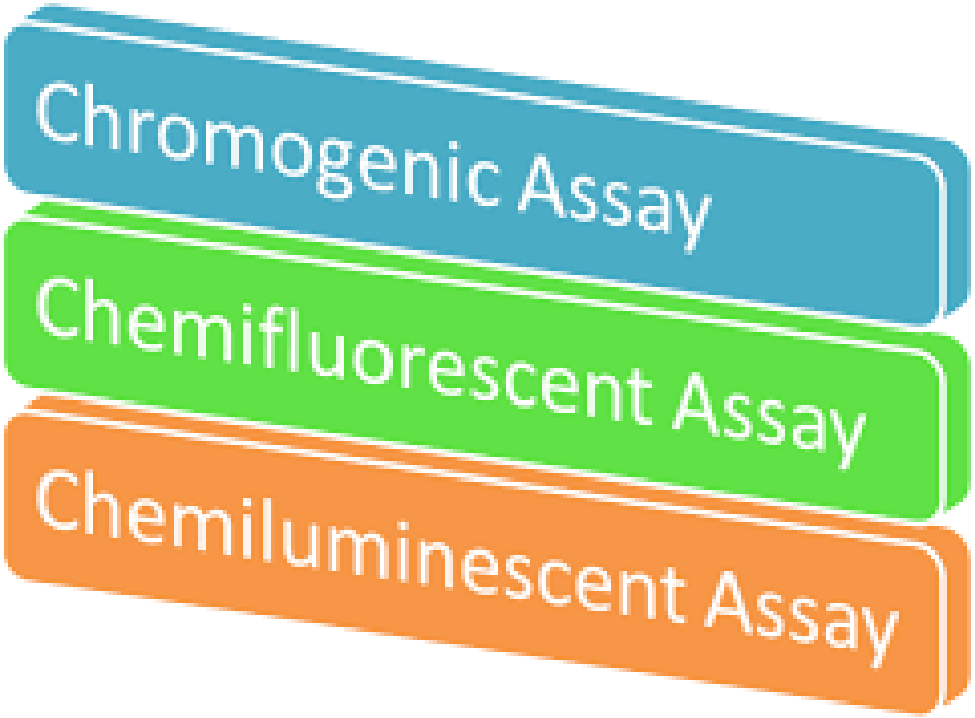
Equipments: ELISA

Mikroplate

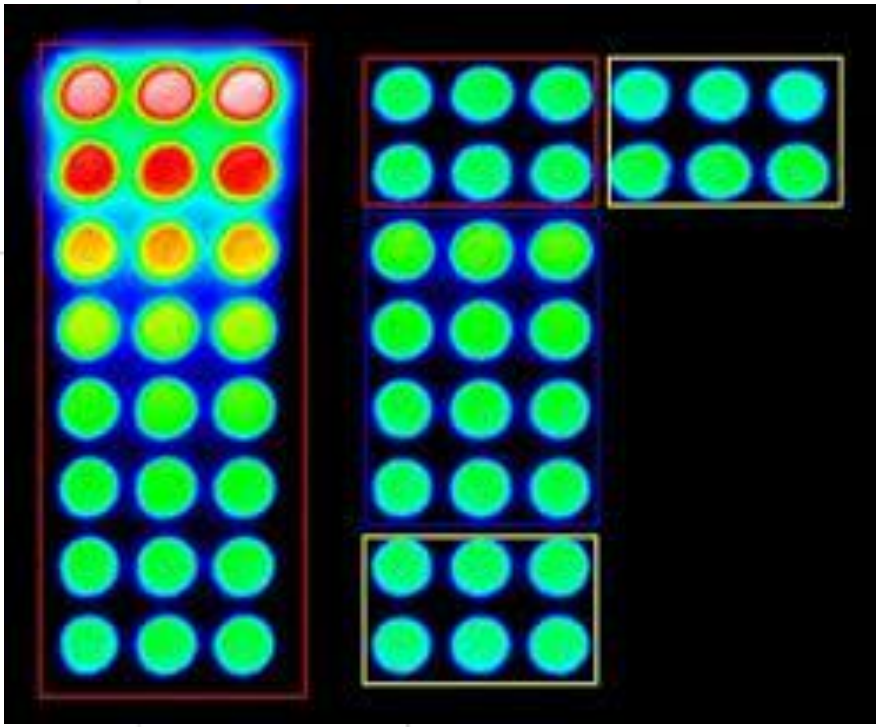
Equipments: ELISA

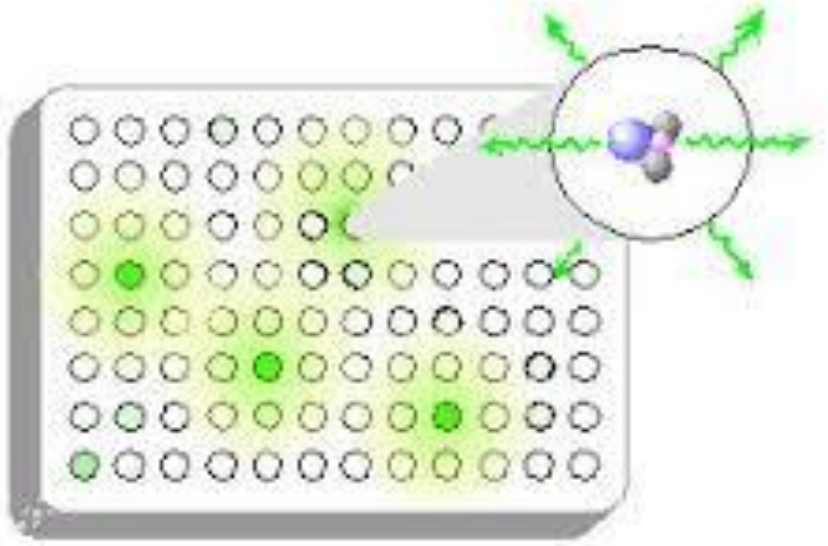
Elisa Multimode REader

Visualisasi ELISA

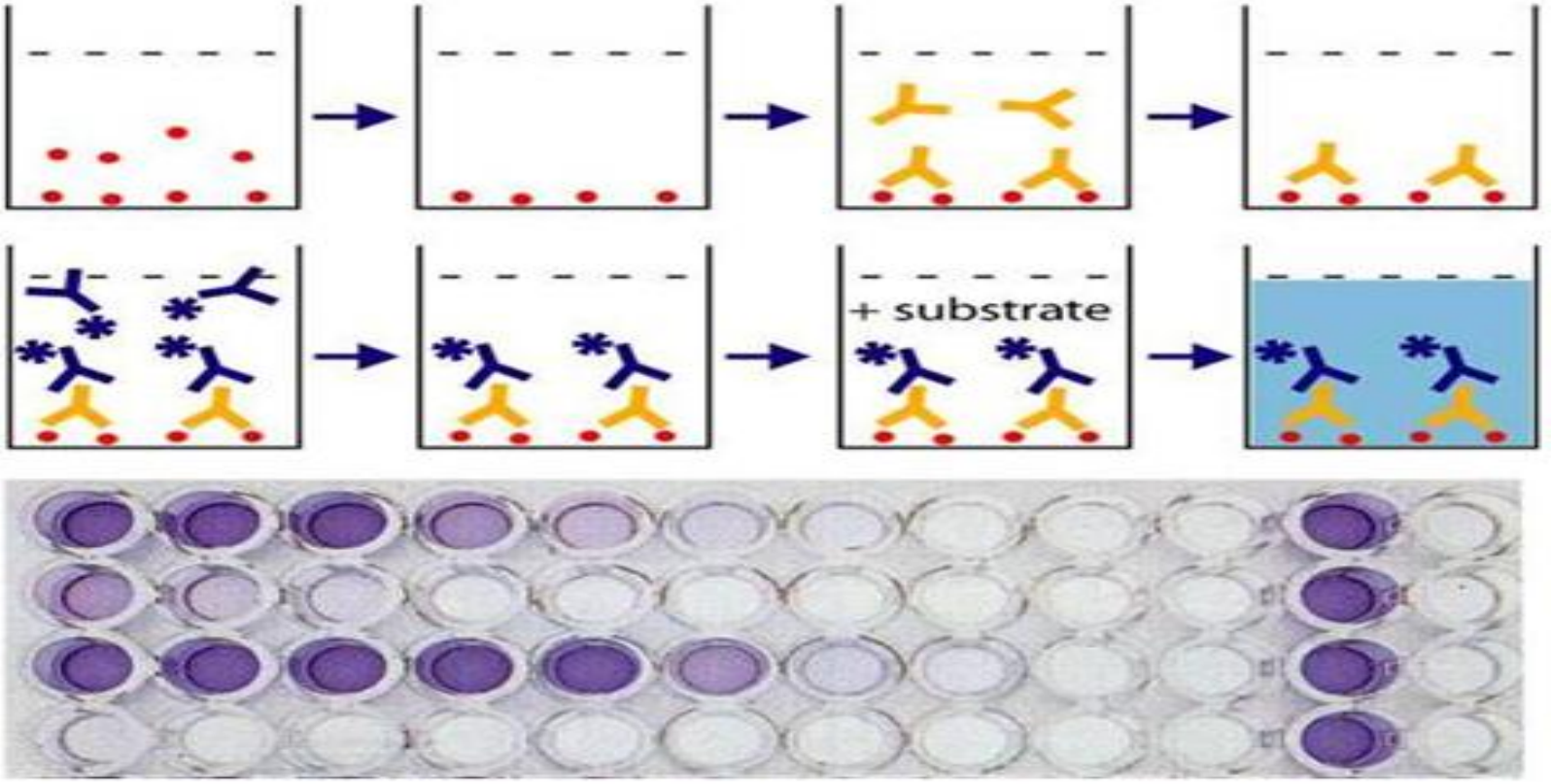


Visualisasi ELISA

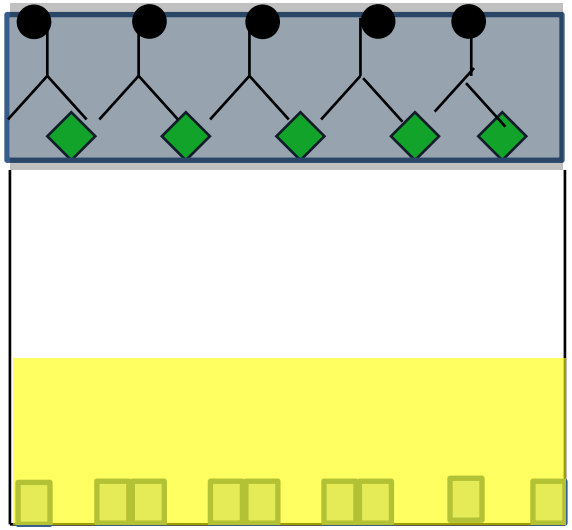




Indirect ELISA /kromogenik



6. Add stop solution

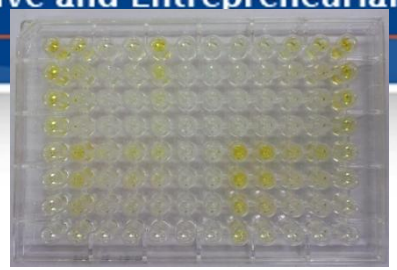


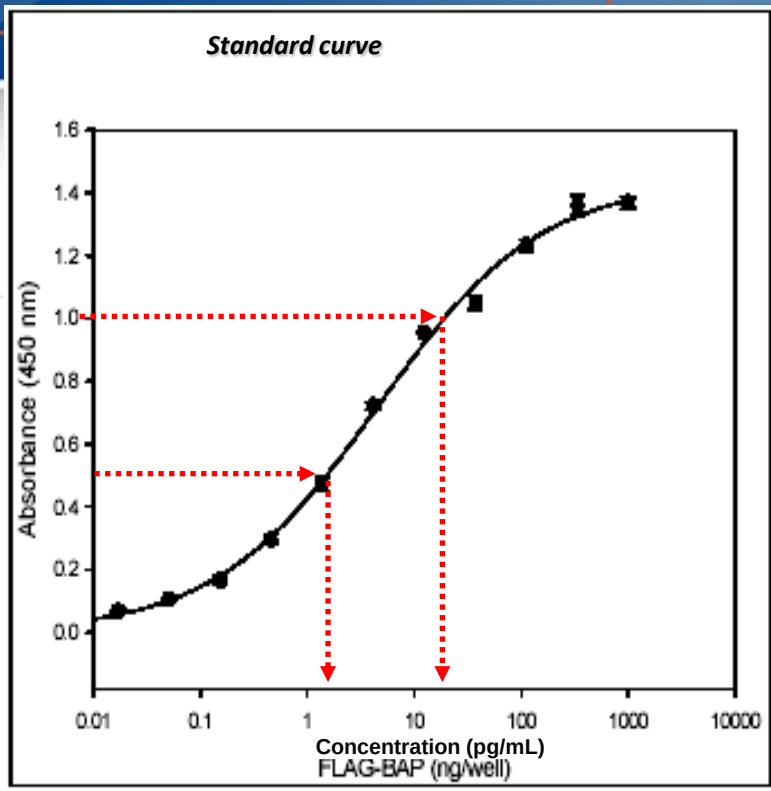
ELISA well

CMV/HFF Antigen

Anti-CMV Ab

HRP Conjugated anti Human IgG





QC (QUALITY CONTROL):
Nilai QC dibandingkan di tiap plate

Blank :
Pelarut tanpa sampel

STANDARD:
Sudah di ketahui konsentrasinya

Result Analysis

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Std 1
B	Std 2	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Std 2
C	Std 3	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Std 3
D	Std 4	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Std 4
E	Std 5	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	Sample 1:300	QC 1:300	Std 5
F	Std 6	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	Sample 1:900	QC 1:900	Std 6
G	Std 7	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	Sample 1:2700	QC 1:2700	Std 7
H	Blank	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	Sample 1:8100	QC 1:8100	Blank

Sampel 1-10

Std

Std

Sampel 11-19

QC

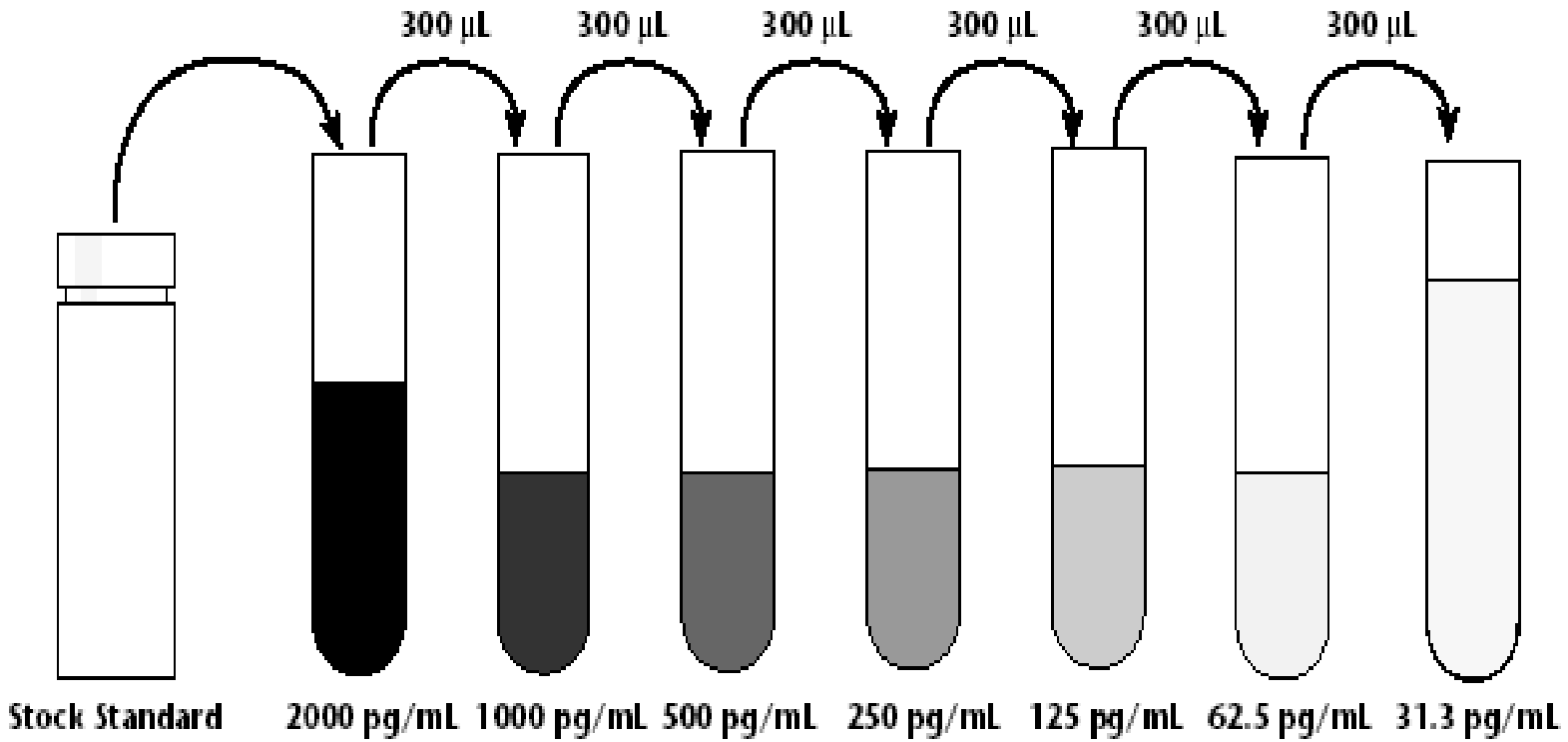
Result Analysis

- ELISA results are reported as a number using a spectrophotometer, spectrofluorometer, or other optical device.
- Unknowns that generate a signal that is stronger than the known sample are called "positive"; those that generate weaker signal are called "negative.

Standard Preparation

- Standards Are Diluted in Blocking Buffer/Tween
- Start By Labeling eight, 1 mL Eppendorf Tubes
- Prepare Highest Conc. Tube (1 mL)
- Fill The Remaining Tubes with 0.5 mL Blocking Buffer
- Serially Dilute From Top To Lowest

Standard Preparation



Advantages of ELISA:

- ✓ It can be used on most type of biological samples like plasma, serum, urine, cell extracts.
- ✓ Less costly and safest.
- ✓ Easy visualization of results with high level of accuracy.
- ✓ Specific and highly sensitive assay that can detect protein at the picomolar to nanomolar range.
- ✓ Easily automated for performance of large numbers of tests.
- ✓ Require minimal reagents.
- ✓ Qualitative detection or Quantitative measurement of either antigen or antibody.
- ✓ Wells can be coated with antigens or antibodies.
- ✓ Can be done by personnel with only minimal training.

Disadvantages of ELISA:

- Measurement of enzyme activity can be more complex than the measurement of activity of some type of radioisotopes.
- Enzyme activity may be affected by plasma constituents.
- Kits are not cheap.
- Very specific to particular antigen but won't recognize other antigens.
- False positive/ negative possible, especially with mutated/ altered antigen.

Applications of ELISA

- Analysis of hormones, vitamins, metabolites, and diagnostic markers.
- Therapeutic drug monitoring.
- Diagnostic procedures for detecting infection.

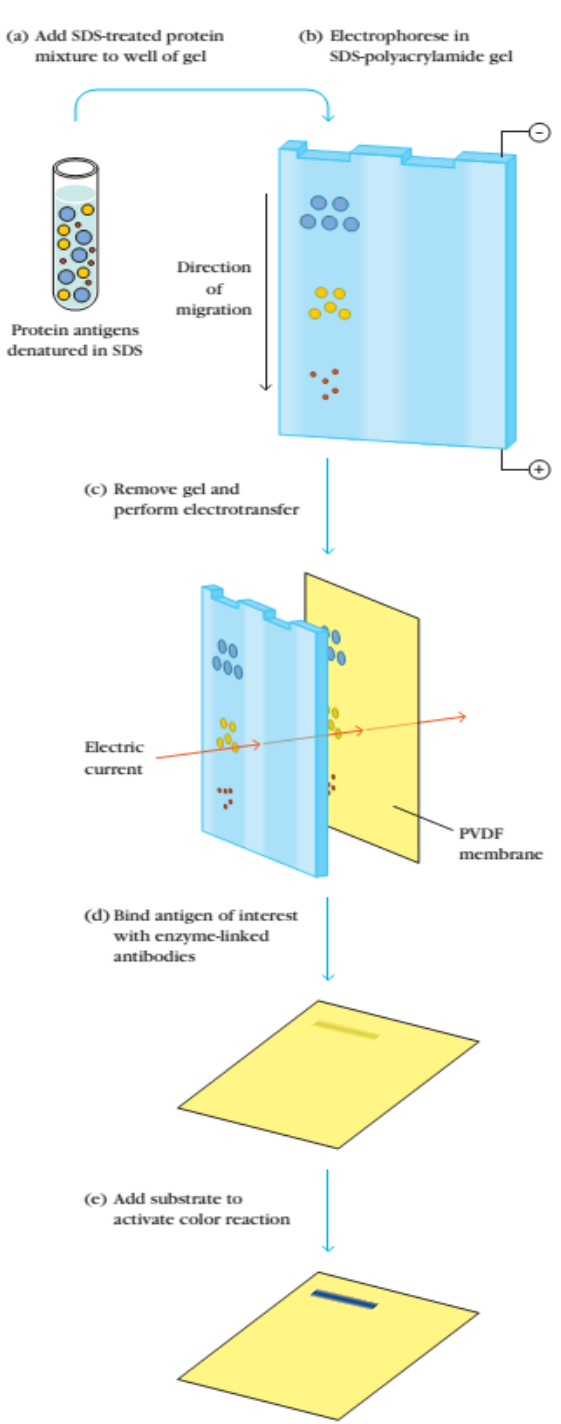
Western Blot

Reaksi antara antibodi dengan antigen pada membrane nitrocellulose dg terlebih dahulu memisahkan antigen tsb menurut berat molekul (MW dalam Da atau kDa)

(Ernawati, 2015)

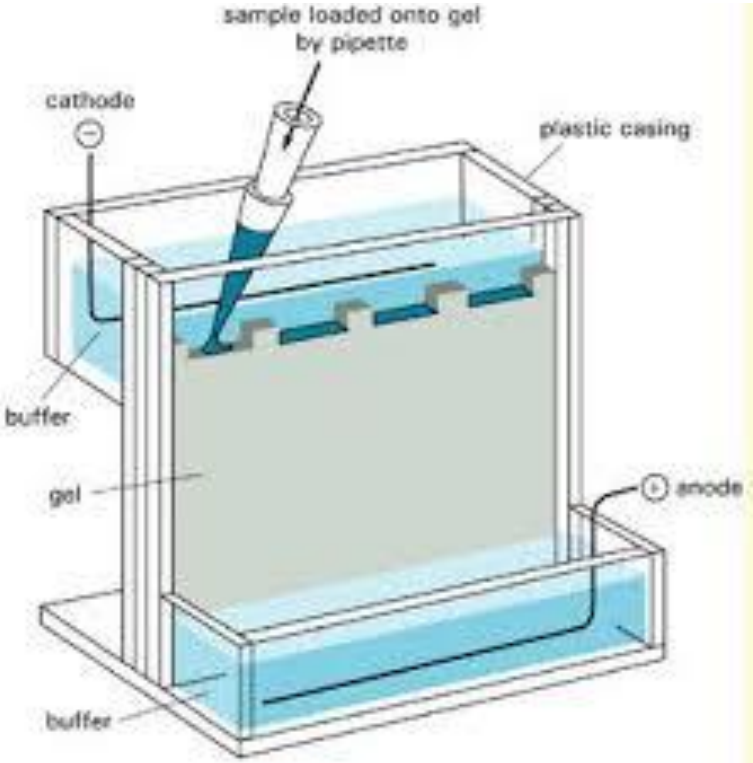
Western blot untuk protein, *Southern blot untuk DNA dan Northern blot untuk RNA*

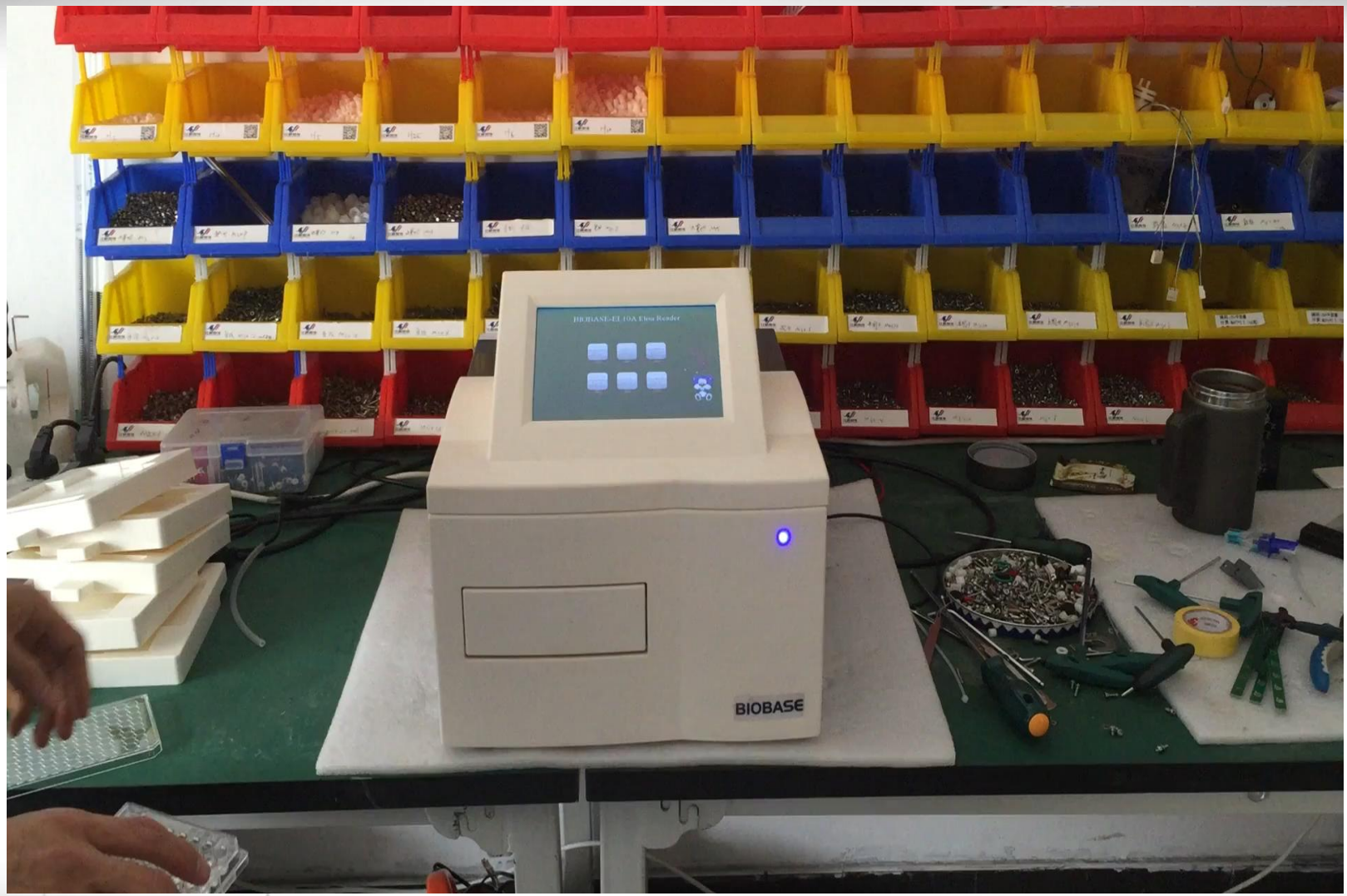
- Tahapan dalam western blot
  - Elektroforesis protein dengan SDS-PAGE (*sodium dodecyl sulfate polyacrylamide gel electrophoresis*)
  - Transfer protein ke membran
  - Deteksi protein dengan antibodi spesifik terhadap protein target. (Antibodi terkonjugasi dengan streptavidin-hrp)
  - Penambahan substrat



Western Blot

SDS PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis


sample loaded onto gel by pipette
cathode
plastic casing
buffer
gel
anode
buffer


BIOBASE

Western Blotting

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Thank You!!!