BCHEM 471

ELISA

Dr. A. Kwarteng
akwarteng@knust.edu.gh

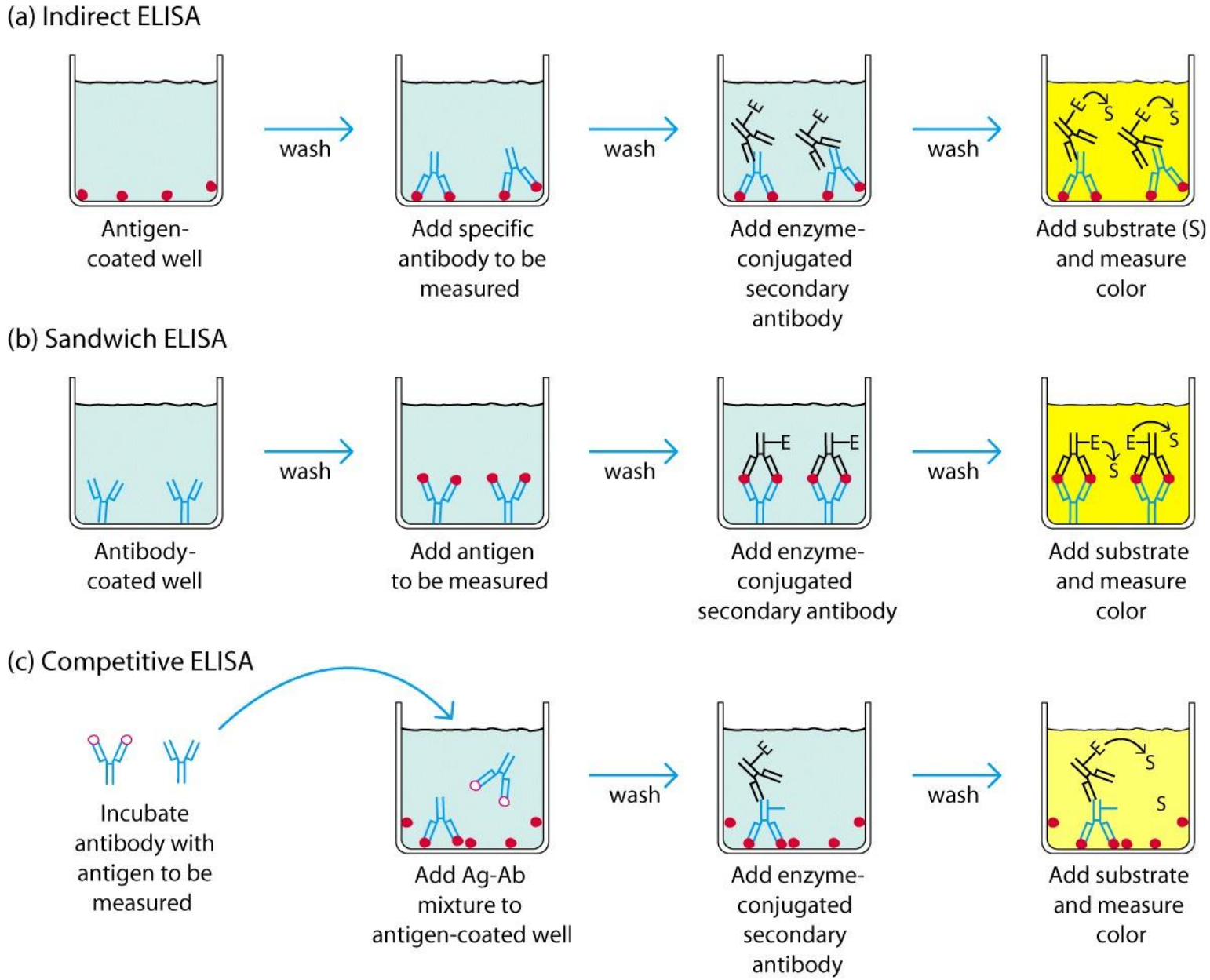
ELISA

- Enzyme Linked Immunosorbent Assay (ELISA)
- Term Was Coined By Engvall and Pearlmann in 1971
- Different Types
  - Sandwich
  - Indirect
  - Competitive
- Can Be Used To Detect Both Antibody and Antigen
- Very Sensitive, pg/mL
- Relies on Monoclonal Abs

- ELISA also has commercial applications,
- including the detection of disease markers and allergens in the diagnostic and food industries.

ELISA microplate reader

ELISA microplate washer

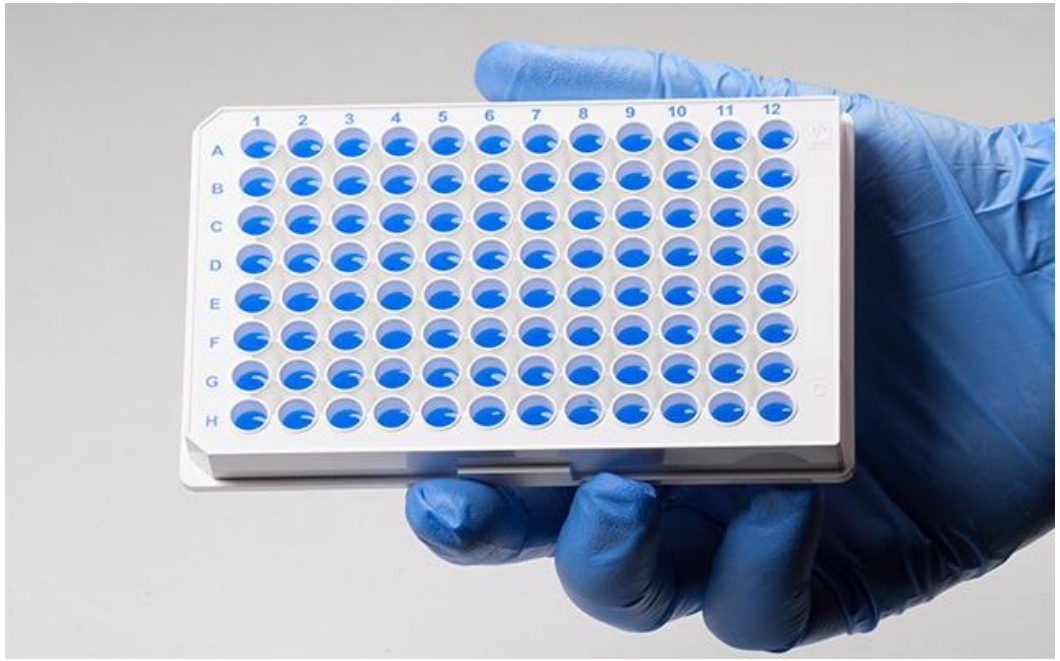

(a) Indirect ELISA
Antigen-coated well
wash
Add specific antibody to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate (S) and measure color
(b) Sandwich ELISA
Antibody-coated well
wash
Add antigen to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color
(c) Competitive ELISA
Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color

Sandwich ELISA

- 2 Antibodies Required
- Must Recognize Different Epitopes
- 1st Antibody Is Referred To As Capture Ab
- 2nd Antibody Detection Ab
- 2nd Antibody Is Biotinylated
- Enzymes Commonly Used: HRP (Horse Radish Peroxidase) And AKP (Alkaline Phosphatase)
- Substrate is TMB (Chromogen)

ELISA Plate

- 96 well plate
- Made of plastic on which protein can be adsorbed (bind) easily
- Usually done overnight @ 4°C
- Special buffer used that will not denature Ab and maximize binding
- Blocking step ensures no empty spaces are left
- Blocking reagent is often 10% FBS



96 well plate

Multi-channel pipette

Standard Curve

- Serial dilutions of the cytokine being measured
- Exact concentration is needed
- A plot of concentration (pg/mL or ng/mL) is plotted against OD (optical density)

Sensitivity Of Elisa

- Typically the lowest cytokine concentration that can be detected above negative control
- 2-3 S.D Above Mean Background Signal
- Depending On Antibody Pair Used Sensitivity Varies
- Ex. 10 pg/mL

Blocking

- Blocking Reagent 10% FBS in PBS
- Alternatively 1% BSA (Immunoassay Grade)
- Why Do We Block?

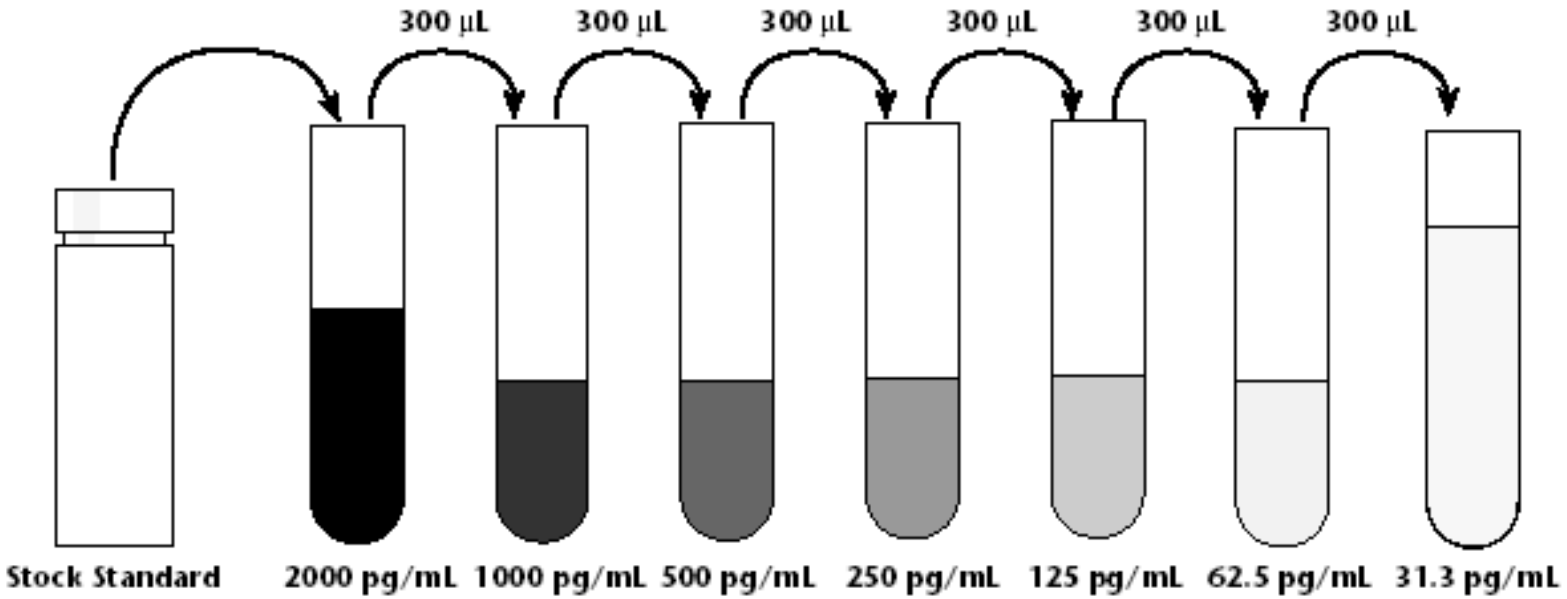
After Blocking

- Wash x3 With PBS/Tween (detergent)
- Add Standards + Samples
- Samples Are Typically Supernatants From Cultures Or Patient Serum/Plasma
- Use 100 μL
- Often Dilution Is Required If Signal Is Too Strong
- Standards?

Standard Preparation

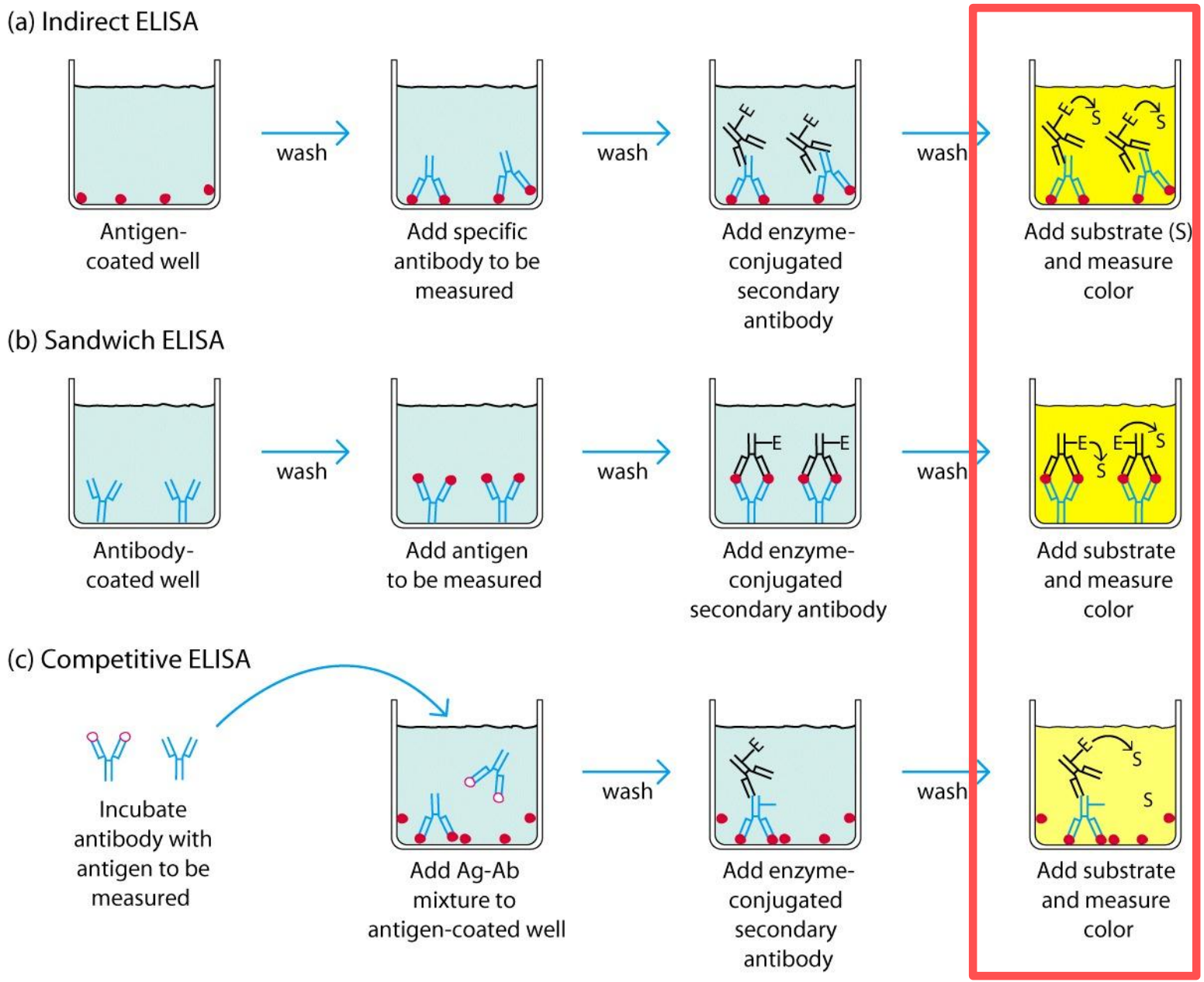
- Standards Are Diluted in Blocking Buffer/Tween
- Start By Labeling eight, 1 mL Eppendorf Tubes
- Prepare Highest Conc. Tube (1 mL)
- Fill The Remaining Tubes with 0.5 mL Blocking Buffer
- Serially Dilute From Top To Lowest

Serial Dilution



After Standard Preparation

- Add Samples, Standards, Negative Control
  - Negative Control Should Be The Buffer You Use Dilute Standard or Culture Medium
- Incubate For 2 Hrs at R.T
- Aspirate And Wash 5x


(a) Indirect ELISA
Antigen-coated well
wash
Add specific antibody to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate (S) and measure color
(b) Sandwich ELISA
Antibody-coated well
wash
Add antigen to be measured
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color
(c) Competitive ELISA
Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
wash
Add enzyme-conjugated secondary antibody
wash
Add substrate and measure color

Addition Of Detection Ab

- Avidin has very high affinity for biotin (B vitamin)
- Add Working Detector @ 100 μL/well
- Incubate for 60 mins @ R.T
- Wash 3x

Addition of Substrate

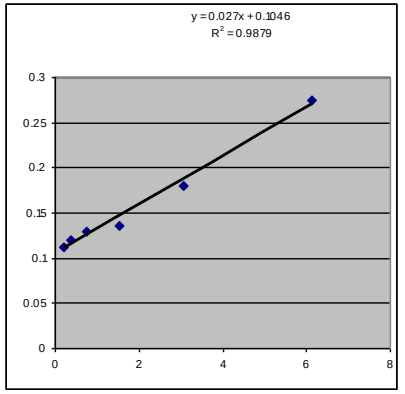
- Add Substrate according to protocol
- Add 100 μL/well
- Incubate for 10 mins,
- Terminate Reaction by Adding 0.5 M H_2SO_4 (color changes from blue to yellow)

Read Plate At Appropriate Wavelength (λ=450 nm)

Data Analysis

	Std 1	Std 2	Dcs	PGE2	LPS	LPS + -5	-6	-7	-8	Neg Ctrl
6.125	0.331	0.275	0.099	0.094	0.315	0.168	0.268	0.289	0.319	0.098
3.0625	0.183	0.18	0.1	0.095	0.31	0.172	0.268	0.285	0.297	0.095
1.53125	0.155	0.136	0.106	0.099	0.286	0.179	0.263	0.263	0.266	0.104
0.765625	0.139	0.13	0.105	0.105	0.322	0.205	0.278	0.298	0.279	0.102
0.382813	0.127	0.12	0.111	0.106	0.324	0.204	0.309	0.353	0.292	0.12
0.191406	0.118	0.112	0.112	0.12	0.31	0.204	0.326	0.308	0.324	0.108
	0.116	0.11	0.045	0.042	0.052	0.052	0.053	0.051	0.042	0.042
	0.123	0.123	0.044	0.052	0.051	0.052	0.054	0.052	0.052	0.053

Dcs	PGE2	LPS	LPS + -5	-6	-7	-8
-0.207	-0.393	7.793	2.348	6.052	6.830	7.941
-0.170	-0.356	7.607	2.496	6.052	6.681	7.126
0.052	-0.207	6.719	2.756	5.867	5.867	5.978
0.015	0.015	8.052	3.719	6.422	7.163	6.459
0.237	0.052	8.126	3.681	7.570	9.200	6.941
0.274	0.570	7.607	3.681	8.200	7.533	8.126



	Med	PGE2 100nM	LPS	NS398 10m	NS398 1mic	NS398 0.1m	NS398 .01microM	
Av	0.033	-0.053	7.651	3.114	6.694	7.212	7.095	
SEM	0.082	0.145	0.206	0.265	0.392	0.458	0.339	

Graph Plotting