Vaccine and sera

lab7

Enzyme linked Immunosorbent Assay ELISA

- **Enzyme linked Immunosorbent Assay (ELISA):** is a plate –based assay designed for detecting and quantifying substances such as, proteins, antibodies, antigens and hormones.
- **ELISA basic principle:-**

1- Substance (antibodies, antigen)

2- Microtiter plates: is a 96 well format are commercially available for use in ELISA.

3-The reactants in the Elisa is attached to a solid –phase, while the separation of the bound is easily made by simple washing procedures.

4-The result of Elisa is a color reaction that can be absorbed by eye and read rapidly using spectrophotometers.

ELISA system major components

1-Antibody: allows for specific detection of substance of interest, e.g. IgG

2-Solid phase (sorbent): allows one to wash away all the material that is not specifically captured.

3- Enzymatic amplification: Allow you to turn a little capture into a visible color change that can be quantified using absorbance plate reader e.g. Horse Radish peroxidase (HRP).

4-substrate: the enzyme act as catalyst to oxidize substrate in the presence of hydrogen peroxide to produce a blue color, reaction stopped with dilute acid to cause complex to turn yellow.

Application:

Measure antibody levels (allergies, vaccine)

Detect viruses(hepatitis ,HIV, venereal diseases)

Types of ELISA assays:

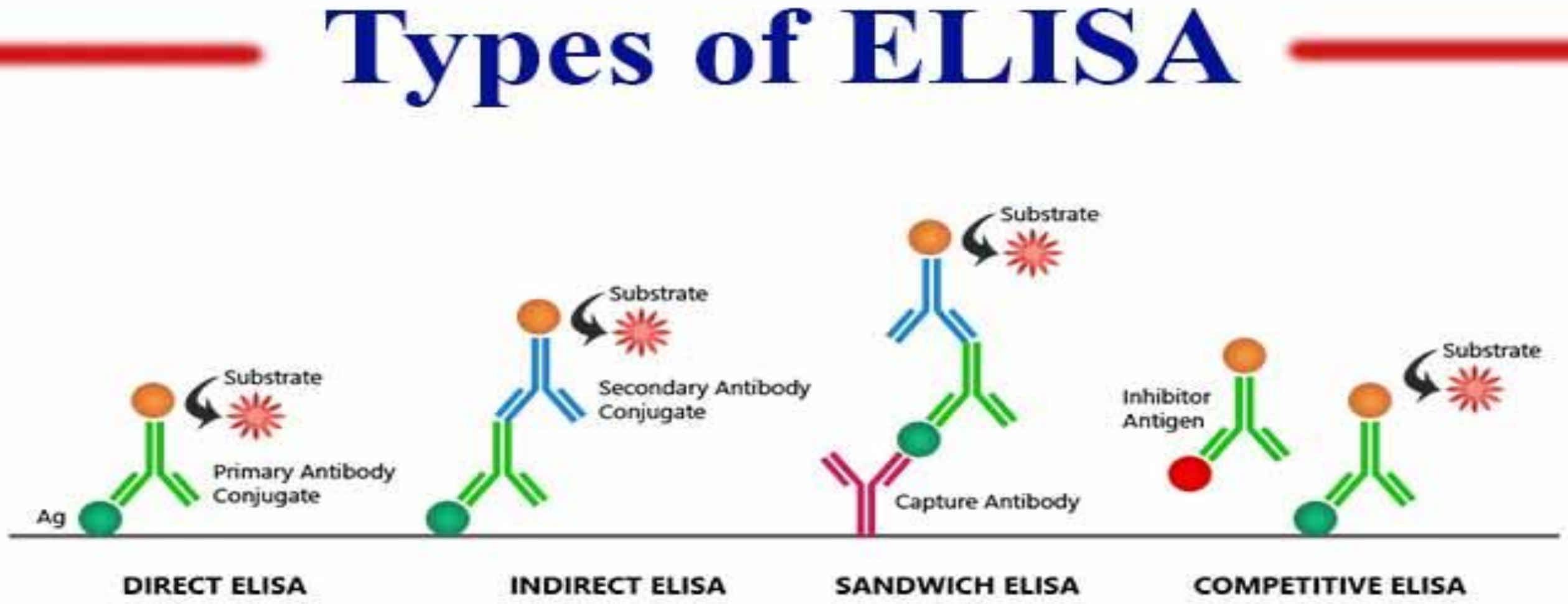
1-Direct ELISA: involve attachment of the antigen to the solid phase, followed by an enzyme-labeled antibody; this type of assay generally makes measurement of crude sample difficult, since contaminating proteins compete for plastic binding sites.

2-Indirect ELISA : involve attachment of the antigen to the solid phase but in this case ,the primary antibody is not labeled .an enzyme-conjugated secondary antibody ,directed at the first antibody, is then added .this format is used most often to detect specific antibodies in sera.

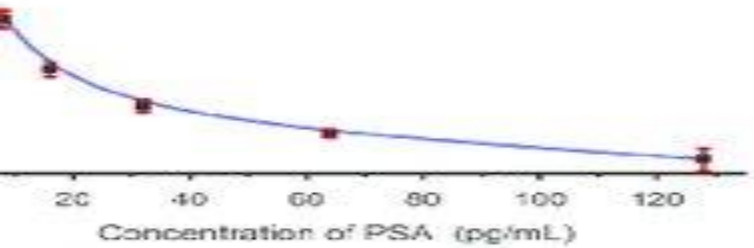
3-Competitive ELISA : involves the simultaneous addition of competing antibodies or proteins, the decrease in signal of sample where the second antibody or protein is added gives a highly specific result.

4- Sandwich ELISA: involve attachment of a capture antibody to a solid phase support. Sample containing known or unknown antigen are then added in a matrix or buffer that will minimize attachment to the solid phase. An enzyme-labeled antibody is then added for detection.

Types of Elisa



c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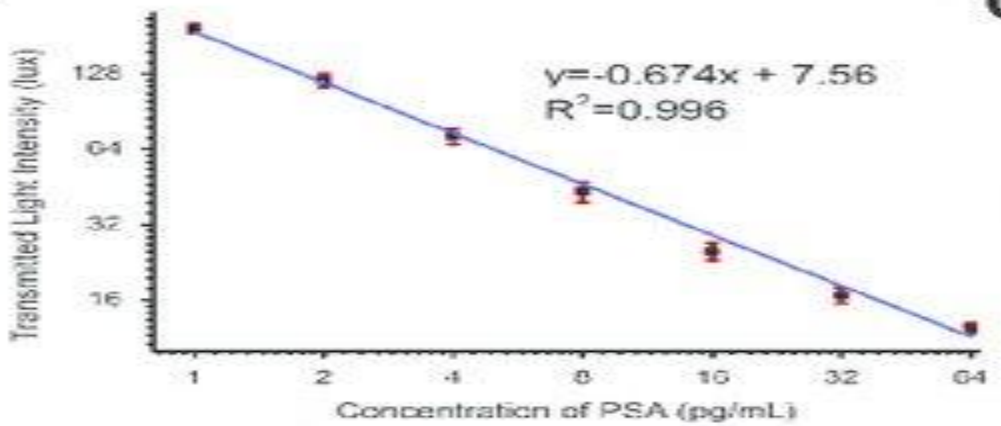
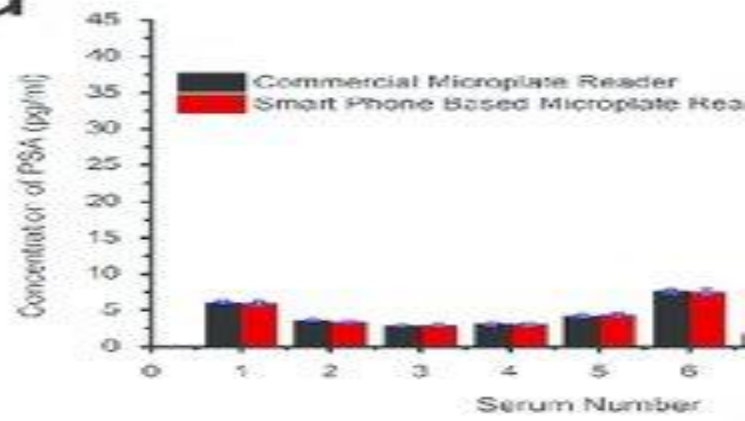
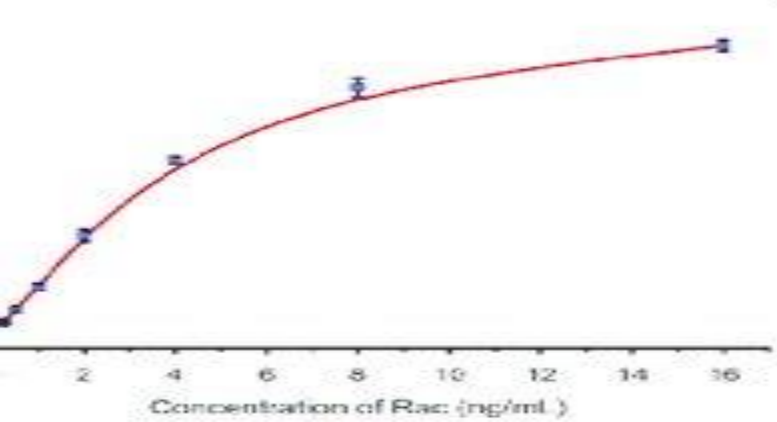
40
60
80
100
120
Concentration of PSA (pg/mL)

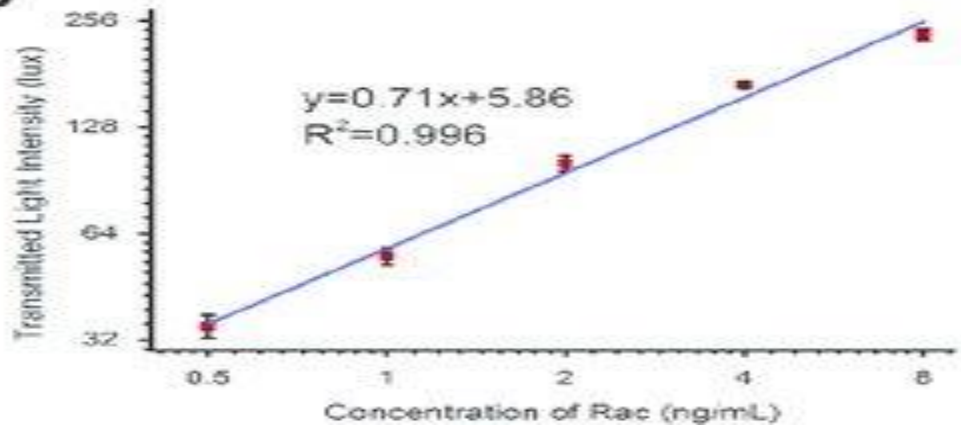

Transmitted Light Intensity (lux)
128
64
32
16
y=-0.674x + 7.56
R²=0.996
1
2
4
8
16
32
64
Concentration of PSA (pg/mL)

d

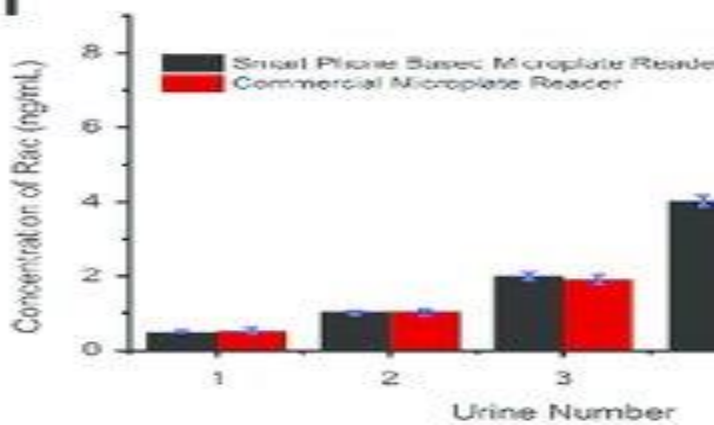

Concentration of PSA (pg/ml)
45
40
35
30
25
20
15
10
5
0
Commercial Microplate Reader
Smart Phone Based Microplate Rea
0
1
2
3
4
5
6
Serum Number

g


2
4
6
8
10
12
14
16
Concentration of Rac (ng/mL)


Transmitted Light Intensity (lux)
256
128
64
32
y=0.71x+5.86
R²=0.996
0.5
1
2
4
8
Concentration of Rac (ng/mL)

h


Concentration of Rac (ng/mL)
8
6
4
2
0
Smart Phone Based Microplate Reade
Commercial Microplate Reader
1
2
3
Urine Number

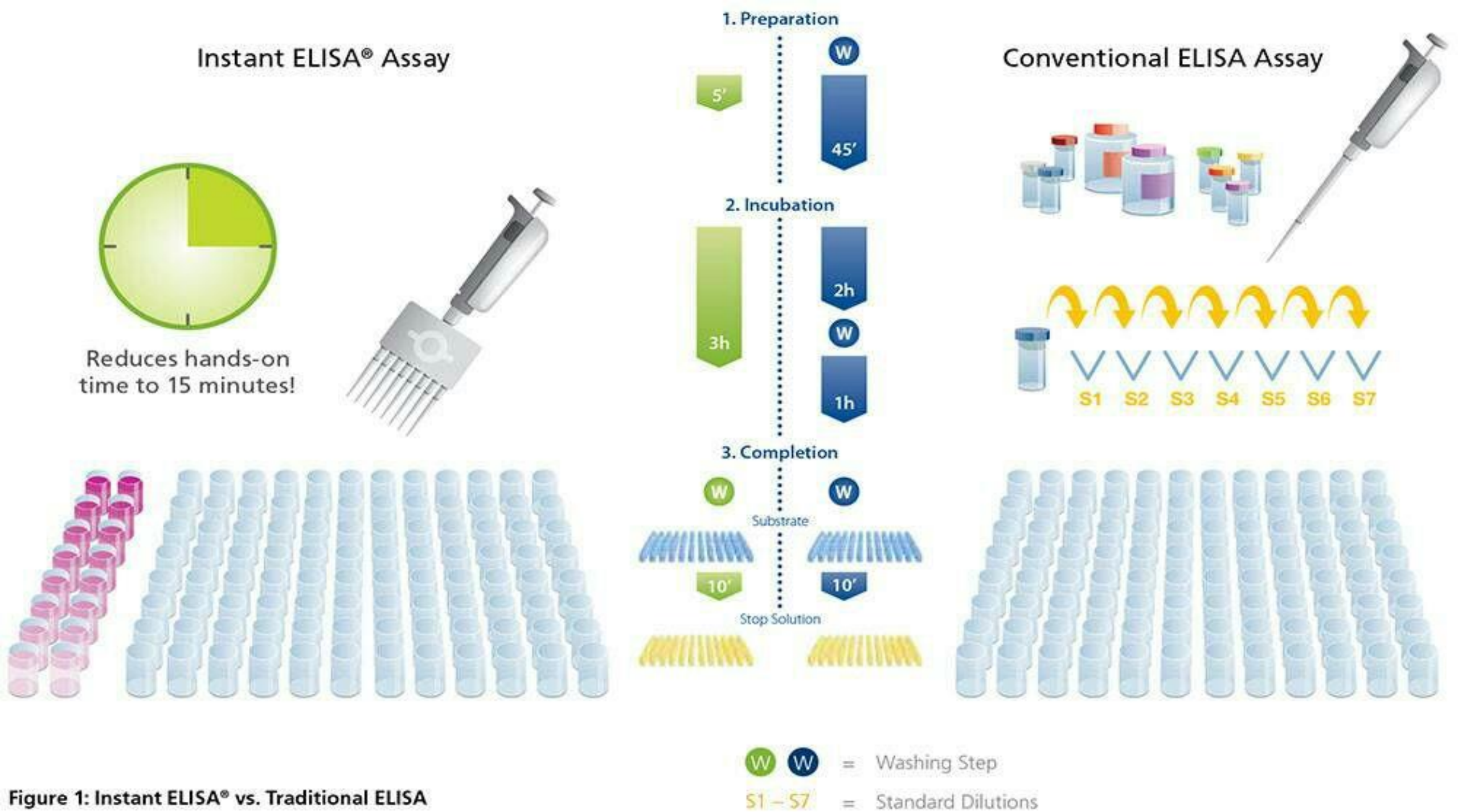


Figure 1: Instant ELISA® vs. Traditional ELISA