
بسم الله الرحمن الرحيم

ELISA technique
(Quality Control and Troubleshooting)

Dr. Masoud Parsania

Associate professor

Department of Microbiology, Faculty of Medicine, Tehran Medical Sciences,

Islamic Azad University, Tehran Iran

IMMUNOASSAYS

Introduction

I. **Definition of an immunoassay**:

- An *immunoassay* is an analytical technique which uses naturally occurring reagents known as antibodies for the selective determination of sample components
- Immunoassays are commonly used in a wide variety of areas, especially in biochemistry and clinical chemistry

II. **Examples of the application of immunoassay include:**

- Drug testing
- Hormone testing (insulin in diabetic patients)
- Bacterial or viral testing (AIDS, hepatitis)

III. **Advantages of immunoassays are:**

- Inexpensive to perform
- Highly selective
- Low limits of detection
- Can have high-throughput. Often done in batch mode
- Applicable to the determination of a wide-range of compounds

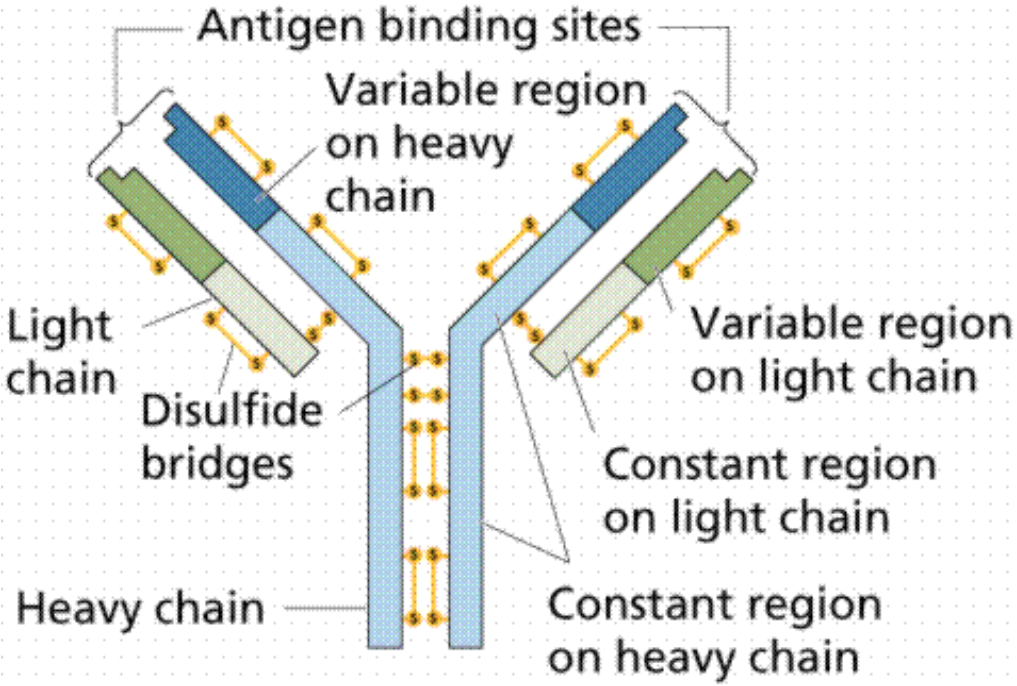
IMMUNOASSAYS

Antibodies

I. Definition of an antibody:

- An *antibody (Ab),* or *immunoglobulin (Ig),* is a member of a family of glycoproteins that make up part of the body's immune system.

II. Basic structure of an antibody:



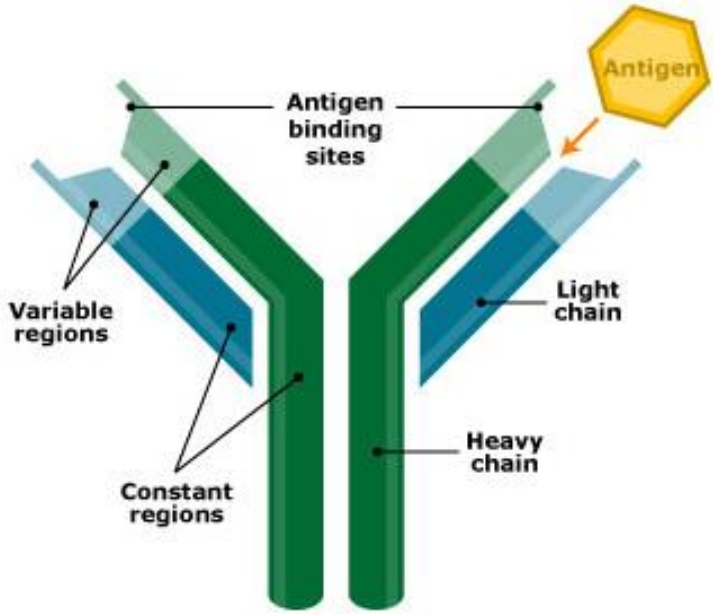
The above antibody consists of four polypeptides-two identical heavy chains (H) and two identical light chains (L) connected by disulfide bonds. These are arranged in a "Y"-shaped structure ending with two identical sites that recognize and bind a given foreign agent or antigen

IMMUNOASSAYS

Introduction

IV. Antibody – Antigen Interactions:

- The body contains between 10^6 and 10^8 types of antibodies
- Each antibody has the ability to bind to a different foreign agent, or ***antigen*** (*Ag*)
- The ability of an antibody to recognize and bind a given antigen depends on the structure of its binding site
  - Determined by the amino acid sequence of the antibody near the N-terminal ends of the heavy and light chains



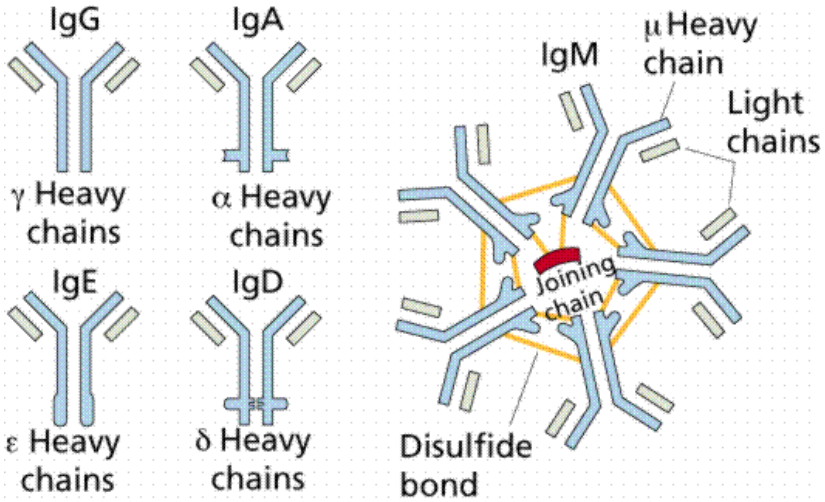
IMMUNOASSAYS

Introduction

V. Antibody Usage:

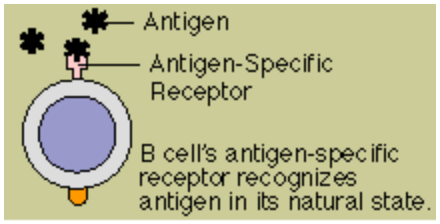
- The selectivity of Ab-Ag interaction makes antibodies useful as analytical reagents for the determination of specific components in mixtures
- Antibodies are useful as analytical reagents since they can be produced to a wide variety of substances:
  - For large analytes (> 5,000 MW), antibodies can be produced by directly injecting the compound into an animal
  - For small analytes (< 5,000 MW), antibodies can also be produced, but require that the compound first be coupled to a larger molecule, such as a protein, prior to injections

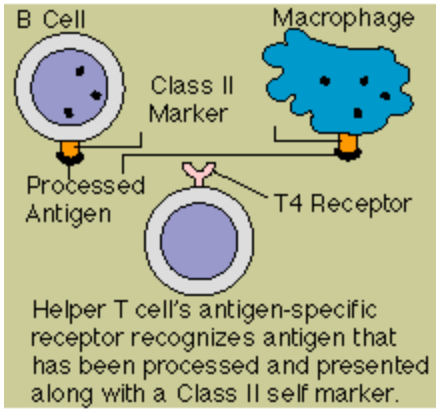
Five classes of antibodies

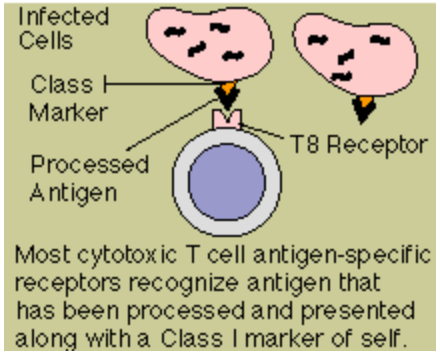


Humoral immunity

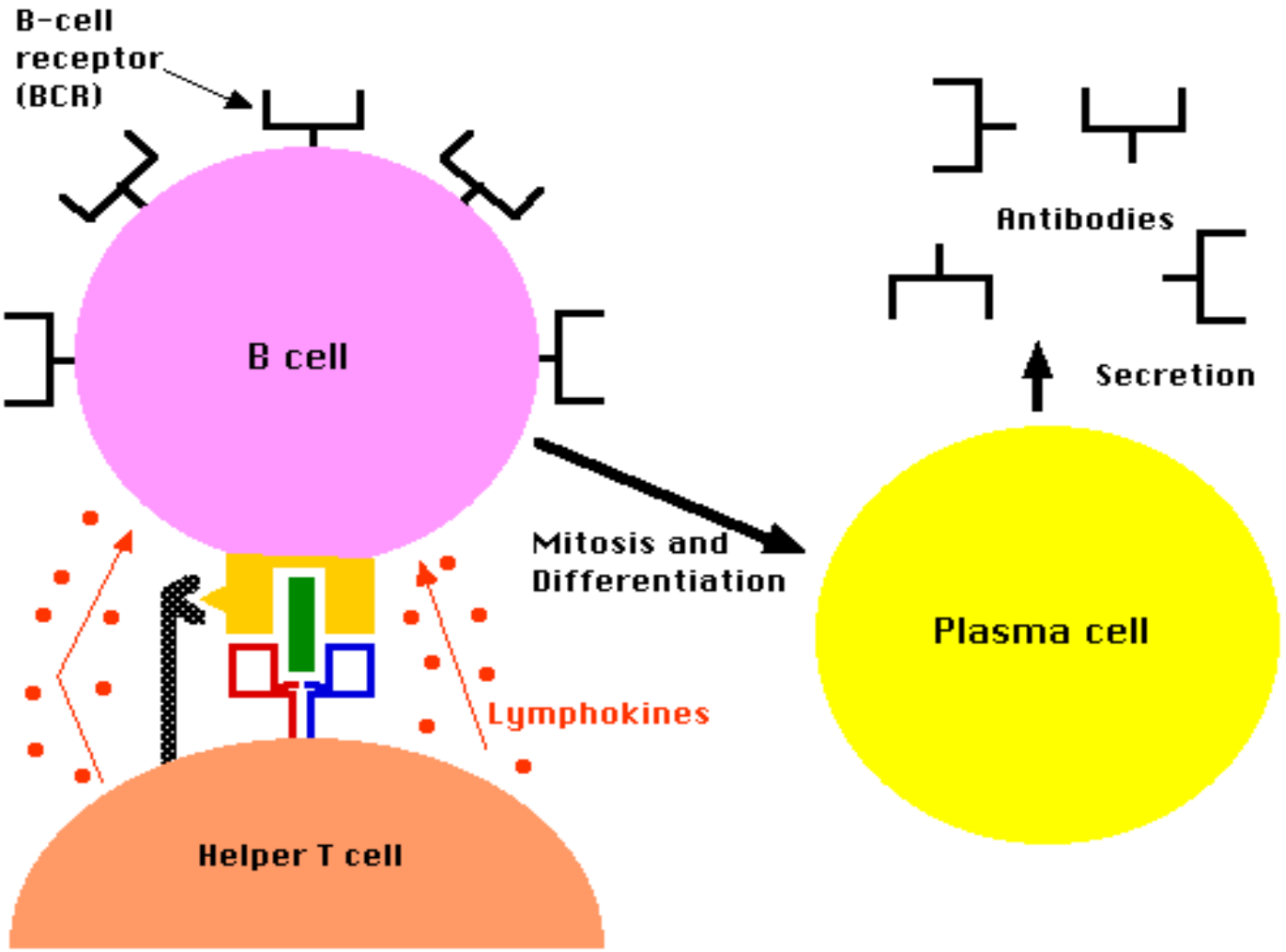
- **Antibody: antigen-binding immunoglobulin (protein), produced by B cells; functions as the <u>effector</u> in an immune response.**







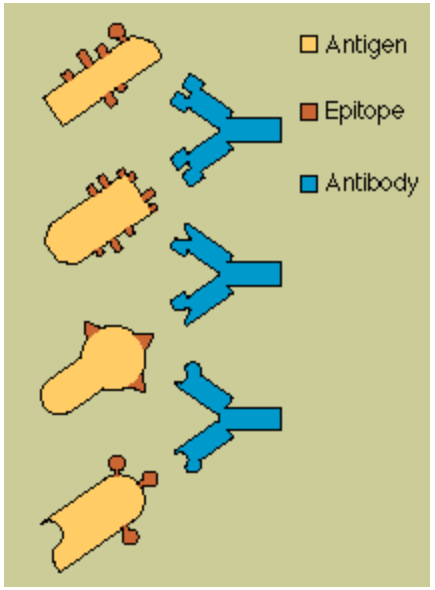
B cell/Helper T cell/Plasma cell



Antigen /Antibody Connection

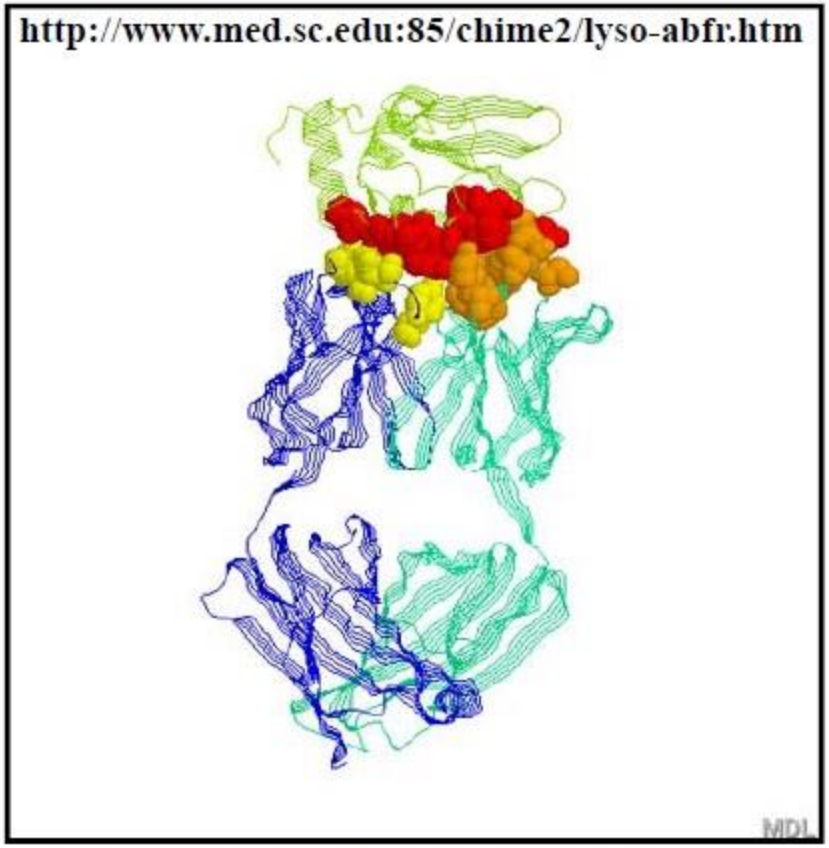
- **Foreign molecules, or antigens, carry distinctive markers, characteristic shapes called epitopes that protrude from their surfaces.**

- **Our Immune system has the ability to recognize many millions of distinctive non-self molecules, and to respond by producing molecules, or antibodies - also cells - that can match and counteract each one of the non-self molecules.**



Nature of Ag/Ab Reactions

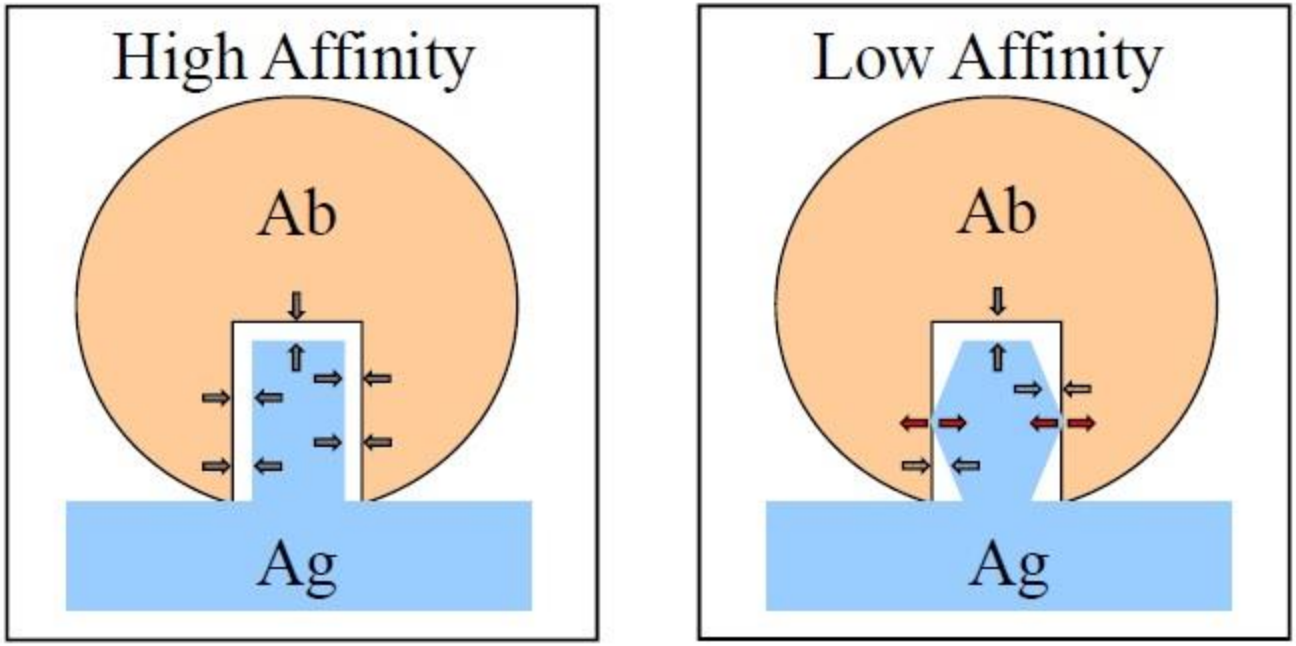
- Lock and Key Concept
- Non-covalent Bonds
  - Hydrogen bonds
  - Electrostatic bonds
  - Van der Waal forces
  - Hydrophobic bonds
- Multiple Bonds
- Reversible



Source: Li, Y., Li, H., Smith-Gill, S. J., Mariuzza, R. A., Biochemistry 39, 6296, 2000

Affinity

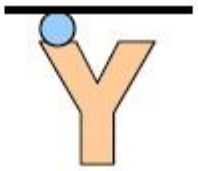
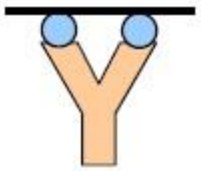
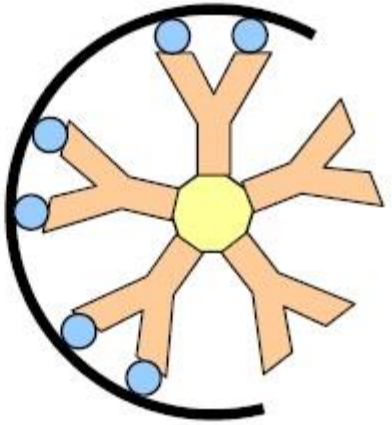
- Strength of the reaction between a single antigenic determinant and a single Ab combining site



Affinity = ☜ attractive and repulsive forces

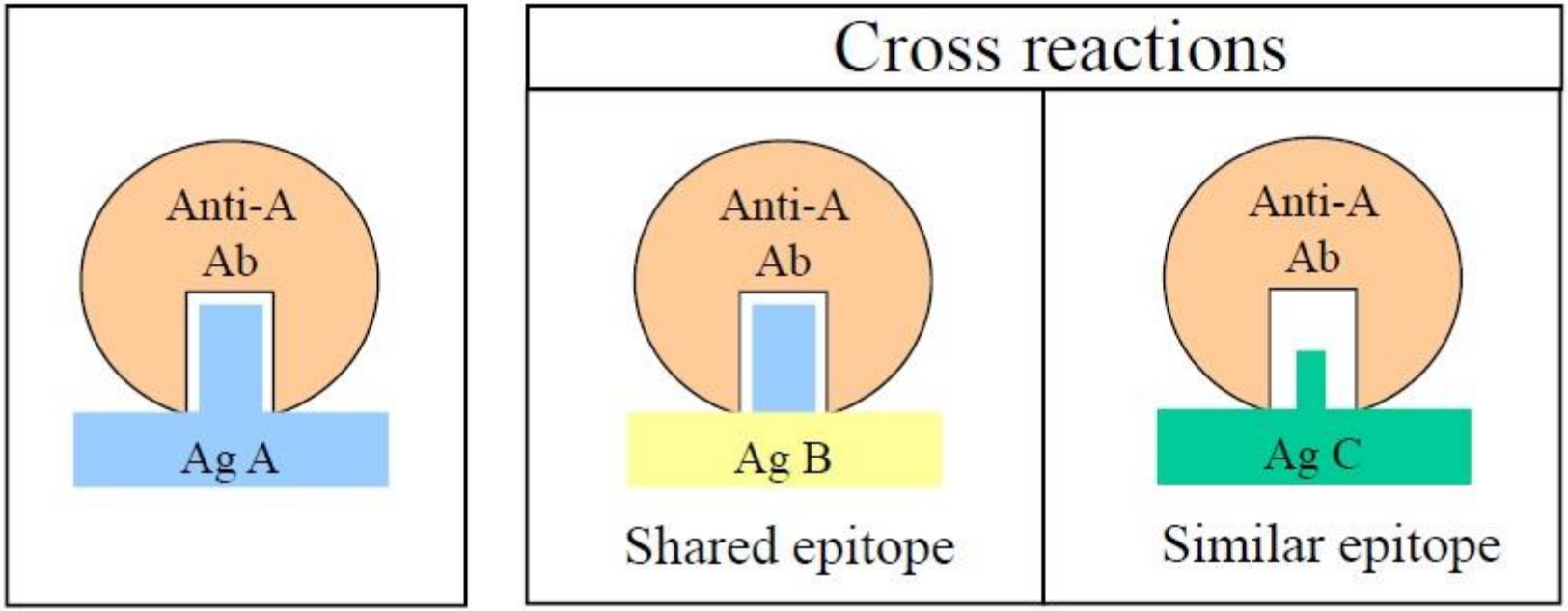
Avidity

- The overall strength of binding between an Ag with many determinants and multivalent Abs

			
K_{eq} =	10^4 Affinity	10^6 Avidity	10^{10} Avidity

Cross Reactivity

- The ability of an individual Ab combining site to react with more than one antigenic determinant.
- The ability of a population of Ab molecules to react with more than one Ag

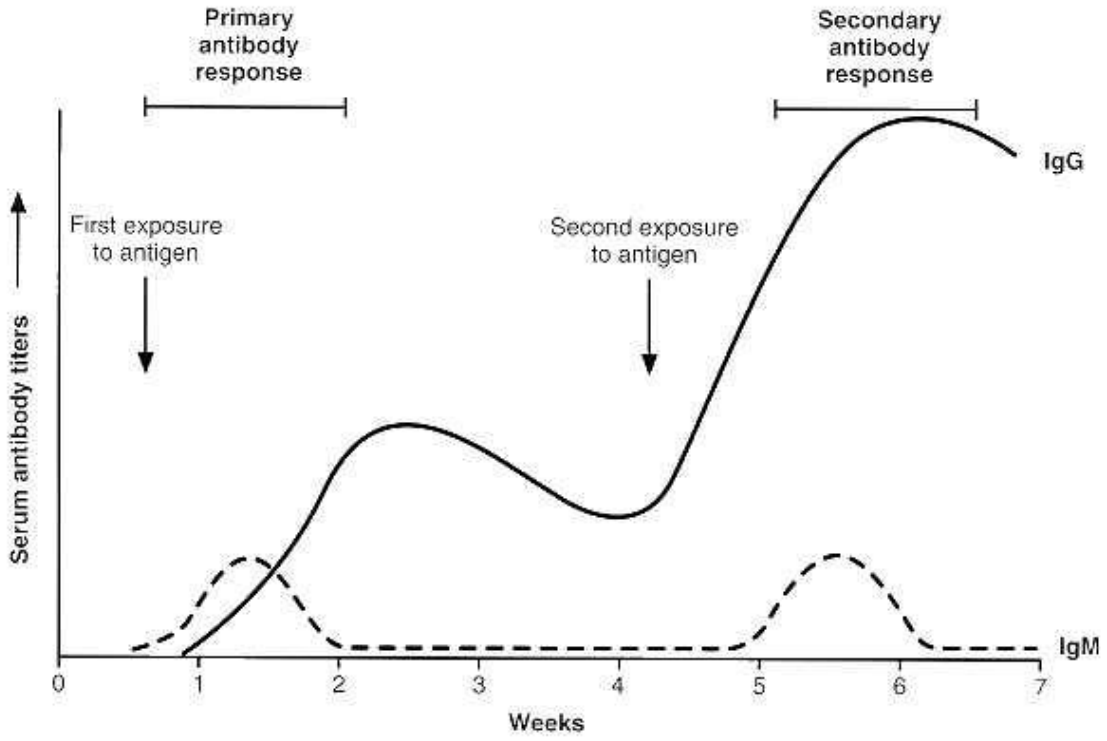


IMMUNOASSAYS

Introduction

VI. Antibody Production - *polyclonal antibodies* :

- One common method for making antibodies to a substance (antigen) is to inject the analyte or analyte-protein conjugate into an animal several times over a period of a few weeks to a few months

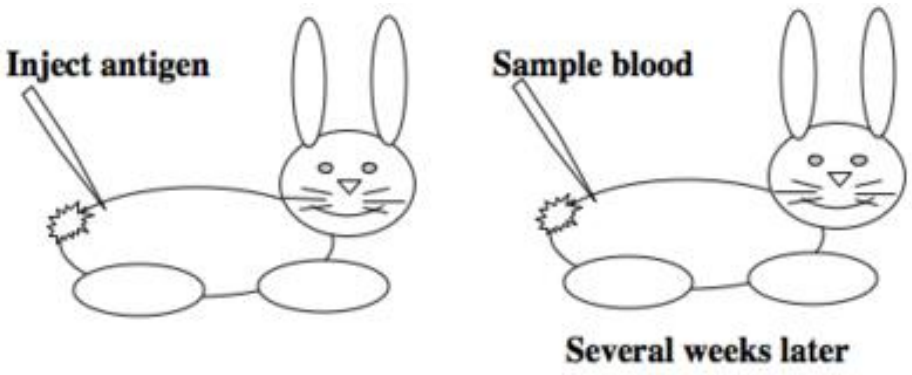


IMMUNOASSAYS

Introduction

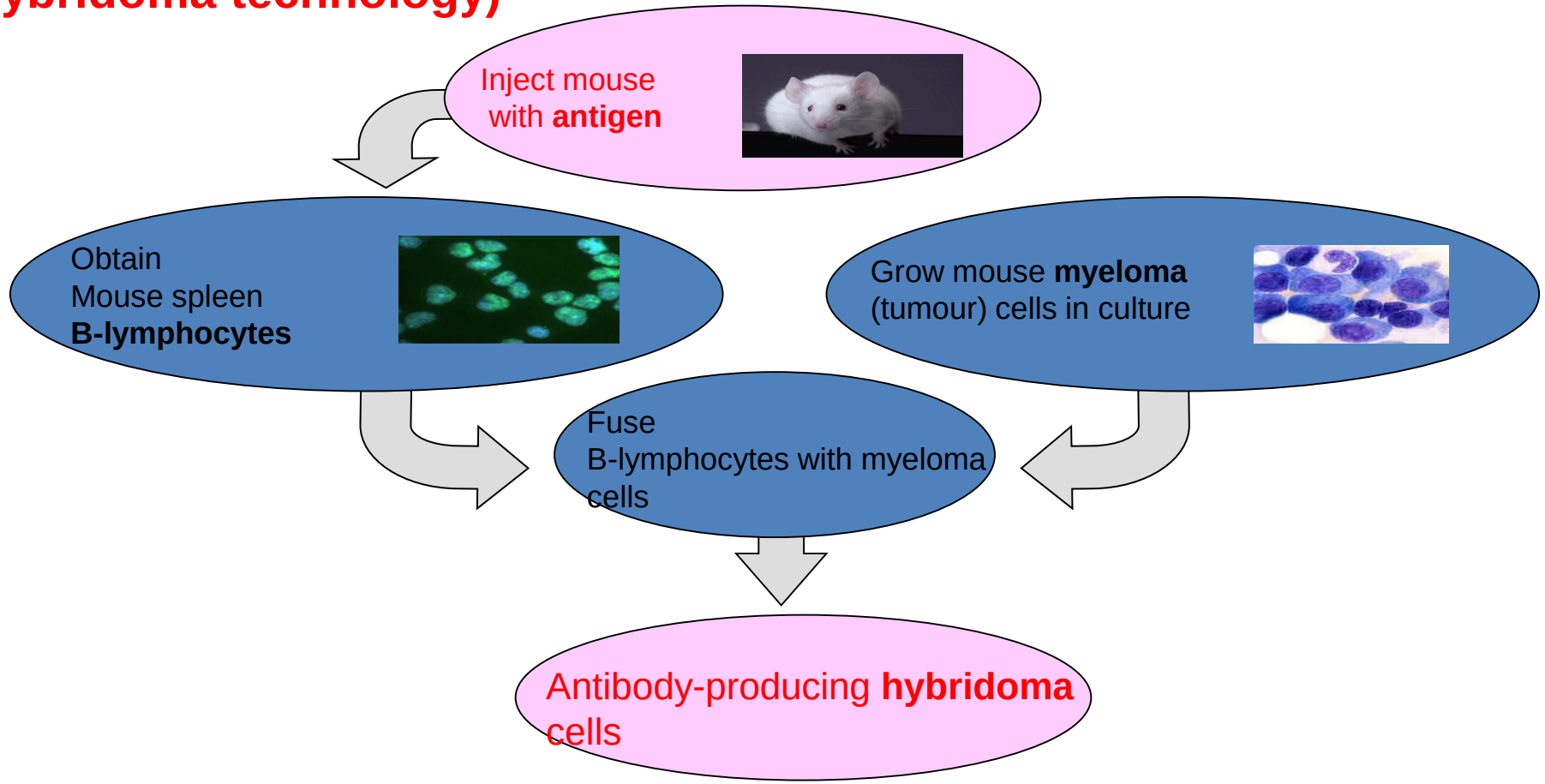
VI. Antibody Production – *polyclonal antibodies*:

- If the agent is a foreign to the animal, the animal will develop antibodies to the agent and release these antibodies into its blood.
- After a few months, blood is removed from the animal and the antibodies produced are collected for use



- Antibodies produced in this fashion are typically very heterogeneous
  - Recognize a number of different sites on the analyte
  - Binding with a range of affinities (K_a)
- Heterogeneous antibodies are known as ***polyclonal antibodies***
  - Arise from several different lines of antibody-producing cells within the animal

Monoclonal antibody production
(hybridoma technology)

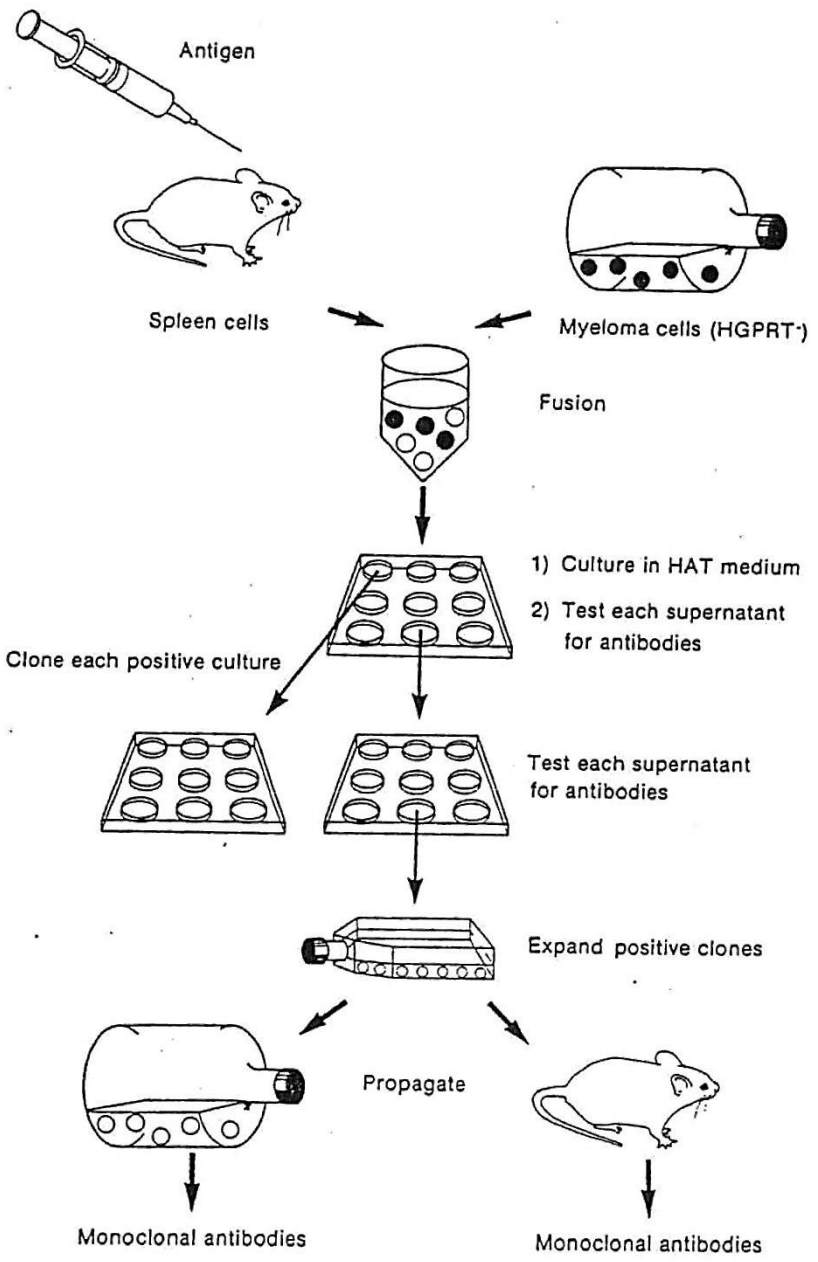


IMMUNOASSAYS

Introduction

VII. Antibody Production - *monoclonal antibodies (mAb)*:

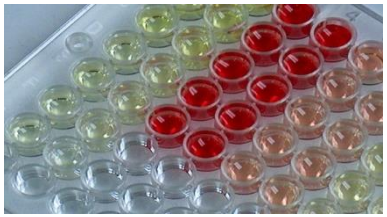
- Monoclonal antibodies differ from polyclonal antibodies in that they are produced by a single cell line within the body
- ***All*** monoclonal antibodies from the same cell line recognize the **same** site on an analyte and bind with an **identical** binding affinity (K_a)



Classification of Ag-Ab interactions

1. Primary serological tests: (Marker techniques) e.g.
   - Enzyme linked immuono sorbent assay (ELISA)
   - Immuno florescent antibody technique (IFAT)
   - Radio immuno assay (RIA)
2. Secondary serological tests: e.g.
   - Agglutination tests
   - Precipitation tests
   - Flocculation tests
   - Complement fixation tests (CFT)
   - Serum neutralization tests (SNT)
   - Toxin-antitoxin test
3. Tertiary serological test: e.g.
   - Determination of the protective value of an anti serum in an animal.

Basic steps of ELISA
Enzyme Linked Immunosorbent Assay



1. Antigen of interest is absorbed on to plastic surface (‘*sorbent*’).

2. Antigen is recognised by specific antibody (‘*immuno*’).

3. This antibody is recognised by second antibody (‘*immuno*’) which has enzyme attached (‘*enzyme-linked*’).

4. Substrate reacts with enzyme to produce product, usually coloured.

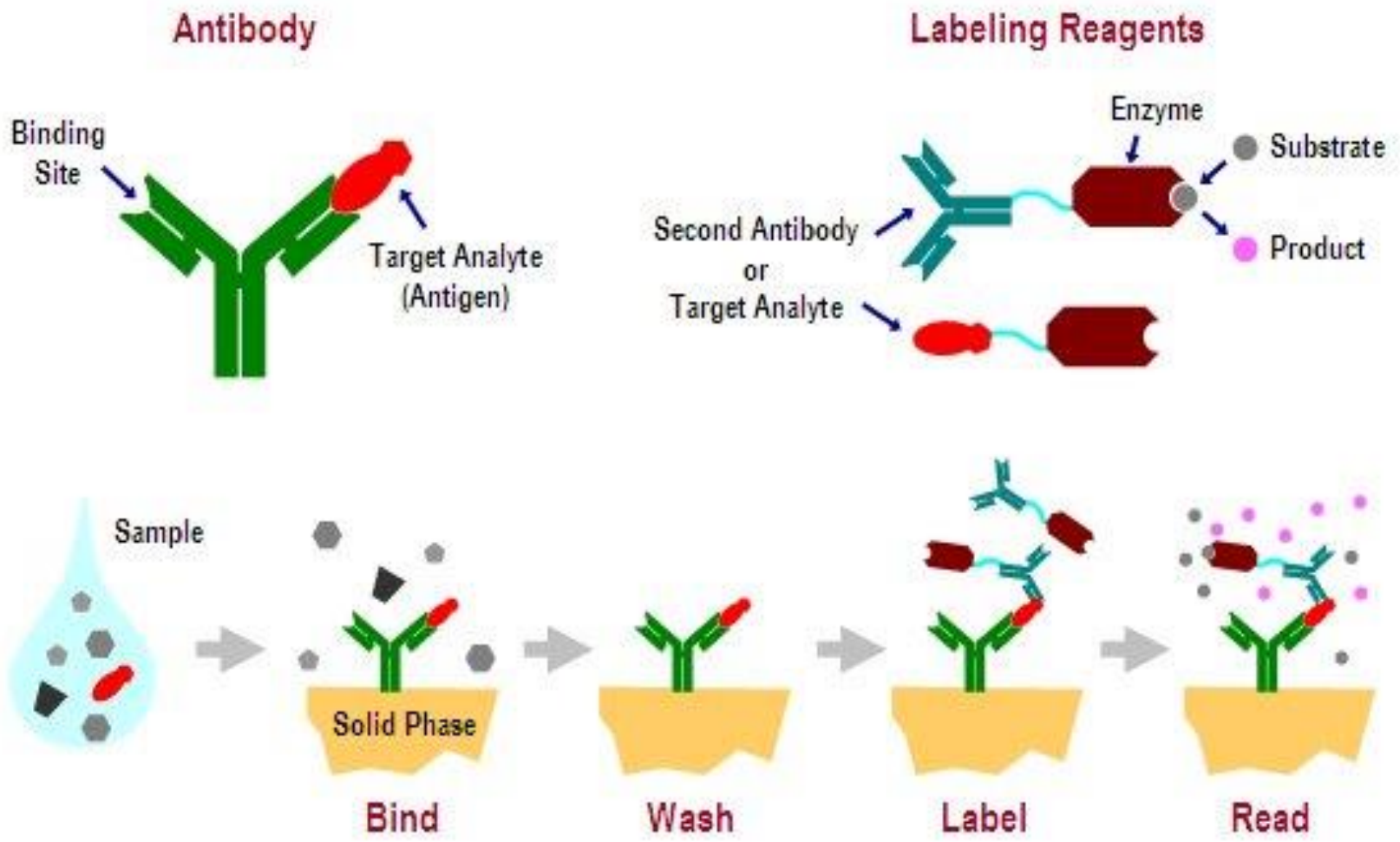
Coloured product = measure (*assay*) of antigen present

What do you measure ?

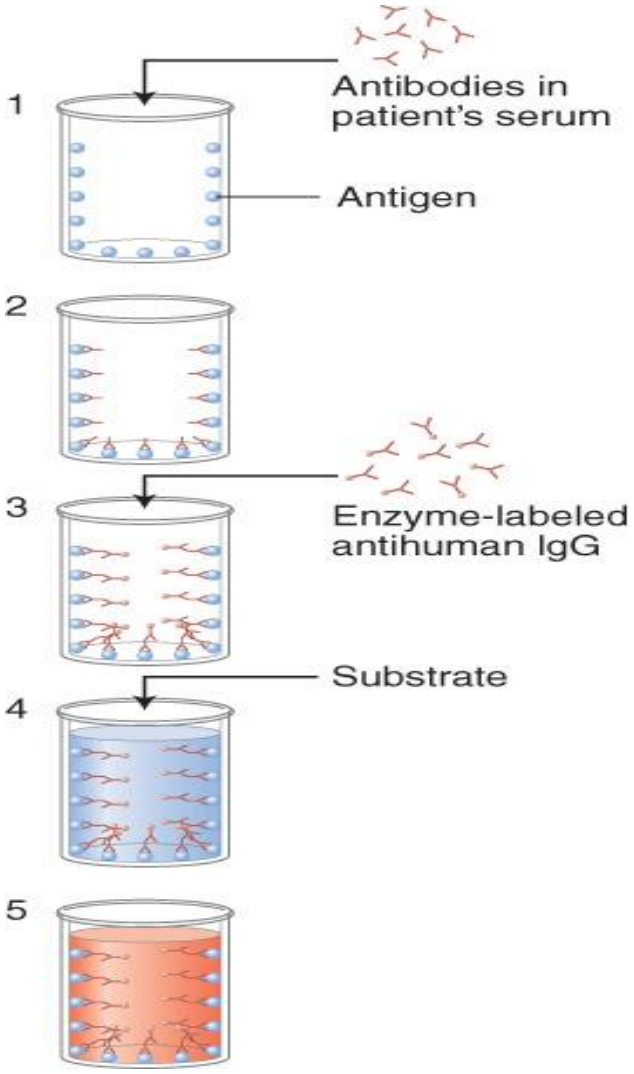
- ELISAs can provide a useful measurement of antigen or antibody concentration.

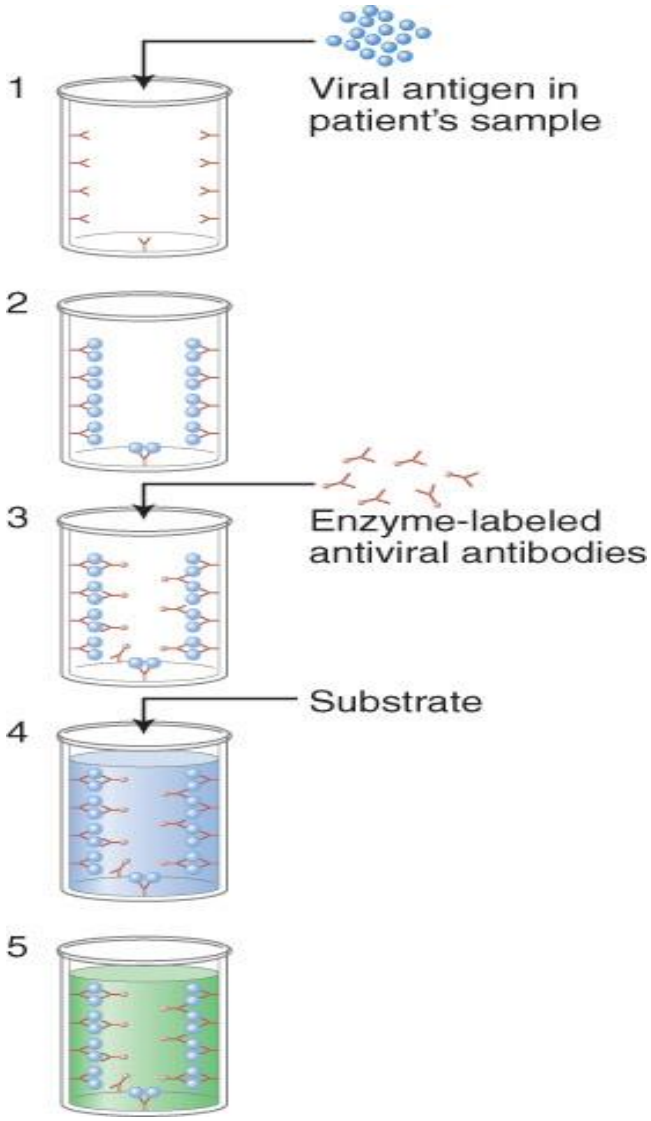
1. detect the presence of antigens that are recognized by an antibody
2. test antibodies that recognize an antigen.

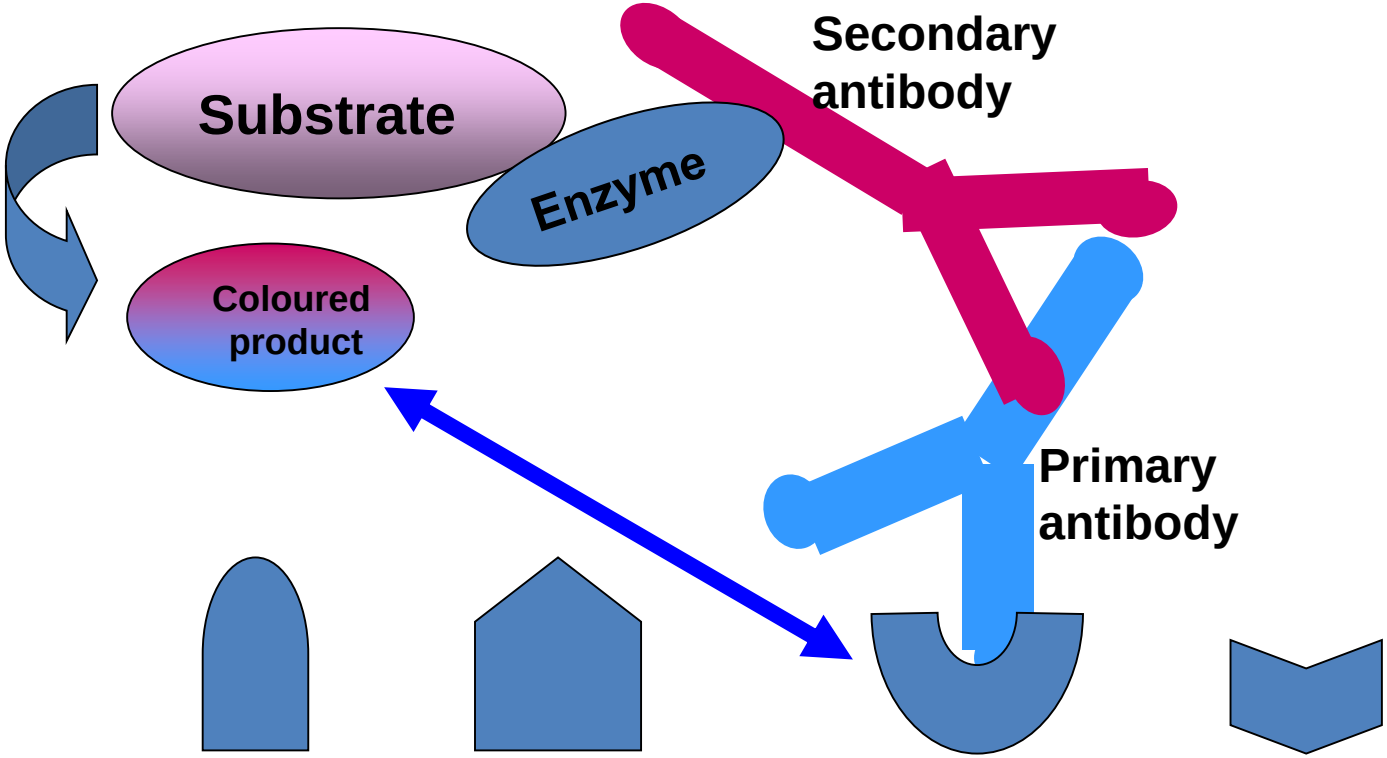
ELISA

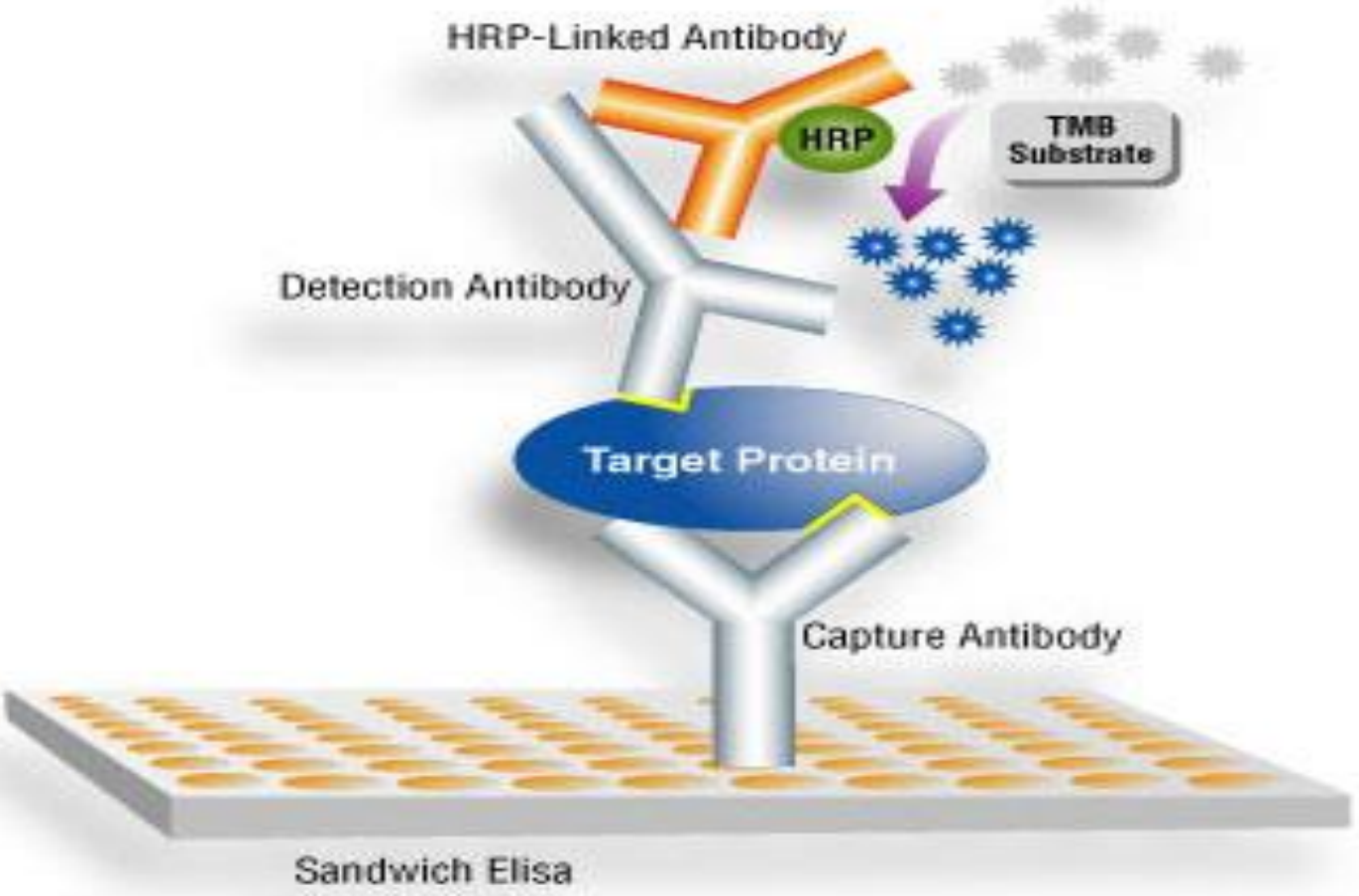

Antibody
Binding Site
Target Analyte (Antigen)
Labeling Reagents
Enzyme
Substrate
Second Antibody or Target Analyte
Product
Sample
Solid Phase
Bind
Wash
Label
Read

ELISA Procedures






Substrate
Enzyme
Secondary antibody
Coloured product
Primary antibody
Different antigens in sample


HRP-Linked Antibody
HRP
TMB Substrate
Detection Antibody
Target Protein
Capture Antibody
Sandwich Elisa

sandwich ELISA

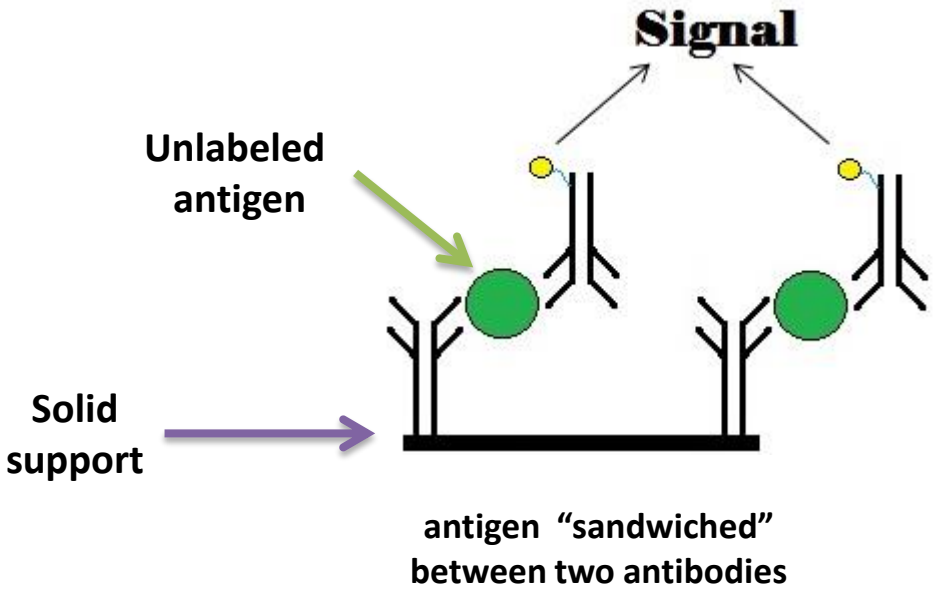
- There are many different types of ELISAs. One of the most common types of ELISA is “sandwich ELISA ”
- The sandwich ELISA measures the amount of antigen between two layers of antibodies.
- The antigens to be measured must contain at least two antigenic sites, capable of binding to antibody, since at least two antibodies act in the sandwich..

IMMUNOASSAYS

Sandwich immunoassays

I. Quantitative method based on use of two antibodies to detect analyte

- First antibody extracts analyte from sample
- Second antibody (containing chemical label) identifies presence of analyte



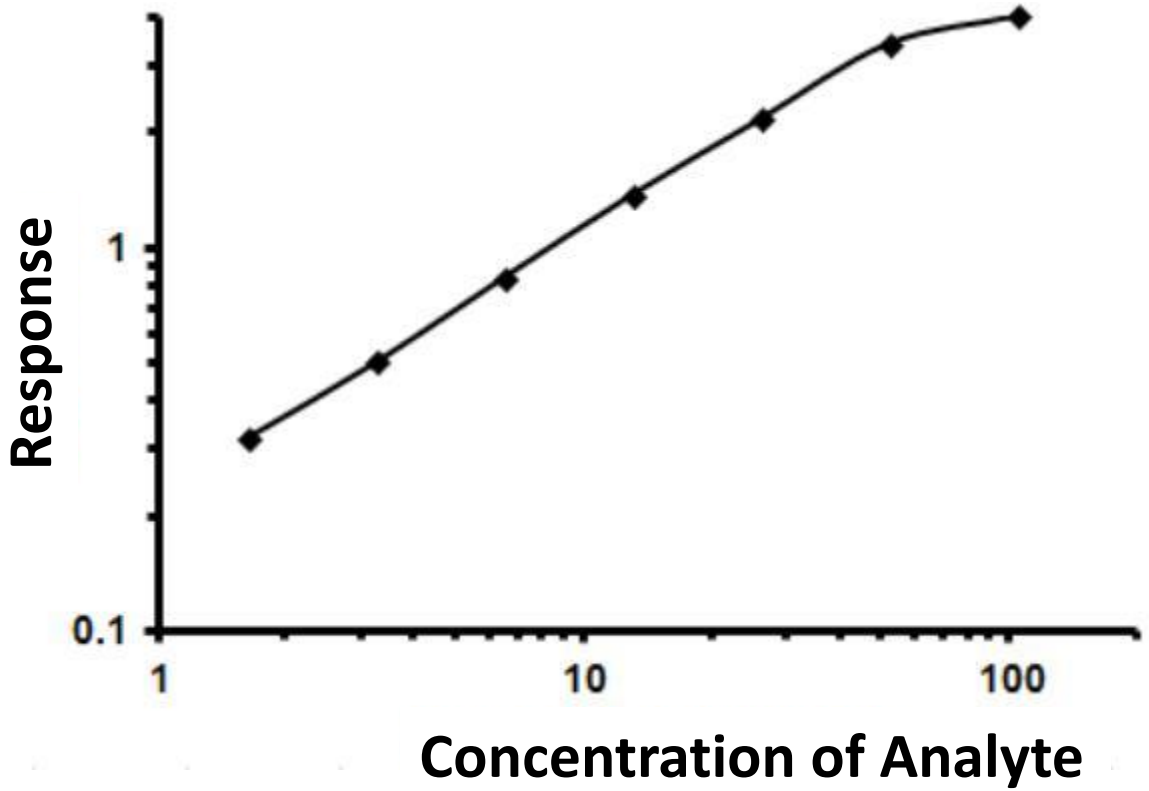
- This type of assay measures the amount of analyte in the sample by looking at the amount of labeled antibody that binds to analyte on the solid support

IMMUNOASSAYS

Sandwich immunoassays

I. Quantitative method based on use of two antibodies to detect analyte

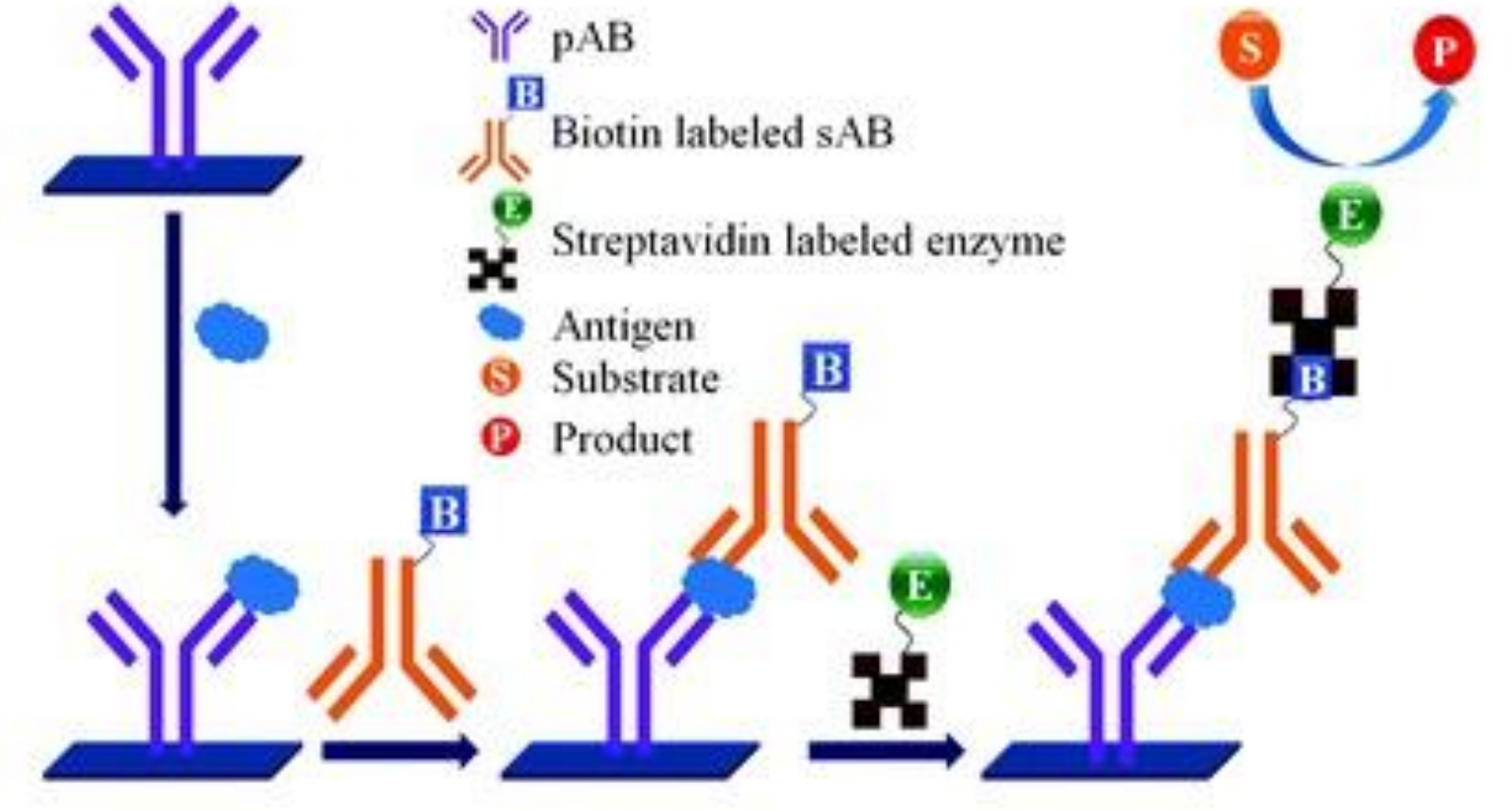
- A typical calibration curve for the assay



- **The sensitivity of the Sandwich ELISA is dependent on four factors:**
- **The number of molecules of the first antibody that are bound to the solid phase.**
- **The avidity of the first antibody for the antigen.**
- **The avidity of the second antibody for the antigen.**
- **The specific activity of the second antibody.**

Signal Amplification Using Chromogenic Detection

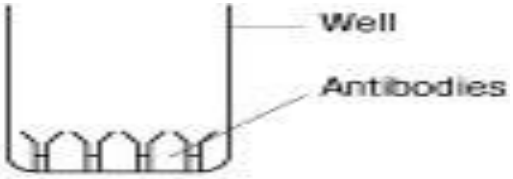
Avidin-Biotin Complex (ABC)



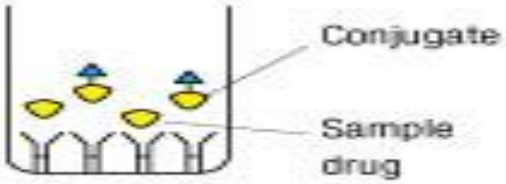
Competitive assay

- Plate is coated with specific antibody
- Test antigen is placed together with labeled antigen
- Both antigens compete to bind to the Antibody

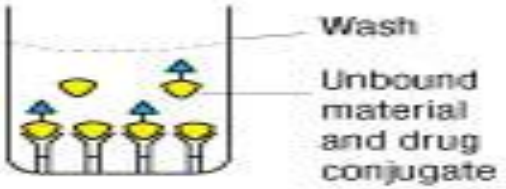
Plates are precoated with the antibody. The plate is ready for use. DO NOT WASH.



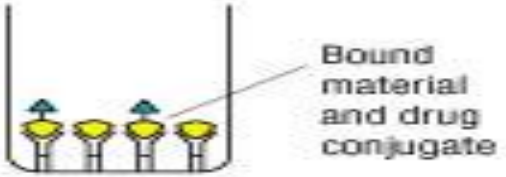
A sample or control is added to each well. Next the drug-enzyme conjugate is added and the mixture is incubated at room temperature.



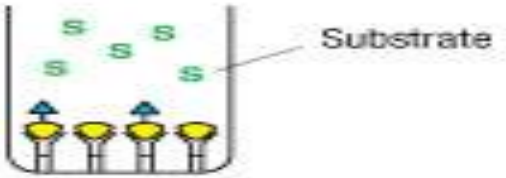
Wash the plate to remove all unbound materials.



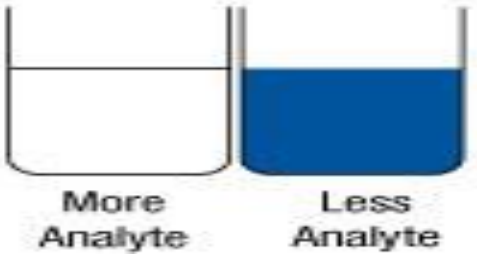
The bound materials now remain in the microplate.



Add TMB substrate to each well and allow the color to develop.



Qualitative results are obtained by measuring the absorbance reading at 650 nm or 450 nm if acid stop is used.

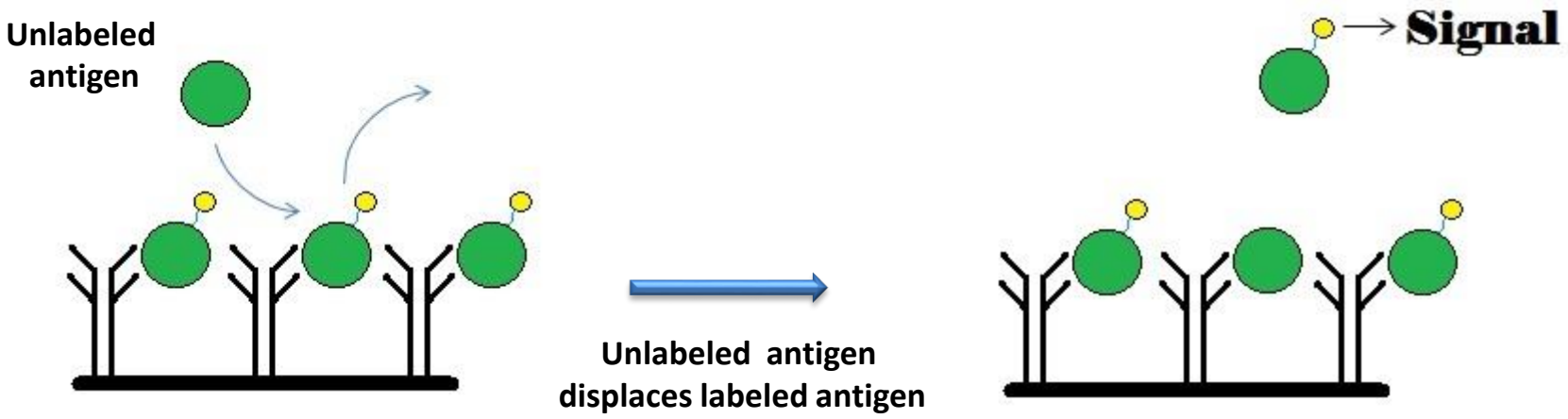


IMMUNOASSAYS

Competitive binding immunoassays

I. Quantitative method based on competition between analyte in sample and a **fixed** amount of labeled analyte for a **limited** number of antibody binding sites (*equilibrium method*)

- Indirectly measures the amount of analyte in the sample by looking at amount of labeled analyte it displaces from the antibody

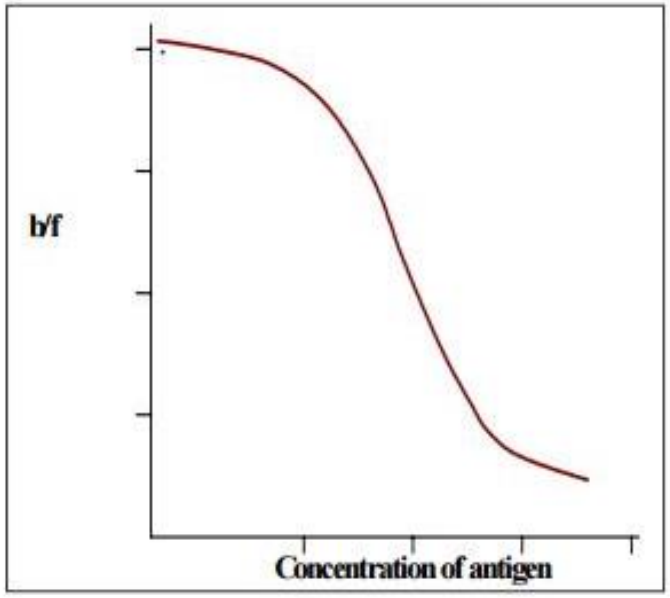


IMMUNOASSAYS

Competitive binding immunoassays

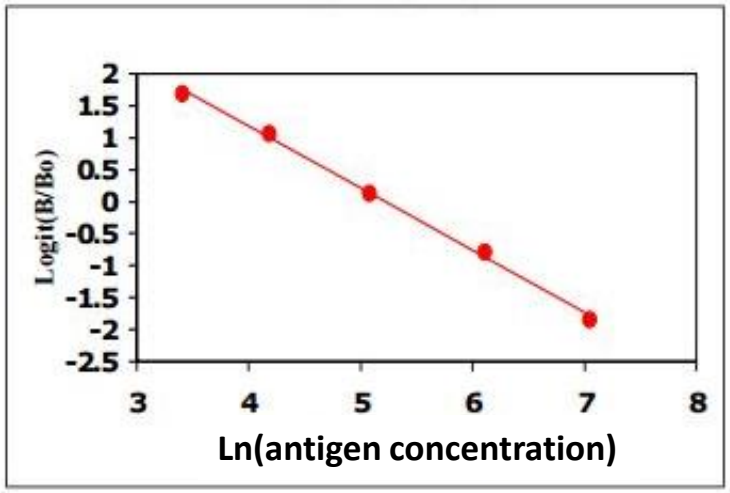
I. Quantitative method based on competition between analyte in sample and a **fixed** amount of labeled analyte for a **limited** number of antibody binding sites (*equilibrium method*)

- A typical calibration curve for the assay



Linear transform

$$Logit\frac{B}{B_o} = In\left[\frac{\frac{B}{B_o}}{1-\frac{B}{B_o}}\right]$$



- **There are commercial kits for assays in different types of Ag or Ab by ELISA.**

The materials for your kit

1. **ELISA plate**
2. **Positive control**
3. **Negative control**
4. **Dilution Buffer (already in dilution tubes)**
5. **Conjugate (secondary antibody)**
6. **TMB Substrate**
7. **Stop solution**

Sandwich ELISA to detect antigen concentration.

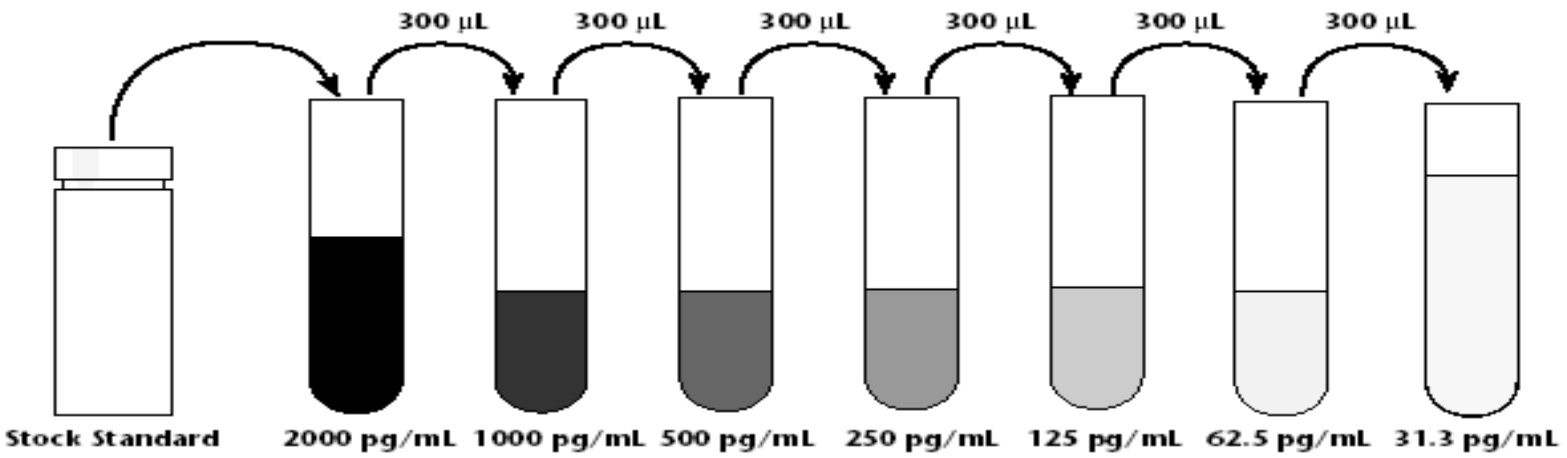
- Use the polyvinylchloride (PVC) micro-titer plate.
- Bind the unlabeled antibody to the bottom of each well by 50 mL of antibody solution to each well (20 mg/mL in PBS). PVC will bind approximately 100 ng/well (300 ng/cm2).
- Incubate the plate overnight at 4° C to allow complete binding

- Wash the wells twice with PBS. A 500 mL squirt bottle is convenient. The antibody solution washes can be removed by flicking the plate over a suitable container.
- The remaining sites for protein binding on the microtiter plate must be saturated by incubating with blocking buffer.
- Blocking buffer - 3% BSA/PBS with 0.02% sodium azide.

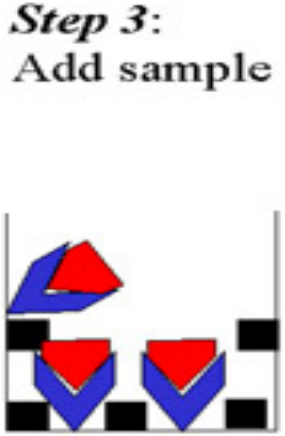
- Incubate for 2hrs to overnight in a humid atmosphere at room temperature.

  (Note: Sodium azide is an inhibitor of horseradish peroxidase. Do not include sodium azide in buffers or wash solutions, if an HRP-labeled antibody will be used for detection).
- Wash wells twice with PBS.

Standard Preparation

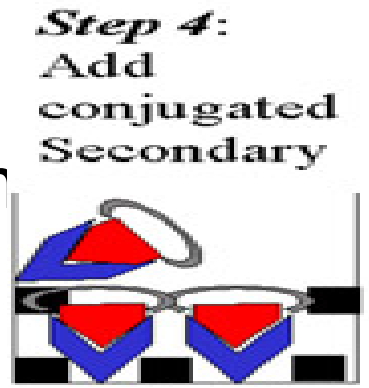


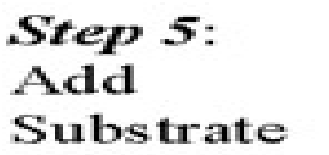
- Add Samples and Standards
- if dilutions are necessary, it should be done in blocking buffer.
- Incubate for at least 2 hrs at room temperature. Wash the plate four times with PBS.

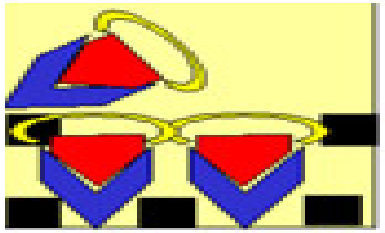


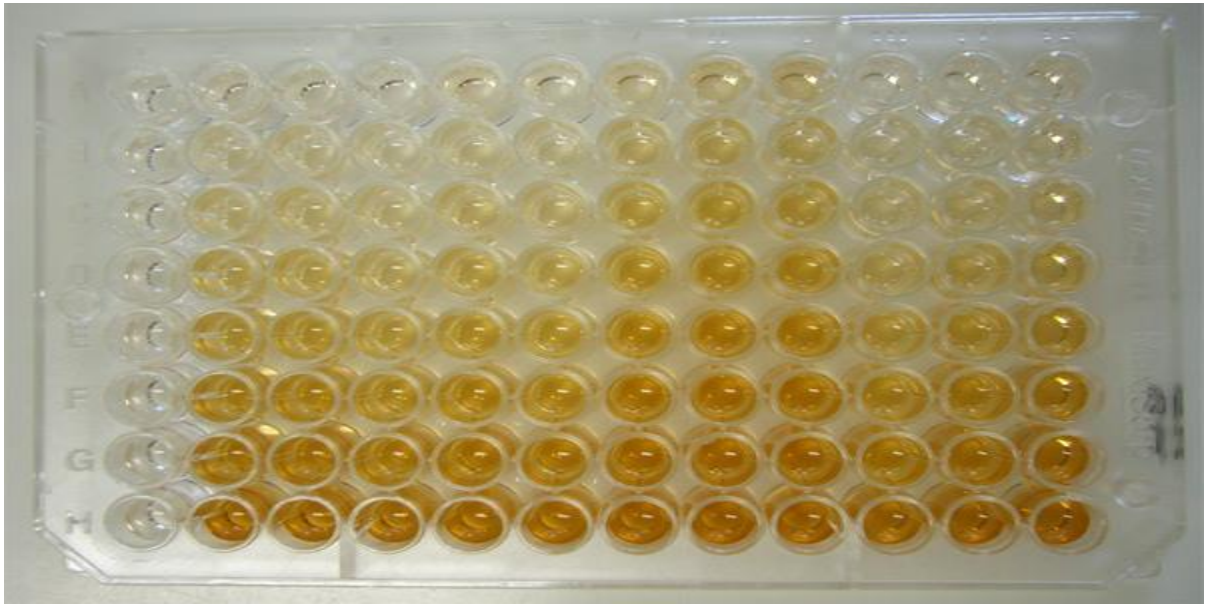

MultiWash
Advantage
corning

- Add the labeled second antibody.
- Incubate for 2 hrs or more at room temperature.
- Wash with several changes of PBS.
- Add substrate as indicated by manufacturer.








A
B
C
D
E
F
G
H

Read Plate At Appropriate Wavelength (λ=450 nm)

Microplate Reader

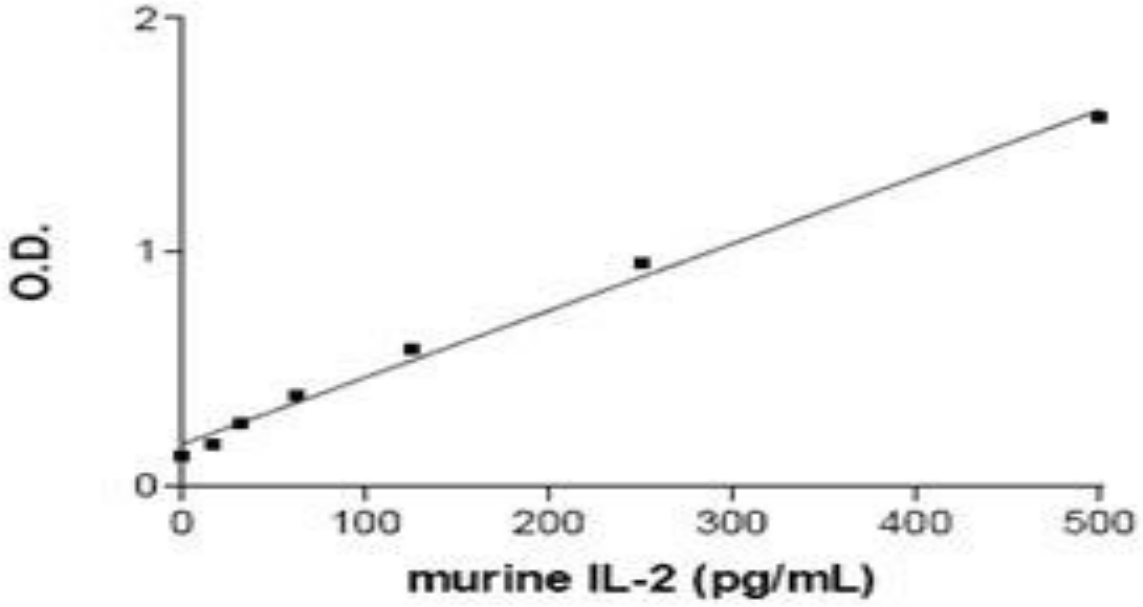
1. wavelength range to that used in ELISA, generally between
2. 400 to 750 nm (nanometres)

3. Some readers (ultraviolet range between 340 to 700 nm.

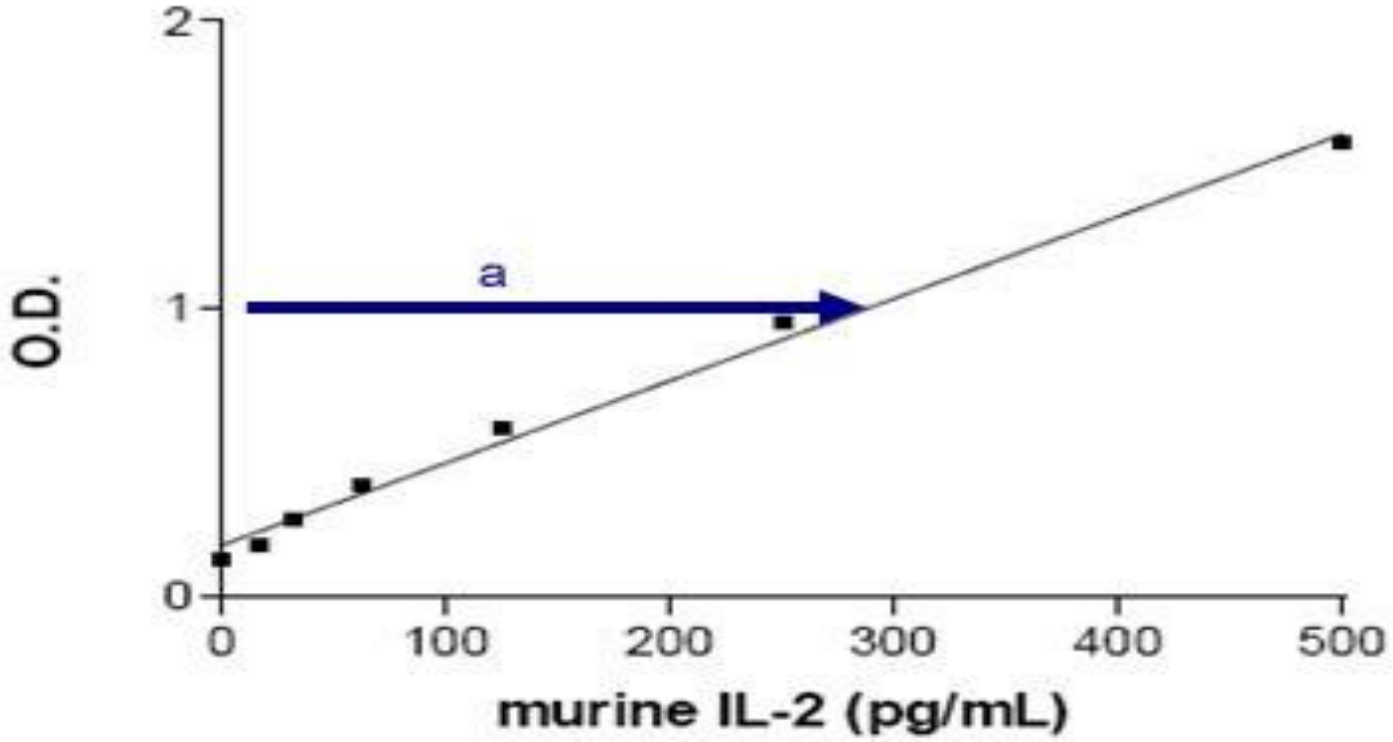
Microplate Reader

INSTALLATION REQUIREMENTS

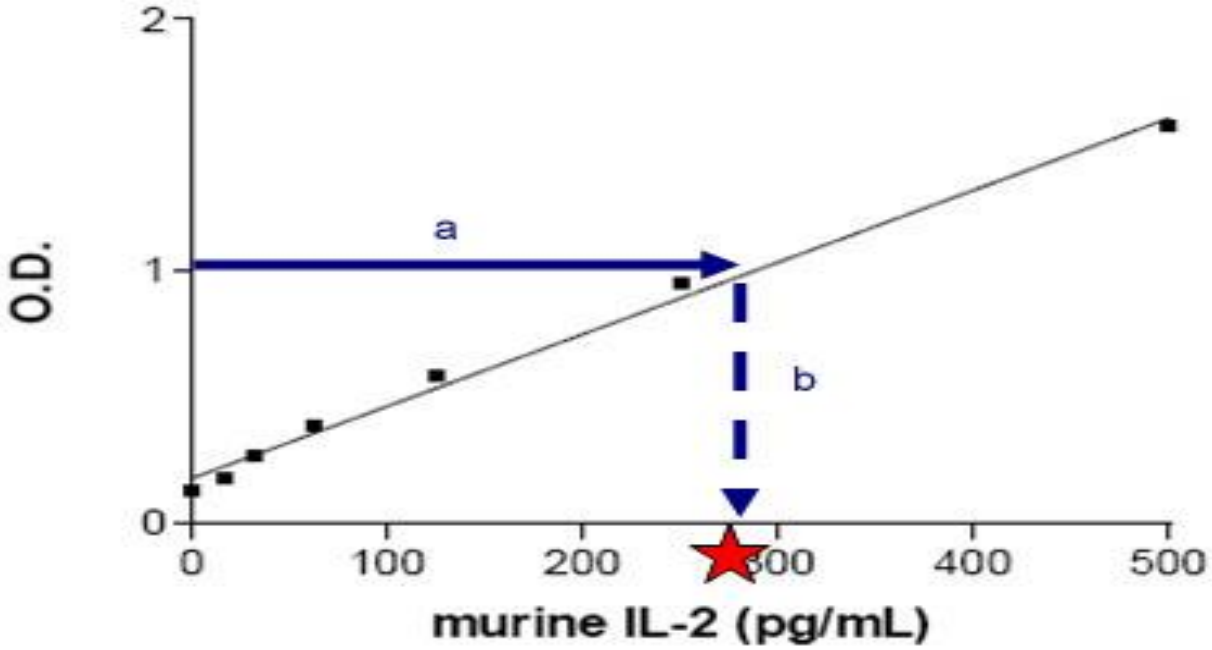
1. A clean, dust free environment.
2. A stable work table away from equipment that vibrates (centrifuges, agitators).
3. An electrical supply source, which complies with the country's norms and standards.



Standard curve murine IL-2 ELISA kit ab46097

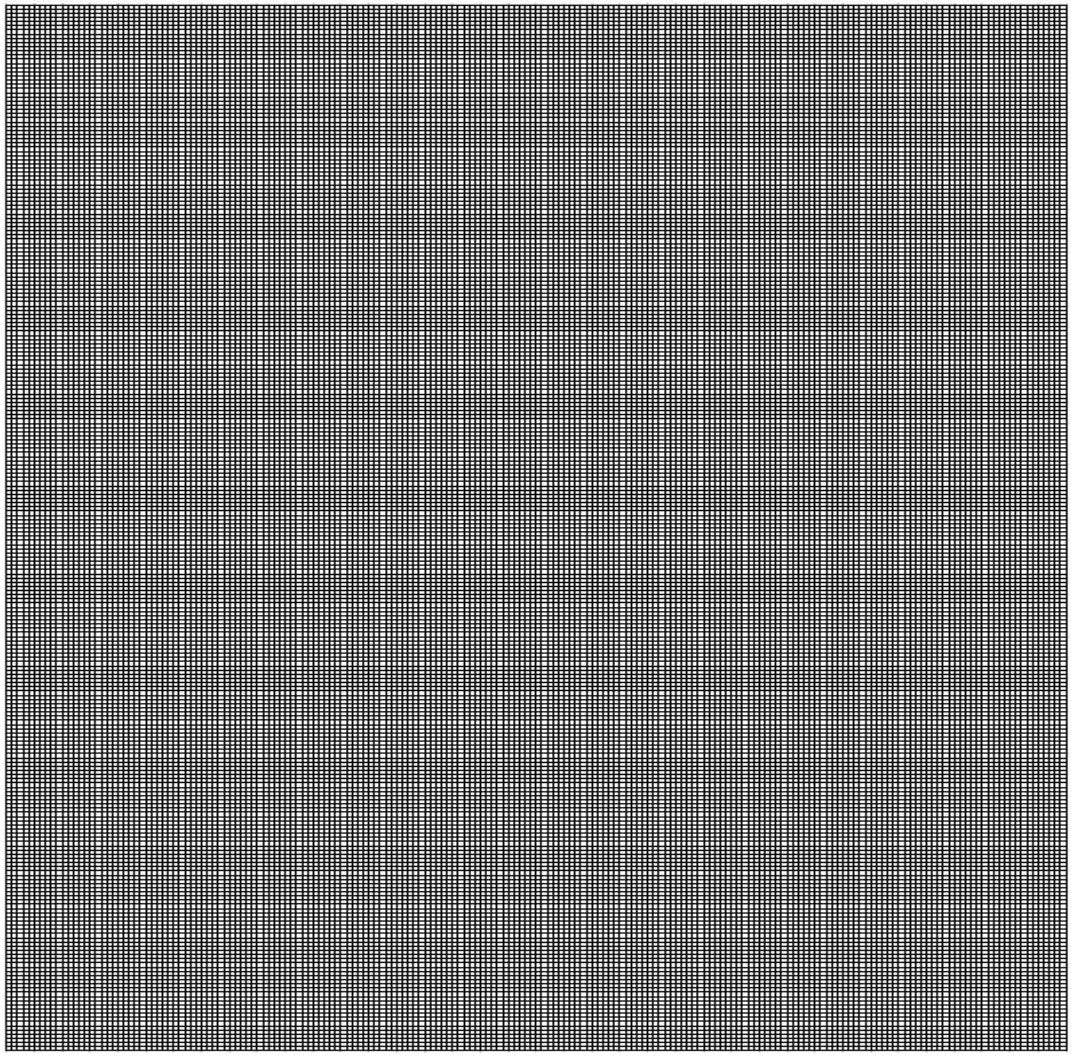


•At the point of intersection, extend a vertical line to the Y axis and read the corresponding concentration

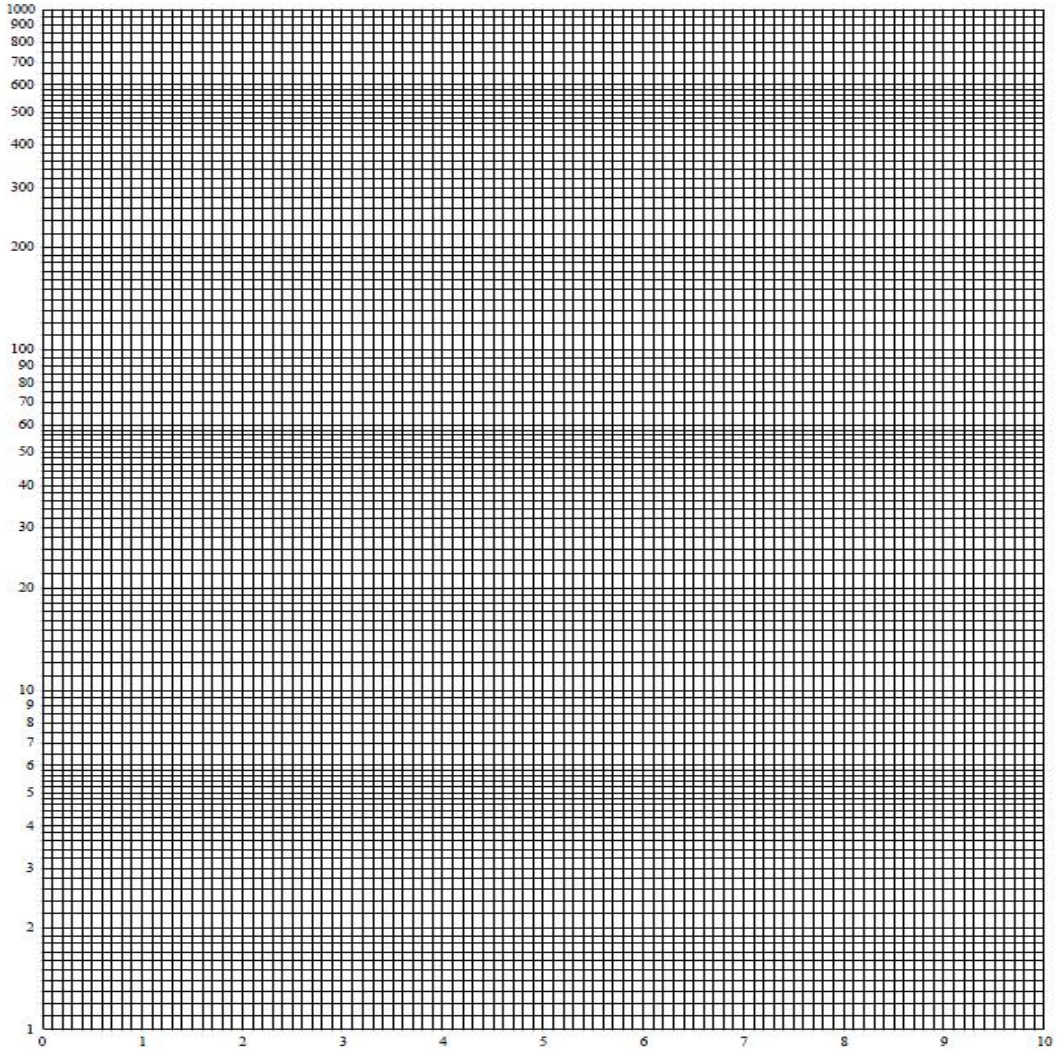


This is the concentration for that sample, approximately 275 pg/ml

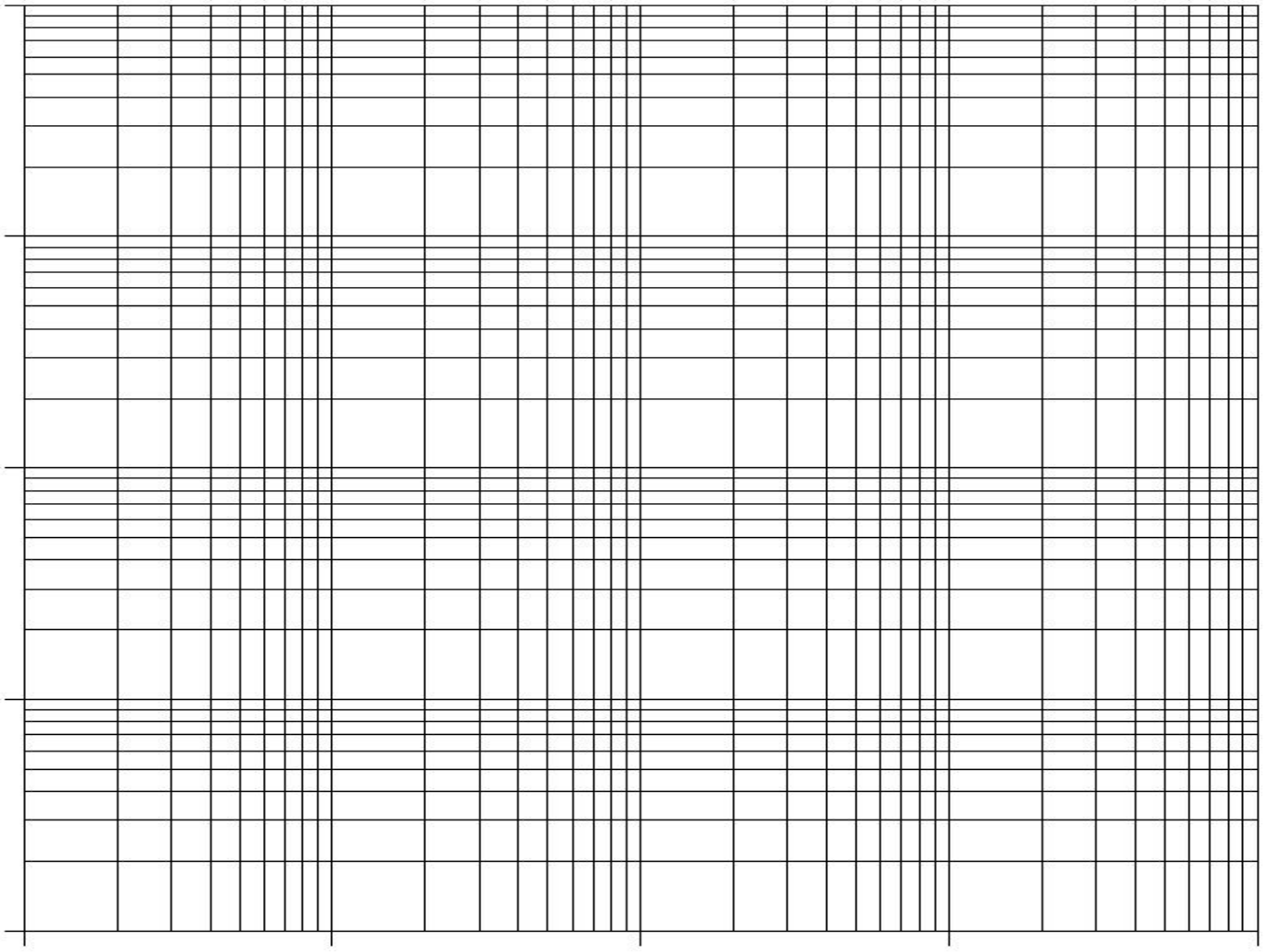
Linear paper



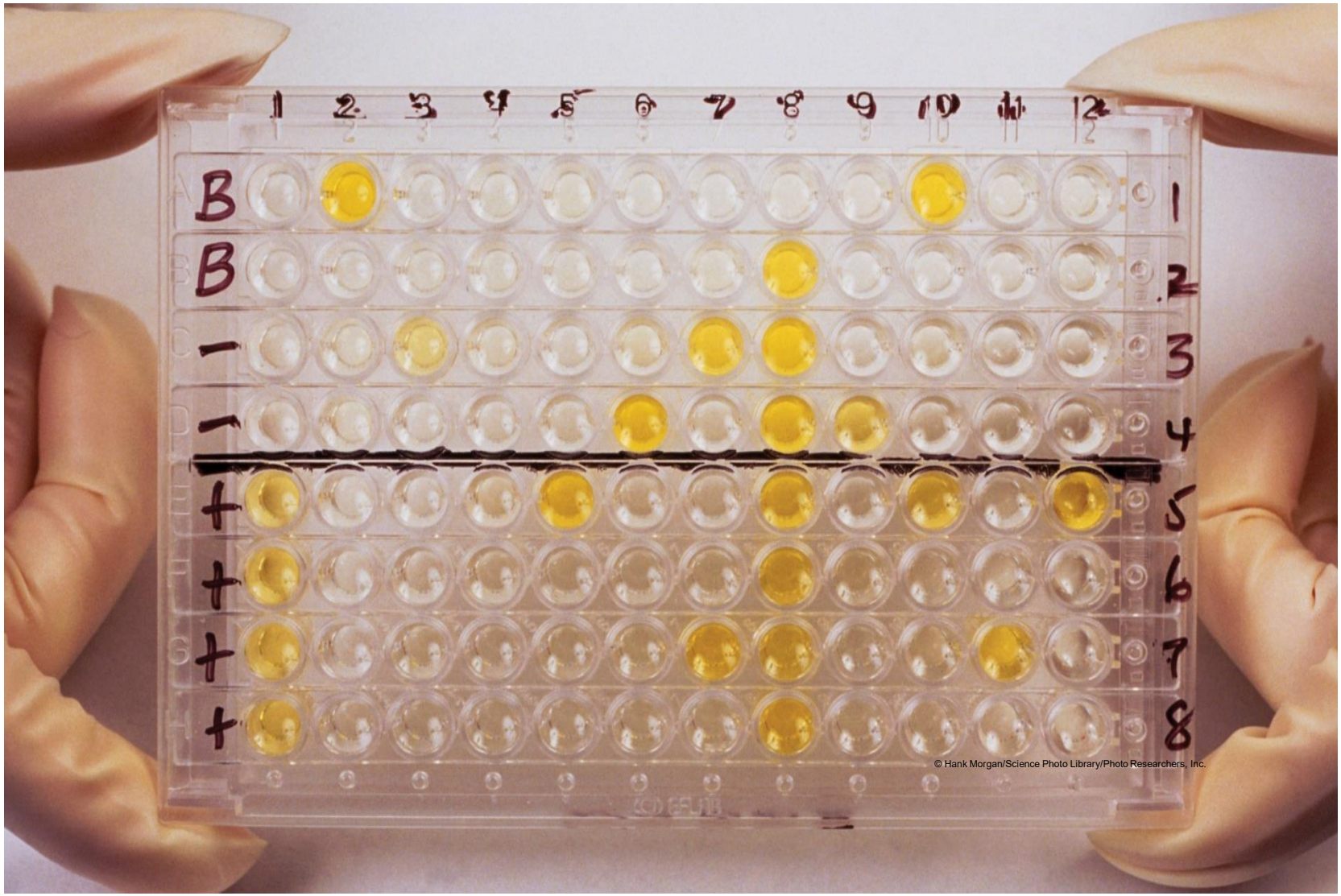
Semi log paper



Log-log paper



Qualitative tests



© Hank Morgan/Science Photo Library/Photo Researchers, Inc.

Calculation of Results

- Average the 2 negative control wells
- Average the 2 positive control wells
- Average 2 wells for each sample

ELISA Results

- **The status of a sample are evaluated by the sample to positive ratio (S/P ratio):**

$$\frac{\textbf{Sample mean - negative control mean}}{\textbf{positive control mean - negative control mean}}$$

(mean of optical absorbance)

S/P ratios of greater than 0.5 are considered positive

Interpretation of Results in Qualitative tests

Negative control = 0.150 or less

The difference between the positive and negative control means

must be greater than 0.075

Example: if negative control mean = 0.100, the positive control

mean must be 0.176 or greater

Expected results:

Blank: OD < 0.1

Negative control: mean OD < 0.15

Positive Control: OD > 0.8

Cut-off Value = Mean negative control + 0.05

Test result calculation: sample/cut-off value

Negative result: sample/cut-off value < 1

Positive result: sample/cut-off value ≥ 1

Troubleshooting: What is the source of the problem?

- Sample?
- Equipment?
- Micro plate?
- Water, Buffer?
- Washing?
- *Substrate?*

نکات عملی در تستهای الایزا

جمع آوری و نگهداری نمونه

- سرم یا پلاسما (توجه به نوع ضد انعقاد)
- عدم استفاده از نمونه های همولیز ولیپمیک
- نگهداری نمونه در دمای مناسب تا قبل از زمان آزمایش
- پرهیز از ذوب و فریز کردن سرم

نحوه صحیح کار با سمپلر

- پیش مرطوب کردن (prewet) سرسمپلرها
- استفاده از سرسمپلر جدا برای هر نمونه
- روش استاندارد استفاده از سمپلر
- تماس دست با سرسمپلر به حداقل برسد
- سرسمپلر را تا حد صحیح در نمونه پایین ببرید (2 تا 5 میلیمتر)
- از سرسمپلر مناسب استفاده شود (بطور کامل فیت با سمپلر)
- از فشار و رهاسازی ملایمی برای کشیدن نمونه ها استفاده کنید
- معرف و نمونه نباید از بالا به داخل چاهک چکانده شود
- سرسمپلر نباید با ته چاهک تماس یابد
- سرسمپلر نباید با زاویه ای شدید به دیواره چاهک تماس داده شود
- سرسمپلر باید با کناره داخلی چاهک و سطح رویی مایع تماس یابد

Pipettes and Best pipetting practice

Manual
single channel

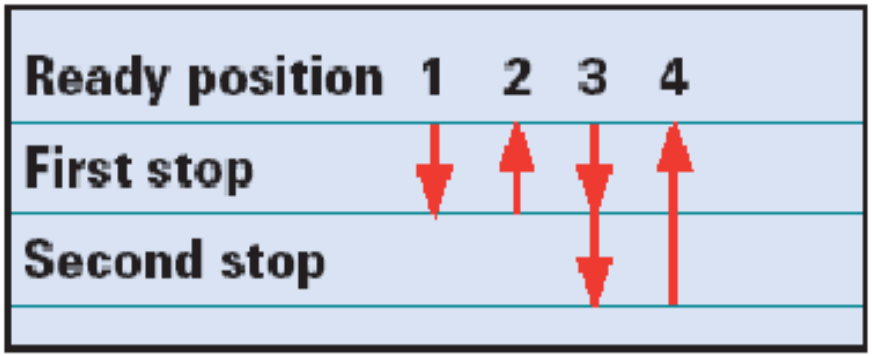
Manual
multi channe

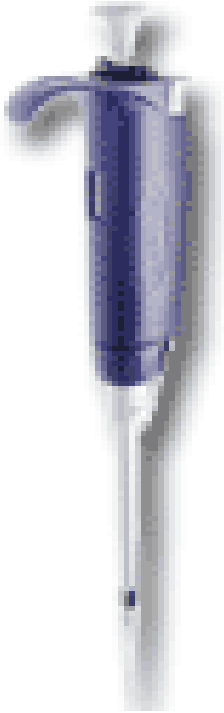
Electronic
multi
channel

Electronic
single channel

Pipetting tips

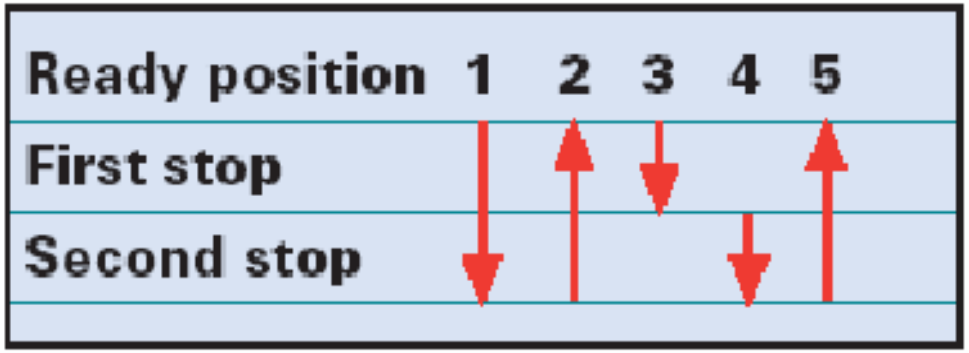
- **Forward Pipetting technique**





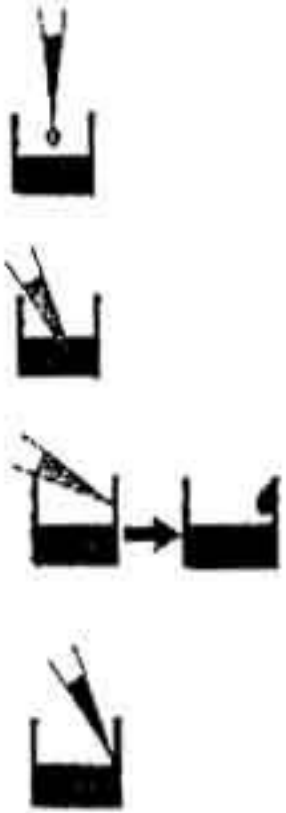
Pipetting tips

- Reverse Pipetting technique
- Highly viscous fluid
- Avoid foaming



GUIDE TO PIPETTING

- **DO NOT DRIP**
- **DO NOT PRESS HARD INTO WELL**
- **DO NOT USE TOO ACUTE AN ANGLE**
- **MAKE SURE TIP TOUCHES SIDE OF WELL AND LIQUID**



PIPETTING

نکات مهم در شستشو

- توجه به روش تهیه محلول شستشو (نحوه رقیق سازی)
- رعایت دقیق تعداد دفعات شستشو مطابق با دستورالعمل کیت
- خالی نمودن محلول اضافی با استفاده از دستمال نم گیر
- محلول شستشو نباید از سر چاهکها سرریز شود
- رعایت زمان انتظار (Soak time) در مواردی که در دستورالعمل کیت اشاره شده است (مثلاً 30 ثانیه)
- در مورد شستشوی اتوماتیک سرعت ریختن و آسپیراسیون محلول باید تنظیم شود
- از ایجاد کف یا حباب باید جلوگیری نمود
- محلول شستشو باید تازه تهیه و استفاده شود

نکات مهم در مورد میکروپلیت

- تا قبل از رسیدن پلیت به دمای اتاق آنرا از کیسه خارج نکنید (همراه بسته نمگیر)
- پس از استفاده از پلیت باقیمانده چاهکها را سریعاً به یخچال منتقل کنید
- در موقع خوانش پلیت با دستمال مرطوب بدون پرز زیر چاهکها را تمیز کنید
- در بین مراحل کار نباید وقفه ای ایجاد شود (جلوگیری از تبخیر) و روی چاهکها با روکش چسب دار پوشیده شود
- در مرحله انکوباسیون سوبسترای TMB از فویل برای پوشاندن سطح پلیت استفاده نکنید

نکات مهم در مورد سوبسترا و کنژوگه آنزیمی

- در موقع تهیه کنژوگه کار به پایداری ذکر شده در بروشور کیت توجه کنید
- سوبستراهای دو محلولی را طبق روش اشاره شده در کیت تهیه نموده و به پایداری آن توجه کنید
- از مخلوط کردن دو سوبسترا با دو سری ساخت مختلف خودداری کنید
- برای تهیه کنژوگه کار و یا سوبسترای کار حتماً از ظروف تمیز استفاده کنید

Process Control:

Quality Control for Quantitative Tests

Quantitative Tests

- Measure the quantity of a particular substance in a specimen
- Quality control for quantitative tests is designed to assure that patient results are:
  - accurate
  - reliable

Implementation steps

- establish policies and procedures
- assign responsibility, train staff
- select high quality controls.
- establish control ranges
- develop graphs to plot control values - Levey-Jennings charts
- monitor control value
- develop procedures for corrective action
- record all actions taken

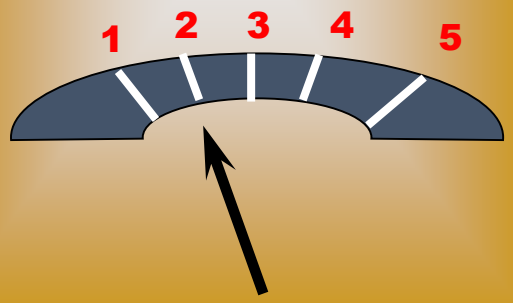
What is a Control?

- material that contains the substance being analyzed
  - include with patient samples when performing a test
- used to validate reliability of the test system
  - run after calibrating the instrument
  - run periodically during testing

Calibrators vs. Controls

Calibrators

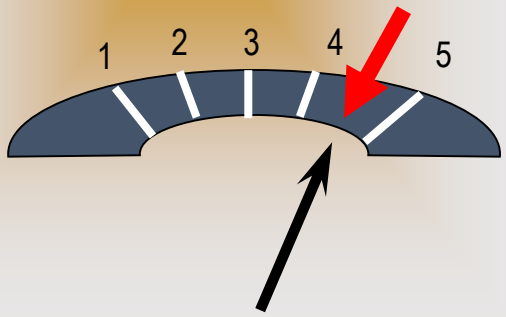
A substance with a specific defined concentration used to set (calibrate) the measuring points on a scale.



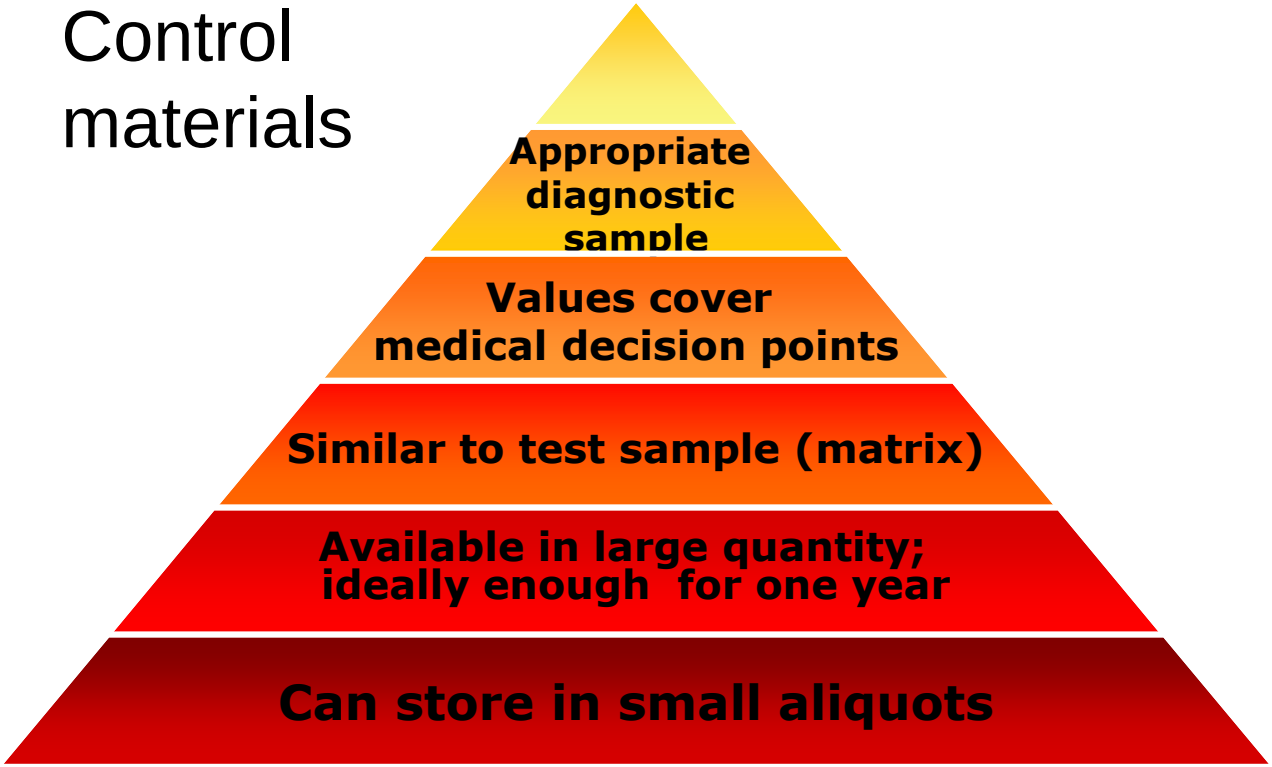
Controls

A substance similar to patients' samples that has an established concentration.

Controls are used to ensure the procedure is working properly.

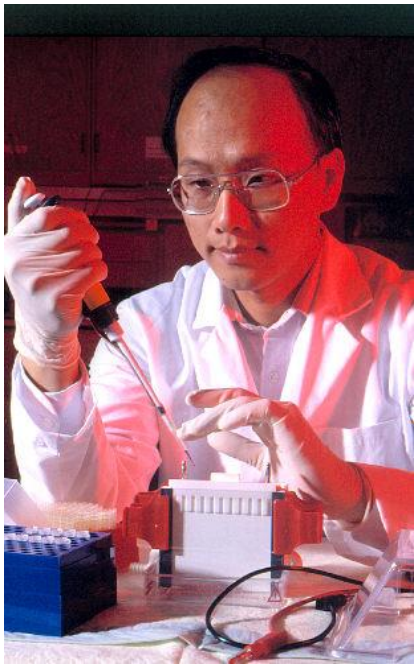


Control materials



Types of Control Materials

- may be frozen, freeze-dried, or chemically preserved
- requires very accurate reconstitution if this step is necessary



Sources of Controls Materials

- commercially prepared
- made “in house”
- obtained from another laboratory, usually central or reference laboratory

Preparation and Storage of Control Material

- adhere to manufacturer's instructions
- keep adequate amount of same lot number
- store correctly

Measurement of Variability

Variability is a normal occurrence when a control is tested repeatedly

Affected by:

- operator technique
- environmental conditions
- performance characteristics of the measurement

The goal is to differentiate between variability due to chance from that due to error

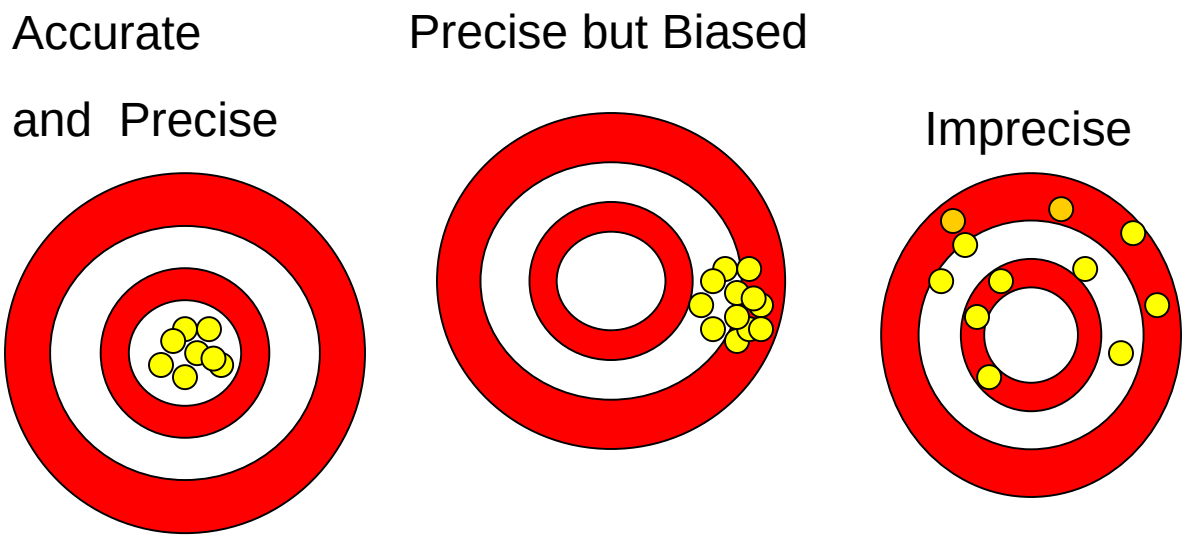
Quality Control is used to monitor the accuracy and the precision of the assay.

What are accuracy and precision?

Definitions

Accuracy	**The closeness of measurements to the true value**
Precision	**The amount of variation in the measurements**
Bias	**The difference between the expectation of a test result and an accepted reference value**

Accuracy and Precision

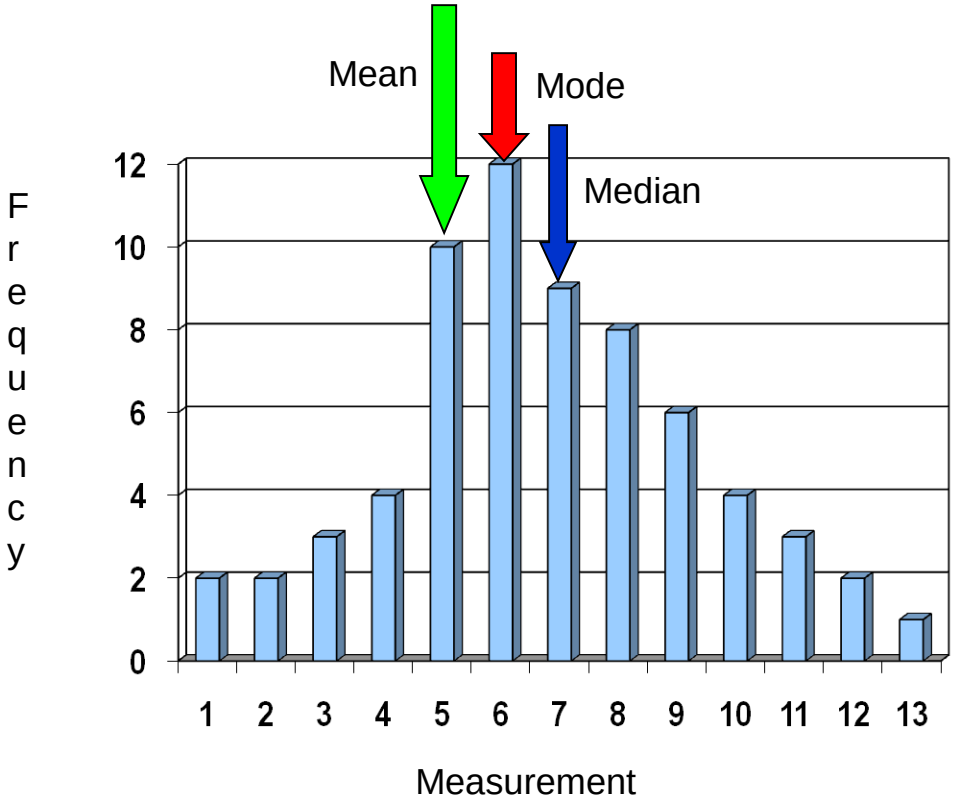


Accurate = Precise but not Biased

Measures of Central Tendency

Mode	the value which occurs with the greatest frequency
Median	the value at the center or midpoint of the observations
Mean	the calculated average of the values

Not all central values are the same



Symbols Used in Calculations

$\sum$ is the sum of (add data points)

n = number of data points

$\mathbf{x_1}$ - $\mathbf{x_n}$ = all of the measurements (1 through n)

$\overline{X}$ represents the mean

Calculation of Mean

$$\overline{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$\overline{X}$ = Mean

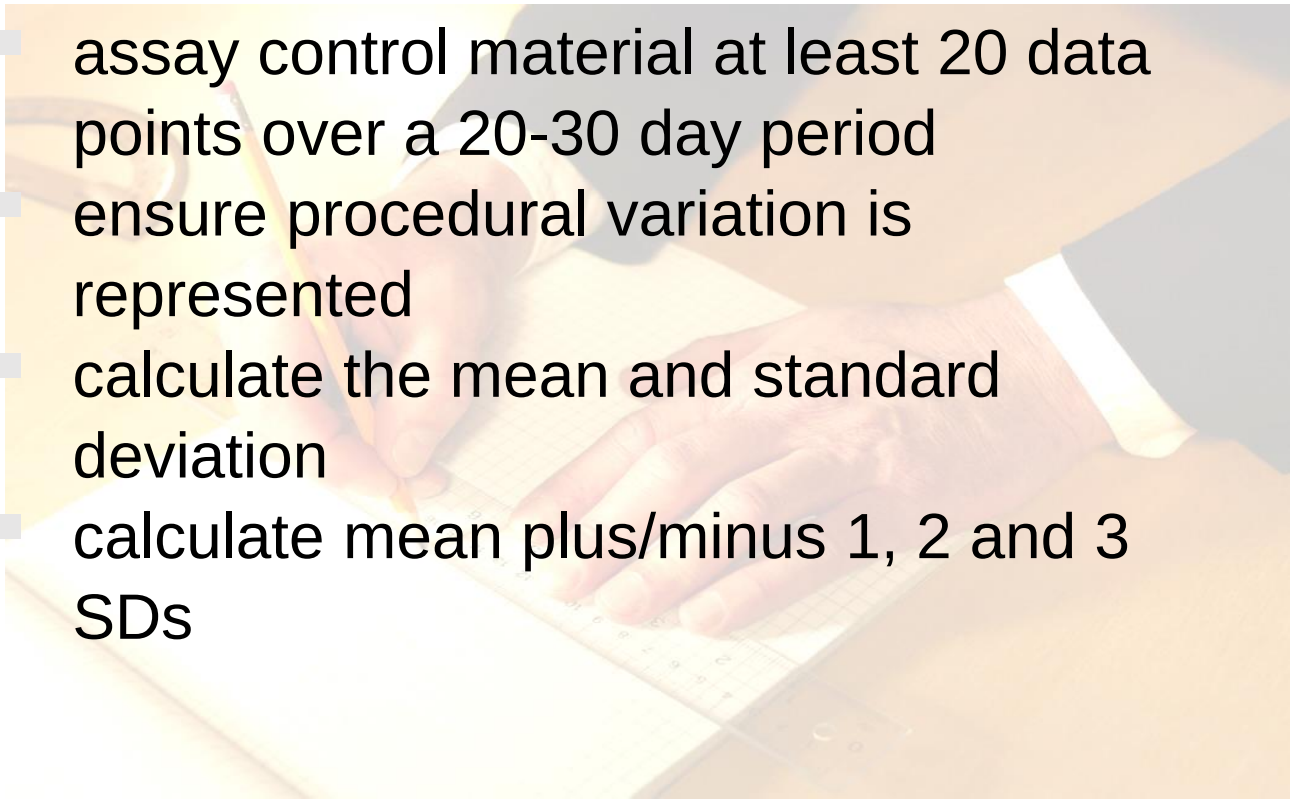
X_1 = First measurement

X_2 = Second measurement

X_n = Last measurement in series

n = Total number of measurements

Statistics for Quantitative QC



- assay control material at least 20 data points over a 20-30 day period
- ensure procedural variation is represented
- calculate the mean and standard deviation
- calculate mean plus/minus 1, 2 and 3 SDs

Data showing outlier

1. 192 mmol/L
2. 194 mmol/L
3. 196 mmol/L
4. 196 mmol/L
5. 185 mmol/L
6. 196 mmol/L
7. 200 mmol/L
8. 200 mmol/L
9. 202 mmol/L
10. **270 mmol/L**
11. 204 mmol/L
12. 208 mmol/L
13. 212 mmol/L
14. 198 mmol/L
15. 204 mmol/L
16. 208 mmol/L
17. 212 mmol/L
18. 198 mmol/L
19. 192mmol/L
20. 196 mmol/L

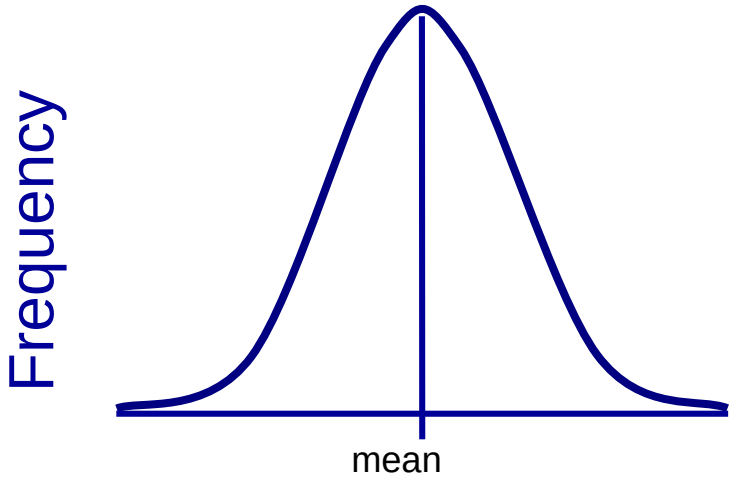
Example

Calculation of Mean: ELISA Tests

- Run controls 20 times in 30 days. Record both OD and cut off (CO) values for each measurement.
- Divide the OD by the CO (OD/CO) for each data point or observation. This standardizes the data.
- Add the ratios and divide by the number of measurements to get the mean.

Normal distribution

- all values symmetrically distributed around the mean
- characteristic “bell-shaped” curve
- assumed for all quality control statistics



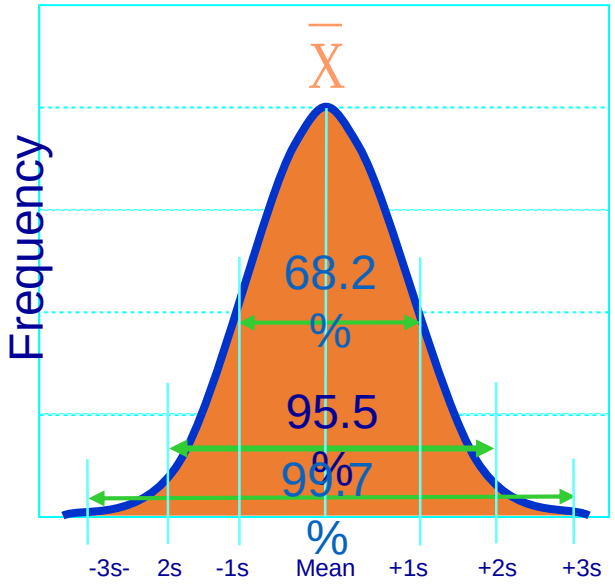
Standard Deviation and Probability

For a set of data with a **normal distribution**, a random measurement will fall within:

+/- 1 SD 68.3% of the time

+/- 2 SD 95.5% of the time

+/- 3 SD 99.7% of the time



Standard Deviation (SD)

SD is the principle measure of variability used in the laboratory

$$SD = \sqrt{\frac{\sum (x_1 - \bar{x})^2}{n-1}}$$

Standard Deviation – Statistical Formula

Coefficient of Variation

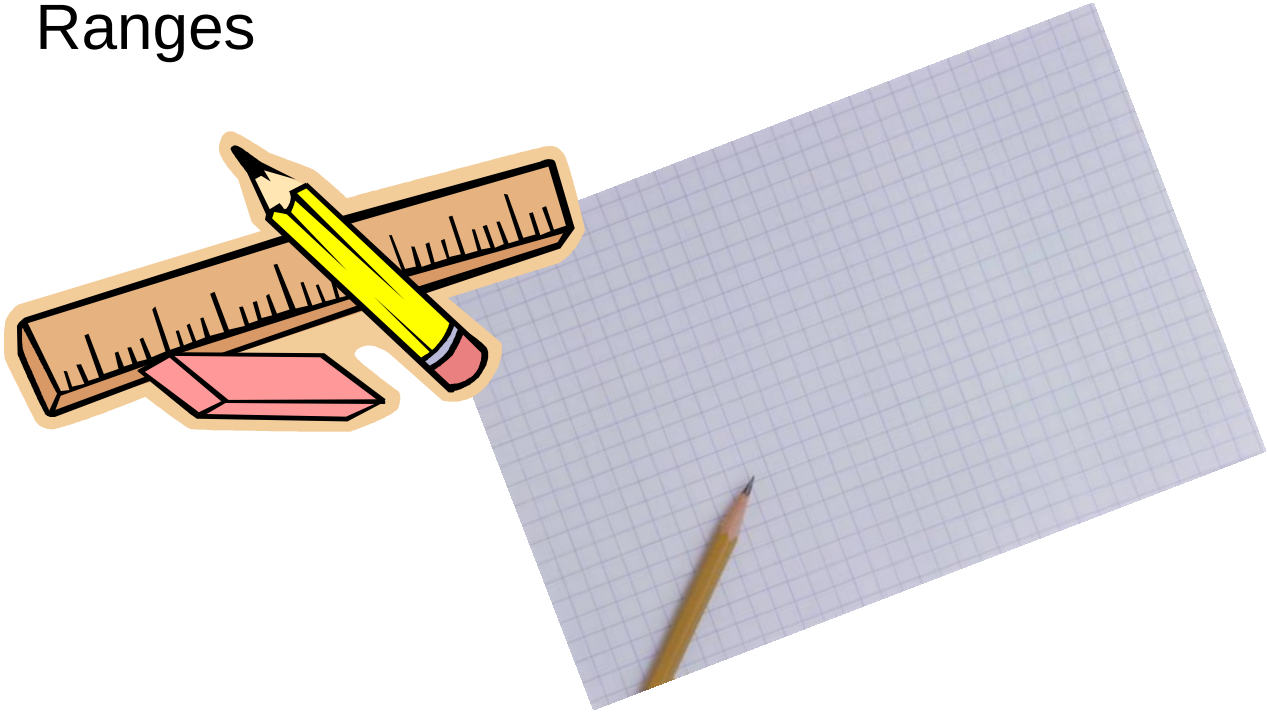
The coefficient of variation (CV) is the SD expressed as a percentage of the mean.

$$CV = \frac{SD}{mean} \times 100\,\%$$

- CV is used to monitor precision
- CV is used to compare methods
- CV ideally should be less than 5%

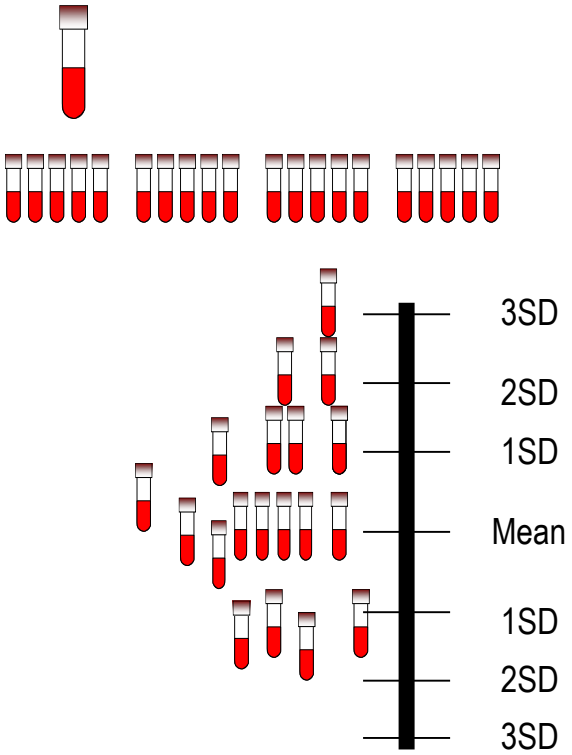
Levey-Jennings Chart

Graphically Representing Control Ranges



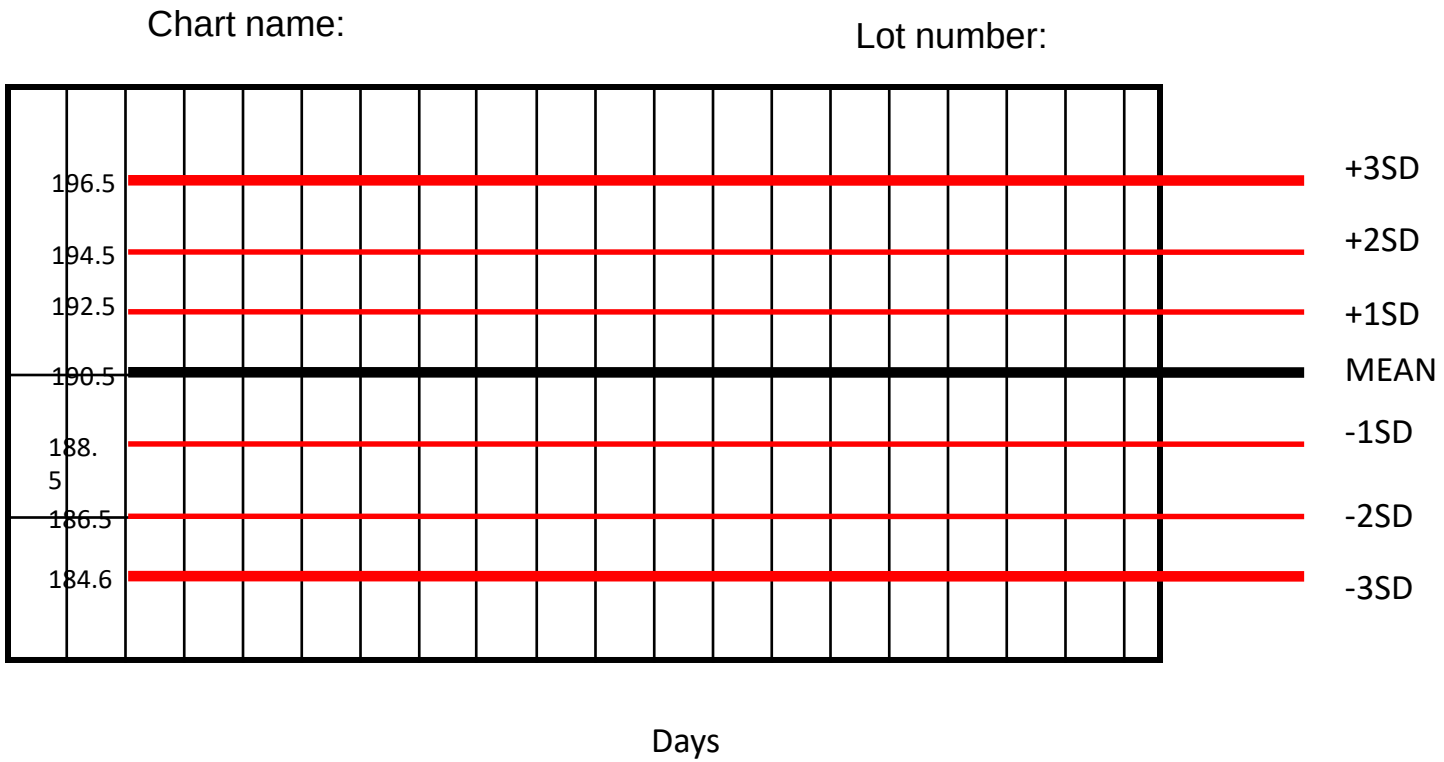
Steps in Implementing Quantitative Quality Control

- obtain control material
- run each control 20 times over 30 days
- calculate mean and +/-1,2,3 Standard Deviations

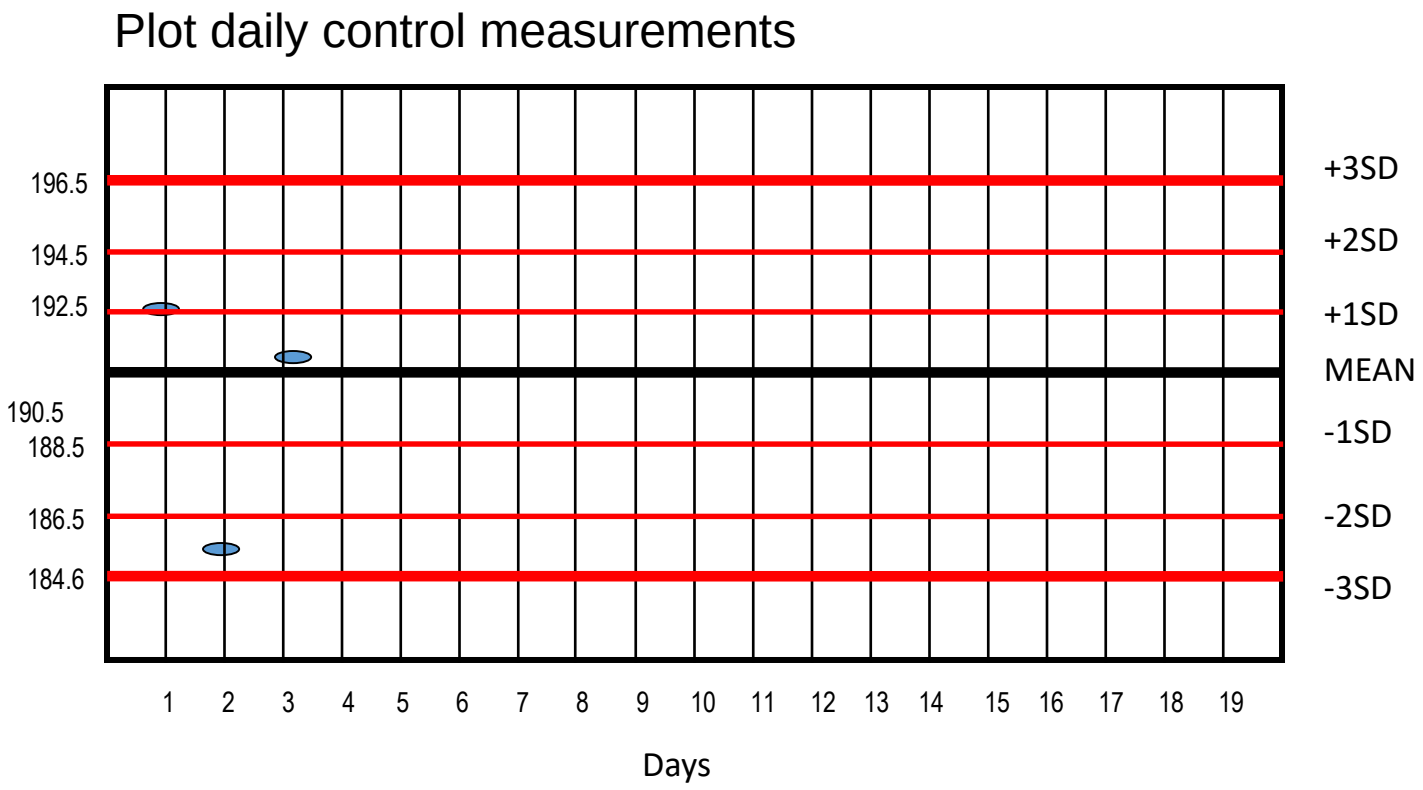


Draw lines for Mean and SDs

(calculated from 20 controls)



Levey-Jennings Chart

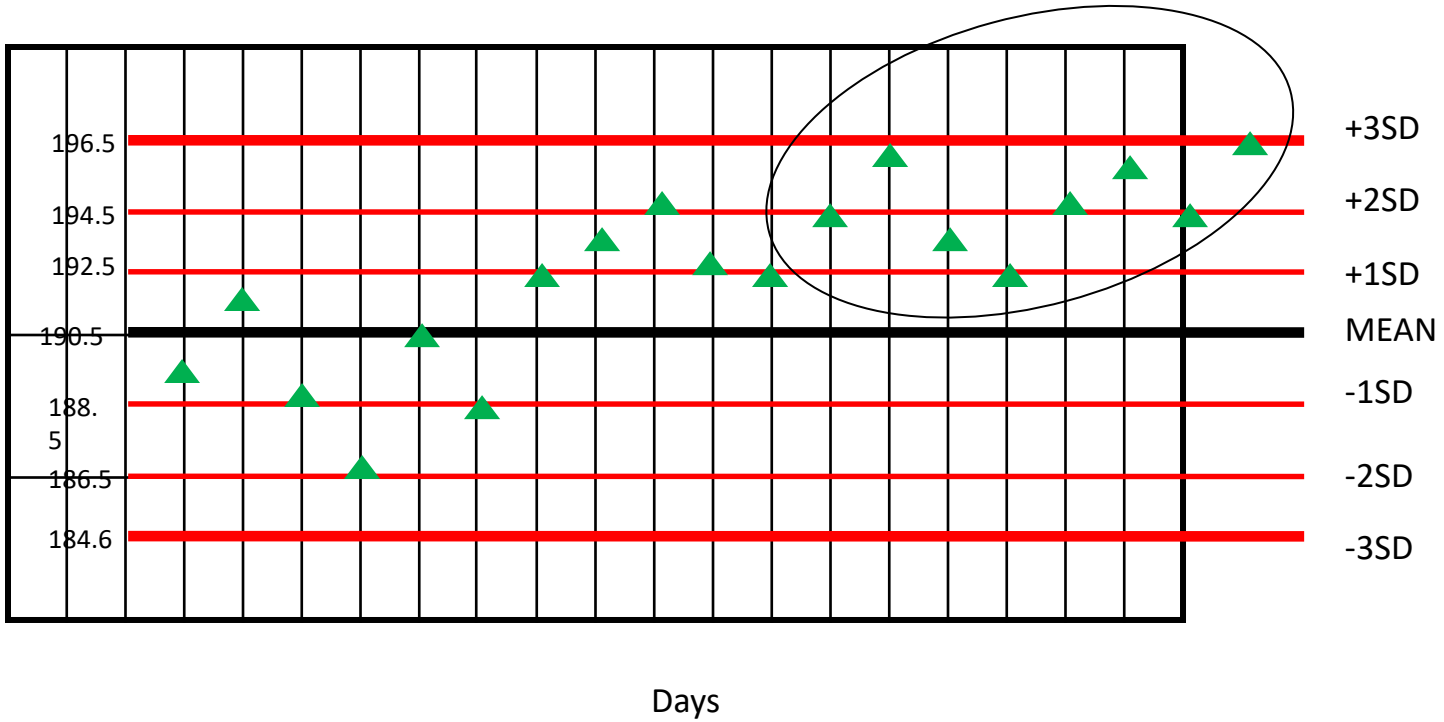


Number of Controls

Interpretation depends on number of controls run with patients' samples.

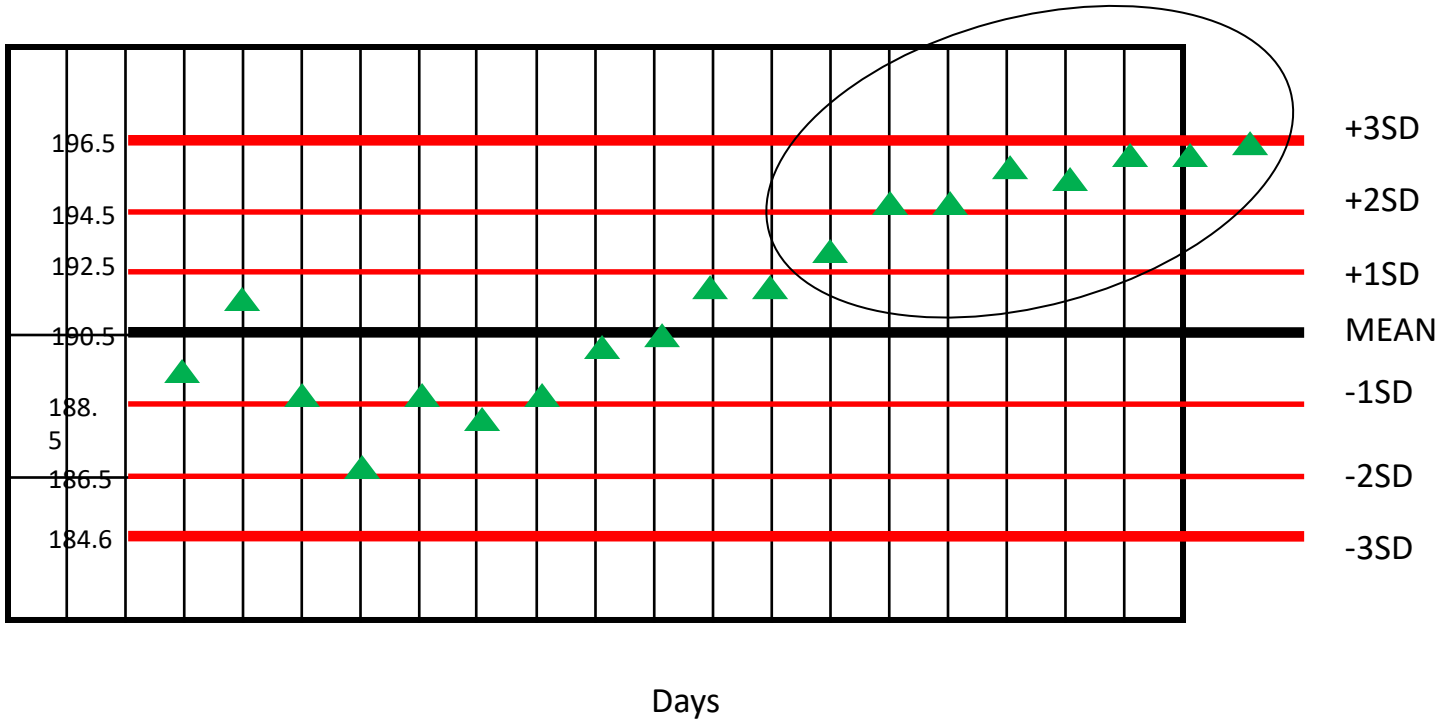
- **Good**: If one control:
  - accept results if control is within ± 2SD unless shift or trend
- **Better**: If 2 levels of controls
  - apply Westgard multirule system

Levey-Jennings Chart
Shift



Levey-Jennings Chart

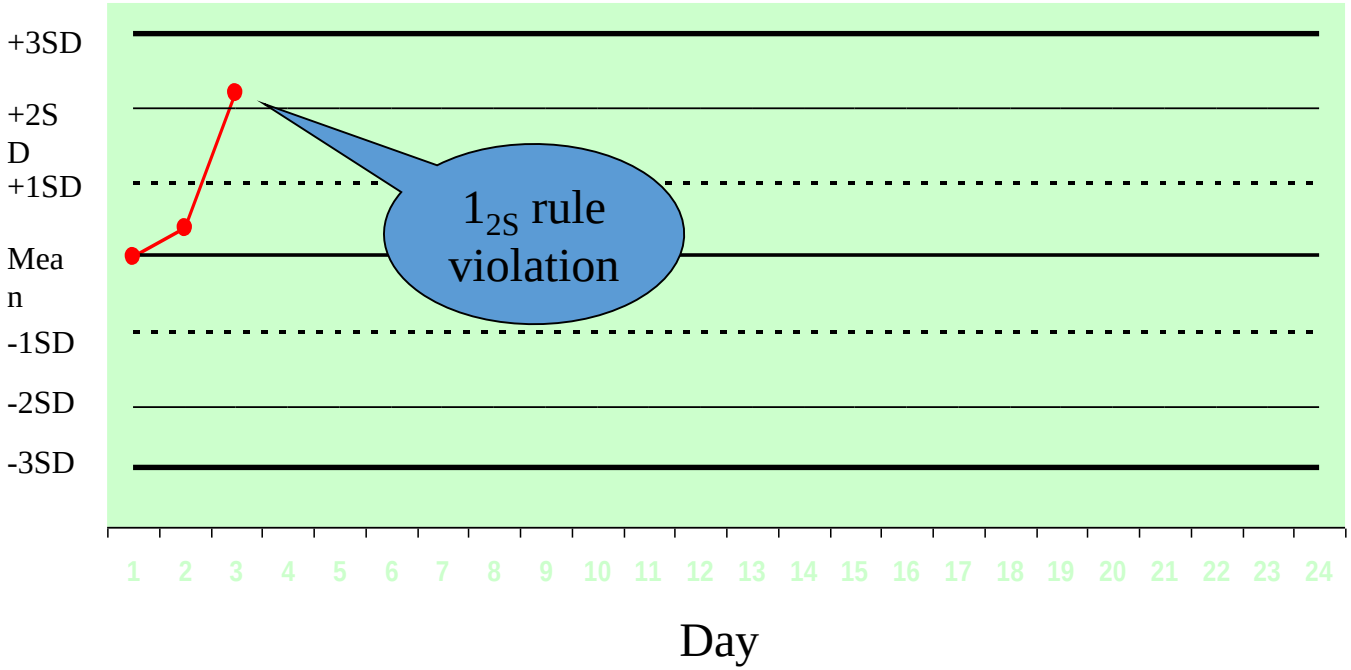
Trend



Westgard – 1_{2S} Rule

- “warning rule”
- One of two control results falls outside ±2SD
- Alerts tech to possible problems
- Not cause for rejecting a run
- Must then evaluate the 1_{3S} rule

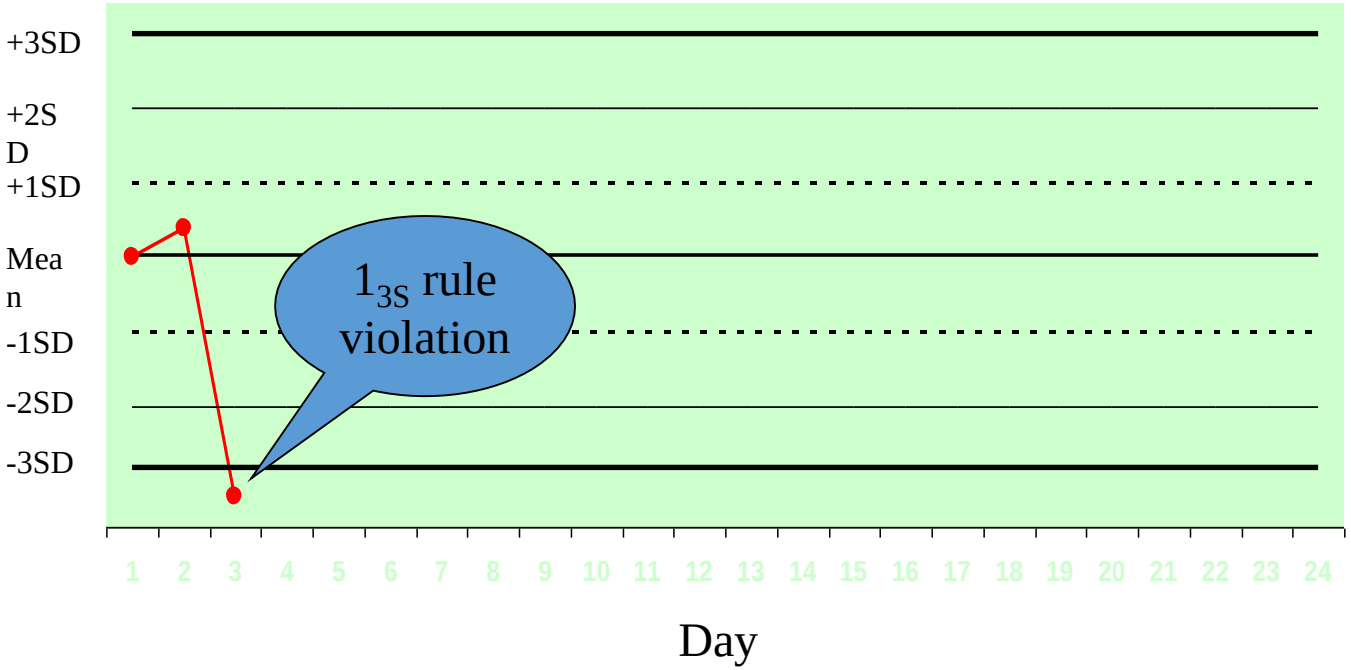
1_{2S} Rule = A warning to trigger careful inspection of the control data



Westgard – 1_{3S} Rule

- If either of the two control results falls outside of ±3SD, rule is violated
- Run must be rejected
- If 1_{3S} not violated, check 2_{2S}

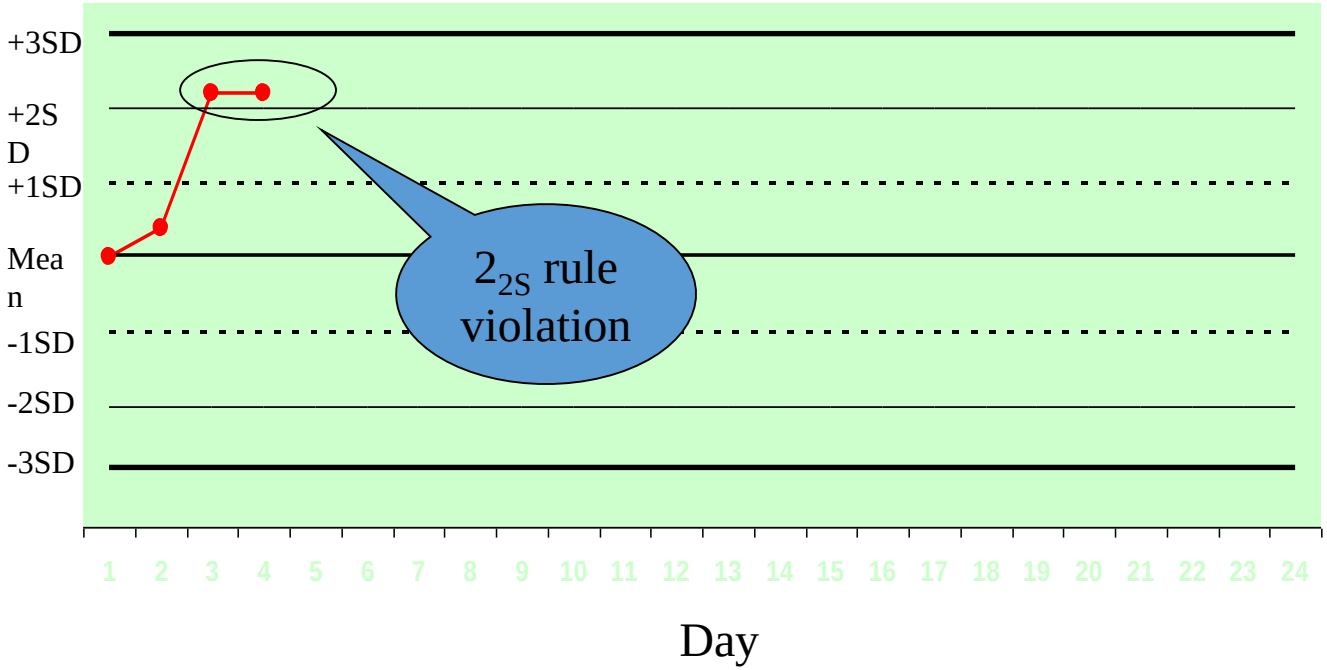
1_{3S} Rule = Reject the run when a single control measurement exceeds the +3SD or -3SD control limit



Westgard – 2_{2S} Rule

- 2 consecutive control values for the same level fall outside of ±2SD in the same direction, or
- Both controls in the same run exceed ±2SD
- Patient results cannot be reported
- Requires corrective action

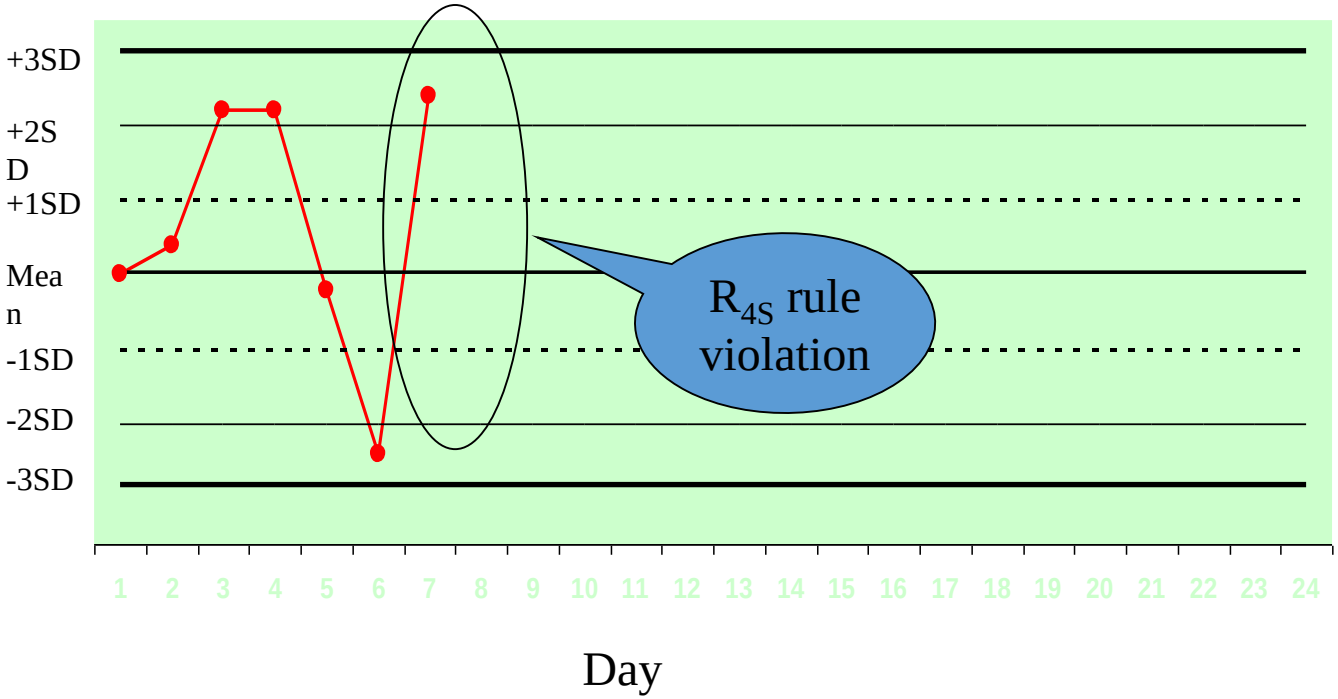
2_{2S} Rule = Reject the run when 2 consecutive control measurements exceed the same +2SD or -2SD control limit



Westgard – R_{4S} Rule

- One control exceeds the mean by –2SD, and the other control exceeds the mean by +2SD
- The range between the two results will therefore exceed 4 SD
- Random error has occurred, test run must be rejected

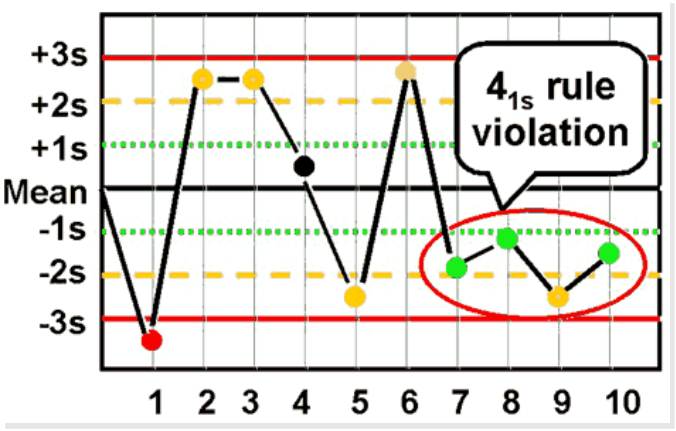
R_{4S} Rule = Reject the run when 1 control measurement exceed the +2SD and the other exceeds the -2SD control limit



Westgard – 4_{1S} Rule

- Requires control data from previous runs
- Four consecutive QC results for one level of control are outside ±1SD, or
- Both levels of control have consecutive results that are outside ±1SD

4_{1s} Rule

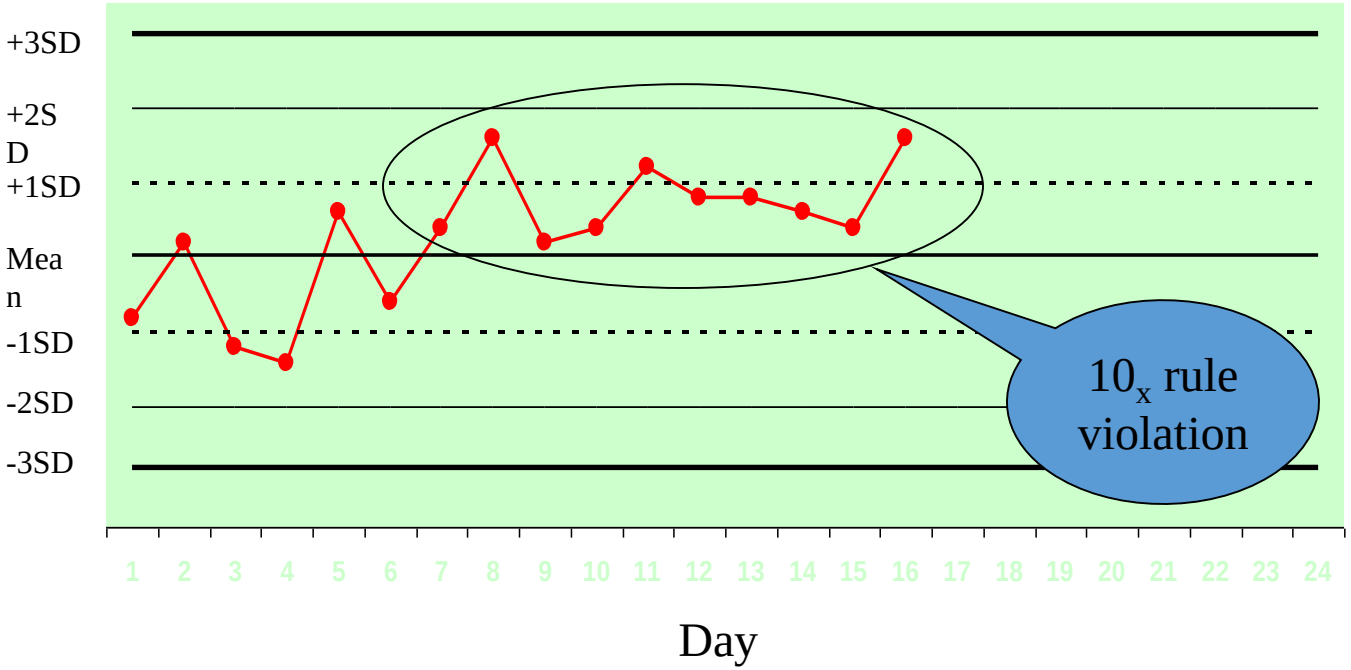


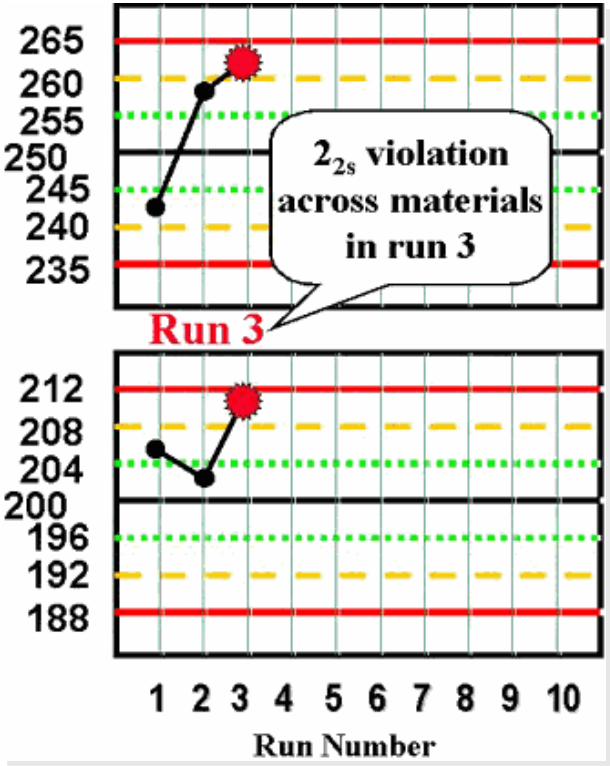
4_{1s} - reject when 4 consecutive control measurements exceed the same mean plus 1s or the same mean minus 1s control limit.

Westgard – 10_X Rule

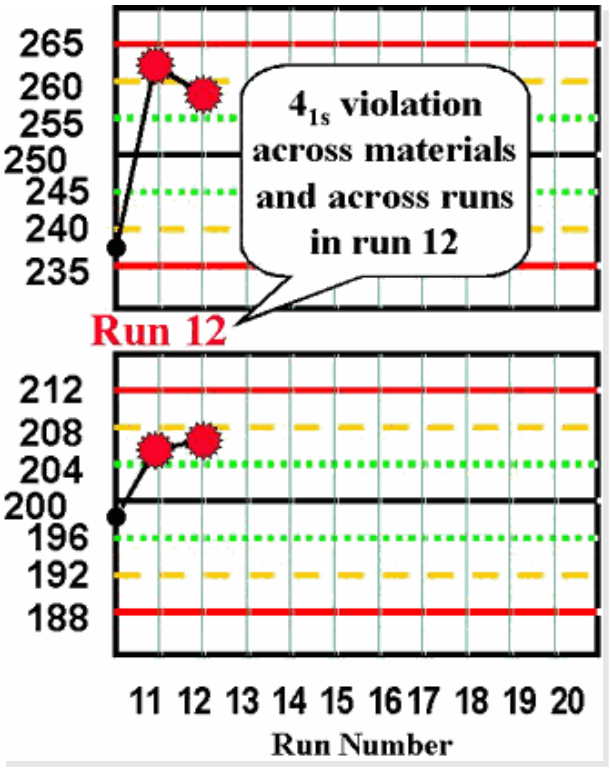
- Requires control data from previous runs
- Ten consecutive QC results for one level of control are on one side of the mean, or
- Both levels of control have five consecutive results that are on the same side of the mean

10_x Rule = Reject the run when 10 consecutive control measurements fall on one side of the mean

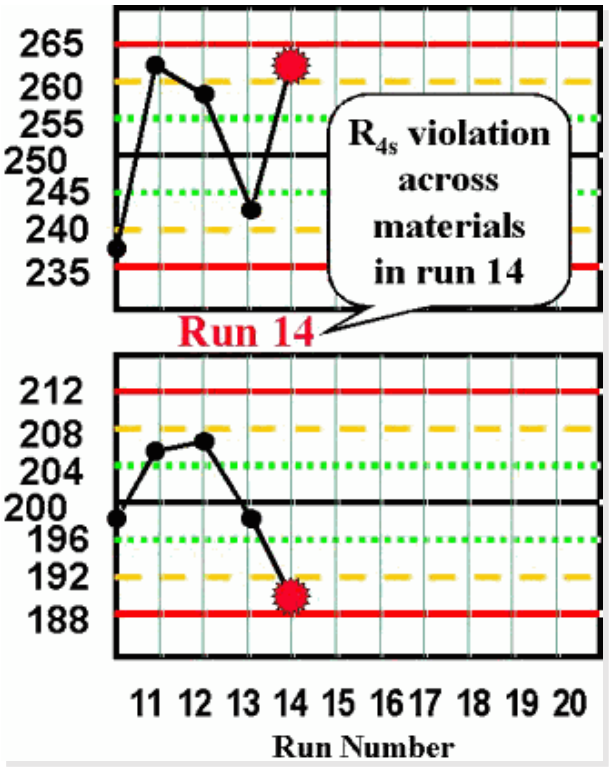




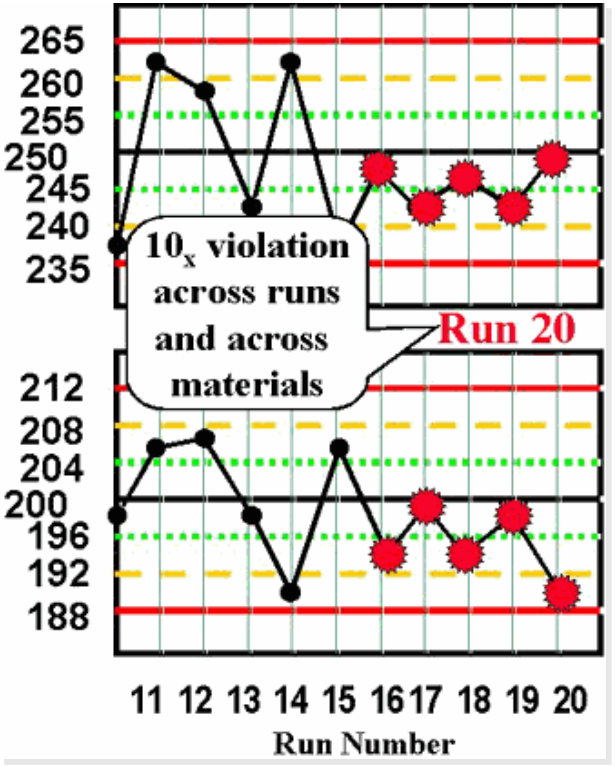
Run 3 Both control results exceed their respective +2s limits, therefore there is a 2_{2s} rule violation across materials. A systematic error is most likely occurring and is affecting the results throughout the critical analytical range from at least 200 to 250 mg/dL.



Run 12 The control charts for the high and low materials show that the last four control observations have exeeded their respective +1s limits, therefore a 4_{1s} rule violation appears to have occured across materials and across runs.



Run 14 The control results for the high material exceeds its +2s limit and the control result for the low material exceeds its -2s limit, therefore an R_{4s} rule violation has occurred. This most likely indicates a random error.



Run 20 The last five control results on the high material and the last five results on the low material all are lower than their respective means, giving a total of ten consecutive control results on one side of the mean. There is a 10_x rule violation across runs and across materials, which indicates that a systematic error most likely has occurred.

مثالهایی از خطاهای راندوم:

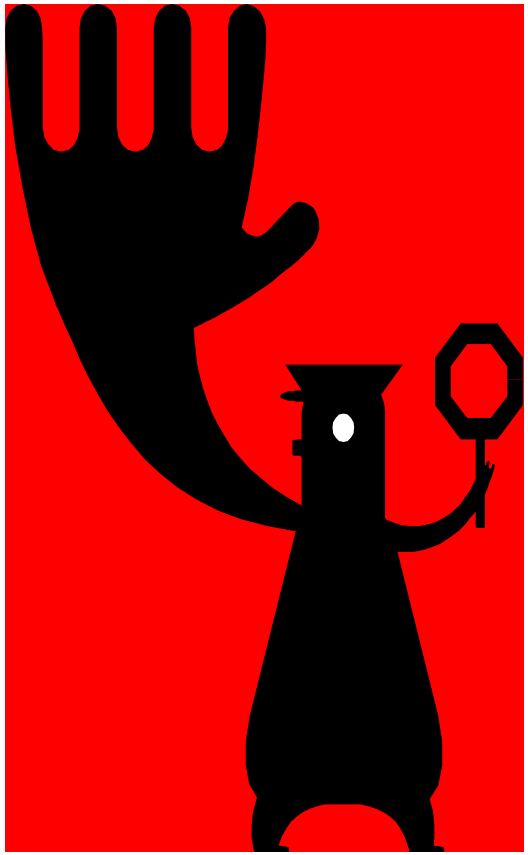
- دمای ناپایدار
- نوسانات جریان الکتریکی دستگاه قرائت کننده
- وجود حباب هوا در زمان انتقال نمونه یا معرف
- عدم رعایت حجم برداشتی از نمونه یا معرف
- عدم رعایت زمان اکوباسیون
- ناپایداری معرف
- عدم رعایت شرایط نگهداری نمونه
- آلودگی ظروف شیشه ای مورد استفاده، نوک سمپلر و ...
- آلودگی نمونه کنترلی، معرف و ...
- اشکال در سیستم قرائت کننده

مثالهایی از خطای سیستماتیک:

- اشکال در کالیبراسیون مانند در نظر گرفتن ارزش نادرست برای کالیبراتور، تهیه نامناسب، آلودگی، افت، تغلیظ، تغییر شماره ساخت و ...
- عوض کردن معرف بدون تغییر در کالیبراسیون
- تخریب تدریجی معرف
- عدم رعایت دستورالعمل سازنده برای تهیه معرف
- تغییر در دمای انکوباسیون
- خطای ثابت در وسایل انتقال دهنده نمونه یا معرف مانند سمپلر

If QC is out of control

- **stop testing**
- identify and correct problem
- repeat testing on patient samples and controls after correction
- **Do not report patient results** until problem is solved and controls indicate proper performance



Solving out-of-control problems

- identify problem
- refer to established policies and procedures for remedial action

Possible Problems

- degradation of reagents or kits
- control material degradation
- operator error
- failure to follow manufacturer’s instructions
- an outdated procedure manual
- equipment failure
- calibration error

راهنمای حل مشکلات در الایزا

عدم تولید سیگنال و یا تولید سیگنال ضعیف

- حذف یا فراموش شدن انجام یک مرحله از الایزا مثلا اضافه نکردن کنژوگه آنزیمی یا سوبسترا
- عدم تهیه مناسب سوبسترا بویژه در مواردی که سوبستراها بصورت دو محلولی هستند.
- محلول شستشو غلیظ تهیه شده است.
- کنژوگه آنزیمی یا سوبسترا غیر فعال شده است.
- درجه حرارت انکوباسیون مناسب نمیباشد و حرارت انکوباسیون کمتر از حد لازم است.
- حجم سوبسترای اضافه شده به چاهک ها کافی نمی باشد.
- استفاده از فیلتر نامناسب برای قرائت نتایج استفاده از فیلتر 405 به جای450 نانومتر باعث کاهش جذب نوری قرائت شده می شود.
- معرف ها به درجه حرارت اتاق نرسیده اند و هنوز برای انجام آزمایش سرد هستند.
- خراشیده شدن ته چاهک با نوک سمپلر
- معرف های کیت منقضی شده اند.
- معرف ها به دلیل نگهداری غیر صحیح با کاهش فعالیت مواجه شده اند.

جذب زمینه ای بالا- افزایش جذب نوری استاندارد ها یا نمونه ها

- غلظت کنژوگه آنزیمی زیاد است و رقت کنژوگه طبق بروشور تهیه نشده است.
- درجه حرارت انکوباسیون نامناسب است و درجه حرارت بالاتر از حد تعین شده برای سنجش می باشد.
- فرایند شستشو کافی نبوده است و یا محلول شستشوبصورت رقیق تهیه شده است.
- آلودگی آنزیمی در نمونه های سرمی وجود دارد.
- آلودگی متقاطع با دیگر نمونه ها ویا باکنترل مثبت اتفاق افتاده است.
- برای قرائت جذب نوری از فیلتر صحیح استفاده نشده است در صورتی که توصیه شده باشد.
- از فیلتر رفرانس استفاده شود و این امر انجام نشود یک جذب زمینه ای بالا مشاهده می شود.
- سوبسترا قبل از مصرف با نور در تماس بوده است.
- چاهک ها در طی انکوباسیون تبخیر شده اند.
- پی پت نوک سمپلر با سوبسترا یا کنژوگه آنزیمی آلوده شده است.
- سوبسترا با یونهای فلزی یا عوامل اکسید کننده آلوده شده است.

شکل منحنی استاندارد نامناسب است

شستشو به خوبی انجام نشده است.

رقیق سازی محلولها به خوبی انجام نشده است و یا خطا در پی پت کردن معرف ها وجود دارد.

معرف ها به خوبی مخلوط نشده اند.

ته چاهک ها از طرف خارج آلوده شده است و کثیف می باشد.

از معرف هائی با سری ساخت متفاوت استفاده شده است.

دقت ضعیف یا دو پلیکیت های ضعیف

خطا در پی پت کردن معرف ها

خراش دادن سطح پلیت با نوک سمپلر

انتقال محلول ها از چاهک های کناری

استفاده از حجم نامناسب محلول ها

وجود رسوب یا پارتیکل در نمونه و کنترل ها

کثیف بودن سطح خارجی چاهک ها

وجود اثر حاشیه ای edge effect

تمام چاهک ها دارای رنگ هستند

سوبسترا آلوده شده است.

محلول شستشو آلوده شده است.

رقیق سازی به خوبی انجام نشده است.

شستشو به خوبی انجام نشده است.

تمام چاهک ها بدون تولید سیگنال هستند و فاقد رنگ می باشند

یکی از مراحل الایزا فراموش شده است.

کنژوگه آنزیمی غیر فعال شده است.(با مخلوط کردن کنژوگه و سوبسترا از فعالیت ایندو معرف اطمینان

حاصل می شود.)

شرایط نگهداری کیت مناسب نبوده است.

شستشوی زیاد چاهک ها ویا غلیظ بودن زیاد محلول شستشو