Laboratory Quality Control

Introduction and statistics

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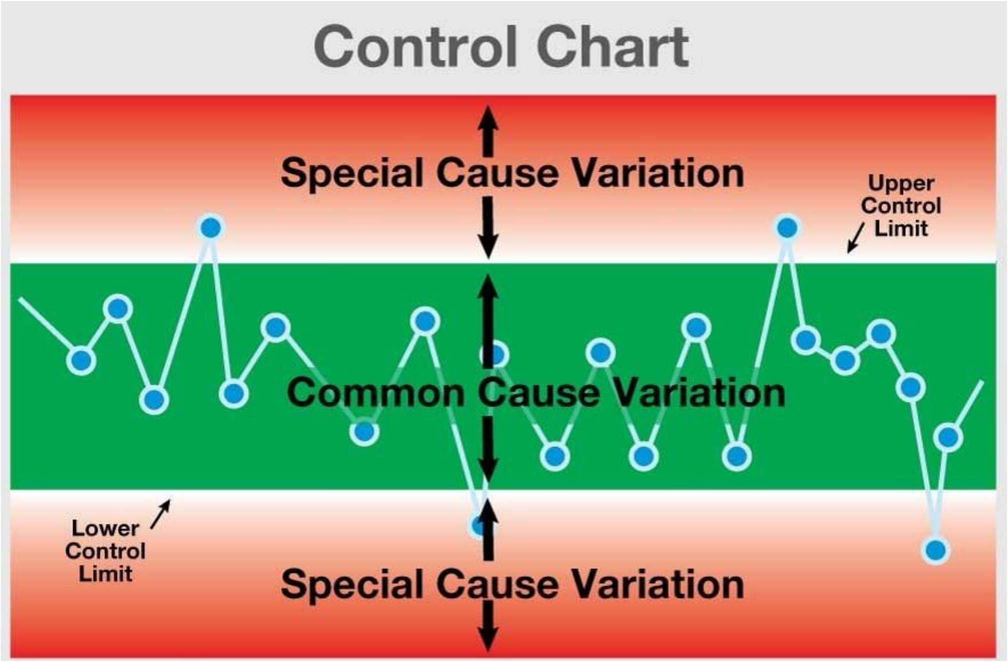
Early Origins of Quality Control

- QC has existed since humans began producing goods
- Became more formal during the **Industrial Revolution (18th–19th century)**
- Early QC = mainly **visual inspection**, no systematic method
- **17th century:** Birth of statistics → foundation for modern QC
- Today, QC heavily depends on statistics
- With computers, QC has become more complex and automated

Origins of Quality Control

- 1920s: Walter A. Shewhart introduced **control charts** at Bell Telephone (1924)
- Foundation of **Statistical Quality Control (SQC)** in industry
- Initially used in manufacturing (automotive, pharmaceuticals)

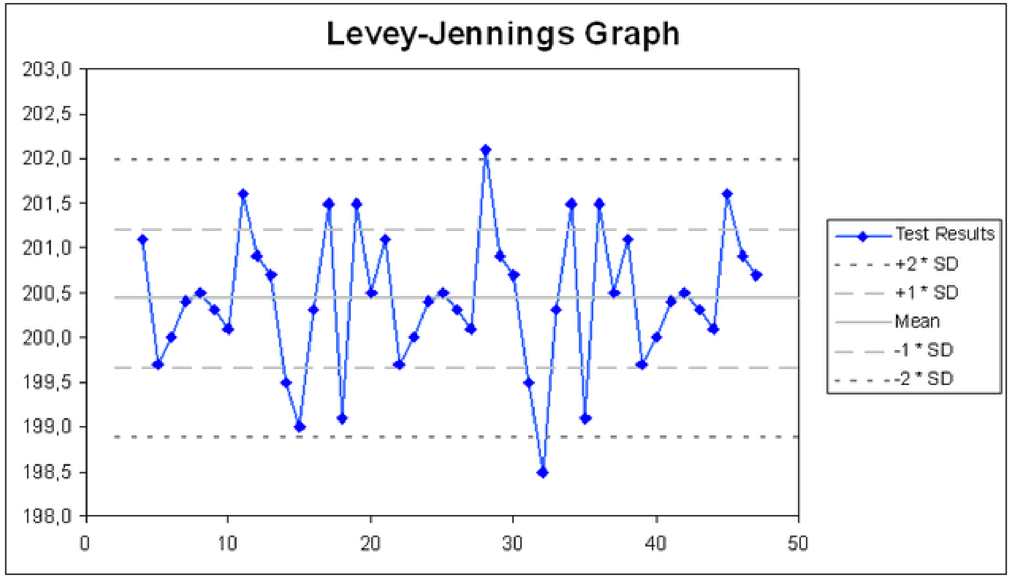


Introduction into Clinical Laboratories

- 1940s–1950s: Concept of QC adopted in clinical chemistry & hematology
- Need to ensure **accuracy and precision** in patient testing
- Early focus on biochemical assays (glucose, urea) and hematology tests (CBC)

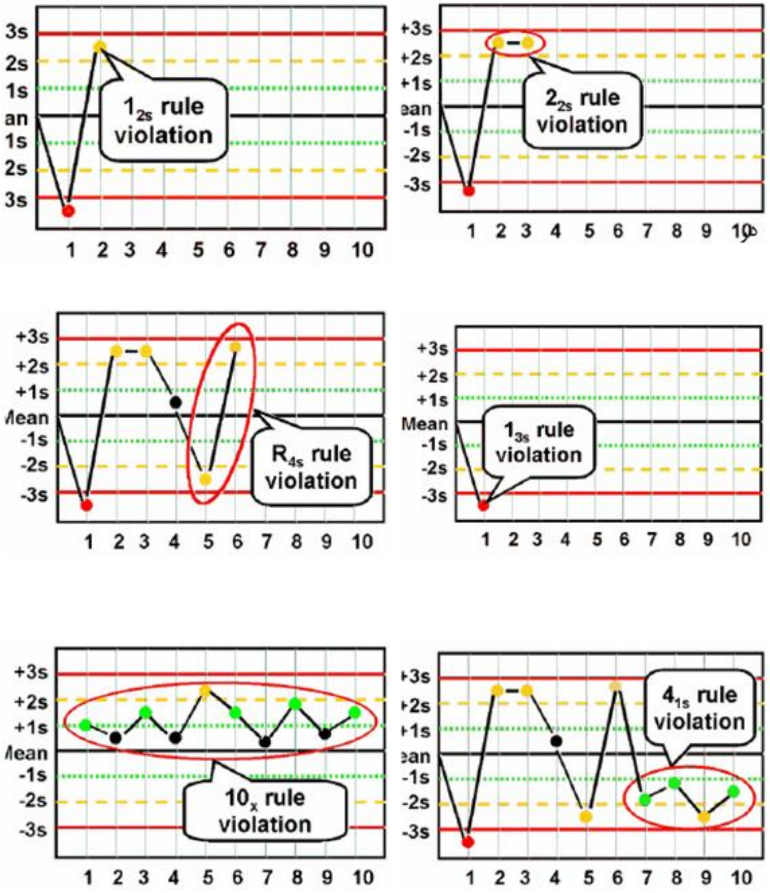
Levey–Jennings Chart (1950)

- 1950: S. Levey and E.R. Jennings adapted Shewhart charts for laboratory QC.
- Became the **Levey-Jennings Chart**, widely used for IQC.
- Provided graphical tool for monitoring precision and accuracy over time.



Westgard Rules (1981)

- 1981: James O. Westgard introduced **multi-rule QC system**
- Improved detection of **random and systematic errors**
- Still the gold standard for laboratory QC today



Modern QC Era

- 1990s–2000s: QC integrated into ISO, CLSI, CAP accreditation standards
- **External Quality Assessment (EQA/PT)** programs became mandatory
- Introduction of **Six Sigma** concepts to laboratory medicine
- Today: Combination of **IQC (LJ, Westgard)** + **EQA/PT** = global standard

Laboratory Statistics

Importance of Statistics in Clinical Laboratories

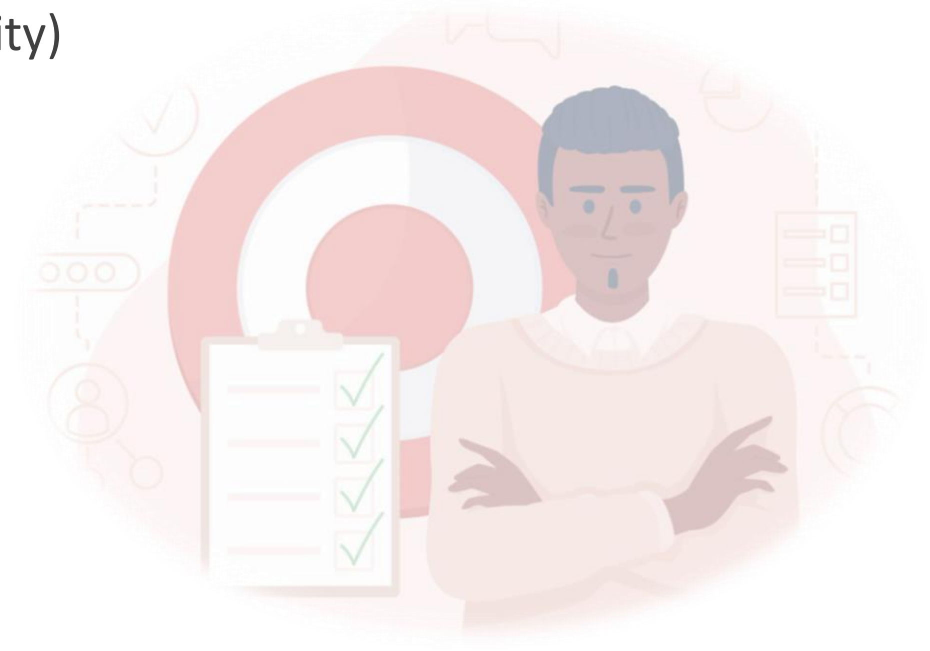
- Establishing reference ranges
- Quality control: precision and accuracy
- Proficiency testing
- Comparing new and existing methods
- Ensuring valid clinical decisions

Objectives

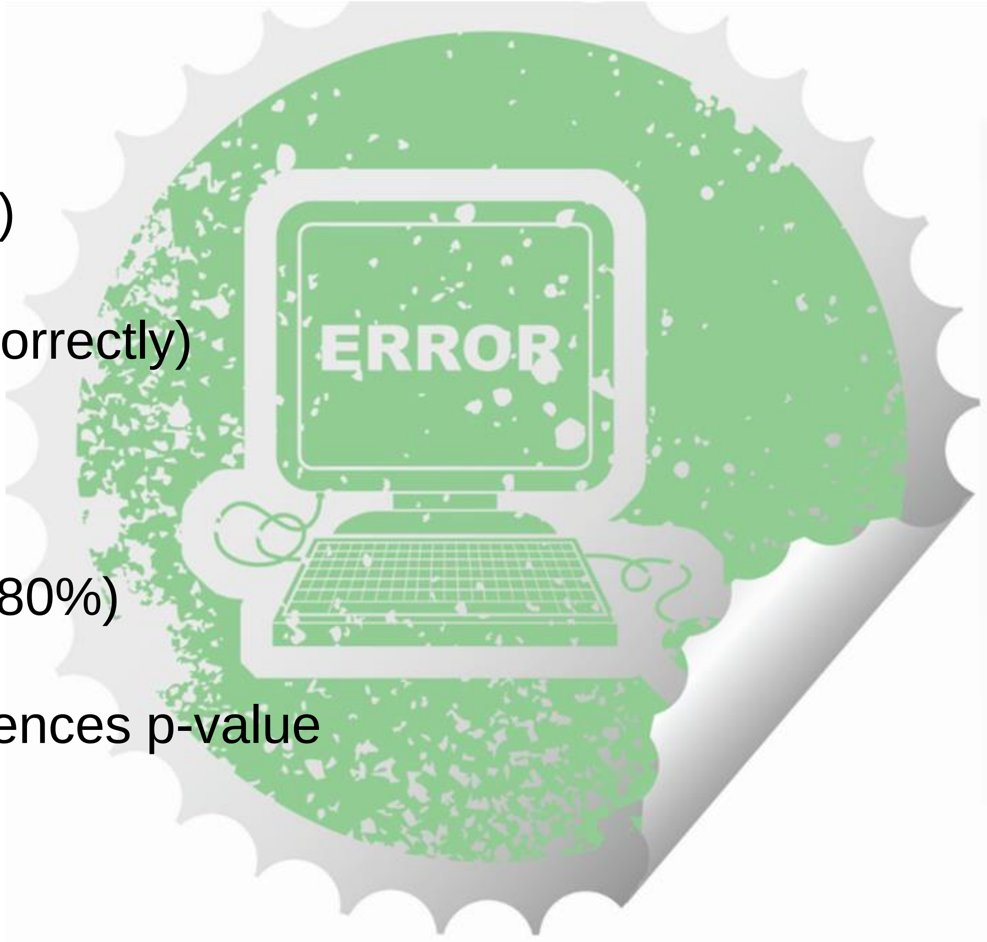
- Define variables and measurement scales
- Understand descriptive statistics (central tendency, dispersion, distributions)
- Apply comparative statistics (t-test, chi-square, nonparametric tests)
- Use regression, correlation, and ANOVA
- Learn method validation: accuracy, precision, sensitivity, specificity
- Recognize pitfalls, misuse of statistics, and false-positive and false-negative risks

Key Definitions

- **Variable:** measurable characteristic that can vary
- **Independent variable:** input/cause (e.g., age, gender, time)
- **Dependent variable:** output/effect (e.g., glucose, enzyme activity)
- **Null hypothesis (H0):** no difference exists
- **Alternative hypothesis (H1):** a difference exists



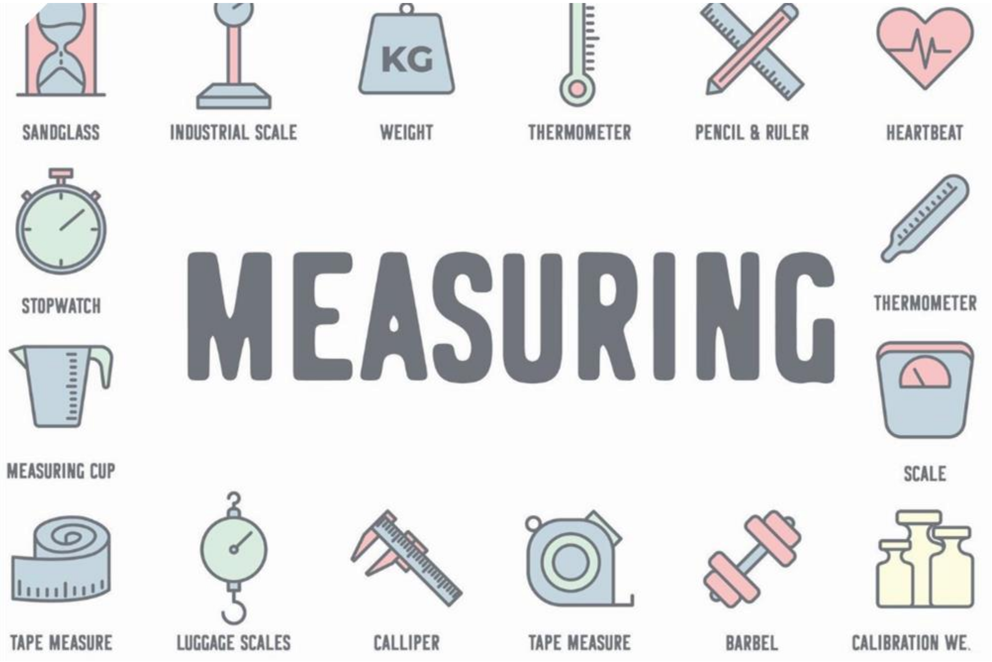
Statistical Errors and Parameters



- **Type I error (α):** false positive (rejecting H0 incorrectly)
- **Type II error (β):** false negative (failing to reject H0 incorrectly)
- **Significance level (p-value):** usually <0.05
- **Power (1−β):** probability of detecting true difference (≥80%)
- **Degrees of freedom (df):** related to sample size, influences p-value

Measurement Scales

- **Nominal:** categories only (e.g., Male/Female, Positive/Negative)
- **Ordinal:** ordered categories (e.g., Trace, 1+, 2+, 3+)
- **Interval:** numerical, equal intervals, no true zero (e.g., °C temperature)
- **Ratio:** numerical, equal intervals, true zero (e.g., glucose concentration)



Sample Size & Power

- Larger sample size → smaller p-value
- Power = probability of detecting true effect
- Usually set at 80% (β=0.20)
- Pilot studies help estimate sample size needed

Measures of Central Tendency

Mean (X̄):

- Arithmetic average of data
- Most common parametric measure
- Valid when data are normally distributed (Gaussian)

$$\text{arithmetic mean} = \frac{\Sigma x}{n} = \frac{\text{sum of data points}}{\text{number of data points}}$$

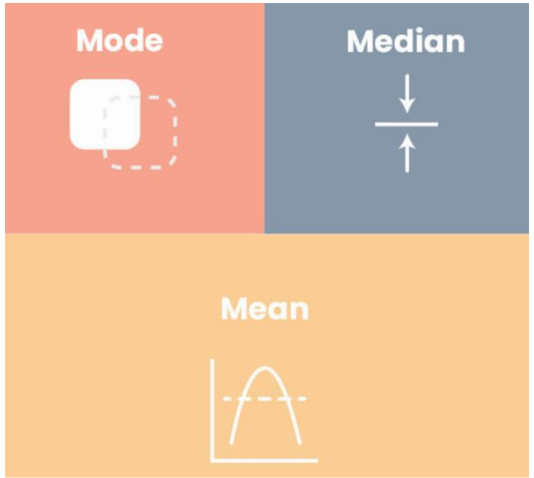
Where
Σ = sum of ...
x = each value of each data point
n = total number of data points

Median (M):

- Middle value of ordered data
- For odd n: middle value
- For even n: average of two central values
- Useful when data are skewed or have large variability

Mode:

- Most frequent value in the dataset
- Common in categorical data or when evaluating frequency by groups (e.g., age groups in hematology)



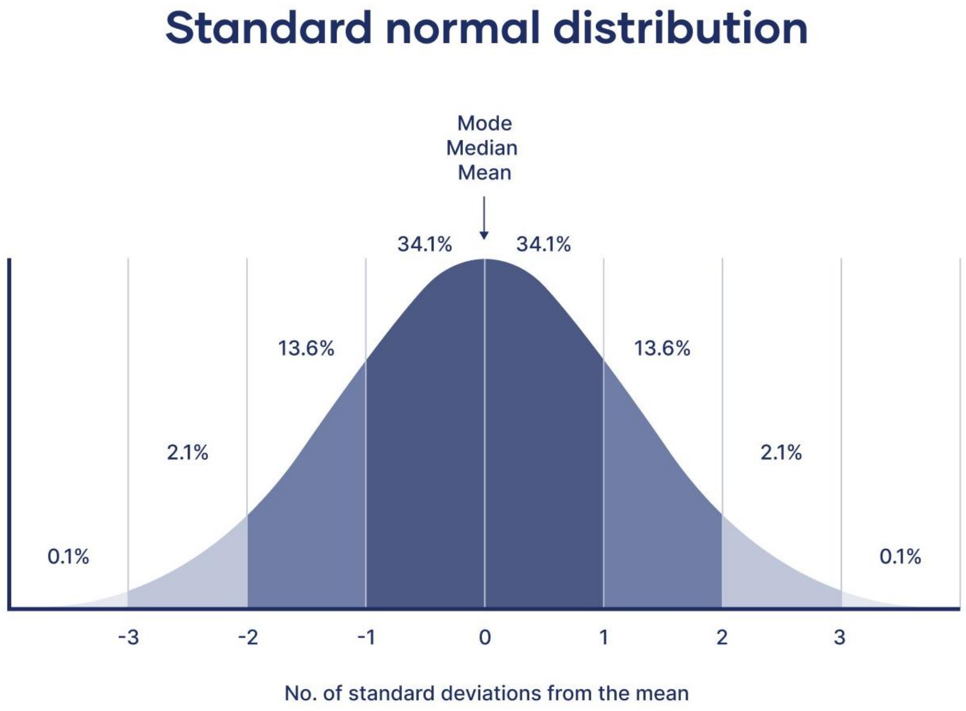
Distribution Patterns of Laboratory Data

Laboratory results always show variability, which can be visualized by plotting values vs. frequency.

Four main types of distributions:

Normal (Gaussian) Distribution

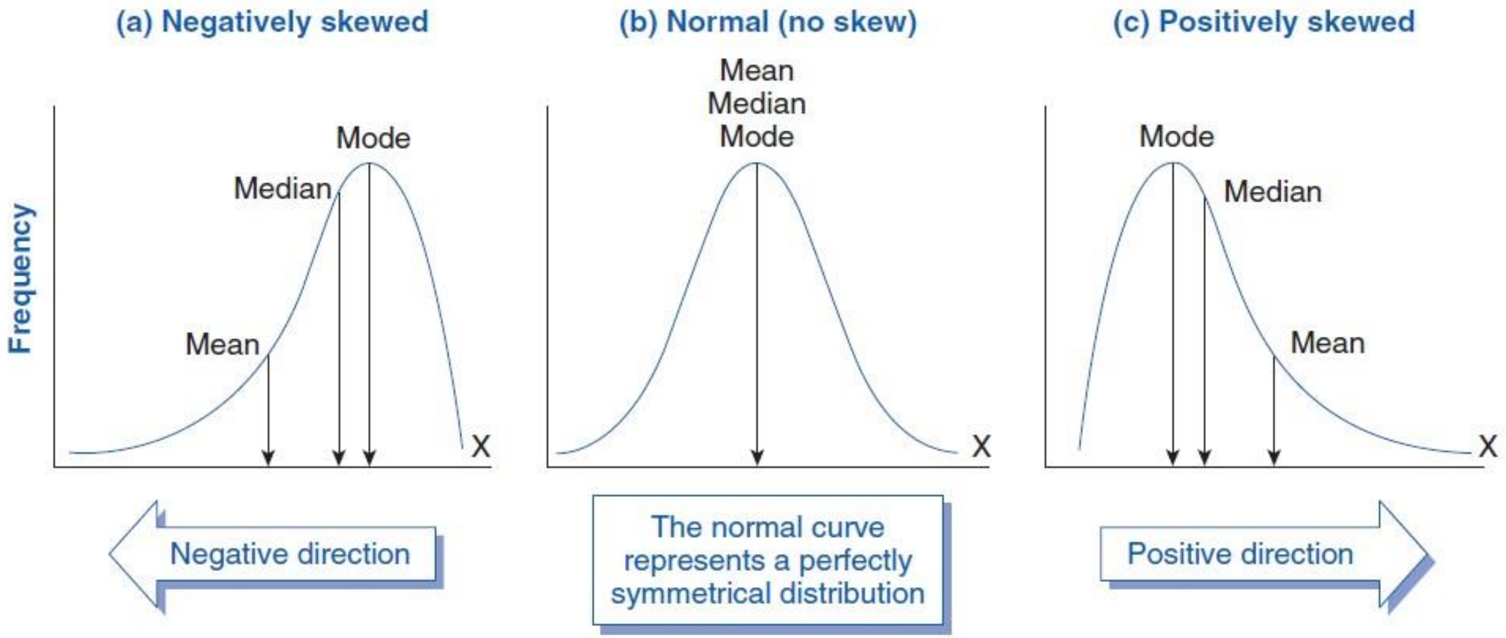
- Symmetrical, bell-shaped curve
- Example: RBC histogram from automated cell counters
- 68.2%, 95.5%, 99.7% of data within ±1SD, ±2SD, ±3SD



Distribution Patterns of Laboratory Data

Skewed Distribution

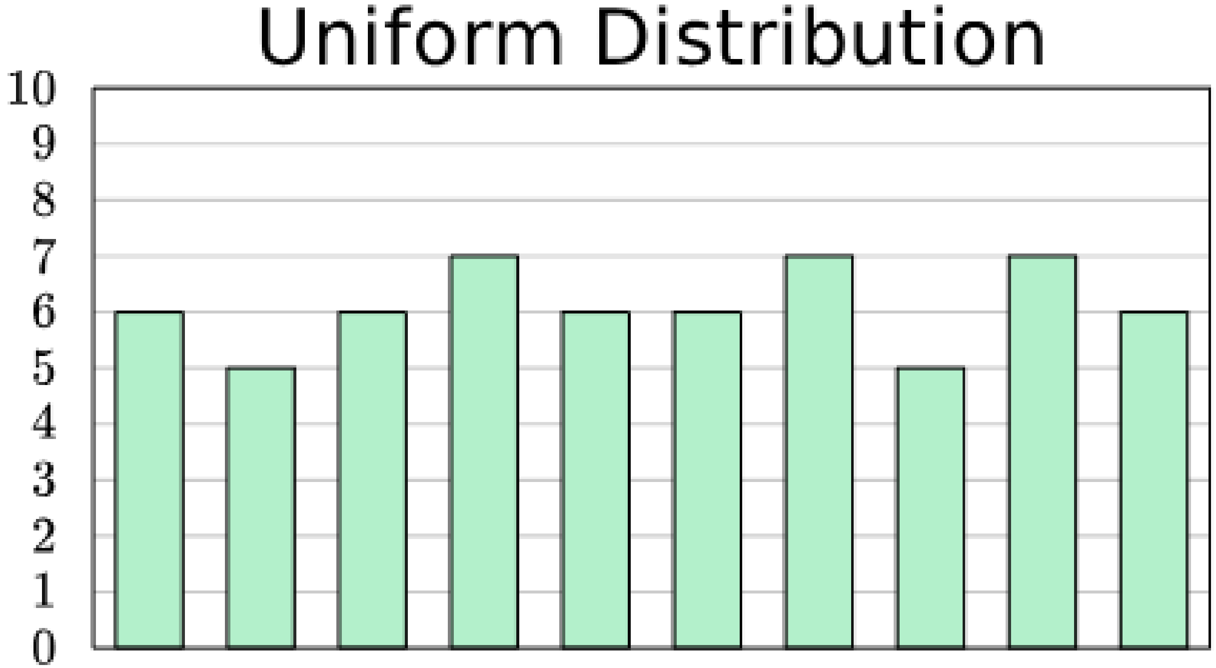
- Asymmetrical curve
- **Positive skew:** tail to the right
- **Negative skew:** tail to the left
- Mean ≠ Median



Distribution Patterns of Laboratory Data

Flat (Uniform) Distribution

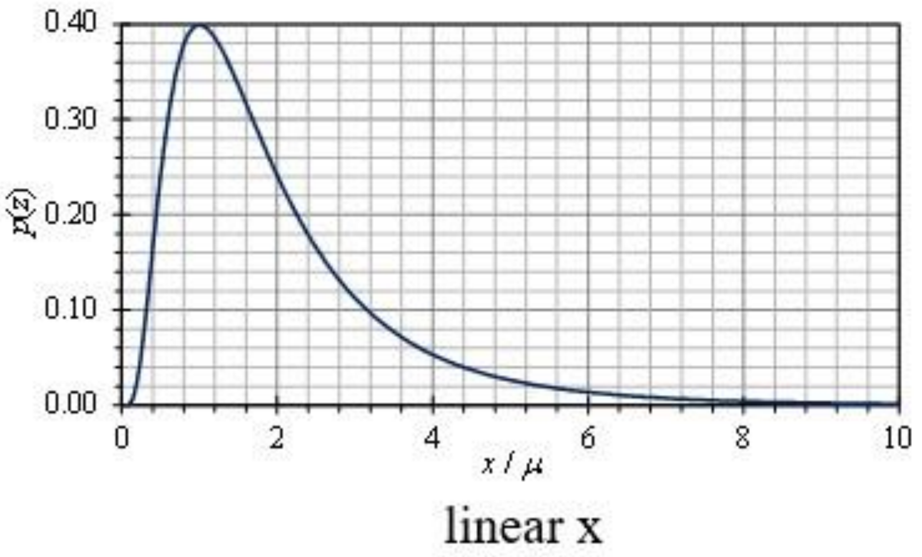
- No dominant peak, relatively even spread of data
- Median is more reliable than mean

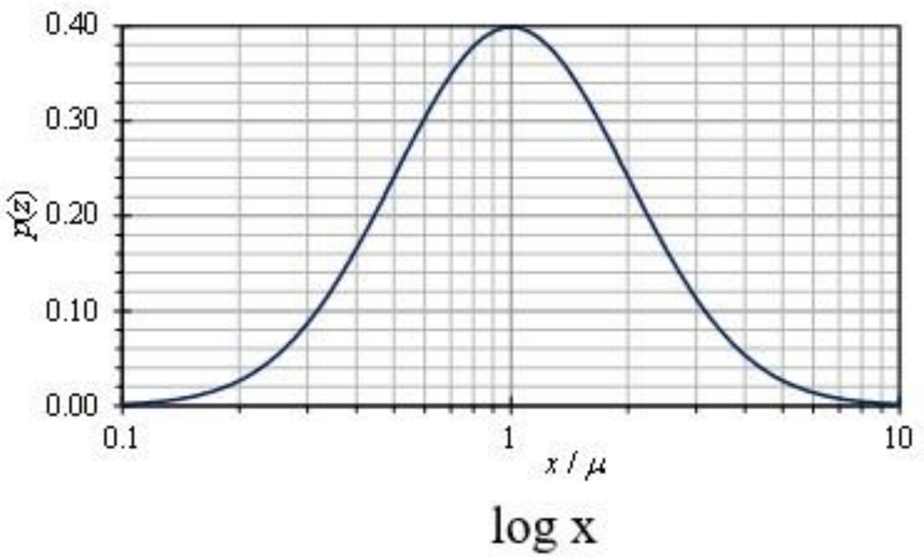


Distribution Patterns of Laboratory Data

Log-normal Distribution

- Skewed data becomes Gaussian after logarithmic transformation
- Example: Platelet volume histogram from hematology analyzers





Measures of Dispersion

Range (R):

- Difference between maximum and minimum values
- Simple to calculate, but sensitive to outliers

$$R = \text{Maximum Value} - \text{Minimum Value}$$

Mean Deviation (MD):

- Average of absolute deviations from the mean
- Reflects how far data deviate on average

$$\text{MD about Mean} = \frac{\sum |X - \bar{X}|}{n} = \frac{\sum |D|}{n}$$

where $|D| = |X - \bar{X}|$ and n is the number of observations.

Variance (S^2):

- Average of squared deviations from the mean
- Used to compare variability between groups or labs

$$\sigma^2 = \frac{\Sigma (x - \mu)^2}{N} \quad \text{Population Variance}$$

$$s^2 = \frac{\Sigma (x - \bar{x})^2}{n - 1} \quad \text{Sample Variance}$$

Measures of Dispersion

- **Standard Deviation (SD):**
- Square root of variance
- Indicates how tightly values cluster around the mean

Population	Sample
$\sigma = \sqrt{\dfrac{\sum (x_i - \mu)^2}{N}}$	$s = \sqrt{\dfrac{\sum (x_i - \bar{x})^2}{n - 1}}$
x_i = elements in population	x_i = elements in sample
μ = population mean	$\bar{x}$ = sample mean
N = population size	n = sample size

- **Coefficient of Variation (CV%):**
- Ratio of SD to mean × 100
- Useful for comparing variability between parameters with different units

$$\text{Coefficient of Variation (CV)} = \frac{\text{Standard Deviation (s)}}{\text{Sample Mean } (\bar{x})} \times 100$$

Standard Error of the Mean (SEM)

Although the mean is a single value, it has variability.

SEM quantifies the dispersion of sample means around the true population mean.

Smaller SEM → mean is a more precise estimate.

$$Standard\ Error\ of\ Mean, SEM = \frac{SD}{\sqrt{n}}$$

Example:

Hemoglobin measured 20 times on the same sample.

Calculate:

- **Mean ($\bar{X}$)**
- **Standard Deviation (SD)**
- **Coefficient of Variation (CV%) = (SD/$\bar{X}$) × 100**
- **SEM = SD / √n**

Comparison of Means

Simple approach:

- Calculate **Mean ± SD** for both groups.
- If the ranges **do not overlap**, the difference is likely significant.
- If the ranges **overlap**, groups may not differ significantly.

Example 1:

Group A: Mean = 40, SD = 3 → Range = 37–43

Group B: Mean = 50, SD = 3 → Range = 47–53

→ No overlap → Significant difference

Example 2:

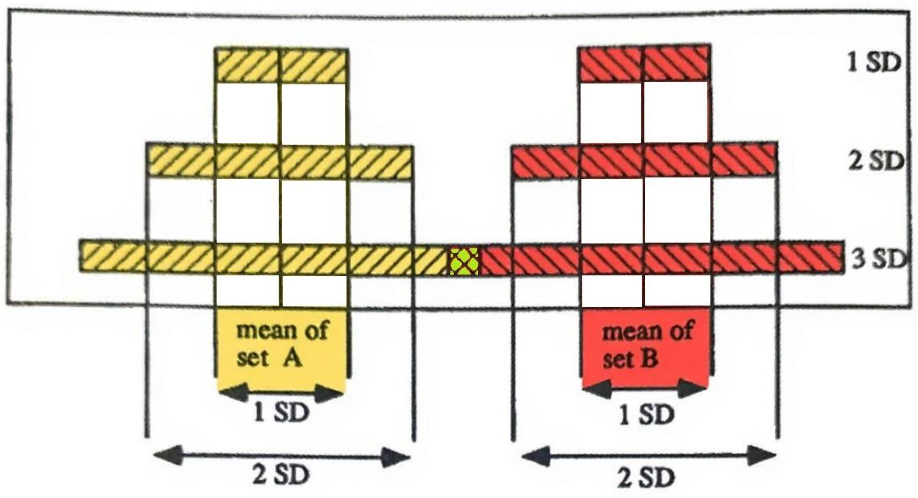
Group A: Mean = 40, SD = 10 → Range = 30–50

Group B: Mean = 50, SD = 10 → Range = 40–60

→ Overlap → Not significantly different

More reliable method:

- Use **Standard Error of the Difference (SEdiff):**
- If difference between means > SEdiff → statistically significant.



$$SEdiff = \sqrt{\frac{(SD_1)^2}{n_1} + \frac{(SD_2)^2}{n_2}}$$

Quality Control

Analysis of Variance (ANOVA)

Purpose:

- Applied in laboratory statistics to compare methods, instruments, or reagents.
- Determines whether differences between groups of data are statistically significant.

Key Tests:

- **Student's t-test:** Compares means of two groups to evaluate if difference is significant.
- **F-ratio Test (Variance Ratio):** Compares variances (SD^2) between groups.

Relation to ANOVA:

- Both t-test and F-test are forms of analysis of variance.
- They indicate whether observed differences between datasets are due to chance or are statistically meaningful.

Conditions for Valid ANOVA

- Random sampling
- Independent observations
- Normal distribution within each group
- Equal variances across groups
- Alternatives if not met: nonparametric tests

Student's t-Test

Purpose:

- Compares the **means** of two or more sets of experimental results.
- Evaluates whether observed differences are **statistically significant**.

Applications in Laboratory Medicine:

- Comparing a **new method** with a reference method.
- Checking **calibration stability** of an analyzer across different days.
- Comparing results of one analyzer against a **calibrated standard analyzer**.

Computation Methods:

- Two approaches are used depending on sample type (paired or independent samples).

t-Test Calculation Using the difference between means

$$SEdiff = \sqrt{\frac{(SD_1)^2}{n_1} + \frac{(SD_2)^2}{n_2}}$$

$$t = \frac{(\bar{X}_1 - \bar{X}_2)}{\sqrt{\frac{(S_1)^2}{n_1} + \frac{(S_2)^2}{n_2}}}$$

Definitions:

X¯1,X¯2: sample means of groups 1 and 2

SD1,SD2: standard deviations of groups 1 and 2

n1,n2: sample sizes of groups 1 and 2

Example: Comparing WBC Counts Between Two Analyzers

- **Study Design:**
- One fresh blood sample measured **10 times** on Analyzer I
- The same sample measured **10 times** on Analyzer II
- **Data (WBC counts):**
- Analyzer I: 12.0, 12.5, 13.0, 12.0, 11.5, 12.0, 11.0, 12.0, 10.6, 12.0
- Analyzer II: 11.5, 12.0, 11.8, 11.5, 11.0, 11.4, 12.0, 11.0, 11.5, 11.0
- **Calculated Values:**
- Mean (Analyzer I) = **12.04**
- Mean (Analyzer II) = **11.48**
- SD1=0.56, SD2=0.34
- SEdiff=0.342
- t=(12.04−11.48)/0.342=1.728
- df=9
- **Interpretation:**
- **Null hypothesis (H_0):** There is no statistically significant difference in WBC counts between Analyzer I and Analyzer II.
- **Alternative hypothesis (H_a):** There is a statistically significant difference in WBC counts between Analyzer I and Analyzer II.

Decision Rule: t-Test for Analyzer Comparison

Objective: Determine if there is a significant difference between two analyzers.

Parameters:

- Paired samples: n=10 → df=n−1=9
- Significance level: p=0.05 (two-tailed)
- Critical value from t-table: **2.262**

Result:

- Calculated t = **1.728**
- Since **1.728 < 2.262**, the value falls **outside the rejection region**.

Conclusion:

- Fail to reject H_0
- No statistically significant difference in WBC counts between Analyzer I and Analyzer II.

t-test table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	1.00	0.50	0.40	0.30	0.20	0.10	0.05	0.02	0.01	0.002	0.001
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

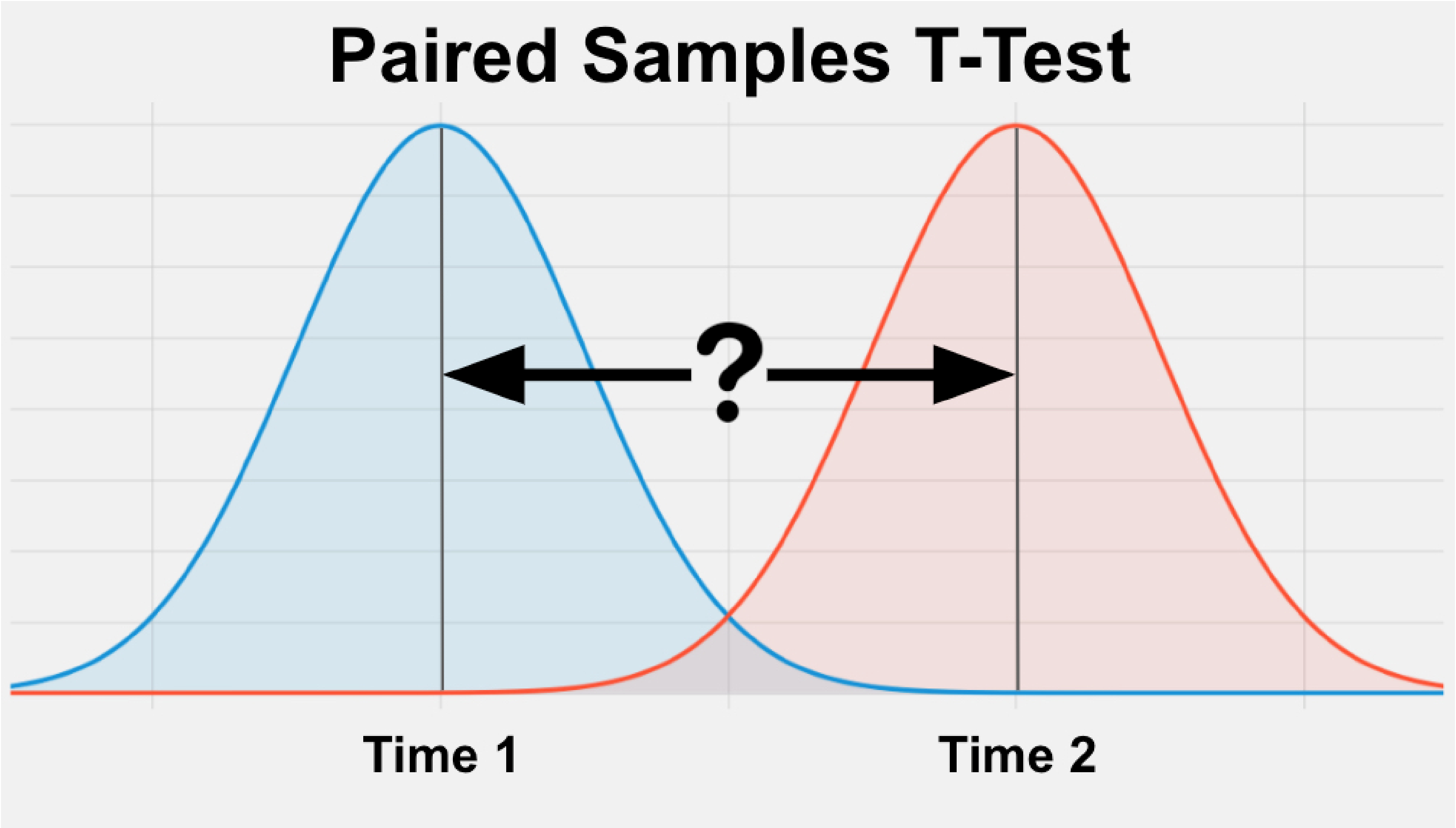
t-Test Calculation Using the difference between paired observations

$$t = \frac{\bar{d}}{S_d / \sqrt{n}}$$

- $\bar{d}$ is the **mean of the differences** between paired observations.

$$\bar{d} = \frac{\sum d}{n}$$

- d=X1−X2 (difference between paired values).
- Sd is the **standard deviation of the differences.**
- n is the **number of pairs.**



Example: Comparing Hb levels by Two methods

? The hemoglobin levels of 13 blood samples were obtained using the X and Y methods as follows. Is there a significant difference between these two methods?

number	Method X	Method Y	d
1	14.2	14.1	0.1
2	14.8	14.6	0.2
3	7.4	7.6	-0.2
4	10.3	10.1	0.2
5	12.8	12.8	0
6	13.2	13.1	0.1
7	6.4	6	0.4
8	15	15.1	-0.1
9	14.9	14.9	0
10	19.4	19	0.4
11	9.2	9	0.2
12	11.4	11.3	0.1
13	17	16.9	0.1

Quality Control

Calculating the average of differences

$$\bar{d} = \frac{\sum d}{n}$$

$$\bar{d} = \frac{0.1+0.2-0.2+0.2+0+01+04-0.1+0+0.4+0.2+0.1+0.1}{13} = 0.115$$

Calculate the standard deviation of the difference and t-test

d (individual)	d (individual)−d¯	[d (individual)−d¯]^2
0.1	-0.015	0.000225
0.2	0.085	0.007225
-0.2	-0.315	0.099225
0.2	0.085	0.007225
0	-0.115	0.013225
0.1	-0.015	0.000225
0.4	0.285	0.081225
-0.1	-0.215	0.046225
0	-0.115	0.013225
0.4	0.285	0.081225
0.2	0.085	0.007225
0.1	-0.015	0.000225
0.1	-0.015	0.000225
The sum of the squares of the differences		**0.356925**

$$s_d = \sqrt{\frac{\sum_{i=1}^{n}\left(d_i - \bar{d}\right)^2}{n-1}}$$

$$s_d = \sqrt{\frac{0.356925}{12}} \approx \sqrt{0.029744} \approx 0.1725$$

$$t = \frac{\bar{d}}{S_d / \sqrt{n}}$$

$$t = \frac{0.115}{0.1725 / \sqrt{13}} \approx \frac{0.115}{0.0478} \approx 2.41$$

Comparison with t table

Result:

- Calculated t = **2.41**
- Since **2.41 > 2.179**, the value is **inside the rejection region**.

Conclusion:

- Reject H_0
- Statistically significant difference in Hb levels measured by Two methods.

t-test table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
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9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

F-Ratio Test

- The F-test is a simple statistical tool to evaluate the precision (repeatability) of two measurement methods.
- It compares the variances of two datasets using the ratio: $F = \frac{Variance\ of\ A}{Variance\ of\ B}$
- Always place the larger variance in the numerator so that $F \geq 1$
- Similar to Student's t-test, critical F values are obtained from statistical tables at 95% (p = 0.05) or 99% (p = 0.01) confidence levels.
- If the calculated F-value exceeds the critical F-value → the difference in precision is statistically significant.

Example: Comparison of the precision of hemoglobin measurement by two hematology analyzers

- Hemoglobin was measured 30 times by two hematology analyzers.
- SD(A) = 2.1 → Variance = 4.41
- SD(B) = 1.8 → Variance = 3.24
- $F = \frac{4.41}{3.24} = 1.36$
- With df=29, the critical value (p = 0.05) is **1.90**.
- Since Fcalc<FcritF, **no significant difference** in precision exists between the two analyzers.

F-table of Critical Values of α = 0.05 for F(df1, df2)

	DF1=1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞
DF2=1	161.45	199.50	215.71	224.58	230.16	233.99	236.77	238.88	240.54	241.88	243.91	245.95	248.01	249.05	250.10	251.14	252.20	253.25	254.31
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45	19.45	19.46	19.47	19.48	19.49	19.50
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.70	8.66	8.64	8.62	8.59	8.57	8.55	8.53
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.77	5.75	5.72	5.69	5.66	5.63
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.53	4.50	4.46	4.43	4.40	4.37
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.84	3.81	3.77	3.74	3.70	3.67
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51	3.44	3.41	3.38	3.34	3.30	3.27	3.23
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.12	3.08	3.04	3.01	2.97	2.93
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.90	2.86	2.83	2.79	2.75	2.71
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85	2.77	2.74	2.70	2.66	2.62	2.58	2.54
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.61	2.57	2.53	2.49	2.45	2.40
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.51	2.47	2.43	2.38	2.34	2.30
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.42	2.38	2.34	2.30	2.25	2.21
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.35	2.31	2.27	2.22	2.18	2.13
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.29	2.25	2.20	2.16	2.11	2.07
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.35	2.28	2.24	2.19	2.15	2.11	2.06	2.01
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.31	2.23	2.19	2.15	2.10	2.06	2.01	1.96
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.19	2.15	2.11	2.06	2.02	1.97	1.92
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.23	2.16	2.11	2.07	2.03	1.98	1.93	1.88
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.08	2.04	1.99	1.95	1.90	1.84
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.18	2.10	2.05	2.01	1.96	1.92	1.87	1.81
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.23	2.15	2.07	2.03	1.98	1.94	1.89	1.84	1.78
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.20	2.13	2.05	2.01	1.96	1.91	1.86	1.81	1.76
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.18	2.11	2.03	1.98	1.94	1.89	1.84	1.79	1.73
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.16	2.09	2.01	1.96	1.92	1.87	1.82	1.77	1.71
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.15	2.07	1.99	1.95	1.90	1.85	1.80	1.75	1.69
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	2.13	2.06	1.97	1.93	1.88	1.84	1.79	1.73	1.67
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	2.12	2.04	1.96	1.91	1.87	1.82	1.77	1.71	1.65
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	2.10	2.03	1.94	1.90	1.85	1.81	1.75	1.70	1.64
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.89	1.84	1.79	1.74	1.68	1.62
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	2.00	1.92	1.84	1.79	1.74	1.69	1.64	1.58	1.51
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.92	1.84	1.75	1.70	1.65	1.59	1.53	1.47	1.39
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.83	1.75	1.66	1.61	1.55	1.50	1.43	1.35	1.25
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.52	1.46	1.39	1.32	1.22	1.00

Non-Parametric Statistics

- Parametric tests (e.g., *Student's t-test* and *F-test*) assume that the variable follows a normal distribution.
- When the assumption of normality is not valid, **non-parametric tests** are applied.
- One of the most widely used non-parametric methods is the **Chi-square test (χ^2 test)**.
- Non-parametric tests are particularly useful when:
- The measurement scale is **nominal** or **ordinal**.
- The sample size is small or distribution is unknown.
- They provide a practical tool for hypothesis testing without strict distributional assumptions.

Chi-Square Test (χ^2 Test)

- The Chi-Square test is a non-parametric statistical test used to evaluate the null hypothesis (H_0).
- It is applied when comparing two or more categorical variables.
- Often used to compare a new measurement method with a reference method.
- Formula:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where:

- **O** = Observed frequency
- **E** = Expected frequency
- A larger χ^2 value indicates a greater difference between observed and expected results.

- The significance of χ^2 is determined by comparing it with the **critical value** from the χ^2 distribution table (based on degrees of freedom).

Example: Chi-Square Test in Hematology

? **Objective:** Compare hematocrit results obtained by an analyzer (Observed, O) with those from the reference microhematocrit method (Expected, E).

E	O	O-E	$(O-E)^2$	$\frac{(O-E)^2}{E}$
38	40	2	4	0.105
49	45	-4	16	0.326
45	43	-2	4	0.088
36	35	-1	1	0.027
40	38	-2	4	0.100
41	37	-4	16	0.390

Steps of Chi-Square calculation

1. Compute the difference between observed and expected values: O-E
2. Square each difference: $(O - E)^2$
3. Divide by the expected frequency: $\frac{(O-E)^2}{E}$
4. Sum across all categories to obtain: $\chi^2 = \Sigma \frac{(O-E)^2}{E}$

- **Results:**

  Calculated $\chi^2 = 1.036$

  Degrees of freedom (df) = 5

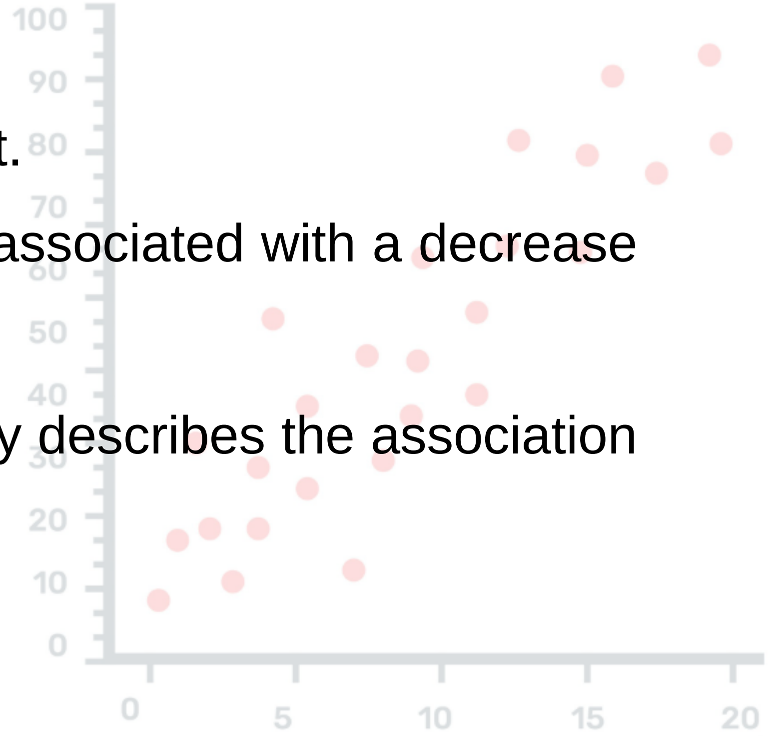
  Critical value χ^2 ($\alpha = 0.05$, df = 5) = **11.07**

	Chi-Square Right-Tail Probability ($\geq \chi^2$)									
DF	0.995	0.99	0.975	0.95	0.9	0.1	0.05	0.025	0.01	0.005
1	---	---	0.001	0.004	0.016	2.706	3.841	5.024	6.635	7.879
2	0.010	0.020	0.051	0.103	0.211	4.605	5.991	7.378	9.210	10.597
3	0.072	0.115	0.216	0.352	0.584	6.251	7.815	9.348	11.345	12.838
4	0.207	0.297	0.484	0.711	1.064	7.779	9.488	11.143	13.277	14.860
5	0.412	0.554	0.831	1.145	1.610	9.236	11.070	12.833	15.086	16.750
6	0.676	0.872	1.237	1.635	2.204	10.645	12.592	14.449	16.812	18.548

- **Conclusion:**
  Since 1.036 < 11.07 → Fail to reject H_0 → **No significant difference** between analyzer and reference method results.

Correlation

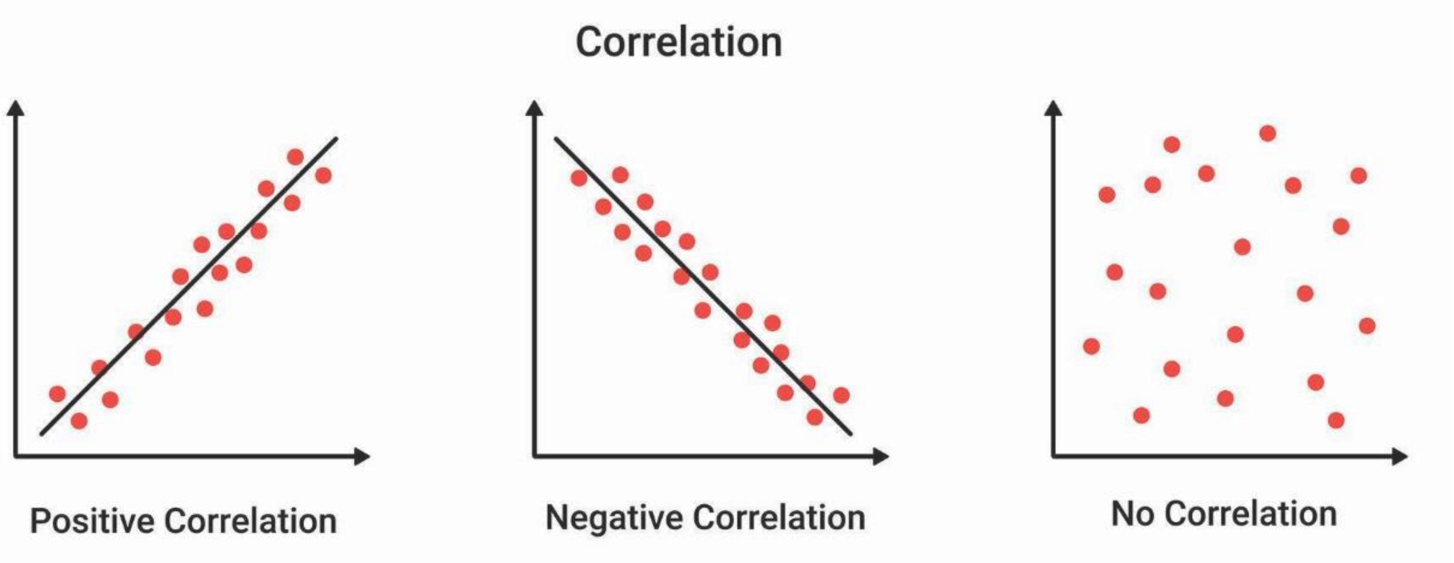
- **Definition:** Correlation describes the relationship between two or more variables.
- **Positive correlation:** When an increase in one variable is associated with an increase in another.
- *Example:* Hemoglobin concentration and red blood cell count.
- **Negative correlation:** When an increase in one variable is associated with a decrease in another.
- **Important note:** Correlation does **not** imply causation; it only describes the association between variables in a population.
- **Notation:** Variables are typically represented as **X** and **Y**.



Correlation and Scatter Plots

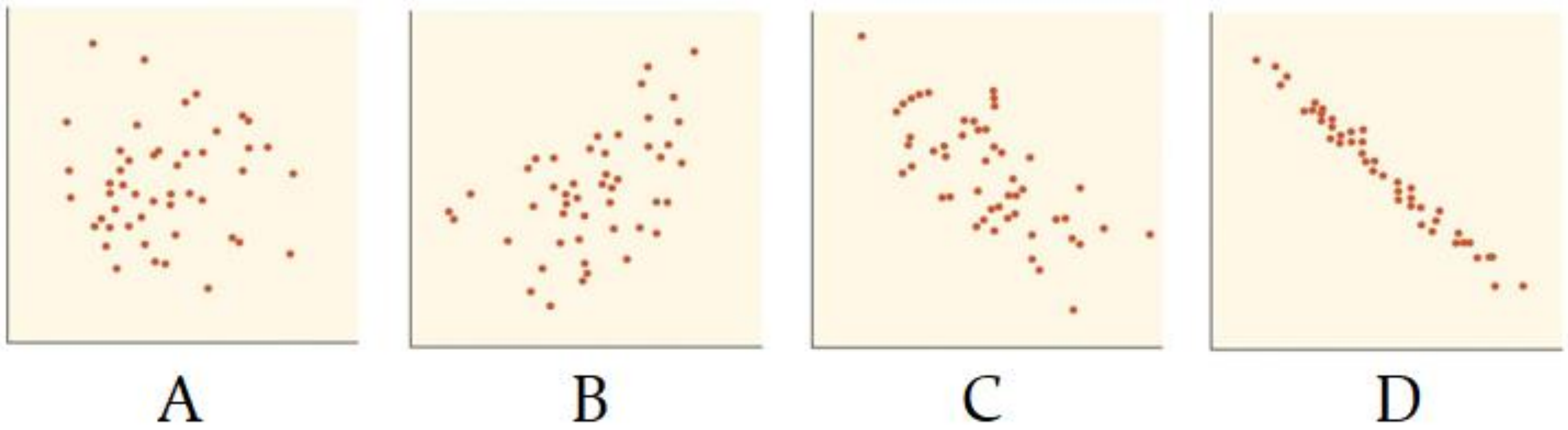
Scatter plot: A graphical method to display the relationship between two variables.

- X-axis: values of one variable
- Y-axis: values of another variable



Types of scatter patterns:

- **No correlation:** Random "shotgun" distribution (Fig A)
- **Positive non-linear correlation:** Elliptical shape extending upward (Fig B).
- **Negative non-linear correlation:** Elliptical shape extending downward (Fig C).
- **Perfect linear correlation:** Data points form a straight line (Fig D).
  - Can be **positive** or **negative** (Fig D is negative)



Correlation Coefficient (r)

- **Definition:**

A statistical measure that shows the strength and direction of the relationship between two variables (X and Y).

- **Range:**

$-1 \leq r \leq +1$

- **r = 0:** No correlation
- **r = +1:** Perfect positive correlation
- **r = -1:** Perfect negative correlation
- Values between -1 and +1 indicate partial correlation

Correlation Coefficient (r) Calculation Methods

1. From raw data:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

2. From deviations from the mean:

$$r = \frac{\sum xy}{\sqrt{\sum x^2 \sum y^2}}$$

Where $x = X - \bar{X}$ and $y = Y - \bar{Y}$

Application:
Used to compare two measurement methods, e.g., RBC count measured by an automated hematology analyzer (Sysmex XE-2100) vs. flow cytometry (FCM).

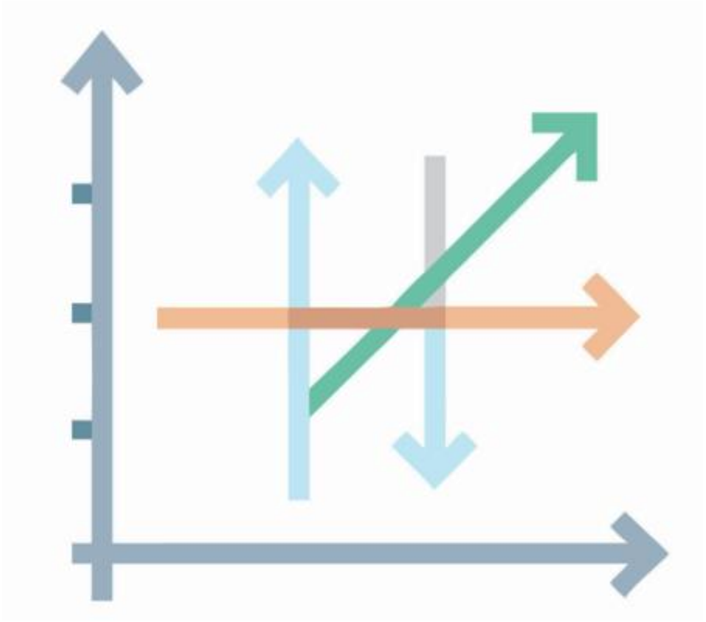
Linear Regression Analysis

Purpose:

- Identifies the best linear relationship between two laboratory methods.
- Provides descriptive information about systematic and random errors.

Equation: $Y = mx + b$

- Y: Dependent variable (new method)
- X: Independent variable (reference method)
- m: Slope (systematic proportional error)
- b: Intercept (systematic constant error)



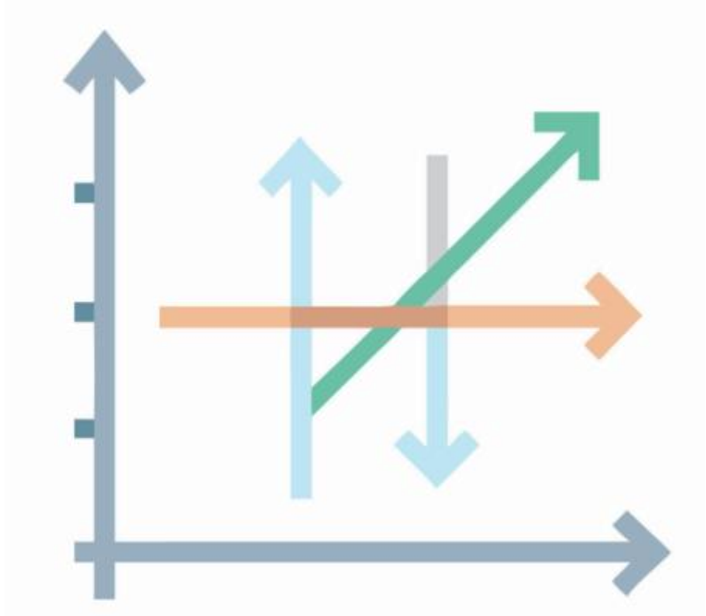
Linear Regression Interpretation

Interpretation:

- Perfect agreement: **m = 1, b = 0**
- **m < 1:** New method underestimates values
- **m > 1:** New method overestimates values
- **b ≠ 0:** Indicates constant systematic bias

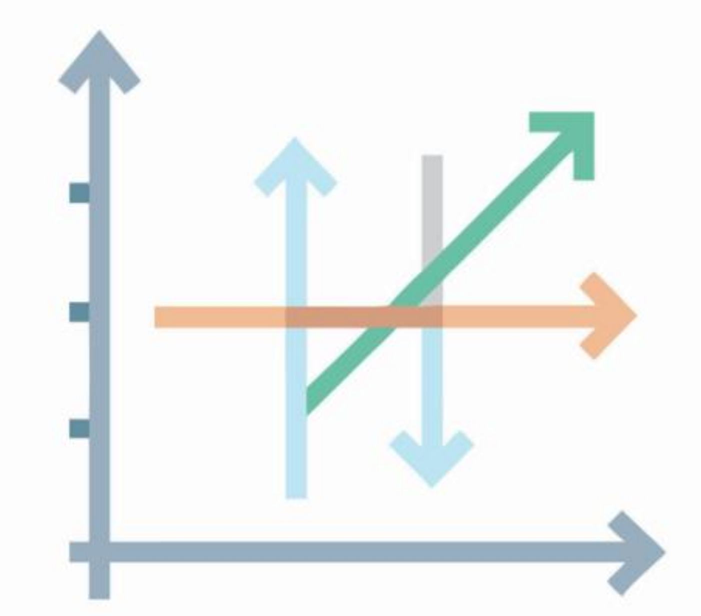
Error types:

- **Random error:** Reflected in scatter around regression line (**Sy/x**)
- **Systematic error:**
    - Constant → shift in intercept
    - Proportional → change in slope



Deming Regression

- Accounts for errors in both x and y
- Minimizes sum of squared distances (both axes)
- Preferred for method comparison in laboratories
- Weighted if precision differs between methods



Youden Plot (XY Plot)

Definition:

- A Youden plot (XY plot) is used when two control samples (e.g., normal and abnormal levels) are measured simultaneously.

Purpose:

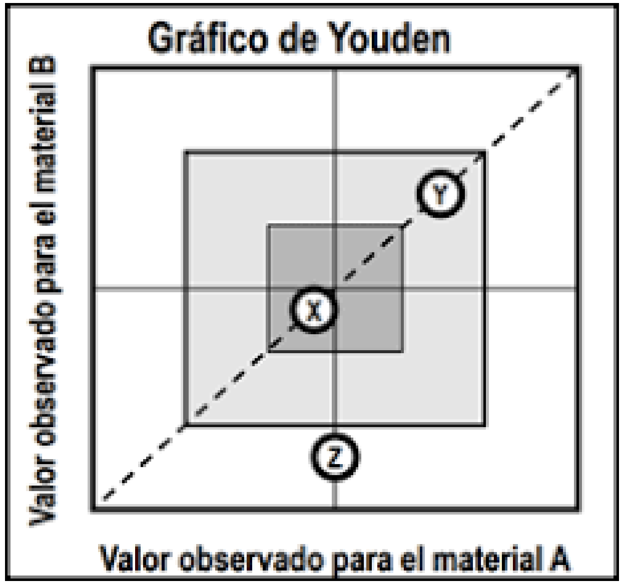
- Distinguishes **random errors** from **systematic errors**.
- Provides a graphical evaluation of control results.

Construction:

- X-axis: SD ranges of the normal control.
- Y-axis: SD ranges of the abnormal control.
- Connecting ±SD lines creates a square (control area).
- The diagonal line represents the **normal scatter path**.

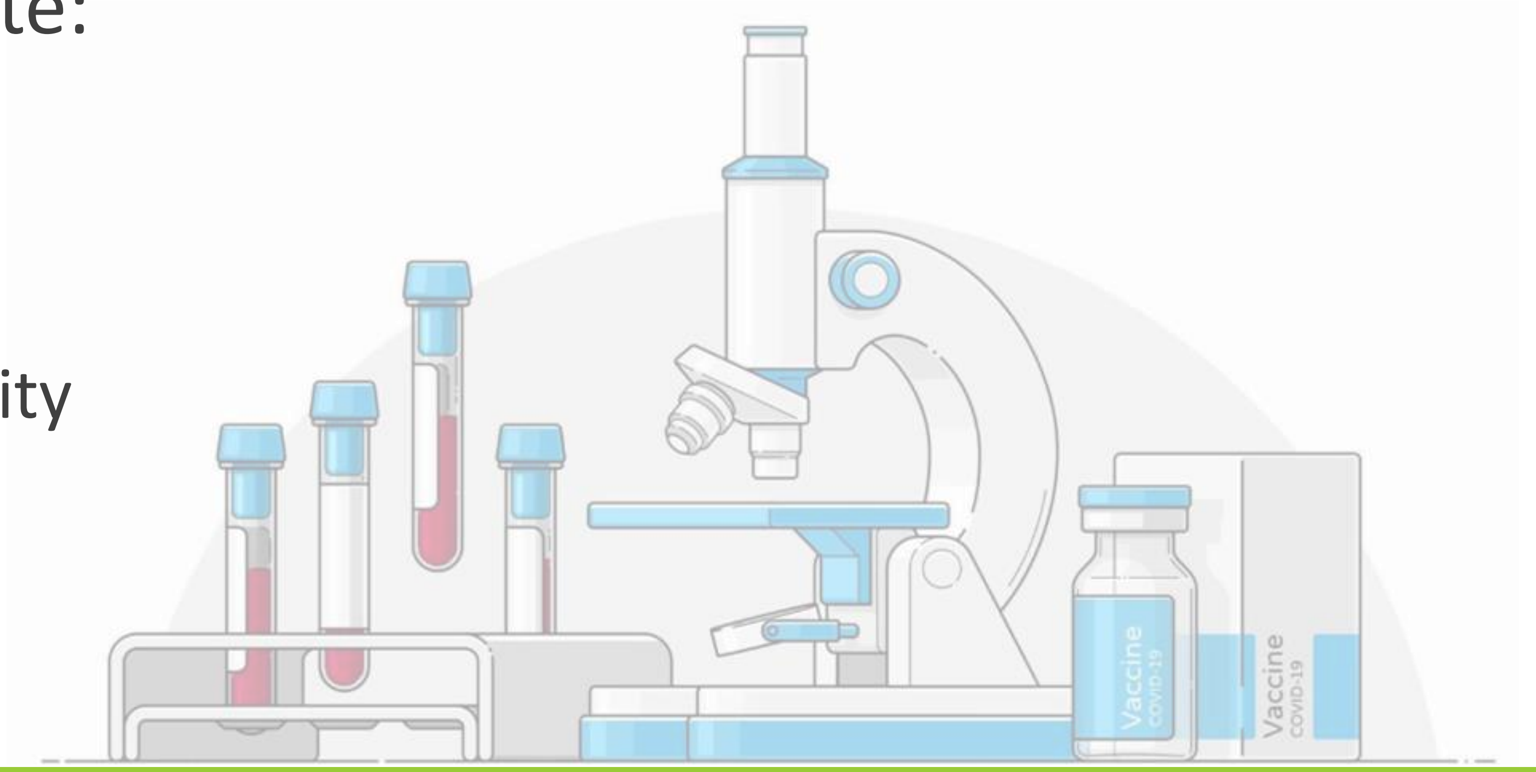
Interpretation:

- Results inside the central square = **acceptable performance**.
- Results in light gray square = **systematic error**
  - Negative bias
  - Positive bias
- Results outside the gray square = **random errors**.



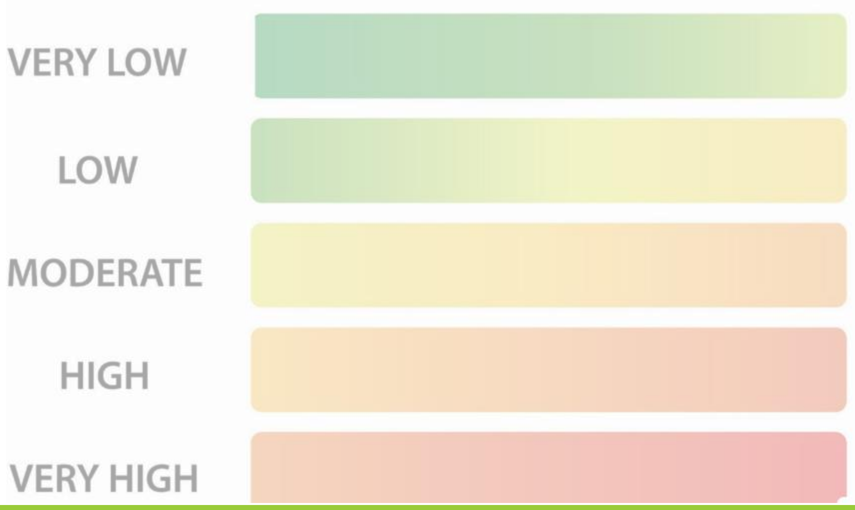
Method Validation in the Laboratory

- Ensures reliability of new methods
- Parameters to evaluate:
  - Reference range
  - Accuracy
  - Precision
  - Sensitivity & Specificity
  - Linearity
  - Interferences



Reference Ranges

- Defined from healthy population
- Usually 95% central values (2.5th – 97.5th percentile)
- Must consider age, sex, ethnicity, physiological state
- Different for inpatients vs outpatients



Accuracy

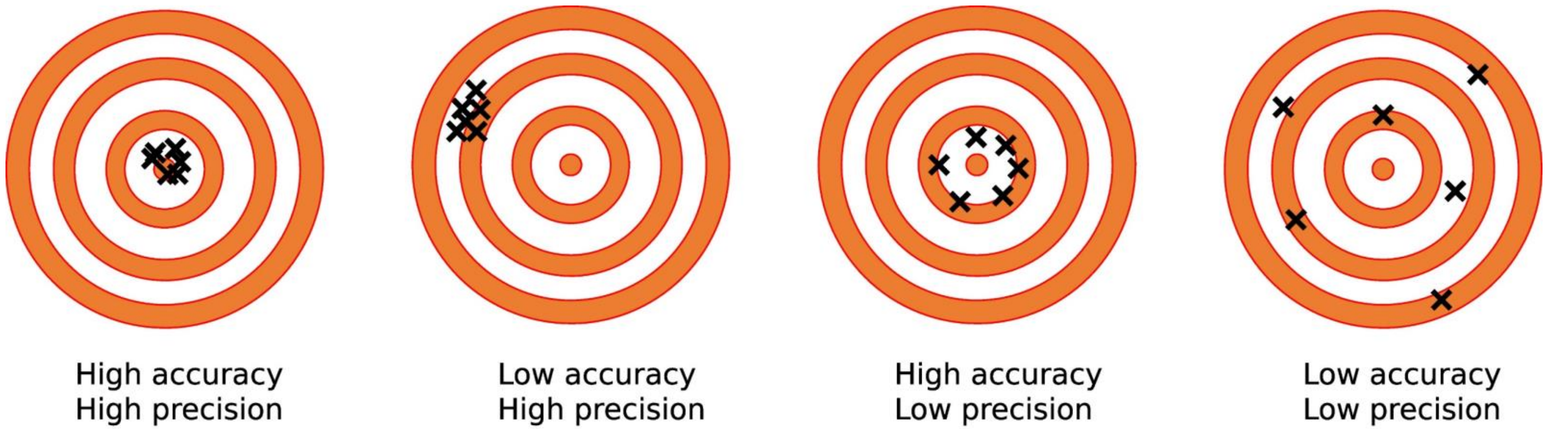
- Closeness to true value
- Determined by comparing to reference method
- Expressed as bias (systematic error)
- Accuracy ≠ precision

Precision

- Reproducibility of repeated measurements
- Measured by standard deviation (SD) or coefficient of variation (CV)
- Within-run precision vs between-run precision
- High precision ≠ high accuracy

Accuracy vs Precision

- Accurate & precise → ideal method
- Precise but not accurate → systematic error
- Accurate but not precise → random error
- Neither accurate nor precise → unreliable method

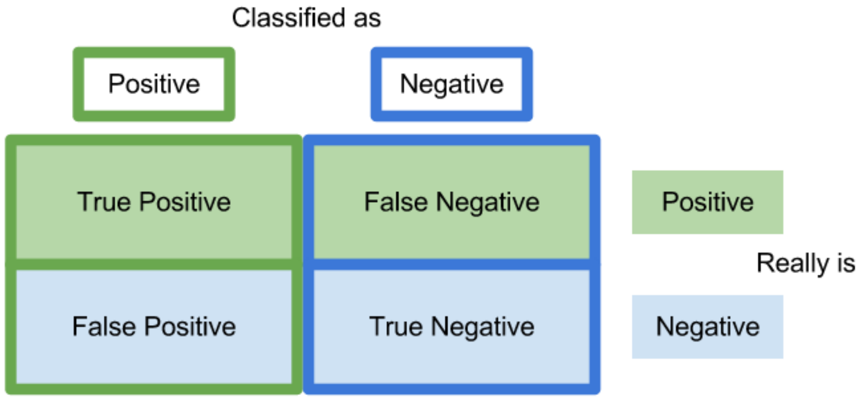


Sensitivity & Specificity

- **Sensitivity:** ability to detect disease (true positives)
- **Specificity:** ability to exclude disease (true negatives)
- Example: HIV ELISA – high sensitivity, lower specificity
- Trade-off between sensitivity and specificity

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}}$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + false positive}}$$

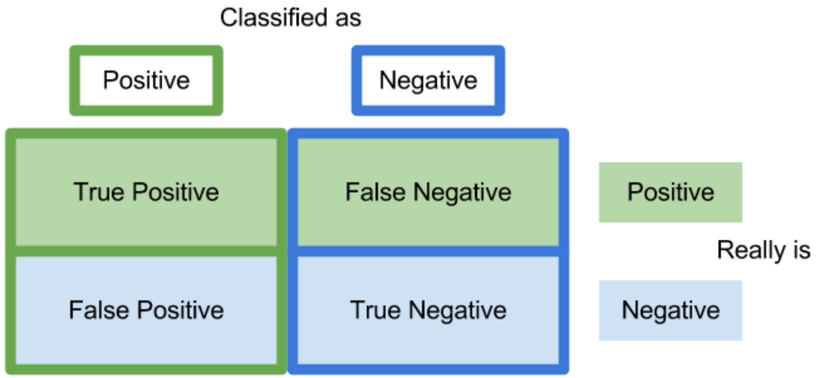


Predictive Values

- **Positive predictive value (PPV):** probability disease present if test positive
- **Negative predictive value (NPV):** probability disease absent if test negative
- Depend on disease prevalence
- Example: same test has lower PPV in low-prevalence population

$$PPV = \frac{\text{True positive}}{\text{True positive + false positive}}$$

$$NPV = \frac{\text{True negative}}{\text{True negative + false negative}}$$



Pitfalls in Statistics

- Misuse of p-values (significance vs importance)
- Multiple comparisons without correction
- Ignoring confounders (e.g., albumin in calcium)
- Small sample size → low power
- Overfitting in multivariate models

Summary

- Laboratory statistics essential for reliable data
- Descriptive: mean, median, SD, CV, distributions
- Comparative: t-test, chi-square, ANOVA, nonparametric tests
- Regression & correlation for method comparison
- Validation: reference range, accuracy, precision, sensitivity, specificity
- Always interpret in clinical context

Thank you

For Your Attention