Metrology

Metrology

Instrumentation and Its

Limits

Science of physical measurement applied to variables such as dimensions

Outline

1. Measurement using balances
2. Precision versus Accuracy
3. Calibration of balances
4. Validation of pipetmen

Types of Balances

Labs are equipped with two types of balances:

- Analytical balance
- Top loading balance

The primary difference between these instruments is Significant Figures

Precision = +/- 0.01g

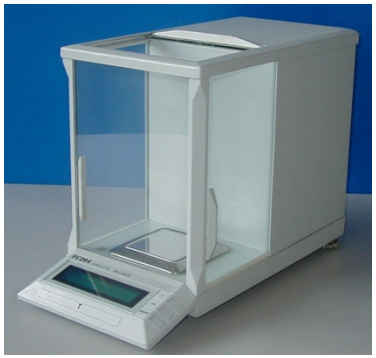
Top Loading Balance

- Used when less quantitative results are required (+/- 0.01g)
  (Capacity < 1200 g)

Analytical Balance

- Used for measurements requiring highly quantitative results i.e. +/- 0.0002 g

  (Capacity < 100 g)



Bio-Rad Pipetmen

- Pipets can measure +/-1% of their largest volume and be accurate

Limits of Measurement

All measurements contain some error and it is calculated by the following formula:

$$\text{Percent Error} = \frac{\text{True Value} - \text{Average Measured Value}}{\text{True Value}} \times 100\%$$

Standard Deviation Gives a Measure of Variability

$$S = \sqrt{\frac{\Sigma (x - \bar{x})^2}{n - 1}}$$

What are Significant Figures?

- The necessary number of figures (digits) required to express the result of a measurement or calculation so that only the last digit in the number is in doubt.

- Measuring gives significance (or meaning) to each digit in the number produced.

Why Consider Significant Figures?

- Science depends upon experimentation which requires numerical measurements.
- Measurements are taken from instruments made by other human beings.
- NO measurement is exact
- Error is always a factor

Determining Significant Figures

- Last figure is estimated in measuring 2.33
- All whole #'s are significant 2.33 = 3 sig figs
- All zeros between 2 numbers are significant 2.03 = 3 sig figs
- Zeros to the right of whole number digits are significant if prec by a decimal point

  203.00 = 5 sig figs
- All zeros to the right of a decimal point & whole number are significant 2.0230 = 5 sig figs
- Zeros to the right of a decimal but to the left of a whole number are not significant 0.0203 3 sig figs

Accuracy

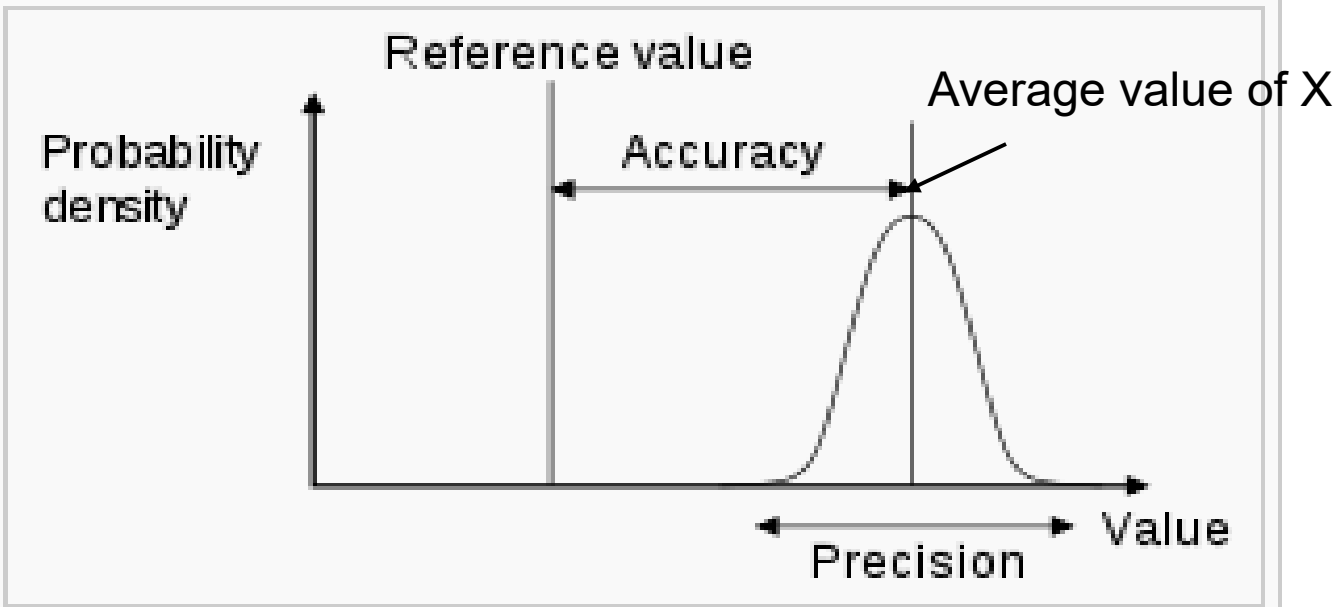
The accuracy of an analytical measurement is how close a result comes to the true value. The analytical method is calibrated using a known standard to determine the accuracy of a measurement.

Precision

- The reproducibility of multiple measurements.

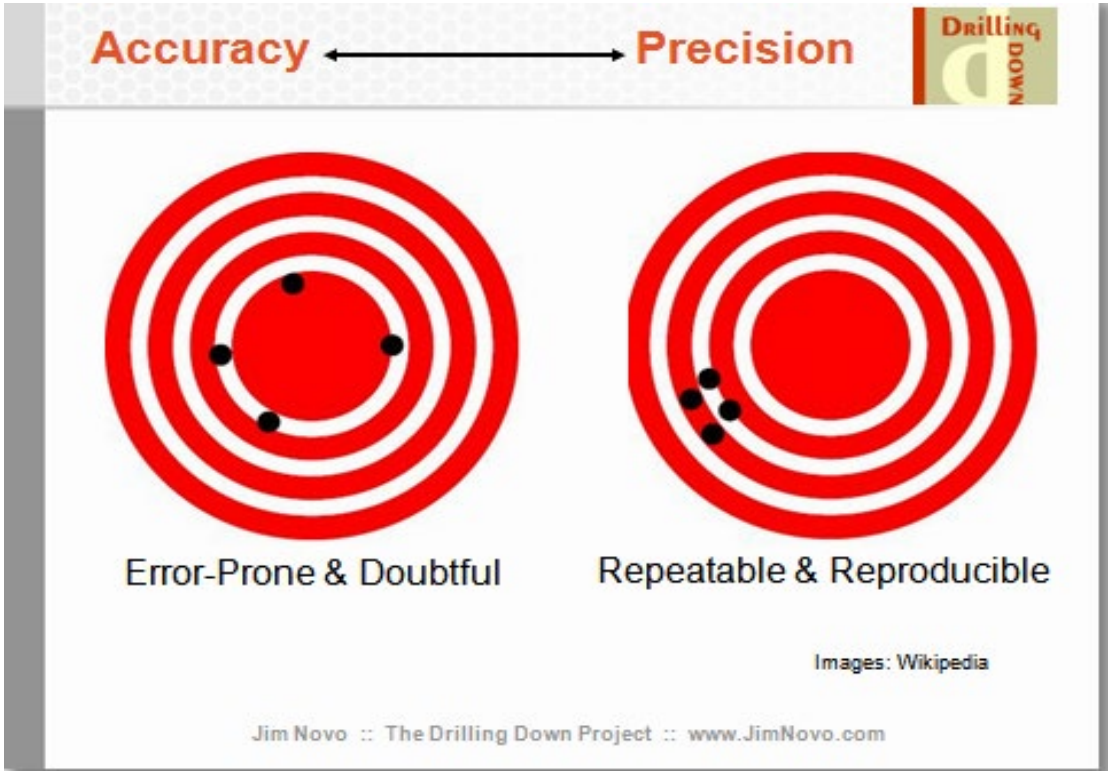
- It is evaluated statistically using standard deviation, standard error, or confidence interval.

Measurement Requires Accuracy and Precision



Accuracy indicates proximity of measurement results to the true value, precision to the repeatability or reproducibility of the measurement

Accuracy vs Precision



Calibration of Balances

- Balance is reset to detect a specific weight according to directions
- A 200.00 g standard is placed on the balance
- After recalibrating, the balance will then show a value equal to the standard

Validation of Pipetmen

- Pipetman is selected and set to a specific volume : 1000 µl or 200 µl
- Water is drawn up to a desired volume and place into weighed small beaker
- Using the equation:

  Density= Mass/Volume

  *calculate the volume using 1 gram/ml for density of water

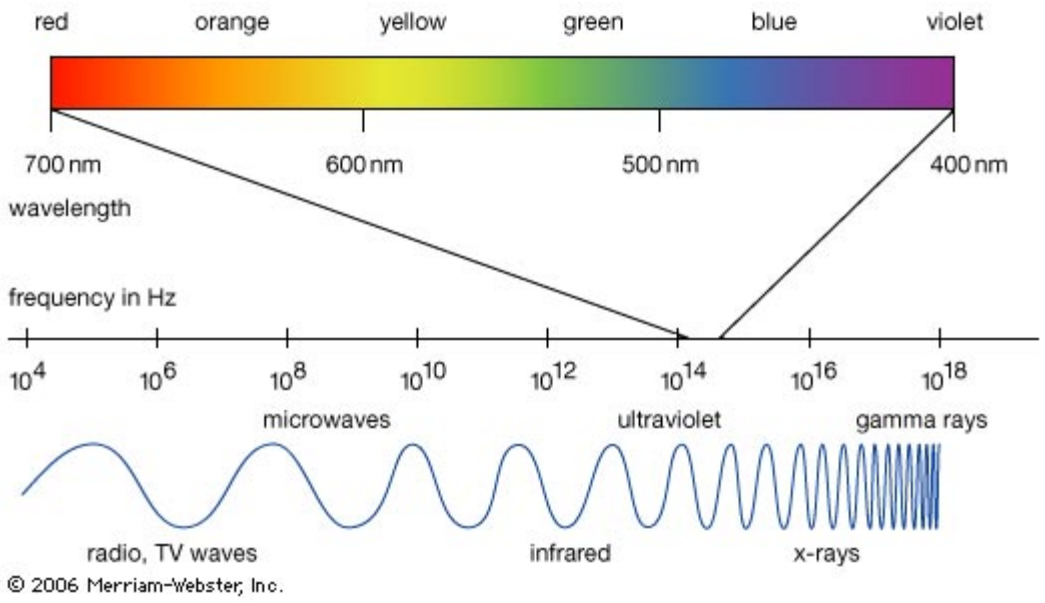
Percent Error

- The deviation from an expected value can be expressed as a Percent Error

- To calculate the Percent Error use the formula below:

$$\% \text{ Error} = \frac{\text{True Value} - \text{Average value}}{\text{True Value}} \times 100$$

Electromagnetic Spectrum



The spectrum of electromagnetic waves ranges from low-frequency radio waves to high-frequency gamma rays. Only a small portion of the spectrum, representing wavelengths of roughly 400–700 nanometers, is visible to the human eye. *© Merriam-Webster Inc.*

Spectroscopy

When light of a specific wavelength interacts with a substance, the subsequent energy transfer results in:

1. Absorption
2. Fluorescence

Absorption

As the light hits a substance, energy is transferred to the substance thereby raising its energy to an excited state.

Fluorescence

Molecular absorption of a photon triggers the emission of another photon with a longer wavelength when the molecule relaxes back to its ground state.

Excitation: $S_0 + h\nu \rightarrow S_1$

Fluorescence: $S_1 \rightarrow S_0 + h\nu$

h = Planck's constant

v = frequency of light

S_0 = ground state of the fluorescent molecule

S_1 = excited state

Examples of Fluorescence

Molecules that are excited through light absorption can transfer energy to a second molecule, which is converted to its excited state and can then fluoresce.

- Fluorescent lights
- Light emitting diodes (LEDs)
- Mercury vapor streetlight
- Glow sticks
- Compact fluorescent lighting (CFL)

UV/Vis Spectrophotometer

Principle:

Absorption of light in the visible and ultraviolet spectrum results in changes electronic structure of molecules

UV/Vis Spectrophotometer

Dual light source:

- Visible range: Tungsten lamp (400 – 700 nm)
- UV range: Deutrium lamp (200 – 400 nm)

Sample cells

Detector

Mirrors

Grating Monochrometer

Application

Lambert-Beer Law $A = \varepsilon cl$ where:

A = absorbance

ε = molar extinction coefficient (L $mmol^{-1}$ cm^{-1})

c = molar concentration (mM)

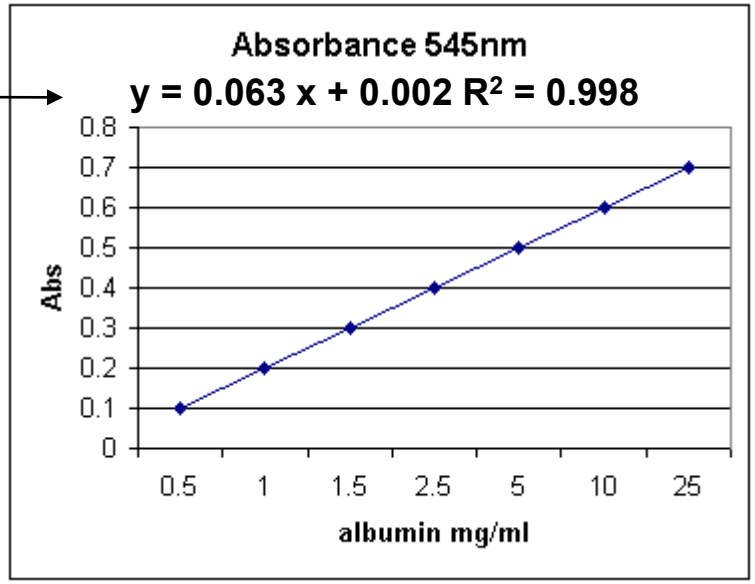
l = pathlength (cm)

The Lambert-Beer law is used to accurately determine the concentration of a substance by measure absorbance at a specific wavelength

Determining Concentration

The Lambert-Beer Law is used to determine the concentration of an unknown using a standard curve.

Equation for a line →



Determining Nucleic Acid Concentration

- Quantitative measurements (µg) for nucleic acids (DNA and RNA) at A_{260}
- $A = \varepsilon cl$
  - ε is specific for each type of nucleic acid
  - 1 OD_{260} of ds-DNA = 50 µg/mL
  - 1 OD_{260} of ss-DNA = 37 µg/mL
  - 1 OD_{260} of ss-RNA = 40 µg/mL

Other Wavelengths

- A_{280}: Protein
- A_{230}: Phenol and peptide
- $A_{260/280}$: Purity of nucleic acid preparation
  (Pure range 1.8 – 2.0)

Terminology

Solution: A homogenous liquid mixture composed of two or more substances

Solute: Substance which dissolves in the solvent

(ex H_20)

Solvent: Liquid in which solute is dissolved

Concentration: The ratio of the mass of a solute to the volume of solution Examples: g/mL, mol/L, %

Dilution (factor, medium, volume):

Serial dilution is logarithmic

Linear dilution using $C_1V_1 – C_2 V_2$

Terminology (cont.)

Aliquot: Equally divided portions of a sample

Buffer: A salt solution which resists change in pH upon addition of acid or base

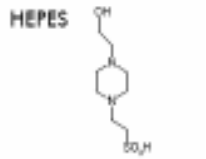
Reagent: A substance which is involved in or consumed during a chemical reaction

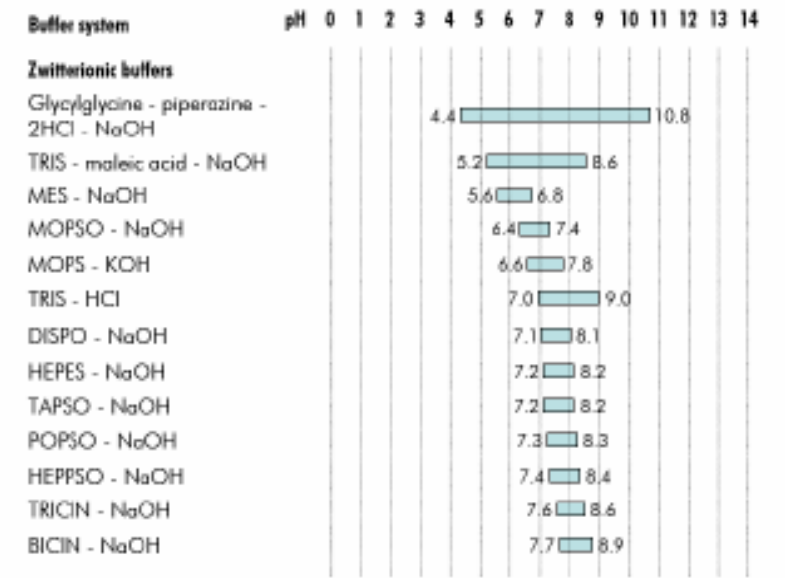
or to detect other substances

Meniscus: A curve in the surface of a liquid which results from interaction with the container

Common Buffers

Optimal pH ranges for common laboratory buffers





pH

$pH = -\log [H^+]$

pH 7: Neutral: $[H+] = 10^{-7}$

pH > 7: Basic: $[H+] > 10^{-7}$

pH < 7: Acidic: $[H+] < 10^{-7}$

A pH meter is an instrument used to measure the pH of a liquid.

Components include:

Probe (glass electrode)

Meter (measures and displays pH)

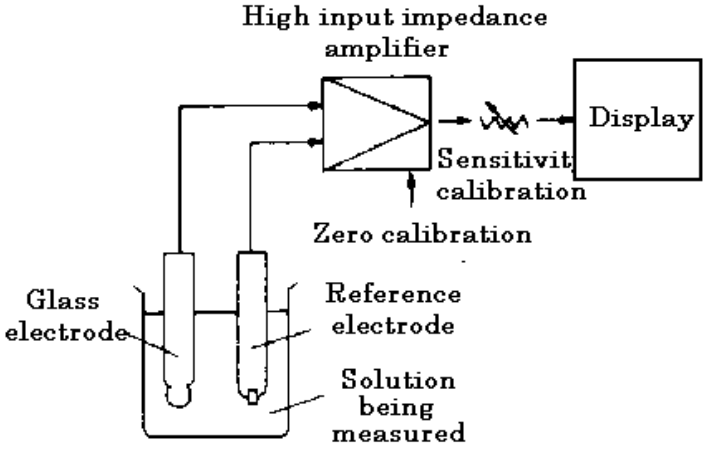
How pH is Measured

- When two solutions with different pH values exist inside and outside a glass membrane, an electromotive force is proportional to the difference between the two pH values. The solution inside the glass membrane has a pH value of 7. The pH value of the solution outside the membrane can be obtained by measuring the electromotive force generated in the membrane.

pH Meter

- The pH meter consists of a glass electrode and a reference electrode. It allows the pH value of the sample to be obtained by measuring the potential difference between the two electrodes with a potential difference meter.

- To calibrate the pH meter, a standard solution with a known pH value is used. As standard solutions, phthalic acid (pH 4.01), neutral phosphate (pH 6.86), and borate (pH 9.18) are mainly used.



Preparing solutions

- Concentration is the ratio of the mass (or volume) of a solute to the mass (or volume) of the solution (or solvent)
  - Mass/volume (%w/v)
  - Volume/volume (%v/v)
  - Molar volume (mol/L or M)

Sample Calculation

Ex. Prepare 1 L of 1.5 M Tris pH 7.5

1. Determine total number of moles of Tris required.
   1 L X 1.5 mol/L = 1.5 mol
2. Tris (MW = 121.1 g/mol)

   1.5 mol X 121.1 g/mol = 181.65 g Tris
3. Dissolve 181.65 g Tris in 700 mL dH_20 (using beaker)
4. Adjust pH to 7.5
5. Transfer to 1 L graduated cylinder. Bring to final volume with dH_20.

Material Safety Data Sheet (MSDS)

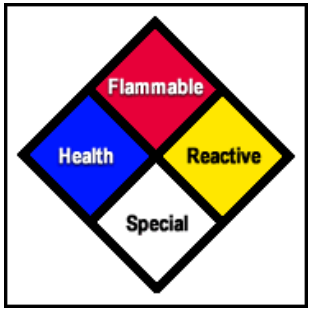
- Detailed information on
  - Physical and chemical hazards
  - Handling procedures
  - Emergency response procedures
- There must be a MSDS for every chemical used and stored in a laboratory
- MSDS for all chemicals must be read and understood before starting a procedure

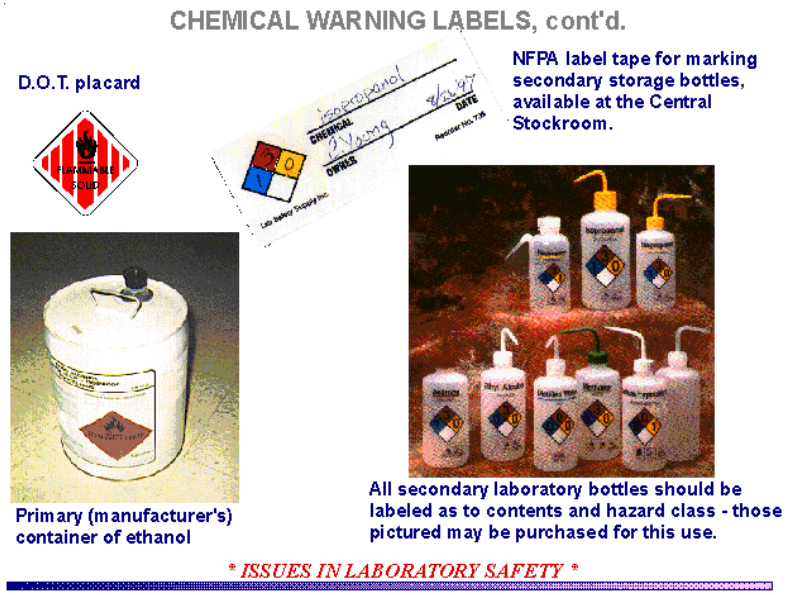
Labels

- Your first line of defense are container labels

Chemical manufacturers post physical and health hazards on container labels

Labels should display this universal biohazard symbol.



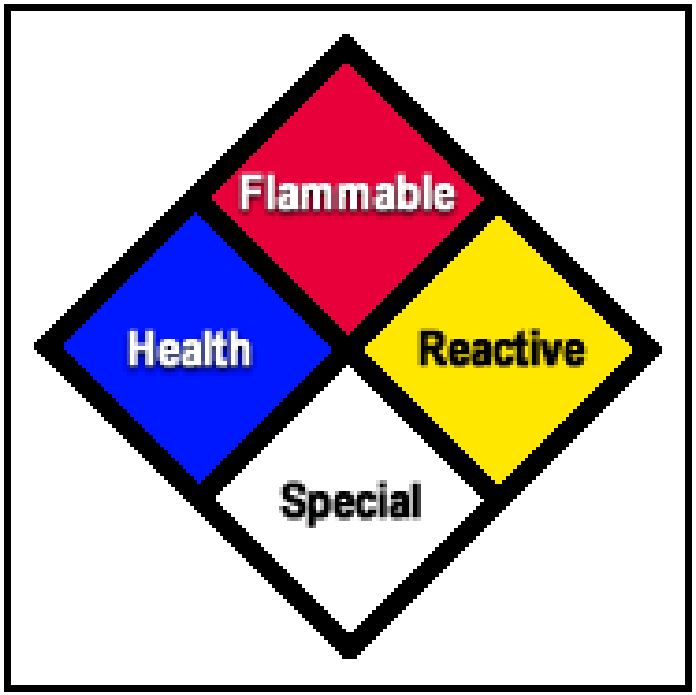


National Fire Protection Association - NFPA CODE

http://www.orcbs.msu.edu/chemical/nfpa/nfpa.html

- **Blue = Health**
- **Red = Flammable**
- **Yellow = Reactive**

White = Special Hazard
Ex : ACID, NO WATER



Scale = 0 – 4

Least Intense to Most Intense

LABELING… Biohazards

What is a Biohazardous material?

- Biological in nature
- Capable of producing harmful effects on other biological organisms, particularly humans

Examples:

Certain bacteria, fungi, viruses, parasites, recombinant products, allergens, cultured human or animal cells and their potentially infectious agents