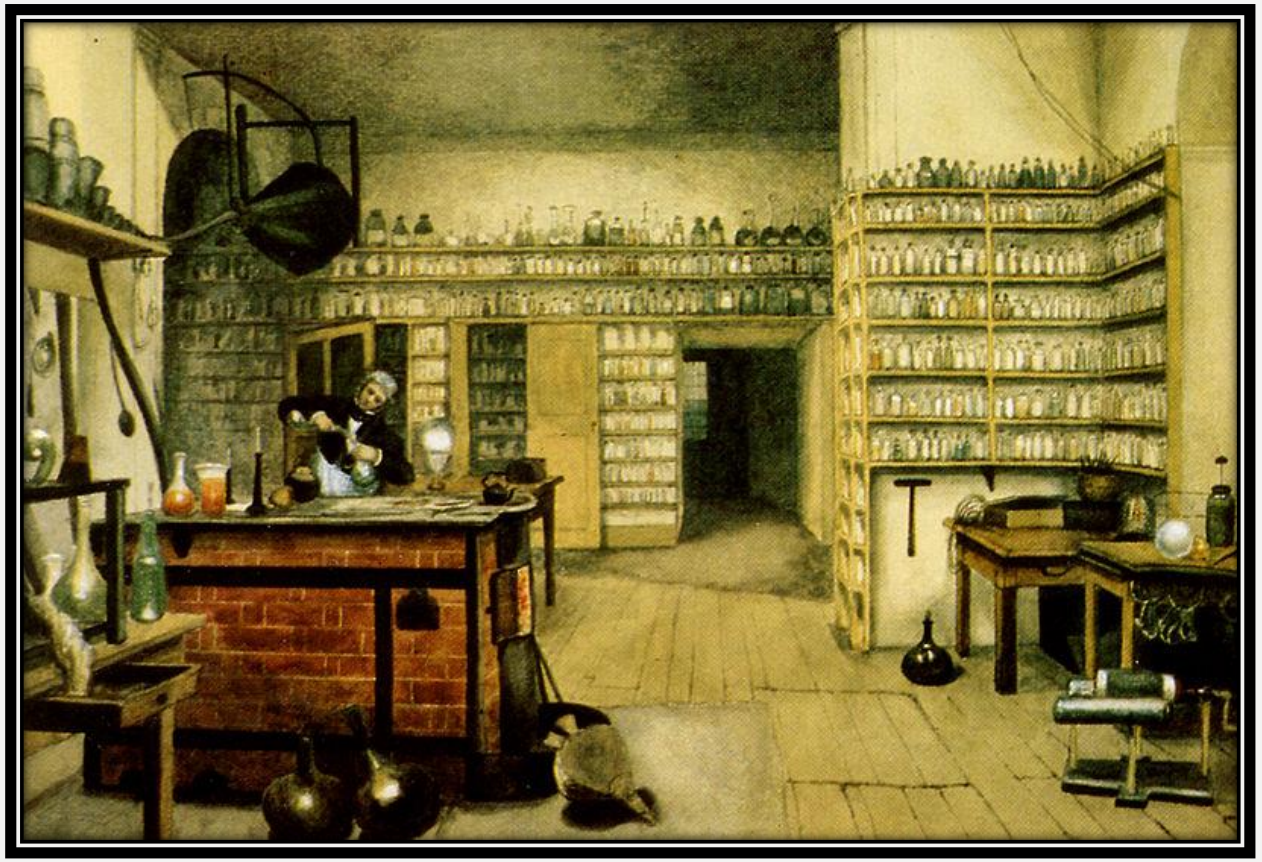
LAB BASICS

RYAN COLLISON, MLS (ASCP)MLS

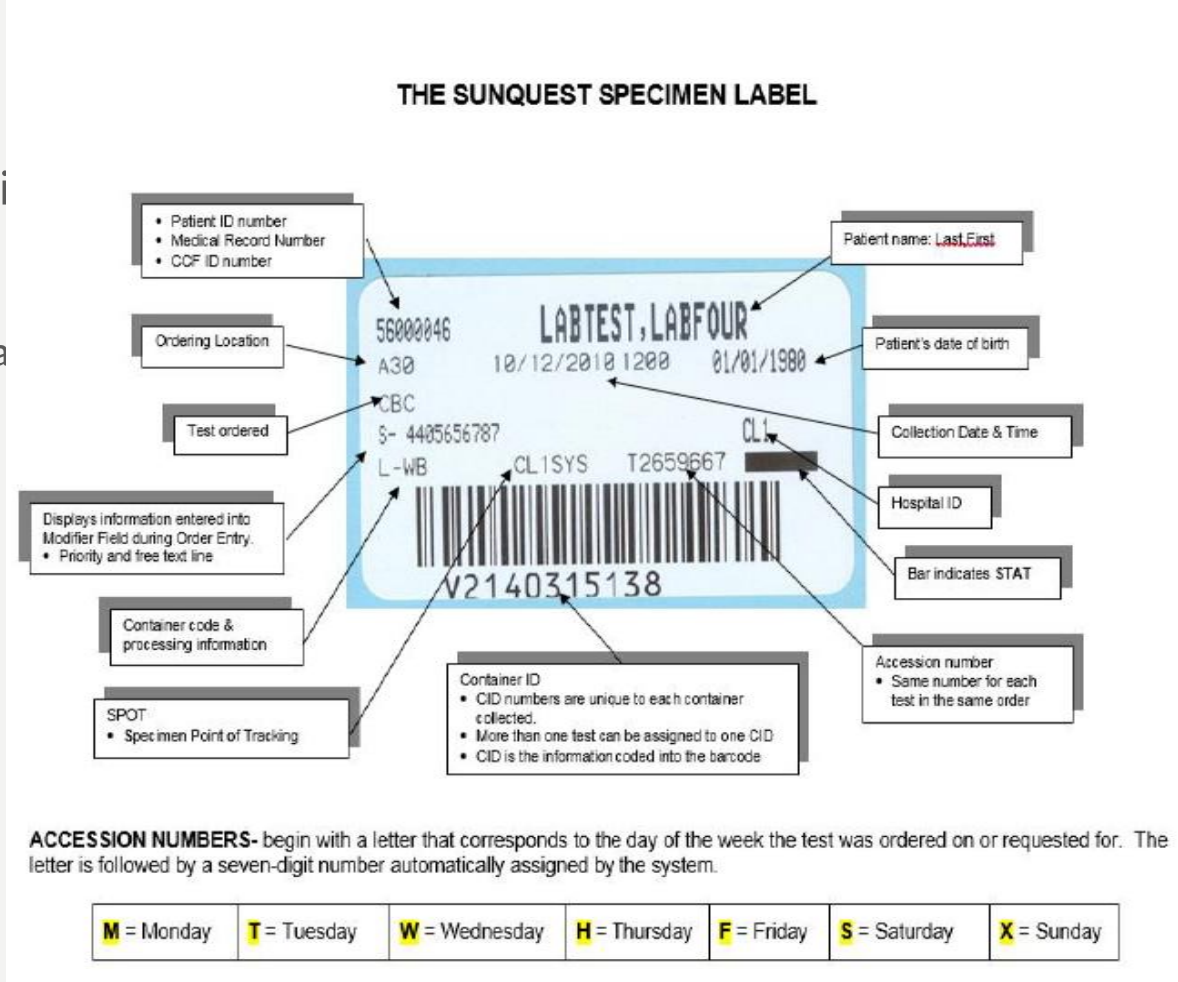
TOPICS

- Specimen Processing
- Laboratory Supplies
- Pipettes
- Reagents



SPECIMEN IDENTIFICATION

- Throughout process specimen labeling is vi
  - 2 unique IDs
    - First and last name, bird date, MRN, Conta
- Making aliquots
  - Secondary container is clean
  - Pipettes clean
  - Good technique
  - Transfer one specimen at a time
  - Visual signals (physically moving tube etc)



SPECIMEN CONTAINERS

- Phlebotomy tubes
  - Whole blood
    - Anticoagulated, kept homogenous or re-mixed
  - Plasma
    - Anticoagulated and spun in centrifuge
    - Citrate, heparin, EDTA, K-oxalate
  - Serum
    - Whole blood clots, spun in centrifuge
- Functional additives
  - NaF, preservatives

Citrate		Sodium citrate, plastic
		Sodium citrate & CTAD, glass
		Sodium citrate, ESR, glass
		ACD
Serum		Serum with clot activator (silica particles)
		Serum thrombin
		BD RST (serum with gel)
		BD SST™ II *Advance* (serum with gel)
Heparin		Lithium & sodium heparin
		BD PST™ II (plasma with gel)
Haematology		EDTA
Crossmatch		Blood banking
PPT		BD PPT™ EDTA with gel
Trace elements		Trace elements with EDTA
		Trace elements serum with clot activator (silica particles)
Glucose		Glucose

SPECIMEN PROCESSING

- Centrifugation
  - Separate particles from solution
  - Concentrate cellular components
  - Enhance Ag-Ab reactions
  - Precipitate protein components
  - Separate liquid phases based on density

CENTRIFUGATION

- Rotor
  - Holds specimen
- Motor
  - Speed regulated by voltage input
  - Tachometer measures, adjusts speed
- Drive shaft
  - Connects motor and rotor
- Timer, Brake
  - Reaches end, slows down rotor

CENTRIFUGATION

- Centrifugal Forces
  - Speed of centrifuge (RPM) does not tell us force
    - Relative Centrifugal Force (RCF) $\mathrm{RCF} = 1.12\ \mathrm{r} \times \left(\frac{RPM}{1000}\right)^2$
      - Radius in mm
        - Smaller radius at same sp
      - Resulting unit is x g (multiples of g-force)
  - Resulting force is high, centrifuge MUST be well-balanced

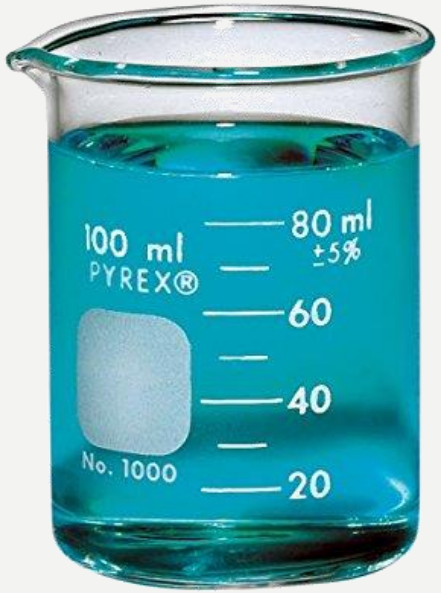
LABORATORY SUPPLIES

- Glassware
  - Borosilicate
  - Aluminosilicate
- Beaker
- Flask
  - Erlenmeyer
  - Volumetric
- Graduated Cylinder

- Pipettes
- Burets
- Plastic
  - The new glass!

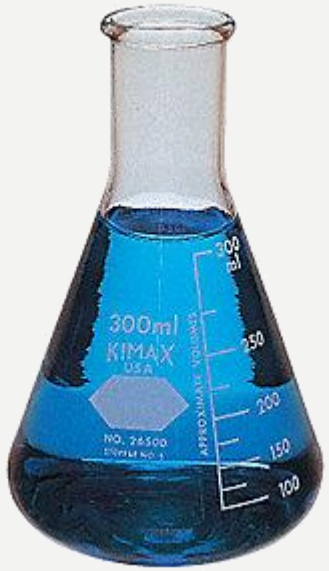
LABORATORY SUPPLIES
HOW TO USE: BEAKERS

- Holding bulk volume of solutions
  - If using small volumes consider using a medicine cup
- Waste containers
- All glassware can be labeled with sharpie
  - Alcohol prep pads clean off sharpie
- Not used for measurement



LABORATORY SUPPLIES
HOW TO USE: FLASKS

- Erlenmeyer
  - Use similar to beakers
  - If sealed by parafilm may mix by swirling
  - Not used for measurement



- Volumetric
  - Holds a single exact volume
  - One calibration mark and tolerance listed
  - Application? i.e. make a 10 g/L NaCl
    - In 1L flask put 10 g NaCl
    - Add almost 1 L of water, say 950 mL
    - Add rest of water dropwise with transfer pipette to meniscus
    - Insert stopper, invert and swirl to mix

LABORATORY SUPPLIES
HOW TO USE: GRADUATED CYLINDERS

- Use class A for measurements
  - These are very inexact
  - Convenient for pouring
  - Filling 50 mL volumetric flask? Pour 45 mL using graduated cylinder
- Short answer?
  - Don't use for important things

PIPETTES

- Class A
  - NIST standards
  - Calibrated
  - CAP requires using calibrated pipettes
- TC versus TD
  - TD: What you think of when you think pipette
  - TC: Requires washing into solution

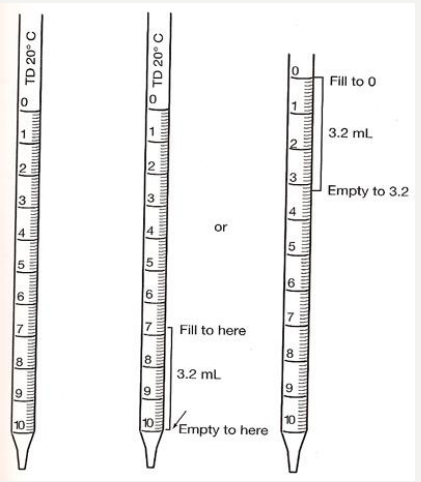
PIPETTES

- Transfer- transfers single known volume
  - Volumetric pipettes
  - Ostwald-Folin ||
- Measuring- labeled in increments
  - Serological ||
  - Mohr
- Micropipette
  - Adjustable or fixed volumes ≤1 mL

Volumetric

Serological

Mohr



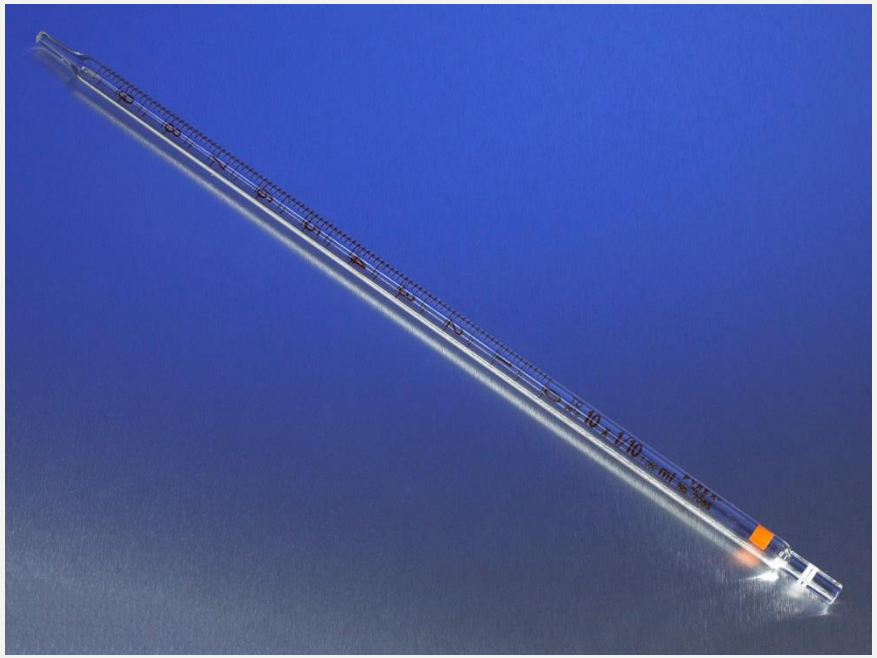
Ostwald-Folin

VOLUMETRIC PIPETTE

- Elongated bulb
- Tapered Tip
- Single Calibration mark
  - Dispenses ONLY the volume stated
- Self draining
- Most precise & accurate pipette
  - Suitable for calibrators

SEROLOGICAL PIPETTE

- Uniform width
- Wider tip
- Measuring pipette
  - Calibrated through the tip
  - Can dispense any volume for which a graduation exists
- Usually has etched ring (or 2) at top of pipette
  - blowout



MOHR PIPETTE

- Similar appearance to serological
- NOTE GRADATIONS
  - Stopping before tip
- Never blowout, never drain to tip
- Why Mohr?

OSTWALD-FOLIN PIPETTE

- Appearance of volumetric pipette
  - Bulb closer to tip
- Frosted rings
  - Blowout
- Why?

PIPETTING PROCEDURE

1. Inspect pipette
    1. Chips, cracks, dirt
2. Using bulb draw liquid up past calibration mark
3. Wipe pipette with kimwipe
4. Holding vertically: allow liquid to drain down to calibration mark (QS'ing)
    1. Place finger to hold meniscus on calibration mark
    2. Move to receiving vessel
5. Allow to drain unrestricted to bottom – IF draining to another calibration mark, stop here
6. Touch tip to side OR blowout depending on pipette

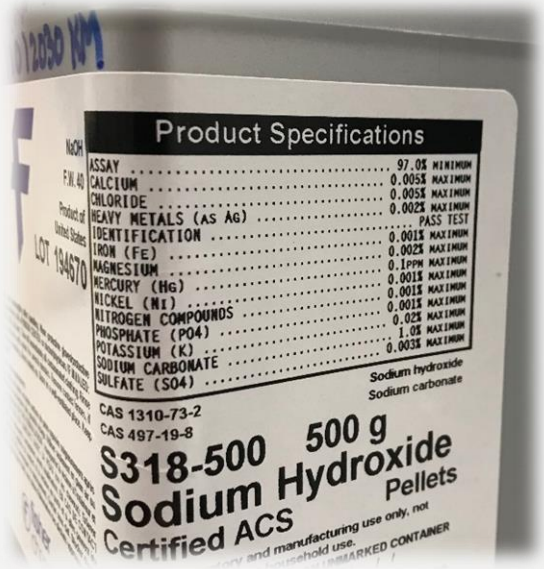
PIPETTING RULES

- Do Not Mouth Pipette
  - Why do we still have to say this? I don't know
- Always check the pipette for chips/cracks/dirt
- Lay pipettes on clean surface
- Make sure there are no bubbles or beads of liquid when using
- Place in pipette soak tip down
- NEVER out of a primary container
  - Use a labeled pourover

AUTOMATIC PIPETTE

- 2 flavors- adjustable and fixed volume
  - Adjustable should be used at top of adjustable range if possible
- Holding pipette vertically depress plunger to first stop
- Place tip in liquid
- Slowly release plunger to top
- Raise pipette, inspect tip for bubbles, wipe outside of tip (DO NOT BLOT ORIFICE)
- In receiving container touch tip to side, depress plunger steadily
  - If second stop, hesitate then depress to second stop.
- Remove pipette, eject tip, release plunger

REAGENTS



- Purity of Inorganics
  - Ultrapure- chromatography, atomic absorption, molecular diagnostics
  - Analytical reagent- Amer. Chem. Soc. Definition actual impurities are listed with %
  - USP/NF- For pharmaceutical use, it won't kill anyone but we don't know purity
  - Chemically pure, pure grade- starting point for manufacture, do not use
- Purity of organics
  - Chromatographic grade- Depends on refinement level
  - Reagent grade- certified to have < % of contaminants
  - Spectroscopic- specroscopically pure
  - CP- almost reagent grade

HANDLING REAGENTS

- Store properly
  - RT = 21°C
- Never sample directly from bottle
  - Pour into cup, beaker, or boat
  - Dispose of according to SDS
  - Expiration dates MUST be honored

WATER PURITY

- Water Water Everywhere, but a not a drop for quantitative clinical chemistry (amiright?)
  - Ultrapure- used for HPLC, pharmaceuticals
  - Type I- Chemistry procedures, cell culture, electrophoresis, reagent/control prep, final rinse of glassware
  - Type II- qualitative chemistry, most heme micro and immunology procedures
  - Type III- used to make Type I, II water, used in first rinses of glassware
- Purity mainly assessed using conductivity