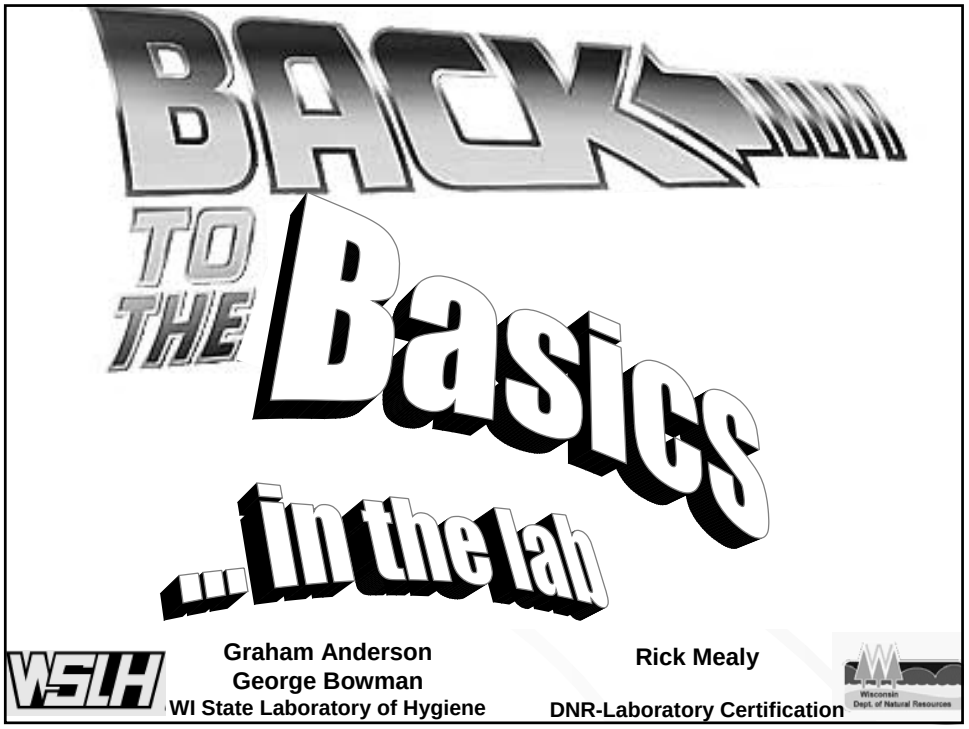


Graham Anderson
George Bowman
WI State Laboratory of Hygiene

Rick Mealy
DNR-Laboratory Certification

Any reference to product or company names does not constitute endorsement by the Wisconsin State Laboratory of Hygiene, the University of Wisconsin, or the Department of Natural Resources.

Disclaimer

General Colin Powell
Chairman (Ret), Joint Chiefs of Staff

LESSON 4

A Leadership Primer

"Don't be afraid to challenge the pros, even in their own backyard."

Learn from the pros, observe them, seek them out as mentors and partners.
But remember that even the pros may have leveled out in terms of their learning and skills.
Sometimes even the pros can become complacent and lazy.
Leadership does not emerge from blind obedience to anyone.
Xerox's Barry Rand was right on target when he warned his people that if you have a yes-man working for you, one of you is redundant.
Good leadership encourages everyone's evolution.

Preparing standards,
verifying analytical balance performance, and
pipetting
are fundamental lab techniques that are often assumed or simply overlooked.

Methods often "cookbook" how to prepare standards in very general terms but rarely provide the "hows" and "whys".
Methods also frequently ignore when is it appropriate to use volumetric pipettes, air-displacement pipettes or large-bore serological pipettes.

Objectives

Glassware Generalisms

Solution Solutions - Preparing Standards Perfectly

Proper Pipetting Principles

Balance Basics

Some Excellent Internet Resources

High School level pipetting
http://www.woodrow.org/teachers/esi/2002/Biology/Projects/lab_skills/ls7/
good close-up of micropipet tip when drawing and dispensing sample
http://www.rainin.com/pdf/edp_plus_manual.pdf

"Oliver's Demos"
http://www.csudh.edu/oliver/demos/pipetuse/pipetuse.htm
http://www.csudh.edu/oliver/demos/bal-use/bal-use.htm

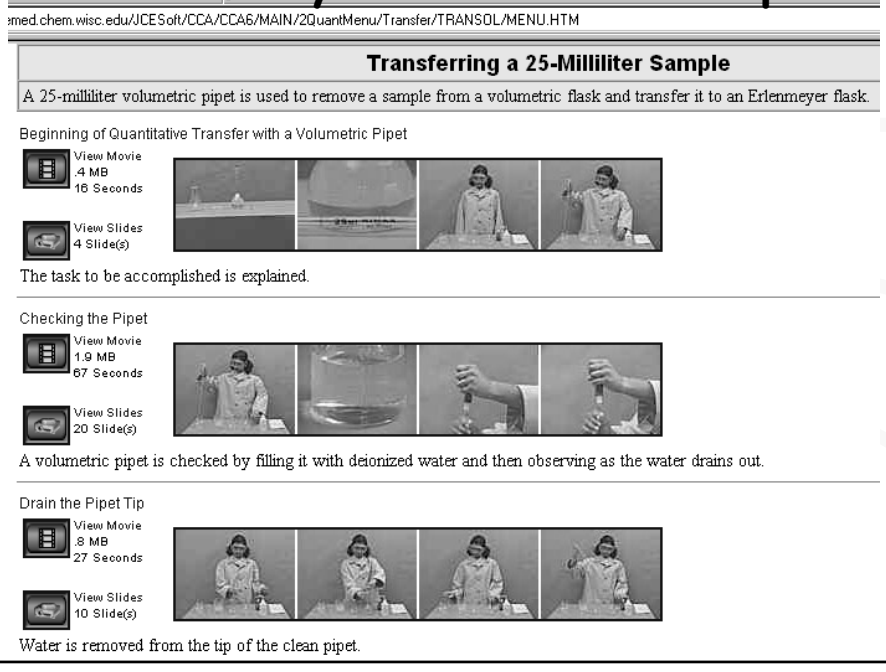
http://acpcommunity.acp.edu/Facultystaff/genchem/GC1/lab/mvolume/piptech.htm

http://chemscape.santafe.cc.fl.us/chemscape/catofp/measurea/volume/pipet/pipet4.htm

http://www.kimble-kontes.com/pdfs/class_a_b_tolerances.pdf
http://www.kimble-kontes.com/pdfs/reading_the_meniscus.pdf
http://www.kimble-kontes.com/pdfs/to_contain_to_deliver.pdf
http://www.kimble-kontes.com/html/FAQ.html
http://www.kimble-kontes.com/html/RelatedLinks.html

***** "Science By (Mr.) Jones"
http://www.sciencebyjones.com
***** "Chemistry Comes Alive!" (alphabetical topic search)
http://jchemed.chem.wisc.edu/JCESoft/CCA/CCA6/MAIN/WORDS/WORDS16.HTM

"Chemistry Comes Alive" sample



"Chemistry Comes Alive" sample

Rinse the Pipet with Solution

View Movie
1.7 MB
61 Seconds

View Slides
18 Slide(s)

The pipet is rinsed with the solution that is to be measured.

Fill the Pipet

View Movie
2 MB
70 Seconds

View Slides
17 Slide(s)

A pipet bulb is used to fill the pipet with the solution to be measured, and then the volume is adjusted to the mark using a finger to control the level of solution.

Transfer the Liquid

View Movie
.8 MB
29 Seconds

View Slides
7 Slide(s)

The liquid is transferred to an Erlenmeyer flask by allowing it to drain naturally from the pipet.

Rinse the Last Drop

View Movie
1.3 MB
44 Seconds

View Slides
10 Slide(s)

The final drop of solution delivered by the pipet is rinsed into the flask.

Glassware Generalisms

TC glassware

- TC = To Contain
  - entire contents = the correct volume
  - transferring the contents to another container requires a quantitative transfer.
  - Example: Volumetric flask

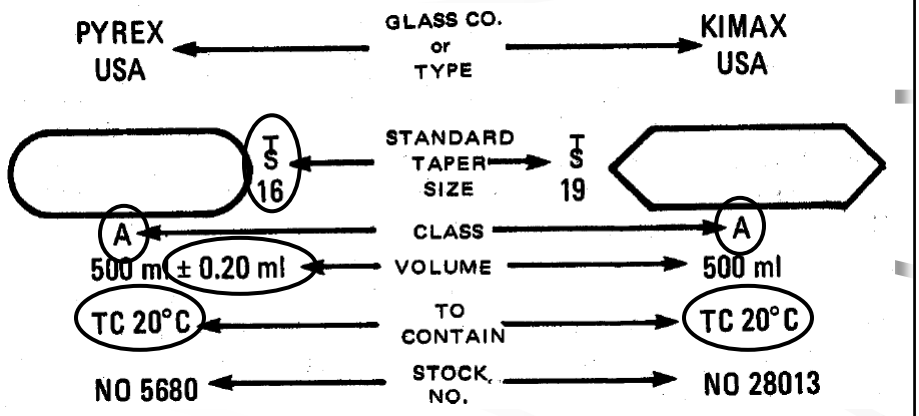
TD glassware

- **TD = To Deliver**
- ***Generally* DO NOT blow out the small amount remaining in the tip**
- **A flowtime must be observed.**
  - Allows all of the water film on the inside of the pipet to drain off, so you get the full accuracy the pipet is capable of.
  - Really accurate pipets, like class A, designed to drain so slowly that the film draining keeps up with the bulk draining.

Flowtimes for TD pipets

Nominal Volume mL	Class A Flowtime (sec)	Class B Flowtime (sec)
1	10	3
10	15	8
25	25	15
50	25	15
100	30	30

Glassware labeling



T = Standard Taper. Finished to a 1:10 taper. A single number following indicates the approximate diameter (mm)of the neck.

P = Product Standard. Used for stopcocks with Teflon plugs. Finished to a 1:5 taper. A single number following indicates the diameter (mm)of the hole in the plug.

Glassware accuracy

Volumetric Flask Tolerances (mL)

Nominal Volume mL	Class A tolerance	Class B tolerance
10	± 0.02	± 0.04
25	± 0.03	± 0.06
50	± 0.05	± 0.10
100	± 0.08	± 0.16
250	± 0.12	± 0.24
500	± 0.20	± 0.40
1000	± 0.30	± 0.60
2000	± 0.50	± 1.00

Pipet Tolerances (mL)

Nominal Volume mL	Class A tolerance	Class B tolerance
1	± 0.006	± 0.012
5	± 0.01	± 0.02
10	± 0.02	± 0.04
25	± 0.03	± 0.06
50	± 0.05	± 0.10
100	± 0.08	± 0.16

Graduated Cylinder Tolerances (mL)

Nominal Volume mL	Class A tolerance	Class B tolerance
10	± 0.08	± 0.1
25	± 0.14	± 0.3
50	± 0.20	± 0.4
100	± 0.35	± 0.6
250	± 0.65	± 1.4
500	± 1.10	± 2.6
1000	± 2.00	± 5.0
2000	----	± 10.0

Pipet Types

Volumetric
No graduated volume scale

Measuring
graduated volume scale

Glass

Mechanical

Mohr
calibrated volume stops prior to the pipet tip

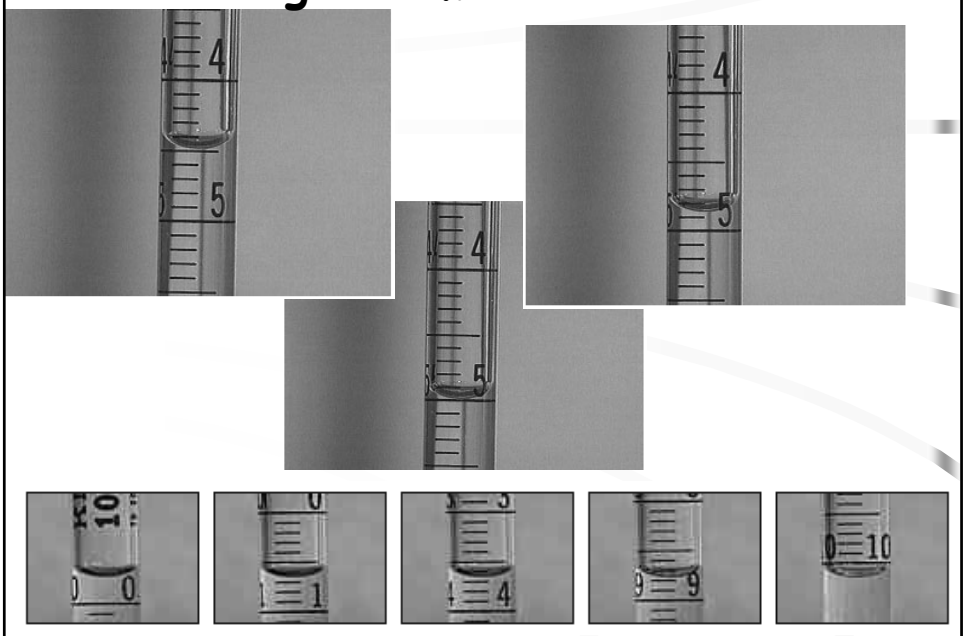
- **Class "A"**
- **Class "B"**

Serological
calibrated volume includes the pipet tip

- Blown Out

Class "B" only

Reading Volumetric Glassware



Pictures courtesy of : http://www.cbu.edu/~mcondren/c214/pipet/pipet4.htm#preparing

What to use…and when

Glassware	Proper Use
Disposable beakers	For pouring out a small volume of standard to warm up and use.
Glass beakers or Erlenmeyer flasks	Digestions. NOT for standard preparation or anything requiring volumetric measurements.
Graduated Cylinders	BOD & TSS when using 100 mLs or more of sample
Micro-bore Volumetric pipets (glass or mechanical)	Standard preparation
Mohr pipets	Color reagent (phosphorus)- if using the NCL modification
Serological pipets (wide-bore)	BOD influents (low volume samples) also preservation of samples for phosphorus or ammonia
Wide bore volumetric pipets	BOD & TSS samples

$$C_1V_1 = C_2V_2$$

(Concentration of existing solution)

x

(Volume of existing solution)

=

(desired concentration of new solution)

x

(desired volume of new solution)

Solution Preparation Example 1

You have the following stock standards of phosphorus available:

50 ppm

5.0 ppm

You need to prepare: 50 mLs of a a 0.1 ppm standard

C_1: Concentration of existing solution × V_1: Volume of existing solution

50 ug/mL (or 5) × V_1: unknown

@

C_2 : Concentration of new solution × V_2 : Volume of new solution

0.1 (ug/mL) × 50 mL (need at least 50 mLs for a std)

$50V_1 = 50 \times 0.1$

$\frac{50V_1}{50} = \frac{5.0}{50}$

$V_1 = 0.1$ mLs

$5V_1 = 50 \times 0.1$

$\frac{5V_1}{5} = \frac{5.0}{5}$

$V_1 = 1.0$ mLs

Thus, 0.1 mLs of a 50 ppm standard, diluted to 50 mLs = 0.1 ppm

Standard Preparation Dilemma

50 mLs final volume

	0						
50. ug/mL	0	0.1	0.2	0.4	0.6	0.75	1.0 mL
25. ug/mL	0	0.2	0.4	0.8	1.2	1.5	2.0
5.0 ug/mL	0	1	2	4	6	7.5	10

0
0.1
0.2
0.4
0.6
~~0.8~~ **0.75**
1.0
ug/mL

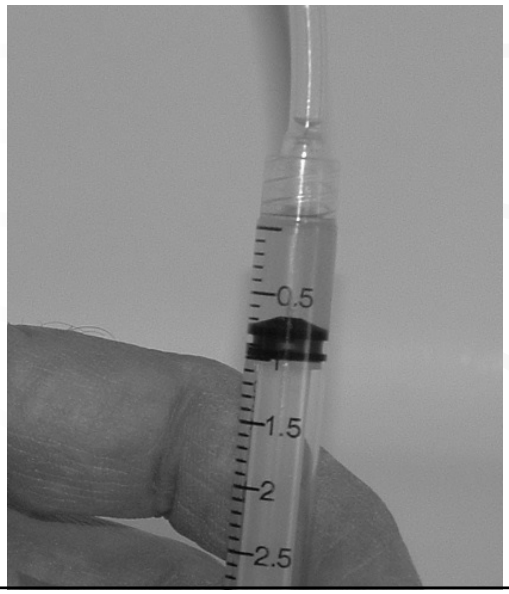
Which pipet(s) will I need?

100 mLs final volume

	0						
50. ug/mL	0	0.2	0.4	0.8	1.2	1.5	2.0 mL
25. ug/mL	0	0.4	0.8	1.6	2.4	3.0	4
5.0 ug/mL	0	2	4	8	12	15	20

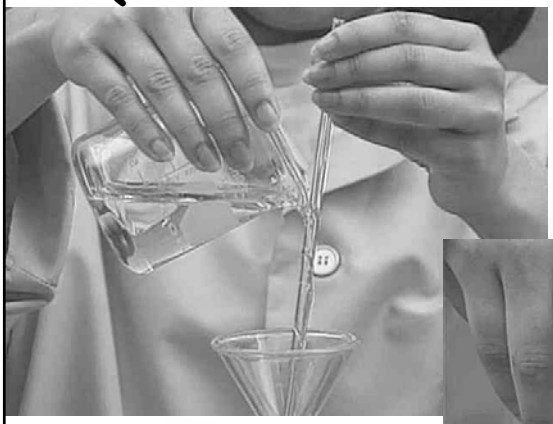
Volume change of a cold solution-1

Volume change of a cold solution-2

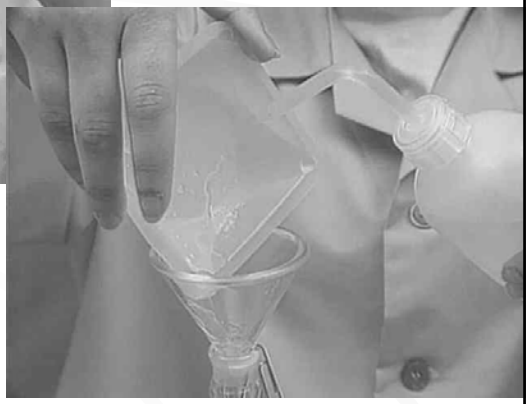


<u>25 +0.8 mLs</u> = + 3.2%
25 mL flask

Quantitative Transfer Techniques

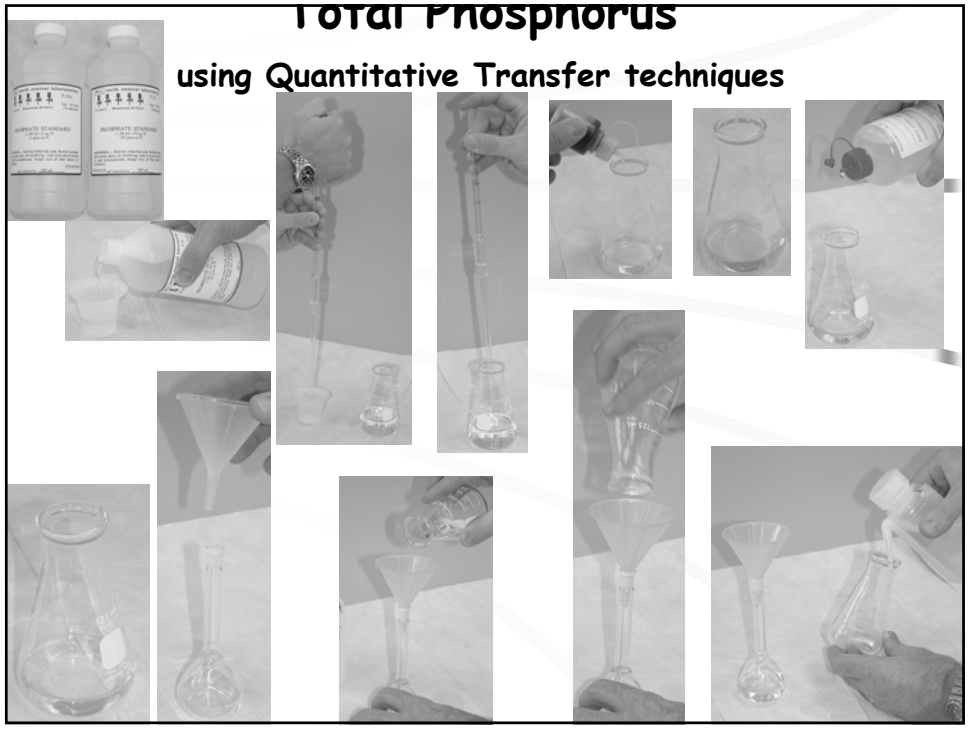


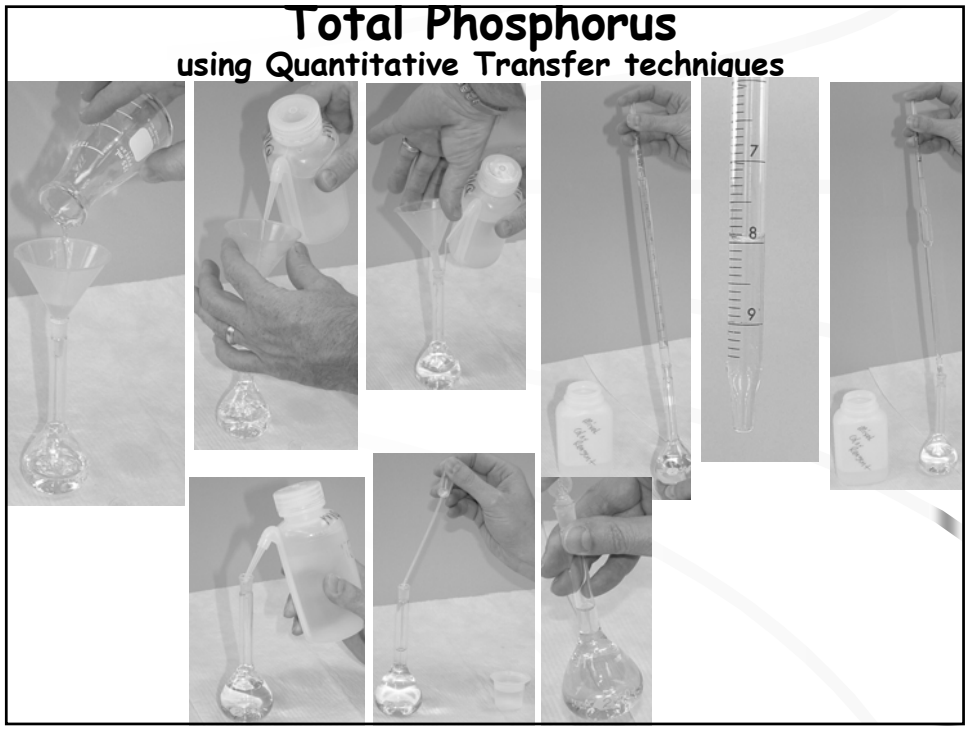
Be careful about the force of the stream…you do not want to cause particles to "fly" off the weighing dish
↓



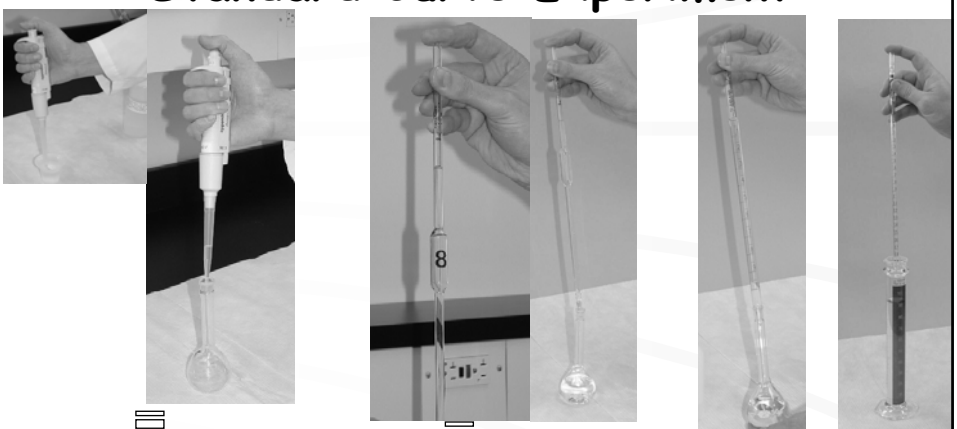
↑ Probably not a good technique for phosphorus due to potential for contamination…but a good general technique

Pictures courtesy of : http://jchemed.chem.wisc.edu/JCESoft/CCA/CCA6/MAIN/1ChemLabMenu/Quantitative Transfer/


Total Phosphorus
using Quantitative Transfer techniques


Total Phosphorus
using Quantitative Transfer techniques

Standard Curve Experiment



Volumetric flask

Volumetric flask

Volumetric flask
+
Graduated Cylinder

Standard Curve Preparation Approaches

Standard mg/L P	Abs	Abs	Abs	Abs
0	-0.001	0.001	0.001	0.007
0.1	0.066	0.065	0.061	0.073
0.2	0.132	0.129	0.114	0.135
0.4	0.262	0.263	0.226	0.279
0.5	0.328	0.329	0.282	0.354
0.75	0.488	0.492	0.423	0.516
1	0.652	0.652	0.55	0.687

ADP/VF	VP/VF	MP/VF	MP/GC
0.6515	0.6534	0.5508	0.6834
0.0007	0.0005	0.0046	0.0050
0.99999	0.99998	0.99984	0.99985

Air-displacement Pipet & 100 mL Volumetric Flasks

Volumetric Pipets with 100 mL Volumetric Flasks

Mohr Pipets with 100 mL Volumetric Flasks

Mohr Pipets with 100 mL graduated cylinders

Standard Curve Preparation Approaches

Air-displacement Pipet & 100 mL Volumetric Flasks

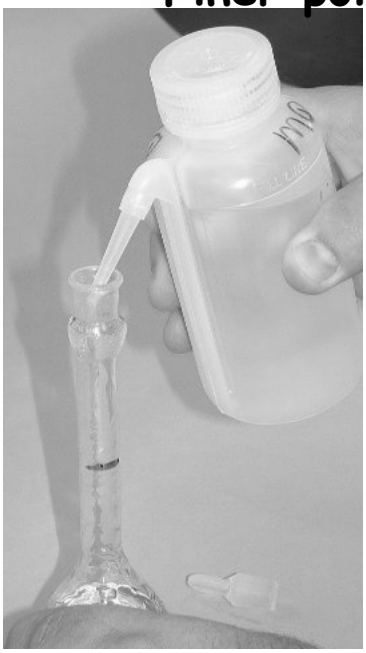
Volumetric Pipets with 100 mL Volumetric Flasks

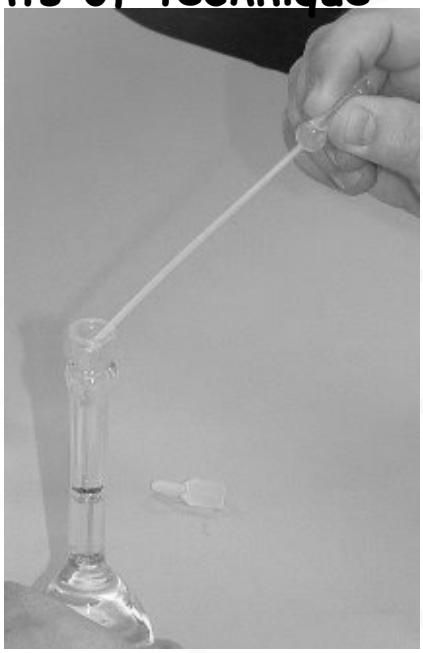
Mohr Pipets with 100 mL Volumetric Flasks

Mohr Pipets with 100 mL graduated cylinders

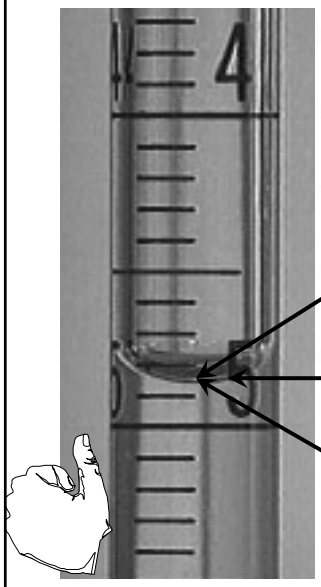
Standard mg/L P	RF	RF	RF	RF
0				
0.1	0.660	0.650	0.610	0.730
0.2	0.660	0.645	0.570	0.675
0.4	0.655	0.658	0.565	0.698
0.5	0.656	0.658	0.564	0.708
0.75	0.651	0.656	0.564	0.688
1	0.652	0.652	0.550	0.687
Range	0.651 - 0.660	0.645 - 0.658	0.550 - 0.610	0.675 - 0.730
mean RF	0.656	0.653	0.571	0.698
stdev	0.003912	0.005064	0.020472	0.019356
%RSD	0.60%	0.78%	3.59%	2.77%

Finer points of technique



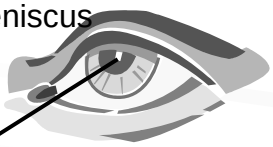


Parallax error...

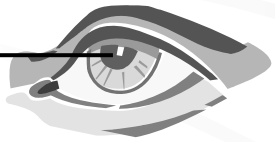


…is a concern whenever reading a meniscus or meter

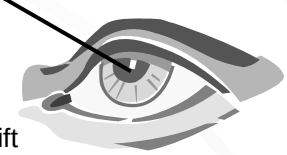
The eye must be perpendicular to the meniscus



Parallax error introduced



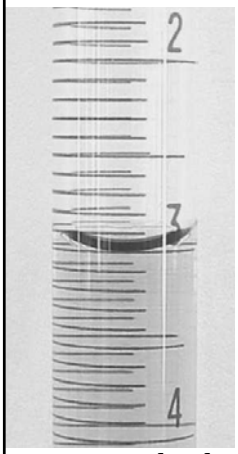
Accurate Reading



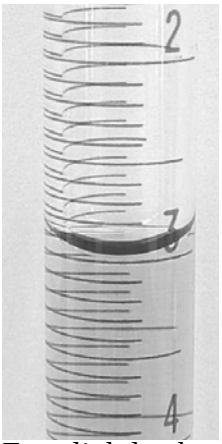
Parallax error introduced

The closer the object is to your eye, the greater the apparent shift

Parallax error…up close

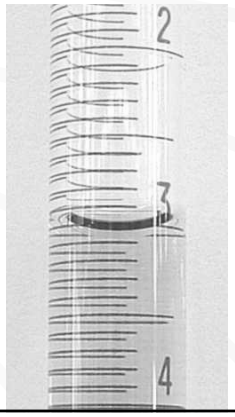


Eye perpendicular

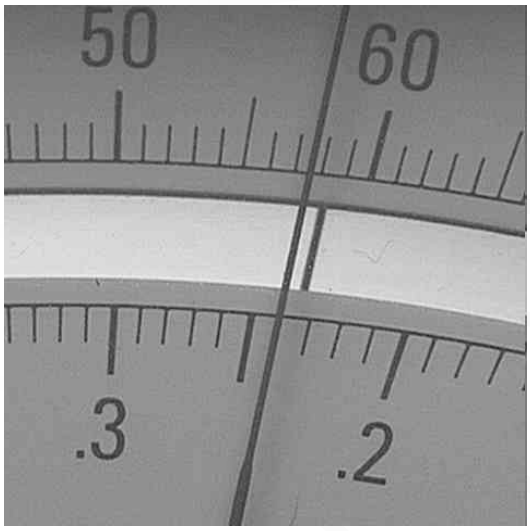


Eye slightly above

Eye slightly below



Parallax error and meters



Pictures courtesy of : http://jchemed.chem.wisc.edu/JCESoft/CCA/CCA6/MAIN/1ChemLabMenu/Measuring/Spectroscopy/spec20_menu/spec20_X_06362610/PICTURE.HTM?3

Value of disposable "beakers"

…a slight added cost to the laboratory, but an excellent way to minimize contamination and the time it takes to come to room temperature

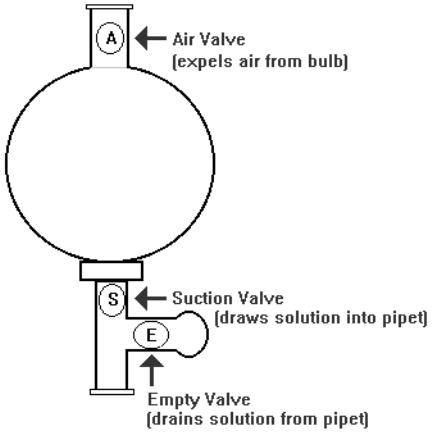
Solution Preparation Summary

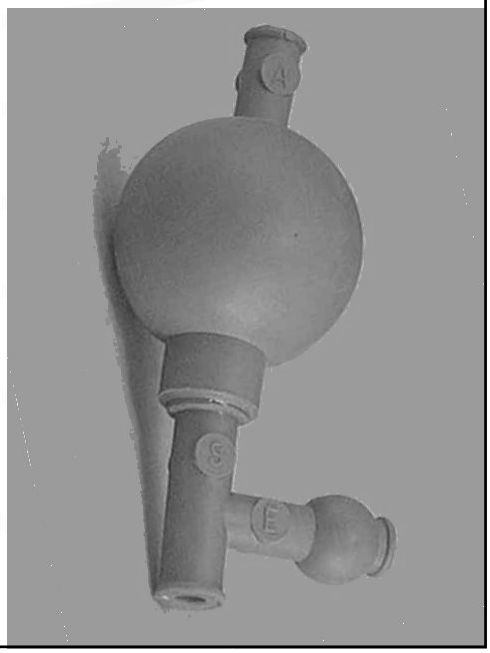
- What equipment / standards do you have to accomplish the task?
- C1V1 = C2V2
- Bring standards/reagents to room temp. before use
- Never pipet from the reagent/standard bottle
- Ensure standards & reagents are properly labeled and linked to un-expired standards
- Read the meniscus properly
- Errors are additive

Using a conventional pipet bulb

Video courtesy of
*ht*tp://jchemed.chem.wisc.edu/JCESoft/CCA/CCA6/MAIN/1ChemLabMenu/*Measuring/Volume/Pipet/PipetBulb/bulbstd_menu/MENU.HTM*

Using a 3-Way bulb



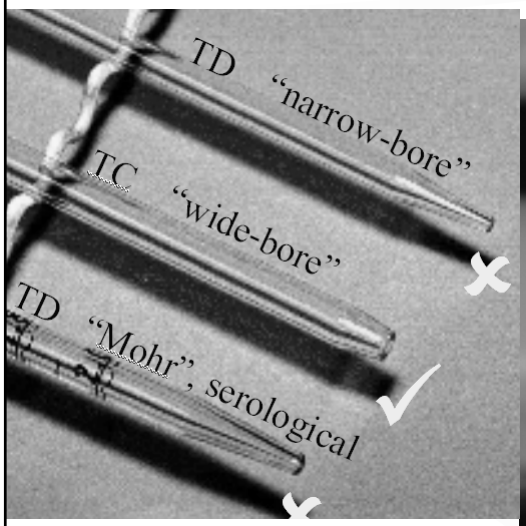


Using a 3-Way bulb

Video courtesy of
*ht*tp://jchemed.chem.wisc.edu/JCESoft/CCA/CCA6/MAIN/1ChemLabMenu/Manipulating/TransferringSamples/Quantitative/TransferringLiquid/mohr_menu/mohr_X_PIPTHREEWAY/THUMBS.HTM

Bulb Alternatives

Widebore pipets



Proper use of volumetric glass pipets

Key points:

- Use class A pipets to prepare standards
- Volumetric pipets are calibrated to deliver (designated -TD) a specific volume
- The inside and outside of the pipet tip must be dry or rinsed with the solution to be transferred before use

Use of a volumetric pipet

- Evacuate the pipet bulb by squeezing.
- Immerse the tip of the pipet into solution to be delivered
- Seat the bulb opening over the top opening of the pipet
- Hold the bulb in place while slowly releasing the squeezing pressure
- Continue to release the pressure while te solution is drawn into the pipet
- Draw the solution up well past the calibration line
- Curse when you go too far and draw the solution up into the bulb
- Quickly remove the bulb and seal the top of the pipet with the index finger
- Keeping the index finer in place, remove the tip from the solution
- Rest the tip of the pipet on the side of the container that held the solution.
- Slowly release finger; allow solution level (meniscus) to drop to calibration line.
- Place tip of the pipet over the receiving vessel and completely release the finger
- Keep the pipet upright and allow to drain completely (Note: Many class A pipets have the drain time imprinted adjacent to the "TD" designation.)

When the draining is complete, touch the tip of the pipet to the inside wall of the vessel and give it a half-twist

Electronic v. mechanical pipet

Glass Volumetric vs. Adjustable volume mechanical

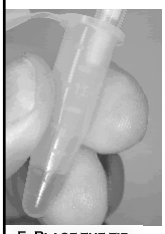
Using an autopipet

1. SELECT THE CORRECT MICROPIPETTE FOR THE JOB. NOTE THE VOLUME RANGES ON THE PLUNGER.

2. DIAL THE VOLUME.

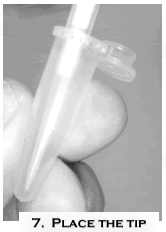
3. PLACE A TIP ON THE MICROPIPETTE WITHOUT TOUCHING THE TIP.

4. DEPRESS THE PLUNGER TO THE FIRST STOP.



5. PLACE THE TIP BELOW THE LIQUID.

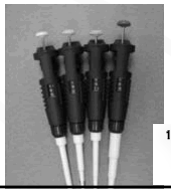
6. SLOWLY RELEASE THE PLUNGER.



7. PLACE THE TIP NEAR THE BOTTOM OF THE EMPTY RECEIVING TUBE.

8. SLOWLY DEPRESS THE PLUNGER PAST THE FIRST STOP TO THE SECOND STOP.

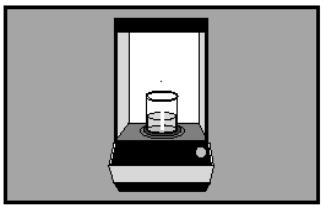
9. RELEASE THE PLUNGER AFTER THE TIP IS REMOVED FROM THE TUBE.



10. EJECT THE TIP INTO A WASTE CONTAINER.

Courtesy of : http://www.fhcrc.org/education/hutchlab/lessons/

Checking autopipet accuracy

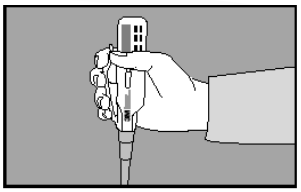


1. Place a disposable weighing dish that can hold 25-50 mLs on the balance.

Prepare a clean disposable beaker containing reagent water at 20-25°C.

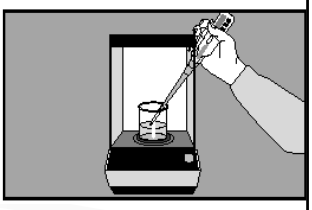
Record the water temperature.

Tare the balance to zero.



2. Aspirate a fixed volume of water from the vessel on the analytical balance. Note its weight.

Tare the balance to zero.



3. Expel the water into the vessel on the analytical balance. Note its weight.
4. Repeat steps 2-3 for a total of 10 (or 20) measurements.
5. Calculate accuracy

Material condensed from: www.3m.com/microbiology/home/products/pipettor/calib.pdf

"Fast is fine… but accuracy is everything"

Wyatt Earp

Table 1: Artel's suggested initial tolerance limits

Pipette Volume[a]	Inaccuracy[b]	Imprecision[b]
2 µL	5.0%	2.0%
10 µL	2.5%	1.5%
20 µL	2.0%	1.0%
100 µL	1.6%	0.8%
200 µL	1.6%	0.8%
1000 µL	1.6%	0.8%

(a) Volumes are for variable volume pipettes at their maximum settings and for fixed volume pipettes. For variable volume pipettes, percentage error increases at lower settings; tolerance limits should be adjusted accordingly.

(b) These inaccuracy and imprecision values are double the specifications given by some of the major pipette manufacturers. Where manufacturers have differing specifications, the higher values were chosen.

Be prepared to have auditors inspect your autopipets!

Information courtesy of : http://www.artel-usa.com/Documents/Reports/report6.htm

Pipet Summary

1. Use ONE pipet for the job (*error is additive*)
   10 mL + 10 mL may get you 20,
   but also gets you double the error

2. LESS volume is MORE chance for error

3. Use the proper pipet for the job
   Mohr vs.
   serological vs.
   volumetric

   Wide-mouth (BOD, TSS) v.
   Microbore (standards, ammonia)

4. Maintain & calibrate your autopipettors

Balance Overview

Types of Balances
Selecting the right balance for the application
Care & Maintenance
Use
- Preliminary Considerations
- Making Absolute Measurements
- Measurements and the Tare function

Select the right balance for the job

Select a balance appropriate for the application.

Accuracy Req'd	
0.01 g	Heavy objects (platinum crucibles, large flasks) - Use a toploader type balance with at least 2 decimal place (0.01 milligram) resolution and accuracy.
0.01 g	Ascorbic acid reagent for Total Phosphorous - Use a toploader type balance with at least 2 decimal place (0.01 milligram) resolution and accuracy.
0.000,0 g	TSS - Use an analytical balance with at least 4 decimal place (0.1 milligram) resolution and accuracy.
0.000,0 g	Testing electronic or air-displacement pipettors - Use an analytical balance with at least 4 decimal place (0.1 milligram) resolution and accuracy.

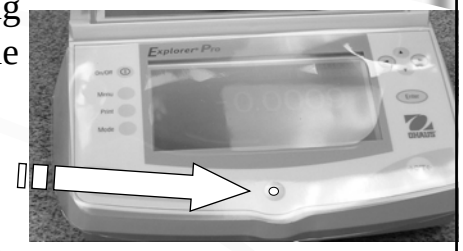
Balance Use 1

Balances are not a "Plug n' Play" device

1. If using an electronic balance, allow to warm up for at least 60 minutes.

Balance must be level to function properly

2. Check the balance leveling gauge to make sure bubble inside the target.



Balances need controlled temperature & humidity
Wide swings in humidity & temperature can damage the sensitive parts and electronics.

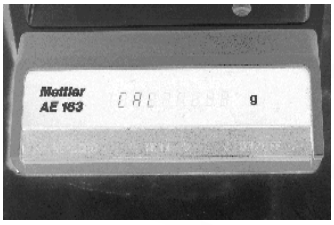
Balance Use 2

The pan and balance floor must be clean.
Use a camel-hair brush (keep near balance).

3. Dust off the balance pan with a clean soft -- *preferably camel-hair*-- brush. Use a mild detergent, DI water and lint free wipe if necessary.

Balance Use 3

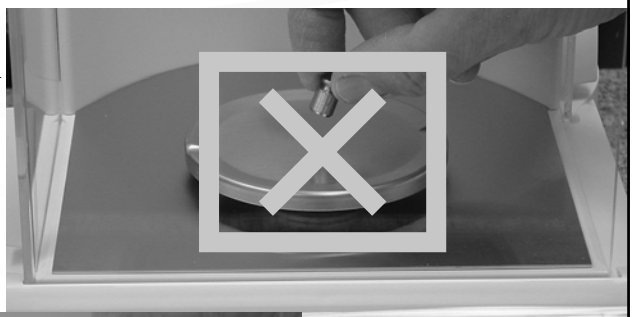
4. Perform the internal calibration process if the balance has an on-board calibration function.



5. Zero the balance by pressing the "tare" bar (or button)

Balance Use 4

6. Place the first Class 1 weight on the clean balance pan ...



... with plastic forceps...

Balance Use 5

….allow the balance to stabilize

...measure and record the observed weight in the logbook.

Balance Use 6

7. Repeat step 6 with the other appropriate weights

8. Compare the observed weights to the acceptance ranges for the Class 1 weights. If any weight exceeds the acceptable range, ***discontinue using the balance and take corrective action.***

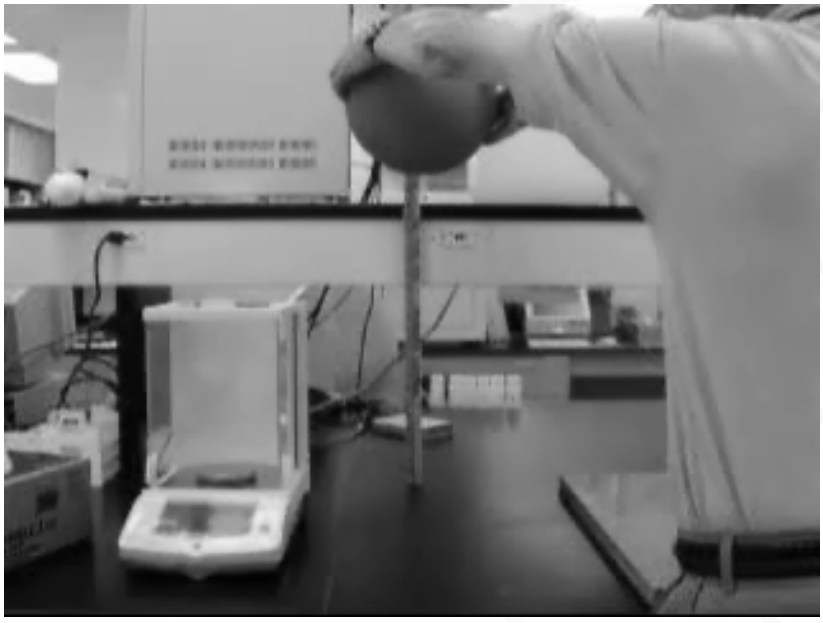
Analytical Balance - Other Considerations

Sample must be at room temperature
warm or hot objects placed in a pan within a closed balance chamber can create air currents that buoy the pan, resulting in erroneous measurements

Sliding doors must be closed.
Avoid the effects of any draft or air currents on the balance

Protect the balance from vibration.
Do not bump the balance or table while making measurements. Vibration can significantly affect measurement accuracy…even leaning on a balance table without a damping pad can affect results by several milligrams.

Vibration Test

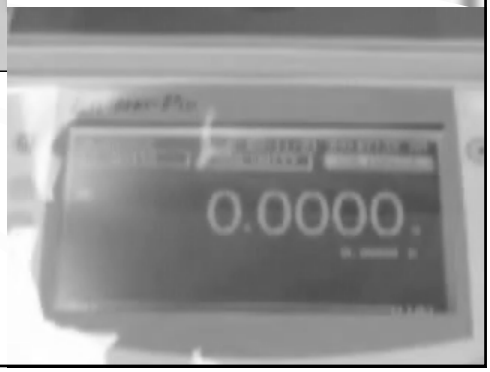


Toploader Vibration

Analytical Balance Vibration

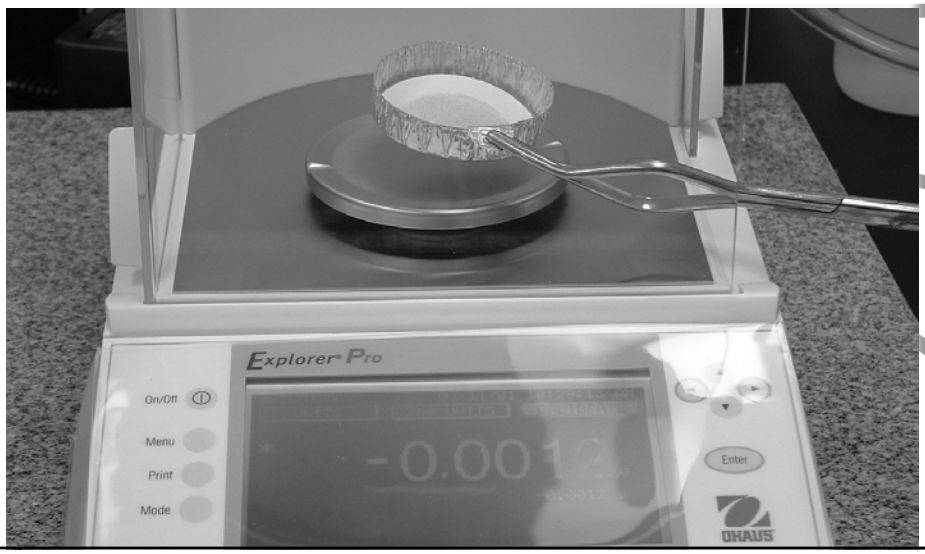
Balance on marble damping pad

Balance on solid countertop only
(no damping)



Analytical Balance Stability

Correct Procedure



Determining Acceptance Ranges for Class 1 Weights Using a Statistical Approach

- Use the following guidelines until statistical limits have been established (*but check w/ your auditor first!):*
  - ± 0.3 mg* for weights <10mg
  - ± 0.5 mg for weights from 10mg to 100mg
  - ± 1-2% for weights >100 mg
- Make 20 replicate measurements of the class 1 weight
- Spread measurements out over several non-consecutive days for best results
- Determine the mean and standard deviation (sd)
- The ***upper acceptable range*** = certified weight + 3 sd
- The ***lower acceptable range*** = certified weight – 3 sd

*Example 1 Determining the Acceptable Range for a 100 mg (0.1000 gm) Class 1 Weight**

Item	Weight (grams)
Mean of 20 measurements	0.09999
Standard Deviation	0.000064
3 Standard Deviations	0.00019
Certified Weight	0.099998
Upper Acceptable Range	0.099998 + 0.00019 =0.1002
Lower Acceptable Range	0.099998 - 0.00019 =0.0998

Range = 0.0998 – 0.1002 g

***Balance readability of at least 0.0001 g**

Summary of Balance Accuracy Verification

- ☞ Calibrate balance daily if it is equipped with an on-board internal calibration feature
- ☞ Check balance accuracy monthly in the g and mg range
- ☞ Use certified class 1 (“S”) weights
- ☞ Have balance serviced annually
- ☞ Have weights re-certified annually or before expiration date
- ☞ Determine the acceptance range using a statistical approach or use the reasonable tolerance guidelines
- ☞ Always record weight measurements, whether they passed or failed and corrective action in the logbook
- ☞ *NEVER* use a balance that fails the verification check!!

Errors are additive

Cold vs. 20° C
Class A or not
TC vs. TD
1 volume➔ 2 pipets
macro v. microbore

±

Cold vs. 20° C
Class A or not
Reading meniscus

±

Cold vs. 20° C
Class A or not
Reading meniscus

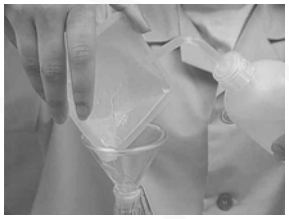
±

Parallax errors

±

Convective errors
Vibrational error
balance/weight accuracy

±



Quantitative transfers
multiple transfers

Acknowledgements

We'd like to thank the following for their assistance in developing this session:

- Mike Raynovic and North Central Laboratories:
  for generously loaning us the equipment used to photograph demonstrations
- State Laboratory of Hygiene staff:
  for dutifully responding to our every wild idea….whether it be to "model" for the camera, prepare some strange concoction, or participate in some (seemingly) weird stunt.

General Colin Powell
Chairman (Ret), Joint Chiefs of Staff

LESSON 7

A Leadership Primer

"Keep looking below surface appearances. Don't shrink from doing so (just) because you might not like what you find."

"If it ain't broke, don't fix it" ...
...is the slogan of the complacent, the arrogant or the scared.
It's an excuse for inaction, a call to non-arms.
It's a mind-set that assumes (or hopes) that today's realities will continue tomorrow in a tidy, linear and predictable fashion.
Pure fantasy. In this sort of culture, you won't find people who pro-actively take steps to solve problems as they emerge.
Here's a little tip: don't invest in these companies.