Calibration of Mechanical Pipettes in the Modern Wastewater Laboratory

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WSLH WISCONSIN STATE LABORATORY OF HYGIENE

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Disclaimer

Class A volumetric pipettes

- Are very accurate and precise
- Do not require periodic calibration
- Have defined volumes
- Are usually broken if dropped
- Require practice to master the technique of use with a bulb

These are fine.

But, maybe it's time to go "Mechanical".

One adjustable volume mechanical pipette can replace a number of single volume pipettes.

Mechanical air-displacement pipettes are becoming more commonly used in the modern wastewater laboratory due to their many advantages over traditional glass pipettes.


ON
MODE
1000 µl
RESET


eppendorf

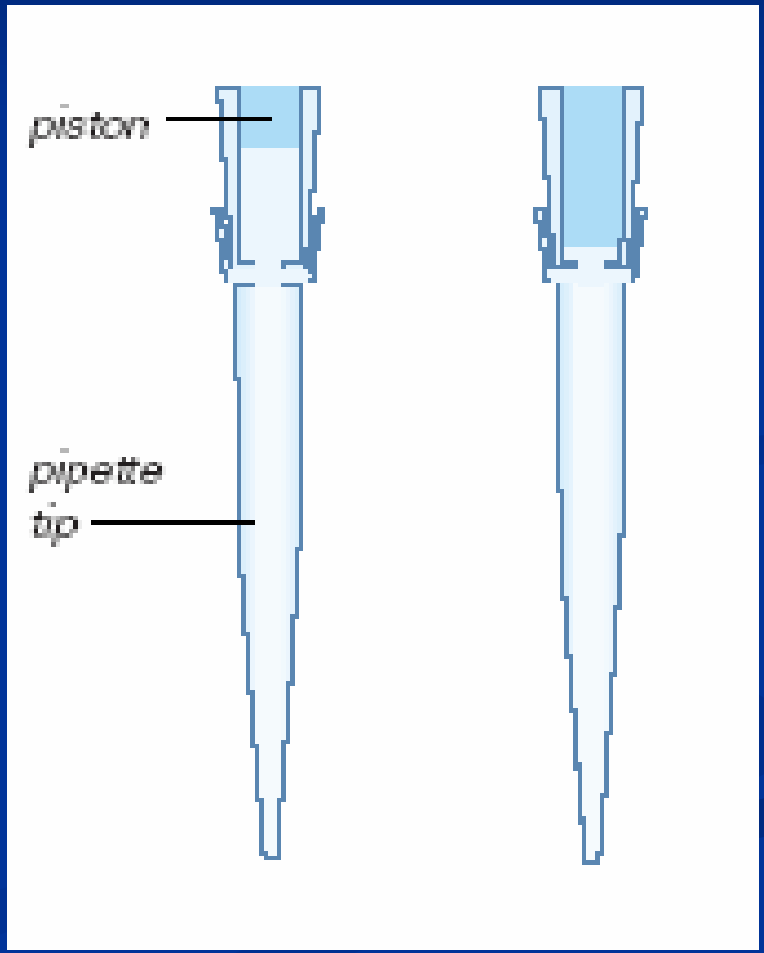

500 - 5000 µl

Where would mechanical pipettes be used in a wastewater laboratory?

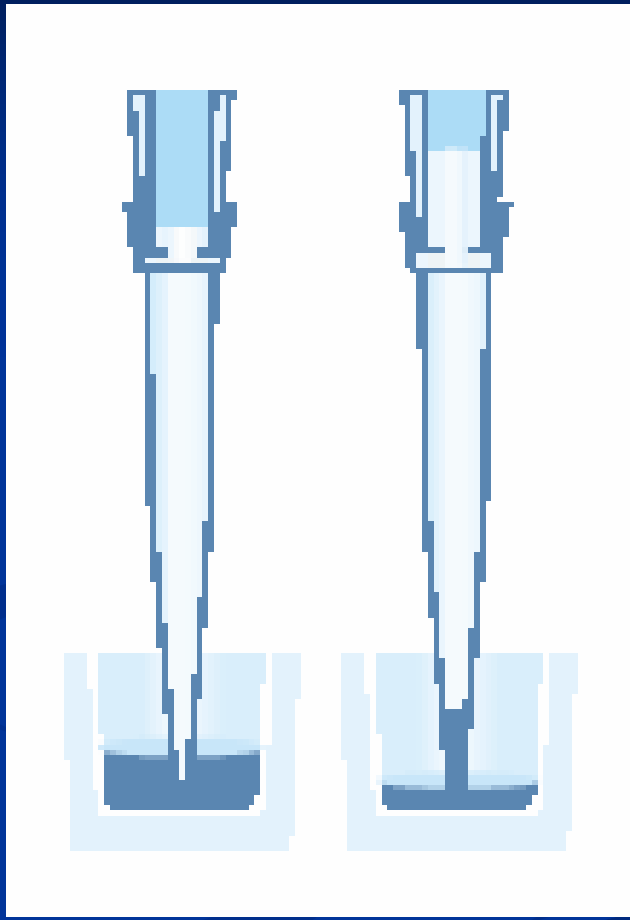
- Chlorine Testing
- Phosphorous Testing, essential if using Test-n-tube® method
- Standards Preparation
- Any pipetting operation up to 10 mL that is repeated often in the laboratory

Mechanical air displacement pipette operation.

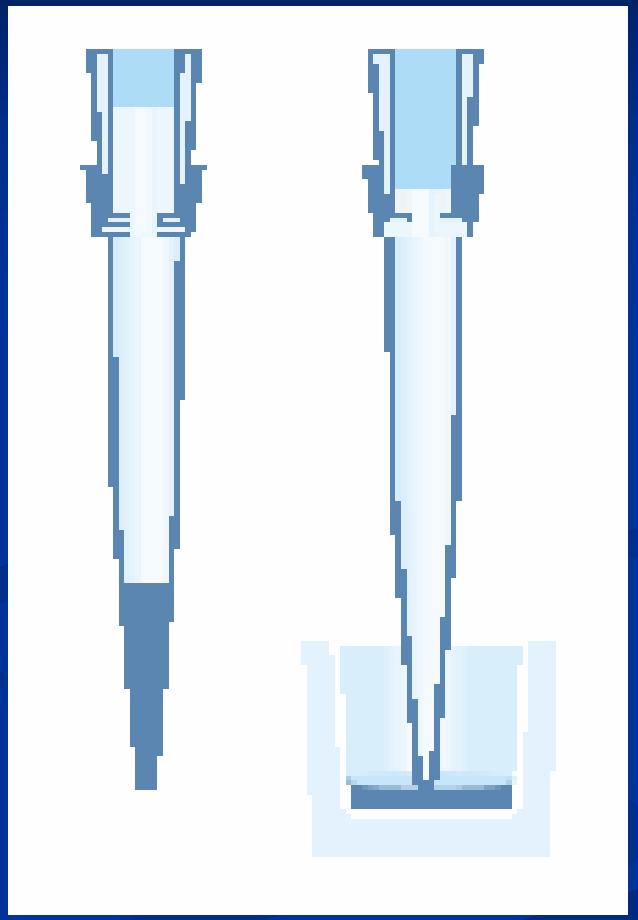
Operating button is pressed to the first stop, moving the piston and expelling a specified volume of air from the tip



After immersing the tip into the liquid, the operating button is released. This creates a partial vacuum and the specified volume of liquid is aspirated into the tip.



When the operating button is pressed to the first stop again, the air dispenses the liquid. To empty the tip completely, the operating button is pressed to the second stop (blow out).



Costs are higher, initially

- Most class A glass volumetric pipettes
  $10 to $40 (depending on the size)
- Mechanical (non-electronic)
  $200 to $400
- Electronic (multi-function)
  $400 to $600

Advantages

- Highly precise and accurate
- Adjustable volumes.
- Disposable tips. No need for washing, saving analyst time. Eliminates possibility of carryover between samples.
- Possible laboratory savings. Despite higher initial investment, cost may be lower over time due to saved analyst time and lower replacement rate than easily broken glass pipettes.

Disadvantages

- Higher initial cost
- Failure to maintain and calibrate may lead to gross errors and poor test results being reported on discharge monitoring reports.
- Failures may be “unpredictable in timing and undetectable by the user.” Periodic calibration and preventive maintenance are, therefore, essential to ensure the integrity of laboratory results.

*George Rodrigues, PhD, ARTEL

Calibration Approaches

- Gravimetric – most common
  - All you need is a calibrated analytical balance capable of measuring down to 0.1 mg (same as for total suspended solids)
  - Thermometer
  - Room temperature distilled water
  - A light weighing vessel for weighing sample replicates
  - Clean, unused pipette tips
  - Spreadsheet or printed form for recording and calculating pipette precision and accuracy

Calibration Approaches

- Photometric – not as common
  - Requires an expensive instrument ($5,000)
  - Expensive reagents

Annual Pipette Calibration (gravimetric)

Date		Analyst		Balance	
Temp. (C)		Z-Factor	#N/A	Serial #	

Pipette #	117205
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Corrective Action Due To Failures? ______________________

Acceptance Criteria
% Inaccuracy must be less than 2.00
No replicate may be greater than 2% from true volume.
%CV must be less than 1.00

Spreadsheet created by:
Donna Johnsen, WSLH

Quarterly Pipette Calibration (gravimetric)

Date		Analyst		Balance	Ae 240
Temp. (C)		Z-Factor	#N/A	Serial #	J52053

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Pipette #	
VOLUME (ml)	WEIGHT (g)
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!

Corrective Action Due To Failures? ______________________

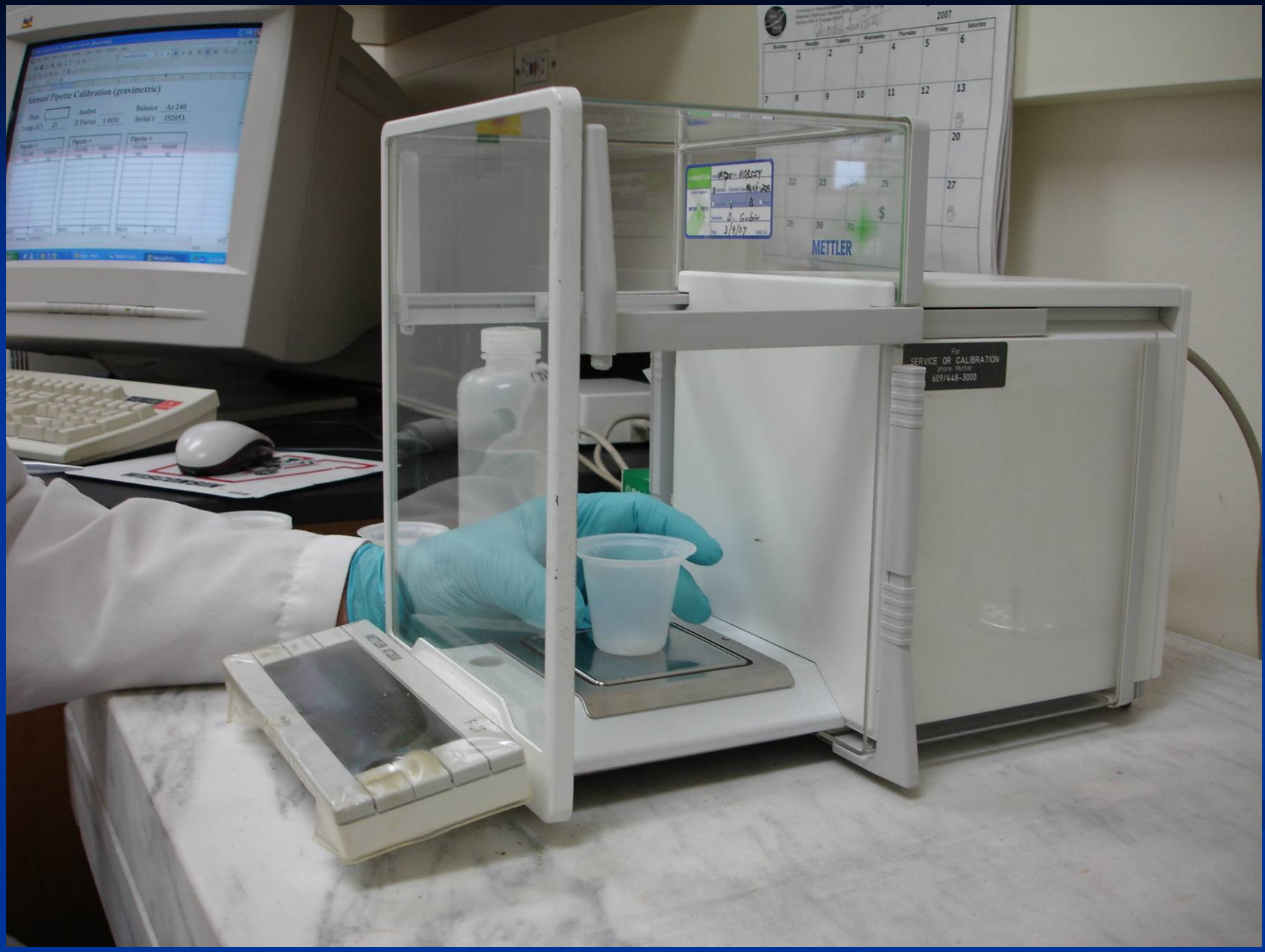
Acceptance Criteria
% Inaccuracy must be less than 2.00
No replicate may be greater than 2% from true volume.
%CV must be less than 1.00

Spreadsheet created by:
Donna Johnsen, WSLH

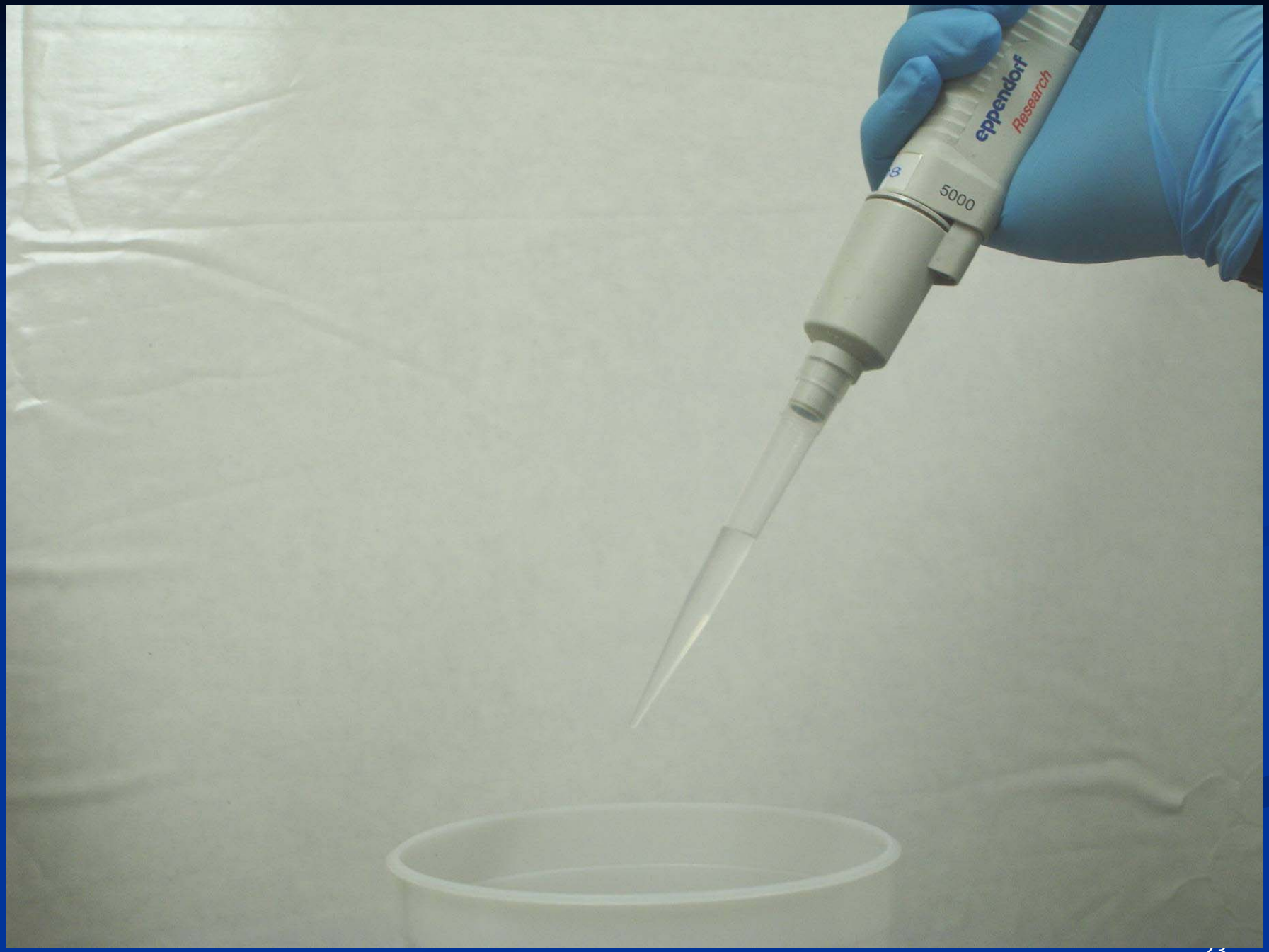
Temperature	Z-Factor
15	1.002
15.5	1.002
16	1.0021
16.5	1.0022
17	1.0023
17.5	1.0024
18	1.0025
18.5	1.0026
19	1.0027
19.5	1.0028
20	1.0029
20.5	1.003
21	1.0031
21.5	1.0032
22	1.0033
22.5	1.0034
23	1.0035
23.5	1.0036
24	1.0037
24.5	1.0038
25	1.0039

Date	**10/25/07**	**Analyst**	**DKP**	**Balance**	**Ae 240**
Temp. (C)	**22**	**Z-Factor**	**1.0033**	**Serial #**	**J52053**

Pipette #	117205
VOLUME (ml)	**WEIGHT (g)**
2.50	
MEAN	#DIV/0!
CORR. MEAN	#DIV/0!
STD DEV	#DIV/0!
% CV	#DIV/0!
% INACC.	#DIV/0!
PASS / FAIL ?	#DIV/0!


Annual Pipette Calibration (gravimetric)
METTLER
For
SERVICE OR CALIBRATION
phone Mettler
609/448-3000


eppendorf
Research
5000


eppendorf
Research
5000


METTLER


METTLER AT200
FACT
Cancel
Configuration
Menu
Select 1
Set
2.5168
g
Re-Zero
On/Off

Pipette #	117205
VOLUME (ml)	**WEIGHT (g)**
2.50	2.511
	2.508
	2.514
	2.517
	2.525
	2.514
	2.521
	2.503
	2.496
	2.517
MEAN	2.51257
CORR. MEAN	2.52086
STD DEV	0.00858
% CV	0.340383825
% INACC.	0.83445924
PASS / FAIL ?	PASS

MEAN	2.51257
CORR. MEAN	2.52086
STD DEV	0.00858
% CV	0.340383825
% INACC.	0.83445924
PASS / FAIL ?	PASS

Acceptance Criteria

% Inaccuracy must be less than 2.00

No replicate may be greater than 2% from true volume.

%CV must be less than 1.00

Calculations

$$\text{Mean Weight} = \frac{\text{sample replicate 1} + \text{sample replicate 2} + \text{(etc.)}}{\text{Number of replicates}}$$

Mean Volume (Corrected Mean) = mean weight × ZFactor

% Inaccuracy = [(Corrected Mean – true value) ÷ true value] × 100

% CV (Coefficient of Variation) = (Standard Deviation÷ Corrected Mean) × 100

Volume	Inaccuracy	Imprecision
1 µl	± 2.5%	≤ 1.8%
2 µl	± 2.0%	≤ 1.2%
5 µl	± 1.5%	≤ 0.8%
10 µl - 15 µl	± 1.0%	≤ 0.5%
20 µl - 40 µl	± 0.8%	≤ 0.3%
50 µl - 90 µl	± 0.7%	≤ 0.3%
100 µl - 200 µl	± 0.6%	≤ 0.2%

Temperature: 20° C - 25° C, constant to ± 0.5° C

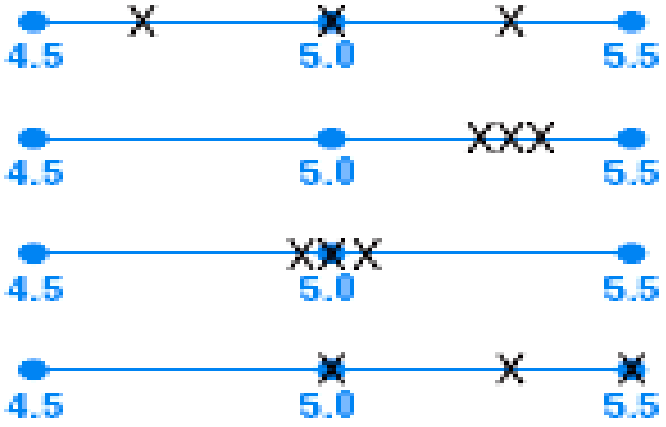
# of measurements: 10

Courtesy of Eppendorf

A pipette is only as good as it's operator.

Technicians in a laboratory are asked to deliver a 5µL sample of solution. Four techs each deliver three samples at the following values:

Tech A	Tech B	Tech C	Tech D
5.3µL	5.3µL	5.0µL	5.0µL
4.7µL	5.35µL	5.05µL	5.3µL
5.0µL	5.25µL	4.95µL	5.5µL



Tech A's values *are accurate but imprecise*

Tech B's values *are inaccurate but precise*

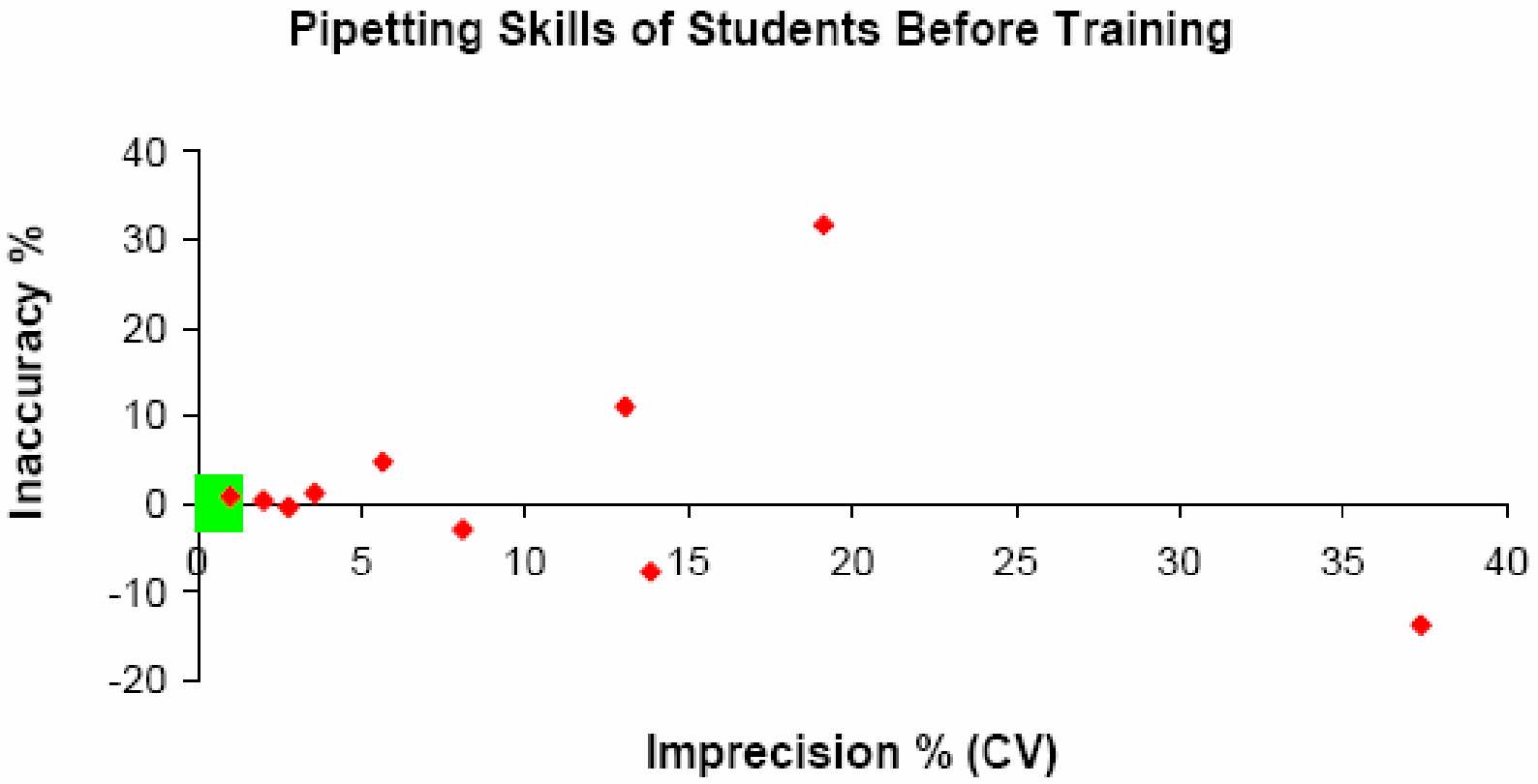
Tech C's values *are accurate and precise*

Tech D's values *are neither precise nor accurate*

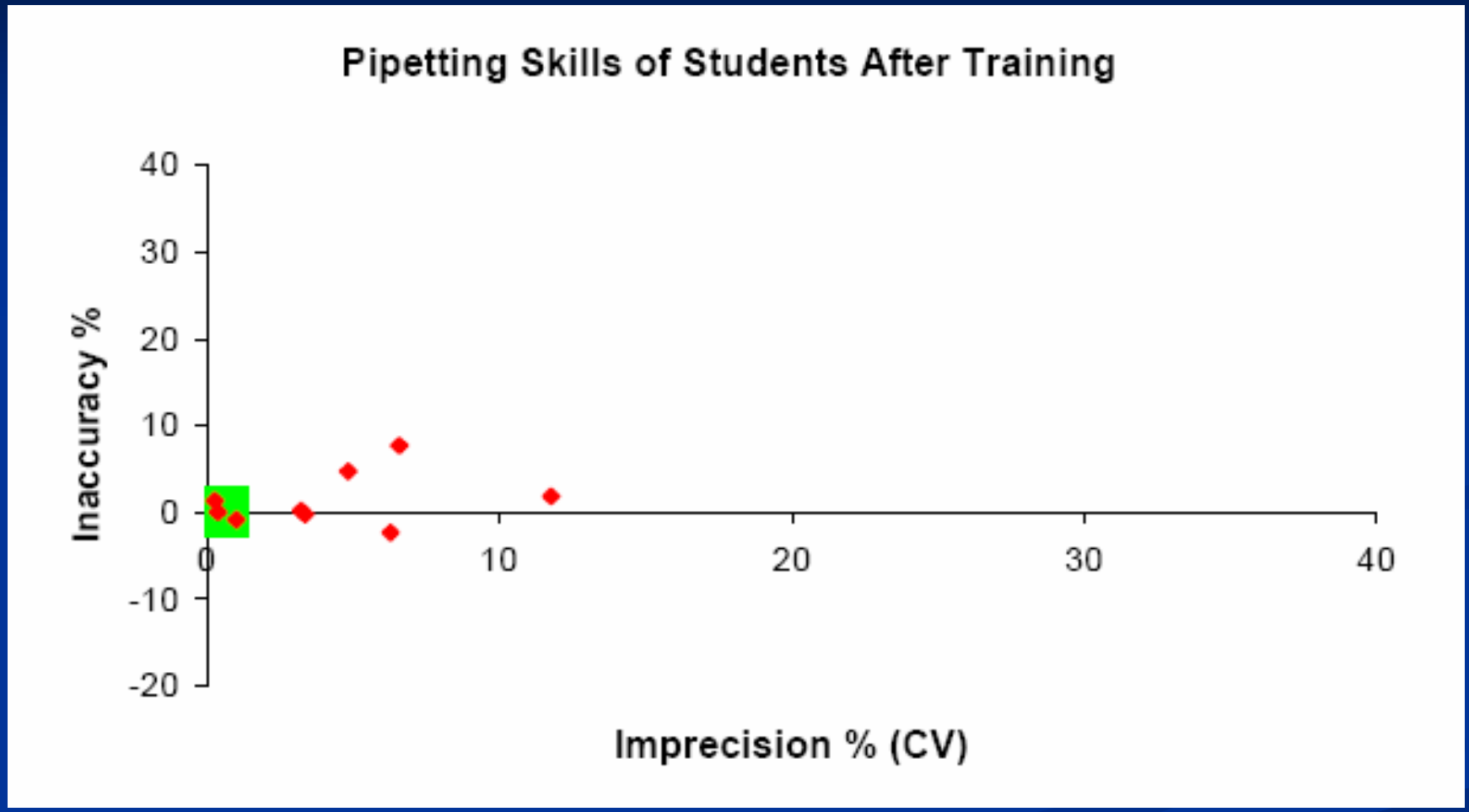
The "clustering phenomenon" observed with Techs B and C is an example of precision.

Courtesy: ARTEL

A little training can go a long way.



Courtesy: ARTEL



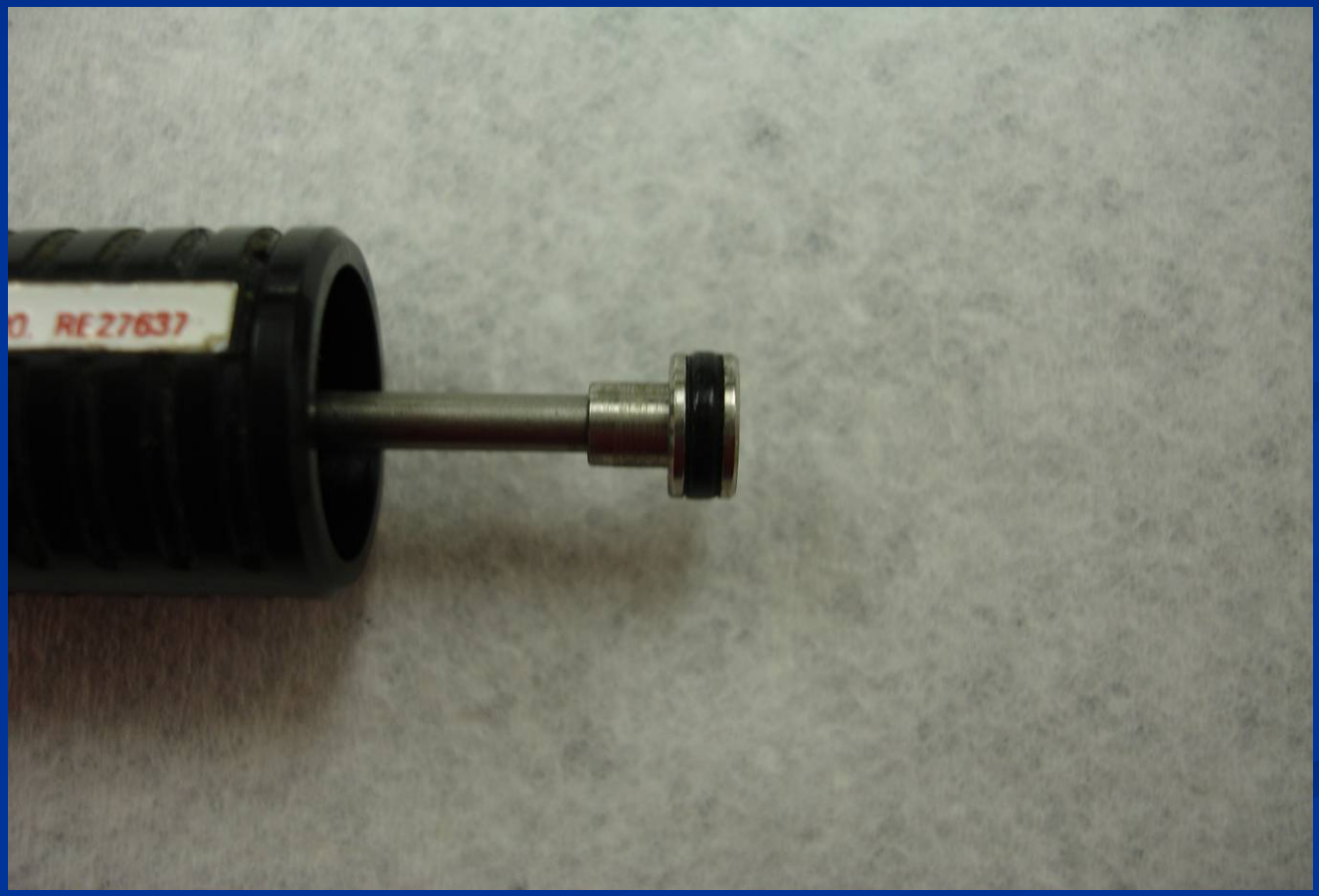
Courtesy: ARTEL

Pipette Maintenance

- Mechanical pipettes must be inspected, cleaned and maintained regularly to assure proper operation.
- Refer to the pipette's instruction manual for guidance on cleaning and maintenance.

- Care must be taken to not aspirate any liquid into the shaft of the pipette. Even non-corrosive liquids can affect the performance of the pipette.
- Do not lay the pipette down when a filled pipette tip is attached as liquid will enter the shaft.
- If liquid accidentally enters the shaft, disassemble the pipette and clean and dry the shaft and piston.

- Inspect all seals and the piston for wear or damage. Replace if necessary



- After cleaning (or replacement), the piston should be lubricated lightly.

In Summary……..

- Air-displacement pipettes are precision instruments that should not be taken for granted.
- Pipettes tend to fail silently and randomly, impacting sample and reagent delivery.
- Periodic calibration and preventive maintenance are, therefore, essential to ensure the integrity of laboratory results.

Summary continued

- Operator technique is also a significant source of pipetting error.
- Pipetting-technique training, especially if it offers the opportunity to obtain immediate feedback, is easy to do and has a significant positive effect on performance.

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- The WSLH will have an instructional CD companion to this presentation available soon. A sign up sheet will be available if you are interested in receiving a copy when it is available.

Thanks for having us!

For More Information

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