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General Biotechnology Laboratory Protocol ©2003
Use and calibration check of Ranin Pipetman
Laboratory #1 in all Biotechnology Classes

Biotechnology Program
Montgomery College
Germantown MD
BSMaT

SOP #: GBT.007
Version: 1.0
Title: Use of Ranin Pipetman

1.0 Purpose

To provide instructions on the proper use of Ranin Pipetman for the accurate and precise delivery of volumes in the microliter (: l) range.

2.0 Scope

This protocol covers the use of P2 through P5000 Ranin Pipetman in the Biotechnology Laboratory. Only non-viscous aqueous solutions are to be pipetted using these instruments. This

3.0 Responsibility

All students, lab assistants and instructors in laboratory courses in the Biotechnology program at Montgomery College shall be instructed in the proper use of Pipetman.

4.0 References

Ranin website (<http://www.rainin.com>)

5.0 Procedures

5.1 Materials

- 5.1.1 Ranin Pipetman P1000, P200, P20
- 5.1.2 Pipetman tips
- 5.1.3 Practice aqueous solution(s) containing food coloring
- 5.1.4 Containers for liquid test articles - disposable weigh boats, disposable beakers, multiwell plates (reusable for this specific exercise)

5.2 Procedure

- 5.2.1 Select the correct pipetman for the volume to be delivered.
- 5.2.2 Set the pipetman to the desired volume by turning the volume adjustment knob counterclockwise to just past the desired volume. Turn backwards (clockwise) until at the exact volume. *Never force the volume adjustment and never exceed the maximum volume for the pipetman.*
- 5.2.3 Select the correct tip for the chosen pipetman and securely seat on the shaft. The tip should be placed on the shaft to just form an airtight seal. A fresh disposable tip is used for each delivery. Do not touch the tip with hands (even if gloved).
- 5.2.4 Double check the volume setting and/or have it verified by your co-worker or supervisor (i.e. instructor).
- 5.2.5 Depress the plunger to the first stop and hold.
- 5.2.6 Place the tip in the liquid to the approximate correct depth and hold the pipetman perpendicular to the surface of the liquid:

<u>Pipetman</u>	<u>Immersion Depth (mm)</u>
P2	1-2
P10	1-2
P20	2-3
P100	2-3
P200	2-4
P1000	2-4
P5000	6-10

Note: Too shallow or too deep will cause volumes to be inaccurate.

- 5.2.7 Allow the plunger to return to the initial starting position *slowly*. Liquid should be drawn into the tip. When the plunger has completed its return wait 1 additional second.

Never allow the plunger to snap back in an uncontrolled manner.

- 5.2.8 Check for air bubbles and splashing in the tip. Be sure the height of the liquid “looks correct” (compared to previous experience).
- 5.2.9 Remove any droplets of liquid on the outside of the tip by either touching the tip against the inside wall of the reagent vessel or wiping with a Kim wipe - be careful not to draw any measured liquid out of the tip by capillary action.
- 5.2.10 Deliver the liquid by placing the tip against the inside wall of the receiving container as close as possible to the liquid surface or just at the bottom if the container is empty. Slowly push the plunger to the first stop. Pause 1-2 seconds and continue to depress to the second stop.
- 5.2.11 With the plunger still depressed to the second stop remove the pipetman and tip from the vessel with the tip pressed against the vessel side for the first few mm of the withdraw. Do not allow any liquid to be sucked up back into the tip.
- 5.2.12 Allow the plunger to *slowly* return to the starting (fully extended) position once it is removed from the vessel.
- 5.2.13 Discard the tip into the proper waste container using the ejector button.
- 5.2.14 Repeat the process using a fresh tip for each delivery.
- 5.2.15 On completion of pipetting return the pipetman to its original position in the pipetman rack.

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SOP#: GBT.007A
Version: 1.0
Title: Protocol for Validation of Ranin Pipetman and Serological Pipette Use

1.0 Purpose

To verify accuracy and precision (i.e. validate) of the pipetman at your lab bench. To verify your ability to correctly deliver known volumes with pipetman and serological pipettes.

2.0 Scope

The P20, P200 and P1000 pipetman at each lab bench will be validated by the first or second lab period by every Biotechnology laboratory class by all students enrolled in the Biotechnology classes.

3.0 Responsibility

All students in the Biotechnology Program must complete this protocol before proceeding to other laboratory exercises.

4.0 Procedures

4.1 Materials:

- 4.1.1 Pipetteman P20, P200 and P1000
- 4.1.2 Pipetteman tips for P20, P200 and P1000
- 4.1.3 Small disposable weigh boats
- 4.1.4 Ultrapure (18.3 Sohm) water
- 4.1.5 Electronic Balance (able to read to nearest 0.001g, e.g. Denver balances in lab)
- 4.1.6 Electronic Pipette aid
- 4.1.7 Serological Pipettes 2, 5 and 10 ml
- 4.1.8 CRC Handbook of Chemistry and Physics or table of water density as a function of temperature

4.2 Procedure :

Overview: For each pipetman at the bench (except the 2: 1 P2) 3 different volumes will be

delivered and weighed at 5 repetitions per volume using water containing food coloring (for visual ease) . The mass will be converted to volume using density. The average of the converted volume will be compared to the volume setting on the pipetman and the coefficient of variance determined.

- 4.2.1 Record the balance number / identification on the data sheet. Certify that the balance is within performance specifications (SOP GBT 006a).
- 4.2.2 Record the temperature and humidity in the laboratory on the data sheet.
- 4.2.3 Look up the density of pure water at the laboratory temperature in the CRC Handbook of Chemistry and Physics and record it on the data sheet.
- 4.2.4 Pour approximately 10 ml of dye solution into a 30 or 50 ml disposable beaker.
- 4.2.5 Obtain a small or medium sized weigh boat and pour about 0.5 ml of the dye solution into a small weigh boat and about 2 ml of the dye solution into the medium weigh boat. The exact volume is not critical.
- 4.2.6 Tare the weigh boat with the liquid in it on the balance.
- 4.2.7 Select a pipette (P20, P200 or P1000) from the rack and record its identity on the data sheet.
- 4.2.8 Place the correct tip on the pipette shaft.
- 4.2.9 Set the volume to the value indicated on the data sheets. Have your setting checked before proceeding (lab partner and/or instructor).
- 4.2.10 Draw up the aqueous solution from the beaker and pipette it into the tared weigh boat.
- 4.2.11 Record the weight to the nearest 0.001 g on the data sheet.
- 4.2.12 After the weight has been recorded tare the balance (display should read 0.000 g).

4.2.13 Repeat steps 4.2.10 through 4.2.12 for a total of 5 times at each specified volume.

4.2.14 Repeat steps 4.2.5 - 4.2.13 for each volume and corresponding pipetteman.

5.0 Calculations

5.1 Principle

A known volume of liquid can be delivered and the mass (weight) of that volume determined using the electronic balance. The electronic balance can be accurately and precisely calibrated using ASTM standards (see SOP GBT 006) so that the mass is accurately known. To verify the delivered volume is accurate the mass is converted back into volume using the density of the liquid at the temperature and pressure of the room at the time of the measurement. Water is most often used as the liquid and its density obtained from a the CRC Handbook of Chemistry and Physics.

5.2 Relevant Equations and Application

$$\text{Density} = \text{mass} / \text{volume} \quad \text{or} \quad d = g / \text{ml}$$

$$\text{ml} = \text{grams} / \text{density}$$

- < grams are obtained by weighing the volume of liquid delivered on the electronic balance
- < density is obtained from the appropriate table in the CRC Handbook of Chemistry and Physics
- < the calculated volume is compared to the volume setting on the Pipetman.

5.3 Sample Calculation

volume setting on P200: 150 : 1

Weights

150: 1	__0.148_g__	__0.147_g__
	__0.148_g__	__0.145_g__
	__0.147_g__	__0.146_g__
average	__0.147_g__	std dev. __0.001__
		CV (%dev) __0.680__

Conversion to volume: assume density of water was 0.9989 g/ml at the conditions of the measurement

$$\begin{aligned} \text{then volume delivered} &= \frac{\text{Grams}}{\text{Density}} = \frac{0.147 \text{ g}}{0.9989 \text{ g/ml}} = 0.147 \text{ ml} \\ &= 147 : 1 \end{aligned}$$

From this you conclude:

Conclusion : Accuracy: PASS

FAIL

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Precision

PASS

FAIL

Comments: Pipetman # _____ must be re-calibrated prior to use.

Protocol and Data Sheet Pipetman Validation

Date : _____

Investigator : _____ Bench # _____

Temperature : _____ °C

thermometer calibration verified : _____

water density : _____ g /ml

Reference source: _____

Relative Humidity: _____ N/D _____ %

Airflow / changes _____ N/D _____ / hr

Balance # : _____ Location : _____

Manufacturer: _____

Model #: _____

Serial Number: _____

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Calibration verified: _____

Data Set for P20 # _____ Bench # _____

**Volume
Setting**

Weight (grams)

5: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

10: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

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18: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

Conclusion : Accuracy: PASS FAIL

Precision PASS FAIL

Comments:

Data Set for P200 # _____

Bench # _____

**Volume
Setting**

Weight (grams)

25: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

75: 1

average _____ std dev. _____

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CV (%dev) _____ volume : _____

150: 1

average _____ std dev. _____

CV (%dev) _____ volume
: _____

Conclusion : Accuracy: PASS FAIL

Precision PASS FAIL

Comments:

Data Set for P200 # _____ Bench # _____

**Volume
Setting**

Weight (grams)

25: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

75: 1

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average _____ std dev. _____

150: 1

CV (%dev) _____ volume : _____

average _____ std dev. _____

CV (%dev) _____ volume : _____

Conclusion : Accuracy: PASS FAIL

Precision PASS FAIL

Comments:

Data Set for P1000 # _____

Bench # _____

Volume

Setting

Weight (grams)

250: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

500: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

900: 1

average _____ std dev. _____

CV (%dev) _____ volume : _____

Conclusion : Accuracy: PASS FAIL
 Precision PASS FAIL

Comments:

Notes on Pipetman : Reading and Setting the volume

Pipetman Selection and volume indicators:

to determine the maximum volume of the pipetman look on the top of the plunger :

Plunger Top	Maximum Volume Capacity		Smallest Displayed Increment	
	_____ : 1	_____ ml	Digital	Vernier Scale
P2	2.0	0.002	0.01 : 1	0.002 : 1
P10	10.0	0.010	0.1 : 1	0.02 : 1
P20	20.0	0.020	0.1 : 1	0.02 : 1
P200	200.0	0.200	1. : 1	0.2 : 1

P1000	1000	1.00	0.01 ml	2.0 : 1
P5000	5000	5.00	0.01 ml	2.0 : 1

Reading the digital volume indicator in the windows

P2-P200

Digital volume indicator is read from **top to bottom**

Black digits indicate microliters (: l)

Red digits indicate tenths or hundredths of microliters (: l)

P1000 - P10000

Digital volume indicator is read from top to bottom

Red digits indicate milliliters

Black digits indicate tenths of a milliliter

examples reading from top down to bottom:

P-2

A	B	C	D
+)) ,	+)) ,	+)) ,	+)) ,
* 1*	* 0*	* 1 *	* 0*
.)) -	.)) -	.)) -	.)) -
+)) ,	+)) ,	+)) ,	+)) ,
* 0*	* 2*	* 7 *	* 0*
.)) -	.)) -	.)) -	.)) -
+)) ,	+)) ,	+)) ,	+)) ,
* 5*	* 1*	* 5*	* 7 *
.)) -	.)) -	.)) -	.)) -

Read 1.05 : l 0.21 : l 1.75 : l 0.07 : l

P-20

	A	B	C	D
	+)), * 1 *	+)), * 0 *	+)), * 1 *	+)), * 0 *
	.)) -	.)) -	.)) -	.)) -
	+)), * 8 *	+)), * 9 *	+)), * 0 *	+)), * 0 *
	.)) -	.)) -	.)) -	.)) -
	+)), * 2 *	+)), * 0 *	+)), * 3 *	+)), * 5 *
	.)) -	.)) -	.)) -	.)) -
Read	18.2 : 1	9.0 : 1	10.3 : 1	0.05 : 1 (out of range for this pipetman)

P-200

A	B	C	D
+)), * 1 *	+)), * 0 *	+)), * 1 *	+)), * 0 *
.)) -	.)) -	.)) -	.)) -
+)), * 8 *	+)), * 7 *	+)), * 0 *	+)), * 0 *
.)) -	.)) -	.)) -	.)) -
+)), * 1 *	+)), * 5 *	+)), * 3 *	+)), * 9 *
.)) -	.)) -	.)) -	.)) -

Read 181 : 1 75 : 1 103: 1 9 : 1 (out of range for this pipetman)

P1000

A	B	C	D
+)) ,	+)) ,	+)) ,	+)) ,
* 1 *	* 0*	* 0 *	* 0 *
.)) -	.)) -	.)) -	.)) -
+)) ,	+)) ,	+)) ,	+)) ,
* 0 *	* 7 *	* 1 *	* 0 *
.)) -	.)) -	.)) -	.)) -
+)) ,	+)) ,	+)) ,	+)) ,
* 0 *	* 5 *	* 3 *	* 9 *
.)) -	.)) -	.)) -	.)) -
Read 1.00 ml	0.75 ml	0.13 ml	0.09 ml (out of range for this pipetman)

Accurate Working Volume Range for Pipetman

Plunger Top	Working Range (: l)	Reproducibility / Precision (: l)
P2	0.1- 2.0	0.014
P10	0.5 - 10.0	0.04
P20	2 - 20	0.06

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P100	10 - 100	0.15
P200	50 - 200	0.30
P1000	100 - 1000	1.5
P5000	500 - 5000	8.0

Vocabulary Term :

the symbol “ λ ” (lambda) is sometimes used in place of : 1

10 λ is the same as 10: 1

Care and Use of Pipetman

- < in some cases a pre-rinse of the tip by drawing up and expelling the liquid in the tip before the actual measurement is made improves accuracy and precision - especially with viscous solvent and “sticky” solutions such as protein solutions
- < the tip quality is critical to accuracy and precision
 - poor tips dirt / dust / other particles (e.g. plastic dust)

Inconsistent diameter of tip

Poor plastic wetting properties

- < Tip should always point towards floor (Never Hold Pipetman upside down)
- < Never drop, slam or throw the Pipetman - Shaft may break or crack
Micrometer may go out of spec/s
Piston may be bent
- < Avoid splashing aerosol formation due to rapid pipetting

Never allow the plunger to “snap” up by quick release after depressing

- < Be sure shaft is contamination free especially after delivering:

Pathogens

Chemicals - toxins, mutagens, teratogens

Radioactivity

Shaft and tip ejector can be washed (aqueous soap solution only) and are autoclavable - the entire Pipetman is *not* autoclavable - however special autoclavable pipetman can be purchased

- < Routine maintenance

Gasket may need to be replaced

Friction ring on volume adjustment may need to be replaced

Wash the shaft and wipe the plunger

Replace lost tip ejectors

Reasons for Loss of Accuracy or Precision

Symptoms:

correct volume not delivered (loss of accuracy)

or

Repeated delivery at same setting gives values that differ by >5%

Possible causes:

- Operator error: be sure operator is properly trained -
Check volume setting
Novices often push plunger to second stop thinking it is the first stop
- Tip seal incomplete: a loose tip is not airtight and will not allow vacuum to form to allow liquid to be drawn up
Be sure tip is firmly seated to form airtight seal
Be sure tip ejector does not prevent airtight seal
- Volume setting is changing: be sure operator does not accidentally move micrometer
Friction ring may need to be replaced
- Gasket needs replacing or is missing: replace gasket
- Shaft is cracked: inspect shaft for damage - loss of vacuum with cracked shaft
- Piston damaged: chipped or corroded due to mechanical or chemical abrasion
Replace piston (factory service)
- Plunger bent: plunger action not smooth / sticky
Replace plunger (factory service)
- Volume setting inaccurate: micrometer damage - overturning, normal wear and tear - Repair micrometer (factory service)

Pipetman Use Problems

1 How many : 1 are in the following volumes and what volumetric piece of equipment would you use to measure this volume ?

: 1 (microliters) Name of Equipment

a. 0.650 ml _____: 1 _____

b.	0.025 ml	_____:	1	_____
c.	1.75 ml	_____:	1	_____
d.	0.175 ml	_____:	1	_____
e.	0.310 ml	_____:	1	_____
f.	0.006 ml	_____:	1	_____
g.	0.062 ml	_____:	1	_____
h.	0.600 ml	_____:	1	_____
i.	0.012 ml	_____:	1	_____
j.	0.001 ml	_____:	1	_____
k.	0.085 ml	_____:	1	_____

2. How many ml are in the following volumes and what device would you use to measure each volume ?

a.	5000 : 1	_____ml	_____
b.	108 : 1	_____ml	_____
c.	12 : 1	_____ml	_____
d.	2.5 : 1	_____ml	_____
e.	785 : 1	_____ml	_____
f.	50 : 1	_____ml	_____
g.	0.2 : 1	_____ml	_____
h.	12.0 ml	_____ml	_____
i.	2 : 1	_____ml	_____
j.	250 : 1	_____ml	_____

3. Below are the “windows” observed when setting a Pipetman. The volume setting and the

Pipetman are indicated - fill in the “window” accordingly.

Example: 75 : 1 P200

+) ,
.) -
+) ,
.) -
+) ,
.) -

A. 12 : 1 P20

+) ,
.) -
+) ,
.) -
+) ,
.) -

B. 85 : 1 P200

+) ,
.) -
+) ,
.) -
+) ,
.) -

C. 175 : 1 P200

+) ,
.) -
+) ,
.) -
+) ,
.) -

D. 340 : 1 P1000

+) ,
.) -
+) ,
.) -
+) ,
.) -

E. 4.5 : 1 P20

+) ,
.) -
+) ,
.) -
+) ,
.) -

F. 970 : 1 P1000

+) ,
.) -
+) ,
.) -
+) ,
.) -

G.	185 : 1	P200	H.	1.5: 1	P2
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
I.	40 : 1	P200	J.	0.2: 1	P2
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
K.	230 : 1	P1000	L.	45: 1	P200
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
M.	7 : 1	P20	N.	1000: 1	P1000
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -
		+) ,			+) ,
		.) -			.) -

4. What should the following volumes of water weigh at 22°C when the density of water is 0.99780 g / ml.

- | | | | |
|----|-----------|---------|----------|
| a. | 0.120 ml | _____ g | _____ mg |
| b. | 185 : l | _____ g | _____ mg |
| c. | 10.0 : l | _____ g | _____ mg |
| d. | 500 : l | _____ g | _____ mg |
| e. | 2.5 ml | _____ g | _____ mg |
| f. | 100.00 ml | _____ g | _____ mg |
| g. | 35.1 : l | _____ g | _____ mg |

5. Calculate the average, standard deviation and coefficient of variance for each of the following data sets (you may use INSTAT):

- 0.145 g, 0.143 g, 0.144 g, 0.145 g, 0.144 g, 0.146 g, 0.145 g
- 0.020 g, 0.020 g, 0.020 g, 0.021 g, 0.021g, 0.020 g
- 0.075 g, 0.071 g, 0.068 g, 0.077 g, 0.071 g, 0.075 g, 0.070 g
- 0.990 g, 0.995 g, 0.991 g, 0.975 g, 0.993 g
- 2.1 mg, 2.0 mg, 1.8 mg, 2.0 mg, 2.0 mg, 2.2 mg

6. Additional volume conversion practice problems

- a. 50 ml = _____ liters
- b. 0.500 liters = _____ ml
- c. 12.5 liters = _____ ml
- d. 250 ml = _____ liters
- e. 0.00089 liter = _____ : l
- f. 6000 : l = _____ ml
- g. 6000: l = _____ liters
- h. 3.7 ml = _____ liters
- i. 0.015 liters = _____ ml
- j. 0.015 liters = _____ : l
- k. 75.0 ml = _____ liters
- l. 695.2 ml = _____ liters
- m. 3.75 liters = _____ ml
- n. 0.450 liters = _____ ml

Pipetman Additional Questions:

1. Why is water placed in the weigh boat and tared before the specified volumes are delivered?
2. What is the consequence of a tip not being placed on the pipetman shaft securely?
3. What is the type and purpose of the filter in the electronic pipette aid?
4. The pipette aid does not draw up liquid - list and explain the remedy for at least three possible reasons for this.
5. What symbol is often used as an alternative to “: I”?
6. Why are three volume settings measured for each pipetman instead of a single volume (e.g. P200 at 30, 90 and 180 instead of P200 at 100)?

Pipetman Additional Questions (answer key):

1. Why is water placed in the weigh boat and tared before the specified volumes are delivered?

To prevent weight changes due to evaporation

2. What is the consequence of a tip not being placed on the pipetman shaft securely?

Incomplete seal and vacuum - volume drawn up and expelled inaccurate

3. What is the type and purpose of the filter in the electronic pipette aid?

Hydrophobic - prevents liquid from getting into the motor and contaminating the body of the pipetteaid

4. The pipette aid does not draw up liquid - list and explain the remedy for at least three possible reasons for this.

No power

plug in / replace battery

pipette not firmly seated so no vacuum

reseat pipette

filter is wet

Replace filter

cotton plug from pipette is drawn into nose piece

*examine nose piece and
remove plug*

5. What symbol is often used as an alternative to “: l”?

8 pronounced lambda

108 is the same as 10: l

6. Why are three volume settings measured for each pipetman instead of a single volume (e.g. P200 at 30, 90 and 180 instead of P200 at 100)?

To be sure the caliper adjustment settings function correctly on the pipette, to be sure the user can be precise over the range of the pipette, and to be sure that no defects in the barrel are present (scratches or particles - e.g. dirt, mold or fungus) that will affect the volume

Name: _____

Date: _____

BT Course # _____ Day / meeting time _____

Pipetteman Use Take Home Quiz

1. How many : 1 are in the following volumes and what volumetric piece of equipment would you use to measure this volume ?

A. 1.50 ml _____ : 1 _____

B. .150 ml _____ : 1 _____

C. 0.015 ml _____ : 1 _____

D. 0.0015 ml _____ : 1 _____

2. How many ml are in the following volumes and what device would you use to measure each volume ?

E. 5.0 : 1 _____ ml _____

F. 50 : 1 _____ ml _____

G. 500 : 1 _____ ml _____

H. 5000 : 1 _____ ml _____

Pipetteman Use Take Home Quiz

3. Below are the “windows” observed when setting a pipetteman. Indicate the correct pipetteman to use and fill in the “window” to indicate the correct volume.

A. 1.5 : 1 P _____
+),
.) -
+),
.) -
+),
.) -

B. 18 : 1 P _____
+),
.) -
+),
.) -
+),
.) -

C. 150 : 1 P _____
+),
.) -
+),
.) -
+),
.) -

D. 810 : 1 P _____
+),
.) -
+),
.) -
+),
.) -

4. Calculate the average, standard deviation and coefficient of variance for the following data set (you may use INSTAT):

0.100 g, 0.101 g, 0.099 g, 0.100 g, 0.098 g, 0.099 g, 101 **mg**

Average: _____

Standard deviation: _____

Coefficient of variance: _____

Serial Dilutions

- recall the equation used for dilution calculations:

$$C_1V_1 = C_2V_2$$

- dilution factor is V_2/V_1

$$DF = V_2 / V_1$$

- concept of 1:10 dilution

In this class a 1:10 dilution means 1 ml + 9 ml = 10 ml so $V_1 = 1$ and $V_2 = 10$

Any multiple of 1:10 dilution can be used:

0.1 + 0.9 same as 100: 1 + 900: 1 use P200 and P1000

0.01 + 0.09 same as 10: 1 + 90: 1 use P20 and P200 or P100

0.5 ml + 4.5 ml (Still 5.0 ml / 0.5 ml = 10 or 10/1)

.03 ml + 0.27 ml

And so on as long as V_2/V_1 is 10 / 1

Can you give examples of this for other dilution factors:

1:20, 1:100, ...

- serial dilution is a repeated dilution in series (one after another)

Draw a picture

Original	→1:10	→ 1:10	→1:10
C0	C1	C2	C3
1	0.1	0.01	0.001

- so can use $C_1V_1 = C_2V_2$ where C2 becomes C1 for next dilution and, in the case of a serial dilution V1 and V2 are constant (or for the mathematically inclined the quotient of V2/V1 is constant - I prefer V2/V1 because it emphasizes the volumes and the dilution aspect of the exercise)

Exercise:

- have the students prepare 1:3, 1:2, 1:4, or 1:9 or 1:20 serial dilutions - each bench a different dilution scheme in duplicate - one partner does one of the duplicates
- do a total of 4 dilutions excluding the starting solution of the dye solution and calculate the concentration of each dilution
- State that the starting concentration of the dye solution is 0.25% (v/v)
- Final volume should be 1 or 4 ml - do in disposable test tubes - should require 5 test tubes
- have them draw the dilution scheme out and calculate the resulting concentrations for each dilution before starting
- Label the test tubes and demonstrate how to keep track of additions
- the protocol should be recorded in the notebook or on piece of paper if no notebook which will be permanently affixed to notebook by week 2 of the lab

Work as pairs at bench

They select the correct measuring device - but check with instructor

Assume the starting concentration of the dye is 0.25% (v/v)

Labeling on test tube with marker

- qualitative observation of results - later plate reader

Dilution Practice Problems

Notes:

1. All dilution problems can be solved using the relation:

$$(C1) \times (V1) = (C2) \times (V2)$$

Where

C1	=	the initial or starting concentration (the larger concentration value)
V1	=	the initial volume or the volume of C1 (the smaller volume value)
C2	=	the final concentration (the smaller concentration value)
V2	=	the final volume or the volume of C1 (the larger volume value)

You must know the number value for three out of the four letters to complete the calculation.

to use this relation C1 and C2 must be in the same units:

M (molarity)
% (percent: e.g. w/v or v/v)
N (normality)

Parts per (e.g. ppm, ppb, ppt)
mg / ml

and V1 and V2 must be in the same units

L (liters)
ml (milliliters)
: l (microliters)

2. Read or listen carefully to figure out which numbers go with each letter.

The words: starting or initial concentration (or concentration unit e.g. initial molarity), stock concentration indicate a C1 value and remember C1 is the largest concentration number

The final concentration, prepare a concentration of, make a solution whose concentration is,... indicate a C2 value.

With respect to volumes be sure to correctly determine V2 :

“Prepare a final or total volume of 500ml” means the $V_2 = 500 \text{ ml}$

“400 ml of water was added to 100ml of stock solution” means
 $V_2 = 400\text{ml} + 100\text{ml} = 500 \text{ ml}$

3. Remember the dilution factor is defined as

$$DF = V_2 / V_1$$

Solution Preparation and Simple Dilutions

1. What is the concentration of a solution prepared by diluting 100ml of 10X amino acid stock solution to a final volume of 1000ml? In theory how many ml of diluent are required?
2. How many mls of a 50X TBE stock solution are required to prepare 250 ml of a 2X working solution?
3. What is the concentration of a 0.350M NaCl solution when 30 ml are diluted with (by adding) 270 ml of water?
4. How many mls of 12.0N HCl are required to prepare 75 ml of 0.100 N HCl ?
5. How much 25% BSA solution is required to prepare a final volume of 1000 ml at 2%.
6. Prepare 500 ml of 1x working solution from a 10X stock solution.
7. Make up 500 ml of 0.15% NaCl from the stock solution. (The stock solution is 5.0M).
8. Make up 2.0L of 0.1 M Tris. (the stock solution is 2.0 M).

9. Prepare 25 ml of a 10 mM solution of EDTA from the 0.5 M stock solution.
10. Prepare 2.5 L of 0.250M magnesium chloride from the 8.0 M stock solution.
11. Prepare 10 ml of 50% Tween detergent. (Pure Tween is available - for a pure liquid the concentration is 100% unless otherwise indicated).
12. Make up 1.0 liter of 10% hydrogen peroxide solution (the commercial concentration is 30%).
13. Prepare 400: 1 of 2.0 mg/ml protein solution from a 12.5 mg/ml sample.
14. Prepare 250: 1 of a 0.50 : g/: 1 DNA sample from a sample at a concentration of 10 : g/: 1.
15. Prepare 250: 1 of a 1.00 : g/: 1 DNA sample from a sample at a concentration of 35 mg/ml.
16. Prepare 2.00 liter of 75% isopropanol.

Dilution Factors:

1. What is the dilution factor when 10 ml is diluted to a final volume of 100 ml?
2. What is the dilution factor when 10 ml is diluted to a final volume 200 ml?
3. What is the dilution factor when 10 ml is diluted to 3000 ml?
4. What is the dilution factor when 5: 1 is diluted to a final volume of 100 : 1?
5. What is the dilution factor when 500 ml is diluted to a final volume of 3.5 liters?
6. What is the dilution factor when 10: 1 is added to 390: 1 of diluent?
7. What is the dilution factor when 5.00 ml is added to 4995 ml?
8. What is the dilution factor when 1: 1 is diluted to a final volume of 5 ml.
9. What is the dilution factor when 20: 1 is brought to a final volume of 100ml?

10. What is the dilution factor when 10ml of solution is mixed with 10 ml of diluent?
11. What is the dilution factor when 50 : 1 is mixed with 0.950 ml?
12. What is the dilution factor when 3 : 1 is mixed with 6: 1 of diluent?
13. What is the dilution factor when 20 : 1 is added to 680: 1 ?
14. 5.5 ml of solution is mixed with 100 ml of diluent - what is the dilution factor?

Multiple Non Serial Dilutions.

1. Calculate the concentration of a DNA solution if 10: 1 of the stock solution (conc = 75 mg/ml) was diluted to a final volume of 200 : 1 and a 5: 1 aliquot of that was again diluted 1:15.
2. Determine the concentration when 1 ml of a protein solution at a concentration of 45.0 mg/ml is diluted to 20 ml with buffer and a 100: 1 aliquot was removed and further diluted by the addition of 200: 1 of buffer.
3. What is the final concentration when 2 ml of 0.25% amino acid solution is diluted by the addition of 8 ml of up water. The diluted solution was then successively diluted by 1:10 and again by 1:50.
4. Determine the concentration when 5.0M NaCl is diluted 1:100 followed by a 1:20 dilution which is followed by a 1:10 dilution which finally is concluded with a 1:3 dilution.
5. Determine the concentration when a 2.5 mg/ml cholesterol solution is diluted 1:50 and the resulting solution is again diluted by removing a 3: 1 aliquot and mixing it with 57: 1 of diluent.

6. Determine the concentration when a 0.75% deoxycholate solution is diluted by mixing 50: 1 with 450 : 1 of up water. The diluted solution was diluted again by 1:10 and finally by diluting a 20: 1 aliquot to 200: 1.
7. A 200: 1 cell culture aliquot was removed and diluted to a final volume of 1.00 ml with HBSS. The diluted cell suspension was again diluted by removing a 50: 1 aliquot and diluting it with an 150: 1 PBS. Finally, 100: 1 of the PBS diluted solution was mixed with 100 : 1 of trypan blue for cell counting. What is the final dilution factor?
8. A 5 : 1 aliquot of an RNA solution (concentration = 20 mg/ml) was diluted by the addition of 95: 1 of DEPC treated water. A 2.0 : 1 aliquot was removed and diluted 1:50. What is the final concentration of the RNA solution and what volume of DEPC water was added for the 1:50 dilution?
9. A peptide solution at 100 mg/ml was diluted by mixing 100: 1 with 0.9 ml of buffer. A 50: 1 aliquot was removed and diluted to 200: 1 with buffer. A final 1:10 dilution to a 25: 1 volume completed. What is the final concentration of the peptide solution ? What are the volumes of peptide and buffer required for the 1:10 dilution.
10. An E. coli culture at 3×10^5 cells/ml was diluted 1:25 to a final volume of 1.00 ml. A 300: 1 aliquot of the diluted cells was diluted by the addition of 2.7 ml of media and mixed. A final dilution was prepared by mixing a 25: 1 aliquot of the diluted cell suspension with 175: 1 of fresh medium. What is the final concentration of E. coli cells ? What volumes of culture and medium were used to prepare the 1:25 dilution?

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Serial Dilutions.

1. Determine the concentration when a 20% Triton X100 solution undergoes a total of four (4) 1:10 serial dilutions.

2. Calculate the final concentration when a 0.5 M EDTA stock solution is serially diluted by 1:4 a

solution?

3. What is the concentration of a 0.350M NaCl solution when 30 ml are diluted with (by adding) 270 ml of water?

4. How many mls of 12.0N HCl are required to prepare 75 ml of 0.100 N HCl ?

5. How much 25% BSA solution is required to prepare a final volume of 1000 ml at 2%.

6. Prepare 500 ml of 1x working solution from a 10X stock solution.

2. What is the dilution factor when 10 ml is diluted to a final volume 200 ml?

3. What is the dilution factor when 10 ml is diluted to 3000 ml?

4. What is the dilution factor when 5: 1 is diluted to a final volume of 100 : 1?

5. What is the dilution factor when 500 ml is diluted to a final volume of 3.5 liters?

6. What is the dilution factor when 10: 1 is added to 390: 1 of diluent?

7. What is the dilution factor when 5.00 ml is added to 4995 ml?

8. What is the dilution factor when 1: 1 is diluted to a final volume of 5 ml.

9. What is the dilution factor when 20: 1 is brought to a final volume of 100ml?

10. What is the dilution factor when 10ml of solution is mixed with 10 ml of diluent?

150: 1 PBS. Finally, 100: 1 of the PBS diluted solution was mixed with 100 : 1 of trypan blue for cell counting. What is the final dilution factor?

8. A 5 : 1 aliquot of an RNA solution (concentration = 20 mg/ml) was diluted by the addition of 95: 1 of DEPC treated water. A 2.0 : 1 aliquot was removed and diluted 1:50. What is the final concentration of the RNA solution and what volume of DEPC water was added for the 1:50 dilution?

9. A peptide solution at 100 mg/ml was diluted by mixing 100: 1 with 0.9 ml of buffer. A 50: 1 aliquot was removed and diluted to 200: 1 with buffer. A final 1:10 dilution to a 25: 1 volume completed. What is the final concentration of the peptide solution ? What are the volumes of peptide and buffer required for the 1:10 dilution.

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10. An E. coli culture at 3×10^5 cells/ml was diluted 1:25 to a final volume of 1.00 ml. A 300: 1 aliquot of the diluted cells was diluted by the addition of 2.7 ml of media and mixed. A final dilution was prepared by mixing a 25: 1 aliquot of the diluted cell suspension with 175: 1 of fresh medium. What is the final concentration of E. coli cells ? What volumes of culture and medium were used to prepare the 1:25 dilution?

Serial Dilutions.

1. Determine the concentration when a 20% Triton X100 solution undergoes a total of four (4) 1:10 serial dilutions.
2. Calculate the final concentration when a 0.5 M EDTA stock solution is serially diluted by 1:4 a total of six (6) times.
3. What is the final concentration when a 25.7 mg/ml protein solution is serially diluted a total of five times by 1:10?
4. What is the final concentration when a 8.50 : g/ml DNA solution is serially diluted 1:3 a total of 8 times ?
5. What is the final concentration when a 2.0 ml aliquot of CHO cells at a concentration of 4.5×10^7 cells /ml is withdrawn from 7 ml of culture in a T-25 flask and serially diluted 1:3 a total of five (5) times.

6. Determine the final concentration when 2×10^7 yeast cells /ml are serially diluted by 1:2 a total of ten (10) times.

7. Show by calculation and in a written protocol how you would prepare a series of dilutions for a protein solution as follows. The initial protein concentration is 13.6 : g/ l. You are to prepare 5 serial dilutions at 1:3 for each dilution starting with a 9.0: g/ l protein solution. Each dilution should have at least a 200 : l final volume.

8. Construct the protocol for preparing a series of viral dilutions according to the following parameters: The viral infectivity is 5×10^8 pfu /ml (pfu = plaque forming units). Prepare 25.0 : l aliquots of virus at dilutions of 1:2 until the concentration is 1×10^3 pfu/ml.

9. How would you serially dilute a 570 : g/ml DNA solution to a final concentration of 0.200: g/ l by making 1:10 dilutions.

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10. Calculate the dilutions required to dilute an enzyme at an activity of 15 units / : 1 to 0.0015 units /ml by 1:10 dilutions. A volume of 300 : 1 is required a each dilution.

Instructor Notes

- < Before class: all pipetman in racks and all tip boxes full
- < Xerox to handout: Ranin Pipetman manual ?
Drummond Pipette Aid instructions
- < Number each dye bottle to correspond to table and write density on the bottle - full label initials, exp date, contents, storage

XYZ Exp. 05/15/00

Dye Solution for Pipetman Calibration

BT *** RT / NS

Demonstrate:

- T bench coat on bench top
- T removing caps / lids
One hand
never place on bench or other surface hold in hand until back on bottle
- T work at least 6" in from edges of bench
- T balance use and taring / weigh boats
- T concept of microliters (liters - ml - : l how to interconvert)
Demonstrate the difference by a factor of 1000 using graduated cylinders -
1000 ml (1.0 liter), 1 ml, 1 : l

Compare : l by factors of 10 1, 10, 100, 1000

Mention use of 8 as an alternative to : l

T calculations

Conversion of liters to ml to : l

Conversion of mass to volume using density

use of INSTANT on computer and statistical calculations

T pipetman use

- position in rack on bench
- size selection P2, P20, P200, P1000 - labeled on plunger top
- tip selection and putting on shaft - full seal
- setting correct volume - ***emphasize not to over extend*** past max volume
- draw up and deliver actions
- disassemble and break down to show seals / barrel cleaning - emphasize ***instructor only does this***
- holding in correct position when in use and when not in use
Do hold at angle or upside down
- replace in original position on rack

Serological pipettes and Pipette Aid use

Instructors Notes

Specific Topics to cover:

- pipette selection - how to choose size

- reading the volumes on the pipette
 - total volume
 - reading exact volumes and significant figures
 - count up or count down
 - delivery of specific volume

- opening plastic - not touching the pipet only plastic wrap

- insertion and removal into pipette aid - straight in straight out

Insert with calibration towards user so can be read

- portable recharge vs turn on separate pump

- buttons
 - top draws up
 - Bottom expels

- disassemble to show filter and how to replace
 - Explain purpose and function of filter - hydrophobic membrane to prevent overdraw into pump mechanism

- reasons for malfunction:
 - No power (unplugged, battery dead)
 - Filter wet

Cotton plugged in nose piece
Pipette clogged - particulate matter in tip or poor quality pipette
Pipette aid is broken

- how to hold the pipette aid when in use and when not in use

Never tilt upside down!

Laboratory Exercise:

- Not a structured experiment - you and students devise your own format
- Start students with a 10 ml serological pipette for Pipetteaid practice

For the adjustable speed pipettes set the speed to “M” for medium or “S” for slow

Just have students practice drawing up and delivering specific volumes
1 ml , 2ml, 3.5ml, ... whatever ...

- have students check their own accuracy and precision using the same concepts presented in the pipetman calibration exercise e.g. weighing delivered volumes - but make or help them figure that out

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Solution preparation - probably best covered second lab:

mass per volume	mg / ml
Percents	w/v and v/v
Molarity	$M = g / (MW * L)$

volumetric flask:

- difference between TC and TD
- powder funnel to add solid solutes
- QSing - not until all solute is dissolved
- standard volumetric sizes for considering when preparing solutions:

1ml, 2ml, 5ml, 10ml, 25ml, 50ml, 100ml, 250ml, 500ml, 1000ml, 2000ml, 4000ml.