# PIPETTING GUIDANCE

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# **1.0 PURPOSE**

The purpose of this guidance document is to lay out the responsibilities, equipment and procedures required for using a pipet aid.

# 2.0 SCOPE

This SOP applies to all persons prescribing and requiring use of a pipettor or a pipet aid.

# 3.0 DEFINITIONS

#### **Aspirate**

The process of drawing the liquid up into the pipette tip.

#### **BSC**

Biological safety cabinet

#### **Dispense**

The process of discharging the liquid from the pipette tip.

#### **Calibration check**

Checking the difference between the dispensed liquid and the selected volume.

#### Adjustment

Altering the pipette settings so the dispensed volume is within the specifications.

# **4.0 ROLES AND RESPONSIBILITIES**

#### **4.1 SUPERVISOR/MANAGER**

- Review this guidance document on a regular basis to consider and mitigate the risks of spill, loss of containment and exposure or other harm.
- Ensure all workers under their supervision are trained and proficient in performing the steps of this guidance.
- Ensure all equipment used for liquid handling are in good repair, calibrated, and decontaminated prior to repair or calibration.

## **4.2 WORKERS**

- Complete all training required for safe operation before starting work.
- Follow this guidance document.
- Take proper care of equipment according to specified use guidelines as set out by the manufacturer.
- Report any broken equipment immediately to their supervisor.

# **5.0 PROCEDURES**

#### **5.1 BEFORE YOU BEGIN**

- Choose the appropriate pipettor, pipet, or pipet aid for the volume and the type of work; review your protocol for the work being undertaken (see Figure 1.0 for the different types of pipets, pipet aids, and pipettors).
- Choose the appropriate tips, or pipets for the type of pipet aid to be used, and that is chemically compatible with the reagents used (e.g. using glass pipets is preferred when dispensing organic solvents). Tips can be filtered or unfiltered. Labware chemical compatibility can be checked here.
- Waste should be disposed of according to the University's <u>Hazardous Waste</u> Standard.
- A container with appropriate disinfectant should be prepared prior to the use of re-useable serological or volumetric pipettes.
- Ensure you have an appropriate disinfecting agent for the biological material to being used. If unsure, use the University's <u>Guide on Developing Chemical</u> <u>Disinfection Protocols</u>.
- Wear the appropriate personal protective equipment (PPE) for the materials you are handling (lab coat, gloves, eye protection etc.).

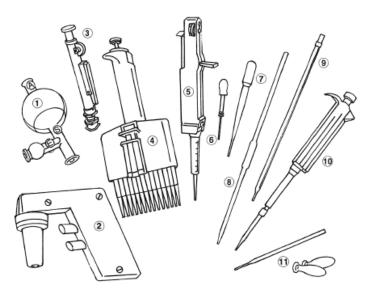


Figure 1: Types of pipettors, pipets, pipet aids

Key: (1) bulb (pipet filler), (2) pipet-aid, (3) pipet pump, (4) multichannel pipettor, (5) repeater pipettor, (6) capillary pipet with bulb, (7) transfer pipet, (8) volumetric pipet, (9) measuring pipet, (10) pipettor, (11) Pasteur pipet, bulb for Pasteur pipet. \*Reprinted from At the Bench: A Laboratory Navigator

#### **5.2 GENERAL SAFETY MEASURES**

Pipetting safely and following sterile technique will reduce the generation of aerosols. Follow the manufacturer's guidelines for the device(s) you are using. A good reference for safety measures can be found at <u>Artel</u>.

- Always work in the appropriate area, biosafety cabinet, fume hood, etc. for the materials you using. For instance, level 2 materials and human blood, bodily fluids and materials should all be pipetted in a BSC.
- Never use your mouth to operate a pipet.
- Always use caution when attaching a tip or pipet (especially glass) to a pipet aid.
- Never hold your pipet aid or pipettor upside down when a used pipet or tip is in place.

#### **5.2.1 REDUCING AEROSOLS & GOOD PIPETTING**

- Do not depress the plunger while the tip is immersed in the liquid (see Appendix
  1); this causes bubbling of the liquid and can generate aerosols.
- Do not forcefully aspirate (draw up liquid) or let go of the plunger while aspirating. This can result in contamination of the interior or the filter of your pipet aid or pipettor, which contributes to inaccurate measurements and causes cross contamination. Liquids drawn up beyond the pipet capacity or through the filter can become aerosolized (see Appendix 1).
- Never forcefully dispense liquids out or let go of micropipette plunger, bulb or other pipet aid devices during dispensing as this can aerosolize your liquids or cause splashing or spilling of hazardous material.
- Do not immerse the tip into liquid while dispensing liquid; this can cause bubbling, generating aerosols.
- Do not forcefully eject tips; eject tips carefully into proper used tip container.
- Remove serological pipettes from pipet aid carefully to prevent the remaining liquid from splashing in the tip.
- Hold pipet aid or pipettor in a vertical position during aspiration. This helps to prevent inaccurate measurements.
- Dispose of all waste solutions according to the University's <u>Hazardous Waste Standard</u>.

Refer to Appendix 1: Aspirating and Dispensing for more information.

#### **5.2.2 ERGONOMIC GUIDELINES**

 Maintain good posture when standing or seated at the bench; ensure you are working at a comfortable height.

- Take regular breaks, as pipetting over a long period (including ejection of tips) can cause a repetitive strain injury.
- Stretch arms and rotate wrists frequently during long periods of pipetting.
- Set up your materials (tips, reagents, disposal container, etc.) so they are within your working space. Follow PHAC BSC Safe Use and Operation Guidelines and do not over-reach. Avoid twisting movements as this can cause a back, hip, or arm injury.
- Avoid resting elbows on the bench, or on BSC grille; this can cause joint damage.
- Never force a glass pipette on the pipet device. The glass can break or fragment creating the potential for a cut injury.

Refer to Appendix 2: Ergonomics of Pipetting for more information.

#### **5.3 INTERNAL OR EXTERNAL CONTAMINATION**

- Never remove the contaminated pipettor or pipet aid from the BSC.
- If liquids have been drawn up into the pipettor or pipet aid, dispose of drawn sample into liquid waste container, dispose tip into appropriate container, and proceed according to manufacturer's directions on how to decontaminate your pipettor/pipet aid. Some pipettors or pipet aids may be disassembled and various parts autoclaved. Follow the guidelines for your equipment.
- Recalibration of pipettor is necessary after disassembly and decontamination.

## **5.4 OPERATIONAL PROCEDURE**

- Determine the appropriate type of pipettor, or pipet aid for your protocol.
- If working with Risk Group 2 material, it is recommended to conduct work inside Biological safety cabinet.
- Follow guidelines for working inside a biological safety cabinet (work from clean to dirty)
- Pipettor racks will prevent placing the pipettor on the Biological safety cabinet work surface.
- Follow pipetting procedure as illustrated in Appendix 1; vertical aspiration helps improve accuracy and reduce aerosols. Tilting the receiving vessel while dispensing liquids improves accuracy and reduces aerosols.
- Monitor pipet aid/pipettor accuracy monthly calibration can be completed either in the lab or sent away to calibration company. Follow manufacturer's recommendations.
- Eject/remove tips carefully into the waste container.
- When removing any device from the BSC, spray the device with disinfectant first.

# **6.0 RESOURCES**

## Types of pipet aids and pipettman

https://www.youtube.com/watch?v=SAGtGk1iTYo

At the bench online link (provides good laboratory practice tips)

http://www.colorado.edu/outreach/BSI/pdfs/AtTheBench.pdf

## **APPENDIX 1: ASPIRATING AND DISPENSING**

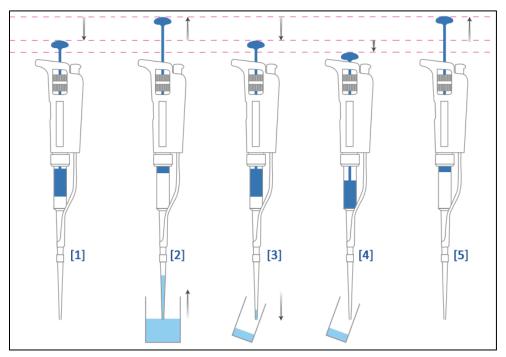


Figure 2: Aspirating and dispensing, taken from Gilson Principals of Pipetting video

- Always depress the plunger to the 1<sup>st</sup> stop prior to immersing the tip into liquid sample.
- Always aspirate sample in the vertical position (see 2 above).
- Dispense sample while tube in held on a slight angle to prevent splashing of sample into tube. The tip should touch the side of the vessel while dispensing. (see 3 above).
- Do not immerse tip into liquid while dispensing (can create aerosols) (see 3 & 4 above).
- When dispensing, carefully depress the plunger to the 1<sup>st</sup> stop; pause, and then depress plunger to the 2<sup>nd</sup> stop to eject (blow-out) remaining liquid (see 3 and 4 above).
- Eject tip carefully into the proper waste container.

# **APPENDIX 2: ERGONOMICS OF PIPETTING**



#### Seated posture

Shoulders elevated X

Upper arm elevated &

Flbow extended X

Wrist in deviation \*



# GOOD

#### Seated posture

Lower back supported by chair 🗸

Upper back and neck upright . .

Upper arm vertical 🗸

Wrist in the same plane as the fore-



#### Standing posture

Upper back and neck stooped #

Lower back and triink stooped X

Elbow flexed X



#### Standing posture

Lower back and trunk upright v

Upper back and neck upright V

Upper arm vertical 🗸

Elbow bent at 90° /

Forearm parallel to the floor &

Wrist in the same plane as the fore-



#### Wrist posture

Upper arm flexed 🗶

Elbow extended X

Wrist deviated downward X



#### Wrist posture

Forearm parallel to the floor /

Wrist and forearm in the same plane /



# Don't Forget these important points when pipetting:

- Do not over reach
- Work at a comfortable height
- Prevent twisting motions
- Vary pipetting activities
- · Alternate with other tasks
- Try using both hands
- · Stretch 'requently
- Grip the pipatte lightly
- Taxe regular short breaks

For technical papers and other pipetting ergonomics information, please visit www.anachem.co.uk or call 01582 747500.





Forearm contact stress on the edge of the bench X



# **APPENDIX 3: PIPETTOR DIAGRAM (GILSON SHOWN)**

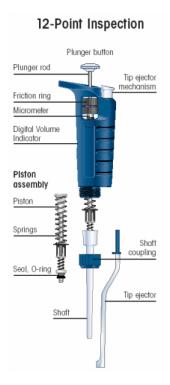


Figure 3: Pipettor diagram

# **APPENDIX 4: AEROSOL GENERATION & PREVENTION USING FILTER TIPS**

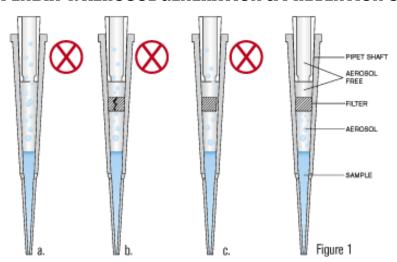
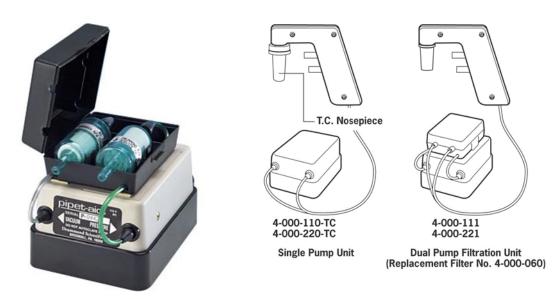


Figure 4: Aerosol generation & prevention using filter tips

- a. Non-filtered tip with aerosols reaching the pipet shaft
- b. Damaged filtered tip, with aerosols reaching the pipet shaft
- c. Filter does not properly fit in the tip, aerosols reach the pipet shaft
- d. Properly fitted filter, blocking aerosols from the pipet shaft

# APPENDIX 5: PIPET-AID® DUAL PUMP FILTRATION UNIT & ORIGINAL PORTABLE PIPET-AID® PIPETTE CONTROLLER



Figures 5-7: Pipet-aid dual pump filtration unit & original portable pipet-aid pipette controller

