



INTEGRATED BIOREPOSITORY OF H3AFRICA UGANDA

MAKERERE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

STANDARD OPERATING PROCEDURE

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| TITLE: PIPETTING GUIDELINES | | PAGE 1 of 7 |
| SOP #: IBRH₃AU-SOP-EQT-006.1 | Effective Date: 09/01/2014 | Next Rev: DEC 2015 |
| Prepared by: _____ | Reviewed by: _____ | Approved by: _____ |
| (Signature & Date) NAME: Musinguzi Henry TITLE: Lab Manager | (Signature & Date) NAME: Dr. Samuel Kyobe TITLE: Coordinator | (Signature & Date) NAME: Prof Moses Joloba TITLE: Principal Investigator |

VALIDATION AND RETIREMENT

| | NAME | DATE |
|---------------|------|------|
| Validated by: | | |
| Retired by: | | |

ACKNOWLEDGEMENT OF READING AND UNDERSTANDING

I have received and understood the training on this SOP. If I have not understood the training I have asked the trainer to retrain me to ensure that I completely understand all the requirements.

| | NAME | SIGNATURE | DATE |
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5.1 INTRODUCTION

Air Displacement Pipettes, used for standard pipetting applications, are highly accurate when well calibrated, used and maintained. However, conditions such as, mishandling, temperature, atmospheric pressure as well as the specific gravity and viscosity of the solution may have an effect on the performance of air displacement pipettes.

5.2 PURPOSE

This SOP is intended to ensure proper, correct, consistent and safe pipetting.

5.3 SCOPE

This SOP applies to all IBRH3AU lab personnel

5.4 RESPONSIBILITY

It's the responsibility of all individuals involved in pipetting to ensure proper, correct, consistent and safe pipetting.

5.5 Pipette terms

- 5.1 **Adjustment** - altering the pipette so that the dispensed volume is within the Specifications
- 5.2 **Air Displacement Pipettes** - are meant for general use with aqueous solutions. In air displacement pipettes, a certain volume of air remains between the piston and the liquid.
- 5.3 **Aspirate** - to draw up the sample.
- 5.4 **Blow-out** - to empty the tip completely.
- 5.5 **Calibration check** - checking the difference between the dispensed volume and the selected volume.
- 5.6 **Dispense** - to deliver the sample.
- 5.7 **Positive Displacement Pipettes** - are used for high viscosity and volatile liquids. In positive displacement pipettes, the piston is in direct contact with the liquid.

6 MATERIALS

- 6.1 Adjustable air displacement pipettes
- 6.2 Pipetting aids
- 6.3 Pipette tips
- 6.4 Weighing scale



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7 PROCEDURES

7.1 General pipetting guidelines

- 7.1.2 Check your pipette at the beginning of your working day for dust and dirt on the outside. If needed, wipe with 70% ethanol.
- 7.1.3 Set the volume within the range specified for the pipette using the adjusting knob.
- 7.1.4 Hold the pipette so the 'grippy finger rest' rests on your index finger.
- 7.1.5 To maximize accuracy, the pipette, tip and liquid should be at the same temperature.
- 7.1.6 Check that you are using tips recommended for this pipette.
- 7.1.7 To ensure accuracy, use only high-quality tips made from contamination-free polypropylene.
- 7.1.8 Tips are designed for single use. They should not be cleaned for reuse as their metrological characteristics will no longer be reliable.
- 7.1.9 Pre-rinsing (1-3 times) the tip with the liquid to be pipetted improves accuracy, especially when using positive displacement tips.
- 7.1.10 Avoid turning the pipette on its side when there is liquid in the tip. Liquid might go to the interior of the pipette and contaminate or damage the pipette.(store in upright position oh holders)
- 7.1.11 Avoid contamination to or from fingers by using the tip ejector and gloves.
- 7.1.12 Store pipettes in an upright position when not in use. Pipette stands are ideal for this purpose.
- 7.1.13 Check calibration regularly, depending on the frequency of use and on the application, but at least once a year

7.2 Preventing cross-contamination

7.2.1 Pipette-to-sample

A contaminated pipette or contaminated tips can cause contamination of samples.

7.2.1.1 Prevention:

- 7.2.1.1.1 Use sterilized tips or sterilized filter tips
- 7.2.1.1.2 Change the tip after pipetting of each sample.

7.2.2 Sample-to-pipette

Samples or aerosols from samples can enter the cone of the pipette.

7.2.2.1 Prevention:

- 7.2.2.1.1 Keep the pipette vertical when pipetting in order to prevent liquid from running into the pipette body.



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7.2.2.1.2 Release the push-button slowly.

7.2.2.1.3 To avoid aerosol contamination use aerosol resistant tips

7.2.2.1.4 Store the pipette vertically.

7.2.3 Sample-to-sample (carry-over)

The remains of sample A can mix with next sample B inside the tip and may cause a false test result.

Prevention:

7.2.3.1 Change the tip after each sample.

7.2.3.2 Use sterile pipettes

7.2.3.3 Use calibrated pipettes.

7.3 Gravimetric testing

Manual pipettes only. This involves weighing samples of distilled water at room temperature (18-22EC) using a reliable electronic microgram balance with a readability of 0.1mgs. It assumes that 1µl weighs 1mg under control conditions.

7.3.1 Fit clean tip, taking care that it is correctly in place.

7.3.2 Set the volume, and pipette distilled water 2 or 3 times to pre-rinse the tip.

7.3.3 Carefully draw in the required volume of distilled water at 18-22EC, keeping the pipette vertical.

7.3.4 Pipette the water into a container placed on a weighing scale and read the change in weight in mgs.

7.3.5 Repeat at least ten times, comparing the results with the table below.

7.3.6 If the mean value of the weights is in the acceptable range the pipette is ready for use.

7.3.7 If there is consistent deviation, recalibrate the instrument accordingly. If results show inconsistency, check all equipment for cleanliness etc. and run a further ten samples taking extra care with your technique until greater precision is obtained. If this does not succeed the instrument should be serviced by a qualified technician.

| Size of Pipette (µl) | Set Volume (µl) | Acceptable range (mg) |
|----------------------|-----------------|-----------------------|
| 0.5 – 10 | 5 | 4.7 – 5.3 |
| 5 – 50 | 20 | 19.7 – 20.3 |
| 50 – 200 | 50 | 49.2 – 50.8 |
| 200 – 1000 | 200 | 198 – 200 |
| 1 – 5ml | 1000 | 990 – 1010 |

As variation of temperature can affect results it's recommended a temperature correction chart is consulted



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7.4 Maintenance

7.4.1 Manual Pipettes

7.4.1.1 Pipette tip cones should be regularly cleaned using ethanol and a soft lint-free cloth or tissue (Biohit tip cones and ejectors are autoclavable at 121EC/ 1atm if required).

7.4.1.2 The casing can be wiped with soft cloth and mild detergent. This is the only maintenance required if the pipette is used correctly.

7.4.1.3 The tip cone is removed for cleaning as follows:

7.4.1.3.1 Hold down the tip ejector.

7.4.1.3.2 Insert tooth of opening tool between tip ejector and its collar to release the locking mechanism

7.4.1.3.3 Gently release tip ejector and remove the tip ejector collar.

7.4.1.3.4 Place the wrench end of the opening tool over the tip cone. Turn anti-clockwise and remove the tip cone.

7.4.1.3.5 Before replacing the tip cone, the piston should be lightly greased using the silicone grease provided.

Note: Tip ejectors are designed to reduce pipette stem abrasion. Some pipettes with metal ejectors may abrade the tip cone if handled incorrectly. Protect the tip cone at all times.

7.5 Operating Temperature

Most pipettes are designed to function at room temperature. For specified performance please make sure that a pipette left in a cold environment overnight is allowed to reach room temperature before use.

7.6 Calibration

7.6.1 Electronic pipettes do not require calibration because they use microprocessor control with optical feedback to monitor piston movement. If giving an inaccurate result, an electronic pipette should be serviced by a qualified technician.

7.6.2 Manual pipettes are adjusted by placing the calibration key into the holes of the calibration adjustment lock under the thumb button and turning the adjustment lock either anti-clockwise (to decrease) or clockwise (to increase) the volume. Repeat the calibration procedure until the pipetting results are consistently correct or allow a qualified technician to service the pipette.

7.6.3 Qualified service should be done annually, and noted on the service report and documented.



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8 REVISION HISTORY

| Revision No | Effective Date | Description of Changes Made from Preceding Revision | Approved by/ Date |
|-------------|----------------|---|-------------------|
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ANNEX 1: DOCUMENTATION OF SUGGESTED CHANGES TO THIS SOP

| CLAUSE | SUGGESTION | BY | DATE |
|--------|------------|----|------|
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