

Ozarks Environmental and Water Resources Institute (OEWRI)
Missouri State University (MSU)

Standard Operating Procedure for:

Operation of Pipettes

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Identification of the method

Operation of Fisherbrand Pipettes

Scope of the method

This standard operating procedure provides OEWRI laboratory personnel with guidance on the procedure for calibrating and operating pipettes.

Summary of method

The procedures follow guidelines provided by the manufacturer. Use of a pipette requires application of correct tip and preparing the tip to deliver the most accurate volume. Checking the calibration of the pipette allows us to assess the accuracy of the pipette delivery volume. This is achieved by averaging the value of 10 deliveries at both the minimum and maximum volumes indicated on the pipette and calculating inaccuracy from the delivered and specified amounts. Manufacturer guidelines for calibration will be followed if the resulting delivery volume is inaccurate beyond specified manufacturer values and the calibration check measurements will be recorded again.

Definitions

There are no phrases or words that are specific to this method.

Health and safety

Operation of a pipette has no unusual risks. Since the pipettes are located in laboratories where other analyses are being conducted, routine laboratory safety practices must be followed when using these instruments.

Personnel qualifications

Laboratory personnel shall have a working knowledge of the pipetting procedures and will have received training from an MSU employee knowledgeable of the proper pipette operation and calibration procedures.

Equipment and supplies

1. Fisherbrand Pipettes
2. Pipette tips (of appropriate size for pipette)
3. Analytical balance for calibration
4. Beaker for calibration

Quality Control

1. The analytical balance used for pipette calibration should be calibrated according to OEWRI-SOP-005 on the Operation of the Sartorius Entris-64-1S Analytical Balance.
2. Pipettes should undergo calibration checks every 3 months or more frequently, depending on their usage frequency.
3. If the delivered volume is found to be inaccurate (beyond the specified inaccuracy volumes in Appendix A or Fisherbrand Manual), the pipette should be recalibrated.
 - a. The calibration procedure should be repeated if the pipette was inaccurate and had to be recalibrated.
4. Deionized (DI) water is to be used during the calibration procedure.
5. The completed pipette calibration log is reviewed by the laboratory manager and QA coordinator.

Calibration and Standardization

1. The pipette use laboratory procedure should be followed when preparing the pipette for calibration.
2. Place a beaker on the balance and tare the balance to get a weight of 0.0g. For pipettes with a volume range over 100 μ l, a readable graduation of 0.1 mg is required.
3. Set the pipette to the maximum volume the pipette allows for. (*It is very important not to force the pipette to a higher volume than the volume printed on the side of the pipette. For example, do not turn the dial past 10 ml if the pipette is a 2 to 10 ml pipette*)
4. Pipette the max volume of DI water into the beaker and record the delivered volume on the Pipette Calibration Bench Sheet.
5. Repeat steps 2-4 until the volume has been recorded 10 times.
6. Repeat steps 2 through 4 for the minimum volume the pipette allows for.
7. Calculate the average of the 10 volume readings for both the maximum and minimum volumes and determine the difference between the selected volume on the pipette and the actual volume dispensed.
 - a. Inaccuracy is the difference between the mean volume actually dispensed and the selected volume on the pipette. It can be calculated using this formula:

$$A = V - V_0$$

Where: A = inaccuracy
 V = mean volume
 V₀ = specified volume

8. If the delivered volume is inaccurate (beyond the specified inaccuracy volumes in Appendix A or Fisherbrand Manual), consult the Fisherbrand manual or guidebook for recalibration of pipettes (Fisher Scientific 2022, Fisher Scientific 2010).

Laboratory Procedure

1. Pipette use

- 1) The pipette tip should be filled and emptied with the solution that will be pipetted 2-3 times before actual pipetting begins.
- 2) Holding the pipette in your hand upright with your thumb on the plunger/delivery knob.
- 3) Attach a clean tip of the correct volume to the pipette.
 - a. To minimize contamination risk, take care in ensuring the tip does not come into contact with external items such as hands, surfaces, or the outside of containers when attaching tip or pipetting.
- 4) By turning the plunger/delivery knob on the top of the pipette, set the desired delivery volume (within maximum and minimum range indicated on pipette).
- 5) Holding the pipette out of the solution, depress the plunger to the first stop.
- 6) With the plunger depressed to the first stop, dip the pipette tip into the solution and slowly release the plunger.
 - a. The tip should only be immersed by a depth of a few mm below the solution surface.
 - b. Liquid should begin to fill the pipette tip as you release the plunger. Try to ensure no air bubbles are present within the tip.
 - c. When removing the tip from the liquid, gently touch the tip to the edge of the container to remove any excess liquid.
- 7) Move the pipette tip from the liquid to the delivery container and place the tip against the inside of the container.
- 8) To expel the liquid, press the plunger to the first stop before continuing to the second stop to empty the tip completely.
- 9) To replace the tip, press the eject button and replace the tip with a clean one.
 - a. The pipette tip should be changed after pipetting each sample or if the tip has come into contact with an external surface.

2. Routine care

- 1) The pipette should be cleaned regularly and checked for cleanliness before use.
- 2) Pipettes should be stored *upright* on the pipette rack when not in use.
- 3) Pipettes should never be turned horizontally or laid down with a tip attached.
- 4) Only 70% ethanol should be used to wipe debris from the pipette.
- 5) Ensure that no liquid is able to enter the pipette, only the pipette tip.

Corrective Actions for Out-of-Control or Unacceptable Data

If a pipette is more inaccurate than the specified inaccuracy values after recalibration, it should be removed from use until it is serviced and able to pass a recalibration within the specified inaccuracy values.

Waste Management

All waste generated when pipetting should be disposed of according to existing waste policies within the MSU Biology Department. Tips will be discarded with paper trash unless sterilization is required before disposal, in which tips will be autoclaved prior to disposal.

References

OEWRI. Standard Operating Procedure for: Operation of Sartorius Entris (64-1S) Analytical Balance. Ozarks Environmental and Water Resources Institute, Missouri State University. OEWRI-SOP-005. 2023.

Fisher Scientific Finnpipette Finntip Good Laboratory Pipetting Guide. 2010.
<https://assets.thermofisher.com/TFS-Assets/LSG/brochures/D16542.pdf>

Fisher Scientific. Finnpipette Single Channel Variable Pipette Use Manual. 2022.
<https://assets.thermofisher.com/TFS-Assets/LCD/manuals/F1%20IFU%201508980-08.pdf>

Tables, diagrams and flowcharts

1. See Appendix A for selected limits of inaccuracy in delivery volume from the manufacturer.
2. See the Pipette Calibration Bench Sheet below. The analyst should make a copy of this sheet when determining pipette accuracy or during calibration.

Appendix A

Range	Volume μl	Inaccuracy	
		μl	%
0,2-2 μ l	2	± 0.080	± 4
	0.2	± 0.080	± 40
0,5-5 μ l	5	± 0.125	± 2.5
	0.5	± 0.125	± 25
1-10 μ l	10	± 0.120	± 1.2
	1	± 0.120	± 12
2-20 μ l	20	± 0.20	± 1.0
	2	± 0.20	± 10.0
5-50 μ l	50	± 0.50	± 1.0
	5	± 0.50	± 10
10-100 μ l	100	± 0.80	± 0.8
	10	± 0.80	± 8.0
20-200 μ l	200	± 1.60	± 0.8
	20	± 1.60	± 8.0
30-300 μ l	300	± 4.0	± 1.3
	30	± 4.0	± 13
100-1000 μ l	1000	± 8.0	± 0.8
	100	± 8.0	± 8.0
0,5-5 ml	5000	± 40.0	± 0.8
	500	± 40.0	± 8.0
1-10 ml	10000	± 60.0	± 0.6
	1000	± 60.0	± 6.0

(Fisher Scientific, 2022)

Pipette Calibration Bench Sheet

Pipette ID: _____

Data Reviewed By:

Date of Calibration: _____

Volume Range: _____

ID	Minimum Volume		Maximum Volume	
	Set Volume (V ₀)	Volume	Set Volume (V ₀)	Volume
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Average Value	V₀ =	V =	V₀ =	V =
Inaccuracy (A = V - V ₀)	A =		A =	