1.0 Purpose

This SOP will address the basic procedure, and frequency for the calibration of the Eppendorf pipettes used for USP analysis.

2.0 Scope

This procedure applies to the USP Eppendorf pipettes. Calibration will be performed on the Eppendorf pipettes by QA Lab personnel on a quarterly basis.

3.0 Responsibility

QA Lab personnel are responsible for performing this procedure.

4.0 Safety Considerations

Safety is a condition of employment. Employees are not authorized to work in an unsafe manner and are prohibited from harming the environment of the facility or community.

5.0 Materials/Equipment

- Eppendorf Pipettes (1000 µL, 5 ml, 10 ml)
- Pipette tips for all pipettes
- Balance-Mettler Toledo X5105Du, B13929Z316
- Thermometer (room temperature range in °C)
- 250 ml Beaker
- Weighing bottle with glass stopper
- Nitrile gloves
- Laboratory Tissue Wipers

6.0 Procedure

1. Fill 250 ml beaker with DI H_2O.
2. Place 250 ml beaker near area where the calibration will take place and place thermometer in beaker.
3. Allow the water and thermometer to come to thermal equilibrium with the surrounding area by letting them set for a few minutes.

All the steps that follow must be performed on each pipette in turn.

4. Check for leaks by holding the pipette vertically for approximately 15 seconds with a tip filled from the 250 ml beaker. Do not touch the pipette tip. Observe the meniscus of the liquid in the tip. If there is a leak, a droplet will be visible on the pipette tip. If the pipette is leaking it will need to be repaired before proceeding.

There is a page in *USP Eppendorf Pipette Calibration Log (L12-PR-100-F014)* for each pipette. These pages list the three volumes to be tested for each pipette. The following steps must be performed for each volume.

5. Enter the temperature of the water in the 250 ml beaker onto the appropriate page of the *USP Eppendorf Pipette Calibration Log (L12-PR-100-F014)* for the pipette to be tested.
6. Enter the air pressure onto the appropriate page of the *USP Eppendorf Pipette Calibration Log (L12-PR-100-F014)* for the pipette to be tested. The air pressure in Waynesville (in inches of Hg) can be found at http://waynesvilleweather.com/Current+Conditions/Waynesville.
7. Attach the appropriately-sized pipette tip onto the nose cone of the pipette.
8. Set the pipette to the volume which is to be tested.
9. Put on nitrile gloves to prevent error from transfer of oil from fingers.
10. Place the weighing bottle with lid on the balance and tare the balance.
11. Pre-wet the tip.
12. Aspirate and dispense the set volume three times and finish with a blow-out (reaching the second stop of the control button).
13. Hold the pipette in a vertical position in the beaker of water.
14. Immerse the tip so that 2-3 mm is in the liquid.
15. Aspirate the test volume slowly and in a uniform fashion. Be sure to allow for a waiting period at the end of the aspiration to ensure completion.
16. Remove the pipette tip from the liquid slowly and again uniformly. Remove any drops that may be on the outside of the tip by wiping the tip against the beaker.
17. Remove weighing bottle from balance, and lid from bottle.
18. Place the filled tip at a 30° angle against the side of the weighing bottle.
19. Dispense the test volume slowly up to the first stop and again allow for a waiting period at the end. Press the button to the second stop to dispense any remaining liquid.
20. While holding the control button at the second stop, slowly drag the tip along the inside of the weighing vessel to remove it.
21. Replace lid on weighing bottle, place bottle back into balance, and close the balance door.

22. Write down the value that appears on the balance display after it has stabilized onto the appropriate page of the *USP Eppendorf Pipette Calibration Log (L12-PR-100-F014)* for the pipette being tested.
23. If necessary, empty the weighing bottle to make room for the next test volume. Use a laboratory tissue wiper to dry the lip of the weighing bottle.
24. Repeat steps 11 – 19 nine more times for a total of ten replicates.
25. Repeat steps 5 – 21 for each of the volumes remaining to be tested for the current pipette.
26. Use a Microsoft Excel worksheet to calculate the systematic error and the random error for each volume and evaluate each error against the error limits in accordance with ISO 8655-2.
27. Enter the results onto the appropriate page of the *USP Eppendorf Pipette Calibration Log (L12-PR-100-F014)* for the pipette being tested.
28. If any of the tests fail, repeat the tests with a fresh pipette tip, paying close attention to the troubleshooting section below. If it fails again, adjustments need to be made to the pipette. Follow the manufacturer's instructions for adjustment.
29. If a pipette failed and was adjusted make a note on the worksheet and perform the calibration procedure again after adjustment.
30. Repeat steps 4 – 23 for all remaining pipettes to be calibrated.

Troubleshooting:

1. If you see droplets of fluid inside the pipette tip, you should attach a new tip as there was uneven wetting of the plastic wall inside the tip.
2. If your pipette drips or dispenses the incorrect volume there are many possible causes:
    a. Loose tip: try pushing the tip on tightly.
    b. Incorrect tip: Check operator's manual for correct tip to use.
3. A leaky pipette or jammed control button may be due to contaminated or damaged seals or piston. Clean and lubricate the piston and replace the seals. Also be sure the nose cone is secured tightly.

For further information, consult your operator's manual.

7.0 Calculations

To calculate the absolute atmospheric pressure at the elevation of Giles Laboratory in kPa ($\mathbf{P_{ABS}}$), from the mean sea level pressure in inches of Hg ($\mathbf{P_{MSL}}$) (as reported at http://waynesvilleweather.com/Current+Conditions/Waynesville), use the following equation:

P_{ABS} = (687.407894148557 * ((P_{MSL} * 25.4) / 760 - 1) + 687.407894148557) * 0.133322368

To calculate **Factor Z (µl/mg)** in accordance with EN ISO 8655 as a function of the temperature and air pressure for distilled water use this table:

Temp.	**Air Pressure (kPa)**						
(°C)	80.0	85.0	90.0	95.0	100.0	101.3	105.0
15.0	1.0017	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0020	1.0021
16.0	1.0019	1.0020	1.0020	1.0021	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0021	1.0022	1.0022	1.0022
17.0	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18.0	1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.5	1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026
19.0	1.0024	1.0025	1.0025	1.0026	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0027	1.0028	1.0028
20.0	1.0026	1.0027	1.0027	1.0028	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0029	1.0030	1.0030
21.0	1.0028	1.0029	1.0029	1.0030	1.0031	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032	1.0032
22.0	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0034	1.0034
23.0	1.0033	1.0033	1.0034	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0036	1.0037
24.0	1.0035	1.0036	1.0036	1.0037	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039	1.0039
25.0	1.0038	1.0038	1.0039	1.0039	1.0040	1.0040	1.0040
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0041	1.0042
26.0	1.0040	1.0041	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0044	1.0044
27.0	1.0043	1.0044	1.0044	1.0045	1.0045	1.0045	1.0046
27.5	1.0045	1.0045	1.0046	1.0046	1.0047	1.0047	1.0047
28.0	1.0046	1.0046	1.0047	1.0047	1.0048	1.0048	1.0048
28.5	1.0047	1.0048	1.0048	1.0049	1.0049	1.0050	1.0050
29.0	1.0049	1.0049	1.0050	1.0050	1.0051	1.0051	1.0051
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0052	1.0053
30.0	1.0052	1.0052	1.0053	1.0053	1.0054	1.0054	1.0054

To calculate each volume dispensed, multiply each mass that appeared on the balance display by the appropriate **Factor Z**.

To calculate the systematic and random errors for each target volume ($\mathbf{V_T}$), take the mean of calculated volumes ($\mathbf{V_M}$), and use the following equations (with all volumes in milliliters):

Systematic Error (± µL) = $|\mathbf{V_M} * 1000 - \mathbf{V_T} * 1000|$
Systematic Error (± %) = 100 * (**Systematic Error (± µL)** / ($\mathbf{V_T} * 1000$))
Random Error (± µL) = 1000 * Standard Deviation of All Calculated Volumes
Random Error (± %) = (**Random Error (± µL)** / ($\mathbf{V_M} * 1000$)

Error limits in accordance with ISO 8655-2 – Research Plus variable multi-channel pipettes					
Model	Testing Volume	Error Limits			
		Error			
		Systematic		Random	
		± %	± µL	± %	± µL
100 – 1000 µL Increment: 0.02 µL	100 µL	± 8.0	± 8.0	± 3.0	± 3.0
	500 µL	± 2.0	± 8.0	± 0.6	± 3.0
	1000 µL	± 0.8	± 8.0	± 0.3	± 3.0
0.5 – 5 mL Increment: 1 µL	0.5 mL	± 8.0	± 40.0	± 3.0	± 15.0
	2.5 mL	± 1.6	± 40.0	± 0.6	± 15.0
	5.0 mL	± 0.8	± 40.0	± 0.3	± 15.0
1 – 10 mL Increment: 10 µL	1.0 mL	± 6.0	± 60.0	± 3.0	± 30.0
	5.0 mL	± 1.2	± 60.0	± 0.6	± 30.0
	10.0 mL	± 0.6	± 60.0	± 0.3	± 30.0

8.0 Reference Documents

USP Eppendorf Pipette Calibration Log (L12-PR-100-F014)

9.0 Change Information

Added **Calculations** section.