The Use of Volumetric Pipets with NIST Handbook 133, *Checking the Net Contents of Packaged Goods*

Purpose: Guidance on the use of Class A pipets for precision measurements in package testing.

Introduction: Laboratory glassware is marked to indicate the volume of fluid it will contain when filled to a specific level. The graduations placed on the glassware by the manufacturer differ greatly in the precision they indicate, depending on the Class of the glassware. Class A pipets are manufactured to provide high accuracy and they are required to meet the requirements and tolerances in ASTM E969 – 02 (Reapproved 2012) "Standard Specification for Glass Volumetric (Transfer) Pipets."

Distilled Water: Use distilled water with Type A pipets to avoid contamination and the buildup of minerals within the glassware.

Safety: Below are a few of the main hazards associated with the use of pipets and other laboratory glassware, and the precautions that should be followed to reduce the risk of injury. Links to additional safety information are provided in the resource section at the end of this paper.

Hazard: Cuts from...

- 1. glass tubing or pipets that break due to forcing plastic or rubber tubing or bulbs onto them;
- 2. the cleanup of broken glass and other sharp items;
- 3. broken glass improperly disposed of in ordinary waste bins;
- 4. flying glass due to explosion or implosion following pressurization or evacuation; and
- 5. broken/flying glass following breakage due to impact or thermal shock.

Minimize risk:

- 1. Before use, check that all glassware is free from cracks, flaws, or scratches that may cause it to fail in use. Dispose of damaged glassware or have it repaired.
- 2. Hold beakers, bottles, flasks and other pieces of glassware by the sides and bottoms rather than by the tops. The rims or necks of these items may break if used as a lifting point.
- 3. Avoid carrying glassware by hand; use a suitable container.
- 4. Thoroughly clean glassware of all chemical residue (especially if the glassware has contained strong corrosives or reagents) before reusing or sending for repair.
- 5. Avoid trying to catch falling glassware.

- 6. Use a brush and dustpan to clean up broken glass. Be especially careful when cleaning broken glass from a sink where water can make sharp edges difficult to see. Use tongs, forceps, or pads of disposable paper towels to pick out pieces.
- 7. Dispose of glass "sharps" in special containers used solely for this purpose and labeled appropriately. Do not overfill. Do not dispose of broken glass in the ordinary waste bins.
- 8. Protect hands with gloves, a towel, or tubing holder when inserting glass tubing into bulbs. Lubricate the tube with water or glycerol. Keep hands on pipets close to the bulb and out of line with end of the tube. Do not use excessive force; NEVER push with the palm of the hand.
- 9. Do not use excessive force. Do not exert force in a direction that will make the glass snap. Think about where the sharp edge of the glass might go if it does break and arrange your grip accordingly. Wrap the glass in a towel or thick layers of paper tissue. Reduce the leverage on pipettes by holding them near the end when fitting fillers. When removing plastic tubing, cut off tubing that does not yield to gentle pressure.
- 10. Pipets should always be covered [or stored in protective sleeves] when not in use.

Pipets: NIST Handbook 133 requires the use of a Class A Mohr (graduated) Pipet or Class A Transfer Pipet for high precision volumetric measurements. Pictured in Figure 1 below are the two pipets referenced in Handbook 133. On the left is a graduated (Mohr) Pipet and on the right is a Transfer (volumetric) Pipet. A Mohr pipet is calibrated in milliliters (and fractions of a mL) to deliver any amount of water in different capacities including 10 mL, 25 mL and 50 mL sizes. A Transfer (volumetric pipet) is calibrated "to deliver" a specific volume in a single delivery and comes in a variety of sizes including 10 mL, 25 mL, 50 mL, and 100 mL. Both types of pipets are calibrated to deliver the specified volumes at 20 °C (68 °F). When you adjust the quantity of water in a pipet, read the meniscus according to "NIST IR7383 – GMP 3 Good Measurement Practice for Method of Reading a Meniscus Using Water or Other Wetting Liquid, April 2013."

http://www.nist.gov/pml/wmd/labmetrology/upload/GMP_3_20130424.pdf

See the last page of this paper for black/white meniscus reader that can be printed out for use.



Figure 1. Class A Pipets

It is important to understand the difference in the way Mohr Pipets and Transfer Pipets are emptied because improper emptying will affect the accuracy of a measurement.

Using a Mohr (Graduated) Pipet

A graduated pipet is filled to its marked capacity and the water is dispensed in the different amounts <u>within</u> the range of graduations. The water that remains below the bottom graduation on the pipet <u>must not be dispensed</u>. Figure 2 shows two ways to use a 10 mL graduated pipet to measure 3.2 mL of water. The drawing on the left illustrates that the pipet is emptied to the lowest graduation and <u>no further</u>. If the water below the lowest graduation is emptied into the receiving vessel, the delivered quantity will be <u>excessive</u>. The right-hand drawing shows how to fill the pipet to the zero mark and dispense the 3.2 mL of water from that point using the graduations.



Figure 2. Dispensing 3.2 mL from a Mohr Pipet

Using a Volumetric (Transfer) Pipet

A volumetric (Transfer) pipet delivers its marked capacity in one delivery (capacity is printed on the bulb). These pipets are filled to a level above the capacity mark and then water is released until the meniscus is aligned with the zero graduation. The pipet is then <u>completely</u> emptied into the receiving vessel.

Drying the Tip of a Pipet: Always keep a low-lint laboratory wipe nearby to enable you to keep the outside of a pipet dry and so you can wipe off the tip of the pipet after it is filled. Hold the pipet in vertical position and gently wipe the pipet from top to bottom to dry it.

Filling the Pipet

Wet-Down Procedure: All pipets are calibrated to deliver the marked capacity after they have been wet down with water. You can either fill and empty the pipette several times or partially fill it and turn it so that its interior surface is wet.

Hold the pipet vertical at eye level and insert the tip into the water. Pipets can be filled with an electric-filler or a rubber bulb such as shown in Figure 3.

Do not insert the pipet too far into the bulb or force the bulb on the pipet. Excessive force may cause the pipet to break or to become stuck in the filler¹.

To use the three valve-type bulb, squeeze the A-Valve and compress the bulb to expel the air. Place the tip of the pipet in the water to be measured, and squeeze the S-Valve to fill the pipet with water (the tip of the pipet must be kept under the surface of the water being measured out during the entire time suction is being applied, or air will be sucked into the pipet). When the water is about 20 mm above the calibration mark of the pipet, release S-Valve to stop. Squeeze the E-Valve gently to the calibration mark.



Figure 3. 3-Way Pipet Filling Bulb

As an alternative, you can also remove the bulb and place your **index finger** on top of the pipet to prevent the water level from falling. By releasing the pressure of your finger, you can allow the water level (meniscus) to fall until it reaches the calibration mark.

DRY THE LOWER OUTSIDE OF THE PIPET AFTER FILLING TO REMOVE ANY EXCESS WATER AT THE TIP.

Dispensing from a Pipet

Place the pipet into the receiving container and release the water to the predetermined amount for Mohr (Graduated Pipets) or the full volume from volumetric (Transfer) pipets.

For volumetric (Transfer) Pipets, place the tip in contact with the wet sidewall of the receiving container (the surface tension created will aid in ensuring a full drainage of the pipet).

Avoid splashing water inside the receiving container (especially when it can end up above the fill capacity or outside of the container).

¹ If the pipet becomes stuck, heat the lower part of the rubber bulb that is around the pipet with hot water until the rubber expands enough to release the pipet. Use extreme care in removing the pipet from the bulb to avoid breaking the pipet.

Drain Time for Volumetric (Transfer) Pipets:

Remove the tip from contact with the sidewall of the receiving vessel within two seconds after the end of the flow of water to complete the delivery. According to ASTM E969², NO AFTER DRAINAGE PERIOD IS REQUIRED.

<u>Do not</u> blow any remaining water out of the lower tip of the pipet. Pipets are calibrated assuming a small amount of liquid will remain due to the surface tension of the water inside the tip.

Resources

ASTM E542-01 (2012) "Standard Practice for Calibration of Laboratory Volumetric Apparatus."

ASTM E969-02 (2012) "Standard Specification for Glass Volumetric (Transfer) Pipets."

Graphic 1 – <u>http://www.borosil.com/images/home_sub_images/7101(2).jpg</u>. Accessed March 14, 2014

Graphic 2 – <u>http://www2.hawaii.edu/~johnb/micro/m140/graphics/use_non-</u> serological.jpg. Accessed March 14, 2014

Graphic 3, Filler

http://chem.wisc.edu/deptfiles/genchem/lab/labdocs/modules/bulb3way/pic/3bulbpic2.gif Accessed March 14, 2014

Care and Cleaning of Pipets

NIST IR7383 – GMP 7 Good Measurement Practice for Cleaning Precision Glassware, April 2013. http://www.nist.gov/pml/wmd/labmetrology/upload/GMP_7_20130424.pdf

Glassware Safety (Wilmad LabGlass)

http://www.wilmad-labglass.com/uploadedFiles/Main_Site/Pages/Support/Safety.pdf. Accessed March 14, 2014

Safe Use of Laboratory Glassware

The information was adapted from: <u>https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/Bulletin/1/al_techbull_al259.pdf</u>. Accessed March 14, 2014

² Note: ASTM E969 states that Class A pipets between 15 mL to 50 mL will typically empty within 25 seconds and 100 mL pipets will empty within 30 seconds.

Meniscus Reader





