

**TROEMNER**

# Pipette Standards Handbook



RAISING  
THE  
STANDARD

# TROEMNER



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# Introduction



SECTION 1:  
INTRODUCTION



Troemner is pleased to offer the Pipette Standards Handbook to help clarify misconceptions surrounding proper techniques, operation, and calibration of these instruments. In a world of powerful analytical

instrumentation, volumetric liquid handling is an area that is sometimes forgotten. Anyone, who uses pipettes consistently in their work, should find this publication a helpful and practical aid.

This publication presents the concepts and practices accepted and employed by a majority of metrological and scientific individuals that are pertinent to current calibration practices and proper pipetting techniques. Obviously, when working in an accredited or other externally regulated environment, not only is

it important for the calibration to be accurate, but it must also have an appropriate data-trail. This is becoming increasingly important as laboratories apply for ISO/EN accreditation, and also in light of the recent classification of pipettes as medical devices by the DIN standard authority, acknowledging their crucial position in the manual performance of clinical diagnostic tests.

## SECTION 1: INTRODUCTION

Troemner was founded over 160 years ago in 1838 by Henry Troemner. The company started as a manufacturer of scales and weights in Philadelphia. Today, Troemner is an ISO 9001 registered company and is the world's leading supplier of precision weights and mass standards. We manufacture and certify weights that meet or exceed the highest tolerance standards of the American Society for Testing & Materials (ASTM) and the International Organization of Legal Metrology (OIML). Since 1995, Troemner's laboratories have been

accredited by the United Kingdom Accreditation Service (UKAS, formerly known as NAMAS) and the National Voluntary Laboratory Accreditation Program (NVLAP) which is sponsored and administered by the National Institute of Standards and Technology (NIST). Troemner offers either NIST/NVLAP or UKAS certification for precision weights.

Troemner's strong background in both metrology and mass calibrations made the decision to transition into the pipette calibration arena an easy one. Troemner is currently the only

company in the United States accredited by NIST/NVLAP for pipette calibrations. Our highly trained technicians perform all calibrations and strive to continue to improve methods and techniques to reduce our measurement uncertainties.

# Regulations and Standards



SECTION 2:  
REGULATIONS  
AND STANDARDS

## SECTION 2: REGULATIONS AND STANDARDS

There are several quality regulations pertaining to volumetric instrumentation and gravimetric pipette calibrations that are worth mentioning, since these documents essentially set the standards. They are as follows:

- 1) DIN Standard 12650<sup>1</sup> - The Standard Committee for Laboratory Devices and Equipment in the DIN (German Institute for Standardization) determines the regulations for conformity testing and certification. The latest draft (4th Standard Proposal, 1996-07) is subdivided into specific sections.

- DIN 12650-1: General Requirements
- DIN 12650-2: Piston-stroke pipettes
- DIN 12650-3: Dispensers
- DIN 12650-4: Diluters
- DIN 12650-5: Piston burettes
- DIN 12650-6: Gravimetric testing for measuring accuracy
- DIN 12650-7: Non-gravimetric test methods
- DIN 12650-8: Multichannel pipettes

The DIN standard describes the work involved with dispensing systems, stipulates requirements in respect to

physical properties, and lists detailed instructions for testing accuracy and precision of dispensing systems. The DIN standard also includes details of admissible limits of error for the various dispensing systems. “Maximum Permitted Errors” is defined as the inaccuracy plus twice the standard deviation.

The error limits are always based on the overall system of the pipette and the tip together. The error limits of pipettes with a nominal volume between the nominal volumes given in the table must correspond to the relative error limits for the next-highest nominal volume. If the

*Foot note:* 1. DIN Standard 12650- Available from: Henry Troemner, LLC, 201 Wolf Drive, P.O. Box 87, Thorofare, NJ 08086-0087 or Deutsches Institut für Normung, DIN/DQS Technorga GmbH, Kamekestr.8, D-50672 Köln

FIXED-VOLUME PIPETTES AND VARIPETTES

Nominal volume	ul	1	2	5	10	20
Error limit	+ ul	0.15	0.2	0.3	0.3	0.4
Nominal volume	ul	50	100	200	500	1000
Error limit	+ ul	0.8	1.5	2	5	10
Nominal volume	ul	2000	5000	10000		
Error limit	+ ul	20	50	100		

MULTI-CHANNEL PIPETTES

Nominal volume	ul	1	2	5	10	20
Error limit	± ul	0.3	0.4	0.6	0.6	0.8
Nominal volume	ul	50	100	200	500	1000
Error limit	± ul	1.6	3	4	10	20
Nominal volume	ul	2000				
Error limit	± ul	40				

DISPENSERS AND MULTIPETTE PIPETTES

Nominal volume	ul	10	20	50	100	500
Error limit	± ul	0.5	0.8	1.5	2	10
Nominal volume	ul	2000	>5000			
Error limit	± ul	10	40			

nominal volume of the pipette is exactly between two nominal volumes given in the table, the relative error limit for the lower volume is acceptable.

The error limits in accordance with the latest version of the DIN 12650 standard based on volume are as follows:

Variable pipettes must meet the standards of fixed pipettes at their maximum volume, no matter what their setting.

Thus, a variable 100ul pipette set at 10ul may have up to a 1.5 ul total error.

*\*Note:* Values given in the DIN Table are for factory or factory workshop testing. Users who test their own pipettes should meet twice the listed standards.

## SECTION 2: REGULATIONS AND STANDARDS

- 2) ASTM E1154-89<sup>2</sup> - This specification covers requirements, operating conditions, and test methods for piston or plunger operated volumetric apparatus.
- 3) ISO Guide 25<sup>3</sup> (proposed revision: ISO/IEC IS 17025) - This guide sets out the general provisions which a laboratory must address to carry out specific calibrations or tests. ISO Guide 25 provides the laboratories direction for the development and implementation of a fundamental quality management system.
- 4) ISO 3696<sup>4</sup> - This standard pertains to the specifications of the water used during the calibration procedure. It sets maximum resistivity, TOC (total organic carbon), absorbance, and silica content values. In order to establish compliance, the water must be within the enumerated parameters.
- 5) GLP Standards<sup>5</sup> - Good Laboratory Practices (GLPs) not only address the organizational aspects of operation and the conditions under which laboratory tests are scheduled, conducted, and monitored; but, also, the aspect of recording and reporting test results. GLP principles are basically used for testing substances and acquiring data on their properties and/or their harmlessness to human health and the environment.
- 6) European Parliament directive on in vitro diagnostic products<sup>6</sup> - This directive was issued in line with the harmonization of individual European state legislation and it stipulates minimum requirements for the free movement of in vitro diagnostic products within its areas of jurisdiction.

*Foot notes:* 2. ASTM E1154-89- Available from: Henry Troemner, LLC, 201 Wolf Drive, P.O. Box 87, Thorofare, NJ 08086-0087 or American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959

3. ISO Guide 25-Available from: Henry Troemner, LLC, 201 Wolf Drive, P.O. Box 87, Thorofare, NJ 08086-0087 or The International Organization for Standardization -Is Irue de Varembe, Case Postak 56, CH-1211 Genève 20, Switzerland

4. ISO 3696-Available from: The International Organization for Standardization -Is Irue de Varembe, Case Postak 56, CH-1211 Genève 20, Switzerland

5. GLP Standards-Available from: FDA (HFE-88), Office of Consumer Affairs, 5600 Fishers Lane, Rockville, MD 20857

6. European parliament directive on in vitro diagnostic products -Available from: Allée du Printemps, B.P. 1024/F, F-67070 Strasbourg Cedex

7) NIST/NVLAP<sup>7</sup> – The National Voluntary Laboratory Accreditation Program (NVLAP), sponsored by the National Institute of Standards and Technology (NIST), assesses and accredits organizations based on their technical ability and quality system, following ISO Guide 25 (proposed revision: ISO/IEC IS 17025).

8) NCCLS<sup>8</sup> – The National Committee for Clinical Laboratory Standards (NCCLS) is a globally recognized, voluntary consensus, standards-developing organization that

enhances the value of medical testing within the healthcare community through the development and dissemination of standards, guidelines, and best practices.

9) CAP<sup>9</sup> – The College of American Pathologists (CAP) is an organization that accredits in order to improve the quality of clinical laboratory services throughout the United States, through voluntary participation, professional peer review, education, and compliance with established performance standards.

10) CLIA<sup>10</sup> – The Clinical Laboratories Improvement Act (CLIA) is a document that outlines minimum standards for personnel, testing, and quality control for clinical laboratories.



7. NIST/NVLAP-Available from: National Voluntary Laboratory Accreditation Program (NVLAP), National Institute of Standards & Technology, 100 Bureau Drive, Stop 2140, Gaithersburg, MD 20899-2141

8. NCCLS-Available from: Henry Troemner, LLC, 201 Wolf Drive, P.O. Box 87, Thorofare, NJ 08086-0087 or National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898

9. CAP-Available from: Henry Troemner, LLC, 201 Wolf Drive, P.O. Box 87, Thorofare, NJ 08086-0087 or The College of American Pathologists, 325 Waukegan Road, Northfield, IL 60093

10. CLIA-Available from: US Department of Health & Human Services, Room 645-F Hubert H. Humphrey Bldg., 200 Independence Ave., S.W., Washington, DC 20201

# Types of Pipettes



SECTION 3:  
TYPES OF  
PIPETTES

## SECTION 3: TYPES OF PIPETTES

There are three basic types of pipettes in use in the laboratory today: glass pipettes, air displacement pipettes, and positive displacement pipettes.

1) Glass pipettes are volumetric pieces of glass calibrated to deliver a specific volume of liquid. They are scribed as either a TD (“to deliver”) or a TC (“to contain”) instrument at a specific temperature which is usually 20°C.

A. Most glass pipettes are scribed “to deliver” which means that the residual sample must **not** be “blown out” when



dispensing a reagent.

B. Pipettes that are scribed “to contain” should be “blown out” with some type of pipette bulb.

These types of instruments are normally only used to deliver

reagents to samples. They are seldom used to dispense an analyte into a flask for dilution purposes. Also, these instruments are usually made of borosilicate glass and are traditionally thrown away after each use. If the instrument is used multiple times, it can not be “calibrated” in the true sense of the word since the graduations



## SECTION 3: TYPES OF PIPETTES

can not be removed. However, this instrument can be tested and “verified”.

2) Air displacement pipettes are pipettes that have a piston in a cylinder or capillary tube that moves to the appropriate position once the volume is set. When the operating button is depressed to the first stop, the piston expels the same volume of air that is indicated on the micrometer setting. Once the tip is immersed into the liquid, the operating button is released, which creates a partial vacuum that aspirates



the specified volume into the tip. When the operating button is depressed to the first stop again, the air dispenses the liquid. In order to empty the tip completely, the operating button is pressed to the second or “blow out” stop. The key feature of an air displacement pipette is the fact that a specified volume of air always remains between the piston and the liquid.

3) Positive displacement pipettes are also pipettes that have a piston in a cylinder or capillary tube that

moves to the appropriate position once the volume is set. However, this type of pipette always has the piston in direct contact with the liquid. Most customers that select these types of instruments do so because the liquids they are pipetting have different characteristics than that of water. Liquids with a high vapor pressure will tend to evaporate inside the pipette and liquids with a higher density or viscosity will tend to expand the column of air inside

the pipette. By using this type of instrument for these types of applications, the user will get a more accurate result than they would if they had used an air displacement pipette. Sample-to-sample and cross-contamination are kept to a minimum by using microsyringe tips that are disposable. The operation of this type of pipette is very similar to an air displacement pipette with a few, very important exceptions. First, the piston moves to the appropriate position when the volume is set, so when the

operating button is depressed to the first stop, the piston descends to the tip opening. When the tip is immersed into the liquid and the button is released, the plunger is raised creating a partial vacuum which causes the liquid to enter the tip. Finally, when the operating button is depressed again, the piston descends, expelling liquid from the tip. Most people do not use positive displacement pipettes routinely because of both the added cost of tips and seals and the inconvenience of changing them.

# Proper Pipetting Techniques and Tips



SECTION 4:  
PROPER PIPETTING  
TECHNIQUES AND TIPS

There are many techniques and tips available that will optimize your pipetting performance and increase the reproducibility of your results.

A brief description of each follows:

### The Equipment

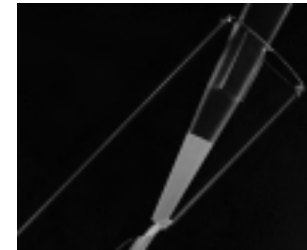
1) Tips - It is advocated that only high quality tips which optimize the pipette's performance be used. A high quality tip is one that has a smooth uniform interior with straight even sides that prevents the retention of liquids and minimizes surface wetting. Also, the tip should have a clean, hydrophobic surface and a

perfectly centered opening in order to ensure the complete dispensing of the sample. These tips should always securely interface with the nosecone, because if they do not fit correctly, the amount of liquid dispensed can be dramatically influenced.

2) Liquid Viscosity - Since the pipette was originally factory calibrated using water, any liquid that has a viscosity higher or lower than water will impact the volume dispensed. Viscosity differentials should be accounted for and taken into consideration in order

to enhance the accuracy of the instrument.

3) Container - The material of construction for the extraction vessel is also important, since some materials tend to force water into a convex configuration while other materials force water into a concave configuration.



## SECTION 4: PROPER PIPETTING TECHNIQUES AND TIPS

Obviously, this can impact the amount of liquid drawn into the tip. A glass container is recommended since it tends to force water into a concave configuration which helps to reduce or eliminate variations due to this effect.

### The Operator

1) Technique - Most end users have a tendency to believe that the volume delivery is completely dependent on the setting of the micrometer dial. Obviously, this is not the case, since many factors associated with pipettes



come into play.

- Position - Pipettes should be held vertical during the aspiration of liquids, however, some end users often hold pipettes at many different angles during a pipetting

interval. Holding a pipette 30° off vertical can cause as much as 0.7% more liquid to be aspirated due to the impact of hydrostatic pressure. Always store pipettes in an upright position when not in use.

- Pre-Wetting/Pre-Rinsing Tips - Failing to pre-wet tips can cause inconsistency between samples since liquid in the initial samples adhere to the inside surfaces of the pipette tip, but liquid from later samples does not. Also, if a new volume is dialed in on the pipette's micrometer, you will receive better results at the new

volume by taking the old tip off and placing a new one on the shaft before you commence pipetting.

- Release of Plunger - Releasing the plunger abruptly can cause liquid to be “bumped” inside the pipette during a liquid transfer application. This can cause liquid to accumulate inside the instrument which in turn can be transferred to other samples causing variability in sample volume and the potential for cross contamination. It is recommended that a smooth, consistent pipetting rhythm be

employed since it helps to increase both accuracy and precision.

After the liquid has been aspirated into the tip, the pipette should be placed against the wall of the receiving vessel and the plunger slowly depressed. This will help all of the liquid in the tip to be dispensed. After a pause of about 1 second, depress the plunger to the bottom or blowout position (if equipped) and remove the pipette from the sidewall by utilizing either a sliding action up the wall or a brief movement away from the wall (called “touching off”).

- Immersion Depth - The pipette tip should only be inserted into the vessel containing the liquid to be transferred about 1-3mm. If the tip is immersed beyond this, the results could be erroneously high. This is due to the fact that liquid could adhere to the tip and be transferred along with the aliquot in the tip. If the tip is not immersed far enough then air could be drawn into the tip which could yield results that are incorrect on the low end.

## SECTION 4: PROPER PIPETTING TECHNIQUES AND TIPS

- **Equilibration Time** - Troemner recommends that the tip, the pipette, the liquid being transferred, and the transfer container itself all be allowed to equilibrate to the same temperature. This is done to lessen the effects of thermal expansion which can dramatically impact the delivered volume.
- **Thermal conductance** - Thermal energy can be transferred from the operator's hand to the air within the pipette (dead air) or even to the internal components themselves. This can have a

dramatic impact on the amount of liquid dispensed due to the effects of expansion and/or contraction. To lessen this effect, it is recommended that some type of thermally insulated gloves like latex or cloth be worn.

- 2) **Pipette Micrometer Setting** - It is important to avoid significantly overdialing or underdialing the recommended range of the pipette. Volume delivery performance may change radically and may become completely undefined.



### **The Environment**

- 1) **Temperature** - The volume delivery performance specifications of pipettes have been referenced by most manufacturers at room temperature which is defined as 20-25°C. Any deviation

from this specification can affect the amount of liquid dispensed due to the expansion or contraction of the internal components. Temperature is probably the most important factor that influences pipette performance. In fact, the density of water in a gravimetric analysis is calculated as a function of temperature.

2) Barometric Pressure - Pressure is reduced by 1.06" Hg for every 1000' of elevation, however, barometric pressure has only a small effect on the density

formula, so the error encountered in not correcting for elevation is often ignored.

3) Relative Humidity - This is the percentage of moisture in the air at a measured dry bulb temperature compared to the amount of moisture that the air can hold at that temperature if the air is 100% saturated. Relative humidity exerts a major influence on taking accurate measurements of volume delivery. Under dry conditions, which are defined as less than 30% RH, it is extremely difficult to ensure an accurate

measurement due to the rapid evaporation rate. Conversely, excessive humidity which is defined as greater than 75% can cause a measurement to be erroneously high due to condensation. Therefore, generally accepted guidelines for pipette volume delivery specify that relative humidity be maintained within the range of 45%-75%. Relative humidity also has an effect on the delivery of air displacement pipettes specifically. This is due to the evaporation of liquid from the upper surface of

## SECTION 4: PROPER PIPETTING TECHNIQUES AND TIPS

the meniscus inside the the tip as the liquid is being aspirated. Thus, you would expect that a pipette which is calibrated in a controlled 60% RH laboratory will test differently at a 30% RH user location. The amount of difference can be up to about 1%, depending on the details of the pipette and the testing method used.

# Methods of Calibration



SECTION 5:  
METHODS OF  
CALIBRATION

## SECTION 5: METHODS OF CALIBRATION

The two most common techniques of calibrating pipettes are the gravimetric and colorimetric (a.k.a. photometric) methods. Of these, the gravimetric method is the most common and the most widespread in use today. This method requires a stringently controlled environment, a high precision balance, a highly skilled pipetting technician, and a rudimentary understanding of statistics. The principle of this method is simple in that, given a certain mass of water with a known specific gravity, its volume can then be predicted. The accuracy and precision of the pipette can then be

assessed by using an appropriate statistical approach. This method can be performed one of four ways: Addition, Addition-Tare, Subtraction, or Subtraction-Tare.

- 1) Addition is perhaps the most common mode of pipette calibration and it is performed by using the cumulative weight of a liquid to determine the volume dispensed.
- 2) The Addition-Tare method is performed by taring the balance each time before dispensing.
- 3) The Subtraction method uses the total subtracted weight of a liquid

to determine the volume aspirated by the pipetting device. In this technique, you tare the balance only once, at the beginning, then you aspirate volumes of liquid from the vessel, take cumulative (negative) weights, and then calculate the volume aspirated based on the difference between the current and previous total weights.

- 4) The Subtraction-Tare method entails taring the balance each time before removing liquid from the vessel.

Since this method is not fool-proof, all variables must be stringently controlled and accounted for in order to produce results that are statistically accurate.

The second most common type of pipette calibration process is the colorimetric or photometric method. This method involves the analysis of volumes of diluted dye in a cell of known path length. According to the Beer-Lambert Relationship, if a beam of monochromatic light passes through homogeneous solutions of equal pathlength, the absorbance measured is proportional to the dye

concentration. So, with this in mind, an unknown volume of dye can be pipetted into a known volume of diluent, the resulting dye concentration can be measured photometrically, and the volume can be calculated.

This method is less prone to environmental influences, but it requires the use of standardized consumables. Obviously, this means that each lot of standardized dye must be very carefully manufactured and calibrated in order to produce results of high accuracy. However, once solutions are prepared, calibrated and

shown to be stable, accurate results can be obtained even at volumes less than one microliter<sup>11</sup>.

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# Factors Affecting Calibration



SECTION 6:  
FACTORS AFFECTING  
CALIBRATION

When performing a pipette calibration using a gravimetric method, there are many factors to consider in order to optimize your results. A brief description of each follows:

### **The Equipment**

*Balance* – Balances with an internal weight can be considered “self calibrating” and periodic servicing of these instruments is extremely important in order to consistently produce quality pipette calibrations. Balances that do not have an internal weight should be calibrated using several external weights for each



range. It is also recommended that the sensitivity of the balance be appropriate for the volume of the pipette. Furthermore, balances must sit level and be placed on a stable

table or platform.

*Tips* - See tip description in Proper Pipetting Technique and Tips section.

*Calibration liquid* - The liquid should only be bidistilled, degassed water. This is the substance that a large majority of pipette manufacturers recommend and what they use in order to recalibrate an instrument.

### **The Operator**

*Technique* - Many factors that are important in proper pipetting techniques also come into play when calibrating a pipette as well. These

## SECTION 6: FACTORS AFFECTING CALIBRATION

include proper pipette position, pre-wetting tips, release of the plunger, immersion depth, the container, equilibration times, and thermal conductance. However, there is one other factor that must be taken into account and it is as follows:

**Number of Measurements** – It is recommended that a minimum of 4 measurements per channel per volume be performed. Anything below this can not be statistically justified and may call the results into question.

### **The Environment**

Many of the same environmental conditions that affect pipette performance also come into play when a calibration is performed such as temperature, barometric pressure, and relative humidity. However, there are still many other factors that must be taken into account to perform a high quality calibration. A description of each follows:

*Vibration* – Since the calibrator is essentially weighing a liquid, the less that a balance is affected by vibration the better.

*Air Movement* – Air movement must be adequate and properly distributed so that it does not cause movement of the balance pan.

*Evaporation* – Evaporation rates must be monitored and accounted for or a humidity trap must be used to minimize the effects of this variable. Evaporation rates are related to relative humidity and can be reduced if RH is controlled effectively, but in order to produce the highest quality calibrations they must be taken into consideration separately as well. This is especially important, since other factors also

influence evaporation besides RH such as the shape of the container, static electricity, drafts, season of the year, and geographic location.

*Air buoyancy* – Air buoyancy is a net upward force due to higher pressures at lower altitudes. Since in a gravimetric analysis, you are essentially converting the “weight” of a liquid to a volume, you must have an air buoyancy correction to get to the true mass.

While the colorimetric technique is less vulnerable to environmental factors than the gravimetric method,

it is not totally free of concerns. The major environmental concerns are temperature equilibrium and cleanliness. These are discussed below.

- A) All materials (e.g. the dye solutions, spectrophotometer, cuvette, pipette, tips, etc.) must be at the same temperature. Thermal equilibrium at room temperature for several hours, or overnight if possible will produce more accurate results.
- B) The cuvette used in the spectrophotometer must be clean and free of scratches or dust. Finger-

prints, smudges or scratches can negatively impact accuracy, while dusty environments will reduce precision.

Additional considerations include proper selection of the spectrophotometer, careful engineering of dye solution properties, and accurate calibration of the dyes:

- A) Photometric noise can be a significant source of measurement uncertainty. Therefore, care must be taken to select a spectrophotometer with suitable performance specifications. Such an instrument should be maintained and

## SECTION 6: FACTORS AFFECTING CALIBRATION

tested periodically to ensure its continued good performance.

B) The dyes used for calibration work must be stable, have a well characterized absorbance – concentration relationship, and have physical properties very similar to water. Most pipettes are calibrated so that the volume setting corresponds to the volume of distilled, degassed water. The dyes must be tested to ensure that an air displacement pipette “handles” the dye like water. This concern is much less important with positive displacement

devices.

C) The absorbance of each dye lot must be carefully measured.

If the dye absorbance changes with temperature, this must be measured and included in a temperature correction calculation<sup>12</sup>.

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# Servicing and Calibration Procedures



SECTION 7:  
SERVICING AND  
CALIBRATION  
PROCEDURES

## SECTION 7: SERVICING AND CALIBRATION PROCEDURES

Several factors to consider when calibrating a pipette or choosing a calibration service:

- 1) If you require the “as found” data, it is advisable to obtain this before any parts or components are replaced since this can drastically change your results.
- 2) Clean and inspect the instrument for any visible signs of wear and tear. Make sure that the instrument can be autoclaved before autoclaving, since this can seriously damage the pipette.
- 3) Replace the pipette’s seals and o-rings and any other part that shows signs of wear. Remember to pay special attention to the piston and replace if it seems especially worn or bent.
- 4) Ensure that the o-rings and seals have seated properly by performing a leak test and a vacuum test.
- 5) Allow the pipette to stabilize in an environmentally controlled, vibration-free room for a 24-hour period to eliminate the effects of thermal expansion.
- 6) Decide which calibration technique that you wish to employ (i.e. Addition, Addition-Tare, Subtraction, or Subtraction-Tare).
- 7) Prepare the balance by “exercising” it and modifying it to accept a liquid containing vessel. It is our recommendation to use a glass container, so that the liquid has a concave meniscus.
- 8) Since most manufacturers originally calibrate their pipettes between 20-25°C while using bidistilled, degassed water, it is our recommendation that these conditions are duplicated.

- 9) Wear some type of thermally insulated gloves to lessen the transfer of heat from your hand to pipette. Latex or cloth seem to work the best.
- 10) Begin the liquid transfer stage of the calibration procedure utilizing the appropriate technique that you have chosen to employ.
- 11) Record the weighings, so that they can be converted into volumetric readings at the end of the calibration procedure.
- 12) Make the conversion taking into account all pertinent environmental conditions. Usually these conditions are used to calculate a Z-factor which is in turn used to convert from a mass reading to a volumetric reading.



## SECTION 7: SERVICING AND CALIBRATION PROCEDURES

- 13) Compare the results to the manufacturers tolerances (or your “in house” tolerances) for the particular instrument in question. If the pipette is out of calibration, adjust it according to the guidelines set forth by each manufacturer and repeat steps 10-12.

(Remember: Accuracy is a function of the adjustment of the pipette and precision is a measure of the quality of the instrument in its particular state. An instrument that is imprecise usually needs to be repaired or replaced.)

# Commonly Asked Questions



SECTION 8:  
COMMONLY ASKED  
QUESTIONS

## SECTION 8: COMMONLY ASKED QUESTIONS

*My pipette seems to leak periodically. What are the causes and the solutions to this problem?*

**Answer:** Pipettes can leak for the following reasons:

- a) The tip is incorrectly attached.
- b) There is a foreign object (i.e. dirt, grit, etc) between the piston, o-ring, and nosecone.
- c) The o-ring has been damaged or warped.

Solutions to the aforementioned problems:

- a) Securely attach the tip to the nosecone.
- b) Clean and grease tip cone module and o-ring; attach new tip.
- c) Change the o-ring.

*My pipette has just been calibrated, but it seems to be dispensing inaccurately. What can I do to resolve this problem?*

**Answer:** The pipette operator may not be observing good pipetting techniques. Based on our experience, as many as 25% of all pipetting errors are directly related to the operator. You may also want to check to see if the tips are securely attached. If this problem persists, then the calibration might have been altered due to misuse (i.e. dropping the instrument, overwinding/underwinding the micrometer, aspirating liquid into the interior of the pipette, etc.)

*Can my pipette be autoclaved?*

**Answer:** Some pipettes can be autoclaved while others can not. It is best to check with the manufacturer or Troemner if there is ever any doubt.

*How many times per year must a pipette be calibrated?*

**Answer:** It really depends on the number of times the pipette is used and on the quality standards of the laboratory. However, ASTM E 1154-89 11.2.3 recommends that pipettes receive a comprehensive evaluation at least on a quarterly basis.

*Normally how many piston positions are there and what are their names?*

**Answer:** Normally there are four piston positions in a one component stroke system pipette and five piston positions in a two component stroke system pipette. They are listed and defined as follows:

Prepare - This is where the plunger is pressed down to the intermediate/ bottom stop at the beginning of the pipetting procedure in order to prepare liquid to enter the tip.

Aspirate - This is the point in the pipetting procedure where the plunger is slowly released and the liquid actually enters the tip.

Deliver - This is where the liquid is delivered to the receiving vessel by pressing the plunger down until the intermediate stop is reached.

## SECTION 8: COMMONLY ASKED QUESTIONS

Blow out - This is the point at which the plunger is fully depressed until the bottom stop is reached in order to “blow out” any residual liquid that remains in the tip. This is an important feature to have on an air displacement pipette, since the entire sample does not leave the instrument at the first step due to the compressibility of air and the adherence of liquid to the pipette tip. (This position is absent in a one component stroke system pipette).

Return - This is the final stage where the plunger is released and allowed to return to its starting position.

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# Terminology



SECTION 9:  
TERMINOLOGY

## SECTION 9: TERMINOLOGY

**Accuracy** - The closeness of agreement between the nominal or accepted value and the measured value.

**Aspirate** - The process of withdrawing a substance with a negative pressure apparatus such as a pipette or syringe.

**Autoclaving (steam)** - The act of placing an instrument inside a machine specifically designed to sterilize by reaching very high temperatures and pressures.

**Dead Volume** - The part of the total liquid volume that is held in

the operational part of the device and not delivered.

**Dilutor** - A measuring instrument designed to take up different liquids and deliver them in combination so that they comprise a predetermined ratio, a predetermined volume, or both. The reservoir of diluent may be integrated with the instrument or connected externally.

**Dispenser** - A measuring device designed to deliver predetermined volumes of liquid from a reservoir. The reservoir may be integrated with the instrument or connected externally.

**Expansion Factor** - The quantification of expansion due to thermal conductance.

$$K = 1 - \alpha(T-20)$$

*Where:*

K = Expansion Factor

$\alpha$  = Cubic expansion coefficient

T = Temperature, degrees Centigrade

**Hydrophilic (polar)** - Any substance that attracts, dissolves in, or absorbs water.

**Hydrophobic (nonpolar)** - Any substance that repels or will not absorb water.

**Isothermal Condition** - This means that the pipette and the environmental temperature are equal. This is accomplished by allowing the pipette to equilibrate to the temperature of the laboratory for a certain period of time.

**Nominal Volume** - the stated volume for which performance is specified.

**Pipette** - A hand held measuring instrument designed to deliver a predetermined volume of liquid from one vessel to another. A pipette is independent of the reservoir.

**Plunger** - A piston-like reciprocating part moving within the cylinder of the pipette.

**POVA** - A piston or plunger operated volumetric apparatus.

**Precision** - The reproducibility of multiple measurements and is usually described by the standard deviation, standard error, or confidence interval.

**Pre-rinsing/pre-wetting**- The action of pre-coating the inside of the liquid contacting parts with a thin film of the same liquid to be pipetted.

**Standard Deviation** - A statistical measure of the degree of variation of a set of quantitative data around its mean.<sup>13</sup>

**Uncertainty** - A measure of the inherent variability of repeated measurements of a quantity.

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13. Duff, L. David, "The Micropipette Primer," June 1998, A-Metrology-Z

