

Bio-Tek Services, Inc.



2008-2009 Pipette Standards Handbook

**Written By:
Kenneth Bonnell
Quality Manager
Bio-Tek Services, Inc.**

**Bio-Tek Services, Inc.
1160 Warwick Park Road
Suite C
Richmond, VA 23231
Toll-Free: 800-792-3625
Phone: 804-222-5833
Fax: 804-222-5360
ken.bonnell@biotekservices.com
www.biotekservices.com**

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

Click
Here

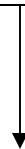


TABLE OF CONTENTS

*****Click on any page number below in order to immediately go to that section*****

Introduction.....Pg. 3

Company History.....Pg. 4

Regulations and Standards.....See Below

ISO 8655 Standard.....Pgs. 5-9

DIN 12650 Standard.....Pgs. 9-10

ASTM 1154-89 Standard.....Pg. 10

ISO/IEC IS 17025 (formerly ISO Guide 25).....Pgs. 10-11

ISO 3696 Standard.....Pg. 11

GMP/GLP Standards.....Pg. 11

European Parliament Directive on In Vitro Diagnostic Products.....Pg. 11

NCCLS.....Pg. 11

CAP.....Pg. 11

CLIA.....Pg. 11

Types of Pipettes.....Pgs. 12-13

Methods of Calibration.....Pgs. 13-15

Components of a Quality Calibration.....Pgs. 16-20

Servicing and Repair Procedures.....Pgs. 20-23

Selecting a Quality Pipette Calibration Provider.....Pgs. 23-24

Glossary.....Pgs. 25-26

Acknowledgement.....Pgs. 27-28

Calculations.....Pgs. 29-31

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

INTRODUCTION

Bio-Tek Services, Inc., a leading metrology company, is pleased to offer this Pipette Standards Handbook in order to help clarify any misconceptions surrounding the proper use, maintenance, operation, or calibration of pipettes and other liquid handling devices. We believe that these instruments are the most misunderstood quantitative tools in use in laboratories today. Pipettes have advanced to the point in terms of technology and price that they should no longer be considered “consumables” or “throw-away” items. These instruments can deliver almost any volume very accurately and precisely if they are maintained and serviced correctly.



Good measurements and quality data can only be realized if these instruments are tested and calibrated on a periodic basis. However, the methods for selecting a quality service provider or the appropriate procedures for testing or verifying these instruments are not so obvious. The Code of Federal Regulations (21 CFR), cGMP (Current Good Manufacturing Practices), cGLP (Current Good Laboratory Practices) and various other regulatory precepts require periodic inspection and maintenance of all liquid handling instruments. Proper care of these devices has also been brought to the forefront by those laboratories seeking accreditation and by the recent classification of pipettes as medical devices by the DIN standard authority, acknowledging their crucial position in both clinical and diagnostic tests. This publication will present the concepts and practices employed by members of the metrological and scientific communities and it will address topics ranging from the proper use and maintenance of these instruments to the criteria that should be used when selecting a quality service provider. This publication should be a useful guide to anyone that utilizes these devices on a regular or periodic basis.



Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

COMPANY HISTORY

Bio-Tek Services, Inc. was founded in 1998 and subsequently became incorporated in 2001. We have become the forerunner in laboratory instrument calibrations through our expertise, precision, attention to detail, modernization, and implementation of six sigma methodologies. All of our processes and procedures are written and designed around the voice of the customer (VOC), ISO/IEC 17025, 21 CFR, and GLP/GMP compliance. These strategies have worked well and the business has continued to expand and develop, because we have adopted a culture of continuous process improvement in respect to our training, education, and quality system.

Bio-Tek Services, Inc. services a diverse and cross-functional group of customers including all of the following:

- Major university research centers
- Pharmaceutical
- Biotech
- Petrochemical companies
- Testing laboratories
- Major manufacturing companies.

There are many factors that have contributed to our success and differentiate us from other calibration service providers including all of the following:

- We utilize the best technology available in the form of state-of-the-art equipment and standards
- All our calibrations are traceable to NIST (the National Institute of Standards and Technology)
- We account for all factors that contribute to measurement error and produce results with very small uncertainties
- We remain educated and current on new and existing FDA and regulatory standards that govern all liquid handling devices

Our calibration service leaders are former scientists that have worked in the areas of chemistry, microbiology, and metrology, which means that we understand the logistical and quality challenges that our customer face on a daily basis. All of our laboratory associates and calibration technicians are well versed in metrology and various gravimetric and photometric measurement systems. This experience has been coupled with extensive training both in-house and by several industry leading manufacturing companies. Our pipette program is truly one of the best in the world and we are always searching for ways to improve the quality of our service in order to further strengthen our core competencies.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

REGULATIONS AND STANDARDS

There are several standards that govern the use and calibration of various liquid handling devices. These standards not only pertain to the instruments themselves, but they also define certain parameters that should be observed during a gravimetric or photometric calibration. The regulations that are listed below are truly significant, since they essentially set the standards to which all regulated companies must comply. They are as follows:



1. ISO 8655 (a.k.a. “The New DIN Standard”)

Part 1: Terminology, general requirements, and user recommendations – This standard governs all piston operated volumetric apparatus (POVA).

- Considerable discussions have taken place surrounding the way that results are expressed, since they can be reported in terms of accuracy (i.e. trueness and precision), inaccuracy (i.e. untrueness and imprecision), or by a mathematical combination of the aforementioned (maximum permitted error or “uncertainty”). Due to the fact that maximum permitted error criteria can be met by a device which has a high precision but less than acceptable trueness, separation of the two parameters has been adopted.

NOTE: “Maximum Permitted Error is defined as the inaccuracy plus twice the standard deviation”.

- Trueness is defined as “the closeness of agreement between the mean of a number of measured values and the nominal value of a parameter.”
- Precision is defined as “the closeness of agreement between results in a series of measurements of the same nominal volume.”

Part 2: Piston Pipettes

- Pipettes may be either fixed volume, which are designed to dispense only one specific pre-set amount, or they may be adjustable volume, which are designed to dispense volumes within a specific range.
- The piston may be in contact with the liquid (positive displacement) or there may be a cushion of air between the sample and the piston itself (air displacement).
- Piston pipettes must be adjusted by the manufacturer or an acceptable service provider before being delivered to the end user. The adjustments must be performed in an environment that has a standard reference temperature between 15°C – 30°C and relative air humidity above 55%. The calibration liquid must conform to ISO 3696, which governs bi-distilled, degassed water.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- If a pipette is out of tolerance after is used or received by the end user, it must be tested and calibrated according to the manufacturer's specifications. This out of tolerance condition must be annotated on the calibration certificate.
- If a pipette is adjusted to accommodate viscous fluids, the pipette must be altered by the user so that an unintentional readjustment cannot be made. Also, the name of the liquid that it was adjusted for should be indicated on the instrument as well as the certificate.
- High quality tips should be used and the "touching off" technique should be employed.
- End users should establish a regular testing routine for their piston pipettes.

The following can be possible sources of error for piston pipettes with an air interface:

1. Variation in air pressure (adjustment vs. use)
 2. Differences in density between the liquid being pipetted and that of the water used for adjustment
 3. Differences in vapor pressure
 4. Viscosity differentials
 5. Leaky piston/cylinder system
 6. Uneven piston movement
 7. Uneven rhythm and timing during pipetting
 8. Depth of plunging of the pipette tip
 9. Angle of pipetting
 10. Variations in pipette temperature and/or liquid temperature
 11. Changes in relative humidity
 12. Failure to pre-wet pipette tip
 13. Failure to wipe tip on the vessel wall ("touching off")
 14. Poorly seated tips/reusing tips/straightness of pipette tips
- User defined tolerances (e.g. "in house tolerances") should not exceed 100% of the maximum permissible error enumerated in the standard
 - Testing should be performed at 10%, 50%, and 100% of nominal volume

Part 3: Piston Burettes

- Piston burettes are used for the accurate delivery of liquids. In contrast with piston operated pipettes, dispensers, and dilutors, which are designed to accurately dispense preselected volumes, piston burettes are required to dispense volumes of liquids until external criteria such as pH or conductivity are met, at which point it is necessary to know the accurate volume dispensed.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- Prior to delivery, the piston system is charged by aspiration with a liquid from some reservoir. After air bubble-free filling of the system, movement of the piston in one direction dispenses the liquid and movement in the other direction recharges the system
- The piston can be operated manually, electronically, pneumatically, or hydraulically. The delivered volume can be indicated mechanically or by electronic means.
- The drive, the piston, and the cylinders can be one unit or modular.
- Piston burettes can be equipped with or without a valve or with several piston/cylinder systems, which dispense continuously.
- Instruments should be tested and calibrated by the manufacturer or an acceptable service provider before use by the end user.
- Testing should be at 10%, 50%, and 100% of the nominal volume.

Part 4: Dilutors

- A dilutor is designed to accurately aspirate a measured volume of a sample liquid and deliver it together with an accurately measured volume of diluent.
- Dilutors may be operated manually, electronically, pneumatically, or hydraulically.
- They may be hand-held, bottle-top, freestanding benchtop instruments, or they may be a component part of an automated analyzer.
- The diluent piston system is charged by aspiration of diluent from a reservoir. After air bubble free filling of the system, diluent is drawn into the volume-measuring cylinder by the diluent piston, either directly, via the uptake and delivery probe, or indirectly, from a reservoir until a volume controlling limit is reached. A measured volume of sample is then aspirated into the uptake and delivery probe.
- Instruments should be tested and calibrated by the manufacturer or an acceptable service provider before use by the end user.
- Testing should be at 10%, 50%, and 100% of the nominal volume

Part 5: Dispensers

- Dispensers are used for the accurate delivery of preset liquid volumes.
- The piston can be operated manually, electronically, pneumatically, or hydraulically.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- Drive mechanism, piston and cylinder can be a single unit or can be separated by simple hand actions, so that different pistons and cylinders can be used with the same drive mechanism.
- During operation, the aspiration duct dips into the reservoir containing the fluid to be dispensed. After the system has been primed with the fluid, the piston aspirates fluid by moving in one direction and delivers the fluid to be measured by moving in the opposite direction.
- Dispenser can be constructed with or without valves.
- During testing and calibration, the instrument should be tested with the filling tube that will be used during the normal operation.
- Instruments should be tested and calibrated by the manufacturer or an acceptable service provider before use by the end user.
- Testing should be at 10%, 50%, and 100% of the nominal volume.

Part 6: Gravimetric Test Methods

- Analytical balances must conform to OIML R76-1 and they must have the appropriate readability for the volume under test.

Volume under Test (μl)

Minimum Readability Required (mg)

0 to 10 μl – Balance Readability 0.001mg

10 to 100 μl – Balance Readability 0.01mg

Above 100 μl – Balance Readability 0.1mg

- The weighing vessel must be capable of holding between four to ten times the total volume of test liquid required during the testing procedure. The height to diameter ratio of the weighing vessel must be at least 3:1.
- All aliquots must be timed. The test cycle time or the time that it takes to completely dispense one aliquot should not exceed 60 seconds. In order to verify this, a stopwatch accurate to 0.1 seconds must be present. (Note: Most pipette calibration software packages that are compliant to ISO 8655 have added this feature – example: Pipette Tracker by Labtronics).
- The test shall be carried out in a draft free room with a stable environment. The relative humidity must be above 55% and the temperature must be between 15°C to 30°C with a stability of 0.5°C. The thermometer must be accurate to 0.1°C and the hygrometer must be accurate to 5%.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- The instrument and the test water must stabilize in the calibration room for at least one hour prior to testing.
- The test water must be degassed, double distilled “grade 3” water that conforms to the ISO 3696 standard.
- Evaporation traps must be employed or the test water must be delivered into a capillary tube assembly to prevent evaporation errors.
- All air displacement instruments must be tested in the “addition” or “addition-tare” mode. (Note: Many ISO/IEC 17025 auditors also believe that this is the only acceptable calibration method).
- The barometric pressure must be monitored and incorporated into the calculation of the Z-factor. The barometer must be accurate to 0.1kPa.
- The instrument must be assessed for both accuracy and imprecision.
- Testing should be at 10%, 50%, and 100% of the nominal volume.

2. DIN Standard 12650

The Standard Committee for Laboratory Devices and Equipment in the DIN (the German Institute for Standardization) determines the regulations for conformity testing and certification. The latest draft (4th Standard Proposal, 1996-07) is very similar to the ISO 8655, since it is also divided into sections.

- ✓ DIN 12650-1: General Requirements
- ✓ DIN 12650-2: Piston Stroke Pipettes
- ✓ DIN 12650-3: Dispensers
- ✓ DIN 12650-4: Dilutors
- ✓ DIN 12650-5: Piston Burettes
- ✓ DIN 12650-6: Gravimetric Testing for Measuring Accuracy
- ✓ DIN 12650-7: Non-Gravimetric Test Methods
- ✓ DIN 12650-8: Multi-Channel Pipettes

The DIN standard is similar to the ISO standard in many other respects including “Maximum Permitted Error” limits for various types of volumetric instruments and parameters for testing. However, there are a few notable differences that are listed below:

- The balance readability specifications are slightly different

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

Maximum Permitted Error (μl) Balance Readability (mg)

0.01 to 0.1 μl – Balance Readability 0.001mg

0.1 to 1 μl – Balance Readability 0.01mg

Above 1 to 10 μl – Balance Readability 0.1mg

Above 10 μl – Balance Readability 1.0 mg

- The temperature of the testing laboratory should be between 20° to 25°C
- Relative humidity specifications were for the air above the surface of the liquid instead of for the entire testing laboratory. Obviously, this was difficult to determine.
- Air displacement pipettes do not have to be tested in the “addition” or “addition-tare” mode.

Note: It is believed that this standard will diminish in importance in favor of the new ISO standard.

3. *ASTM E1154-89*

This standard is very similar to the two aforementioned standards. This document includes specifications applicable to all types of piston or plunger operated volumetric apparatus (POVA). The major differences between this standard and the other two are as follows:

- Immersion depths are different
- Balance readability requirements are more stringent.
- This standard does not define “Maximum Permitted Error” limits.
- The relative humidity specification is 45 to 75%.
- The recommended reference temperature is 21.5°C
- This standard makes allowances for forward and reverse mode pipetting.

4. *ISO/IEC IS 17025 (formerly ISO Guide 25)*

This is an international standard that contains all of the requirements that testing and calibration laboratories have to meet in order to carry out specific testing activities. This particular standard encompasses and governs two important concepts: the calculation of the measurement uncertainty (and best measurement capability) and assuring the quality of the measurement results. This standard contains an array of specific parameters to which companies must comply such as training, traceability, documentation, internal audits, etc. This document is also used as the framework for accreditation.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

5. ISO 3696

This standard enumerates the mandatory physical and chemical characteristics that water must meet in order to be classified as a certain grade. It sets maximum resistivity, TOC (total organic carbon), absorbance, and silica content values. In order to establish compliance, the water must be within the specified parameters. The quality attributes of this water are detailed below:

pH value at 25°C – 5.0 to 7.5

Electrical conductivity $\mu\text{S}/\text{cm}$ 25°C, max – 5.0

Oxidizable matter Oxygen (O_2) content mg/L max – 0.4

Residue after evaporation on heating at 110°C mg/kg, max - 2

6. GMP/GLP Standards

Good Laboratory Practices (GLP's) are a set of quality standards and concepts designed to govern the design, implementation, and organization of studies and laboratory testing. GLP standards define the conditions under which studies are planned, performed, recorded, reported, and monitored. The FDA recently published a GLP/GMP (Good Manufacturing Practices) Guide for API's (Active Pharmaceutical Ingredients) in which they stated that all laboratory equipment calibrations must be traceable to some nationally recognized standard.

7. European Parliament Directive on In Vitro Diagnostic Products

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.

8. NCCLS

The National Committee for Clinical Laboratory Standards (NCCLS) is a globally recognized, voluntary consensus, standards-developing organization comprised of over 2,000 member organizations worldwide that enhances the value of medical testing within the healthcare community through the development and dissemination of standards, guidelines, and best practices.

9. CAP

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 within established performance standards.

10. CLIA

The Clinical Laboratories Improvement Act (CLIA) is a document that outlines minimum standards for personnel, testing, and quality control for clinical laboratories. This standard was probably one of the earliest in existing that governed liquid handling instrumentation.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

TYPES OF PIPETTES

There are three basic types of pipettes in use in laboratories 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. There are two basic types of glass pipettes in use in laboratories today: volumetric pipettes and transfer pipettes.

The volumetric pipette is calibrated to deliver a specific volume of liquid. It is usually scribed as TD (“to deliver”) at a specific temperature (normally 20°C). The transfer pipette is traditionally used to deliver reagents to samples. It should not be used to dispense an analyte into a flask for dilution purposes.

Volumetric Pipette Transfer Pipette

Most pipettes are designed “to deliver” which means that the residual must not be “blown out” when dispensing the reagent. However, pipettes that are scribed as TC (“to contain”) should be blown out with a pipette bulb. Obviously, no liquid should ever be transferred by mouth pipetting. These instruments are usually made of borosilicate glass and are traditionally thrown away after each use. If the instrument is used multiple times, it cannot be “calibrated” in the true sense of the word since the graduations cannot 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 characteristics that are different than 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

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

3. Positive displacement pipettes (Continued)

depressed again, the piston descends, expelling liquid from the tip. Most people do not use positive displacement pipettes because of the added costs of tips and the inconvenience of changing them. Liquid comes in direct contact with piston.

METHODS OF CALIBRATION

There are several methods that can be used to calibrate pipettes and they include: the gravimetric method, the photometric method (a.k.a. colorimetric method), the titrimetric method, and the isotopic method. The two most common methods employed today are the gravimetric and photometric methods, so these are the only two techniques that will be addressed here. The gravimetric method is extremely involved and requires a stringently controlled environment, a high precision balance, a highly skilled technician, grade 3 bi-distilled water, and a rudimentary understanding of statistics and metrology. 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. (Note: Several accrediting and quality organizations have recently reaffirmed that addition and addition-tare are the only two “acceptable” methods for calibrating liquid handling instrumentation).

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 tarring 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 tarring 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 statistically accurate results.

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 pathlength. According to the Beer-Lambert Law, if a beam of monochromatic radiation 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.

Bio-Tek Services, Inc. 2008-2009 Pipette Standards Handbook

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. In fact, the photometric method appears to perform better at the lower volumes than it does at the higher volumes. The photometric method is a far better choice for any volume range below 10 μ l. It is equivalent to the gravimetric system in terms of measurement uncertainties up to and including 100 μ l. However, any measurement above 100 μ l should be performed utilizing the gravimetric system, since it is the far better choice for this particular range.

The following are results from an “in-house” study that Bio-Tek Services, Inc. performed recently comparing the gravimetric method to the photometric method.

Photometric						Gravimetric					
	Mean	Inaccuracy	Imprecision	S.D.	Uncertainty		Mean	Inaccuracy	Imprecision	S.D.	Uncertainty
0.1 μ l	0.114	13.90%	20.72%	0.0236	0.0472		0.0947	-5.2762%	12.8659%	0.0121	0.0242
	0.112	11.80%	4.06%	0.0046	0.0092		0.0932	-6.7878%	8.8362%	0.0082	0.0164
	0.107	7.10%	22.15%	0.0237	0.0474		0.0909	-9.1055%	12.0306%	0.0108	0.0216
	0.111	10.50%	7.14%	0.0079	0.0158		0.0913	-8.7024%	11.8191%	0.0107	0.0214
	0.103	3.40%	9.90%	0.0102	0.0204		0.0884	-11.6247%	13.1431%	0.0115	0.0230
0.2 μ l	0.202	0.95%	4.01%	0.0081	0.0162		0.1965	-1.7492%	5.0152%	0.0098	0.0196
	0.202	1.05%	4.69%	0.0095	0.0190		0.1980	-0.9935%	7.9179%	0.0156	0.0312
	0.204	1.95%	7.37%	0.0150	0.0300		0.1954	-2.3035%	6.9570%	0.0135	0.0270
	0.212	5.75%	5.77%	0.0122	0.0244		0.2054	2.6846%	7.0072%	0.0143	0.0286
	0.209	4.65%	6.96%	0.0146	0.0292		0.1952	-2.4043%	9.0624%	0.0176	0.0352
0.5 μ l	0.476	-4.78%	2.27%	0.0108	0.0216		0.4738	-5.2403%	2.5479%	0.0120	0.0240
	0.495	-1.02%	5.14%	0.0255	0.0510		0.4803	-3.9378%	4.7216%	0.0226	0.0452
	0.504	0.71%	3.46%	0.0174	0.0348		0.4802	-3.9579%	2.6295%	0.0126	0.0252
	0.481	-3.72%	4.47%	0.0215	0.0430		0.4804	-3.9178%	4.9311%	0.0236	0.0472
	0.487	-2.68%	6.03%	0.0293	0.0586		0.4779	-4.4187%	7.6263%	0.0364	0.0728
1 μ l	0.974	-2.63%	1.13%	0.0110	0.0220		0.9732	-2.6754%	1.2182%	0.0118	0.0236
	0.976	-2.40%	0.96%	0.0094	0.0188		0.9847	-1.5333%	1.6932%	0.0166	0.0332
	0.986	1.41%	2.41%	0.0237	0.0474		0.9926	-0.7418%	2.2987%	0.0228	0.0456
	0.994	0.60%	1.78%	0.0177	0.0354		0.9916	-0.8420%	1.1167%	0.0111	0.0222
	1.013	1.28%	3.85%	0.0390	0.0780		1.0119	1.1919%	2.2074%	0.0223	0.0446



Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

2.5ul	2.590	3.56%	0.99%	0.0260	0.0520	2.5190	0.7603%	0.3955%	0.0100	0.0200
	2.680	7.12%	1.57%	0.0420	0.0840	2.6035	4.1416%	0.4414%	0.0115	0.0230
	2.640	5.64%	0.60%	0.0160	0.0320	2.5486	1.9448%	0.3623%	0.0092	0.0184
	2.620	4.84%	2.49%	0.0650	0.1300	2.5640	2.5610%	0.4087%	0.0105	0.0210
	2.600	4.16%	1.75%	0.0460	0.0920	2.5511	2.0448%	0.4937%	0.0126	0.0252
5ul	5.070	1.34%	0.65%	0.0330	0.0660	5.0133	0.2661%	0.3763%	0.0189	0.0378
	5.030	0.58%	0.70%	0.0350	0.0700	4.9940	-0.1201%	0.2625%	0.0131	0.0262
	5.040	0.88%	0.69%	0.0350	0.0700	4.9951	-0.0981%	0.2101%	0.0105	0.0210
	5.060	1.12%	0.54%	0.0270	0.0540	4.9991	-0.0180%	0.3031%	0.0151	0.0302
	5.070	1.42%	0.34%	0.0170	0.0340	5.0119	0.2381%	0.1553%	0.0078	0.0156
10ul	10.180	1.59%	0.80%	0.0810	0.1620	10.1447	1.4471%	0.2987%	0.0303	0.0606
	10.100	0.97%	0.65%	0.0650	0.1300	10.1427	1.4271%	0.4129%	0.0419	0.0838
	10.110	1.07%	0.51%	0.0510	0.1020	10.1537	1.5372%	0.3835%	0.0389	0.0778
	10.120	1.23%	0.83%	0.0840	0.1680	10.1617	1.6172%	0.6191%	0.0629	0.1258
	10.110	1.10%	0.52%	0.5200	1.0400	10.1667	1.6672%	0.6365%	0.0647	0.1294
20ul	19.820	-0.92%	1.15%	0.2270	0.4540	19.7902	-1.0491%	1.0350%	0.2048	0.4096
	20.050	0.24%	0.60%	0.1200	0.2400	19.8985	-0.5076%	0.4922%	0.0979	0.1968
	20.050	0.23%	0.31%	0.0610	0.1220	19.9350	-0.3250%	0.2745%	0.0547	0.1094
	19.980	-0.09%	0.46%	0.0920	0.1840	19.9276	-0.3620%	0.2830%	0.0564	0.1128
	20.060	0.32%	0.50%	0.1010	0.2020	19.9908	-0.0460%	0.4080%	0.0815	0.1630



50ul	49.900	-0.24%	0.18%	0.0900	0.1800	50.3767	0.7534%	0.3015%	0.1513	0.3026
	49.900	-0.14%	0.42%	0.2100	0.4200	50.3086	0.6171%	0.3452%	0.1730	0.3460
	49.900	-0.28%	0.59%	0.3000	0.6000	50.1347	0.2895%	0.1791%	0.0895	0.1790
	50.300	0.58%	0.43%	0.2200	0.4400	50.2736	0.5473%	0.1876%	0.0940	0.1880
	50.400	0.88%	0.81%	0.4100	0.8200	50.4999	0.9999%	0.2742%	0.1380	0.2760
100ul	100.000	-0.02%	2.31%	2.3100	4.6200	100.7535	0.7535%	0.2947%	0.2959	0.5918
	100.600	0.58%	0.41%	0.4200	0.8400	100.9022	0.9022%	0.2171%	0.2183	0.4366
	101.100	1.08%	0.76%	0.7700	1.5400	100.8543	0.8543%	0.3078%	0.3094	0.6188
	100.500	0.54%	0.74%	0.7400	1.4800	100.7312	0.7312%	0.1701%	0.1707	0.3414
	100.800	0.85%	0.32%	0.3200	0.6400	100.8109	0.8109%	0.0916%	0.0921	0.1842
500ul	502.000	0.48%	0.35%	1.8000	3.6000	505.2893	1.0579%	0.1813%	0.9129	1.8258
	505.000	0.90%	0.74%	3.7000	7.4000	504.7831	0.9566%	0.3501%	1.7608	3.5216
	504.000	0.72%	0.62%	3.1000	6.2000	503.2117	0.6423%	0.1345%	0.6741	1.3482
	504.000	0.72%	0.40%	2.0000	4.0000	505.6468	1.1294%	0.1388%	0.6992	1.3984
	496.000	-0.74%	3.58%	17.8000	35.6000	503.2911	0.6582%	0.1989%	0.9973	1.9946
2500ul	2487.000	-0.52%	0.55%	13.6000	27.2000	2519.8854	0.7954%	0.0829%	2.0804	4.1608
	2486.000	-0.57%	0.50%	12.4000	24.8000	2514.3635	0.5745%	0.0958%	2.4008	4.8016
	2491.000	-0.35%	0.40%	10.0000	20.0000	2514.5104	0.5804%	0.1199%	3.0037	6.0074
	2447.000	-2.14%	5.38%	131.6000	263.2000	2514.3245	0.5730%	0.1164%	2.9159	5.8318
	2484.000	-0.66%	0.83%	20.7000	41.4000	2500.5349	0.0214%	0.2785%	6.9387	13.8774



Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

COMPONENTS OF A QUALITY CALIBRATION

When performing a pipette calibration using the gravimetric method, the calibrator must consider and account for all of the factors that impact the measurement uncertainties in order to produce a quality calibration. These factors can be grouped into three broad categories: the equipment, the operator, and the environment.

The Equipment

Balances – Balances must be properly designed in order to facilitate having the pipette move in and out of the balance chamber. Some designs cause the operator to precariously position the pipette inside the chamber and create significant errors. Some recommended models would be: the Mettler AX Series, the Mettler XP Series, the Mettler MX Series, the Sartorius Genius Series, and the Sartorius RC Series.

The balance must have the appropriate readability for the volume under test (please refer to the “Regulations and Standards” section for more details). If the balance has internal weights, the balance still should be calibrated externally using traceable mass standards. There is a significant amount of debate in the metrology community about the traceability of a balance that has been only been calibrated with the internal weights. Balances must sit level and be placed on a stable table or platform.

Tips – All calibrators are encouraged to use only manufacturer’s tips, since this is what was used to initially calibrate the instrument and to set the performance specifications. Tips should still be analyzed with a stereomicroscope in order to ensure that they are of high quality and free from flash that may be present after the molding process. The entire checklist of characteristics that should be used to determine the quality of a tip is as follows:

- ✓ Smooth interior
- ✓ Straight even sides
- ✓ Perfect-centered opening
- ✓ Absence of flash anywhere on the tip
- ✓ Fits the nosecone tightly without applying too much pressure

Calibration Liquid – The only calibration liquid that should *ever* be used is grade 3, bi-distilled, degassed water. This is the only substance recommended; since this is the liquid that is used by every pipette manufacturer in order to define the performance of their instrument.

Calibration Container – The material of construction for the extraction vessel is very important, since some materials tend to force water into a convex configuration while other materials force water into a concave configuration. 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 hydrostatic pressure effects.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

The Operator

Technique is probably the most important parameter in a quality calibration, yet it is the most difficult to control.

Dialing the micrometer – When dialing in the desired amount on the micrometer, it is recommended that the volume be *slightly* overdialed and then dialed back. This action helps to prevent mechanical backlash and helps to improve the consistency of the results.

NOTE:

Significantly overdialing or underdialing the micrometer can damage any pipette.

Position – Pipettes should be held vertical during the aspiration phase of a pipetting interval. Holding a pipette just 30° off of 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 – It is highly recommended that tips be pre-wetted before beginning a calibration. Failure to pre-wet tips can cause significant inconsistencies between samples since liquid in the initial samples tend to adhere to the inside surfaces of the pipette tip, but liquid from later samples do not. Also, if a new volume is dialed in on the 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 commencing the measurement series.

Release of the 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 blow out 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. 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.

Equilibration Time – It is recommended 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. The recommended minimum stabilization time is two (2) to four (4) hours.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

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, nitrile, or cotton be worn.

Number of Measurements – It is recommended that a minimum of four (4) measurements per channel per volume be performed for a verification check. It is recommended that a minimum of ten (10) measurements per channel per volume be performed for a full calibration. Anything below four (4) measurements cannot be statistically justified.

The Environment

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 terms of the environment. In fact, the density of water in a gravimetric analysis is calculated as a function of temperature.

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.

Vibration – Since the calibrator is essentially weighing a liquid, the less that a balance is affected by vibration the better. Vibration can be significantly reduced by placing the balance on a table constructed of marble or a table specifically designed to be used in conjunction with a balance. The balance table should be placed near a load-bearing wall away from any instrument that mixes, shakes, or stirs.

Air movement – Air movement must be adequate and properly distributed so that it does not cause movement of the balance pan. Air movement can be appropriately controlled and directed with the use of diffusing screens.

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 has an effect on the delivery of air displacement pipettes specifically. This is due to the evaporation of liquid from the upper surface of the meniscus inside the tip as the liquid is being aspirated. Thus, you would expect that a pipette, which is calibrated in a controlled 60% RH laboratory, would test differently at a 30%

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

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.

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 shape of the container, static electricity, drafts, season of the year, and geographic location.

Static Electricity – Static electricity can usually be controlled by wiping all of the containers down with alcohol or placing small radioactive strips in the balance chamber.

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 perform an air buoyancy correction to get to the true mass.

The Photometric Method

When performing a pipette calibration using the photometric method, there are still concerns that must be addressed. The colorimetric technique is less vulnerable to environmental factors than the gravimetric method, but there are concerns relating to temperature equilibrium and cleanliness.

Temperature Equilibrium – All materials (e.g. the dye solutions, the spectrophotometer, the cuvette, the pipette, the tips, etc.) must all be at the same temperature. Thermal equilibrium at room temperature for several hours or overnight, if possible, will produce more accurate results.

Cleanliness – The cuvette in the spectrophotometer must be clean and free from scratches or dust. Fingerprints, smudges, or scratches can negatively impact accuracy, while dusty environments can reduce precision. The cuvettes can be cleaned with alcohol and then wiped dry with a lint free cloth. Be sure to inspect each cuvette before placing it in the spectrophotometer. Additional considerations include proper selection of the spectrophotometer, careful engineering of dye solution properties, and accurate calibration of the dyes:

- ✓ Photometric noise can be a significant source of measurement uncertainty. Therefore, care must be taken to select a spectrophotometer with suitable performance specifications.
- ✓ The dyes used for calibration 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 bi-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.
- ✓ 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.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- ✓ Such an instrument should be maintained and tested periodically to ensure its continued good performance.

NOTE: Many accrediting organizations have asserted that any photometric device used for pipette calibrations must yield the same number of significant digits as a comparable balance would after the Z-factor correction. **Artel** is the only company at this point that has made this modification in order to satisfy this requirement. The photometric testing system available from **Artel, Inc.** is highly recommended.

SERVICING AND REPAIR PROCEDURES

Although it is virtually impossible to specify repair and servicing procedures that will encompass every possible scenario, it is possible to enumerate general procedures that should be followed in order to resolve common problems that arise. This guide is not intended to replace the experience and expertise of your service provider; however, it can be useful in resolving minor repair issues that occur frequently with these instruments.

Air Displacement Pipettes **Mechanical**

Calibration Error – If an air displacement pipette is out of calibration, it could require one or more of the following: a stroke adjustment, a new seal or o-ring, a new piston spring, a new piston (most pistons can be re-polished), or a new nosecone assembly.

- Stroke Adjustment – Usually there is some sort of adjustment or setscrew that will allow you to modify the piston stroke length. Many of these adjustments can be made with everyday tools; however, there are a few manufacturers that require you to purchase special calibration adjustment devices. Please refer to your operator's manual or call us at Bio-Tek Service, Inc. if you cannot find the calibration adjustment mechanism. A stroke adjustment should be the last thing that is considered, since mechanical failures are usually the culprit behind inaccurate measurements
- Seal and/or O-Ring – These items are usually located inside the nosecone or they are incorporated into the piston assembly. A faulty seal or o-ring can cause air to leak through and render the instrument inaccurate. A leak test or vacuum check will allow you to assess the performance and condition of these components. If the instrument fails either of these tests, then these parts should be replaced.
- Piston Spring – The piston spring envelops the piston and sits inside the nosecone. If a spring loses its compression strength due to wear or stress fractures, this can yield inaccurate aliquots and extremely non-linear results. If the spring is “clicking” or “grinding” inside the housing or if you notice extreme swings in linearity or precision, then this component should be replaced.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- Piston – The piston moves up and down inside the nosecone in order to aspirate and dispense the test fluid. If the piston is scored or damaged, it can create vary large errors. Most stainless steel pistons can usually be re-polished which should return them to a usable condition. However, if the piston is ceramic, it normally needs to be replaced. Most pistons need to be lubricated when they are inserted into the pipette. The lubricant is usually some sort of silicon grease. Be careful not to over grease or undergrease this part. The proper amount should leave a thin film across the entire surface area of the piston. It is best to check with the manufacturer or with Bio-Tek Services, Inc. in order to ascertain the correct lubricant for your particular instrument. This is very important, since most lubricants vary in terms of composition, viscosity, and modulus of flow.
- Nosecone Assembly – The nosecone assembly can become blocked or clogged with contaminants and create an out of tolerance condition. Most nosecones can be cleaned and reattached, however, if there is any sign of damage this component should be immediately replaced.

Pipette is leaking – Occasionally you will encounter an instrument that will appear to aspirate the test liquid correctly, but before it can be transferred into the receiving vessel the liquid begins to drip from the tip. This is a problem that can be easily fixed, since the root cause is usually either a faulty seal or o-ring or too much/too little grease on the piston.

- Seal and/or O-Ring - These items are usually located inside the nosecone or they are incorporated into the piston assembly. A faulty seal or o-ring can cause air to leak through and render the instrument inaccurate. If these components are severely damaged or cracked, they will actually cause the pipette to leak. A small leak usually cannot be detected with the naked eye especially when you are dealing with small volumes and micropipettes, so a leak and vacuum analysis should be performed. These tests will allow you to accurately assess the performance and condition of these components. If the instrument fails either of these tests, then the parts should be replaced.
- Piston Lubricants – If a piston is damaged or scored causing the instrument to leak, it should either be re-polished or replaced. It should not be “fixed” by applying a few extra layers of grease. This action will force the pipette to function properly temporarily in terms of creating a partial vacuum and a suction lift; however, the instrument could fail at any time. Conversely, not enough of the lubricant can create undue friction and damage the piston over time. The appropriate amount of lubricant should leave a thin film across the entire surface area of the piston.

The Numbers on Micrometer Shift While Pipetting – If you are using a Gilson Pipetman or other similar instrument, this is an indication that the friction ring needs to be replaced. It is recommended that you allow a service provider perform this replacement for you, since it entails removing the entire spindle assembly and then replacing it correctly.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

The Numbers on Micrometer Shift While Pipetting (continued) - However, if you are using a model similar to an Oxford Benchmate that utilizes a plastic locking mechanism, a replacement or repair can be performed quite easily. The locking handle can usually just be pulled off, since it remains in place due to a friction fit. The plastic locking mechanism is screwed into the center of the instrument and surrounds the plunger or shaft. Simply unscrew it and replace it with a new one if it is damaged. If it is not damaged, simply tighten it and replace the lock handle.

Electronic

Calibration Error – The calibration errors that are encountered with electronic pipettes are far fewer than with traditional mechanical pipettes, however, they still exist. Most (but not all) electronic pipettes do not allow you to enter the software in order to make a calibration correction; so the adjustments must be made by replacing a series of parts. For example, if an out of tolerance condition is encountered with an electronic pipette, the calibration technician would try to replace or recondition the parts that fail most frequently. These include the nosecones, pistons, seals, and o-rings. The pipette would be tested for conformance after each part was serviced and/or replaced. If the instrument still did not comply with the appropriate specifications, then it must be assumed that the programmed electronic and digital components have failed and must be replaced. Most electronic pipette manufacturers install a “hidden” diagnostic subroutine into their software. This means that you can invoke this test, which will help guide you to the appropriate malfunctioning part. The electronic components that usually need to be replaced include the pico board (the small printed circuit board that controls the operation of the instrument), the stopper assembly, or the optical encoder.

- Pipette is Leaking – The servicing procedures for leaky electronic instruments are identical to mechanical pipettes. Please refer to the previous section in order to review these servicing procedures in detail.

- The LCD/LED Display Doesn't Illuminate Properly – The displays on all electronic instruments eventually malfunction, since they all have finite lives. This failure will usually be denoted by missing characters or a gradual fading of the display intensity. Unfortunately, this should only be corrected by a qualified service provider, since the display usually has to be replaced.

Positive Displacement

Calibration Error – Since positive displacement instruments work very similar to a syringe, it is acceptable to make a stroke adjustment immediately after inspecting the instrument for signs of wear and damage.

- Stroke Adjustments – A stroke adjustment is not that easy to make on a positive displacement instrument. The first step is to remove the plunger button. This part is usually composed of two pieces that fit together via a friction fit. If you pull up on the top piece and pull down on the bottom piece, they should separate after you apply a modicum amount of force. The bottom

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

- Stroke Adjustments (continued) - housing must then be removed, so that the plunger can be extricated. After you have totally disassembled the instrument, you should be able to see the calibration screw that is inserted into the internal part of the housing. In order to adjust the calibration, simply decrease or increase the insertion depth of this screw in order to decrease or increase the piston stroke. After this has been completed, simply reassemble the instrument and retest.
- Pipette is Leaking – Positive displacement instruments utilize a disposable piston and capillary assembly, which means that most leaks can be attributed to them if they are improperly sized or poorly fitting. If you do experience this problem, ensure that the piston is secure and that the capillary tube fits the nosecone properly.
- The Numbers on Micrometer Shift While Pipetting – Most positive displacement instruments utilize “staked” micrometers, which means that they cannot be replaced. If the micrometer is shifting during a pipetting interval, the gears inside the micrometer are probably worn or damaged and the entire upper housing must be replaced.

SELECTING A QUALITY PIPETTE CALIBRATION PROVIDER

Selecting an appropriate service provider is a difficult task; however, it is something that can be done if you are properly informed. Any quality calibration company should be incorporating the following elements into their overall calibration program. If they are missing even one of the following, then they are truly doing you a disservice and you are not getting your money’s worth:

1. Some sort of quality system should be in place. Documentation should be available for your review.
2. SOP’s (e.g. work instructions) should be readily available for perusal – sometimes a non-disclosure agreement must be signed. Companies that classify these documents as “proprietary” usually don’t have any.
3. Cubic expansion coefficients (a.k.a. thermal expansion coefficients) should be incorporated into the Z-factor calculation. This is a number that attempts to “quantify” the hand warming effect.
4. An uncertainty budget should be calculated and available for review. This will help you determine what the prospective company’s “best measurement” capabilities are.
5. All balances should be calibrated daily in-house with a set of mass standards traceable to NIST and then at least yearly by an outside source. All of this activity should be documented and available for review.
6. Testing should be performed at three different volumes not two. It is extremely easy to get any volumetric instrument to pass using only two volumes.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

7. High quality service providers will offer a “complaint” service that includes both “as found” and “as left” data included on the certificate. This will alert you to any “out of tolerance” pipette that might have been used during a critical process or application.
8. The calibration facility should always adjust every instrument towards the center of its tolerance band even if it passes the “as found” test. This ensures that the instrument will remain “in tolerance” until it is due for recalibration.
9. Technicians should be certified by some sort of training programs and documentation should be available to substantiate that claim.
10. The pipette certificate should list the standards used to calibrate the test equipment as well as the environmental monitoring devices. These standards should be traceable to NIST.
11. The calibration company should routinely return all replaced parts to you. If they are reluctant, then they probably didn’t replace them.
12. The pipette certificate should contain statistical data for every channel and for every test volume. Be careful when sending in multi-channels for calibration, since some service providers will pick only one channel and use it to calibrate the entire instrument. Each channel should be evaluated independently and the certificate should reflect that.
13. Be sure that the price quoted includes parts. If not, obtain a price list of parts in order to ensure that there are no surprises in the future.
14. Be sure that the service provider is leak testing and vacuum testing at every visit. This is the **ONLY** way to ensure that the seals and o-rings are seated properly. The leak test should be a “true” leak test utilizing negative pressure not positive pressure. Positive pressure tests can cause a pipette with a leaky o-ring or seal to yield a false pass.
15. If you contract a company to perform service at your facility, the company is not actually performing a calibration unless they are bringing measurement instruments such as balances onsite with them. This may seem very basic, but many pipette service companies promote the fact that they can “service” instruments onsite without actually mentioning the word “calibration”. If the pipette is disassembled and an internal component is replaced, the instrument ***MUST*** be checked either gravimetrically or photometrically to ensure that it performs according to specifications. This tactic is employed by the largest pipette service company in the industry, so please read the fine print and ask the right questions.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

GLOSSARY

Accuracy – The degree or conformity of a measurement to a standard or a true value.

Aspirate – The process of withdrawing a substance with a negative pressure apparatus such as pipette, syringe, or other piston operated volumetric instrument.

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 connect 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.

Exercising the Balance – This is a procedure that should be incorporated daily before any measurements are taken. It involves taking weights that are 50% and 100% of the maximum load capacity of the balance and repetitively loading and unloading them. The smaller weight should be used first and then the larger one until at least 10 repetitions are performed for each mass. This helps to “warm up” the balance’s internal weighing mechanism while helping to improve linearity and precision.

Expansion Factor – The quantification of expansion due to thermal conductance.

$K=1-(T-20)$ Where: K=Expansion Factor, α = Cubic Expansion Coefficient, T=Temperature, degrees Centigrade

Flash – Residual pieces of plastic that may remain around the opening of a pipette tip after the molding process is complete. This is usually an indication that the tip is of poor quality and/or the mold was either defective or unclean.

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

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

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

GLOSSARY (continued)

Humidity Traps – Specially designed glass vessels that are placed inside the balance chamber in order to create a “micro-climate” during pipette calibrations.

Isothermal Condition – This means that the temperature of 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 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 it 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.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

ACKNOWLEDGEMENT

Bio-Tek Services, Inc. wishes to acknowledge the following sources, since they were used as resources during the preparation of this publication.

1. ISO/IEC IS 17025 (formerly ISO Guide 25) – Available from:
Bio-Tek Services, Inc., 1160 Warwick Park Road, Suite C, Richmond, VA 23231 or The International Organization for Standardization – Is Irue de Varembe, Case Postak 56, CH-1211 Genève 20, Switzerland.
2. Artel, Inc., 25 Bradley Drive, Westbrook, ME 04092.
3. ASTM E1154-89 – Available from: Bio-Tek Services, Inc., 1160 Warwick Park Road, Suite C, Richmond, VA 23231 or American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959.
4. Biohit, 3535 Route 66, Building 4, P.O. Box 308, Neptune, NJ 07754-0308.
5. Brandtech Scientific, 25 Middlesex Turnpike, Essex, CT 06426-1476.
6. Brinkman Instruments, One Cantiague Road, Westbury, NY 11590-0207.
7. CAP Standards – Available from: Bio-Tek Services, Inc., 1160 Warwick Park Road, Suite C, Richmond, VA 23231 or The College of American Pathologists, 325 Waukegan Road, Northfield, IL 60093.
8. CLIA Standards – Available from: U.S. Department of Health and Human Services, Room 645-F, Hubert H. Humphrey Building, 200 Independence Avenue, S.W., Washington, D.C. 20201.
9. David Duff, A-Metrology-Z, 2851 S. Senour Road, Indianapolis, IN 46239.
10. European Parliament on In Vitro Diagnostic Products – Available from: Allée du Printemps, B.P. 1024/F, F-67070 Strasburg Cedex.
11. GLP Standards – Available from: FDA (HFE-88), Office of Consumer Affairs, 5600 Fishers Lane, Rockville, MD 20857.
12. ISO 3696 – Available from: Bio-Tek Services, Inc., 1160 Warwick Park Road, Suite C, Richmond, VA 23231 or The International Organization for Standardization – Is Irue de Varembe, Case Postak 56, CH-1211 Genève 20, Switzerland.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

ACKNOWLEDGEMENT (continued)

13. Mettler-Toledo, Inc., 1900 Polaris Parkway, Columbus, OH 43240.

14. NCCLS Standards- Available from: Bio-Tek Services, Inc., 1160 Warwick Park Road, Suite C, Richmond, VA 23231 or The National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

15. Nichiryo America, Inc., P.O. Box 198, 230 Route 206, Flanders, NJ 07836.

16. NIST (National Institute of Standards and Technology), 100 Bureau Drive, Gaithersburg, MD 20899-2141.

17. VistaLab Technologies, Inc., 27 Radio Circle Drive, Mt. Kisco, NY 10549.

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

CALCULATIONS

Definitions

W = mean weight

V = mean volume

V₂₀ = volume at 20 degrees, in required units of ml or µl

W_e = weight corrected for evaporation, in measured units of g or mg

W_c = current cumulative weight

W_p = previous cumulative weight

T = time interval between W_c and W_p, units can be seconds or msec

x_e = time of the current event

t_p = time of the previous event

E_c = evaporation rate for the current blank

Z = Environmental Correction factor in cm³/g

T = temperature of the liquid in C

P_a = air density at T, in g/cm³

P_w = density of the test liquid at T, in g/cm³

P_b = density of the balance weights, equals 8.0 g/cm³ always

P = barometric pressure, in mm of Hg

H = relative humidity in %

α = Cubic expansion Coefficient in units of g/cm³/deg C

K = expansion factor, no units

SD = standard deviation

CV% = coefficient of variation

Accuracy:

$$E\% = \frac{(V - V_e) \times 100}{V_e}$$

Where V_e = Expected Volume

Precision:

$$CV\% = \frac{SD \times 100}{V}$$

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

CALCULATIONS (Continued)

Density of Test Liquid:

$$P_w = \frac{0.001(999.83952 + 16.94576T - 7.9870401 \times 10^{-3}T^2 - 46.170461 \times 10^{-6}T^3 + 105.56302 \times 10^{-9}T^4 - 280.54253 \times 10^{-12}T^5)}{(1 + 16.879850 \times 10^{-3}T)}$$

Air Density:

$$P_a = \left[\frac{0.46460[P - 0.0037960(H_{e_s})]}{273.15 + T} \times 10^{-3} \right]$$

where:

P_a = Density of air, g/cm³

P = Barometric Pressure, mmHg

H = Relative Humidity, percent

T = Temperature, degrees Centigrade

$E_s = 1.3146 \times 10^9 \times \text{EXP}(-5315.56/(T+273.15))$

Expansion Factor:

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

where:

K = Expansion Factor

α = Cubic expansion Coefficient

T = Temperature, degrees Centigrade

Environmental Correction Factor:

$$Z = \left(\frac{1}{P_w - P_a} \right) \left(1 - \frac{P_a}{P_b} \right) \times K$$

Time Intervals:

$$t = t_c - t_p$$

Bio-Tek Services, Inc.
2008-2009 Pipette Standards Handbook

CALCULATIONS (Continued)

Evaporation Correction:

$$Ec = \frac{(Wc - Wp)}{t}$$

→

Absolute
Value

Note: If it is less than zero (due to negligible evaporation and balance error) it will be set to zero.

Volume:

$$V_{20} = (W_e \times Z)$$

Mean Weight:

$$W = \frac{\sum W_e}{N}$$

Mean Volume:

$$V = W \times Z$$

Standard Deviation:

$$SD = \sqrt{\frac{\sum (W_e - W)^2}{n-1}} \times Z$$

This will be set to "NA" if $(n-1) \leq 0$

F-Error:

$$F = |e| + 2 \times SD$$