HAMILTON®

Liquid Handling Reference Guide

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1 About This Guide

This guide introduces the concept of liquid handling and explains the fundamentals of how to perform liquid handling on an automated liquid handler. It is great for both new learners, who need to understand liquid handling basics, as well as experienced liquid handlers, who can benefit from best practices and troubleshooting tips.

This guide offers insights into liquid properties and how different liquids can affect the results of your pipetting. You will learn how automated liquid handling works and the correct methodology to use when aspirating and dispensing with an automated liquid handler. This guide describes method settings and liquid classes to make sure you have the right process setup for your assay, as well as best practices and common examples for understanding Monitored Air Displacement (MAD) and Total Air Displacement Monitoring (TADM) for liquid transfers. Utilizing the information in this guide will ensure your liquid handling measurement, aspiration, and dispensing techniques are precise and accurate.

The concepts in this guide are intended for use on all Hamilton automated liquid handlers and their related software. As a result, no detailed steps are provided for individual platform and software combinations.

2 About Liquid Handling

Liquid handling is the act of transferring liquid from one location to another in a laboratory, usually for testing purposes.

This section introduces liquid handling, different ways liquid handling can be performed, and the important physical aspects of liquids.

2.1 How It's Used

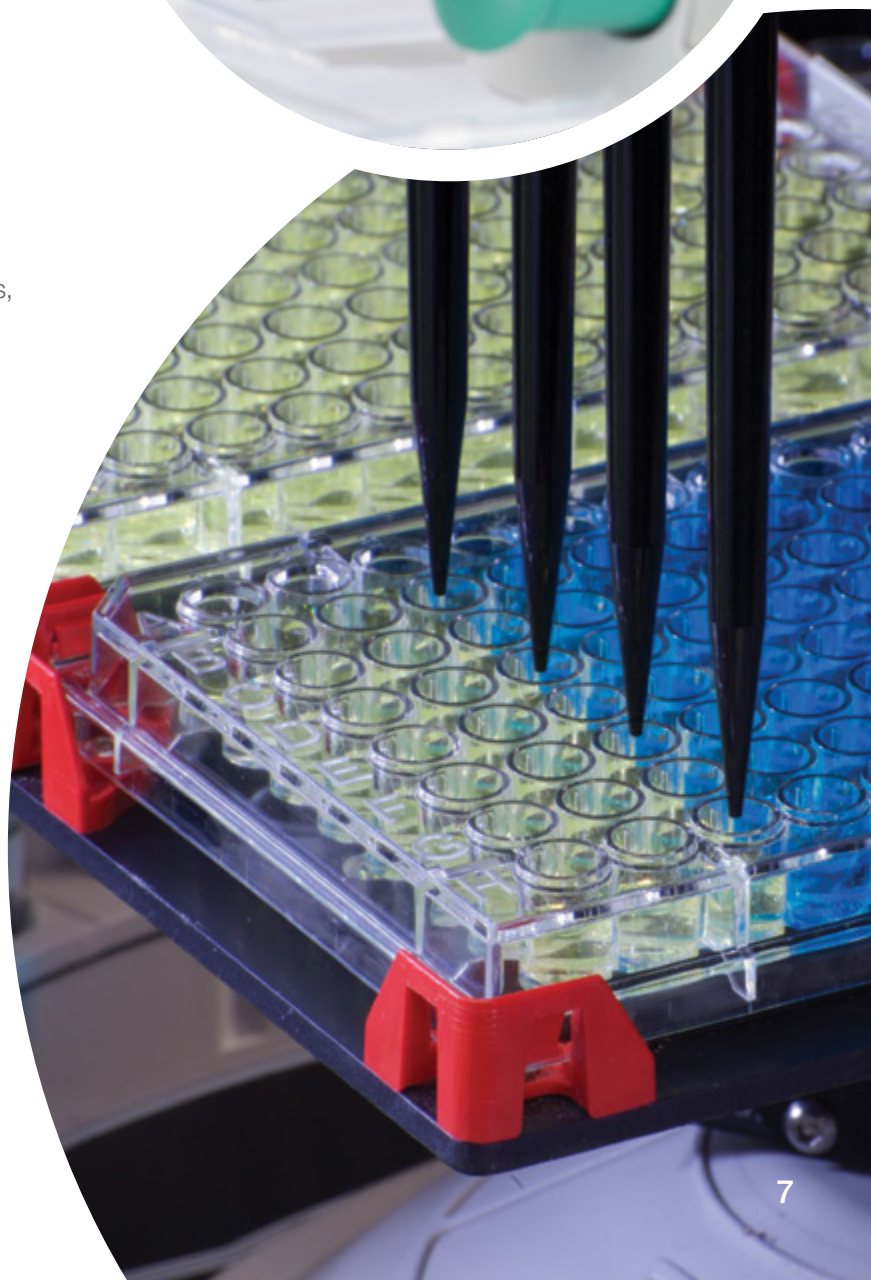
Simple though it seems, liquid handling is important to laboratories around the world. Most testing involves checking countless, tiny samples of liquid for certain attributes. Samples can be smaller than 1 microliter (µL) and still help the lab detect chemicals, screen for diseases, and multiply DNA for further testing.

However, liquid handling can be a time-consuming process for a lab. While a 1 µL sample may be an efficient use of a liquid for a single test, testing equipment usually provides room for dozens if not hundreds of samples, with each one requiring a transfer of liquid.

Imagine a lab technician using a hand pipettor to carefully transfer 96 1 µL samples onto a single microplate. Precision is critical to good test results, so each transfer of liquid needs to be exact. An exact liquid transfer has everything to do with the type of liquid that's being transferred and the technique of the technician as the liquid is dispensed. It's both a science and a technique.

To save time, a Hamilton automated liquid handler can automate the science and the technique. Instead of a lab tech carefully dispensing a sample into 96 individual wells, the automated liquid handler is programmed to use settings that facilitate proper and consistent transfers. Programming an automated liquid handler means taking everything that was done manually by the lab tech and defining it literally in a set of instructions for the automated liquid handler to follow.

Labs often use a mixture of hand and automated pipetting to achieve their goals involving liquid handling.



2.2 Manual Pipetting vs. Semi-Automation vs. Automation

Both hand pipetting and automation can be used effectively in a lab that is managing liquid samples. The method to choose depends on the application. See below for some key factors to help you decide which method is best for your application.

2.2.1 When to Use Manual Pipetting

Hand pipetting is fast in small applications and only requires the hand of a practiced lab tech instead of extra hardware. While manual pipetting is straightforward, it does introduce risks of technician-to-technician variability.

2.2.2 When to Use Semi-Automation

Semi-automation offers a way for labs to incrementally scale up production and increase reproducibility through liquid handlers that automate certain aspects of sample preparation. Instead of a technician manually setting volumes on a pipette or keeping track of steps in a method, semi-automated liquid handlers (like Hamilton's Microlab 600) automate those aspects of the work, only requiring the technician to move a hand probe from vessel to vessel.

2.2.3 When to Use Automation

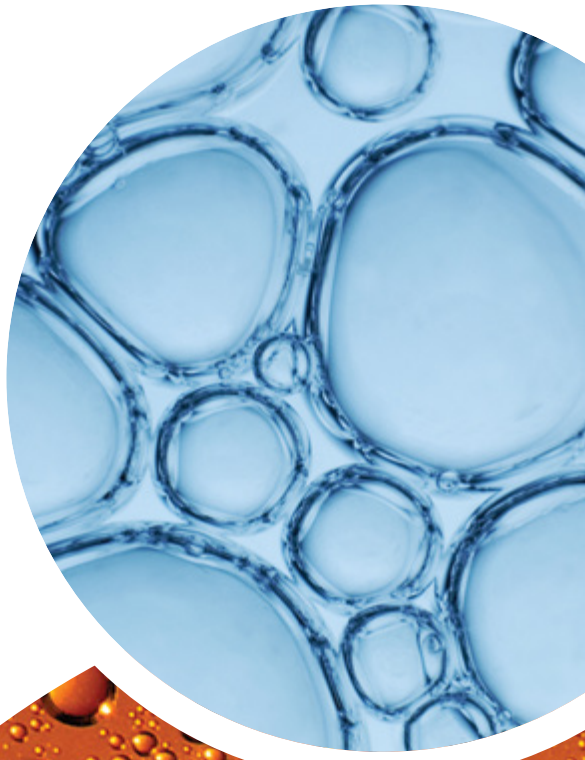
Full automation is most valuable in high-throughput applications that benefit from completely removing human movements. Liquid handling platforms process hundreds of samples at a time and follow highly complex methods without deviation.

When to Use Manual Pipetting vs. Semi-Automation vs. Automation

	Manual Pipetting	Semi-Automation	Automation
Sample Involved	Few	Dozens	Hundreds/Thousands
Throughput Needed	Low (5–10 samples per hour)	Moderate (11–100 samples per hour)	High (100+ samples per hour)
Dead Volume Allowed?	Small amount	Small to none	High Amount
High Reproducibility Needed?	No	Yes	Yes
Are labor costs a concern?	No	Moderately	Yes
Chance of repetitive stress injuries?	Yes	Some	No
Protection from hazardous/ infectious samples?	No	No	Yes

2.3 Liquid Properties

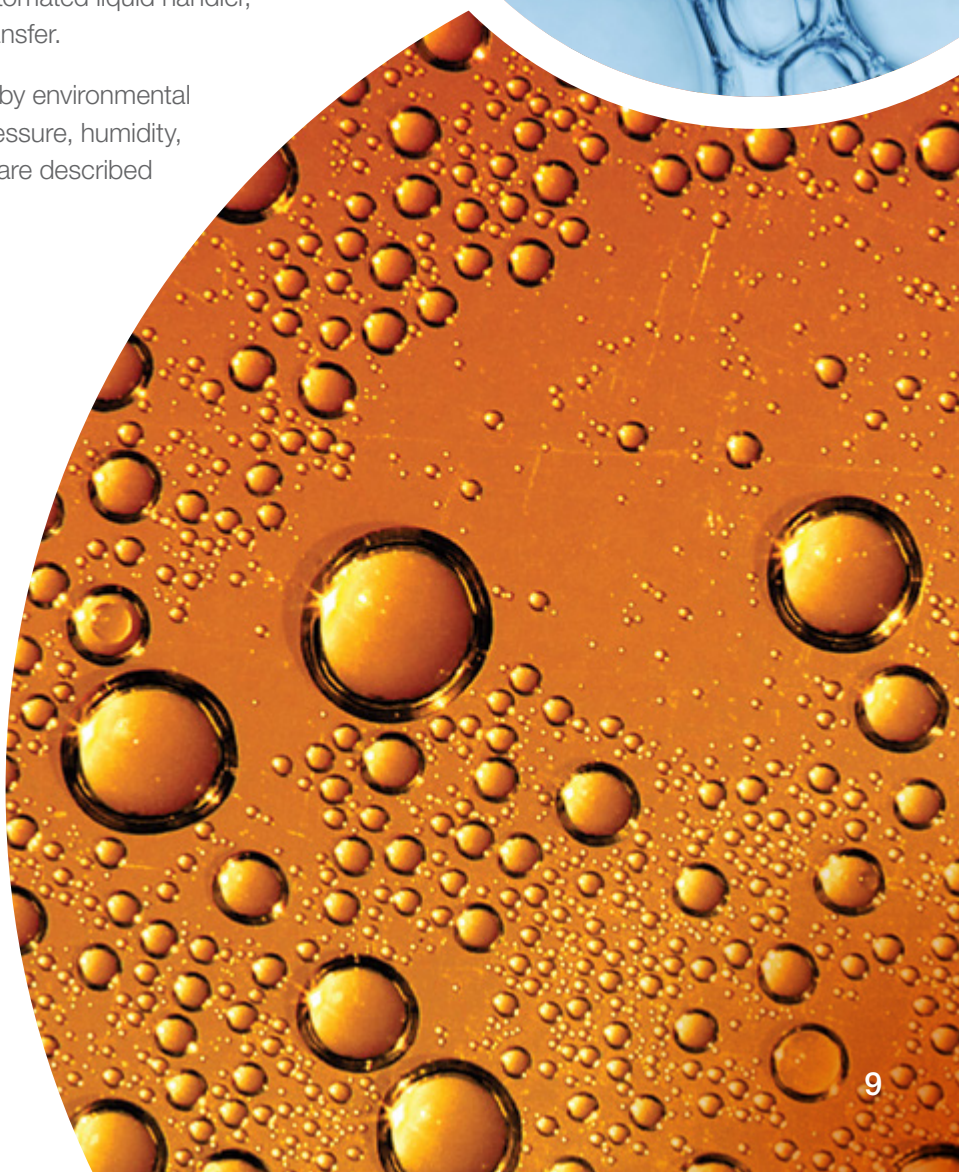
Liquids tested in labs are as varied as the industries they appear in—everything from sticky honey to a fast-flowing petroleum. Depending on what the lab needs to test, these varied liquid types can be transferred by either hand pipettors or Hamilton automated liquid handlers.



Successful liquid transfers involve knowing the type of liquid and accounting for its behavior in a particular environment. A lab tech can rely on experience and intuition to inform how each liquid should be treated. The lab tech learns where to position the tip to make sure there are no bubbles as the liquid dispenses, where to hold the tip to prevent contamination between samples, and how to position the tip and for how long to make sure the liquid dispenses completely.

While humans can readily assess the liquid's attributes and adapt accordingly, an automated liquid handler must know everything about the liquid in advance. The properties in this section all have an impact on the liquid handling information given to the automated liquid handler, which makes all the difference in a successful transfer.

All liquid properties are affected to some degree by environmental conditions such as temperature, atmospheric pressure, humidity, etc. These conditions along with their influences are described in the following sections:



2.3.1 Viscosity

What Is It?

Viscosity describes the flow behavior of a liquid.

High viscosity liquids have a thicker flow and are less fluid. Examples of high viscosity liquids are honey and glycerin.

Low viscosity liquids have a thinner flow and are more fluid. Examples of low viscosity liquids are water and petroleum.

Why It's Important

Viscosity impacts the way a liquid is transferred in very specific ways. The spectrum below shows two example liquids and how to convey the property to the automated liquid handler. The descriptions use common liquid handling terms that are defined in detail in Section 5 Method Settings and Liquid Class.

High Viscosity Liquid

Use a **lower flow rate**. If you try to aspirate too quickly, suction in the tip prevents the fluid from entering the tip, resulting in inaccurate volumes.

Use a **surface dispense mode**. Jet dispensing isn't as effective with high viscosity liquids.

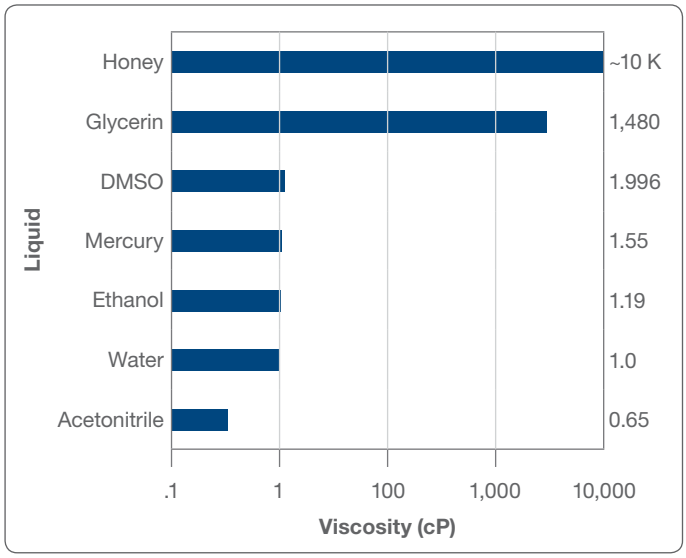
Low Viscosity Liquid

Use a **higher flow rate**. The goal is to get the liquid into the tip as fast and effectively as possible. Low viscosity liquids don't resist entering the tip, so the flow rate can be higher.

Spectrum of Viscosity

The following chart shows a spectrum of viscosity for a variety of liquids. Note that many liquids such as water and ethanol have similar values for viscosity, but when compared to liquids such as glycerin and honey, the scale of the difference is stark.

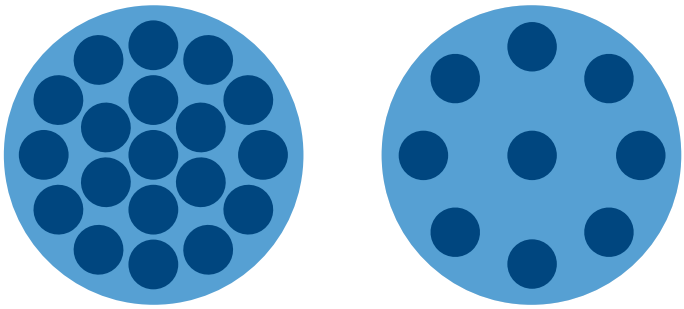
Viscosity of Sample Liquids at 20˚C / 86˚F



2.3.2 Density

What Is It?

Density is a measurement of how much of a substance can occupy a space. A material's density gives the impression of heaviness or lightness. Density is affected by temperature and atmospheric pressure.



High Density **Low Density**

Why It's Important

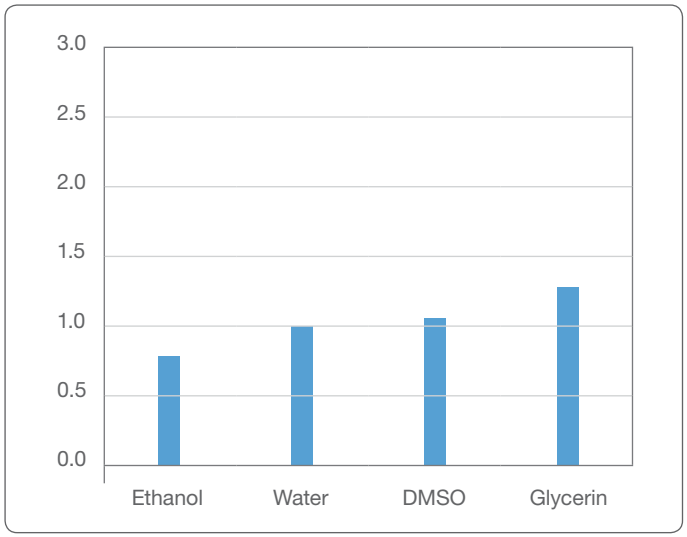
The density value for a liquid is required for measuring liquid transfers gravimetrically. The volumetric mass density of a substance is its mass per unit volume. If the weight of a liquid transfer is determined gravimetrically, then the transfer volume can be determined. Density is often provided on material safety data sheets, but can also be determined empirically.

Mixtures of liquids with different densities may not remain in suspension, which can make it hard to transfer a mixture properly. A suspension is a mixture of two or more substances that essentially become one homogenous liquid. However, some suspensions eventually settle and separate. Liquids with less density rise to the top, while the heavier density liquids fall to the bottom. Many applications require the addition of one liquid to another with the eventual transfer of a single solution. Awareness of the density of different liquids can guide you to mix your samples before aspirating, or aspirating quickly before settling can occur.

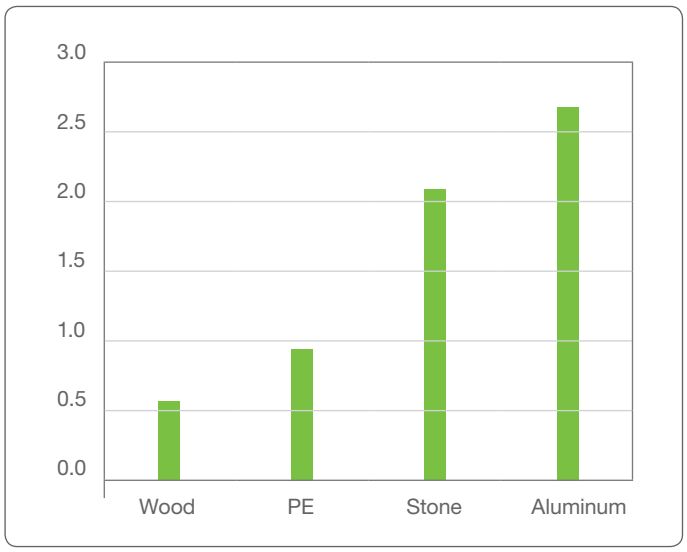
Density Ranges

The density of different liquids and solids is shown below to visualize the range (in g/cm3):

Density of Various Liquids



Density of Various Solids



2.3.3 Adhesion / Cohesion

What Is It?

Adhesion and cohesion are two different ways to describe the stickiness of a liquid. Liquids can be high in one or both with different impacts on the liquid transfer.

Adhesion is the measure of how much the liquid wants to stick to other substances. A high adhesive liquid like glue wants to bind to substances nearby, like tips and labware. A low adhesive liquid like mercury resists nearby substances.

Cohesion is the measure of how much the liquid wants to stick to itself. A high cohesive liquid like mercury wants to bind to itself and remain together. A low cohesive liquid like methane disperses and moves away from itself.

The adhesive and cohesive properties of a liquid are affected by the chemical compatibility of the tips and labware used to make a liquid transfer. Some liquids and materials are attracted to one another while others are inert when placed together. Chemical compatibility information is important to understanding how a liquid will interact with the various parts of the automated liquid handler and labware involved in the transfer.

The pressure of adhesion and cohesion results in capillary action, which causes the liquid to work against gravity. Capillary action occurs when the adhesion of the liquid to the tips or labware is stronger than the cohesive forces between the liquid molecules.

Why It's Important

Adhesion and cohesion impact the way a liquid is transferred in very specific ways. The spectrum below shows two example liquids and how to convey the property to the automated liquid handler. The descriptions use common liquid handling terms that are defined in detail in Section 5 Method Settings and Liquid Class.

High Adhesion Liquid

Increase the blowout volume. More force is needed to get a sticky substance out of the tip.

Low Adhesion Liquid

Decrease the blowout volume. The liquid doesn't resist being removed from the tip, so not as much force is needed to remove the liquid from tip.

High Cohesion Liquid

Decrease the air transport volume. Since the liquid wants to stick to itself, less force is needed to hold the liquid in the tip.

May need to increase the blowout volume.

Low Cohesion Liquid

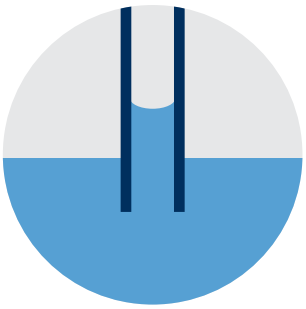
Increase the air transport volume to protect from drips. The increased air transport volume ensures that the liquid has more of an air buffer inside the tip during transport.

2.3.4 Capillary Action

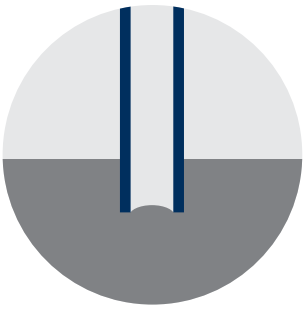
What Is It?

Capillary action is the tendency of a liquid to work against gravity and atmospheric pressure. Liquids with high capillary action can actually climb the tip in small volumes. Capillary action occurs when the adhesion and cohesion qualities of a liquid work together to move the liquid upward. Other properties can impact capillary action, including vapor pressure and environmental variables.

Liquids with low capillary action or any liquid in larger volumes generally are drawn downward by gravity and atmospheric pressure.



In this image, the capillary action of **water** is shown. For water, the adhesion is greater than the cohesion, which results in a meniscus that turns downward and could cause the liquid to move up inside a tip.



Mercury, on the other hand, has high cohesion and low adhesion. It results in a meniscus that turns downward and will not climb into the tip without help from the channel.

Why It's Important

Capillary action impacts the way a liquid is transferred in very specific ways. The spectrum below shows two example liquids and how to convey the property to the automated liquid handler. The descriptions use common liquid handling terms that are defined in detail in Section 5 Method Settings and Liquid Class.

High Capillary Action Liquid

Be careful with low volumes. Especially when working with 10 or 50 µL tips, capillary action can have a bigger effect. More liquid can be aspirated than intended.

Low Capillary Action Liquid

No changes to make. Since the liquid has no tendency to climb the tip, nothing extra needs to be taken into account for this factor.

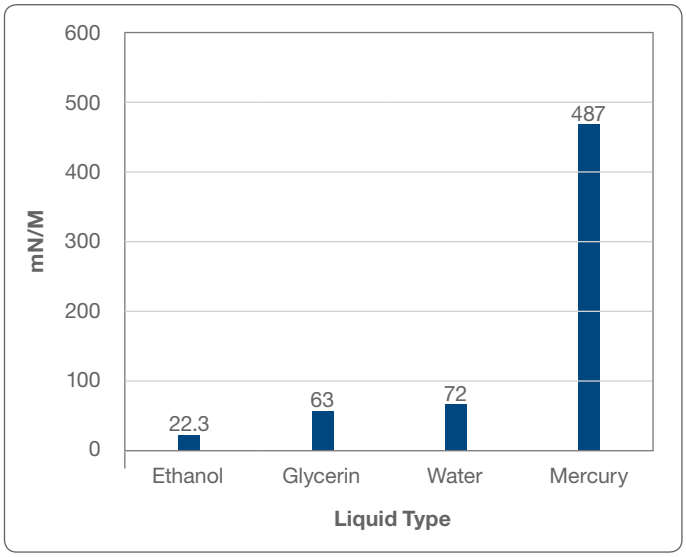
2.3.5 Surface Tension

What Is It?

Surface tension is the elastic tendency of liquids that makes the liquid acquire the least surface area possible. In terms of liquid handling, the surface tension affects how well the molecules at the surface of the liquid cohere to each other and to the walls of the tip.

The chart shows the surface tension of various liquids. The higher the number, the higher the surface tension and the more likely the liquid is to form a strong cohesion at the surface of the liquid.

Surface Tension of Various Liquids



Why It's Important

Surface tension impacts the way a liquid is transferred in very specific ways. The spectrum below shows two example liquids and how to convey the property to the automated liquid handler. The descriptions use common liquid handling terms that are defined in detail in Section 5 Method Settings and Liquid Class.

High Surface Tension Liquid

Use a **lower air transport volume**. Liquids with high surface tension stay in the tip and don't require a large air buffer to hold the liquid in.

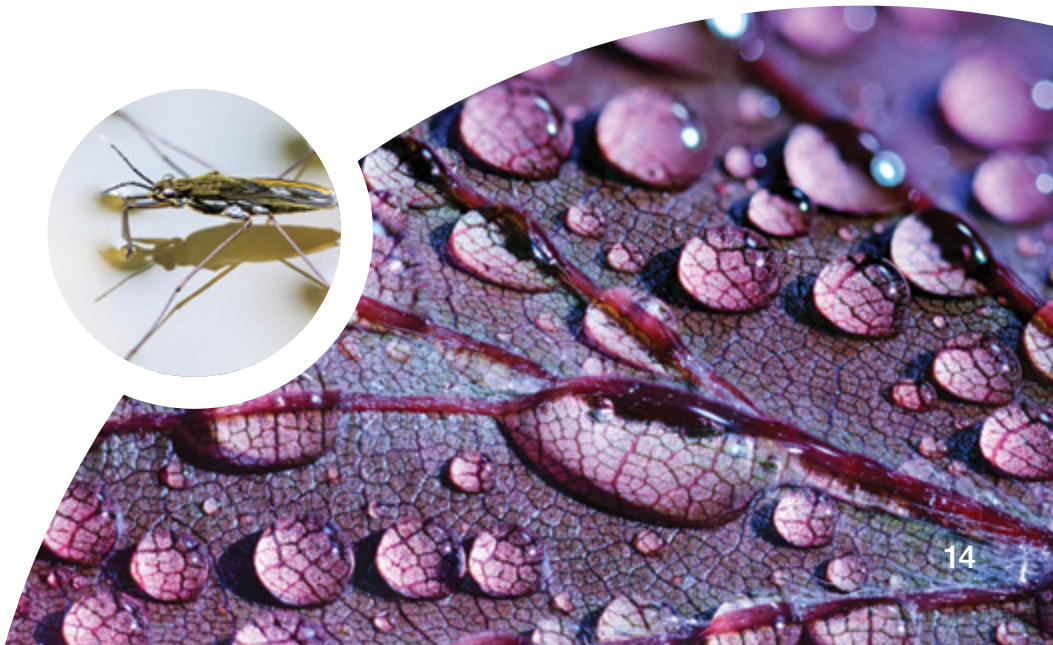
Use a **high swap speed** to break the connection between the liquid in the well or tube and the tip.

Use **side touch** or **minimize the distance from the end of the tip and the labware** to allow the liquid to be more easily removed from the tip.

Low Surface Tension Liquid

Increase the air transport volume. Since the liquid wants to leave the tip, increasing the transport volume provides a greater air buffer in the tip.

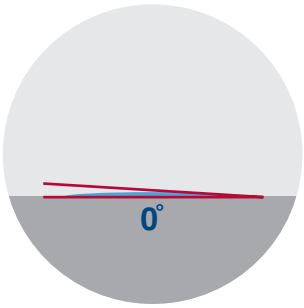
Decrease the settling time to minimize the amount of time the tip is in contact with the liquid and prevent time for droplet formation.



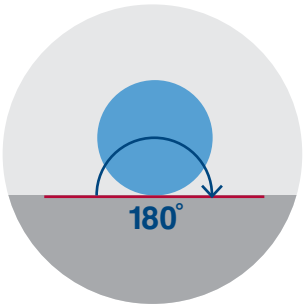
2.3.6 Contact Angle

What Is It?

The contact angle is the angle the liquid makes to the surface of the labware.



A low contact angle like 0° makes it harder for low volumes to be transferred, since the liquid spreads out very thinly.

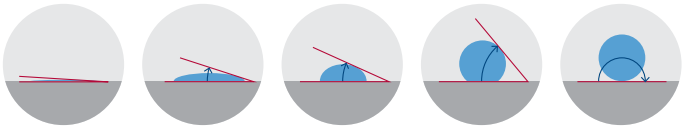


A high contact angle like 180° causes the liquid to stand taller in smaller volumes, but can make it difficult to aspirate and dispense since the cohesion can be repellent.

The contact angle is also affected by the type of surface where the liquid sits. See below for how different surfaces are characterized:

- On a hydrophilic surface, the contact angle will be low (0°).
- On a hydrophobic surface, the contact angle will be high (90°).
- On a super hydrophobic surface, a contact angle will be higher than 160°.

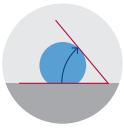
The figure below shows a variety of contact angles, ranging from 0° to 180°.



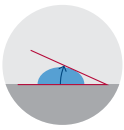
0° Angle **180° Angle**

Examples of Contact Angles

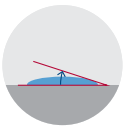
The below images show varying contact angles on different surfaces.



100 µL water on hydrophobic coated plastic has a contact angle of ~130°.



100 µL water on untreated plastic has a contact angle of ~90°.



Water on aluminum surface.

Ethanol on aluminum surface. The contact angle is lower than water.

Why It's Important

The contact angle impacts the way a liquid is transferred in very specific ways. The spectrum below shows two example liquids and how to convey the property to the automated liquid handler. The descriptions use common liquid handling terms that are defined in detail in Section 5 Method Settings and Liquid Class.

High Contact Angle

Make sure the tip enters the liquid properly. If the contact angle is high, the liquid might deform around the tip and cause issues with the volume.

Low Contact Angle

Be careful with low volumes. A liquid with a low contact angle may be hard to aspirate in low volumes because the height of the liquid is minimal.

Use **bottom touch** off during aspiration and dispense to reach the very bottom of the well or tube.

Consider using **side touch** during dispense.

2.3.7 Vapor Pressure

What Is It?

Vapor pressure is the pressure created by vapor above a liquid's surface. As atmospheric pressure pushes downward, the liquid's vapor pressure is pushing upward.

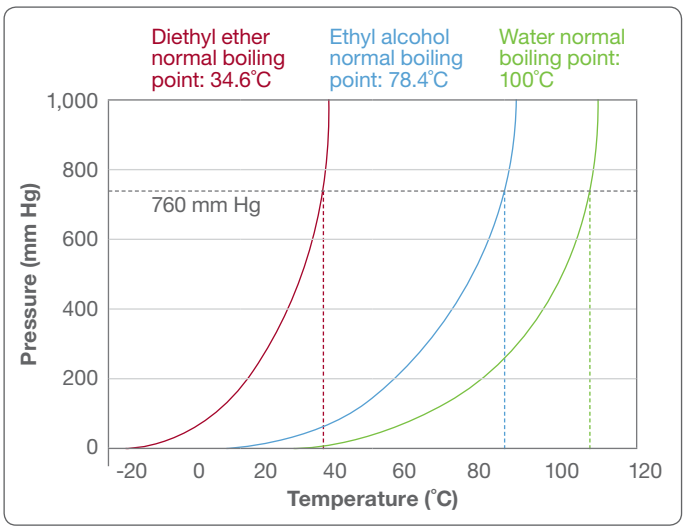
Liquids with **high vapor pressure** off-gas since the atmospheric pressure isn't enough to hold them down.

Liquids with **low vapor pressure** don't off-gas or try to escape into the air.

Liquids with high vapor pressure also tend to evaporate quickly. A substance with a high vapor pressure at normal temperatures is referred to as volatile. Liquids with high vapor pressure include alcohols and ethers.

The chart below shows a temperature and pressure comparison for three liquids. Note that 760 mmHg is atmospheric pressure.

Vapor Pressure Curves



Environmental properties in the lab can have a large impact on vapor pressure. Changes in the elevation alter the atmospheric pressure, which can in turn change when the liquid begins to off-gas.

Additionally, if the temperature of the liquid increases, the vapor pressure also increases, just as water boils and turns to steam.

Why It's Important

Vapor pressure impacts the way a liquid is transferred in very specific ways. The spectrum below shows two example liquids and how to convey the property to the automated liquid handler. The descriptions use common liquid handling terms that are defined in detail in Section 5 Method Settings and Liquid Class.

High Vapor Pressure Liquid

Use **Anti-Droplet Control**. Liquids with high vapor pressure are more likely to drip.

Pre-wet the tip. The processing of pre-wetting involves aspirating and immediately dispensing some liquid to allow the tip's vapor pressure to adjust, then aspirating enough volume for the liquid transfer.

Use a **larger blowout volume** to allow room for the vapor.

Low Vapor Pressure Liquid

No changes to make. Since the liquid isn't likely to drip based on off-gassing, nothing extra needs to be taken into account for this property.

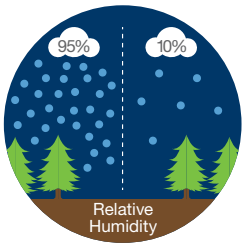
2.3.8 Environmental Influences

What Is It?

Environmental influences are properties of the space around the liquid that impact the behavior of the liquid itself.

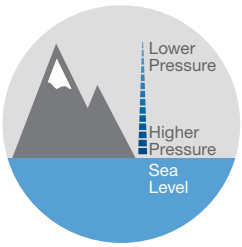
Here are some of the important environmental influences to consider along with their effects on liquids:

Humidity



- Low humidity results in a higher evaporation rate since there is less liquid in the air. There may also be higher instances of static electricity, which could impact low volume pipetting.
- High humidity results in a lower evaporation rate since the air is already saturated.

Atmospheric Pressure

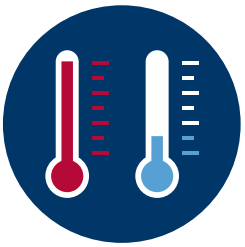


- A decrease in pressure increases the rate of evaporation.
- Closely linked to vapor pressure.
- Atmospheric pressure changes with the elevation. For example, if creating a liquid class at sea level, test the liquid class again before using it at a lab at 6,000 feet elevation.

Radiation

- Changes the chemical properties of a substance.

Temperature



- All liquid properties can shift with temperature, even when the change is just a few degrees: vapor pressure, atmospheric pressure, density, viscosity.
- It is recommended to control for temperature in the lab.
- Always develop liquid classes at common lab temperatures. Record the temperature during development.

Vibrations

- Vibrations can cause drops to form.
- Ideal to have no vibrations, especially during gravimetric measurements.

Why It's Important

The automated liquid handler can only execute a programmed sequence of activities for a set liquid class. Any changes in the environment that alter the liquid properties without the automated liquid handler knowing jeopardize the quality of the liquid transfer.

For example, if the temperature in a lab is usually controlled, but one day spikes due to an issue with the air conditioner, there could be a change in the liquid transfer due to the increased heat.

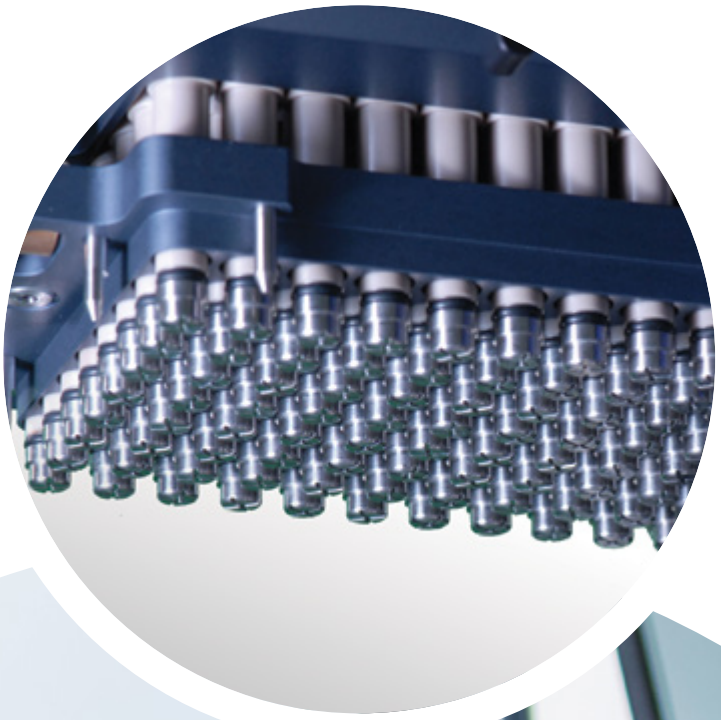
If the environmental conditions change, the performance of the liquid transfers must be confirmed again.

3 How Automated Liquid Handling Works

This section describes how automated liquid handling on a Hamilton automated liquid handler platform executes automated programs to move and process liquids. There are two key aspects that are covered in detail:

3.1 Hardware

The automated liquid handler's hardware is the first and most important part of the automated liquid handling process. The hardware does the physical work of transferring liquids between different types of labware. Each automated liquid handler is relatively customizable, so that the size and transfer capabilities can be configured as needed for most lab applications. No matter the configuration, lab techs can easily load labware, samples, and other liquids onto the automated liquid handler's deck.

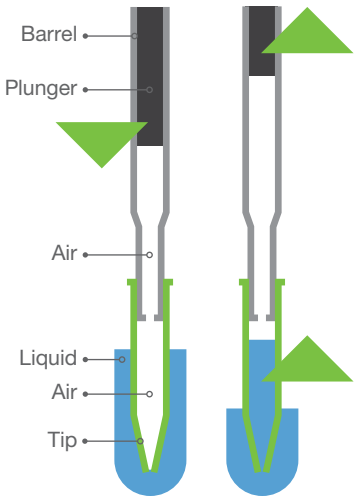


3.1.1 Channel Technology

Within each automated liquid handler, the same component manages the actual transfer of liquid: the channel.

The channel is a pipette equipped with high precision motors and electronics for movement on the y- and z-axis of the automated liquid handler. The pipette is used to pick up disposable tips to then aspirate and dispense liquid from its source to its destination labware.

Channels can be added to an automated liquid handler in different sizes and configurations to best serve the need. An automated liquid handler can have anywhere from 1 independent pipette channel to 384 pipette channels on a Probe Head. Channel sizes control how much volume can be aspirated and dispensed, ranging from channels that allow less than 1 µL to the 5 mL channels for significantly more volume.

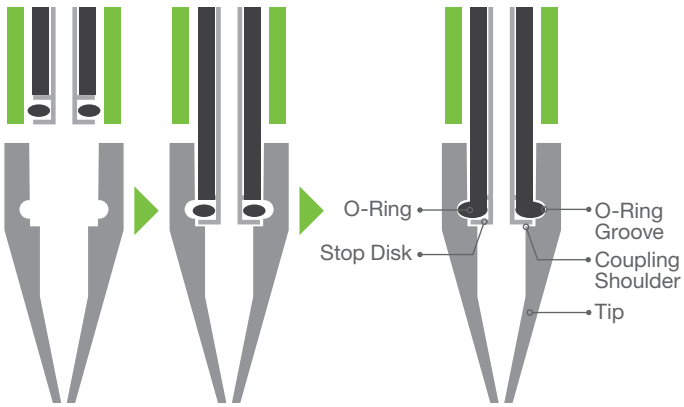


Hamilton channels work similarly to a hand pipettor, using air to displace the liquid. Since there is no liquid in the channel itself, there's no risk of carryover between different samples.

The channel picks up a tip, then aspirates liquid into the tip only. The plunger and barrel are used to control the aspirate and dispense using air. The channel itself never comes in contact with the liquid.

The independent channels (1,000 µL, 5 mL) have two ways to detect the liquid level: by capacitance and pressure. Capacitive liquid level detection works for liquids that are conductive, while pressure liquid level detection is useful for liquids that aren't conductive. For the capacitance liquid level detection to work, it is necessary to use a specific type of Hamilton-made disposable tip or reusable needles that are conductive. Refer to Section 3.2 Tips and Reusable Needles for more information on tip types. Note that for Multi-Probe Heads, only capacitance liquid level detection is available.

Hamilton channels use a special proprietary method to pick up tips, called CO-RE. CO-RE stands for Compressed O-Ring Expansion. This means that every channel has an o-ring near the bottom that fits into a small groove in the tip. When the channel is ready to pick up the tip, the channel compresses the o-ring to fill the groove, allowing the tip to be lifted with little pressure except on the o-ring.



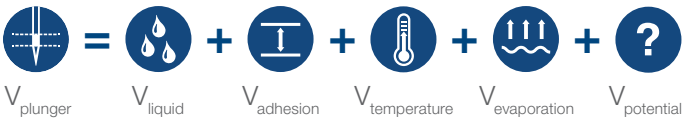
Benefits of CO-RE technology

- Allows for more precise and consistent tip attachment. CO-RE technology creates a lock and key functionality which prevents the tip from falling off during transfers.
- Allows for gentler tip detachment. Tips are not ejected by force into the waste (creating possible aerosol contamination), but instead fall off when the o-ring is released.
- Lessens the impact on the hardware from the fit of the tip. This allows for more sensitive monitoring of z-step losses. It also ensures that the hardware doesn't wear out as quickly.
- Enables pressure monitoring because of the tight seal between the channel and the tip.
- Allows for the interchange of multiple tip sizes within the same process.

3.1.1.1 Inside the Pipette Head

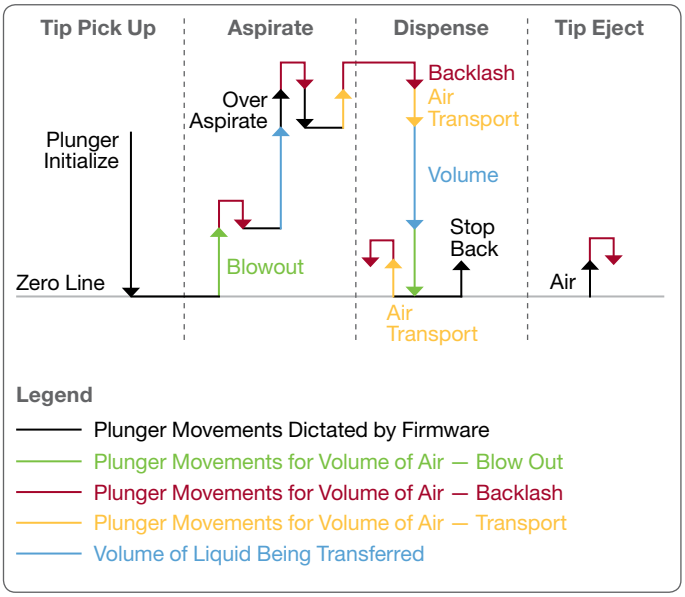
The volume of air displaced by the plunger inside the channel is not always equivalent to the volume of liquid that is aspirated. Reasons for this include:

- Adhesive qualities of the liquid
- Temperature of the liquid
- Rate of evaporation
- Hydrostatic pressure due to the force of gravity



A liquid class must be defined to overcome these variables, ensuring accuracy and precision of the final liquid transfer. For example, a correction value is applied to the intended target value, which moves the plunger more or less than the intended volume. In addition, other adjustments to the flow rate, air volumes, over-aspirate volume, and others can be made.

The next image shows in more detail the plunger movements that occur during each of the main transfer steps: Tip Pickup, Aspirate, Dispense, and Tip Eject. The transfer volume in the aspirate and dispense steps are only part of an overall process for the plunger.



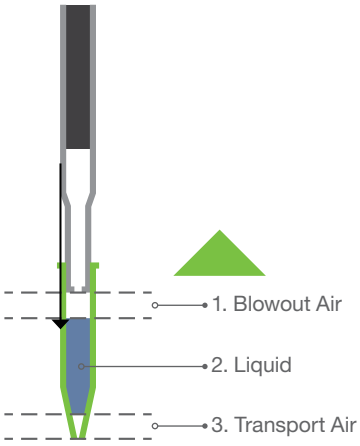
Parameters like the volume, blowout, over aspirate, and air transport are all configurable.

One behavior that is not controllable is the backlash of the plunger. The backlash is observed at the end of an upward plunger movement and is a mechanical recoil in the plunger mechanism. The recoil is minimal and does not have a large effect on the majority of transfers, but can play a role in lower volume transfers as plunger movement is so minimal to begin with.

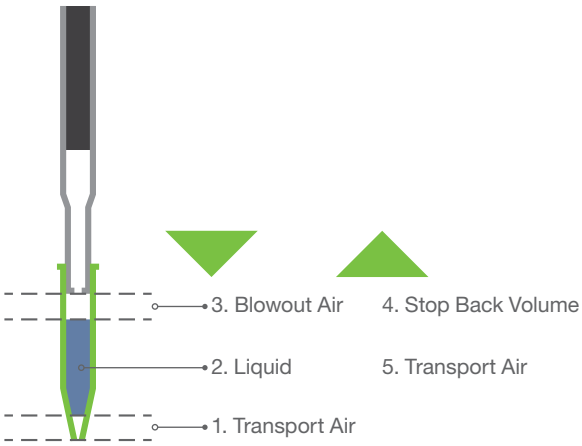
3.1.1.2 Inside the Tip

The activity inside the tip is affected by certain liquid class parameters. During an aspirate step, the blowout volume of air must be aspirated prior to the transfer volume. On the dispense, the blowout volume is then dispensed after the transfer volume.

Aspirate



Dispense



3.1.1.3 1,000 µL Channels

The 1,000 µL channel is the most common channel type across all Hamilton automated liquid handlers. Up to 16 1,000 µL channels can be added to one automated liquid handler, depending on what other modules are on the automated liquid handler, like the 5 mL channels or the camera channel. The 1,000 µL channel can accommodate a wide range of volume transfers from 0.5 to 1,000 µL. The different volume sizes can be made by selecting the appropriate tip size. Each channel can act independently and transfer different volumes, handle different liquid types, and pick up different sized tips within the same transfer step.

The 1,000 µL channel can fit multiple sizes of disposable tips. Review the table for trueness and precision specifications by tip size.

Pipetting Specifications for Disposable Tips

	Disposable Tip Size	Volume	Trueness |R| (%)	Precision CV (%)
Individual 1,000 µL Pipetting Channels	10 µL	0.5 µL	10.0%	6.0%
	10 µL	1 µL	5.0%	4.0%
	10 µL	5 µL	2.5%	1.5%
	10 µL	10 µL	1.5%	1%
	50 µL	0.5 µL	10.0%	6.0%
	50 µL	1 µL	5.0%	4.0%
	50 µL	5 µL	2.5%	1.5%
	50 µL	50 µL	2.0%	0.75%
	300 µL	10 µL	5.0%	2.0%
	300 µL	50 µL	2.0%	0.75%
	300 µL	200 µL	1.0%	0.75%
	1,000 µL	10 µL	7.5%	3.5%
	1,000 µL	100 µL	2.0%	0.75%
	1,000 µL	1,000 µL	1.0%	0.75%

For pipetting of less than 10 µL, use 10 µL / 50 µL Volume Disposable Tips to achieve the highest precision.

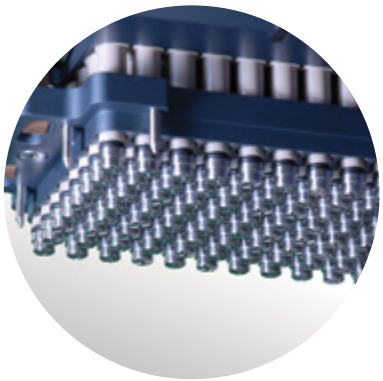
3.1.1.4 5 mL Channels

The 5 mL channel accommodates the largest transfer volume possible on Hamilton automated liquid handlers. Up to eight 5 mL channels can be added to one automated liquid handler, depending on other modules. 5 mL channels can be included alongside 1,000 µL channels on the same automated liquid handler. The 5 mL channel can accommodate a range of volume transfers from 50 µL to 5 mL. Like the 1,000 µL channel, each 5 mL channel can act independently, transfer different volumes, and handle different liquid types.

Pipetting Specifications for 5 mL Disposable Tips

	Disposable Tip Size	Volume	Trueness |R| (%)	Precision CV (%)
Individual 5 mL Pipetting Channels	5 mL	50 µL	5.0%	2.5%
	5 mL	500 µL	2.0%	1.5%
	5 mL	1,000 µL	1.5%	1.0%
	5 mL	5,000 µL	1.0%	0.5%

3.1.1.5 1,000 µL CO-RE 96-Probe Head



The 1,000 µL CO-RE 96-Probe Head contains 96 1,000 µL channels. Each channel aspirates or dispenses the same volume from all channels simultaneously, making it a useful tool for high throughput since the 96-Probe Head can fill an entire 96-well microplate with the same liquid in one transfer step. The 1,000 µL CO-RE Probe Head can accommodate a wide range of volume transfers from 1 to 1,000 µL.

If more flexibility is needed, individual 1,000 µL or 5 mL channels should be used.

The 1,000 µL CO-RE 96-Probe Head can fit multiple sizes of disposable tips. Review the table for precision specifications by tip size.

CO-RE 96-Probe Head Pipetting Specifications for Disposable Tips

	Disposable Tip Size	Volume	Trueness |R| (%)	Precision CV (%)
1,000 µL CO-RE 96-Probe Head **Maximum Pipetting Volume: 1,000 µL**	10 µL	1 µL	5.0%	5.0%
	10 µL	5 µL	2.5%	2.0%
	10 µL	10 µL	1.5%	1.5%
	50 µL	1 µL	5.0%	5.0%
	50 µL	5 µL	2.5%	2.0%
	50 µL	50 µL	1.5%	1.0%
	300 µL	10 µL	3.0%	2.0%
	300 µL	50 µL	1.5%	1.0%
	300 µL	300 µL	1.0%	1.0%
	1,000 µL	10 µL	7.5%	3.5%
	1,000 µL	100 µL	2.0%	1.0%
	1,000 µL	1,000 µL	1.0%	1.0%

For pipetting of less than 10 µL, use 10 µL / 50 µL Volume Disposable Tips to achieve the highest precision.

3.1.1.6 50 µL CO-RE 384-Probe Head

The 50 µL CO-RE 384-Probe Head contains 384 50 µL channels. Each channel aspirates or dispenses the same volume simultaneously, making it a useful tool for high throughput work since the 384-Probe Head can fill an entire 384-well microplate with the same liquid in one transfer step. The 50 µL CO-RE Probe Head can accommodate a range of volume transfers from 0.5 to 50 µL.

Due to the smaller channel size, the 384-Probe Head is optimal for low volume transfers.

The 50 µL CO-RE 384-Probe Head can fit one size of 50 µL disposable tips, but the tips can be arrayed in sets of 96 or 384. In addition, the use of Hamilton's Rocket Tips converts the CO-RE 384-Probe Head into a 96 pipetting channel head with a volume range of up to 300 µL.

CO-RE 384-Probe Head Pipetting Specifications for Disposable Tips

	Disposable Tip Size	Mode: Volume	Precision CV (%)
50 µL CO-RE 384-Probe Head **Maximum Pipetting Volume: 50 µL**	50 µL	Surface: 0.1 µL	8.0%
	50 µL	Surface: 0.5 µL	6.0%
	50 µL	Surface: 1 µL	3.5%
	50 µL	Jet: 1 µL	15.0%
	50 µL	Surface: 5 µL	3.0%
	50 µL	Jet: 5 µL	4.0%
	50 µL	Surface: 10 µL	2.0%
	50 µL	Jet: 10 µL	3.0%
	50 µL	Surface/Jet: 50 µL	2.0%

Disposable Tips with 50 µL CO-RE 384-Probe Head as 96-Probe Head

	Disposable Tip Size	Mode: Volume	Precision CV (%)
Maximum Pipetting Volume: 300 µL	300 µL Rocket	2 µL	4.0%
	300 µL Rocket	5 µL	2.0%
	300 µL Rocket	10 µL	2.0%
	300 µL Rocket	100 µL	2.0%
	300 µL Rocket	300 µL	2.0%

3.2 Tips and Reusable Needles

Tips are critical to the liquid handling process, since they are the only part of the automated liquid handler that comes in contact with the liquid. Multiple sizes and material types are available. The automated liquid handler's hardware, specifically its channels, determine which tip sizes can be used.

3.2.1 Tip Properties

Use the following properties to help you choose the correct tip for your application.

Size

The size of the tip determines the maximum transfer volume and affects how the liquid flows out of the tip.

Common Tip Sizes:

Material

Tips come in different materials for different applications. The material affects the wetting and adhesion of the liquid.

Disposable Tips

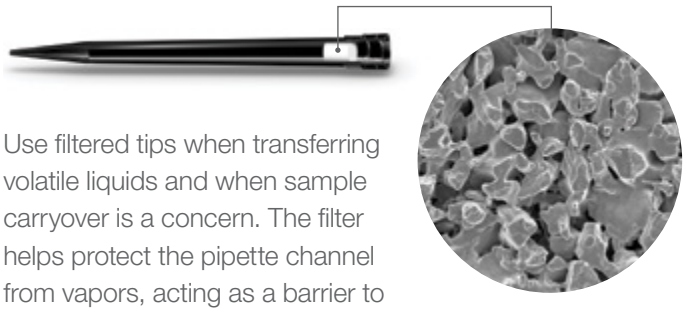
- Black plastic: Conductive. Contains carbon to make the tip conductive, which allows for capacitive liquid level detection.

- Clear plastic: Non-conductive.

Reusable Steel Needles:

- Require washing and proper procedures for maintenance and periodic replacement.

Filtered or Non-Filtered

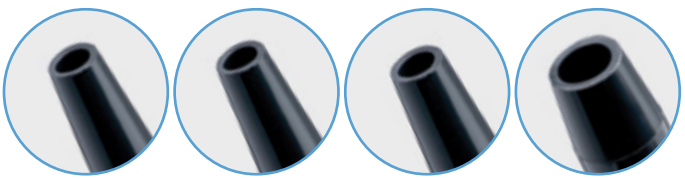


Use filtered tips when transferring volatile liquids and when sample carryover is a concern. The filter helps protect the pipette channel from vapors, acting as a barrier to prevent cross contamination of sample.

Note that the use of a filtered tip can affect the flow and maximum allowed correction volume due to the extra physical barrier it introduces between the channel and tip and the height of the filter itself. For all Hamilton tip types except 5 mL, the tip can still pipette the maximum volume stated, for example 1,000 µL filtered tips can still pipette 1,000 µL.

Normal or Wide Bore

For especially viscous liquids, liquids containing cells, or liquids containing clots, it may be necessary to use a wide bore tip, because the orifice size of normal tips may be too small. Wide bore tips are available in several orifice sizes, which reduce the maximum volume capacity. The larger the orifice, the shorter the tip and the less the total volume.



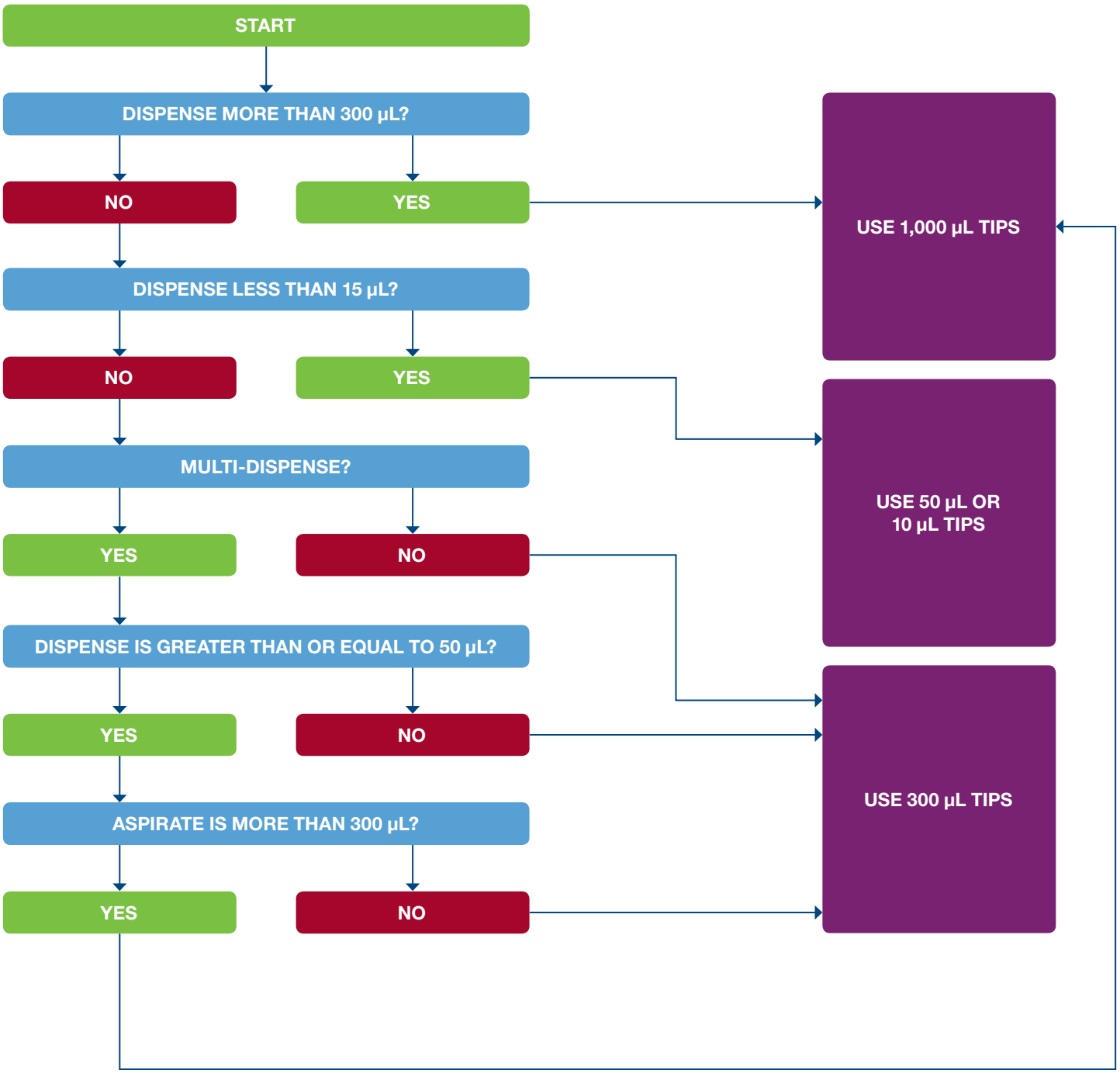
Geometry or Shape

Observe how each tip size also has a different geometry. The differences affect how liquid flows and collects in the tip. For example, thinner tips are more susceptible to capillary effect.

3.2.2 How to Choose a Tip Size

Use the chart to determine which tip size to use. Tip size is determined by the amount of volume that needs to be pipetted. In general, the tip size selected should be in the volume range of approximately 5 to 100% of the intended volume to be transferred. If the transfer volume is less than 5% of the tip volume capacity, the trueness and precision of the transfer could be negatively affected.

Determine Which Tip Size to Use



4 Automated Liquid Handling Methodology

The same general process applies when developing liquid handling methods across all labs, industries, and applications. Even though the details change, the liquids fundamentally need to be defined and optimized for use on the automated liquid handler.

The methodology explored in the subsequent pages walks you through the high level steps needed to develop optimized liquid handling performance, including best practices for common liquid handling activities.

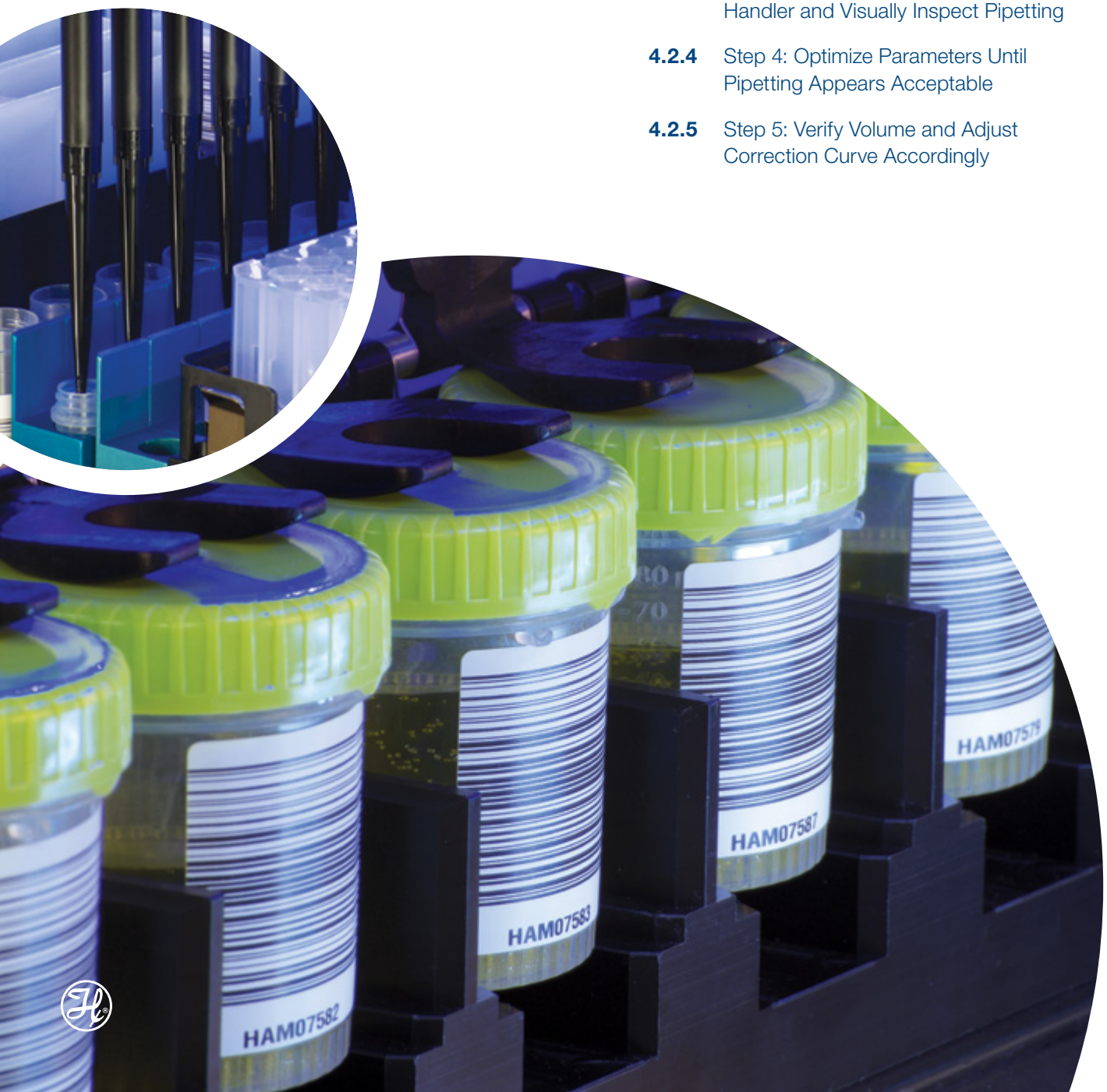
4.1 Before You Begin

Make sure that the following items are ready to go before getting started:

- The automated liquid handler needs to be installed, set up, and ready for use.
- The liquids for the application should be known, available, and brought to the desired temperature for the application.
- The tips and labware for the application should be known and available.

4.2 Steps for Setting Up Automated Liquid Handling

See the next sub-sections for a more in depth look at these steps for setting up automated liquid handling.



4.2.1 Step 1: Understand Properties of Liquid

The first step in the methodology is a careful examination of the physical properties of the liquid to be transferred. The purpose of the examination is to determine how the liquid behaves so you can get a sense for how to teach the automated liquid handler to handle it. For example, you could use a hand pipettor to pipette the liquid and get a feel for how it transfers before testing it in the automated liquid handler.

You can also refer to related reference documentation to get the specifics. Common resources include the MSDS (Material Safety Data Sheets) and online resources like wolframalpha.com.

For a detailed list of physical properties to analyze and their implications, refer to Section 2.3 Liquid Properties.

4.2.2 Step 2: Select a Predefined Liquid Class

All Hamilton software platforms come with predefined liquid classes. These liquid classes can be implemented in methods or used as a starting point for developing a more precise liquid class for a specific application.

When selecting a liquid class for use in a method or developing a new liquid class, always choose the liquid class with the closest match to the liquid. A suitable liquid class allows the automated liquid handler to know how to interact with the liquid and prevent mishandling.

For example, if developing a new liquid class for Master Mix, use the existing water definition as a starting point (green row) since both liquids are part aqueous and will have similar properties.

Examples of Synonymous Liquid Classes

Aqueous Solution	Volatile Organic Solvents	Involatile Organic Solvents	Viscous Liquid	Blood Products
Water	*Ethanol*	*DMSO*	*Glycerol*	*Serum*
PBS	Isopropanol	Alkane	Beads	Plasma
Master Mix	Acetone		Oil	Whole Blood
TRIS	Formaldehyde			Red Blood Cell

4.2.3 Step 3: Run Test and Visually Inspect Pipetting

Once the predefined liquid class is selected, it's time to test the liquid transfer on the robot. The predefined liquid class is used as a baseline to speed up the process of testing.

1. Set up the automated liquid handler with the appropriate source and destination labware.
2. Fill the labware with the liquid that you intend to transfer. If the liquid in question is costly or in short supply, use water for initial testing and optimization.
3. In the automated liquid handler's software, build a simple method to transfer the liquid that will mimic the step in the actual process. Focus on one transfer step in the process before testing the next. Make sure that the method settings are defined as you want them for the actual transfer. For more information about the available settings, see Section 5 Method Settings and Liquid Class.
4. Run the method and observe the transfer. Look for the following:
   a. Is the aspirate height or liquid level submerge depth too high/low?
   b. Are there any droplets on the end of the tips after aspiration?
   c. Is the dispense height or liquid level submerge depth too high/low?
   d. Are there any droplets on the end of the tips after dispense?
   e. Are the channels properly following the liquid level during aspiration and dispense? Should following be turned off?

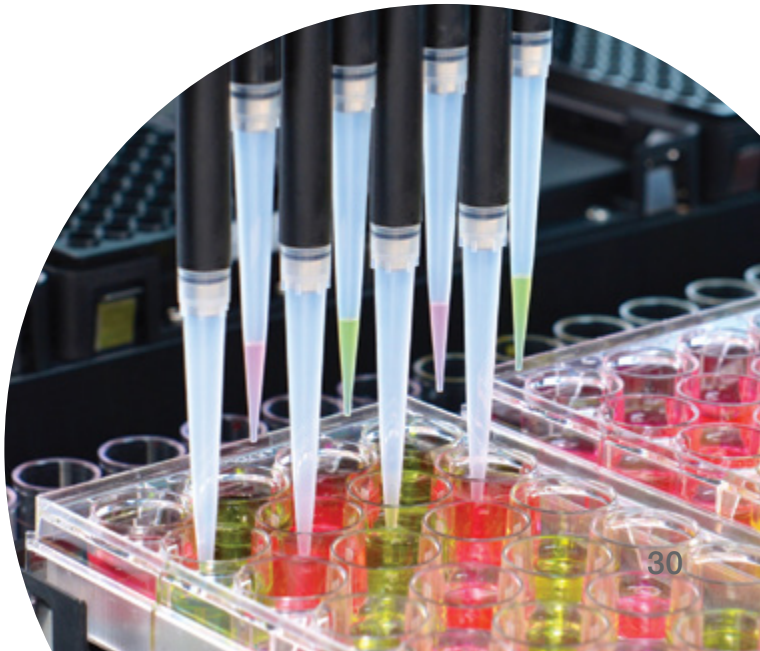
4.2.4 Step 4: Optimize Parameters Until Pipetting Appears Acceptable

Continue to run the simple method, observe the pipetting, and make adjustments based on what you see. The goal is to make sure that the pipetting looks correct. For example, there should be no dripping from the tip and no bubbles on dispense.

Start by making modifications to method settings such as enabling cLLD on the aspirate or adjusting the fixed height. For more information about method settings, see Section 5.1 Method Settings.

If the method settings are optimized, but the transfer still appears inconsistent, you can then focus on modifying the liquid class settings to improve performance. Follow these steps to adjust the liquid class:

1. Save the liquid class under another name. Now it can be modified to work for the specific application.
2. Change one liquid class parameter at a time to see its effect on the liquid transfer. For more information about liquid class parameters, see Section 5.2.1 Liquid Class Parameters.
3. Inspect transfers.
   a. Visually inspect for consistent transfers.
   b. Spot check with a handheld pipette to give an indication of consistency and if the transferred volume is short or in excess
4. Once the transfers look consistent, move on to step 5.



4.2.5 Step 5: Verify Volumes and Adjust Correction Curve Accordingly

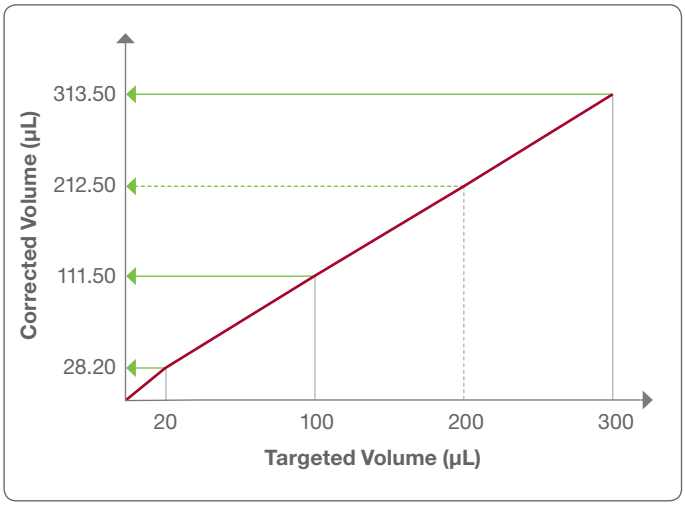
Once the pipetting appears to be acceptable, the transferred volumes can be quantified to determine the precision and the trueness of the liquid transfers.

Before measuring, it is important to know your application's pipetting requirements to make sure that the final optimizations meet the need. If the requirements are unknown and you want to minimize the amount of variability that pipetting contributes to your application, you can strive to match the specifications set for the pipetting device you are using. See Section 3.1.1 for pipetting specifications by device.

Once you know the pipetting requirements, use the tools and processes described in Section 8 Monitor Liquid Transfers to verify the transferred volumes. These verification steps can be used to assess and optimize transfers before testing them in your method. You might measure volumes gravimetrically to make sure the transfers are both accurate and precise. Then, you can continue to adjust liquid class settings until optimal precision is achieved.

When precision is achieved, the correction curve of the liquid class can be adjusted to ensure trueness of all volumes of interest for your application. For example, if you are transferring a volume of 300 µL, but are measuring a value of 295 µL, then you can increase the corrected value in the liquid class by an additional 5 µL. Continue to adjust until the proper target volume is achieved. For more information about correction curves, see Section 5.2.3 Correction Curve.

Corrected Volume and Targeted Volume



Once verification is complete, make sure the updated liquid class is implemented in your method. The liquid class can then be used by lab technicians running liquid transfers for experiments. Periodically check the performance of the liquid transfers to make sure that no changes are needed.

Keep in mind that the correction curve for any default liquid classes cannot be modified.

5 Method Settings and Liquid Class

Pipetting behavior in automated liquid handling is controlled through the method and liquid class settings. This section provides a detailed description of each setting, including when to use each one and best practices for how to optimize it.

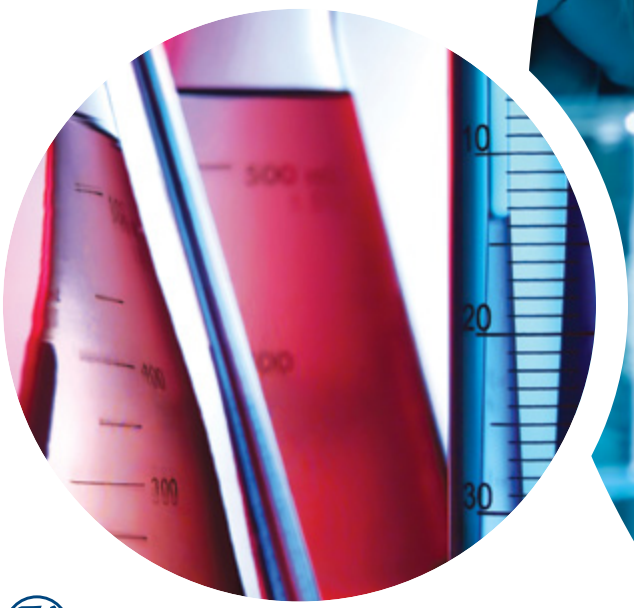
5.1 Method Settings

Method settings are defined for every individual aspiration and dispense in a method. These settings control the physical approach of the channel and tip based on the requirements for an individual transfer.

Part of defining method settings involves selecting a liquid class for each aspiration and dispense. The same liquid class is generally used for both aspiration and dispense cycles within the same step.

5.2 Liquid Class Settings

Liquid class settings are defined globally for a liquid, allowing the liquid to be used across many methods. These settings are developed based on the liquid's properties and control the channel's plunger during aspiration and dispense.



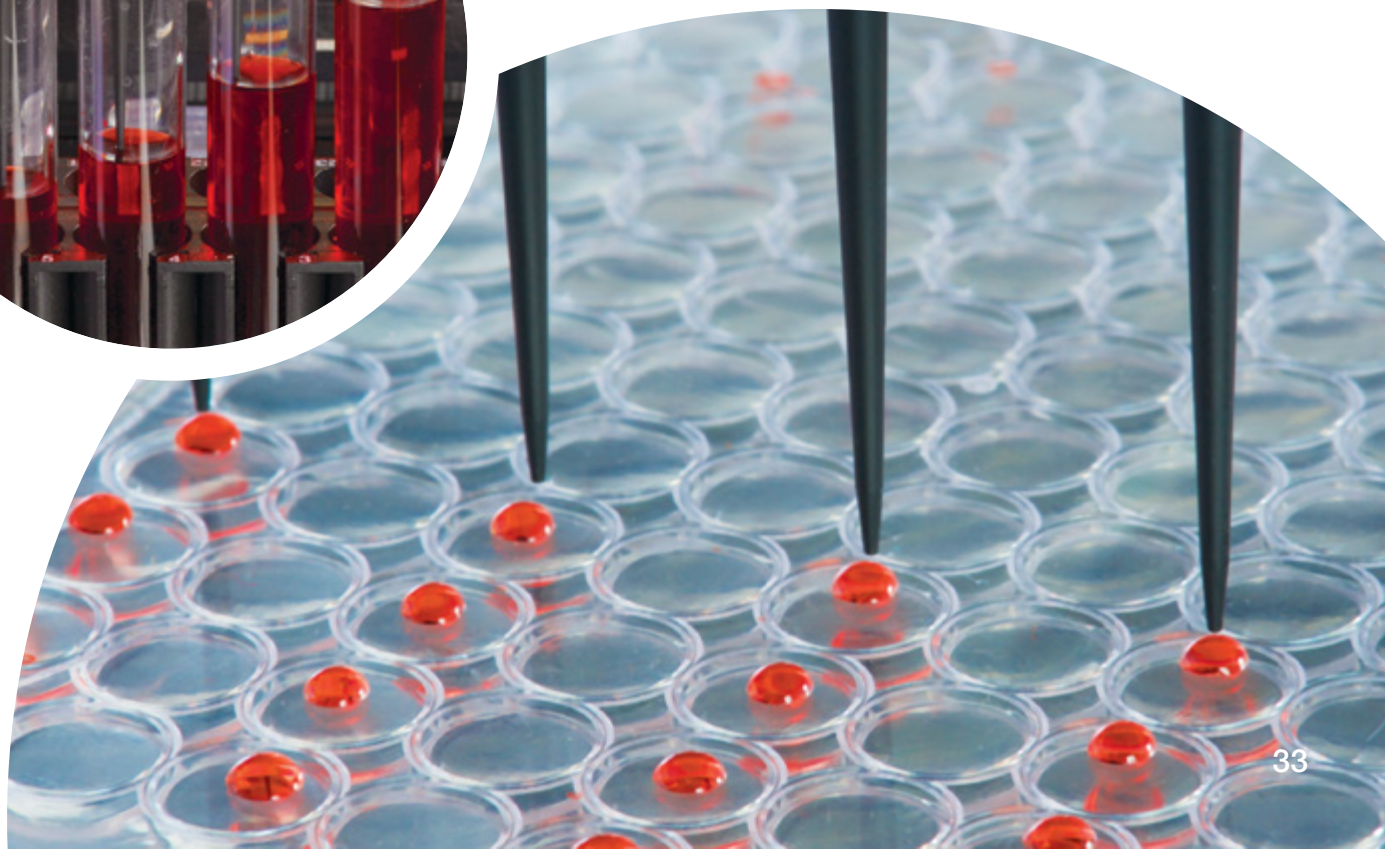
5.1 Method Settings

Method settings customize the pipetting behavior during an individual liquid transfer. These settings are defined in each method, for each aspiration and dispense, making them highly specific. The settings enable everything from liquid level detection to tip placement during aspiration and dispense.

Here are the liquid handling settings available for customization during method development:

- Aspirate mode and dispense mode
- Liquid level detection (LLD)
- Fixed height
- Anti-droplet control (ADC)
- Single vs. multiple uses of tips
- Pre-wetting
- Liquid following
- Minimize z-move
- Dispense on the fly

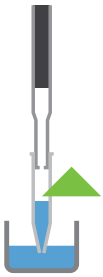
Keep in mind that Hamilton has multiple software platforms, and each platform has a different set of steps for how to access these settings. For more information, refer to Section 1 About This Guide that includes information about the relevant manual for each platform.



5.1.1 Aspiration Modes

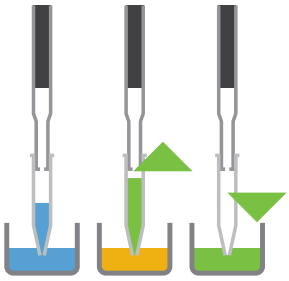
The aspiration mode controls how the liquid is pulled up into the tip. There are three options:

Aspiration



Default mode for the first aspirate in any pipetting cycle.

Consecutive Aspiration

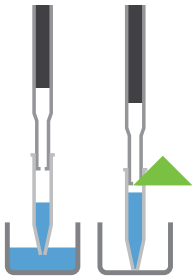


If several aspirations are done with the same tips before dispensing, set the first aspiration to Aspiration mode, and any of the other aspirations before dispensing to Consecutive Aspiration.

A blowout volume of air will not be aspirated during this consecutive aspiration step.

There are some applications where consecutive aspirations make sense and may save time, but it can be complex to fully optimize.

Aspirate All



Aspirates the complete, requested volume without an error. Monitoring is disabled as well as volume estimation during this transfer.

X Error Tracking

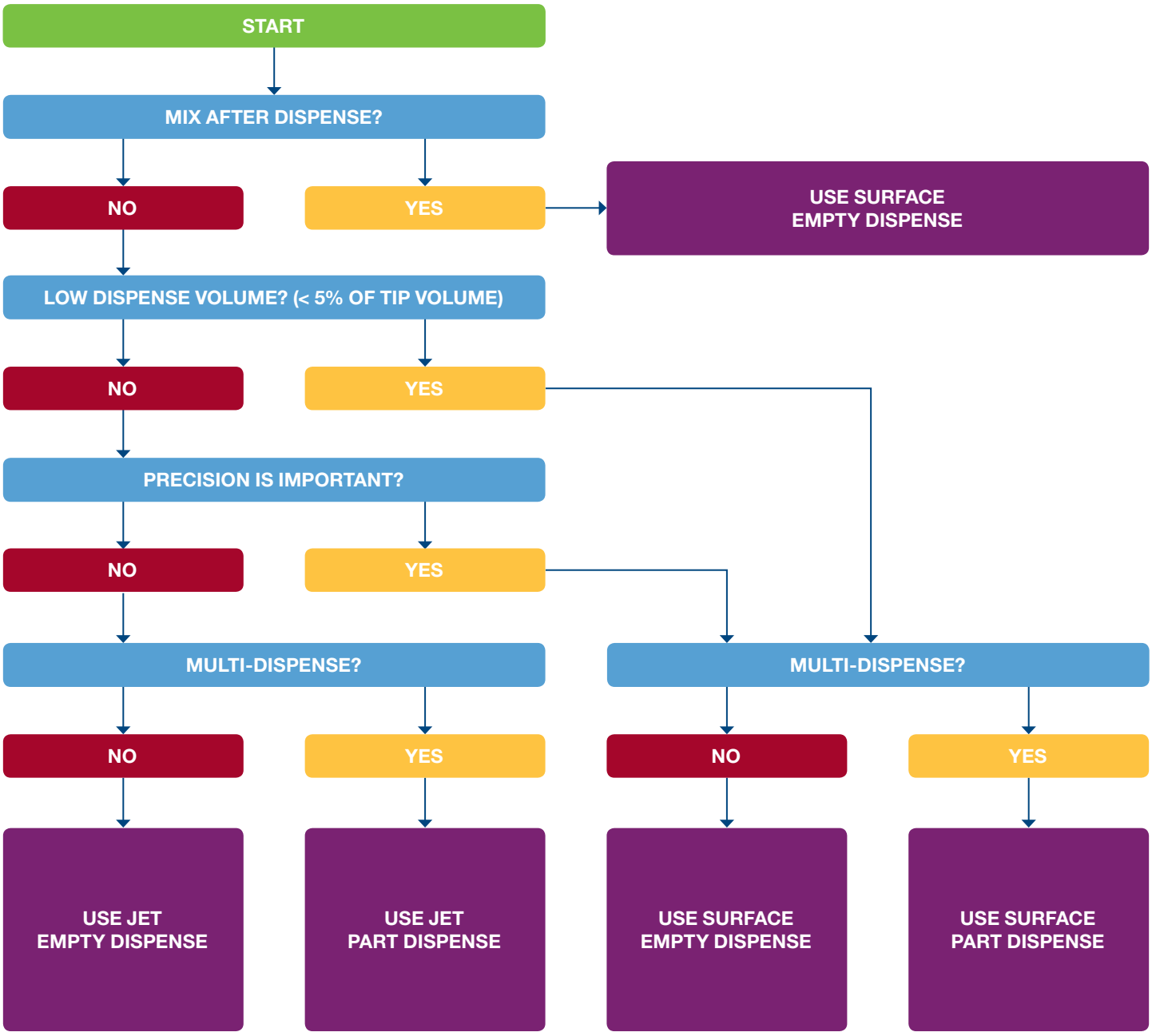
This setting is intended to be used to aspirate all the liquid in a container when specifying an aspiration volume larger than what may be present in the container. In this case the tip will follow the falling liquid level to the bottom of the container and stay there until the defined volume has been aspirated.

In addition, this function is useful when LLD is used but the volume estimation indicates a false insufficient amount of liquid. Aspirate All would ignore the error in this case and allow the benefit of using LLD.

5.1.2 Dispense Modes

The dispense mode controls how the liquid is released from the tip. There are two categories of dispense, jet and surface, with a few customizations for each category. Use the chart below to determine which mode to use for your application then read about each mode on the following page.

Determine Which Mode to Use



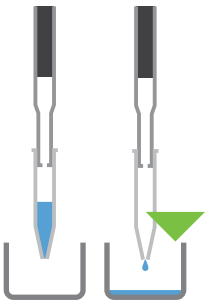
Jet Dispense

When set to a jet dispense mode, liquid from the tip is released from above the liquid level. Jet dispense is useful for keeping the tips out of the liquid already present in the well or tube.

When using jet dispense, make sure to properly set the dispense height, flow rate, and blowout volume to prevent foam as a result of dispensing. Also, keep in mind that jet dispense isn't effective for high viscosity liquids.

Jet Dispense Empty Tip

Empty the entire contents of the tip above the liquid level or into an empty container

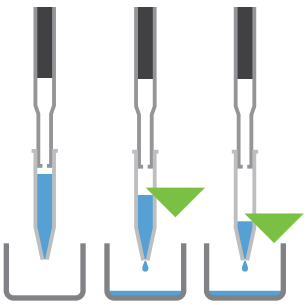


When to Use

- Pipetting higher volumes into empty containers
- Re-using tips and cannot touch liquid already present

Jet Dispense Part Volume

Dispense partial amount of the volume in the tip above the liquid level or into an empty container.



When to Use

- Multi-dispensing is required into empty containers or when the tips cannot touch liquid already present

Surface Dispense

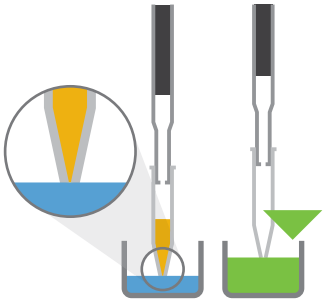
When set to a surface dispense mode, liquid from the tip is released at the liquid's surface or below the liquid level. Surface dispense is useful for all volumes of liquid, especially small volumes of 20 µL or less. High viscosity liquids need to be dispensed using surface dispense.

When using surface dispense, make sure to properly calibrate the other liquid handling settings to prevent bubbles and get accurate volumes.

- Be cautious when you use air transport volume. Any air in the transport volume can cause a bubble to form during dispensing.
- Use a blowout volume on aspiration, but not on dispense for low volumes. The air can help minimize the capillary effect.

Surface Dispense Empty Tip

Empty the entire contents of the tip into the liquid.

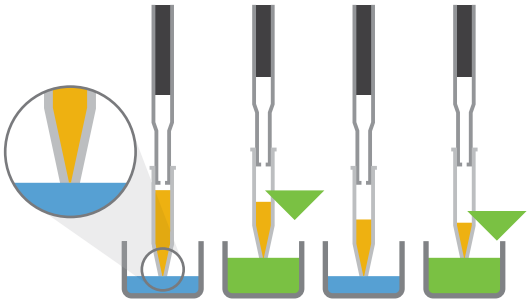


When to Use

- Optimal performance is required
- Pipetting low volumes
- Mixing is required during the dispense step

Surface Dispense Part Volume

Dispense partial amount of the volume in the tip into the liquid.



When to Use

- Multi-dispensing is required into liquid filled containers and re-use of tips is acceptable
- Pipetting via multi-dispensing is normally is done by jet part dispense mode. However, if the target containers are empty and there's no risk of cross-contamination, surface part dispense can be used to dispense small amounts of liquid to the bottom of the container that tend to adhere to the tip.

5.1.3 Liquid Level Detection

What Is It?

Liquid level detection, often abbreviated LLD, is the ability of the channel and tip to determine where the liquid surface is in a well or tube. Once the liquid level is found, an additional submerge depth can be specified to ensure the tip is in the liquid once it begins aspirating or dispensing.

Hamilton automated liquid handlers can detect the liquid level in two ways:

Capacitive Liquid Level Detection (cLLD)

A small charge is held in the channel until the tip reaches a conductive liquid. The charge disperses after coming in contact with the liquid, which indicates to the channel that the liquid level has been reached. This form of LLD is only possible with conductive liquids and conductive tips. Involatile organic solvents can't be detected.

A minimum volume has to be available to measure the capacity (e.g. 30 µL in a 384 MTP). The signal depends on the labware and carrier.

Pressure Liquid Level Detection (pLLD)

The channel aspirates a small amount of air as the tip travels downward. As the tip nears the liquid, the liquid causes a slight resistance, allowing the pressure sensor in the channel to detect the liquid level. pLLD is generally available for liquid level detection on all types of liquid.

Liquid level detection with the pressure sensor requires that the liquid has a sufficient resistance.

Always use dry tips with pLLD. If the tip has already been used, the precision with small volumes can be affected due to carryover.

Why It's Important

Confirms liquid is present prior to the aspirate or dispense step.

Prevents aspiration of air.

Prevents excessive carryover when used in conjunction with a submerge depth and liquid following.

Returns height of found liquid, which is used to determine the estimated volume.

In general, cLLD is used more often than pLLD as it allows for the re-use of tips and can be enabled on the dispense step. The sensitivity of both LLD functions can be adjusted. As the optimal sensitivity to use is greatly influenced by the labware, the default sensitivity value is set in the labware definition. However, it can be overwritten to adjust the settings from low to high. See Section 1 About This Guide for information about the relevant manual for each platform.

A combination of cLLD and pLLD can be used to detect foam. If the measured difference during the execution of the aspiration is greater than the maximum specified difference, an error can be generated.

If liquid level detection isn't an option for a liquid, a fixed height should be set to determine where the aspirate and dispense should physically occur within the well or tube. Both types of LLD require a minimal amount of liquid in the container to work reliably. If that volume is insufficient, then LLD won't perform robustly and a fixed height is recommended. The amount of liquid needed for proper LLD varies based on container type.

5.1.4 Fixed Height

What Is It?

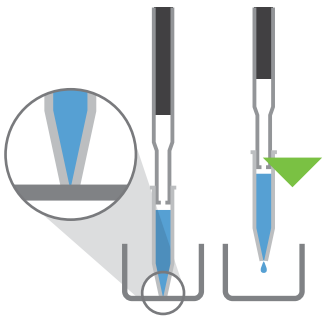
Fixed height is a set position from the bottom of a well or tube. Liquid can be aspirated to or dispensed from the fixed height.

Why It's Important

If the volume is too low, LLD may not work reliably and fixed height must be used. Fixed height helps to minimize dead volume and improve speed.

Fixed height has additional settings that can optimize the liquid transfer. These settings are optional and are disabled by default:

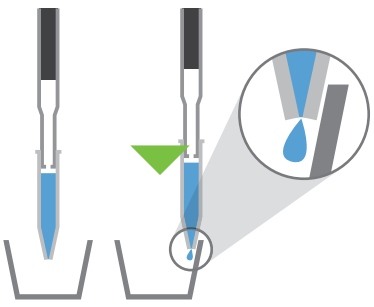
Touch Off



When Touch Off is enabled, the tip gently lowers to the bottom of the well or tube until it locates the bottom. The tip then raises up to a specified height from the bottom.

Touch Off is available in both aspirate and dispense steps and helps to ensure consistent heights. This function is particularly helpful in cases where labware becomes warped due to heating, making the bottom of each container vary slightly by height.

Side Touch

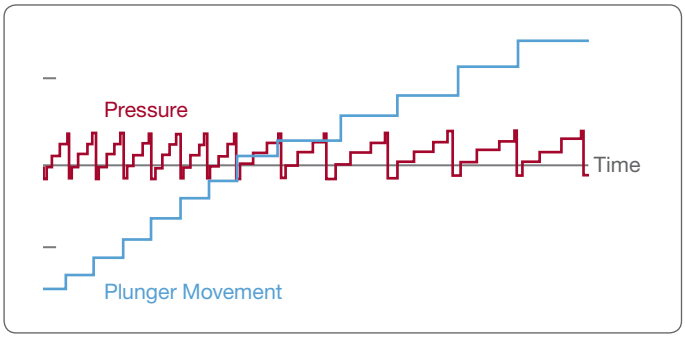


When Side Touch is enabled, the tip dispenses at the side of the container wall. The distance for movement to the side of the container and the dispense height is defined within the labware definition. This setting helps ensure proper dispense of liquids that have residual droplets or foam.

5.1.5 Anti-Droplet Control

What Is It?

Anti-droplet control, often abbreviated to ADC, is the ability of the channel to continuously monitor the pressure inside the barrel, detecting tiny increases in pressure (red line). When pressure reaches the point where dripping is likely, the channel's plunger movement is incremented to equalize the pressure to keep liquid inside tip and prevent dripping (blue line).



When using ADC, set the liquid class parameters to the following values for robust performance:

- Set air transport volume close to zero.
- Set stop back volume lower than 10 µL.
- Set a low swap speed (e.g. 2 mm/s).

Why It's Important

ADC is useful for volatile liquids with extremely low viscosity and high vapor pressure like acetone and methanol.

Limitations

- Don't use ADC if the liquid has a low vapor pressure or if the same tips are used multiple times, like in a multi-dispense. If ADC is used in these conditions, there can be errors in precision of the pipetting.
- ADC can only help in transferring liquids where droplets are formed due to a pressure increase due to evaporation. It will not assist in cases where the liquid drips due to a pressure decrease due to gravity or lack of surface tension.
- ADC can only be used for 1,000 µL and 5 mL channels. The function is not available for Multi-Probe Heads.

5.1.6 Single vs. Multiple Uses of Tips

For optimal performance, use tips only once (single use). Using new tips for every pipetting step helps avoid carryover between different wells or containers.

If carryover is not a concern, tips can be re-used for multiple steps. Keep in mind that a re-used tip may transfer a slightly larger volume than intended due to residual volume.

5.1.7 Pre-Wetting

Pre-wetting is a mixing step during aspiration to saturate the vapor pressure and reduce drop formation. It can also be used as a mixing step for a serial dilution or for homogenizing a mixture before aspiration.

Keep in mind:

- It may not be possible to use pLLD with a pre-wet tip. Any residual liquid left in the tip may interfere with the pLLD function.
- A pre-wet tip may transfer a slightly larger volume than intended.
- The correction curve for a pre-wet tip is likely different from an unused tip.

5.1.8 Liquid Following

When liquid following is enabled, the tip follows the decreasing liquid level according to the aspirated volume and the container geometry. If enabled on the dispense step, the tip moves up as the liquid level increases. The distance covered while following the liquid level is computed from the known geometry of the liquid container.

Liquid following can help prevent or minimize the tip from being submerged in the liquid while it is aspirating or dispensing, which limits carryover effects.

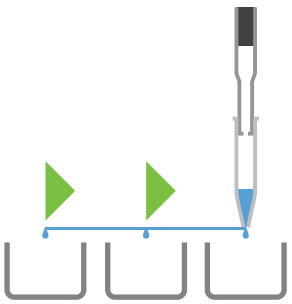
5.1.9 Minimize Z-Move

If minimize z-move is selected on the dispense step, the automated liquid handler moves the channels to a lower traverse height above the labware as opposed to the higher, default traverse height. Enabling this parameter can help speed up a multi-dispense step.

Make sure to prevent collisions when enabling minimize z-move. If enabled, the channels will use the minimal set traverse height of labware as the start and end position of the step. Ensure there is no taller labware placed between these steps or else a collision may occur.

5.1.10 Dispense on the Fly

Similar to the minimize z-move function, the dispense-on-the-fly function can speed up a multi-dispense step. If enabled, the tip raises to a fixed height above the labware and dispenses to the wells while continually moving in the x-direction. Unlike the normal dispense step or the minimize z-move function, the channels do not stop to move back to a traverse height.



This function can be challenging to optimize. The proper x-speed of the channels must be set together with an x-offset that will work for the volume being dispensed. Start the dispense at the beginning of the well so that it is captured properly.

5.2 Liquid Class Settings

Liquid classes are global definitions for a liquid that can be used in any method. The liquid class provides detailed instructions for how a channel's plunger should manipulate a liquid. A liquid class needs to be selected for each aspiration and dispense step in a method, and that liquid class needs to match the tip type and mode. This means that the liquid class needs to be defined in detail ahead of time before the method can be built.

Generally, there is one liquid class for each unique combination of the liquid type, tip type, and dispense mode. For example, a variety of default liquid classes are available for water, including the following classes:

Examples of Default Liquid Classes for Water

Liquid Class	Liquid	Tip Type	Dispense Mode
StandardVolume_Water_DispenseJet_Empty	Water	300 µL	Jet Empty Tip
StandardVolume_Water_DispenseJet_Part	Water	300 µL	Jet Part Volume
StandardVolume_Water_DispenseSurface_Empty	Water	300 µL	Surface Empty Tip
StandardVolume_Water_DispenseSurface_Part	Water	300 µL	Surface Part Volume
LowVolume_Water_DispenseSurface_Empty	Water	10 µL	Surface Empty Tip
LowVolume_Water_DispenseSurface_Part	Water	10 µL	Surface Part Volume

Hamilton provides predefined liquid classes for common liquid types.
Any predefined class can be modified to meet the needs of a specific liquid.

Modification of a liquid class involves adjusting multiple settings:

- Liquid class parameters control the tiny movements of the plunger that affect the movement of the liquid in the tip.
- The correction curve is a manual adjustment to the plunger's calibration to ensure that you can flexibly transfer any volume needed. Without a correction curve, there could be errors in a transfer unless the method transfers the exact same volume that was defined in the liquid class.

5.2.1 Liquid Class Parameters

Liquid class parameters are critical in liquid handling, because they affect the precision and trueness of every liquid transfer. When a new liquid class is being developed, each of these values may need to be adjusted.

Liquid Class Parameter Values

Parameter	Definition	Aspirate	Dispense
Flow rate	Liquid flow rates in µL/s, corresponding to plunger speed.	✓	✓
Mix flow rate	Liquid flow rates in µL/s, corresponding to plunger speed for mixing.	✓	✓
Air transport volume	Volume of air in µL is aspirated at the end of the aspiration and dispense step.	✓	✓
Blowout volume	Volume of air in µL that is aspirated first during the aspiration step. If dispensing in empty tip mode, part or all of the air is dispensed.	✓	✓
Swap speed	Speed in mm/s which the pipette head is moved out of the liquid.	✓	✓
Settling time	Time in seconds that the pipette head remains in the liquid after aspiration or dispense.	✓	✓
Over-aspirate volume	After aspirating the required volume an additional volume in µL is aspirated and dispensed again immediately.	✓	✗
Clot retract height	A parameter for clot detection that determines how high the pipette head is allowed to move out of the liquid while there is still a liquid detection signal after aspiration.	✓	✗
Stop flow rate	Dispense flow rate in µL/s at which the dispense step terminates abruptly.	✗	✓
Stop back volume	Volume in µL which is aspirated again immediately after the dispense.	✗	✓

5.2.1.1 Flow Rate

Aspirate Dispense

What Is It?

Liquid flow rate in µL/s that correspond to plunger speed for aspirate and dispense steps. The optimum setting for flow rate allows the liquid to enter or leave the tip as fast as possible while still allowing for consistent volumes to be transferred. The flow rate is heavily dependent on liquid properties.

Influenced by:

- Tip geometry
- Density
- Viscosity
- Vapor pressure

Best Practices for Aspirate

Fast

- Volatile liquids

Slow

- Smaller tip diameters
- High viscosity liquids

Best Practices for Dispense

Fast

- Jet dispense mode
- Volatile liquids

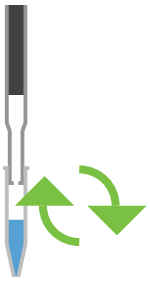
Slow

- Smaller tip diameters
- Surface dispense
- High viscosity liquids

5.2.1.2 Mix Flow Rate

Aspirate Dispense

What Is It?



Liquid flow rates in µL/s, corresponding to plunger speed for mixing.

Influenced by:

- Tip geometry
- Density
- Liquid viscosity
- Vapor pressure

Best Practices for Aspirate

Fast

- Volatile liquids

Slow

- Smaller tip diameters
- High viscosity liquids

Best Practices for Dispense

Fast

- Jet dispense mode
- Volatile liquids

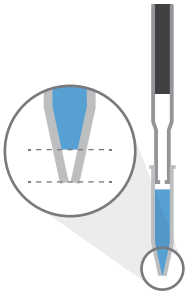
Slow

- Smaller tip diameters
- Surface dispense
- High viscosity liquids

5.2.1.3 Air Transport Volume

Aspirate Dispense

What Is It?



Volume of air in µL that is aspirated at the end of the aspiration and dispense step and is automatically dispensed again as an extra volume at the beginning of the dispense step. This extra air volume helps prevent droplet formation and dripping.

Keep in mind that using surface dispense mode plus air transport volume could cause bubbles to form. Do not use an excessive amount of volume for the air transport.

Influenced by:

- Tip geometry
- Liquid vapor pressure

Best Practices

- Set air volume to be less than liquid volume.
- Only use the volume that's needed to do the job. Too little or too much volume can cause leaks from the tip.

Large Air Transport Volume

- Volatile liquids

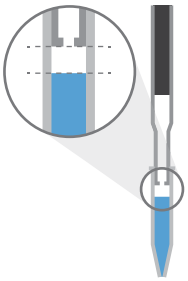
Small Air Transport Volume

- Smaller tips

5.2.1.4 Blowout Volume

Aspirate Dispense

What Is It?



Volume of air in µL that is aspirated first during the aspiration step. If dispensing in empty tip mode, part or all of the blowout volume of air is dispensed.

Influenced by:

- Liquid viscosity
- Liquid vapor pressure

Best Practices for Aspirate

- Set equal to or greater than the blowout volume set for the dispense.
- Set higher than the value set for the dispense when pipetting volatile liquids.

Best Practices for Dispense

- Be careful using blowout volume in surface dispense mode. Bubbles and foam can be created.
- Be careful using blowout volume in jet mode. If set too high, the liquid could be turned into an aerosol and cause contamination.
- When transferring a small volume, use blowout volume to push all the liquid out of the tip.

Large Blowout Volume

- Jet dispense mode
- High viscosity liquids

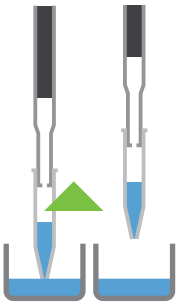
Small Blowout Volume

- Surface dispense

5.2.1.5 Swap Speed

Aspirate Dispense

What Is It?



Speed in mm/s at which the pipette head is moved out of the liquid after aspiration and dispense.

After aspiration and dispense, the tip speed must be carefully controlled to prevent droplet formation for various liquid types.

Influenced by:

- Liquid viscosity
- Liquid vapor pressure

Best Practices for Aspirate

Fast

- Volatile liquids

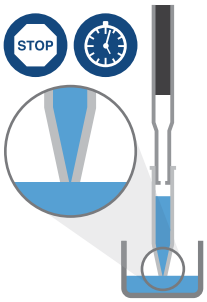
Slow

- High viscosity liquids

5.2.1.6 Settling Time

Aspirate Dispense

What Is It?



Time in seconds that the pipette head remains in the liquid after the aspiration and dispense step before moving out of the liquid. Some liquids take longer to settle into the tip than others.

If a liquid that requires a long settling time is programmed to use a short settling time, then an insufficient amount might be aspirated or dispensed.

Influenced by:

- Tip geometry
- Liquid viscosity

Best Practices

Long

- Viscous solutions
- Surface dispense into empty containers

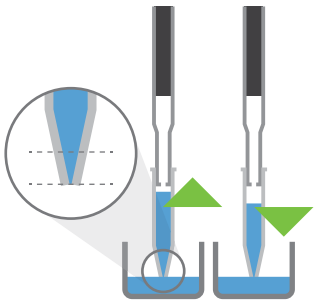
Short

- Aqueous solutions
- Highly volatile solutions
  (Keep the settling time very short in this case)

5.2.1.7 Over-Aspirate Volume

Aspirate Dispense

What Is It?



After aspirating the required volume, an additional volume is aspirated and dispensed again immediately.

The over-aspirate volume works as a pre-conditioning or pre-wetting of the tip and can help minimize any capillary effect.

Influenced by:

- Liquid viscosity
- Vapor pressure

Best Practices

- Useful at low volumes
- Set lower than the aspirate volume

Large Over-Aspirate Volume

- High viscosity liquids
- Volatile liquids

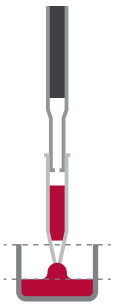
Small Over-Aspirate Volume

- Dilution steps (set the volume to zero in this case)

5.2.1.8 Clot Retract Height

Aspirate Dispense

What Is It?



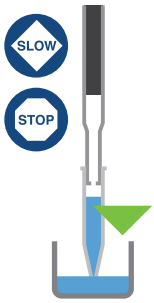
A parameter for clot detection that determines how high the pipette head is allowed to move out of the liquid while there is still a liquid detection signal after aspiration. It is measured from the liquid surface upwards. If this distance is exceeded, an error message is generated.

Only used to help detect clots after aspiration, and doesn't affect liquid transfers.

5.2.1.9 Stop Flow Rate

Aspirate Dispense

What Is It?



Dispense flow rate in µL/s in at which the dispense step terminates abruptly.

Influenced by:

- Liquid viscosity

Best Practices

- Set equal to or less than dispense flow rate

Fast

- If using jet dispense, set stop flow rate generally equal to dispense flow rate, especially when multi-dispensing.

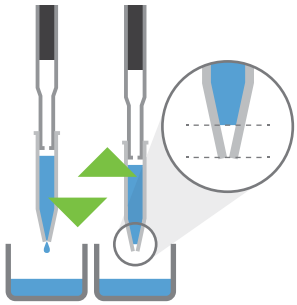
Slow

- If using surface dispense, set stop flow rate generally less than the dispense flow rate.

5.2.1.10 Stop Back Volume

Aspirate Dispense

What Is It?



Volume in µL which is aspirated again immediately after the dispense. This volume is aspirated as quickly as possible. The stop back volume helps prevent droplets after dispense in jet part volume mode by acting as an air transport volume in between transfers.

Influenced by:

- Liquid viscosity

Best Practices

- Pay attention to dispense height if using this setting. If set too low, the volume of dispensed liquid could be aspirated inadvertently instead of aspirating air.

5.2.2 Example Parameters

This section contains tables with common liquid types and examples of how the liquid class parameters vary by liquid, tip size, and dispense modes. Each table shows the default values for liquid classes provided by the software for 1,000 µL channels. The values shown are useful for getting a sense of how the liquid class parameters change by liquid type. In general, the following rules are true:

- **Larger tip sizes** result in faster aspirate flow rates, larger air transport volumes, and larger blow out volumes.
- The **more viscous the liquid**, the slower the aspirate flow rate and the larger the blowout volume.
- The **more volatile the liquid**, the larger the air transport volume and the faster the aspirate flow rate.
- **Jet Empty mode** requires faster dispense flow rates and larger blowout volumes compared to Surface Empty mode.

50 µL Surface Empty Liquid Class

	Ethanol	Water	Glycerin 80
Aspirate			
Flow rate (µL/s)	100	100	50
Air transport volume (µL)	2	0	0
Blowout volume (µL)	2	1	2
Dispense			
Flow rate (µL/s)	75	120	50
Air transport volume (µL)	2	0	0
Blowout volume (µL)	2	1	2

300 µL Surface Empty Liquid Class

	Ethanol	Water	Glycerin 80
Aspirate			
Flow rate (µL/s)	100	100	50
Air transport volume (µL)	3	5	0
Blowout volume (µL)	0	0	0
Dispense			
Flow rate (µL/s)	150	120	10
Air transport volume (µL)	5	5	0
Blowout volume (µL)	0	0	0

1,000 µL Surface Empty Liquid Class

	Ethanol	Water	Glycerin 80
Aspirate			
Flow rate (µL/s)	250	250	150
Air transport volume (µL)	5	5	0
Blowout volume (µL)	10	0	30
Dispense			
Flow rate (µL/s)	120	120	120
Air transport volume (µL)	15	5	10
Blowout volume (µL)	10	0	30

1,000 µL Jet Empty Liquid Class

	Ethanol	Water	Glycerin 80
Aspirate			
Flow rate (µL/s)	250	250	200
Air transport volume (µL)	5	5	5
Blowout volume (µL)	0	40	50
Dispense			
Flow rate (µL/s)	400	400	300
Air transport volume (µL)	15	5	15
Blowout volume (µL)	0	40	50

5.2.3 Active Parameters

Depending on which aspirate or dispense mode is selected when defining a liquid class, some liquid class parameters may or may not be available. Use the charts in this section to understand how the mode affects the parameters. For example, if the Aspirate All mode is selected for an aspirate step, the mix flow rate is disabled because the entire contents of the container will be aspirated and no extra mix step is needed.

Parameters by Aspirate Mode

	Simple Aspirate			Consecutive		Aspirate All		
LLD Setting	**cLLD ON**	**pLLD ON**	**LLD OFF**	**cLLD ON**	**LLD OFF**	**cLLD ON**	**pLLD ON**	**LLD OFF**
Flow Rate	✓	✓	✓	✓	✓	✓	✓	✓
Mix Flow Rate	✓	✓	✓	X	X	X	X	X
Transport Volume	✓	✓	✓	✓	✓	✓	✓	✓
Blowout Volume	✓	✓	✓	X	X	✓	✓	✓
Swap Speed	✓	✓	✓	✓	✓	X	X	X
Settling Time	✓	✓	✓	✓	✓	✓	✓	✓
Over Aspiration	✓	✓	✓	X	X	X	X	X
Clot Retract	✓	X	X	✓	X	X	X	X

✓ Editable

X Not Editable

Parameters by Dispense Mode

	Jet		Surface			
	Part Volume	Empty Tip	Part Volume		Empty Tip	
LLD Setting	**cLLD OFF**	**LLD OFF**	**cLLD ON**	**pLLD OFF**	**cLLD ON**	**LLD OFF**
Flow Rate	✓	✓	✓	✓	✓	✓
Mix Flow Rate	✗	✗	✗	✗	✓	✓
Transport Volume	✓	✓	✓	✓	✓	✓
Blowout Volume	✗	✓	✗	✗	✓	✓
Swap Speed	✗	✗	✓	✓	✓	✓
Settling Time	✗	✗	✓	✓	✓	✓
Stop Flow Rate	✓	✓	✓	✓	✓	✓
Stop Back Volume	✓	✗	✗	✗	✗	✗

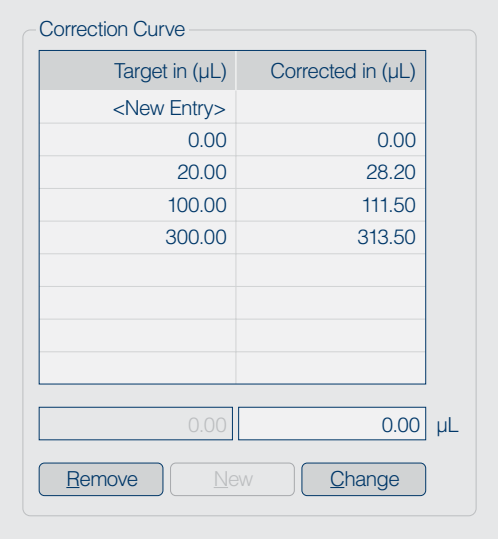
✓ Editable

✗ Not Editable

5.2.4 Correction Curve

While the parameters help determine the precision of the liquid transfer, the liquid class correction curve affects the trueness. The correction curve makes sure that the liquid is pipetted correctly across a range of volumes.

As the liquid class is defined, the correction curve also needs to be set, by defining target and corrected values. In the screenshot below, a correction curve with 4 points is defined. The target value is the amount of liquid that actually needs to be transferred. The corrected value is an adjusted volume to control the plunger, ensuring that the target amount is hit. For more information about the plunger's movement, see Section 3.1.1 Channel Technology.

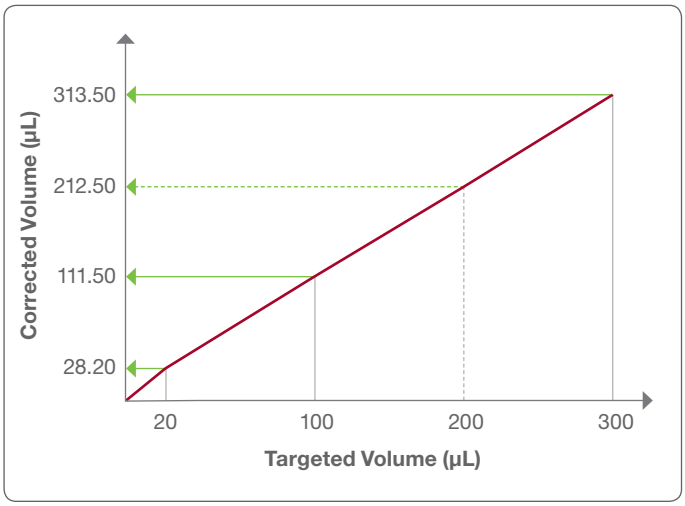


Including more points on the curve ensures truer volume transfers across a larger dynamic range. As a result, when the instrument is used to run a new method on a volume that's not specified as a target volume, the software can rely on a corrected value derived by linear interpolation of the values already provided.

For example, the chart and table (right) show a correction curve for a 300 µL tip. In addition to 0, the correction curve has three target volumes up to 300 µL. If you used this liquid class's correction curve to pipette 200 µL, the corrected value would be interpreted as 212.5 µL. Note that this does not mean that 212.5 µL of liquid is transferred. Instead, the channel's plunger is moving in increments equal to 212.5, but physically only 200 µL of liquid is transferred. Refer to Section 3.1.1 Channel Technology for more detail.

Keep in mind that values in the correction curve can be modified in increments as low as .1 µL.

Corrected Volume and Targeted Volume



To improve a correction curve, use the data generated from liquid testing and calculate the appropriate changes to points on the curve. To determine the new correction value, make a relative correction adjustment using the measured value, the current correction value, and the target value:

$$\left(\frac{\text{Current Corrected Volume}}{\text{Measured Volume}}\right) \times \text{Target Volume} = \text{New Corrected Value}$$

Change the value to the new corrected value in the liquid class and perform additional testing to confirm. For a step-by-step walkthrough on fine-tuning a correction curve, refer to the companion spreadsheet "Improving a correction curve."

6 Common Examples and Best Practices

Certain types of liquid handling activities are common across most labs and can be a challenge to resolve. This section contains best practices and troubleshooting advice for these types of common liquid handling activities.

If you're not familiar with the names of the settings and what they mean, see Section 5 Method Settings and Liquid Class to learn their background.

6.1 Best Practices for Pipetting Low Volumes Using 1,000 µL Channels

Low volume pipetting using the 1,000 µL channels is defined as any volume between 0.5 and 20 µL.

Method Settings

- Use 50 µL tips
- Single dispense if possible
- Use surface empty mode
- Keep distance between tip and pipetting surface minimal without blocking the tip
- Liquid following turned off
- Use new tips for each transfer

Liquid Class Parameters

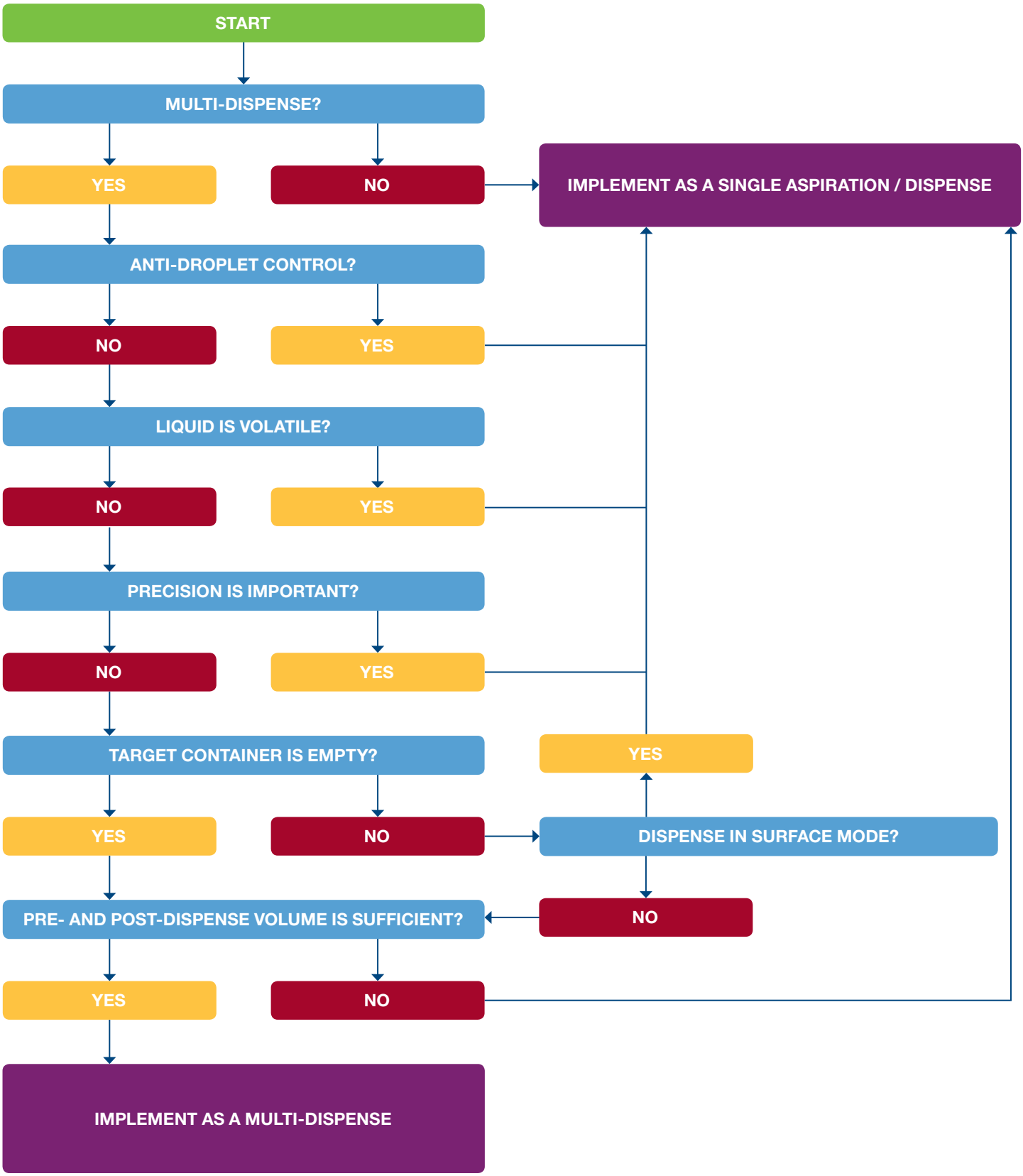
- May require an increase in settling time
- Slow swap speeds
- Increase blowout volume on aspiration, but minimize on dispense. Prevents capillary action by creating an overpressure

6.2 Best Practices for Multi-Dispensing (Aliquot)

Multi-dispensing, also known as aliquotting, is the act of aspirating enough total volume to dispense more than once from the same tip. While multi-dispensing can save time and tips, it can take more effort to control for the trueness and precision for each liquid transfer.

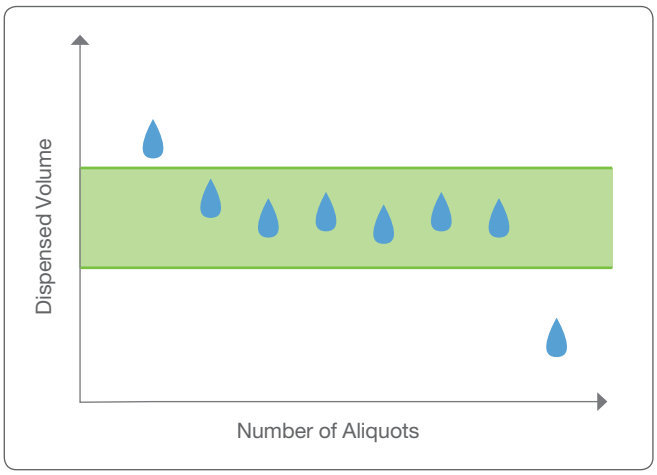
To decide if pipetting should be implemented as a multi-dispense, refer to the chart on the next page.

Determine if Pipetting Should Be Implemented as a Multi-Dispense



Method Settings

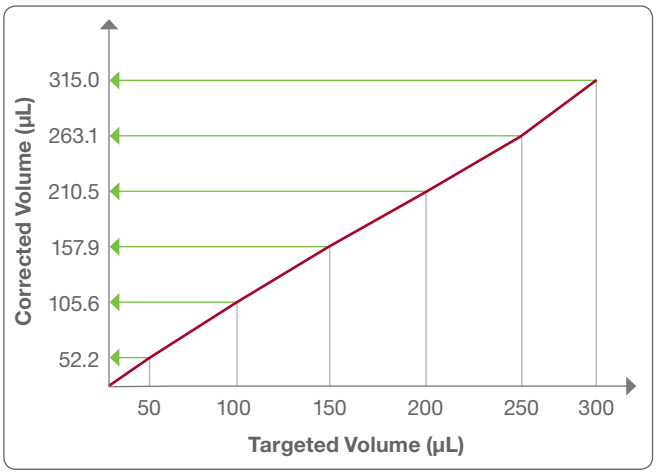
- Only use 50 µL tips or higher.
- The first and last aliquots are often inaccurate and should be discarded in order to maintain good precision overall amongst the transfers. Some liquids may require 2 pre-dispenses. See the image below for an example of a pre- and post-aliquot transfer that are discarded, ensuring that the transfer in between are within an acceptable range.



- Limit total number of aliquots per cycle.
  - The limit can vary based on transfer volume and tip size, but in general, it is recommended to keep the number of transfers to a minimum for easier control and more consistent liquid handling.
  - Recommend restricting the number of transfers for ease of method programming. For example, if pipetting to a 96-well format plate with an 8-channel automated liquid handler, limit the number of transfers steps to 6 (half the plate) or 12 (one whole plate).
- Pre-wet the tip on first aspirate. This action can nullify the effects of the correction curve and allow for consistent transfers.
- Use “minimize z-move step” function to prevent the pipettes from moving to a default traverse height in between transfers, increasing the speed of the transfers.
  - Set dispense height equal to the clearance height.
- To maximize speed, use “dispense on the fly” function to prevent the pipettes from stopping during the x and z-movement in between transfers. This function can be challenging to optimize. See Section 5.1.10 About the Dispense on the Fly Feature for more information.

Liquid Class Parameters

- Increase stop back flow rate to match dispense flow rate.
- Use a small stop back volume (dry dispense only).
- For the correction curve, it is important to note the total volume aspirated and then use a step down approach to determine the correction for each transfer.
  - For example, to transfer six aliquots of 50 µL using 300 µL tips, do not adjust for trueness at the 50 µL target volume like one would for a single transfer. Instead, adjust the target volume of 300 µL for the first transfer, 250 µL for the next, and so on. Refer to the table below.
  - Use more correction curve points to increase the trueness of every individual liquid transfer. The overall precision amongst all transfers would then improve.



Correction Curve

Target in (µL)	Corrected in (µL)
<New Entry>	
0.00	0.00
50.00	52.2
100.00	105.6
150.00	157.9
200.00	210.5
250.00	263.1
300.00	309.0

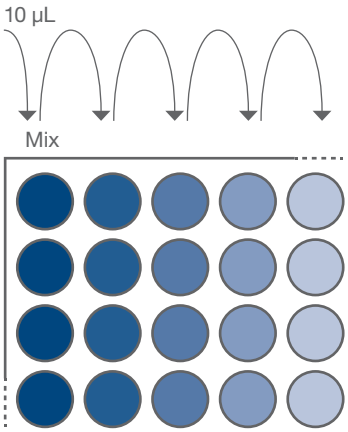
250.0 | 263.1 µL

Remove | New | Change

6.3 Best Practices for Serial Dilution

A dilution is one of the more common liquid transfers performed and occurs when a sample liquid is added to another liquid, decreasing its concentration. A serial dilution repeats this process over multiple steps in order to test samples at a variety of concentrations and/or to arrive at very low final concentration.

For example, 90 µL of buffer solution is added to all of the wells in a 96-well plate. 10 µL of sample is then added to column 1, mixed, and then 10 µL of that mixture is transferred to column 2, mixed, and so on. Each step dilutes the sample by a factor of 10.



Method Settings

- Use a new tip for each transfer.
- Dispense to surface if possible.
- Mix very well before and/or after each transfer. Start with many mix cycles and decrease only to optimize for speed if performance allows.
- Mix at a fixed position to optimize the dispense and mix heights.
- If using LLD and liquid following, properly defined labware is critical to ensure proper following and mixing.
- Mix volume should be less than 80% of total volume in the well. If the mix volume is larger than 80%, it increases the chance of aspirating air and creating bubbles.
- Be mindful of retract distance for air transport if dispensing at fixed height. If the retract distance and the fixed height are set too low, then it is possible that the tip may not be removed from the liquid before it aspirates the air transport volume. This results in the possibility of extra liquid being aspirated instead of air.

Liquid Class Parameters

- Depending upon dilution factors, different liquid classes for each step may be required.
- Remove the over-aspirate volume parameter, because it could lead to excess carryover after subsequent dispense and mixing.

7 Monitor Liquid Transfers

Once proper liquid transfers are established, it is important to implement process controls to help monitor for any exception. Hamilton automated liquid handlers provide a variety of process controls that monitor for proper liquid transfers. These controls are optional and can be used in tandem with each other or disabled altogether. The controls rely upon the pressure and capacitance sensors located on the pipetting channels.

With the pressure sensors, Hamilton automated liquid handlers can monitor liquid transfers for errors using two methods:

7.1 Monitored Air Displacement (MAD)

- Built into every Hamilton automated liquid handler for no extra cost. Only checks for errors on aspiration.

7.2 Total Air Displacement Monitoring (TADM)

- Extra cost for TADM software that records liquid transfers and performs monitoring. Checks for errors on both aspiration and dispense.

Both of these methods are useful to confirm that the transfer occurred without problem. While a human can observe the transfers as they happen when using a hand pipettor, the automated liquid handler only follows instructions without deviation. Monitoring tools like MAD and TADM can help inform the lab tech if anything has gone wrong with the liquid transfer without them having to actually watch each pipetting step the automated liquid handler makes. Automated error recovery for such exceptions can also be implemented.

Important Caveats

- Neither MAD nor TADM can be used to measure the precision or volume of the liquid transfer. Refer to Section 8 Measure Liquid Transfers.
- MAD can only be used on the 1,000 µL and 5 mL channels.
- TADM can only be used on 1,000 µL channels, 5 mL channels, and the CO-RE 96 Probe Head TADM.
- MAD and TADM cannot be used at the same time.

Comparison of MAD and TADM Functionality

	MAD	TADM
Monitors Pressure Over Time	✓	✓
Detects Aspiration of Air	✓	✓
Detects Clots	✓	✓
Monitors Aspiration	✓	✓
Monitors Dispense	✗	✓
Lower Volume Limit	50 µL	10 µL (for 1,000 µL Channels)
Scope	General process control that covers a range of volumes	Tolerance bands required that are specific to each liquid type and volume
Pressure Exception Checks	Checks for a minimal pressure difference between the start and end of aspiration	Monitors the pressure continuously over the aspiration and dispense for tolerance exception
Liquid Class	Liquid class independent	Liquid class dependent
Recording	No recording of pressure curves	Records all pressure curves in Recording or Monitoring mode
Re-use of Tips	Acceptable if contents of tip emptied for each transfer	Acceptable, but separate liquid class and tolerance bands may be necessary
Activation	Globally or specifically set via commands in the method	Globally set to Recording or Monitoring mode; Only TADM enabled liquid classes are activated

Hamilton automated liquid handlers can also use the capacitance sensors built into the cLLD technology to monitor liquid transfers. Capacitance monitoring is not as robust a process as MAD or TADM and should only be used if the other two are unavailable. Do not use capacitance sensors together with the pressure sensors.

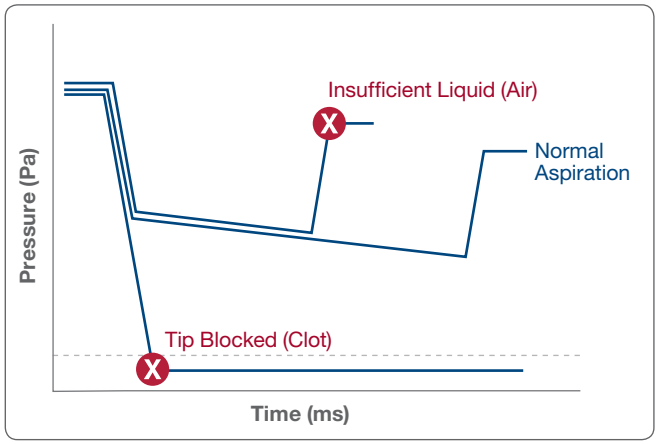
- Aspiration Monitoring with cLLD: During the aspiration step, the capacitance signal can be monitored to see if it is maintained. If the signal is lost, it means that the tip is no longer in the liquid and an improper aspiration must have occurred. cLLD must be enabled on the aspirate step to use this form of monitoring. You cannot aspirate from a fixed height and use cLLD monitoring.
- Clot Detection Monitoring with cLLD: After the aspiration phase, the capacitance signal is monitored to see if it is maintained as the tip is removed from the liquid. If the signal is still maintained past a specified clot retract height, a clot detected error will occur. This clot retract height parameter is set in the liquid class for the transfer step.

7.1 Monitored Air Displacement (MAD)

Monitored Air Displacement (MAD) monitors aspiration of a liquid in real time. The automated liquid handler's software monitors the pressure curve during aspiration to detect errors in aspiration, like clots, foam, and lack of liquid.

Using MAD during the aspiration, pressure changes are monitored for the following attributes:

- Pressure increase/spike due to aspiration of air
- Pressure decrease due to aspiration of clot. The pressure drops below a lower clot threshold indicating a clot was detected.
- Minimum pressure difference between the start and the end of the liquid transfer



When to Use

- MAD is optimized for aqueous solutions and unused disposable tips.
- MAD should be used when aspiration monitoring is needed, and dispense monitoring is not.
- MAD is best used as a general process control as its monitoring can cover a range of aspiration volumes. TADM can only monitor specific volumes (see Section 7.2).

When Not to Use

- If complete aspiration and dispense monitoring is required, use TADM instead of MAD.
- If tips are re-used or needles are used for pipetting, MAD may not perform consistently as it could generate intermittent "false" improper aspiration errors.
- If a recording of the pressure curve needs to be made, it is not recommended to use MAD. MAD only stores the most recent aspiration pressure curve on the channel board. It is only accessible through the use of the Hamilton Service Software and therefore is only meant for troubleshooting purposes.
- If using liquid types other than aqueous solutions, the use of MAD may not be optimal.
- If the aspiration volume is below 50 µL, the default threshold values may need to be adjusted and tested with real liquids.
- If using low aspiration volumes 10 µL or lower, the pressure difference may be too minimal for MAD to work properly.

7.2 Total Air Displacement Monitoring (TADM)

Total Air Displacement Monitoring (TADM) monitors aspiration and dispense of a liquid in real time. The pressure sensor in the channel is used during both aspiration and dispense steps to detect errors, like clots, foam, and lack of liquid. Upper and lower tolerance bands can be established and monitored for exception.

TADM can be used in two different modes:

- **Recording mode:** The pressure inside the pipetting channel is recorded and stored.
- **Monitoring mode:** The recorded pressure is compared to a user-defined tolerance band in real time. If the measured value leaves the tolerance band, the plunger movement stops immediately and the software-dependent error handling is executed. In Monitoring mode, the recorded pressure curves will be stopped after last defined TADM tolerance band point.

When to Use

TADM should be used if both aspiration and dispense monitoring is required. TADM is optimized for unused disposable tips during:

- Single aspirations and dispenses for volumes above 10 µL.
- Multiple dispenses for individual aliquot volumes above 10 µL.
- Transfers of various liquid types. TADM is not limited to the use of aqueous solutions only.

It is possible to use TADM with used disposable tips and washable steel needles, but success with these tip types depends on the consistency of their generated pressure curves. Residual liquid from used tips and residual wash fluid from steel needles could cause some irregularity in the pressure curves, making it a challenge to set meaningful guardbands.

TADM is especially useful for regulated environments and development purposes since a recording of every transfer step is made. The recording of the pressure profile allows the automated liquid handler's operator to review and compare the pressure profiles of liquid transfers during and after a method run. The recordings can be helpful in troubleshooting if the profile is not stable or some transfers show atypical behavior.

When Not to Use

- If the volume to pipette is less than 10 µL, TADM may not be able to be used for monitoring as the pressure profiles for low volumes may not be consistent.
- If you need to determine the pipetted volume, TADM cannot determine the volume.
- If you need to determine how much volume is missing from a sample, TADM cannot determine this volume.
- If you need to make a statement on trueness and precision, TADM cannot confirm the precision of the transfer.
- If variable volumes are transferred in the method, it may not be practical to create tolerance bands for every possible volume. The volumes could still be recorded, but not monitored for exception.

In the scenarios listed above, TADM doesn't offer a benefit or might not even be possible to implement. A method using TADM should be tested for maximum repeatability and robustness to ensure proper transfers before putting TADM into regular use.

7.2.1 How to Read an Aspiration TADM Curve

- Built into every Hamilton automated liquid handler for no extra cost. Only checks for errors on aspiration.

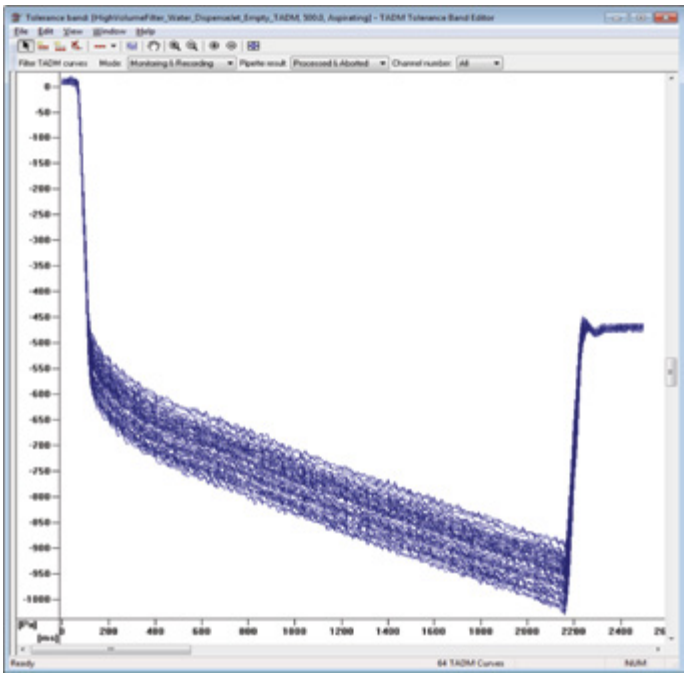
7.2.2 How to Read a Dispense TADM Curve

- Extra cost for TADM software that records liquid transfers and performs monitoring. Checks for errors on both aspiration and dispense.

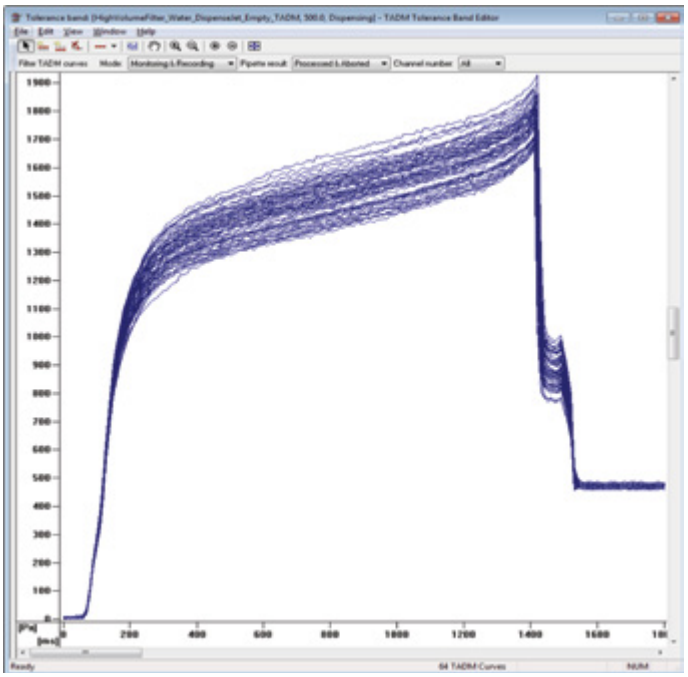
7.2.3 Use TADM Guardbands

- Set guardbands to actively monitor for any exception during runtime.

Example of 96 Recorded TADM Aspiration Pressure Curves



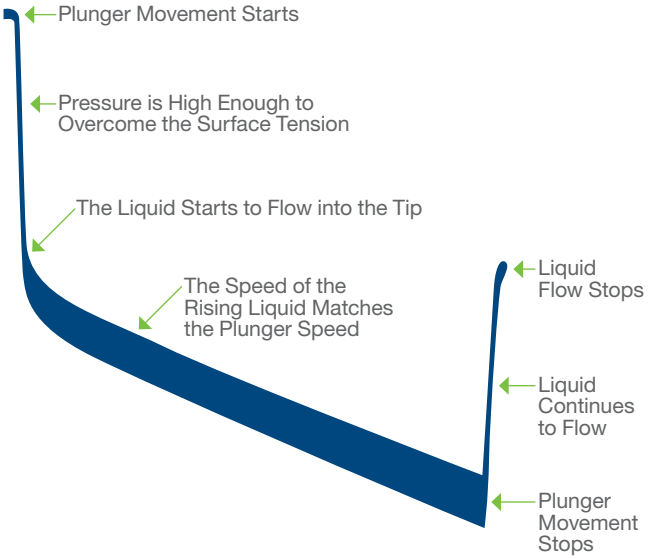
Example of 96 Recorded TADM Dispense Pressure Curves



7.2.1 How to Read an Aspiration TADM Curve

During aspiration, TADM monitors the pressure in the channel and reports it in a curve. The curve can be used to detect anomalies in the aspiration.

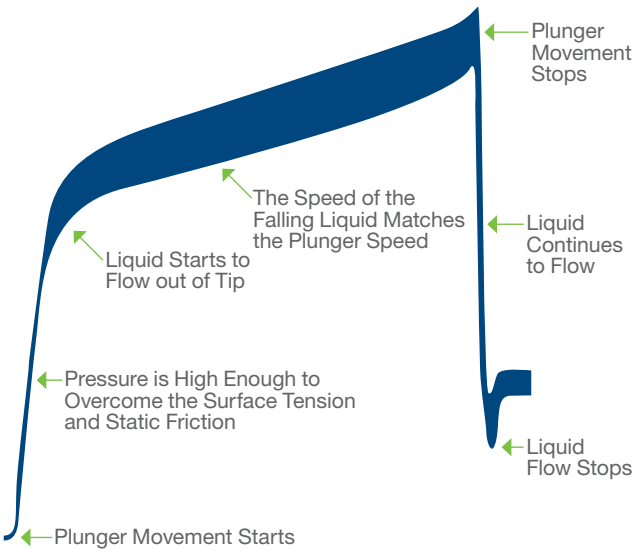
Use the figure to read the different aspects of the TADM curve.



7.2.2 How to Read an Dispense TADM Curve

During dispensing, TADM monitors the pressure in the channel and reports it in a curve. The curve can be used to detect anomalies in the dispense.

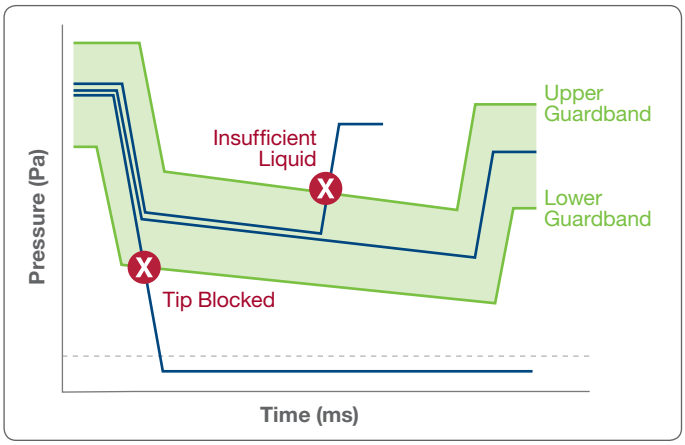
Use the figure to read the different aspects of the TADM curve.



7.2.3 Use TADM Guardbands

When collecting TADM curves, look for consistency in the pressure profiles for each volume transferred. If the profile for any individual transfer differs from the others, it indicates that a poor liquid transfer may have occurred. Once enough information is collected, you can set guardbands to actively monitor for any exception during runtime.

In the example image below, the green lines are guardbands put in place to monitor aspirations. Any aspiration that results in a line within the guardbands executed as expected. An error occurs when aspiration crosses outside of the guardbands. User or automated error recovery steps could be enabled to address the error.



8 Measure Liquid Transfers

Once the liquid handling is complete, it's valuable to be able to double check the automated liquid handler and make sure that the liquid was transferred at the correct volume. This Section describes how to measure the liquid transfer to ensure success. Liquids can be measured through a wide range of tests, from simple visual checks to more complex measurements that involve dyes or special scales.

Keep in mind that measurement described in this Section only provides you with a measure of the volume transferred. For information about the success of the transfer and prevention of clots, see Section 7 Monitor Liquid Transfers.

Visual — A qualitative approach and not a trustworthy measure.

Using just your eyes, confirm that the transfers appear to be uniform.

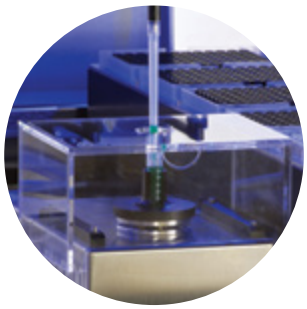
Manual — A qualitative approach and not always a trustworthy source.

Using a hand pipette, aspirate what was dispensed by the instrument. Visually inspect for confirmation.

Photometric / Fluorometric — A valid and quantitative approach.

Add dye to the liquid and verify using a plate reader. Testing can generally be done in the actual assay labware (plates).

For more information, refer to Section 8.1 About Photometric Measurement and Section 8.2 About Fluorometric Measurement.



Gravimetric — A valid and quantitative approach.

Use an analytic balance to measure the mass of material dispensed.

Refer to Section 8.3 for detailed instructions.

The purpose of measuring liquid transfers is to check for a "true" and "precise" result. Each automated liquid handler listed in Section 3.1 has expected performance at various liquid volumes and tip sizes. These specifications can be used as a reference during verification, but keep in mind that differences in laboratory conditions, pipetting approaches, and liquid types can result in different performance. It is a good practice to consider the pipetting specifications to be the best case scenario and to consider your own method's pipetting performance requirements when evaluating verification results.

Trueness $\%R = 100 \times \left(\frac{V_M - V_T}{V_T} \right)$

Trueness is defined as Yield (% trueness / bias). It is a percent error between the average volume of solution measured and the expected or accepted value. A result of high trueness is equivalent to a small percent error.

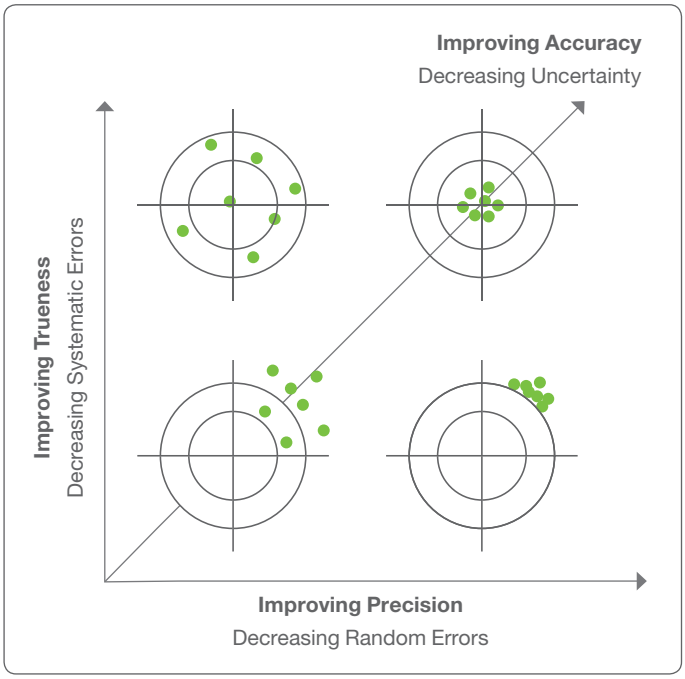
Precision $\%CV = 100 \times \frac{SD}{Mean}$

Precision is defined as Reproducibility (% CV). It is the closeness of a set of values obtained from identical measurements of the same volume. A high amount of precision is equivalent to a small percent coefficient of variation.

In addition to trueness and precision, the term "accuracy" is often used in discussions about automated liquid handlers. In 2015, liquid handler manufacturers defined and standardized the terms to be used across the industry. The manufacturers also standardized the volumetric performance determination for Automated Liquid Handling Systems (ALHS). For reference, the standardization decisions are logged in ISO International Workshop Agreement (IWA) 15 titled "Specification and method for the determination of performance of automated liquid handling systems."

ISO IWA 15 describes accuracy as the relationship between trueness and precision. Definitions from the ISO document include the following:

- **Trueness** — An average which is very close to the true value. As trueness improves, there is a decrease in systematic errors.
- **Precision** — Highly consistent results. As precision improves, random errors decrease.
- **Accuracy** — Knowing that each measurement correctly represents what is present in the sample. As accuracy is obtained, uncertainty is decreased.



8.1 Photometric Measurement

A photometric approach uses a dye and a plate reader to analyze results. Add dye to the liquid and verify using a plate reader. Testing can generally be done in the actual assay labware (plates). Keep in mind that it can be difficult to choose an appropriate dye for the specific test, and dyes can alter physical characteristics of the solutions being tested.

Advantages

- Allows testing to be done in the actual labware used in the process, which makes for a truer comparison.
- Is good at gathering large amounts of data. For example, photometric measurement is useful for testing with a 96 or 384 multi-probe head since all transfers can be performed and measured at the same time. Each well in a 96- or 384-well plate corresponds to a channel on the multi-probe head. Since an absorbance value is collected per well, the transfer volume can be determined per channel.
- Is the only method that works for liquid filled systems because of a potential dilution effect that cannot be detected by weighing the sample. In contrast to the gravimetric approach, the plate reader absorbance read could detect unwanted dilution relative to reference standard or manually pipetted transfer of the sample. Since the photometric approach is required for liquid filled systems, it is therefore a popular and accepted standard of measurement for any type of automated liquid handling system.
- Works with off-the-shelf products and readily available laboratory equipment. Options exist to purchase a ready-made solution or create your own at less cost.

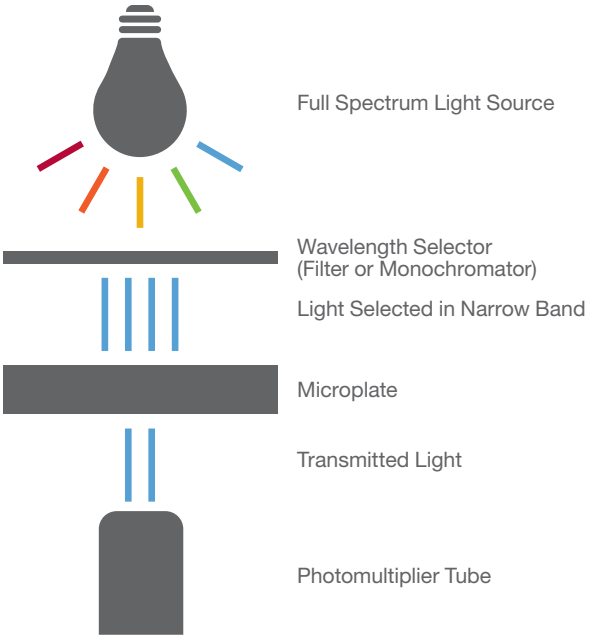
Disadvantages

- Can be challenging to develop a test from scratch, since it requires identifying the optimal wavelength, using and/or acquiring an appropriate reader, and setting up the necessary calculations. For reference, the photometric measurement process is described in detail below.
- Can be difficult to choose an appropriate dye for the specific test. The addition of dyes to the liquid can alter the physical characteristics. This makes the liquid type not truly representative of the one you are trying to measure.
- Can limit the amount volume to be tested. The transfer volume may be limited to the labware required for analysis in the plate reader. A microplate typically holds about 300 µL of liquid. If more volume is required, additional testing procedures must be used that are different from the liquid transfer settings in the method.
- Requires the use of a plate reader and further calculations in order to obtain results. Data collection can therefore be slower using this approach.
- Not ideal for small volumes under 10 µL. The path length for the light to travel through the liquid would be minimal and the approach may not be sensitive enough for reliable measurement. This depends especially upon well geometry. To avoid this issue, small volumes may require additional buffer, which creates additional variables. Such variables include the potential for uneven path lengths and variable path length and concentration which complicates the application or Beer's Law.

Alternatives to the photometric measurement include the fluorometric approach, which can help address some of these disadvantages. Refer to Section 8.2 for more information.

How to Perform Photometric Measurement

1. Obtain a dye and known optimal wavelength and concentration for use. If unknown, determine it using spectral analysis of the color. Measure with scanning spectrometer (i.e. Molecular Devices Spectramax®) when possible to determine optimal wavelength and concentration. If not possible, guess based on dye color: (Yellow dye transmits yellow light, so it absorbs blue (450 nm)).
2. Use a flat bottom plate for testing. For example, a round well plate can cause issues with the test due to light reflection and refraction on the curved part of the well. If needed, other plate types can be accommodated, but it takes modification in the reader software and equation.
3. Test several concentrations to determine reader saturation point and aim for a test concentration 50% to 75% of saturation.



4. Apply Beer's Law to determine volume: Absorbance (Abs) = eLc. Refer to the instructions below for details on the application of Beer's Law. Use it to create a standard curve around the value of interest.

Beer's Law

Abs = eLc
e = extinction coefficient
L = pathlength (depth of liquid in well)
c = concentration of dye

5. Create a standard curve with same dye solution that is being used for unknown volumes. This allows constants to cancel out. The curve is obtained by collecting data below and above the volume of interest and fitting a linear curve to determine the slope and offset.

6. Additional detail on Beer's Law:

    1. Because e and c are constants that do not change from well to well, they can be viewed as a single proportionality constant, k, so Beer's Law is simplified to: **Abs = k* Volume**

    2. Using the curve fit parameters, the slope is the value for k.

    3. There also exists an offset due to absorbance within the plate material itself that is the y-intercept.

7. Apply the curve to calculate unknown data.

    1. Read a plate containing wells of liquid transfers of the volume to be tested. These are considered the unknowns in this test.

    2. Apply curve values to calculate unknown data.

Example Where the Transfer Volume of Interest is 50 µL:

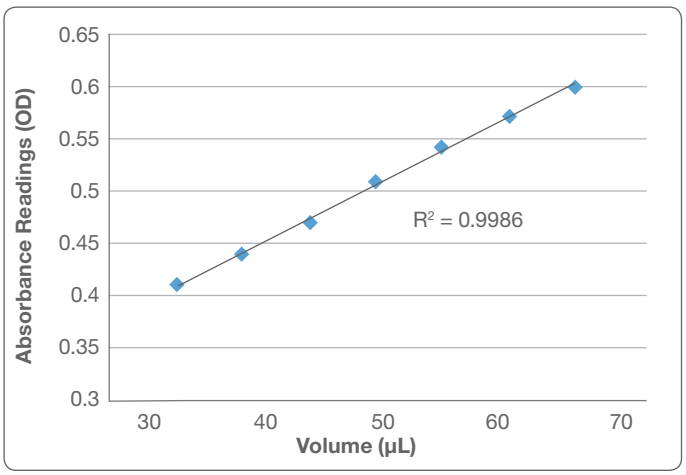
1. First, absorbance data is collected for several volumes of dye transfers below and above 50 µL. Specifically, 30, 40, 50, 60, and 70 µL.

Raw Data From Reader and Average of Replicates

Volume	Row	Rep 1	Rep 2	Rep 3	Average
35	A	0.4076	0.4121	0.4113	0.41033
40	B	0.4381	0.4412	0.4421	0.44046
45	C	0.4701	0.4693	0.4692	0.46953
50	D	0.5072	0.5102	0.5093	0.50890
55	E	0.5418	0.5406	0.5399	0.54076
60	F	0.5721	0.5693	0.5704	0.57060
65	G	0.6013	0.6004	0.5989	0.60020

2. A linear curve is fitted to determine the slope and offset.

    y = 0.0064x + 0.184 where **y** is the Absorbance and **x** is the Volume



3. A plate of unknown 50µL liquid dye transfers is read collecting absorbance values for each transfer.

Raw Data From Reader

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	0.5176	0.5153	0.5040	0.5045	0.5029	0.5102	0.5015	0.5090	0.5133	0.5164	0.5143	0.5048
B	0.5050	0.5087	0.5042	0.5146	0.5015	0.5078	0.5144	0.5151	0.5162	0.5011	0.5006	0.5017
C	0.5075	0.5145	0.5142	0.5091	0.5096	0.5051	0.5176	0.5169	0.5064	0.5076	0.5133	0.5143
D	0.5178	0.5047	0.5035	0.5130	0.5014	0.5033	0.5117	0.5095	0.5058	0.5032	0.5013	0.5113
E	0.5035	0.5180	0.5073	0.5131	0.5120	0.5158	0.5128	0.5066	0.5127	0.5055	0.5088	0.5096
F	0.5181	0.5099	0.5176	0.5140	0.5074	0.5035	0.5138	0.5152	0.5120	0.5110	0.5058	0.5108
G	0.5030	0.5177	0.5090	0.5079	0.5176	0.5048	0.5176	0.5172	0.5101	0.5021	0.5171	0.5025
H	0.5142	0.5149	0.5059	0.5022	0.5170	0.5057	0.5057	0.5005	0.5046	0.5044	0.5119	0.5126

4. The curve values are applied to the unknown absorbance values to determine the volume for each transfer.

Average	50.76435
STDEV	0.841395
% CV	1.657453
% Trueness	1.5287

Calculated Data and Summary Statistics

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	50.08	49.96	51.73	51.55	51.56	50.92	50.71	51.10	51.85	52.26	50.26	51.88
B	50.50	51.82	50.36	51.40	51.49	51.61	51.02	49.74	52.26	52.13	50.50	50.57
C	49.97	49.65	50.08	50.11	51.23	49.55	49.80	49.97	51.20	50.84	51.49	50.67
D	51.19	51.39	52.18	49.65	49.48	50.33	51.08	49.77	51.66	51.40	49.71	52.03
E	51.42	51.54	50.62	51.62	50.79	52.03	49.62	50.37	51.53	49.53	49.97	50.04
F	50.44	50.48	50.81	49.49	50.34	51.79	50.23	51.39	50.41	51.61	51.16	50.48
G	49.62	50.52	52.09	49.80	51.57	51.66	50.49	50.43	50.48	50.81	49.72	52.20
H	51.99	49.44	49.66	49.87	50.01	49.85	49.61	52.10	51.38	49.87	50.25	50.56

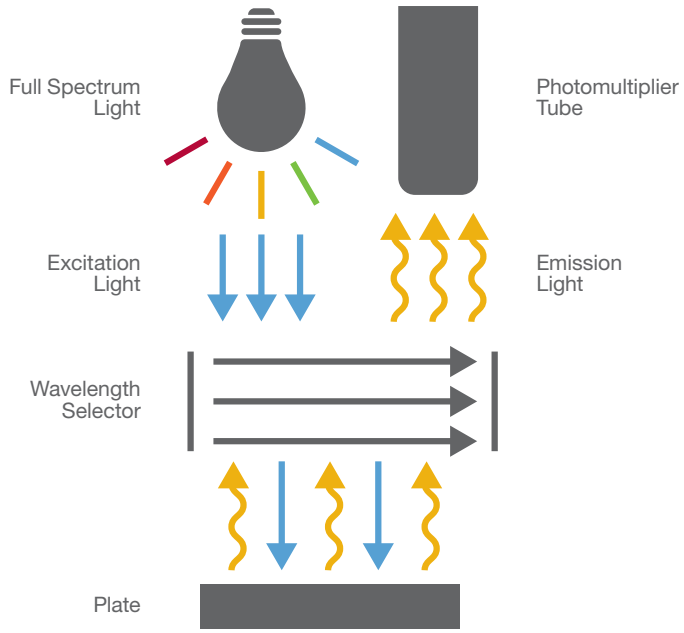
8.2 Fluorometric Measurement

Fluorometric measurement is similar to photometric approaches, because both types of measurement require light to be directed onto the sample well and then measured using a photomultiplier tube. However, there are some significant differences in the approach that can lead to one being a better option than the other depending on the circumstances.

In photometric measurement, the amount of light transmitted is subtracted from the amount of light generated at the source. This allows you to determine the total amount of light absorbed.

In contrast, when using fluorometric measurement, the dye is illuminated at a specific wavelength and absorbs light energy. The light energy then emits at a different wavelength. Since the light emits directly from the dye molecule, it emits in all directions equally. This means that a variety of plates can be used, for example black, opaque plates without an optically clear bottom. In fact, using black plates during testing reduces background interference, because they absorb scattered light.

To perform fluorometric measurement, follow the same general steps and data handling as used for photometric measurement.



Advantages

- Allows testing to be done in the actual labware used in the process, which makes for a truer comparison.
- Is good at gathering large amounts of data and is the only method besides photometric that works for liquid filled systems.
- Is not effected by well geometry because the measurement is only dependent on the total amount of fluorescent molecule residing in the area illuminated by the light source.
- Is effective for small volumes, but may still need a diluent. Fluorescent dyes tend to be sensitive and often require substantial dilutions from a stock solution to remain in the dynamic read range for a plate reader. This ensures accurate measurements of low volumes. Fluorescent measurement examines the total amount of fluorophore in the area that is excited, eliminating the effect of varying diluent volumes.

Disadvantages

- Can be difficult to choose an appropriate dye for the specific test. The addition of dyes to the liquid can alter the physical characteristics. This makes the liquid type not truly representative of the one you are trying to measure. Fluorescent dyes are more expensive than the dyes used for photometry and also tend to be sensitive to light and sometimes temperature. In addition, fluorescent dyes do generally come with known excitation and emission wavelengths.
- Can limit the volume to be tested. The transfer volume may be limited to the labware required for analysis in the plate reader. A microplate typically holds about 300 µL of liquid. If more volume is required, additional testing procedures must be used that are different from the liquid transfer settings in the method.
- Requires the use of a plate reader and further calculations in order to obtain results. Data collection can therefore be slower using this approach. Plate readers for fluorescence measurements tend to be more expensive than those that just read absorbance.

8.3 Gravimetric Measurement

The gravimetric approach uses an analytical balance integrated directly on the automated liquid handler. Once the liquid is weighed, the known density of the liquid can be used to calculate the volume.

Advantages

- Uses only the liquid of interest with no dyes or other additives.
- Covers a large range of pipette channel volume. It can cover a range of volumes used by all Hamilton pipetting devices from 0.5 µL to 5 mL.
- Provides immediate feedback from the balance which allows for quicker optimization of liquid classes.

Disadvantages

- Doesn't use the actual labware used in the assay. Instead, you may be pipetting to a large tube on a balance instead of a plate or specific tube type.
- Requires you to know the density of the liquid in order to correlate the weight to a volume. While it is easy to look up the density of common liquid types, it may be unknown for less common types or for mixtures.
- Affected by temperature. The temperature can impact the density of the liquid, so the temperature needs to be monitored to properly check for trueness. See Section 2.3.2 for more information.
- Allows only one transfer at a time to be weighed. This prevents the testing of multiple channels at the same time to collect individual measurements. Specifically, this limitation prohibits testing of all channels in a multi-probe head (96- and 384-) at the same time.
- With small volumes, the balance quality and readability sensitivity can play a factor. It may not be possible to obtain significant digits with low volume transfers given the sensitivity of the balance and laboratory conditions.

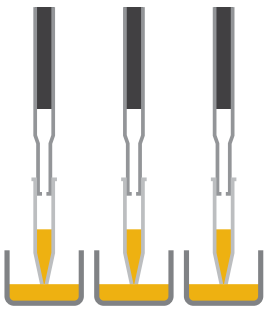
Hamilton makes a gravimetric measurement system, known as the Liquid Verification Kit (LVK). The LVK is made up of a graphical user interface that directs liquid handling tests on an analytical balance that is placed on the automated liquid handler's deck. The same Mettler Toledo WXS 205S analytical balance is used by customers who purchase the LVK and Hamilton field service engineers to conduct liquid testing. During execution, the LVK program displays data in real-time and creates reports on the pipetting performance. It is also possible to program custom methods using the Hamilton software that control the balance to verify liquid transfers without the use of the LVK interface.

Industry standard processes for gravimetric testing are defined in ISO 8655-6.

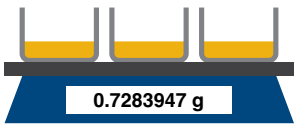
8.4 Combined Photometric and Gravimetric Measurement

It is also possible to combine the photometric and gravimetric approaches. The first liquid transfer containing dye can be weighed, then later be analyzed on a plate reader to determine the volume per well. This approach is used by Hamilton service engineers to perform installation and operational qualification testing of the automated liquid handlers as part of the Field Verification Kit 2. This process allows both the independent pipetting channels and the multi-probe heads (96- and 384) to be verified in a similar fashion.

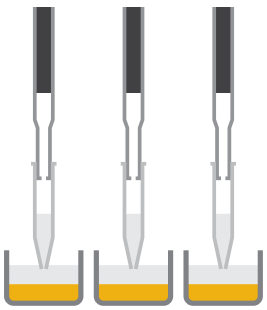
With the weight measurement and the obtained optical density (OD) values, the volume of each well can be determined.



1. Pipette dye liquid in a microplate.



2. Weigh the plate.



3. Add clear liquid like Borate Buffer, to make sure that the liquid sufficiently covers the bottom of the well. Mix the clear liquid and dye together.

4. Measure the optical density by reading the plate with a photometric reader.

9 Hamilton Parameter Glossary

Hamilton Parameter	Definition	Other Industry Terms
Flow Rate	Liquid flow rates in µL/s, corresponding to plunger speed	Aspiration Speed Dispense Speed
Mix Flow Rate	Liquid flow rates in µL/s, corresponding to plunger speed for mixing	Aspiration Speed Dispense Speed
Air Transport Volume	Volume of air in µL is aspirated at the end of the aspiration and dispense step	Trailing Air Gap (TAG)
Blowout Volume	Volume of air in µL that is aspirated first during the aspiration step. If dispensing in empty tip mode, part or all of the air is dispensed	System Trailing Air Gap (STAG)
Swap Speed	Speed in mm/s which the pipette head is moved out of the liquid	Retract Speed
Settling Time	Time in seconds that the pipette head remains in the liquid after aspiration or dispense	Delay
Over-Aspirate Volume	After aspirating the required volume an additional volume in µL is aspirated and dispensed again immediately	Conditioning Volume
Clot Retract Height	A parameter for clot detection that determines how high the pipette head is allowed to move out of the liquid while there is still a liquid detection signal after aspiration	Exit Signal Detection
Stop Flow Rate	Dispense flow rate in µL/s at which the dispense step terminates abruptly	Dispense Speed Break-off Speed
Stop Back Volume	Volume in µL which is aspirated again immediately after the dispense	No equivalent, could be done but would need to be done as a new aspiration action immediately following the dispense into vessel with the z-travel set at the dispense location

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