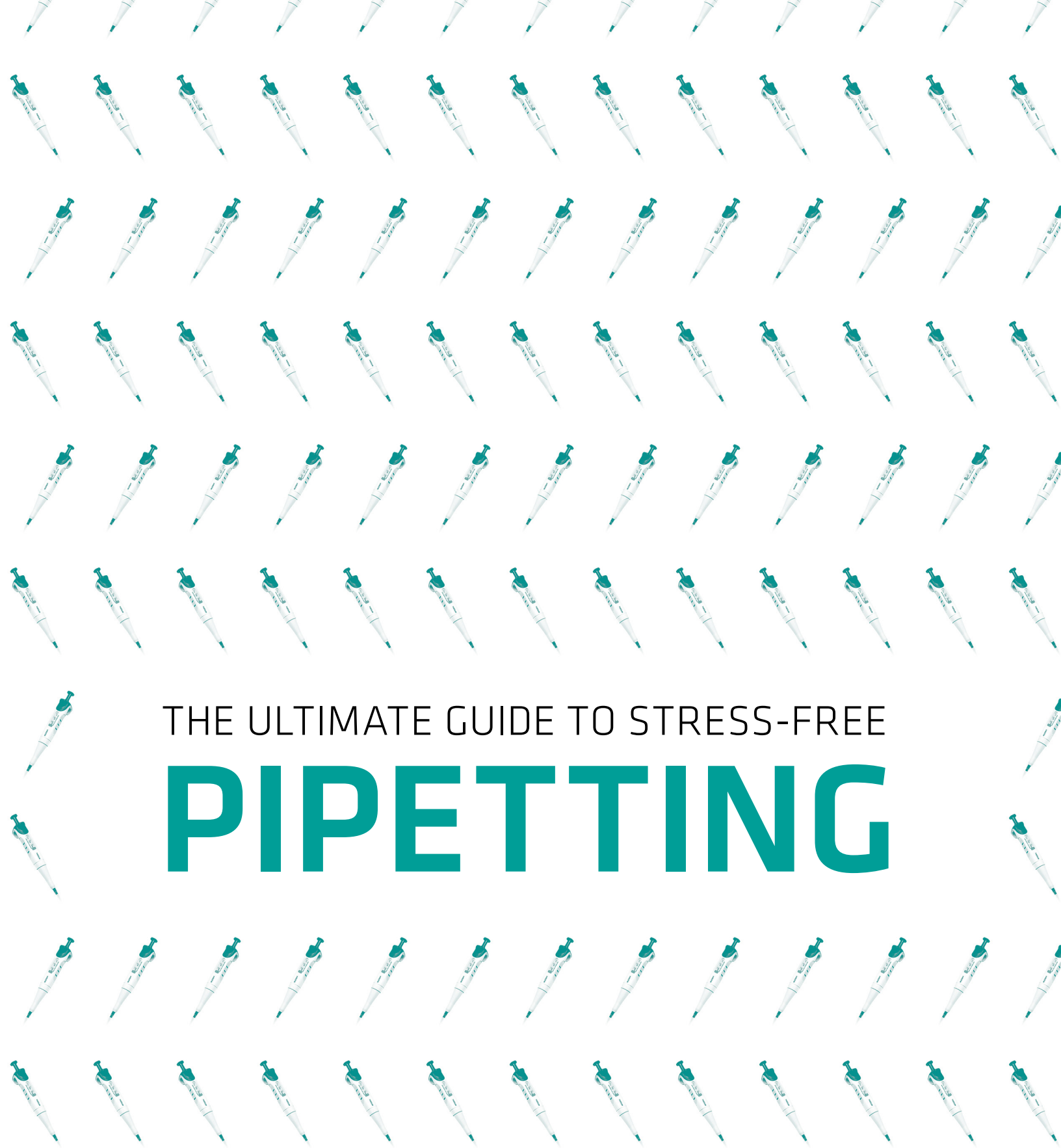

THE ULTIMATE GUIDE TO STRESS-FREE

PIPETTING

INTEGRA

FOREWORD

Pipetting is a fundamental skill in any laboratory as liquid handling protocols form the backbone of many life sciences workflows. However, pipetting improperly can lead to inaccurate results, repetitive strain injuries, and wasted resources. This eBook aims to equip you with the knowledge and skills you need to turn pipetting from a tedious necessity into an efficient, accurate and enjoyable part of your lab work.

Our educational articles and practical tips and tricks will help you to choose the right pipette and pipette tip for your application while addressing crucial ergonomic considerations and pipetting best practices, with a focus on achieving high accuracy and precision even when handling challenging liquids. We also present valuable information covering essential maintenance and calibration routines to prolong your pipettes' lifespans and ensure consistent results. This eBook additionally contains in-depth application notes illustrating how INTEGRA Biosciences' solutions can support you in optimizing your liquid handling protocols. We're confident that this comprehensive guide will play a significant role in helping your facility to achieve stress-free pipetting once and for all.

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CHAPTER 1:
What you need to know about pipettes

There are numerous pipettes on the market today, all with their unique attributes, key uses, and pros and cons. In this chapter, we will cover some of the main differences between the various types of pipettes and tips, what to consider when choosing the right solutions for your application, and how to protect yourself against injuries caused by extensive manual pipetting.

1.1 Everything you need to know about the different types of pipettes

Pipettes are essential laboratory tools for scientists but, with so many available options, knowing which one to choose can create confusion. What are the differences between the various types of pipettes? What attributes to look for when purchasing a new one? How to use it correctly, and ensure that it stays reliable for years to come? This comprehensive article will cover all these questions and many more.

What is a pipette?

Pipettes are one of the most extensively used lab tools. They are designed to measure small amounts of liquid and/or transfer it between containers. They're available in a wide variety of types, from simple serological and Pasteur pipettes to more complex instruments containing a body, with a plunger and piston. The latter are often referred to as micropipettes, of which there are several kinds, with many different features. All these will be covered below.

Different types of pipettes and how they work

Air displacement vs. positive displacement pipettes

Air displacement pipette

Air displacement pipettes have an air cushion between the piston and the sample. To aspirate liquid, the piston first descends, pushing out a volume of air corresponding to the required volume of liquid, before moving up again and creating a partial vacuum that will be filled by the liquid.

Positive displacement pipette

In positive displacement pipettes, the piston is in direct contact with the sample, so is part of the disposable tip, not the pipette itself, to avoid cross-contamination. The aspiration process is similar to air displacement pipettes: the piston moves up, creating a partial vacuum that is filled by liquid.

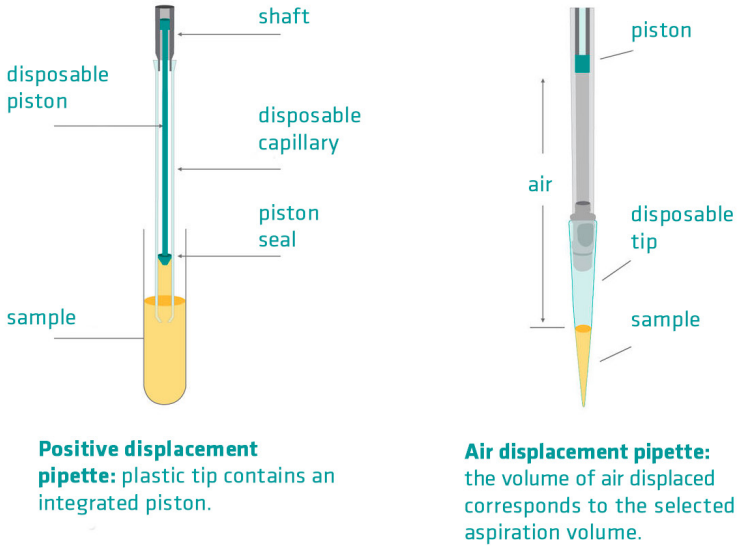


Figure 1: Positive vs. air displacement pipette.

Labs typically use air displacement pipettes, as they're less expensive and well suited to most liquids, including viscous and volatile liquids – as long as the correct technique and pipette tips are used (see section *The influence of the right pipette tip*). However, if you regularly need to pipette very viscous or very volatile liquids, a positive displacement pipette might be better suited to your needs. Positive displacement tips are more costly because of the integrated pistons but, as there's no air cushion inside the tip, volatile liquids can't evaporate and viscous samples are pushed out more efficiently. In addition, positive displacement pipettes are more accurate when pipetting hot or cold samples, as they are not affected by temperature variations.[1]

Adjustable vs. fixed volume pipettes

As the name suggests, adjustable volume pipettes cover a certain volume range, while fixed volume pipettes are designed to pipette a specific volume only. When purchasing a pipette, laboratories usually opt for adjustable volume pipettes, because they offer greater flexibility and can be used for many different applications. However, their accuracy is highest at the maximum pipetting volume, and it decreases slightly with the pipetting volume, so a fixed volume pipette may be more appropriate for some applications.[2]

Because adjustable volume air displacement pipettes are usually the first choice of labs, the following chapters will focus on this pipette type.

How to choose a new pipette

Searching for the right pipette can be confusing. To help you with this decision, we have outlined a few things to bear in mind when selecting a new pipette.

Manual vs. electronic pipettes

The first thing you should decide is whether you need a manual or an electronic pipette. Manual pipettes are widely used and are great tools, but if you have the budget, then an electronic pipette will pay for itself in the long term, because it offers 5 significant advantages:

- **Improved ergonomics** – minimizing the risk of repetitive strain injuries, as there's no plunger to press down to move the piston, and no need to twist knobs or the plunger to adjust the volume.
- **Better precision and accuracy** – as they are less operator-dependent. You can also save pipetting protocols to reduce manual errors and prevent operator-to-operator variability.
- **Multiple pipetting modes** – avoiding the need for separate repeater, dilutor and titrator pipettes.
- **Straightforward calibration** – with built-in gravimetric performance validation. Simply enter the dispensed versus target volume, and it will self-calibrate. Many electronic pipettes also offer calibration reminders.
- **Integrated pipetting protocols** – with pre-set or custom programs to minimize operator errors.

Reliability

The volume you transfer can significantly influence the reliability of pipetting. As explained before, you should always choose the smallest pipette capable of handling the required volume as the accuracy of adjustable volume air displacement pipettes decreases with the set volume.

Reliability can also be negatively affected if the volume is accidentally changed during the pipetting procedure. You should therefore choose a pipette with a mechanism designed to avoid inadvertent volume changes. Another common problem is tips that loosen, leak, or fall off, so selecting a pipette with tips specifically designed for it is better than one that uses universal tips.

Efficiency

Filling microplates with a single channel pipette can quickly become a very tedious and error-prone task. Using multichannel pipettes allows you to transfer multiple samples at once, increasing efficiency and preventing errors and repetitive strain injuries. Multichannel pipettes can even be used for transfers of samples between different labware formats, if you buy one with adjustable tip spacing.

The number of channels you require, and if you need adjustable tip spacing, depends on the labware types you're planning to use. The table on the next page lists the most common pipette formats, and the labware types they're best suited for.

PIPETTE FORMAT	LABWARE TYPE
8 channel pipette	Transferring samples from reservoirs or 96 well plates to entire columns of a 96 well plate
12 channel pipette	Transferring samples from reservoirs or 96 well plates to entire rows of a 96 well plate
16 channel pipette	Transferring samples from reservoirs or 384 well plates to entire columns of a 384 well plate
4 channel adjustable tip spacing pipette	Transferring samples from different labware formats to entire rows of a 12 well plate or entire columns of a 24 well plate
6 channel adjustable tip spacing pipette	Transferring samples from different labware formats to entire rows of a 24 well plate
8 channel adjustable tip spacing pipette	Transferring samples from different labware formats to entire columns of a 96 well plate
12 channel adjustable tip spacing pipette	Transferring samples from different labware formats to entire rows of a 96 well plate

Ergonomics

When choosing your pipette, you should make sure that it's lightweight, well-balanced and fits comfortably into the hand, for both left- and right-handed users. Additionally, tip loading and ejection forces should be as low as possible to reduce the strain on operators.

How to use a pipette

The very first thing you should do is make sure that you choose the right pipette for your experiment. As explained above, adjustable volume air displacement pipettes are most accurate when pipetting at their maximum capacity, so you should always choose the smallest pipette capable of handling the required volumes.

If your experiment allows, you should also make sure that the pipette, tips and liquids are equilibrated to room temperature before you begin your work. This will prevent temperature-dependent expansion or contraction of the air cushion inside of the pipette tip, which can negatively impact the accuracy and precision of your results.

While pipetting, there are a few additional things to keep in mind:

- Pre-wet the pipette tip by aspirating and dispensing the nominal volume 3 times to humidify the air cushion.
- Always hold the pipette at an angle not exceeding 20 degrees to ensure that the hydrostatic pressure inside the tip stays consistent.
- When immersing the tip into a liquid, it shouldn't be more than 2-3 mm below the surface, to minimize the amount of liquid retained on the outside of the tip.
- Following each dispense, there may be a liquid droplet clinging to the end of the tip. To remove it, you should perform a touch off.
- When you're repeat dispensing multiple aliquots, you should discard the first and last dispense of the series, as they have the largest associated volume errors.

The influence of the right pipette tip

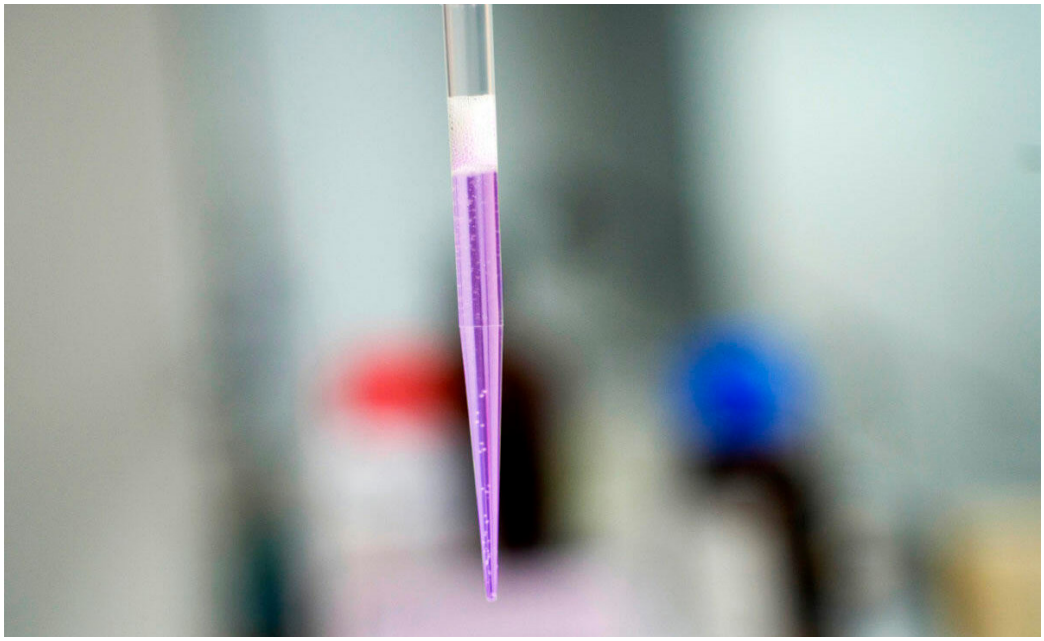
It's not just using the right pipetting technique that has an impact on the accuracy and precision of your results, but also using the right tip. For example, for low surface tension or viscous solutions, low retention tips produce significantly better results than standard tips. To learn more about when to use which tip, check out chapter 1.4 of this eBook.

How to pipette viscous and volatile liquids

Pipetting viscous and volatile liquids with an adjustable volume air displacement pipette can be challenging but mastered with the right technique and pipette tip. Viscous liquids should be aspirated and dispensed slowly using reverse pipetting. Using this technique, a larger volume than needed is aspirated, which compensates for the retained liquid adhering to the inside of the tip. Low retention tips are the ideal option for viscous liquids, and for very high viscosity liquids, or those that tend to foam, wide bore tips are recommended.

When pipetting volatile liquids, be sure to pre-wet the tip and use fast pipetting speeds for both aspiration and dispensing to minimize the effects of evaporation. Do not pause unnecessarily between aspiration and dispensing and use reverse pipetting to further reduce the effect of evaporation on the actual volume to be delivered.

Pipettes are usually calibrated with distilled water, at room temperature. It may be useful to recalibrate them when pipetting liquids with different physical properties (specific gravity and vapor pressure).



How to prolong the lifespan of your pipette

Besides correct use, the proper storage and cleaning of your pipettes – as well as regular calibration and maintenance – are crucial to ensure that they will yield reproducible results for many years.

Storage

Never lay a pipette down on the bench. Instead, store it vertically on a stand. This will ensure that any liquid residue trapped inside the pipette body drains out, and it prevents piston misalignment or lubricant accumulation on one side of the pipette. As pipette tips may retain liquid residues, you should always eject them when you finish pipetting. If not, this residual liquid can evaporate into the pipette body. Last but not least, you should always set your pipette to its maximum volume (if you're using a manual pipette), to allow the spring to return to its least stressed position.

Cleaning

Always consult the operating manual of your pipette before cleaning it. It often contains detailed information about the chemical compatibility of your pipette with common cleaning agents, and tells you how to disassemble and reassemble it.

Cleaning the outside of the pipette should be part of your daily routine. Simply wipe it with a lint-free cloth lightly soaked with 70 % ethanol.

Cleaning the interior of your pipette is a more complex and time-consuming process but can usually be done by the operator for single channel pipettes. First, you have to take the pipette apart. Depending on whether you're cleaning it as a matter of routine, or because it has been contaminated, you'll need to clean the components not just with distilled water, but also with a suitable decontaminant. Afterwards, you should check the components for visible damage, let them air dry, and lubricate the piston, before reassembling the pipette. Finally, briefly check the pipette's functionality by performing a leak test and volume validation.

Calibration and service

The last aspect that can increase the lifespan of your pipette is regular calibration and service. You should have it calibrated and serviced every 6 to 12 months to ensure that it remains accurate and precise, and that potential problems are detected and addressed before costly repairs or replacement becomes inevitable. Performing routine checks on a regular basis is also recommended, so that you can be confident in your results between calibrations.

Summary

You now know all the tips and tricks to find out which pipette suits you best, how to correctly use it and how to make sure that it serves you faithfully for many years.

1.2 Are you using the right type of micropipette?

Are you one of those lab professionals that spends numerous hours a day with a micropipette in hand? Or do you run a lab, and are frequently confronted with questions about how to improve pipetting efficiency or how to guarantee reliable results? If so, selecting the right pipette type could be key to the success of your work. It not only ensures the performance of your experiments, it can also boost your efficiency.

Factors increasing reliability

Scientists rely on pipettes that deliver accurate and reproducible pipetting results to guarantee the success of an experiment. Consider the physical properties of your liquid (aqueous, viscous, volatile) as well as the pipette's accuracy and precision to improve your pipetting results.

Physical properties of your liquid

Most liquids are of the aqueous type, making air displacement pipettes the first choice. Although a majority of liquids will work perfectly well using this pipette type, you may wish to consider positive displacement pipettes if you are working with very viscous or volatile liquids.

Regardless of the liquid type, the correct pipetting technique is essential to achieve excellent results with air displacement pipettes.

The most critical aspects affecting pipetting results are accuracy and precision which can be defined as follows:

Accuracy

Accuracy is the ability of a measuring instrument to give responses to a true value. A pipette is accurate to the degree that the volume delivered is equal to the specified volume.

Precision

Precision expresses the degree of reproducibility, or agreement between repeated measurements. If you perform multiple dispenses of a certain volume with a pipette, and it dispenses actual volumes very close to each other, the pipette is precise and your results are reproducible.

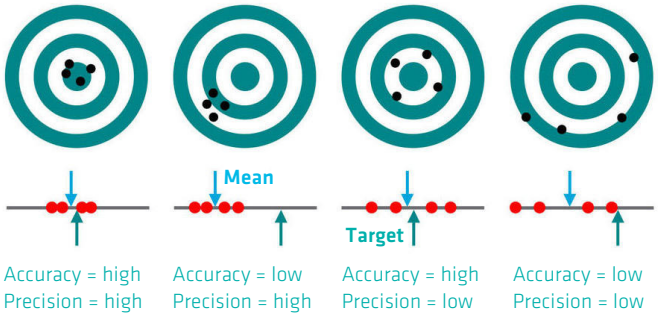


Figure 1: The difference between accuracy and precision.

For maximum pipetting accuracy and precision, we advise you to look at the following criteria:

Micropipette size

Micropipettes are available in different volumes ranging from 0.2 µl to 5,000 µl. As a rule of thumb, always choose the smallest pipette capable of handling the required volume. This is important because accuracy decreases when the set volume is close to the pipette's minimum capacity. For example, if you dispense 50 µl using a 5,000 µl pipette, you will get rather poor results. Using a 300 µl pipette will give you better results, whereas using a 50 µl pipette would be ideal.

Easy calibration

Your micropipettes should be easy to calibrate. Some electronic pipettes have useful features, such as setting a calibration reminder or saving the calibration history.

Volume adjustment locking

Volumes set on traditional manual pipettes can change while pipetting, due to unintentional plunger turns. However, some pipette manufacturers have developed volume adjustment designs that prevent inadvertent volume changes while pipetting.

High quality pipettes and tips

Do your pipette tips ever loosen, leak or fall off? This is a common issue in laboratories, caused by the use of universal pipette tips. Such tips require ‘hammering on', which stretches the pipette tip rim. This can cause leaking or misaligned tips, or even cause the pipette tips to fall off the pipette completely! Choosing micropipettes which were designed together with the tips ensures secure connections and tips that do not leak or fall off.

Color coded pipettes and pipette tips

Color coding helps you to choose the right tips for your pipette.

Factors increasing efficiency

In a high throughput setting, it is important to be as efficient as possible while keeping your pipetting processes reliable and consistent. There are many ways to improve your pipetting efficiency, including the use of multichannel and/or electronic pipettes. Whether or not a multichannel or electronic pipette could benefit your application depends on the following criteria:

What vessels are you using?

Transferring samples between labware of different formats can quickly become very tedious and error-prone using single channel pipettes. Multichannel pipettes allow you to transfer multiple samples at once. This helps you to be much more efficient and also to prevent pipetting errors and repetitive strain injuries (RSI).

Some pipettes are even able to change the tip spacing during pipetting, enabling parallel transfer of multiple samples between different labware sizes and formats.

Figure 2: VOYAGER is the only automatic adjustable tip spacing pipette on the market.

Repetitive tasks

If you dispense multiple aliquots of the same volume, an electronic pipette could be a great help, allowing repeat dispensing without refilling the tips.

Versatility

Electronic pipettes usually offer multiple different modes – such as reverse pipetting, variable dispensing, programmed serial dilutions and many more – making your pipetting tasks more efficient.

Factors increasing ergonomics

Pipetting is one of the most common tasks carried out in laboratories, and lab professionals often spend several hours pipetting each day. This can cause discomfort and, in more serious cases, even lead to hand or arm injuries. To avoid these potential risks, consider the following features when choosing a pipette:

Weight

Use micropipettes that are lightweight and well-balanced, with the mass in the center for better stability.

Tip loading and ejection force

Tip loading and ejection often requires more force than pipetting, and presents a potential risk for injuries, especially in high throughput settings. Pipette tips should snap into place with minimal force, provide a secure connection, and eject just as easily.

Grip design

The pipette should fit comfortably into the hand, for both left- and right-handed users.

Volume adjustment

Adjusting the volume should be as comfortable and fast as possible, to avoid unnecessary strain on the hands.

Summary

The pipette you use for liquid handling tasks can have a significant impact on the accuracy and reproducibility of your results. The correct pipette type can also improve your overall lab efficiency for greater sample throughput. Therefore, before purchasing a new pipette, it's worthwhile to consider factors like the viscosity and volume of the liquids you are working with, as well as the number of samples you may have to process on a regular basis. We hope the information and tips in this article will assist you in making the right choice of pipette in the future.

1.3 Ergonomic pipettes and pipetting guidelines

Your health is important, so take care! Prolonged repetitive tasks such as manual pipetting put you at risk of developing hand, neck or shoulder issues and may cause complications such as RSI. You can prevent RSI by following a few simple guidelines.

Are you someone that needs to stretch and mobilize your wrist muscles after manually pipetting into a 96 or 384 well plate? During plate set-up you are totally focused on dispensing the right sample into the right well, and only after ejecting the last tip do you realize that your wrist and thumb are pretty strained. Even if you are lucky enough to be pain free, you will have noticed your colleagues complaining about it.

This empirical evidence that repetitive pipetting or an awkward posture can cause pain is supported by studies that report a significant increase in the risk of developing musculoskeletal problems when pipetting for more than 300 hours a year.[1] Assuming you work between 180 and 200 days a year, pipetting for 1 or 2 hours a day results in an increased risk of injury.

This data is 25 years old, so what's changed? Evolution hasn't changed our bodies during this time, but lab technicians' workloads and sample throughputs have definitely increased.

Automation is certainly more widespread than before but, nevertheless, pipetting for more than 2 hours is still common. And in today's lab, when you are not pipetting you are sitting in front of a computer. Congratulations, you have full-time exposure to ergonomic hazards during your working day!

Ergonomic pipetting – RSI and related terms

There are many different terms and abbreviations that buzz around the subject of ergonomic pipetting, such as RSI, WRULD, MSD, CTD. To cut a long story short, they all essentially refer to pain in the hands, neck and shoulders caused by prolonged and repetitive movements. Typical examples of conditions caused by repeated exposure to ergonomic hazards include carpal tunnel syndrome, tendonitis, tenosynovitis, and tennis elbow.

GLOSSARY

RSI	Repetitive strain injury: Term covers a range of health problems brought on by frequent,highly repetitive actions
WRULD	Work-related upper limb disorder: Conditions of the upper body caused by activities performed during the working day
MSD	Musculoskeletal disorder
CTD	Cumulative trauma disorder

What causes RSIs during pipetting?

The likelihood of developing an RSI is dependent on various risk factors. The 3 major ones are posture, force and repetition. Awkward body postures, heavy pipettes, stiff plunger buttons as well as highly repetitive processes may cause RSI.

1. Posture

In many labs, pipetting is done sitting at the lab bench. Just like sitting in front of your computer, it is important to keep your back straight and your head and shoulders aligned whenever possible, at the same time keeping your shoulders relaxed.

Your legs should be placed beneath the lab bench with your feet flat on the floor or, if necessary, a footrest. Ideally, your arms should be kept close to your body; organize your workplace so that all your reagents and labware are in close proximity to avoid stretching.

Furthermore, lab tools, such as pipettes, have to be lifted up to be operated. The weight of the pipette affects how you hold it, and its size and the positioning of components – including the plunger, and tip ejection and volume change mechanisms – determines whether or not it can be held ergonomically.

2. Force

With manual pipetting, the plunger is thumb operated, allowing liquids to be aspirated and dispensed in a controlled way, with additional force needed to ensure all the liquid is dispensed. The thumb force is further increased when pipetting high viscosity liquids.

Force is also needed to lift up a pipette and to hold it in an upright position during use, stabilizing the hand grip with the help of the thumb muscles.[2] The weight of the pipette contributes to these forces.

3. Repetition

We all know that accurate and precise pipetting can be a challenge, involving numerous highly monotonous movements: attach the tip(s) to the pipette; depress the plunger with the thumb; move to the source liquid; raise thumb to aspirate; move to the target; depress the plunger with the thumb to dispense; and, finally, eject the tip. These movements are repeated over and over again – several hours a day, and several days a week.

How to prevent RSI

Even if you are lucky enough to be pain free at the moment, adapting your working environment early on can avoid problems later. Start now!

Talk to your employer

In many countries, employers are legally obliged to evaluate the RSI risk of each workspace by undertaking a Risk Assessment Test.[3]

The main goal of all employers is to have healthy staff who are in the lab, not with the doctor or physiotherapist. So be confident – the ergonomic improvements in your lab workflow will be appreciated by your colleagues and your employer!

Listen to your body!

The initial symptoms of an RSI include:

- painaching or tenderness
- stiffness
- throbbing
- tingling
- numbness
- weakness
- cramping

… of the hand, shoulder, neck, wrist or thumb.

If you notice any of these early symptoms, first try to identify the repetitive motion causing the pain. If possible, avoid this motion in the future, or adapt your process and never forget to take regular breaks.

Visit a doctor if necessary

If the pain persists even without performing the repetitive motion, visit a doctor, as treatment will be necessary. RSIs can be treated in various ways depending on the symptoms, from pain killers and steroid injections to physiotherapy. In severe cases, it may be necessary to take sick leave until the pain subsides.

Take time to recover

How long it takes to recover from an RSI varies from case to case. But RSIs develop over a long period and, unfortunately, it can take a long time to get rid of them. Sadly, once you have been severely affected by an RSI, the risk of a reoccurrence is high. If the problem is ignored, it may result in long-term disability.

The graphic below shows some of the preventive measures that you can apply in your lab processes to avoid any RSI pipetting issues.

Thumb

Problem

Manual pipettes require force to depress a plunger, which can cause problems such as thumb tenosynovitis. Manual tip ejection can lead to the same condition when the ejection force is too high.

What can you do?

Select a manual pipette with minimal plunger force and stroke distance. Electronic pipettes are ideal because the plunger is controlled by a microprocessor, requiring minimal thumb movement. Use tips that need the lowest possible ejection force for removal.

INTEGRA's ergonomic pipetting solutions

- EVOLVE manual pipettes are lightweight with low plunger force.
- VIAFLO/VOYAGER electronic pipettes feature 1-button plunger control and an intuitive thumbwheel design. Simply press the button to aspirate and dispense using zero force.
- GRIPTIPS® pipette tips have the lowest ejection forces on the market.
- The ASSIST pipetting robots automate handheld electronic pipettes, eliminating repetitive manual tasks.
- The VIAFLO 96 or VIAFLO 384 electronic pipette dramatically reduces the time spent pipetting and offers a computer-controlled plunger and electronic tip ejection.

Hand

Problem

Hammering on tips can lead to long-term hand pain. In addition, holding the pipette too tightly for extended periods can lead to nerve damage and, potentially, carpal tunnel syndrome.

What can you do?

Don't hold the pipette too tight. Choose a pipette with a finger hook to rest your hand, and low tip loading forces to reduce the need to hammer on tips.

INTEGRA's ergonomic pipetting solutions

- EVOLVE manual pipettes are lightweight with low plunger force.
- VIAFLO/VOYAGER electronic pipettes, and EVOLVE manual pipettes, use GRIPTIPS, which snap effortlessly onto the tip with no hammering on required.
- The ASSIST PLUS pipetting robot features automatic tip loading and takes the pipette out of the hands of the user.

- The VIAFLO 96 or VIAFLO 384 electronic pipette significantly reduces the time spent pipetting by dispensing/mixing an entire plate at a time, as well as offering electronic tip loading.

Wrist

Problem

Twisting and turning the wrist too much and too often can lead to muscle injuries. Bending the wrist is especially problematic, as it places it in an unnatural position.

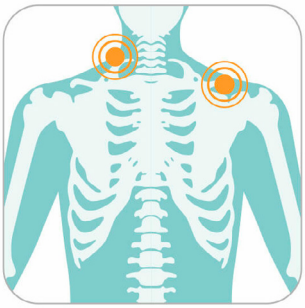
What can you do?

Choose a pipette that allows easy volume changes and keeps the wrist in a neutral position. Pipette handles that can be rotated help with this, allowing the user to choose the most comfortable position for them.

INTEGRA's ergonomic pipetting solutions

- EVOLVE manual pipettes use 3 separate dials for straightforward volume changes, avoiding the typical approach of twisting the plunger. This allows quick volume changes that don't affect the wrist.
- VIAFLO/VOYAGER electronic pipettes have handles that can be turned to allow each user to find the most comfortable position for them. Volume changes are very easily done using a comfortable thumbwheel.
- The ASSIST pipetting robots automate handheld electronic pipettes, removing the need to move the wrist. All volume changes and liquid handling tasks are carried out via a simple-to-use program.
- The VIAFLO 96 or VIAFLO 384 electronic pipette can be operated in automatic mode, reducing the required hands-on time and wrist movements.

Neck and shoulders



Problem

Pipetting over long periods of time results in the neck and shoulders being hunched forward. In general, bad posture when pipetting can cause strains and restrict blood flow to the muscles. Awkward posture is commonly associated with working in a laminar flow air cabinet.

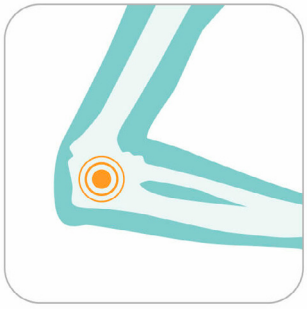
What can you do?

Be aware of your posture. The head should be directly above the shoulders, and the shoulders in line with the hips. Sit in the middle of your chair/stool with your back straight.

INTEGRA's ergonomic pipetting solutions

- The ASSIST pipetting robots completely solve posture issues by removing the need for manual pipetting.
- The VIAFLO 96 or VIAFLO 384 electronic pipette allows you to keep your neck in an upright position and your shoulders relaxed during the pipetting process. The motor assistance for moving the pipetting head enables an effortless workflow, while the electronic tip positioning guarantees optimal targeting of the wells without visual control.

Elbow



Problem

Tennis elbow, or lateral epicondylitis, can occur when pipetting over long periods of time, especially when the elbow needs to be fully extended. Resting the elbow on a hard surface while pipetting can compound the problem.

What can you do?

Don't fully extend the elbow and keep labware as close to the pipetting area as possible.

INTEGRA's ergonomic pipetting solutions

- The ASSIST pipetting robots eliminate elbow problems by automating handheld electronic pipettes, removing the need for manual pipetting.
- The VIAFLO 96 or VIAFLO 384 electronic pipette is designed in such a way that the elbow is never fully extended, and can be automated to aid in pipetting processes.

Summary

How to pipette ergonomically?

- Choose a pipette with minimal plunger force and stroke distance.
- Use tips with the lowest possible ejection force.
- Don't hold the pipette too tight. Pipettes with finger hooks and low tip loading forces can help with this.
- Choose a pipette that allows easy volume changes.
- Keep your wrist in a neutral position. Pipettes with handles that can be rotated can help with this.
- Don't fully extend the elbow when pipetting and keep labware as close to the pipetting area as possible.
- Your head should be directly above the shoulders, and the shoulders in line with the hips.
- Sit in the middle of your chair/stool.
- Keep your back straight.

1.4 The different types of pipette tips (and when to use them)

The first thing you ask yourself in the morning, after pondering if you can hit snooze one more time, is: “What should I wear today?” Your decision is influenced by many internal and external factors, such as your mood, the weather or whom you are going to meet.

Similarly, many factors have to be considered when choosing the right pipette tips for your task. Hopefully, this time the decision is not influenced by your whims, but by relevant factors such as the property of the liquid you are pipetting, and the type of application. But how do you know which pipette tip is best to pick? Here, we discuss the importance of sterility, quality and fit.

Different types of pipette tips

Non-sterile, sterile, filtered, unfiltered, long, short, low retention, wide bore – the variety of pipette tips available can be overwhelming. So let's explore what each of these options is good for:

Non-sterile vs. sterile

It's common sense that sterile pipette tips should be used for applications where sterility is important. But can you buy non-sterile tips and autoclave them yourself to save money? In theory, the answer is yes. However, you need to make sure that the manufacturer declares them as autoclavable, and be aware of the following:

- **Lack of quality control** - Tip manufacturers validate their sterilization process, and perform regular quality checks. In contrast, individual labs don't usually test the effectiveness of their autoclaving process, which can lead to contaminated samples.

- **Autoclaved tips aren't free of RNase and DNase** - Tips you've autoclaved yourself are sterile, which means that they are free from living organisms, but not necessarily from RNase and DNase. If you need to perform sensitive assays where this is required, you should opt for sterile pipette tips from a manufacturer who can certify that their tips are free of RNase and DNase.

Filter tips

Every time you aspirate liquid, aerosols are generated inside the pipette tip. If you don't use filter tips, these aerosols may contaminate your pipette and, consequently, your next samples – even if you change tips in between.

For example, when performing PCR applications, the cross-contamination of samples by aerosols in the pipette could lead to false positive results, as even the smallest quantities of DNA from a previous sample could be amplified. It's particularly important to use filter pipette tips when handling liquids that could damage your pipette, such as radio-labeled or corrosive samples, for both your own safety and lifetime of your pipette.

Here's a list of liquids that should always be pipetted with filter tips, as they could either contaminate or damage your pipette:

- RNA/DNA solutions
- Infectious samples
- Radio-labeled samples
- Volatile, corrosive or viscous samples
- Strong acids or bases

And last but not least, filter pipette tips can be useful for training new lab staff. Spending extra money on filter tips until your colleagues get used to your instruments is a good idea that usually pays for itself, as you can avoid pipette contamination or damage from liquid entering the lower end of the pipette.

Long tips

Have you ever risked cross contamination by putting the shaft of your pipette into a tube for a standard tip to reach the bottom? To eliminate this risk, many manufacturers offer extended length pipette tips suitable for labware such as microcentrifuge tubes or deep well blocks.

Short tips

Short pipette tips offer 2 advantages. First, they support the targeting of small wells, e.g. when manually pipetting into a 384 or 1536 well plate with a multichannel pipette. And second, they offer improved ergonomics by allowing you to pipette closer to the bench, reducing the strain on your arm.

Figure 1: Multichannel pipette with filter tips.

Low retention tips

As the name suggests, low retention pipette tips retain less liquid, providing more accurate and consistent results while saving precious reagents. However, they are more costly than standard tips, so you should know exactly when it is worth using them. Our in-house application scientist demonstrated, in a series of tests, that both standard and low retention tips provide ideal liquid recovery when pipetting water, but produce significantly different results when viscous or low surface tension solutions are handled.[1] Therefore, low retention tips are ideal when pipetting highly concentrated, and consequently viscous, samples during:

- PCR, cloning, sequencing or other DNA and RNA applications
- SDS-PAGE, protein purification or other protein analysis applications

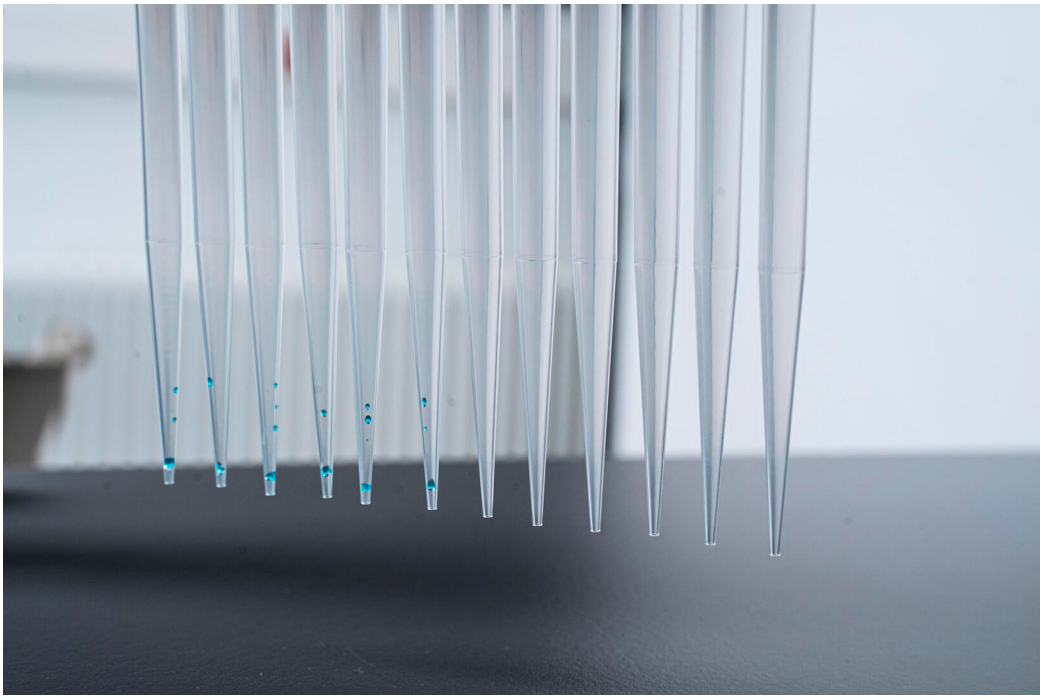


Figure 2: Liquid retention in standard tips (left) and low retention tips (right).

Are all low retention tips the same?

When producing low retention pipette tips, manufacturers typically use either a different polypropylene blend to their standard tips, or add a silicone coating. Both techniques prevent viscous or low surface tension liquids from spreading out and 'wetting' the inner wall of the tips, however, the latter has one major disadvantage – a silicone coating can wash or leach out with your sample. So you should always choose pipette tips with a polypropylene blend offering heightened hydrophobic properties to ensure that liquid-repellents can't contaminate your samples.

Wide bore tips

Fragile cellular samples can get damaged when they are forced through the narrow orifice of standard pipette tips. Therefore, you should use wide bore tips when transferring cellular samples, such as fragile cell lines, or other viscous materials. The wider orifice of these tips prevents (cell) shearing and reduces flow resistance.

Why tip quality and fit are crucial

Now that you know when to choose each type of tip, we need to address tip quality and fit. Low quality or poorly fitting pipette tips can negatively impact the reproducibility of your results. This means that you will need to repeat experiments, wasting valuable time and money. On top of that, repeating experiments means even more pipetting, which increases the strain on your arm, wrist and fingers – especially if you use poorly fitting tips that require high attachment and ejection forces – and can ultimately lead to repetitive strain injuries.

How to assess tip quality

The first factor influencing the quality of pipette tips is the polypropylene blend. High quality tips are made from virgin polypropylene, which is free from plastic and/or metal additives that could contaminate your samples. Checking that a manufacturer doesn't use metal additives is especially important when buying colored tips, as metal additives can often be found in dyes.

The second factor influencing quality is the injection molding machine. The slightest batch-to-batch or within batch variations – such as differences in straightness, molding flash or streaking – negatively impact the accuracy and precision of your results. These irregularities often can't be seen by the naked eye, so it is better to avoid buying the cheapest tips on the market, to reduce the risk of inaccurate and imprecise results.

How to find a properly fitting tip

Tip shapes are not universal, so not every tip will fit your pipette. Ideally, you should only use the tips recommended by your pipette manufacturer. If you use tips that haven't been tested and validated by your manufacturer, you should always gravimetrically test their performance to ensure that they produce reliable results.

Summary

Buying high quality, properly fitting pipette tips might seem more expensive at first glance, but will save you a lot of time, money and health issues. However, the question of whether you can work with non-sterile standard tips, or whether you need to invest in tips with features such as heightened hydrophobic properties or wider orifices, depends on your requirements. Make sure to answer this question every time you start a new application by following our hints above, so that your pipette tips will never compromise your lab work again.

CHAPTER 2:
Pipetting best practices

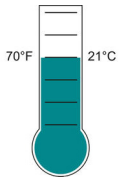
Inconsistencies in liquid handling technique can lead to poor data and reproducibility, compromising the integrity of results. However, it can be difficult to consistently operate pipettes, whether they are manual or electronic, even with years of experience under your belt. On top of this, certain liquids prove especially problematic, requiring particular skills to handle successfully. This chapter explains different ways you can easily improve your technique for greater precision and accuracy.

2.1 Proper pipetting: 10 tips on how to use a pipette

Are you annoyed with inaccurate pipetting results? Learn how to correctly use a pipette and improve your results instantly.

Before you start

1. Ensure temperature equilibrium



To improve accuracy and precision when using an air displacement pipette, the pipette, tips and liquids need to be equilibrated to room temperature if the experiment allows.

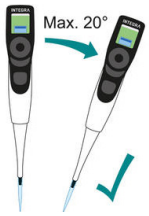
Accuracy refers to how close the dispense volume is to what you set the pipette to dispense. It is affected by pipette calibration and pipetting technique.

Precision measures how true a pipette is to the target volume over multiple dispenses. Precision can be corrected with proper pipetting practices.

Note: Temperature differences lead to volume contraction or expansion of the air cushion inside the pipette tip and pipette, which can negatively impact the accuracy and precision of the dispense.

How to pipette

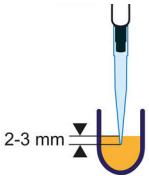
2. Maintain consistent pipette angle



When pipetting, the angle of the pipette can play an important role in obtaining good results. If possible, hold the pipette at a constant angle throughout the entire process. Ideally, the angle at which the pipette is held should not exceed 20 degrees. For very small volumes of 30 microliters or less, the straighter the pipette, the better.

Note: With changing the angle, the hydrostatic pressure inside the tip varies. As a result, the aspiration volume will be inconsistent.

3. How to aspirate



A common mistake made during aspiration is to immerse the pipette tip as deep as possible in the source vessel. As this increases the risk of liquid droplets clinging to the outside of the pipette tip, it is best to immerse the pipette tip just below the liquid's surface. It is recommended to insert the tip only 2-3 mm into the source liquid to allow the desired volume to be aspirated. An exception to this is the aspiration of extremely low volumes such as 0.2 microliters.

Note: Liquid retained on the outside of the tip can result in an inaccurate dispense.

4. Touch off after dispense

After a dispense, you will often see a droplet at the end of your tip. As this droplet belongs into the dispense, you should use 1 of the following 3 methods to remove your pipette from the target vessel.

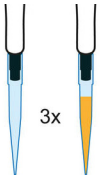
- **Side wall touch off** - Remove the pipette tip by sliding the tip end along the sidewall of the vessel. This is the standard method and recommended to achieve the most accurate dispense.
- **Surface touch off** - Remove the tip by touching off the liquid droplet on the surface of the liquid in the container. This technique is recommended when dispensing less than 1 microliter as a neat transfer. Touching the droplet to the liquid draws the small droplet out of the pipette tip which ensures accurate delivery.
- **Into liquid dispense** - If the dispense was made directly into the liquid it is considered a wet-dispense and a touch off is not required. This method is ideal for small volume dispensing to ensure the liquid does not remain on the side of the vessel.

Note: After a dispense, residual liquid often clings to the tip end. The above techniques remove this residual liquid from the tip.

Optimizing pipetting performance

5. Pre-wet

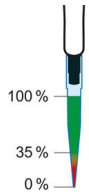
After loading tips onto your pipette, aspirate and dispense the nominal volume 3 times. This will equilibrate temperature differences and humidify the dead air space inside the pipette and tip. When neglecting the pre-wetting procedure, the first few dispenses tend to deliver less volume due to evaporation.



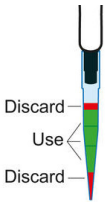
Note: The evaporation can also cause droplet formation on the tip end, as vapor pressure increases and liquid is forced out of the tip.

6. Optimize the volume range

The volume range of your pipette can have an impact on both accuracy and precision. Air displacement pipettes show the best performance between 35 % and 100 % of their nominal volume. The closer your dispense volume is to the total volume of the pipette, the better your results. Below 35 %, the volume of dead air in the pipette becomes quite large, and the risk of inaccurate and imprecise dispensing increases. On top of that, pipetting within the optimal volume range is less technique dependent and reduces user related errors.



7. Discard first and last dispense



When using the repeat dispense mode of an electronic pipette, it is recommended to discard the first and last aliquot of the series. Here's why:

- When the electronic pipette is finished aspirating the liquid, the mechanics inside the pipette need to change direction before starting to dispense. This could result in the first dispense being too low in volume.
- As no pipette is 100 % accurate, each dispense might be slightly off the target volume. The last dispense will include the accumulated error of all previous dispenses.

Note: The first and the last dispenses should not be used for the assay because they contain the largest errors.

8. Viscous liquids

Viscous samples can be challenging to pipette. They usually enter the tip more slowly than other liquids and tend to stick to the tip wall when being dispensed because of their low elasticity. Therefore, you should apply the following techniques when pipetting viscous liquids:

- Viscous samples should be aspirated and dispensed at slower speeds. Moverover, pausing after every aspiration or dispense gives the liquid more time to smoothly move into or out of the tip.
- To avoid that you dispense volumes that are too low because of liquid adhering to the tip's inside wall, you need to use the "Reverse pipet" mode. As it aspirates the selected volume plus an extra dispense that will be discarded, it is ideal to compensate for the retained liquid. An additional benefit of reverse pipetting is that there is no blow-out at the end. This prevents that viscous liquids start to foam or form air bubbles.

9. Volatile liquids

Volatile liquids can be difficult to pipette accurately. As they evaporate faster than aqueous solutions, tips often start to drip. To prevent dripping tips, you should pre-wet your pipette tip to humidify the dead air space. This can be achieved by aspirating and dispensing the full volume of your tip 3 times using the liquid you will be pipetting. Additionally, volatile solutions should be pipetted quickly and in “Reverse pipet” mode. The "Reverse pipet" mode incorporates a larger sample volume to minimize the effect of evaporation on the actual volume to be delivered.

10. Calibrate based on liquid density

Significant errors can occur when liquids other than aqueous solutions are used. Recalibrate your pipette if the liquid has a considerably different density than water.

Note: Pipettes are normally tested and calibrated at the factory with distilled water at room temperature. Pipetting liquids with different densities results in inaccurate dispenses.

Summary

In summary then, there are many simple ways you can improve your pipetting technique to quickly see more accurate and precise results.

- Ensure temperature equilibrium: Make sure the pipette, tips and liquids are at room temperature (if experiment allows)
- Maintain consistent pipette angle: Hold the pipette at a consistent angle not exceeding 20 degrees
- How to aspirate: Aspirate by immersing the tips just below the liquid's surface (2-3 mm)
- Touch off after dispense: Touch off after each dispense when removing the pipette from the target vessel
- Pre-wet: Pre-wet the pipette tip by aspirating and dispensing the nominal volume 3 times
- Optimize the volume range: Pipette within the optimal volume range of your pipette (35-100 % of the nominal volume for air displacement pipettes)
- Discard first and last dispense: When dispensing multiple aliquots, it is recommended to discard the first and last dispense of the series
- Viscous liquids: Pipette viscous liquids at slower speeds and in "Reverse pipet" mode
- Volatile liquids: Pipette volatile liquids quickly and in "Reverse pipet" mode
- Calibrate based on liquid density: Recalibrate your pipette if the liquid has a considerably different density than water

If you've already integrated these tips into your daily routine, you're officially a pipetting pro. If not, it is only a matter of time before they become your daily habit and you reap the benefits of a proper pipetting technique.

2.2 Pipetting tips to increase accuracy and precision

Pipettes are routinely used in life sciences laboratories, yet some researchers still have inconsistencies in their liquid handling technique, even when following the same protocol every time. This can lead to poor data and reproducibility concerns, compromising the integrity of results. While quality assurance efforts in liquid handling typically place emphasis on pipette calibration, repair and maintenance, ensuring correct and consistent pipetting technique is just as critical for efficient workflows and successful projects.

At INTEGRA, our application team has decades of combined experience in liquid handling, so we have put together a video series to provide you and your laboratory colleagues with general rules, tips and tricks for correct pipetting. Following these simple guidelines will help to ensure:

- Accuracy and precision for every aspiration and dispense, further increasing the reproducibility and reliability of results
- Consistent operation of both manual and electronic micropipettes
- Improved pipetting of ‘problem liquids' – such as viscous and volatile liquids
- Correct maintenance for long-term operation

Watch the video series

2.3 How to pipette viscous and volatile liquids

Pipetting viscous and volatile liquids – such as glycerol, DMSO, ethanol or Tween® 20 – can pose serious challenges for several reasons. Calibration of air-displacement micropipettes is done in a controlled environment with water, and pipetting of non-aqueous liquids may affect the accuracy of results. You therefore need to use the proper pipetting technique to ensure the best results.

Some general recommendations are valid for all pipetting tasks:

- Keep the micropipette as upright as possible for every pipetting step.
- Do not immerse the pipette tip too far into the sample reservoir, to reduce the risk of sample carry-over.
- Adjust the pipetting speed to suit the liquid.

Pipetting common non-aqueous liquids

So, what can be done? In cases where only one type of non-aqueous solution is pipetted, it is possible to calibrate the pipette to this liquid to improve results. However, the experience and technique of the user – including their subjective impression of manual pipetting speeds – can significantly influence pipetting of these liquids, and is often an underestimated factor.

Here at INTEGRA, the most common non-aqueous solutions have been tested with our pipettes. Below you'll find an explanation of the challenges of liquid, best practice recommendations for how to handle them, and optimal settings for our electronic pipettes. Using these recommendations should allow you to set up your micropipette faster, getting the best results with less testing (although further optimization may be required for specific workflows).

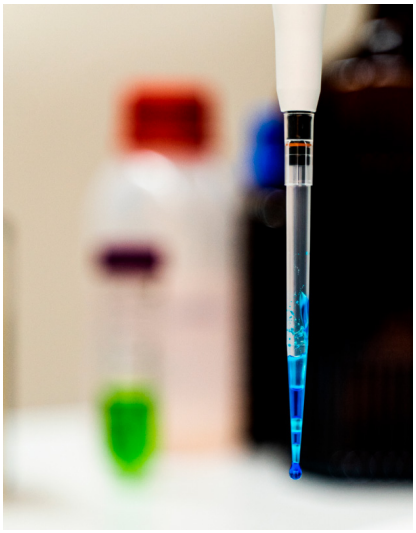
How to pipette viscous liquids

DMSO

Dimethyl sulfoxide (DMSO) is commonly used as cryoprotectant in cell culture, or to prevent the formation of secondary structures in DNA templates or primers during PCR. The following recommendations can improve the pipetting accuracy for viscous DMSO using VIAFLO pipettes:

- **Use low retention tips** – as viscous liquids attach to the wall of the tip, making it difficult to expel the full volume of liquid
- **Use reverse pipetting** – as viscous liquids can make it difficult to completely empty the tip. Aspirate more liquid than you need, dispense the desired amount, and discard the rest.
- **Keep the tip in the solution for longer** – wait 2-3 seconds after aspiration and dispensing, as the small pipette tip orifice and elasticity of the air column may prevent viscous samples from being pipetted completely otherwise
- **Adjust the speed** – use a pipetting speed of ~450 µl/sec (Speed 6 on 50-1250 µl VIAFLO electronic pipettes) or less for higher DMSO concentrations. Higher pipetting speeds may be used for lower DMSO concentrations

Glycerol



Glycerol is commonly used as cryoprotectant in cell culture, or as a carbon source in microbial fed-batch fermentations. The following recommendations can improve the pipetting experience for viscous glycerol with VIAFLO pipettes:

- **Use wide bore tips** – especially for 80 % glycerol, as these will enable the liquid to enter the tip more easily
- **Use reverse pipetting** – as it may be difficult to completely empty the tip with normal pipetting methods, due to the attachment of the liquid to the tip wall
- **Keep the tip in the solution for longer** – wait 2-3 seconds after aspiration and dispensing, as the small pipette tip orifice and elasticity of the air column may prevent viscous samples from being pipetted completely otherwise

- **Adjust the speed** – use a pipetting speed of ~300 µl/sec (Speed 4 on 50-1250 µl VIAFLO electronic pipettes). Low glycerol concentrations are less viscous, and can be pipetted at higher speeds

Tween 20

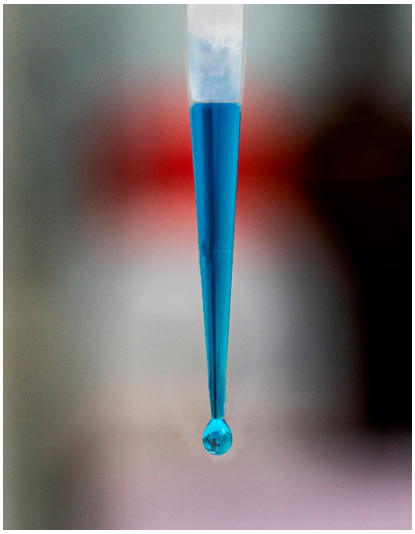
Tween 20 is a common washing agent for immunoassays, as it prevents non-specific antibody binding. The following recommendations can improve pipetting results with viscous and foaming Tween 20 using VIAFLO pipettes:

- **Use wide bore tips** – as these will enable the liquid to enter the tip more easily
- **Use reverse pipetting** – to reduce the risk of dispensing air during blow out into the sample, which may lead to further foaming
- **Adjust the speed** – use a pipetting speed of ~100 µl /sec (Speed 2 on 50-1250 µl VIAFLO electronic pipettes)

How to pipette volatile liquids

Ethanol

Ethanol is used for a broad spectrum of applications, including for immobilization of microbes, as a carbon source, and as a disinfectant, solvent or precipitant for DNA and RNA. The following recommendations can improve the pipetting accuracy of volatile ethanol using VIAFLO pipettes:



- **Pre-wet the tips with ethanol** – by performing 2-3 aspirations/dispenses of the full volume of the pipette. This ensures that the environment inside the pipette tip is saturated with ethanol vapor, reducing evaporation of the target liquid. For VIAFLO pipettes, the ‘Pipet/Mix' mode is ideal for this, and you can set it as the first pipetting step for your custom programs.
- **Use reverse pipetting** – to further reduce the effects of evaporation, as any loss will be in the discarded post-dispense, not the volume dispensed

- **Adjust the speed** – Use a pipetting speed of ~1100 µl/sec (Speed 10 on 50-1250 µl VIAFLO electronic pipettes) for aspiration and a pipetting speed of ~343 µl/sec (Speed 5 on 50-1250 µl VIAFLO electronic pipettes) to dispense. This speed allows the ethanol film attached to the inside of the tip to run down and catch up with the dispense. Lower ethanol concentrations are less volatile and can be pipetted at lower speeds

Summary

Viscous and volatile liquids can be problematic to pipette. Knowledge about the sample enable pipette settings to be optimized to suit the needs of each liquid, ensuring the highest accuracy and reproducibility of results. These 4 examples are designed to give you an idea of the best settings to use for a range of non-aqueous solutions, and can be applied and adjusted to other liquids with similar properties or pipettes with different volume ranges.

CHAPTER 3:
Maintaining and calibrating your pipettes

You need to recalibrate and maintain even the best quality liquid handling products to keep them in top condition and providing reliable and reproducible results. In this section, we discuss how you can clean, decontaminate and store your pipettes correctly for optimal performance. We also cover the importance of regular calibrations for confidence in your data and explain how you can check the accuracy and precision of your pipette.

3.1 How to clean, decontaminate and store pipettes

You undoubtedly chose a career in research because of your insatiable curiosity and love for problem solving – not because you're passionate about pipettes. But now, your daily work includes countless hours of pipetting, and getting precise and accurate results depends on your pipettes not letting you down mid-experiment. To ensure that you can fully rely on them for years to come, you should spare a thought for how to properly take care of them. As with everything in life, it's a bit of give and take.

Find out how you can clean, decontaminate and store your pipettes correctly, and why regular calibration is crucial.

How to clean a pipette

When did you last clean your pipettes? It's more likely that you'll be thinking of running your next experiment, rather than spending time cleaning your pipettes, but you shouldn't put it off for too long. They are some of your most important tools and deserve some care to ensure that they work smoothly throughout their entire lifespan.

Exterior cleaning

Cleaning the outer surface of your pipette is quick and easy, and should be part of your daily routine. Wiping them with a lint-free cloth lightly soaked with 70 % ethanol should be sufficient, but do check the chemical compatibility of the pipettes beforehand.

Interior cleaning

Most manufacturers recommend that multichannel pipettes should be disassembled and cleaned by trained personnel. For single channel pipettes, however, you can and should clean them yourself regularly by following these simple steps:[1,2,3,4]

1. **Disassemble the pipette -** Check the operating instructions to learn how to disassemble your pipette. Most models require you to disconnect the upper and lower parts before removing the O-ring and piston.
2. **Rinse with distilled water** - Clean the various components with distilled water and use a cotton swab to remove any clogs in the air passages.
3. **Perform a maintenance check** - Once you've cleaned all the components, check them for visible damage and, when necessary, replace any damaged parts. It's recommended that you replace the O-rings and seals each time.
4. **Dry all components** - Place all the components on a clean surface to air dry.
5. **Grease the piston** - Check the operating instructions of your pipette to learn where to apply lubricant, and what type of lubricant you should use. If no recommendation is available, use a grease made especially for pipettes. Common lubricants have a variety of different compositions, and some may not be appropriate to use on your pipette.
6. **Reassemble the pipette** - Ensure all the components are free of lint or dust particles before reassembling your pipette.
7. **Test your pipette's functionality** - To make sure that your pipette is working as intended after cleaning, you should perform a leak test and validate the pipetting volumes.

Decontamination

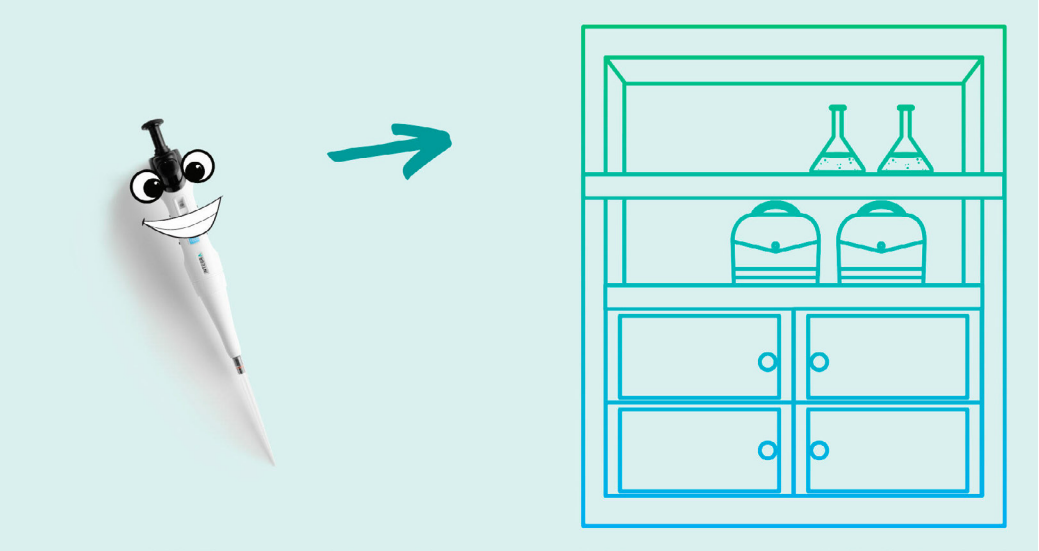
Even though your pipette shouldn't come into direct contact with any liquids you are pipetting, it is possible that they may occasionally become contaminated, e.g., when more liquid than the tip volume allows is aspirated, when a pipette is placed on the bench with liquid inside its tip, or when unfiltered tips are used with RNA/DNA solutions.

To avoid cross-contamination, you should decontaminate your pipette immediately. To do so, follow the cleaning procedure above, but add some additional decontamination steps after disassembly. The table below lists the most common contaminants, and the different decontamination steps to be taken for each of them. Don't forget to check the chemical compatibility of your pipette for the decontamination reagents before use.

CONTAMINANT	DECONTAMINATION STEPS
Aqueous solutions	Decontaminate all components with 70 % ethanol, rinse with distilled water, and allow to air dry.
Organic solvents	Decontaminate all components with a detergent, rinse with distilled water, and allow to air dry.
Radioactive solutions	Decontaminate all components with a high-strength radioactivity decontamination solution, rinse several times with distilled water, and allow to air dry. Check that the pipette has been successfully decontaminated with a Geiger counter, and dispose of all cleaning materials in the radioactive waste.
Proteins	Decontaminate all components with a detergent, rinse with distilled water, and allow to air dry. **Important:** Don't use alcohol, as it coagulates proteins.
RNase	Decontaminate all components with 95 % ethanol, and rinse with distilled water. Soak in 3 % hydrogen peroxide for 10 minutes, rinse with distilled water, and allow to air dry.
DNA / RNA	Decontaminate all components with a 10 % bleach solution, rinse with distilled water, wipe with isopropyl alcohol, and allow to air dry.

Pipette storage

Proper pipetting techniques, combined with careful handling, deliver accurate results and prevent excessive wear and damage. But what about storage? Have you ever thought about what happens to your pipettes when you're not using them? Appropriate storage can be just as important as careful handling, and implementing the tips below will only take you a few seconds a day.



Use a pipette stand

You should get into the habit of always storing your pipettes on a stand. Laying them down on the bench can lead to several problems:

- Piston lubricant may accumulate on one side, subjecting your pipette to excessive wear.
- The piston can get misaligned, compromising calibration.
- If there is any liquid inside, it can run up further into the pipette body and cause corrosion.

Not yet convinced? Pipette stands have even more advantages. They help protect your pipettes from spills and from getting knocked off the bench, as well as keeping your workspace organized. If you use a stand, you'll also immediately notice when a colleague picks up one of your pipettes. Additionally, for electronic pipettes, stands can double up as charging stations, which means that you won't need to remember to charge them.

Remove the tips

Never store your pipette with a tip attached to it. Any liquid residue present inside the tip could evaporate into the pipette body. You should always remove the tips, even if you're just putting the pipette aside for a few minutes.

Set it to its maximum volume

If you're using a manual pipette, set it to its maximum volume once you are finished with your work. This allows the spring to return to its least stressed position, increasing your pipette's lifespan.[5]

Why regular calibration is essential

Another way of extending the lifespan of your pipettes is to calibrate them regularly. Even when pipettes are carefully handled, appropriately stored, and regularly cleaned, they should be calibrated periodically by either the manufacturer or a specialized calibration company. We recommend service intervals of 12 or 6 months, depending on the needs of your lab. Keeping a record of past calibration dates helps to ensure that the next one won't be missed. Some electronic pipettes and pipette software packages allow you to set calibration reminders or record its service history to help you with this.

Regular calibration of your pipettes will not only ensure that they deliver precise and accurate results but can also help you to detect potential problems or damaged parts at an early stage. As a result, they can often be fixed before more costly repairs or replacement become inevitable.

Summary

Now that we've explained what you can do to keep your pipettes delivering reliable results for many years, it's your turn. Plan a time to check if your pipettes need cleaning, if you have enough pipette stands to store them, and when they were last calibrated.

3.2 Calibration check: How to calculate the accuracy and precision of a pipette

What happens to your pipette's performance between annual calibrations? Follow the best practice guidelines below to perform a routine check, from what you need to prepare beforehand, to performing the measurements and calculating the pipette's accuracy and precision.

Why a routine check?

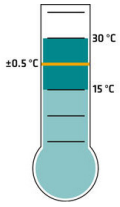
Pipettes should be calibrated annually to maintain their performance. In the interim, it is recommended to perform a quick routine check to test the functionality of your pipettes in order to be confident with your pipetting results during important experiments.

Note: On INTEGRA's electronic pipettes you can set a calibration reminder either in days or cycles.

1. Environment and materials

Environment

Draft free, constant temperature between 15 °C and 30 °C, max. ±0.5 °C deviation during the measurements.



Materials

Balance

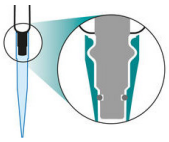
Equipped with draft protection and evaporation trap.

Alternative trap: 4 containers filled with water in each corner of the windshield.

Number of required digits is determined by the nominal volume of your pipette:

NOMINAL VOLUME	DIGITS (READABILITY)
0.5 µl ≤ V < 20 µl	Single channel balance: 6 (0.001 mg) Multichannel balance: 5 (0.01 mg)
20 µl ≤ V < 200 µl	5 (0.01 mg)
200 µl ≤ V ≤ 10 ml	4 (0.1 mg)

Weighing container



Preferably use a metal container to minimize build-up of static charges.

Alternative: 1.5 ml microcentrifuge tube.

Test liquid

Distilled water

Pipette tips

Use pipette manufacturer's recommended tips for best results.

Note: INTEGRA GRIPTIPS® pipette tips snap on and never loosen, leak or fall off.

2. Before you start

Place pipette, tips and test liquid in the test room 2 hours before starting measurements to reach equilibrium with room conditions.

Note date, ambient temperature and air pressure. If no barometer is available, search the internet for atmospheric pressure at a local weather station.

3. Leak test

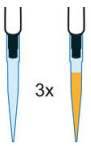
Before performing any gravimetric measurements, test if the pipette is leaking:

- **Pre-wet** the tip(s) by aspirating and dispensing the nominal volume **3 times**.
- Using the same tip(s), aspirate the nominal volume.
- With the tip(s) immersed 2 mm in liquid, hold the pipette vertically for 30 seconds then, with tip(s) immersed in liquid, dispense all remaining liquid from the tip(s).
- If liquid level does not drop and no air bubbles are present, continue with validation. A decreasing liquid level indicates a leak. Contact the manufacturer to discuss further steps.

4. Gravimetric measurement

The weighing container should not be dry. Add some distilled water.

Number of measurements



Perform at least 4 measurements each at 100 % and at 10 % of the nominal volume. Start at 100 %.

Validate the first and a middle channel on multichannel pipettes.

Procedure of the gravimetric measurement

- **Tare the balance** with the weighing container.
- Load a **new pipette tip.**
- Perform a **pre-wet** (see section 3).

- **Dispense** liquid into the weighing container. Ensure to dispense along the inner container wall and finish by drawing the tip end along wall to remove residual liquid.
- **Record** the weight in a table.
- Using the same tip, repeat steps 4 to 5 at least 4 times. Tare the balance after each reading.
- Eject the tip and load a new tip.
- **Repeat** steps 1 to 7 for the second test volume.

5. Data analysis

1. Convert balance readings for each measurement (mg) to volume (µl) using the Z factor (www.bit.ly/z-factor):

$$V_i = m_i \times Z$$

V_i = Single volume in µl
m_i = Single weighing in mg
Z = Z factor

2. Calculate the mean volume per test volume and per channel using the calculated V_i measurements:

$$\overline{V} = \frac{\sum_{i=1}^{n} V_i}{n}$$

$\overline{V}$ = Mean volume
$\sum_{i=1}^{n}$ = Sum of values
n = Number of weighings

3. Calculate the accuracy (systematic error) in % per test volume and per channel:

$$e_s = \frac{100\ (\overline{V} - V_s)}{V_s}$$

e_s = Systematic error in %
V_s = Selected test volume

4. Calculate the precision (random error) in % per test volume and per channel:

$$s_r = \sqrt{\frac{\sum_{i=1}^{n} (V_i - \overline{V})^2}{n-1}} \qquad CV = 100\ \frac{s_r}{\overline{V}}$$

s_r = Repeatability standard deviation
CV = Coefficient of variation in %

Compare the calculated accuracy and precision with the manufacturer's specifications. If the calculated values are not within specifications, the pipette needs to be calibrated. If the pipette has passed the routine check, it is working as intended.

Note: INTEGRA electronic pipettes can be easily calibrated. Simply enter target volume and actual volume you have measured and then click calibrate.

Summary

Here's a summary of how to calculate the accuracy and precision of your pipette to determine if it needs to be calibrated:

- Perform the routine check in a draft free environment with a constant temperature between 15 °C and 30 °C.
- Prepare a balance equipped with draft protection and evaporation trap, a metal weighing container, distilled water and tips.
- Place the pipette, tips and distilled water in the test room 2 hours before starting measurements.
- Note the date, ambient temperature and air pressure.
- Test if the pipette is leaking.
- Perform at least 4 gravimetric measurements each at 100 % and at 10 % of the nominal volume.
- Calculate the accuracy and the precision and compare them with the manufacturer's specifications.

CHAPTER 4:
INTEGRA Biosciences' pipetting solutions

Learn how our extensive range of liquid handling solutions can empower you to create faster and more efficient pipetting workflows with fewer errors.

Handheld pipettes

Manually transferring multiple liquid samples between different labware formats can be tedious and highly error prone, and may lead to repetitive strain injuries (RSI) in the long term. Our VOYAGER adjustable tip spacing pipettes feature motorized tip spacing adjustment, enabling parallel transfer of multiple samples between different labware formats to increase pipetting speeds, ensure higher reproducibility and eliminate transfer errors. Tip spacing can be changed by the simple push of a button – no manual adjustments or 2-handed operations are necessary. This not only boosts your pipetting productivity, but it also reduces the risk of RSI.

Our range of ultra lightweight VIAFLO electronic pipettes is also suitable for lower throughput liquid handling applications, featuring unsurpassed multichannel pipetting ergonomics, even during prolonged pipetting sessions. Color screens and user-friendly touch wheel interfaces make set-up and operation easy, enhancing pipetting productivity.

Both VIAFLO and VOYAGER pipettes feature 10 predefined pipetting programs covering the most common liquid handling tasks, helping to accelerate and simplify set-up of your specific workflows. For more elaborate pipetting protocols, up to 40 user defined custom programs can be created and saved on the pipette. View our series of video tutorials to learn valuable pipetting tips, tricks and best practices for the VIAFLO and VOYAGER handheld pipettes.

Learn more about **VOYAGER**

Learn more about **VIAFLO**

Scan to view **video tutorials**

Our EVOLVE single and multichannel manual pipettes feature a quick set design, with 3 separate dials for accurate and rapid volume adjustment. This both speeds up manual liquid handling steps – for greater lab productivity – and prevents inadvertent volume drift due to plunger movements, leading to better reproducibility. The range covers pipetting volumes from 0.2 to 5000 µl, with single, 8, 12 and 16 channel models available, and has been built to reduce repetitive strain injuries, with its ultra-lightweight, well-balanced designs.

Learn
more about
EVOLVE

96 and 384 channel pipettes

The VIAFLO 96 and VIAFLO 384 electronic pipettes are benchtop liquid handling systems for high throughput applications, and allow you to fill 24, 96 and 384 wells simultaneously. They offer interchangeable pipetting heads, providing high flexibility for current and future applications. The MINI 96 portable electronic pipette is the ideal solution for laboratories that need to rapidly and precisely perform whole or partial plate filling of 96 and 384 well plates. It is extremely affordable and compact, so fits into any budget and workspace, and is available in 4 volume ranges to suit a variety of applications or batch sizes. Compare the different options to find out which pipette is best to skyrocket your lab's productivity.

Learn more about **VIAFLO 96** and **VIAFLO 384**

Learn more about **MINI 96**

Compare the different options

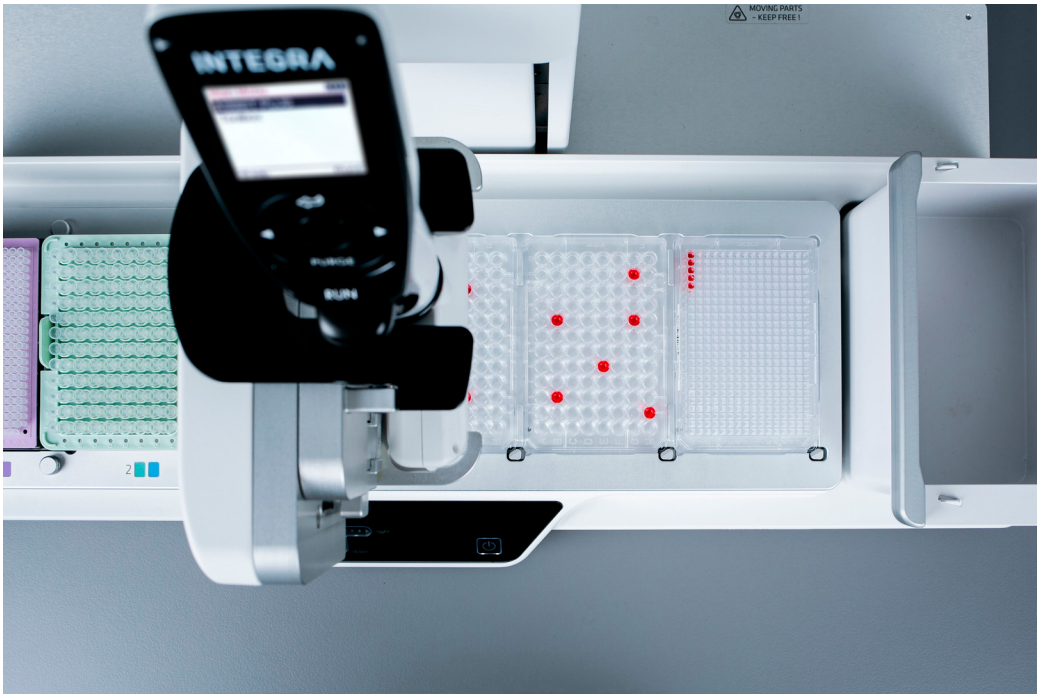
Pipetting robot

The ASSIST PLUS pipetting robot can free you from routine pipetting, providing flexibility and optimized processing for superior lab throughput and pipetting consistency. VIAFLO and VOYAGER multichannel pipettes as well as the D-ONE single channel pipetting module can be automated on the ASSIST PLUS for reliable walk-away processing in unlimited applications. Moreover, our pipetting robot can be programmed by anyone; no special training is required to use the intuitive VIALAB software which guides the user step by step through the set-up of individual protocols.

Learn more about **ASSIST PLUS**

Learn more about **D-ONE**

Learn more about **VIALAB**



High quality consumables

GRIPTIPS® pipette tips complement the INTEGRA range of pipetting solutions, fitting perfectly onto all pipettes to eliminate the risk of loosening, leaking or falling off. All tips are precisely aligned horizontally on multichannel pipettes, enabling accurate touch-offs and consistent pipetting results, even when pipetting with 384 tips.

INTEGRA also offers a range of multichannel, divided, 12 well and automation-friendly reagent reservoirs in a variety of volumes. Unlike traditional reagent reservoirs that have hard to see graduations, all of our reservoirs fit into a reusable base with bold, crisp, clearly visible markings. This unique design leads to more accurate measurements, no over pouring and less waste.

Learn more about **GRIPTIPS®**

Learn more about **reagent reservoirs**

CHAPTER 5: Application notes

At INTEGRA, we always endeavour to share our knowledge and experience with the wider community to support scientific advancements and discoveries. In this chapter, we've included a selection of key application notes demonstrating how some of our electronic pipettes, pipetting robots and reagent reservoirs are used in a broad spectrum of life sciences projects.

5.1 Fast and efficient sample transfer from plate to plate with INTEGRA's electronic pipettes

Effortless sample reformatting

The transfer of samples between same (e.g. from 96 well to 96 well plate) or different plate formats (e.g. from 96 well to 384 well plate) is a common process in life science applications, from cell culture, seeding and staining to drug testing, PCR, protein analysis and nucleic acid purification. However, it is a slow, tedious and highly error prone procedure when performed manually with a single channel pipette. INTEGRA's electronic pipettes allow precise and efficient transfer of multiple samples in parallel, significantly speeding up the reformatting process. For example, the VOYAGER adjustable tip spacing pipette is an ideal tool when the source and target plate formats differ, and can be used either manually or automated on the ASSIST PLUS pipetting robot. For even higher throughput reformatting, the VIAFLO 96, VIAFLO 384 and MINI 96 offer a fast solution for whole plate transfers.

Key benefits

- Replicate or reformat of all kinds of plates – including 12, 24, 48, 96, 384 or 1536 well plates – quickly and easily.
- Transfer 384 samples at once to significantly accelerate pipetting productivity.
- Pipetting errors and transfer mistakes are eliminated using INTEGRA's electronic pipettes.
- Reproducibility and accuracy of liquid handling steps increased – the VIAFLO 96 handheld electronic pipette is 4 times faster than pipetting with an 8 channel electronic pipette. Sample transfers with the ASSIST PLUS pipetting robot are 12 times faster than using a single channel pipette.

Overview: How to transfer samples from plates to plates

This application note shows some examples of how to transfer samples from plate to plate **(Figure 1).**

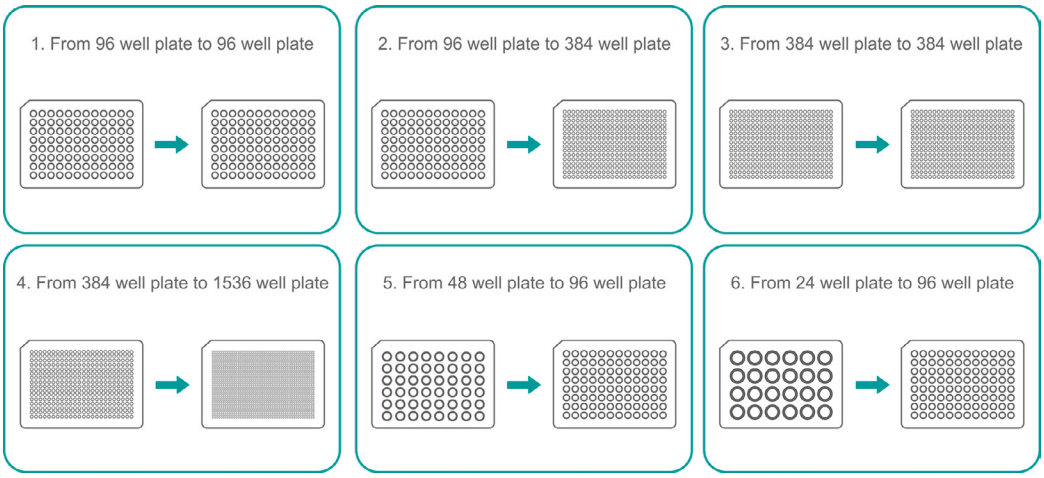


Figure 1: Most common plate to plate transfer procedures.

Step-by-step procedure

1. 96 well plate replication

Transferring samples from one 96 well plate to another 96 well plate.

It is possible to replicate 96 well plates with all INTEGRA's electronic pipettes.

The MINI 96 portable electronic pipette, the VIAFLO 96 or VIAFLO 384 handheld electronic pipettes offer the easiest and fastest solution to replicate a 96 well plate. These instruments transfer 96 samples in 1 pipetting step.

The 12 channel VOYAGER and the VIAFLO electronic pipettes can be used – either manually or on the ASSIST PLUS pipetting robot – to transfer samples from a 96 well plate to a 96 well plate.

When working manually with the VOYAGER adjustable tip spacing pipette, it is important to set the right well to well distance:

- 26 mm for 12 well plates
- 19 mm for 24 well plates
- 14 mm for 48 well plates
- 9 mm for 96 well plates
- 4.5 mm for 384 well plates.

This can be done by selecting 'Tip spacing' in the main menu of the pipette, followed by choosing 'Position 2', and setting the tip spacing accordingly. Once saved, the tip spacing is available at any time.

Pairing the VIAFLO or VOYAGER electronic pipettes with the ASSIST PLUS pipetting robot allows a hands-free replication process.

2. Transfer from 96 well plates to 384 well plate

Transferring samples from 96 well plates to a 384 well plate.

It is possible to transfer samples from 96 well plates to a 384 well plate with all INTEGRA's electronic pipettes. For example, the VOYAGER 8 and 12 channel electronic pipettes can be used, or they can be paired with the ASSIST PLUS pipetting robot.

When deciding which instrument to choose for sample transfer from four 96 well plates to a single 384 well plate, the sample order in the destination plate should be considered (see **Figure 2**). The sample order from the source plates can easily be maintained when transferring the samples with a VOYAGER (with or without the ASSIST PLUS), but using the VIAFLO 96 or the MINI 96 will change the sample order.

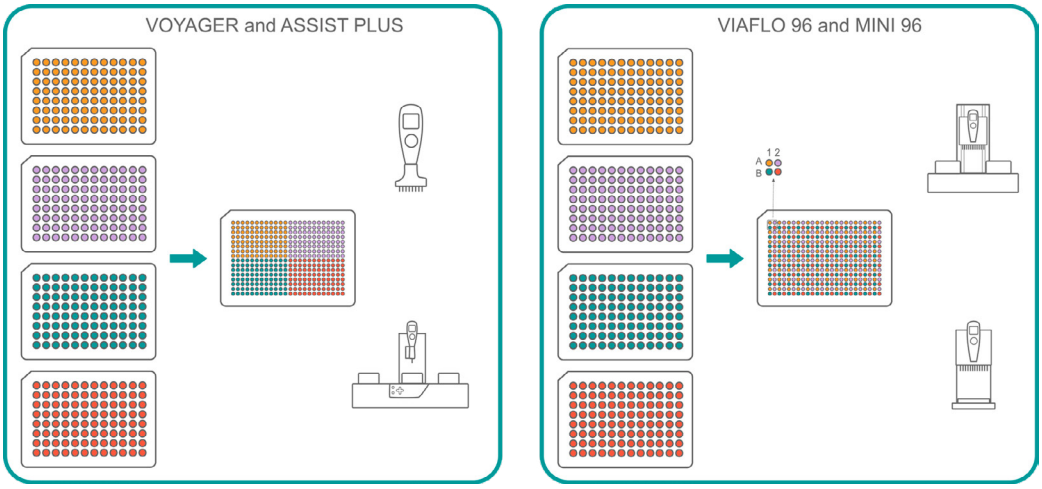


Figure 2: Sample transfer from four 96 well plates to one 384 well plate with the VOYAGER, ASSIST PLUS, VIAFLO 96 and MINI 96.

3. 384 well plate replication

Transferring samples from one 384 well plate to another 384 well plate.

Replication of 384 well plates is possible with all INTEGRA's electronic pipettes.

The fastest option is the VIAFLO 384 handheld electronic pipette, which allows the transfer of 384 samples in one go. Alternatively, the VIAFLO 96 handheld electronic pipette enables sample transfer in 4 steps.

On the ASSIST PLUS pipetting robot, the 12 channel VOYAGER or 16 channel VIAFLO electronic pipettes are the fastest options. The 384 well plates should be placed in a portrait orientation on the deck when using the 12 channel VOYAGER, or in a landscape orientation when using the 16 channel VIAFLO **(Figure 3)**.

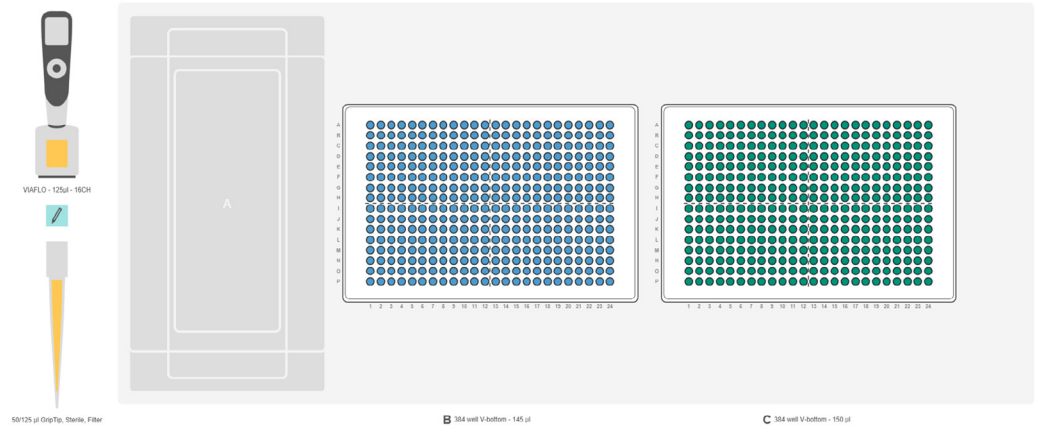


Figure 3: 384 well plate replication with the ASSIST PLUS pipetting robot and 16 channel VIAFLO electronic pipette.

4. Transfer from 384 well plates to 1536 well plates

Transferring samples from 384 well plates to a 1536 well plate.

The VIAFLO 384 is the ideal instrument to transfer samples from 384 well plates to a 1536 well plate. However, the resulting sample order in the destination plate should be taken into account (see **Figure 4** and **Figure 5**).

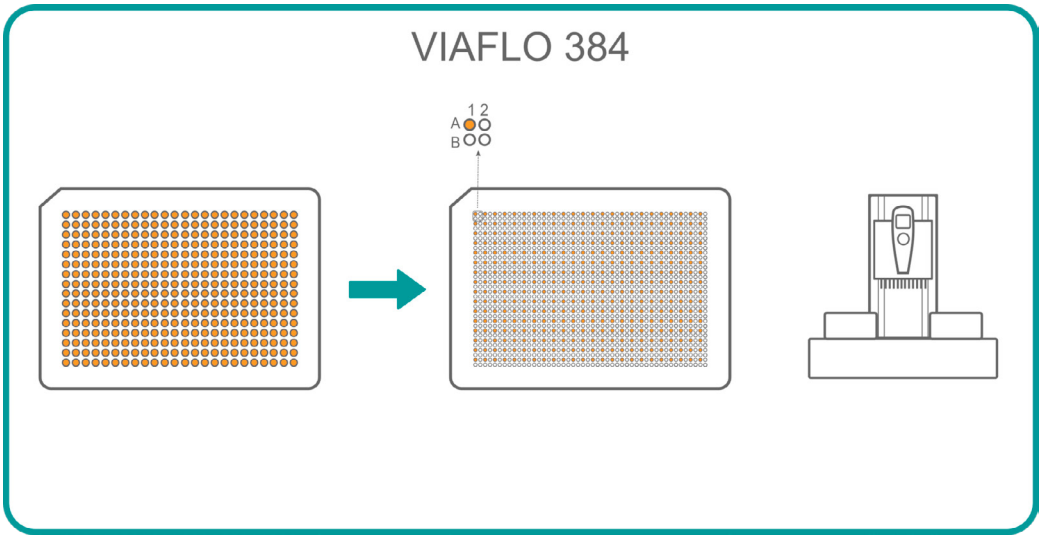


Figure 4: Sample transfer from a 384 well plate to a 1536 well plate with the VIAFLO 384.

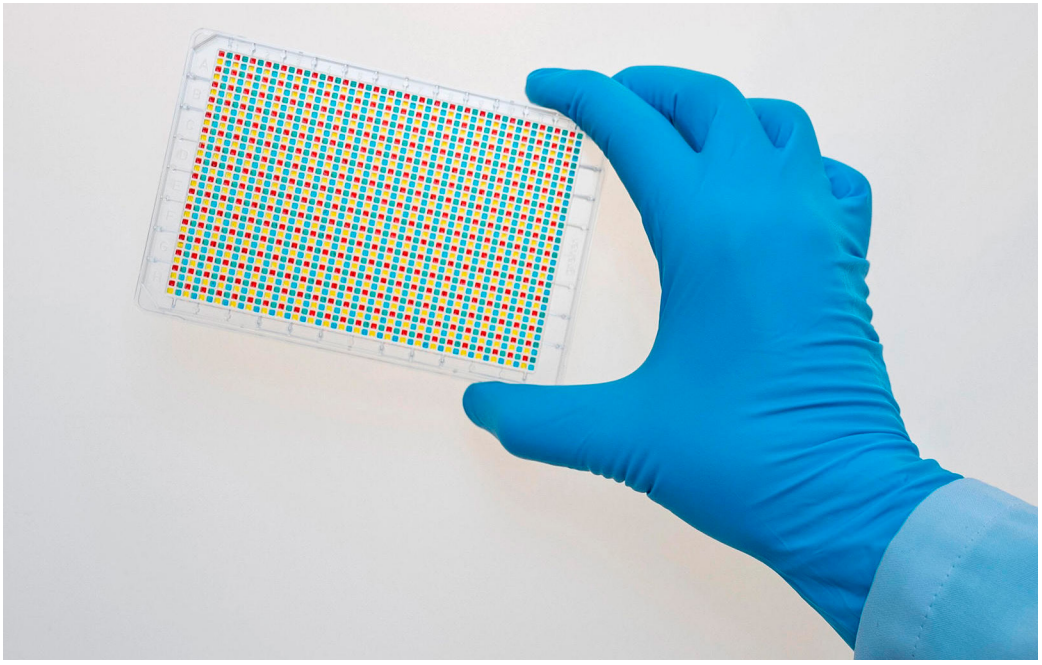


Figure 5: Result of pipetting samples from four 384 well plates to a 1536 well plate with the VIAFLO 384.

5. Transfer from 24 well plates to 96 well plate

Transferring samples from 24 well plates to a 96 well plate.

Transferring samples from 24 well plates to a 96 well plate can be performed manually with the VOYAGER adjustable tip spacing pipette or can be automated by pairing the VOYAGER with the ASSIST PLUS pipetting robot. Alternatively, the VIAFLO 96 and VIAFLO 384 handheld electronic pipettes can be equipped with a 24 channel pipetting head.

The VOYAGER 6 channel electronic pipettes can be used to transfer samples from a 24 well plate to a 96 well plate and, when using the ASSIST PLUS, the right plate orientation is very important. The 24 and the 96 well plates should be placed in portrait orientation on the deck **(Figure 6)**.

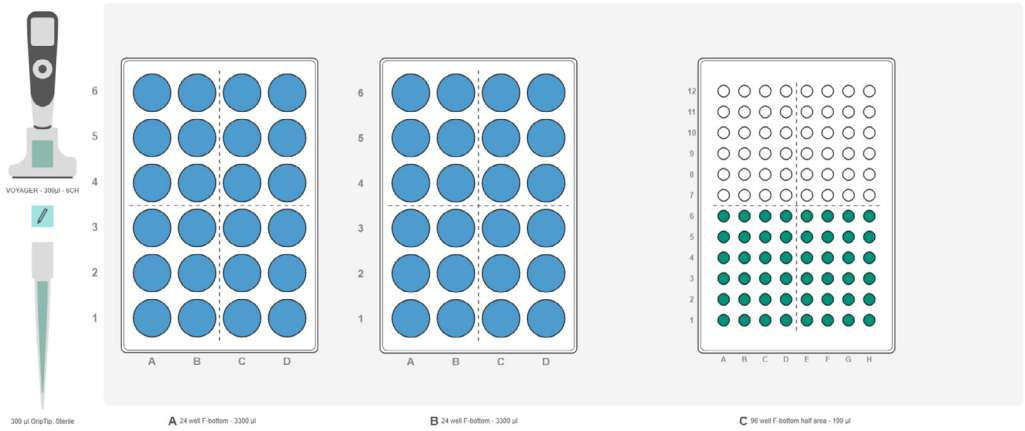


Figure 6: Sample transfer from two 24 well source plates (blue wells) to a 96 well destination plate (green wells) with the ASSIST PLUS pipetting robot.

When a VIAFLO 96 or VIAFLO 384 with a 24 channel pipetting head is used to transfer samples from 24 well plates to a 96 well plate, the user should be aware of the sample order in the destination plate (see **Figure 7**).

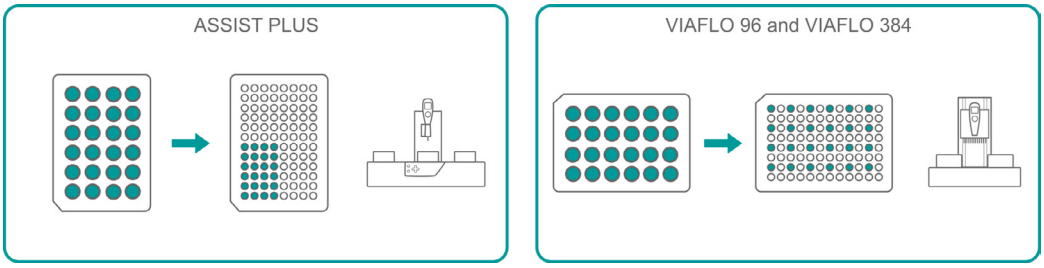


Figure 7: Sample transfer from a 24 well plate to a 96 well plate with either the ASSIST PLUS pipetting robot or the VIAFLO 96 and VIAFLO 384 handheld electronic pipettes.

6. Processing 48 well plates

Transferring samples from 48 well plates.

Transferring samples from 48 well plates to 12, 24, 48, 96 or 384 well plates can be performed manually with a VOYAGER adjustable tip spacing pipette, or automatically by using the VOYAGER in combination with an ASSIST PLUS pipetting robot.

Depending on the target plate, 4, 6 or 8 channel VOYAGER pipettes can be used to transfer samples from 48 well plates.

When the ASSIST PLUS pipetting robot is used for transfers from a 48 well plate to 12 well plates, both plates should be in portrait format. For transfers from a 48 well plate to 24 well plates, the 48 well plate should be in landscape orientation, while the 24 well plate should be in portrait. When a 48 well plate is replicated, both plates should be in portrait orientation. For transfers from a 48 well plate to a 96 or 384 well plate, the 48 well plate has to be in portrait orientation, and the 96 or 384 well plate in landscape **(Figure 8)**.

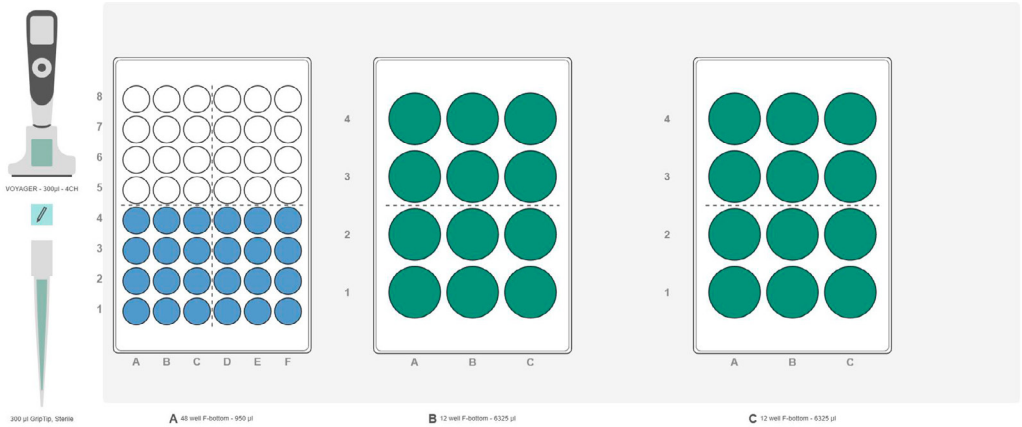


Figure 8a: Sample transfer from a 48 well source plate (blue) to two 12 well destination plates (green) with the ASSIST PLUS pipetting robot.

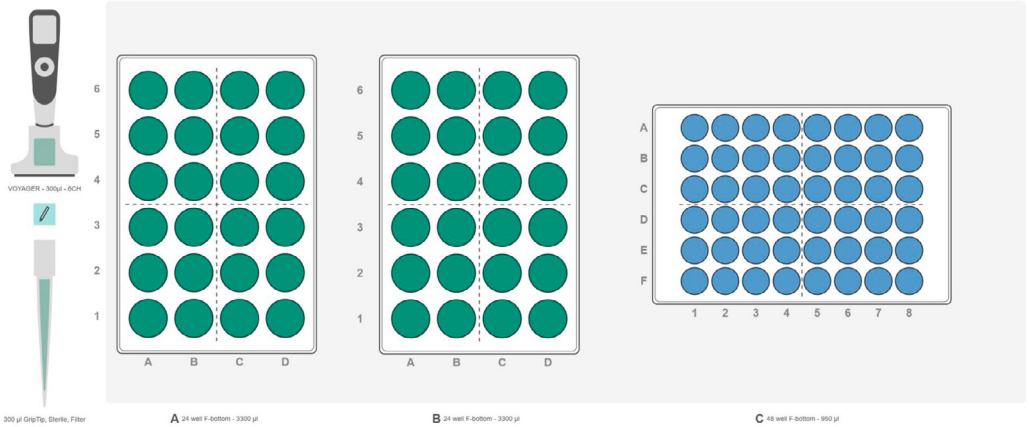


Figure 8b: Sample transfer from a 48 well source plate (blue) to two 24 well destination plates (green) with the ASSIST PLUS pipetting robot.

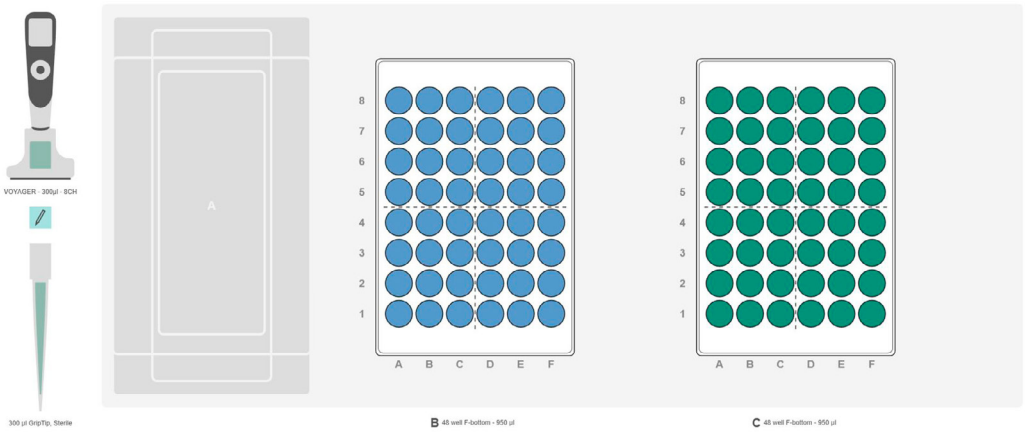


Figure 8c: Sample transfer from a 48 well source plate (blue) to another 48 well destination plate (green) with the ASSIST PLUS pipetting robot.

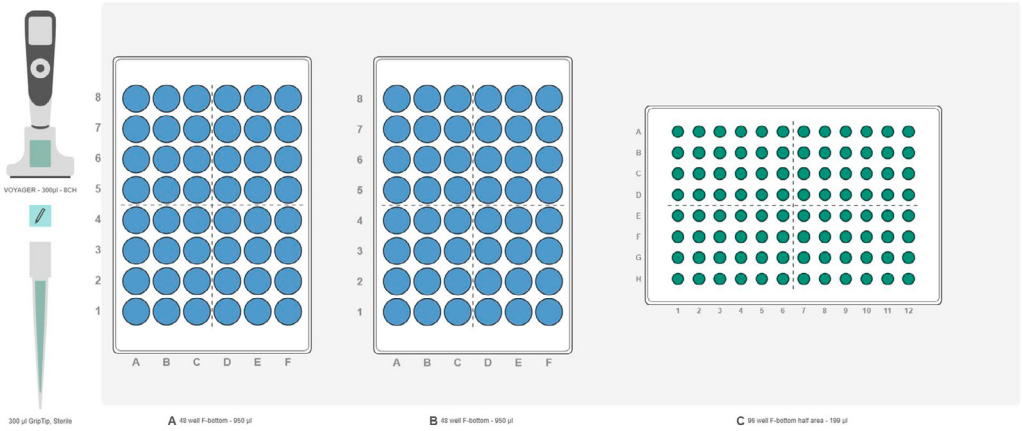


Figure 8d: Sample transfer from two 48 well source plates (blue) to a 96 well destination plate (green) with the ASSIST PLUS pipetting robot.

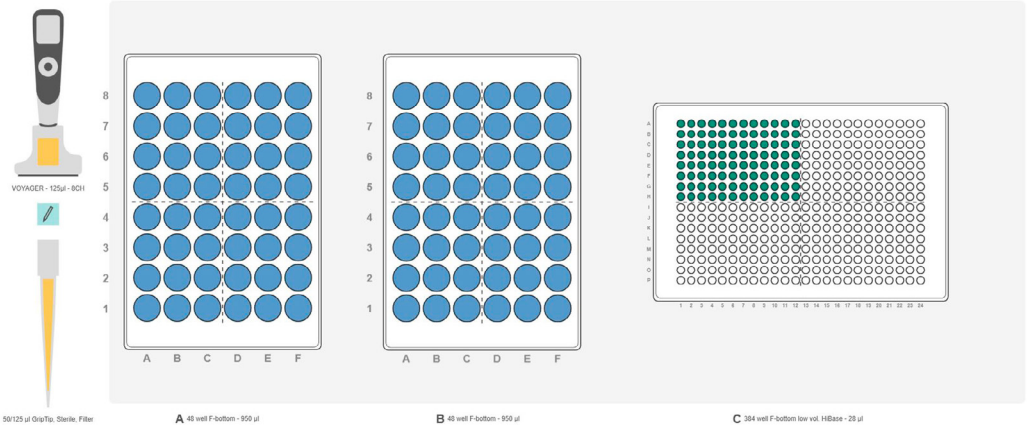


Figure 8e: Sample transfer from two 48 well source plates (blue) to a 384 well destination plate (green) with the ASSIST PLUS pipetting robot.

7. 24 well plate replication

Transferring samples from one 24 well plate to another 24 well plate.

Replicating a 24 well plate is possible with the VOYAGER adjustable tip spacing pipettes, an ASSIST PLUS pipetting robot paired with a VOYAGER pipette, or with the VIAFLO 96 and VIAFLO 384 handheld electronic pipettes equipped with a 24 channel pipetting head.

A 4 or 6 channel VOYAGER pipette can be used for the transfer. When the sample transfer is automated with the ASSIST PLUS pipetting robot, the plates have to be in landscape orientation if a 4 channel VOYAGER pipette is used, whereas a 6 channel VOYAGER pipette requires plates to be placed in portrait.

For processing of a full 24 well plate in one go, the VIAFLO 96 and VIAFLO 384 handheld electronic pipettes can be used with a 24 channel pipetting head **(Figure 9)** and 24 channel plate holders. With this set-up, it is possible to load GRIPTIPS® from a tip box 4 times. Always check the well to well distance of the 24 well plate, and align the pipetting head according to your plate.

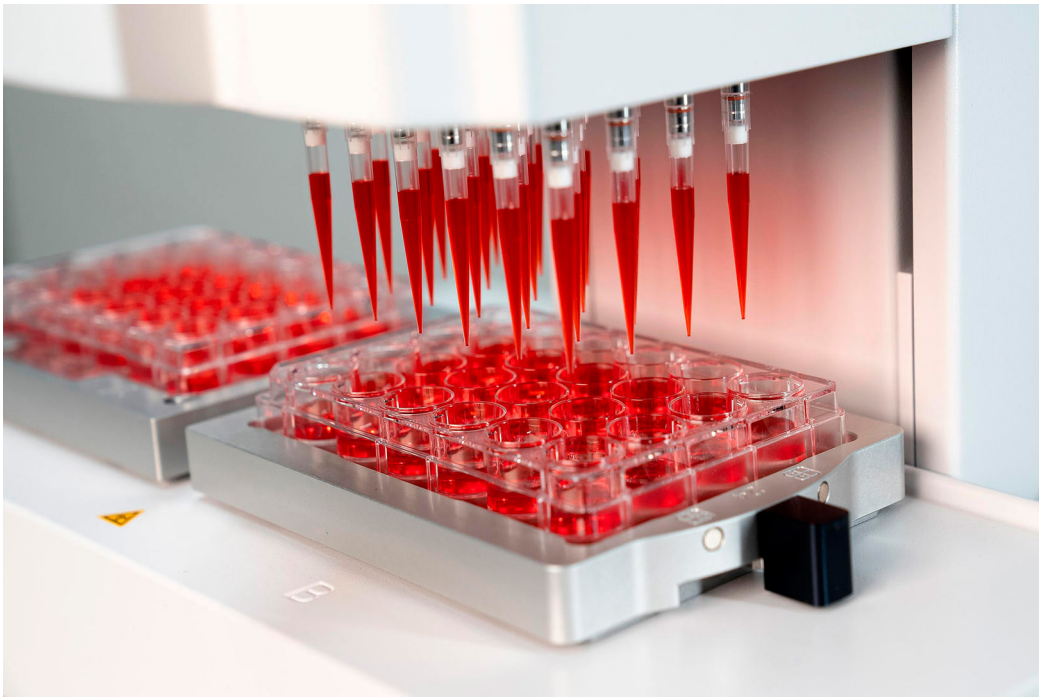


Figure 9: Sample transfer between 24 well plates with the VIAFLO 96 handheld electronic pipette equipped with a 24 channel pipetting head.

8. Processing of 12 well plates

Transferring samples from 12 well plates.

Transferring samples manually from 12 well plates to 12, 24, 48 or 96 well plates is possible with a VOYAGER adjustable tip spacing pipette, or automatically with the ASSIST PLUS pipetting robot equipped with a VOYAGER pipette.

For transferring samples from a 12 well plate to any other plate format, a 4 channel VOYAGER electronic pipette is required **(Figure 10)**.

Figure 10: Sample transfer from a 12 well plate to a 24 well plate with a 4 channel 1250 µl VOYAGER adjustable tip spacing pipette.

When using the ASSIST PLUS pipetting robot, the 12 well plate has to be placed on the deck in portrait orientation **(Figure 11)**. 12 well plate to 24 or 96 well plate transfers require the destination plate to be in landscape orientation, whereas 12 well plate to 48 well plate transfers require the 48 well plate to be in portrait. Two 12 well plates can be processed without any manual intervention, giving more walk-away time.

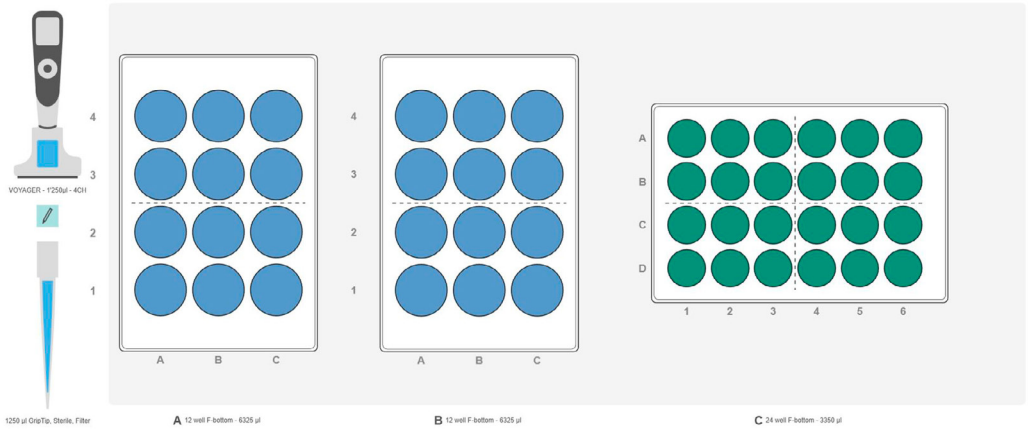


Figure 11a: Sample transfer from two 12 well source plates (blue) to a 24 well destination plate (green) with the ASSIST PLUS pipetting robot.

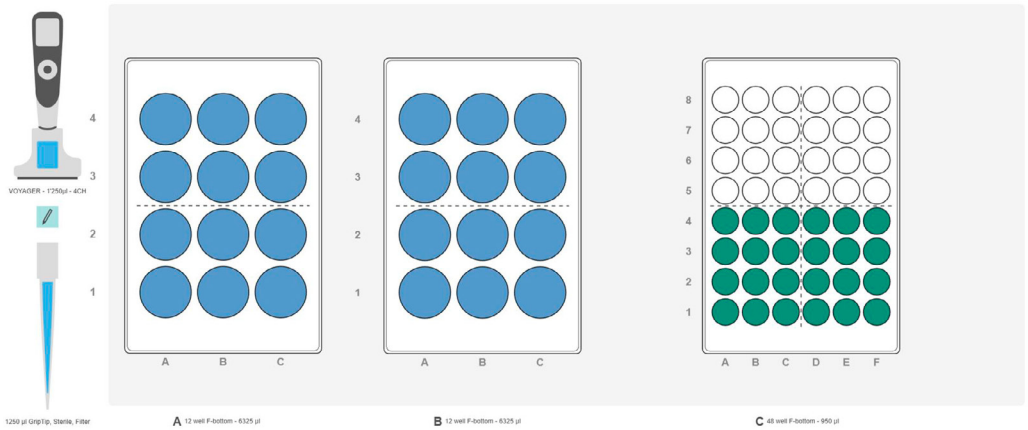


Figure 11b: Sample transfer from two 12 well source plates (blue) to a 48 well destination plate (green) with the ASSIST PLUS pipetting robot.

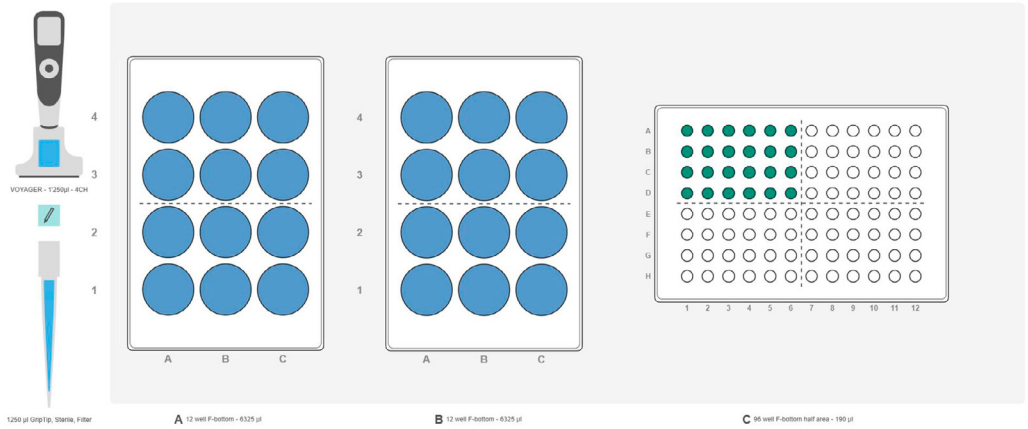


Figure 11c: Sample transfer from two 12 well source plates (blue) to a 96 well destination plate (green) with the ASSIST PLUS pipetting robot.

From / To well plates	≤ 12	24	48	96	384	1536
≤ 12	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS		
24	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS VIAFLO 96 VIAFLO 384	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS VIAFLO 96 VIAFLO 384	VOYAGER ASSIST PLUS	
48	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	
96	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS VIAFLO 96 VIAFLO 384	VOYAGER ASSIST PLUS	VIAFLO VOYAGER ASSIST PLUS MINI 96 VIAFLO 96 VIAFLO 384	VOYAGER ASSIST PLUS (MINI 96) VIAFLO 96 VIAFLO 384	
384	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS	VOYAGER ASSIST PLUS (MINI 96) VIAFLO 96 VIAFLO 384	VIAFLO VOYAGER ASSIST PLUS (MINI 96) VIAFLO 96 VIAFLO 384	(ASSIST PLUS) VIAFLO 384

Table 1: Summary of possible plate to plate transfers with INTEGRA electronic pipettes. Product names in black: handheld solutions. Product names in turquoise: automated solutions. Product names in orange: high-throughput solutions. Product names in brackets: it is possible to use them for the respective transfer but there are other solutions that are better suited.

Summary

- Sample transfers from plate to plate, even between different formats, is no longer a cumbersome task using INTEGRA's VOYAGER, ASSIST PLUS, MINI 96, VIAFLO 96 or VIAFLO 384.
- Reformatting has never been easier, quicker and more reproducible.
- Thanks to the compact footprint of the instruments, they can be placed in a laminar flow hood when sterile working conditions are required.
- If high throughput and fast pipetting is needed, the MINI 96, VIAFLO 96 and VIAFLO 384 are the best solutions. Alternatively, an ASSIST PLUS equipped with a VOYAGER pipette provides the most versatile, fully automated set-up for plate to plate reformatting.
- Sample transfers from and to all possible plate types is achievable. The VOYAGER adjustable tip spacing pipette is available with various numbers of channels, and in different volumes. The VIAFLO 384 handheld electronic pipette can be equipped with 24, 96 or 384 channel pipetting heads with different pipetting volumes, the VIAFLO 96 can be used with 24 and 96 channel pipetting heads, and the MINI 96 portable electronic pipette is available in 4 volume ranges.

For more information and a list of materials used, please refer to our website.

5.2 Fast and efficient automated sample transfer from tubes to plates with the ASSIST PLUS pipetting robot

Streamline your sample reformatting

When processing samples in tubes, transfers are often performed manually with a single channel pipette. This is time consuming, error prone and draining for the operator. The ASSIST PLUS pipetting robot – in combination with a VOYAGER adjustable tip spacing pipette – provides a novel solution for accurate and efficient transfer of liquids to and from a variety of tube types. This unique pipetting robot provides all the benefits of the VOYAGER's adjustable tip spacing – reformatting samples from tube to plate up to 12 times faster compared to using a single channel pipette – as part of a walk-away, automated protocol.

Key benefits

- When using the ASSIST PLUS, the right sample is always transferred to the right well, eliminating the chance of human errors.
- INTEGRA's extensive range of tube racks, with the option to place labware in either landscape and portrait orientations, ensures the highest degree of versatility for sample transfers.
- Automated transfer of hazardous samples using the ASSIST PLUS protects operators from potential exposure to harmful substances, while extending walk-away times.
- The VOYAGER adjustable tip spacing pipette can be equipped with 300 µl long GRIPTIPS® to avoid the risk of cross contamination by touching the wall of long tubes.

Overview: How to transfer samples from tubes faster

In this application note, we show 6 examples of different sample transfers from either tubes to a plate, or from tubes to tubes (See **Figure 1**):

- Transfer from 0.5 ml microcentrifuge tubes to a 96 well plate.
- Transfer from 1.5 ml microcentrifuge tubes to HPLC vials.
- Transfer from cryogenic vials to a 96 flat bottom plate.
- Transfer from swab tubes to 96 deep well plate.
- Transfer from test tubes to 96 deep well plate.
- Transfer from 15 ml centrifuge tubes to 96 deep well plate.

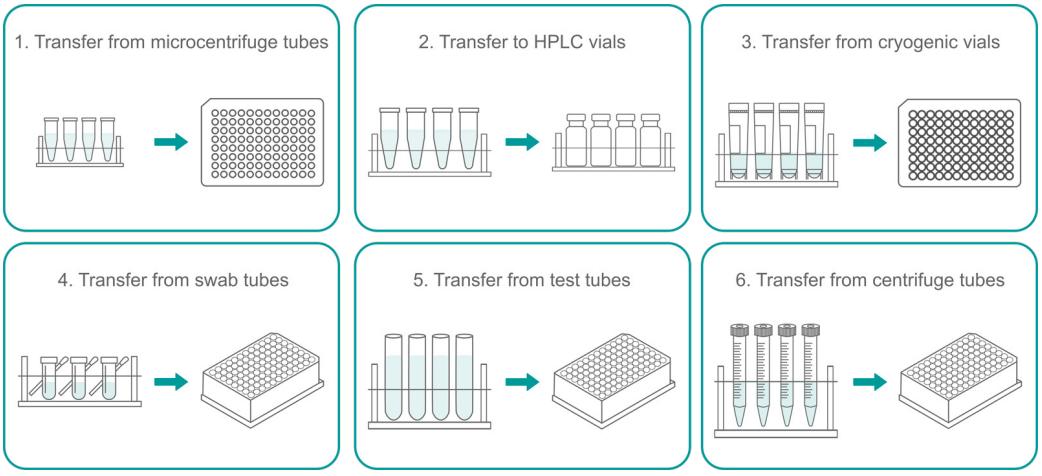


Figure 1: 6 example sample transfer procedures.

Step-by-step procedure

1. Transfer from microcentrifuge tubes

Sample transfer for qPCR analysis from 0.5 ml microcentrifuge tubes to a 96 well PCR plate placed on a cooling block.

Place the INTEGRA rack for 0.5 ml microcentrifuge tubes on deck position B, and load with microcentrifuge tubes containing the samples for qPCR (**Figure 2**, blue). Next, place the INTEGRA cooling block on position C, and place a 96 well PCR plate with the qPCR master mixes onto the cooling block (**Figure 2**, green).

Select and run the VIALAB program 'Transfer_from_microcentrifuge_tubes' using an 8 channel 12.5 µl VOYAGER pipette equipped with 12.5 µl sterile filter GRIPTIPS. The pipette transfers eight 2.5 µl samples to the PCR plate in parallel. A 0.5 µl pre- and post-dispense ensures precision while pipetting, and the tips are automatically changed after each dispense to avoid cross contamination.

After completing the 6th dispense step, the ASSIST PLUS informs the operator to change the sample rack. With 6 further aspirations and dispenses, sample transfer into the 96 well PCR plate is complete.

Tip:

The procedure parameters are easily modified in the VIALAB software as required. If the sample volume for qPCR is different, this can also be set up rapidly with ease.

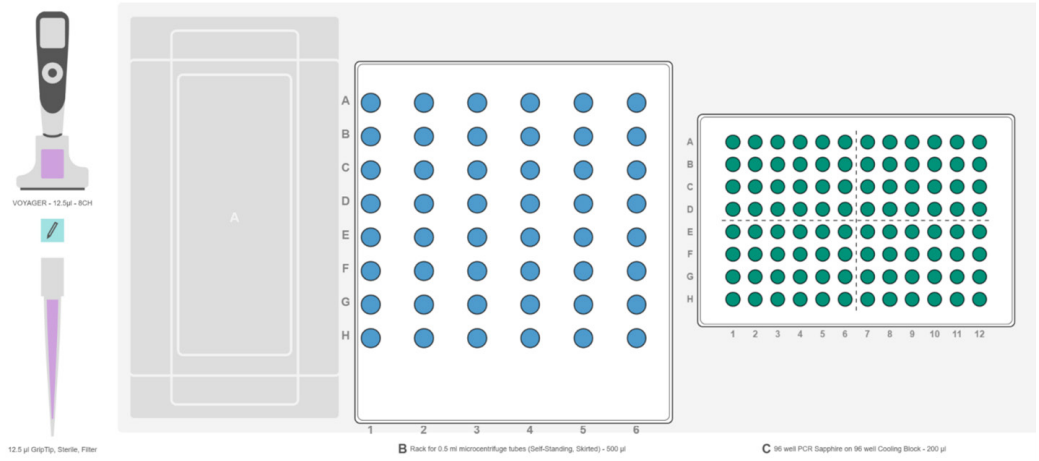


Figure 2: Deck set-up for sample transfer from 0.5 ml microcentrifuge tubes to a 96 well PCR plate placed on a cooling block. A: Empty. B: INTEGRA rack for 0.5 ml microcentrifuge tubes (blue). C: 96 well PCR plate placed on INTEGRA cooling block (green).

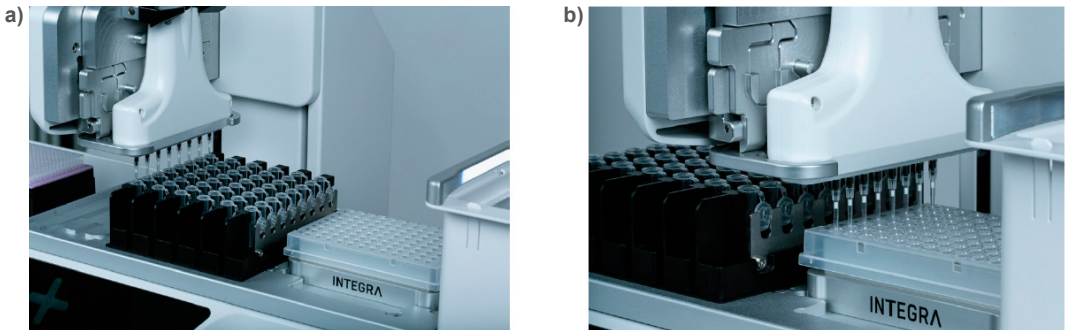


Figure 3: Sample transfer from **a)** 0.5 ml microcentrifuge tubes to **b)** a 96 well PCR plate.

2. Transfer to HPLC vials

Sample transfer from 1.5 ml microcentrifuge tubes to HPLC vials.

Place the INTEGRA rack for 1.5/2 ml microcentrifuge tubes on deck position B, then fill with sample tubes (**Figure 4**, blue). Next, place the INTEGRA rack for HPLC tubes on deck position C (**Figure 4**, magenta).

Select the VIALAB program 'Transfer_to_HPLC_vials' and press Run. Using an 8 channel 300 µl VOYAGER pipette with 300 µl sterile filter GRIPTIPS, the system transfers 200 µl samples from the microcentrifuge tubes to the HPLC tubes for analysis. A 15 µl pre- and post-dispense step is used to increase the accuracy and precision of pipetting.

Tip:

The pre- and post-dispense volume should be 3-5 % of the nominal pipette tip volume.

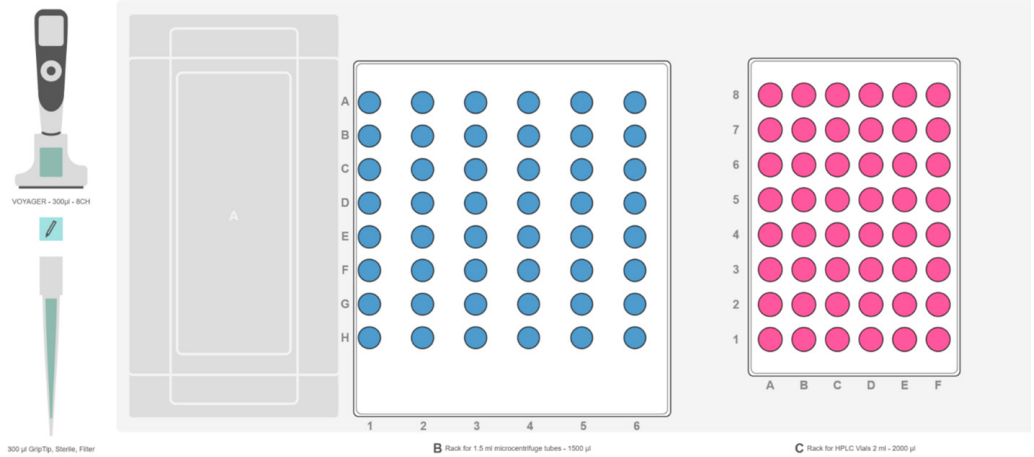


Figure 4: Set-up for sample transfer from 1.5 ml microcentrifuge tubes to HPLC vials. Position A: Empty. Position B: INTEGRA rack for 1.5/2 ml microcentrifuge tubes (blue). Position C: INTEGRA rack for HPLC tubes (magenta).

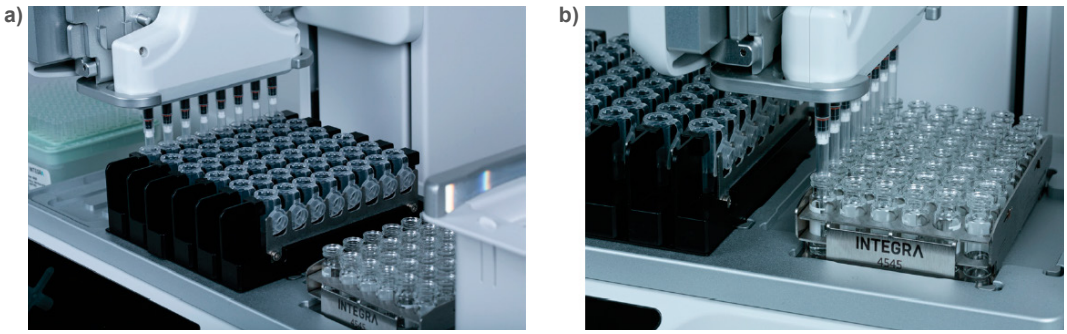


Figure 5: Sample transfer from **a)** 1.5 ml microcentrifuge tubes to **b)** HPLC tubes with a VOYAGER pipette.

3. Transfer from cryogenic vials

Sample transfer from cryogenic vials to a 96 well flat bottom plate.

Place INTEGRA cryogenic vial racks containing the samples to be transferred for analysis on deck positions A and B (**Figure 6**, blue), then set a 96 well flat bottom plate on position C (**Figure 6**, magenta).

Select and run the VIALAB program 'Transfer from cryogenic vials' on the pipette. An 8 channel 300 µl VOYAGER pipette fitted with 300 µl sterile filter GRIPTIPS then transfers 200 µl samples from cryogenic tubes to 96 well flat bottom plate, changing the tips after each transfer. A 15 µl pre- and post-dispense step is included to increase the accuracy and precision of pipetting.

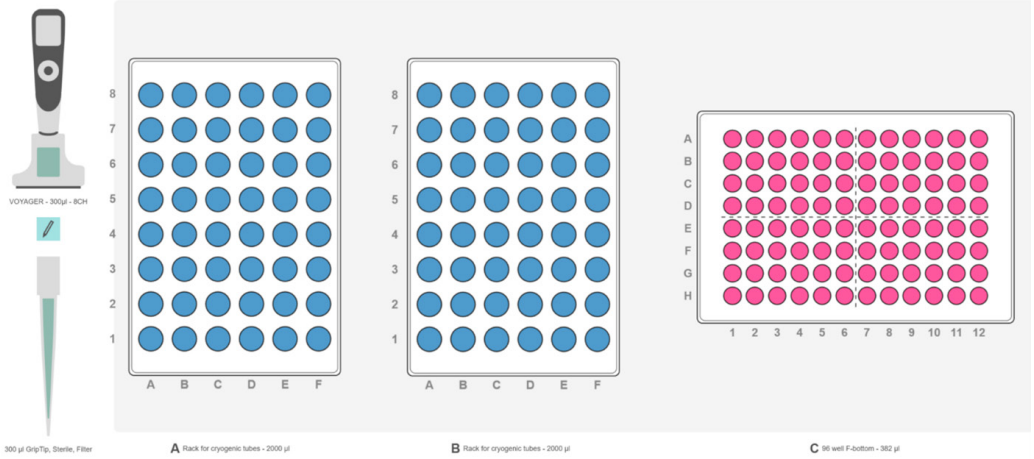


Figure 6: Example set-up for sample transfer from 96 cryogenic vials to a 96 well flat bottom plate. Position A: INTEGRA rack for cryogenic tubes (blue). Position B: INTEGRA rack for cryogenic tubes (blue). Position C: 96 well flat bottom plate (magenta).

a) b)

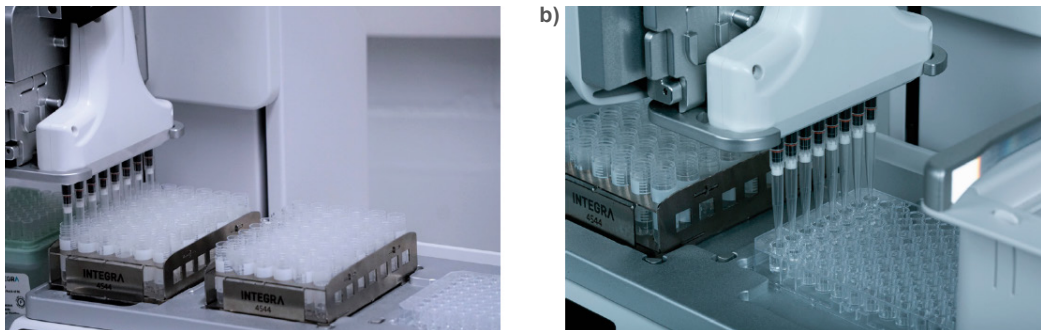


Figure 7: Sample transfer from a**)** cryogenic tubes to **b)** a 96 well flat bottom plate with VOYAGER pipette.

4. Transfer from swab tubes

Sample transfer from swab tubes, to 96 deep well plate.

First, place an INTEGRA swab tube rack containing the patient's samples on deck position B (**Figure 8**, blue) and an empty 96 deep well plate on deck position C (**Figure 8**, magenta).

Select and run the VIALAB program 'Transfer_from_swab_tubes'. A 6 channel 1250 µl VOYAGER pipette with 1250 µl sterile filter GRIPTIPS aspirates 1000 µl sample from the swab tubes, then dispenses into the 96 deep well plate. Tips are automatically changed between samples to avoid cross contamination. A 25 µl pre- and post-dispense is also set to ensure accurate and precise liquid handling, even if the preservation medium tends to form bubbles during pipetting. After filling the first 6 columns of the 96 deep well plate with samples, the pipette informs the operator to change the swab tube rack. The VOYAGER then continues to fill the second half of the 96 deep well plate with samples.

Tip:

A 25 µl air gap has been set at the end of the aspiration to eliminate the risk of cross contamination.

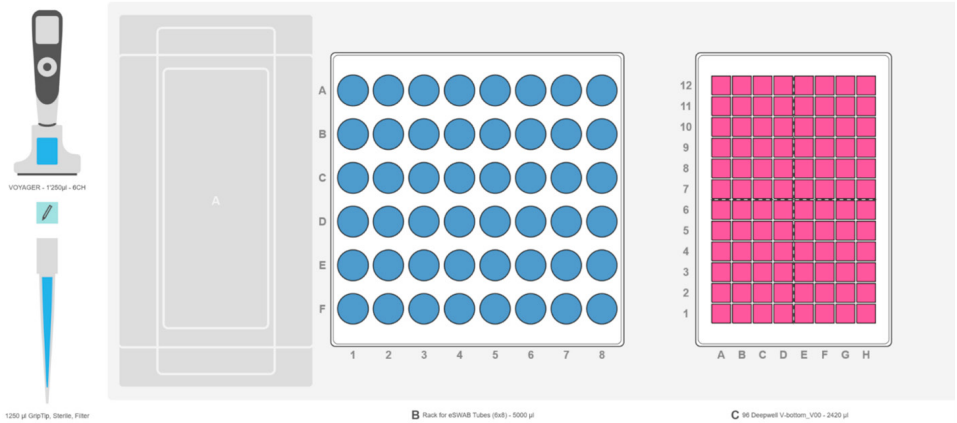


Figure 8: Deck set-up for sample transfer from swab tubes to 96 deep well plate. Position A: Empty. Position B: INTEGRA rack for swab tubes. Position C: 96 deep well plate.

a) b)

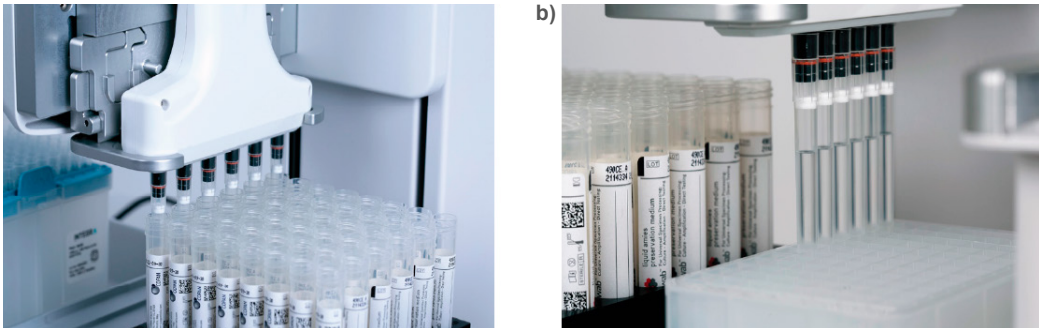


Figure 9: Sample transfer from **a)** swab tubes to **b)** a 96 deep well plate.

5. Transfer from test tubes

Sample transfer from 5 ml test tubes to a 96 deep well plate.

First, put 2 INTEGRA 5 ml test tube racks with samples on deck positions A and B (**Figure 10**, blue), and an empty 96 deep well plate on position C (**Figure 10**, magenta). For this application, use an 8 channel 1250 µl VOYAGER pipette with 1250 µl sterile filter GRIPTIPS.

Select the custom VIALAB program 'Transfer_from_test_tubes' on the pipette, and press Run. The pipette transfers 1000 µl of each sample from the test tubes to the 96 deep well plate after mixing 3 times.

Tip:

Proper mixing of samples prior to transfer can be critical for certain sample types. The mixing volume, speed and number of cycles can be easily set up in the VIALAB program.

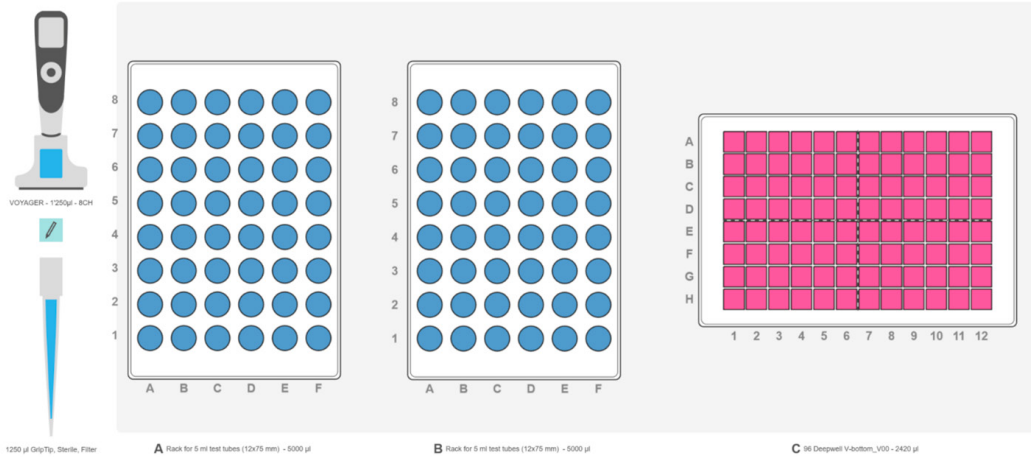


Figure 10: Deck set-up for sample transfer from 5 ml test tubes, to a 96 deep well plate. Position A: INTEGRA rack for 5 ml test tubes (blue). Position B: INTEGRA rack for 5 ml test tubes (blue). Position C: 96 deep well plate (magenta).

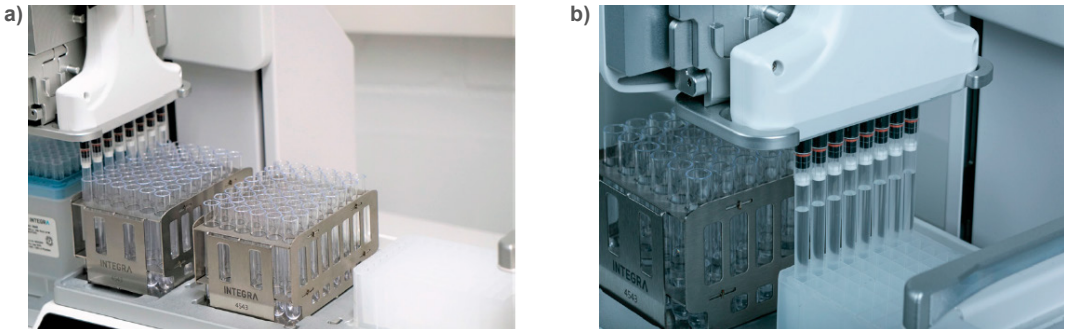


Figure 11: Sample transfer from **a)** 5 ml test tubes to **b)** a 96 deep well plate.

6. Transfer from centrifuge tubes

Sample transfer from 15 ml centrifuge tubes to a 96 deep well plate.

Equip the ASSIST PLUS with a 6 channel 1250 µl VOYAGER pipette using 1250 µl sterile filter GRIPTIPS. Place an INTEGRA 15 ml centrifuge tube rack on deck position B (**Figure 12**, blue), and load all the sample tubes into the rack. Place an empty 96 deep well plate, in portrait orientation, on deck position C (**Figure 12**, magenta).

Choose and run the VIALAB program 'Transfer_from_centrifuge_tubes' on the VOYAGER pipette. Samples in the centrifuge tubes are mixed 3 times, then 1000 µl of each sample is transferred to the deep well plate.

Tip:

The tip travel function is used during the mixing steps to optimize tip immersion depth.

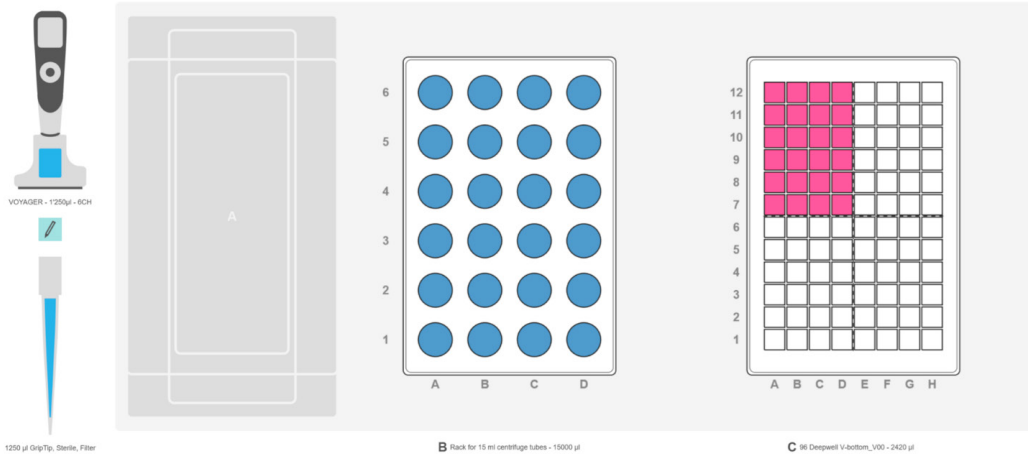


Figure 12: Set-up for sample transfer from 15 ml centrifuge tubes to a 96 deep well plate. Position A: Empty. Position B: INTEGRA rack for 15 ml centrifuge tubes (blue). Position C: 96 deep well plate (magenta).

a) b)

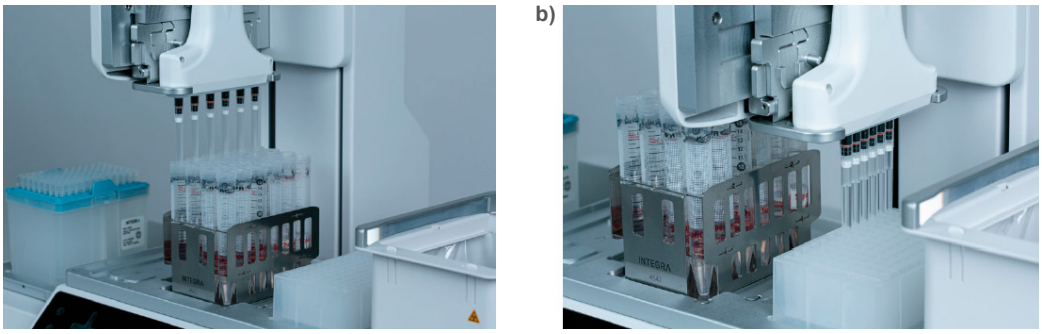


Figure 13: Sample transfer from **a)** 15 ml centrifuge tubes to **b)** a 96 deep well plate.

Remarks

VIALAB software

The VIALAB programs can be easily adapted to your specific protocols.

Partial plates

Programs can be easily adapted to accommodate different number of samples, providing complete versatility to meet current and future laboratory workflow demands.

Summary

- The ASSIST PLUS pipetting robot, equipped with a VOYAGER adjustable tip spacing pipette, is the perfect solution to precisely transfer samples to and from different tube types, without the risk of transcription errors.
- The VOYAGER adjustable tip spacing pipette enables processing of multiple tubes in parallel, and is available with various channel options and volumes. This allows the user to pipette to the tube or plate of their choice, speeding up the transfers.
- The preprogrammed automated protocols provide simple, fast and ready-to-use solutions for 6 possible sample transfer processes to maximize walk-away time.
- If sterile conditions are required during sample transfer, the compact overall size and footprint of the ASSIST PLUS mean it can be placed in a laminar flow hood.
- The ASSIST PLUS is indispensable for users wishing to increase their throughput in sample processing.

For more information, a list of materials used and VIALAB programs, please refer to our website.

5.3 Automated reagent and sample aliquoting with the ASSIST PLUS pipetting robot

Aliquoting samples made easy

Aliquoting is a routine pipetting procedure that refers to the transfer of one or multiple portion(s) of a liquid to a different vessel. It is a useful technique to create space-saving back-up plates of primary tubes, or to split expensive reagents into smaller volumes to increase overall open vial stability. When done manually, aliquoting can be time consuming and error prone. Combining the ASSIST PLUS pipetting robot with the VOYAGER adjustable tip spacing pipette or D-ONE single channel pipetting module provides unique solutions for the user. With the VOYAGER, the pipetting robot offers adjustable tip spacing when aliquoting between different types of labware, while the D-ONE pipetting module guarantees precise aliquoting from a single tube.

Key benefits

- The ASSIST PLUS pipetting robot ensures accurate sample transfers to help prevent human errors.
- Using the VOYAGER adjustable tip spacing pipette to aliquot samples between different labware formats in high throughput makes the process simple and easy.
- Reagent aliquoting from tubes provided in a kit saves money and time when using the D-ONE single channel pipetting module.
- The operator gains more walk-away time and is protected from exposure to harmful substances by using the ASSIST PLUS pipetting robot.

Overview: How to aliquot reagents and samples faster

In this application note, we show 6 examples of reagent and sample aliquoting into different labware types (**Figure 1** shows single and multiple aliquoting):

- Sample (from swab tubes) aliquoting for space-saving, long-term storage
- Aliquoting of compounds dissolved in DMSO for cryogenic storage
- Purified nucleic acid aliquoting from deep well extraction plate
- Sample (bronchial lavage, urine, etc.) splitting for biobanking
- Reagent (primers, probes, etc.) splitting to increase overall open vial stability
- Aliquoting reagents (buffers, master mixes, etc.) for kit preparation

Experimental set-up

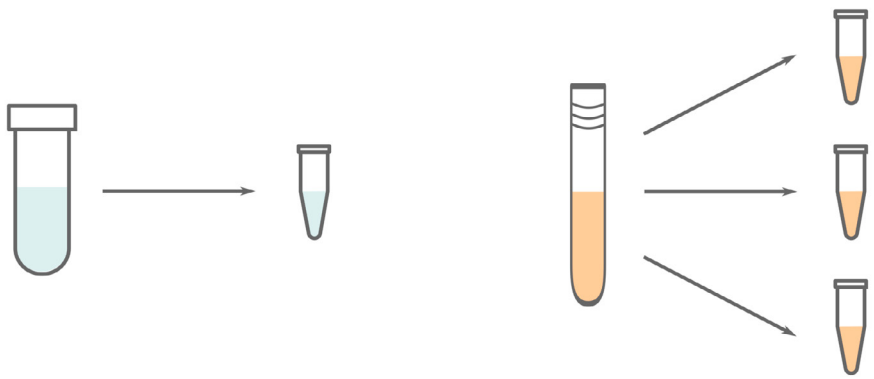


Figure 1: Illustration of a single aliquoting versus a multiple aliquoting procedure.

Step-by-step procedure

1. Sample (from swab tubes) aliquoting for space-saving, long-term storage

Aliquoting from swab tubes to a 96 deep well plate.

Place an INTEGRA rack for swab tubes with patient samples on deck position B (**Figure 2**, blue) and a 96 deep well plate on deck position C (**Figure 2**, magenta).

Run the VIALAB program 'Aliquoting_from_swab_tubes'. The 6 channel 1250 µl VOYAGER adjustable tip spacing pipette with 1250 µl sterile, filter, wide bore GRIPTIPS® aspirates 1000 µl of each sample from the swab tubes (**Figure 3a**) and dispenses them to the 96 deep well plate (**Figure 3b**), automatically changing the tips between different samples. After filling up the first half of the 96 deep well plate, the pipette prompts the user to exchange the INTEGRA rack for swab tubes on deck position B (**Figure 2**, blue). After loading the next 48 swab tubes, the VOYAGER continues to fill up the second half of the 96 deep well plate.

Tips:

- A 50 µl air gap at the end of every aspiration step minimizes the risk of cross-contamination.
- Wide bore tips and slower aspiration/dispense speeds can be used when samples tend to be more viscous.

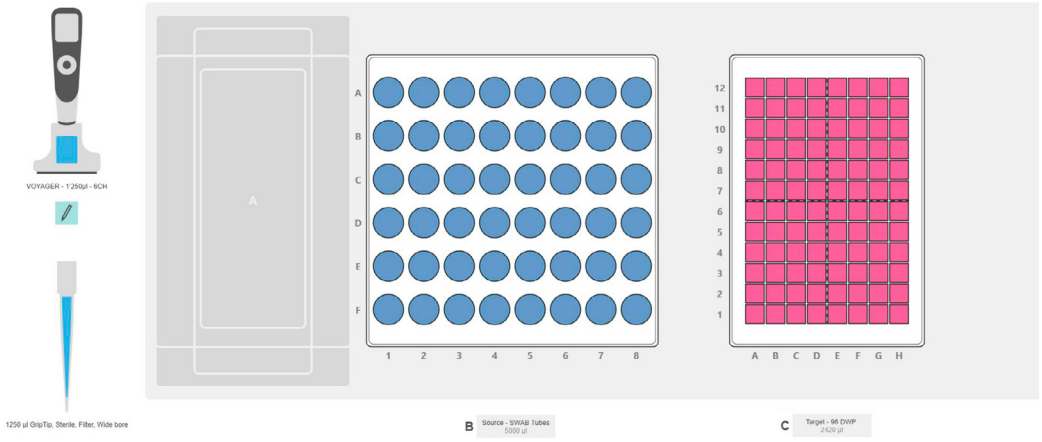


Figure 2: Deck set-up for sample transfer from swab tubes to 96 deep well plate. Position A: Empty. Position B: INTEGRA rack for swab tubes (blue). Position C: 96 deep well plate (magenta).

a) b)

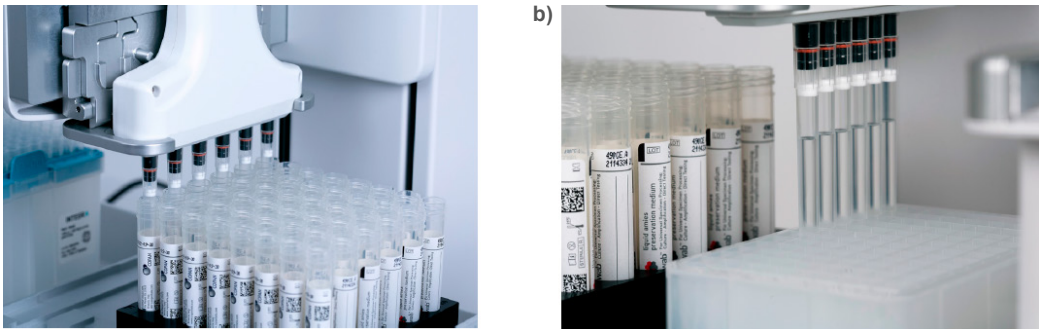


Figure 3: Sample transfer from **a)** swab tubes to **b)** 96 deep well plate with VOYAGER pipette.

2. Aliquoting of compounds dissolved in DMSO for cryogenic storage

Aliquoting from a flat bottom 96 well plate to cryogenic vials.

Place a flat bottom 96 well plate with samples on deck position B (**Figure 4**, blue) and an INTEGRA rack for cryogenic tubes on deck position C (**Figure 4,** magenta).

Run the VIALAB program 'Aliquoting_from_f-bottom_plate'. The 8 channel 300 µl VOYAGER pipette with 300 µl sterile, filter, low retention GRIPTIPS aspirates 200 µl of each sample from the flat bottom 96 well plate and dispenses them to 1.5 ml cryogenic vials. Tips are changed automatically between samples. The pipette will inform the user to change the INTEGRA rack for cryogenic vials on deck position C (**Figure 4**, magenta) when the first half of the 96 well plate has been aliquoted.

Tips:

- Setting an aspiration/dispense delay ensures that viscous liquid enter and exit the GRIPTIPS properly.
- Low retention GRIPTIPS and slower dispense speeds can be used to ensure the full volume of liquid is expelled.

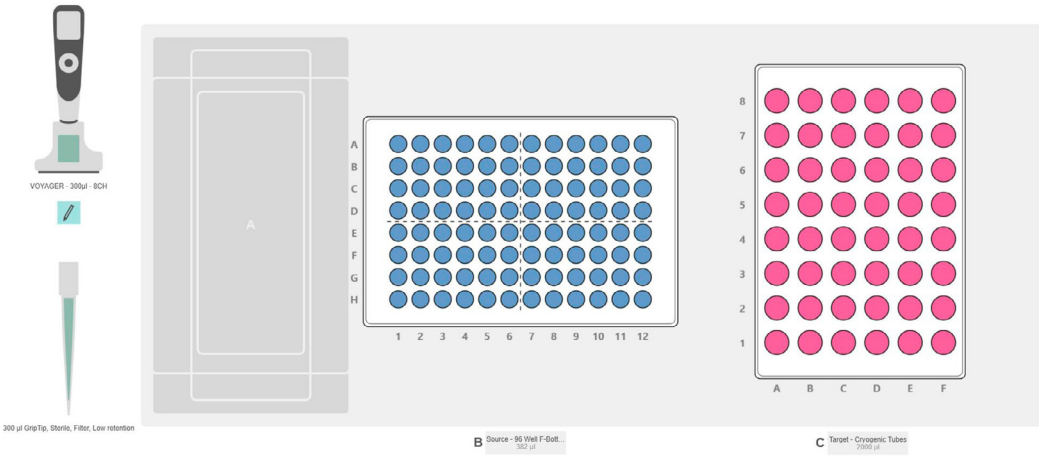


Figure 4: Set-up for sample transfer from flat bottom 96 well plate. Position A: Empty. Position B: flat-bottom 96 well plate (blue). Position C: INTEGRA rack for cryogenic tubes (magenta).

3. Purified nucleic acid aliquoting from deep well extraction plates

Aliquoting from deep well extraction plate to 1.5 ml microcentrifuge tubes.

Place a deep well extraction plate on deck position B (**Figure 5**, blue) and an INTEGRA rack for 1.5 ml microcentrifuge tubes on deck position C (**Figure 5**, magenta).

Run the VIALAB program 'Aliquoting_from_extraction_DWP'. The 8 channel 125 µl VOYAGER pipette with 125 µl sterile, filter GRIPTIPS aspirates 50 µl of each sample from the elution plate and dispenses them into 1.5 ml microcentrifuge tubes. Tips are changed automatically between samples. After half of the samples have been transferred, the pipette will inform the user to load a new INTEGRA rack with empty 1.5 ml microcentrifuge tubes on deck position C (**Figure 6**, magenta) and continue aliquoting.

Tip:

The procedure parameters can be easily modified in the VIALAB software. If the volume of the purified nucleic acid is different, this can be adjusted rapidly.

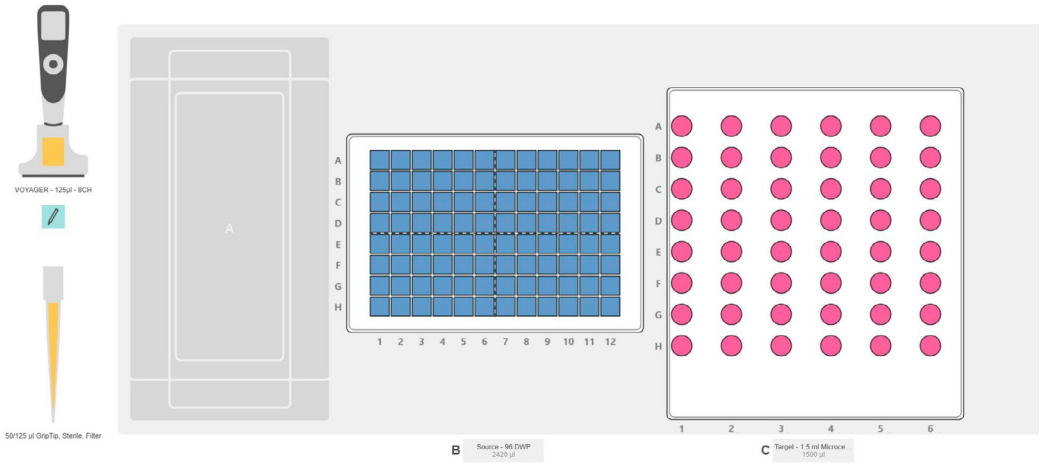


Figure 5: Deck set-up for purified nucleic acid transfer from deep well extraction plates. Position A: Empty. Position B: Deep well extraction plate (blue). Position C: INTEGRA rack for 1.5 ml microcentrifuge tubes (magenta).

4. Sample (bronchial lavage, urine, etc.) splitting for biobanking

Multiple sample aliquoting from centrifuge tubes to FluidX™ tubes.

Place an INTEGRA rack for centrifuge tubes with patient samples on deck position A (**Figure 6**, blue), and a FluidX 96-format tubes on positions B and C (**Figure 6**, magenta).

Run the VIALAB program 'Aliquoting_from_centrifuge_tube'. The 6 channel 1250 µl VOYAGER pipette with 1250 µl sterile, filter GRIPTIPS aspirates the samples from the centrifuge tubes, and dispenses 250 µl of each sample to FluidX tubes until every sample contains eight 250 µl aliquots, with automatic changing of tips between different samples.

Tip:

The repeat dispense function of an electronic pipette speeds up this process, making it 4 times faster than using a mechanical multichannel pipette.

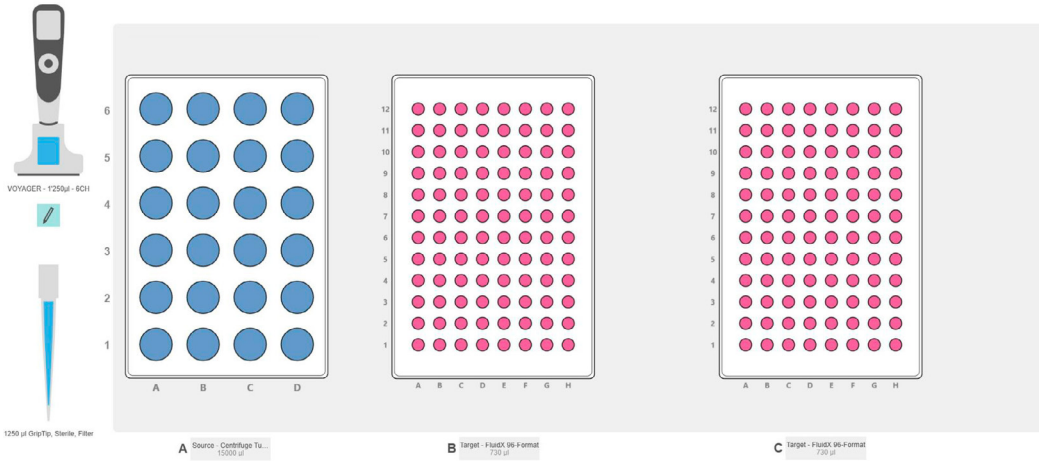


Figure 6: Deck set-up for sample aliquoting from centrifuge tubes to FluidX™ tubes. Position A: INTEGRA rack for centrifuge tubes. Position B: FluidX 96-format tubes (magenta). Position C: FluidX 96-format tubes (magenta).

5. Reagent (primers, probes, etc.) splitting to increase overall open vial stability

Multiple aliquoting from a 2 ml manufacturer's tube to 0.5 ml microcentrifuge tubes.

Place an INTEGRA rack for 2 ml tubes with the manufacturer's tube on position A1 on deck position B (**Figure 7**, blue) and an INTEGRA rack for 0.5 ml centrifuge tubes on deck position C (**Figure 7**, magenta).

Run the VIALAB program 'Aliquoting_from_manufacturer_tube'. The 5-1250 µl D-ONE pipetting module with 1250 µl sterile, filter GRIPTIPS aspirates the reagent from the manufacturer's tube and dispenses 50 µl into every 0.5 ml microcentrifuge tube **(Figure 8)**.

Tip:

This protocol is optimized for speed. If the reagent needs to be aliquoted at high precision, the tip type can be switched to the 125 µl GRIPTIPS in VIALAB, and the system will automatically adjust the volume that needs to be aspirated.

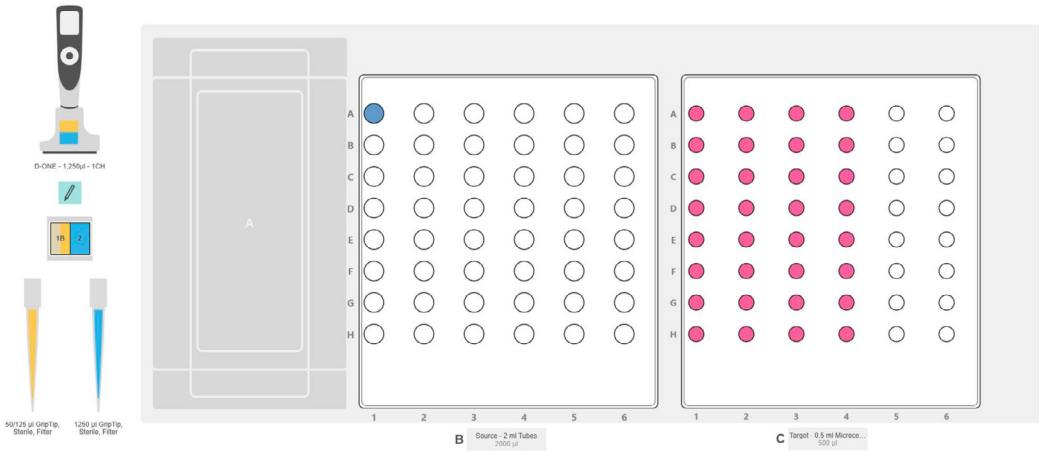


Figure 7: Set-up for reagent transfer from manufacturers' tubes to microcentrifuge tubes. Position A: Empty. Position B: INTEGRA rack for 2 ml tubes. Position C: INTEGRA rack for 0.5 ml microcentrifuge tubes.

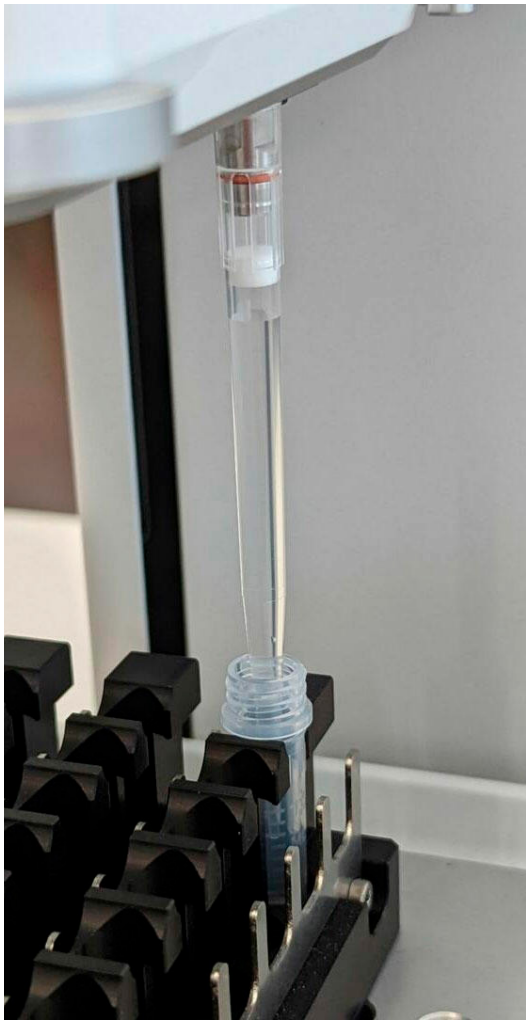


Figure 8: Reagent aliquoting from a manufacturer tube with the D-ONE pipetting module.

6. Aliquoting reagents (buffers, master mixes, etc.) for kit preparation

Multiple aliquoting from 100 ml INTEGRA reservoir to 2 ml tubes.

Place a 100 ml INTEGRA reservoir on deck position A (**Figure 9**, blue), and INTEGRA racks for 2 ml tubes with screw caps on deck positions B and C (**Figure 9**, magenta).

Run the VIALAB program 'Aliquoting_from_reservoir'. The 8 channel 1250 µl VOYAGER pipette with 1250 µl sterile, filter GRIPTIPS aspirates the reagent from the reservoir, and dispenses 1000 µl into each 2 ml tube.

Tip:

If the aliquoting volume needs to be smaller than 500 µl, the dispense type can be switched in VIALAB, speeding up the process.

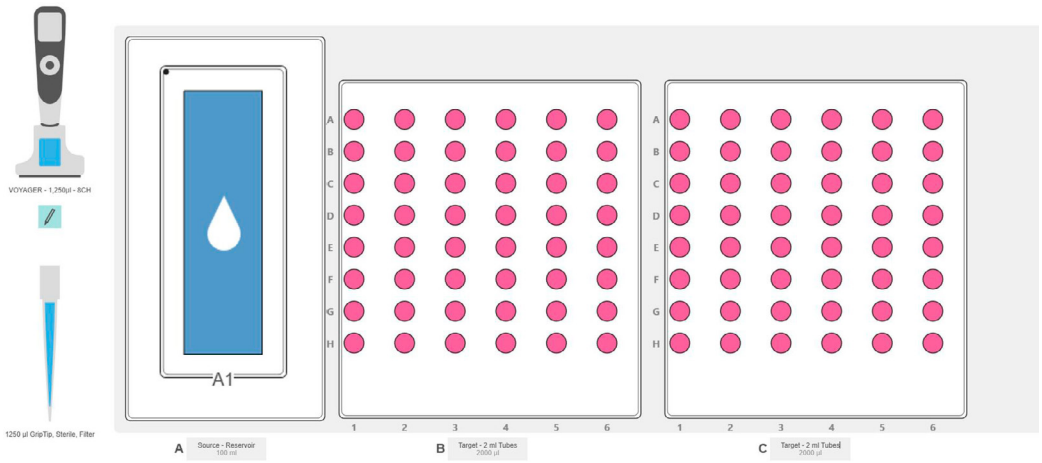


Figure 9: Deck set-up for reagent aliquoting from an INTEGRA reservoir to tubes. Position A: INTEGRA 100 ml reservoir. Position B: INTEGRA rack for 2 ml centrifuge tubes (magenta). Position C: INTEGRA rack for 2 ml centrifuge tubes (magenta).

Remarks

VIALAB software

The VIALAB software gives the option to change protocols and labware according to workflow needs, adapting easily to future requirements.

Partial plates

Programs can be adapted to accommodate a different number of samples at any time.

Summary

- The ASSIST PLUS pipetting robot equipped with the VOYAGER adjustable tip spacing pipette is the perfect solution to transfer samples and reagents between different labware formats using single or multi-dispensing.
- The D-ONE pipetting module enables fast and easy multiple aliquoting from a single manufacturer's tube to any other labware type.
- The automated protocols provided offer simple, fast, and ready-to-use solutions for 6 common aliquoting procedures with maximum walk-away time.
- Thanks to its compact footprint, the ASSIST PLUS can be placed in a laminar flow hood whenever sterile conditions are required during aliquoting.

For more information, a list of materials used and VIALAB programs, please refer to our website.

5.4 Efficient 2-way sample pooling automated with the ASSIST PLUS

How to minimize the number of samples that have to be confirmatory tested by a smart sample pooling approach

In diagnostics, sample pooling is an efficient method to increase sample testing while reducing reagent use. Traditional pool construction requires combining equal portions of specimens into 1 vessel. Positive sample pools trigger retesting of each individual sample in that pool. In 2-way pooling[1], the specimens are pooled in a grid pattern. Samples are first pooled horizontally, and then vertically. In this way each sample is tested in 2 separate pools. Positive samples will yield a positive result in 2 pools, allowing direct identification of the individual positive sample, minimizing the need for confirmatory testing. With the ASSIST PLUS pipetting robot, the whole pooling process – including transferring samples from individual tubes to plates and the pooling steps – can be automated to eliminate any source of error.

Key benefits

- The ASSIST PLUS allows automated, high throughput and efficient transfer of samples from tubes to plates using the VOYAGER adjustable tip spacing pipette.
- The deck of the ASSIST PLUS can accommodate all sizes of tubes using a variety of INTEGRA tube racks.
- Automatic tip changes ensure contamination-free transfer of samples.
- Automation of sample pooling eliminates the possibility of pipetting errors caused by operator fatigue or inattention.
- The compact footprint of the ASSIST PLUS enables placement in a biosafety cabinet, allowing it to be used with potentially infectious samples.

Overview: How to automate sample pooling

2 sets of 8 pools are constructed from 64 samples (**Figure 1**). The ASSIST PLUS is equipped with a VOYAGER 8 channel 1250 µl electronic pipette using 1250 µl sterile, filter GRIPTIPS®. Samples may consist of respiratory specimens, urine, serum, or other uniform liquid. Depending on the sample type, the pipetting speed might need to be adapted, or other types of tips should be used (e.g. wide bore).

Prior to pool construction, samples are transferred from specimen collection tubes (2.0 ml cryogenic vials) into a deep well plate, while maintaining appropriate sample identification practices.

The protocol is divided into 3 parts:

- **Program 1:** Transfer samples from cryogenic vials to a 96 deep well plate (Tube_to_plate_ transfer).
- **Program 2:** Column pools – Construct 8 pools from 64 samples in columns 1-8 (Column_ pools).
- **Program 3:** Row pools – Construct 8 pools from 64 samples in rows A-H (Row_pools).

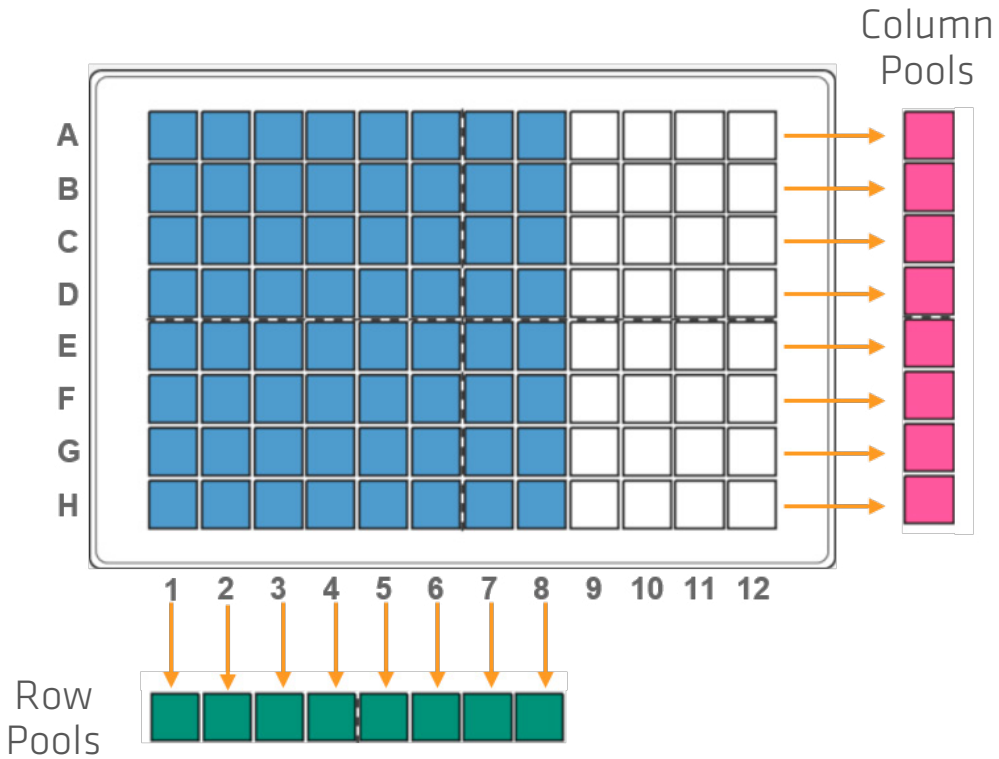


Figure 1: Schematic of 2-way pooling showing column pools (magenta) with 8 samples from columns 1 to 8, and row pools (green) with 8 samples from rows A to H.

Experimental set-up: Program 1

Deck position A:	INTEGRA rack for cryogenic vials containing 32 samples (1-32, **Figure 2**, blue).
Deck position B:	Empty 96 deep well V-bottom plate (Greiner Bio-One, **Figure 2**, pink).
Deck position C:	INTEGRA rack for cryogenic vials containing 32 samples (33-64, **Figure 2**, blue).

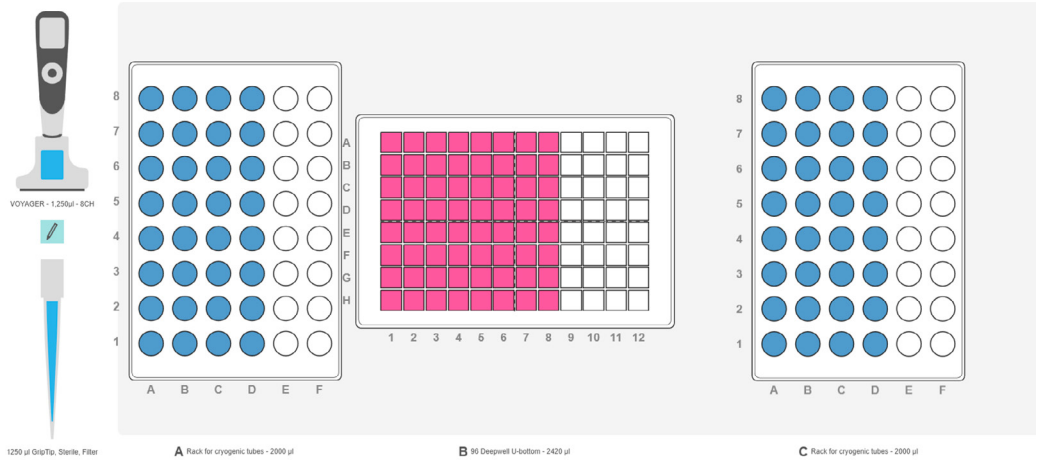


Figure 2: Set-up for transferring samples from cryogenic vials to 96 deep well plate.

1. Transfer samples from cryogenic vials to a 96 deep well plate

Transferring 1000 µl samples from cryogenic vials to a 96 deep well plate.

Load 2 INTEGRA racks for cryogenic vials on to deck positions A and C, with 32 samples in each. Place an empty 96 deep well plate in landscape orientation on deck position B **(Figure 2)**. Select and run the VIALAB program 'Tube_to_plate_transfer' on the VOYAGER pipette. The pipette transfers 1000 µl of each sample from the cryogenic tubes to the deep well plate. High accuracy and precision are ensured with 25 µl pre- and post-dispense. The tip travel function is used for both aspiration and dispensing to prevent contamination. The pipette tip does not go to the bottom of the tubes and plate, but is only immersed a few mm below the liquid surface, and moves the set distance down during aspiration and dispense to avoid contamination.

Tips:

- A 25 µl air gap has been added at the end of the aspiration to prevent cross-contamination risk. For the same purpose, the racks with the cryogenic vials are placed on decks A and C, minimizing the travelling of the pipette tips containing the samples above other samples.
- The pipetting speed is easy to set in the VIALAB program, with options from 1 till 10. This has to be adjusted based on the sample type (e.g. viscous sample should be pipetted slower).

Experimental set-up: Program 2

Deck position A:	Empty
Deck position B:	96 deep well V-bottom plate containing 64 samples (**Figure 3**, blue) in landscape orientation
Deck position C:	Empty 96 deep well V-bottom plate

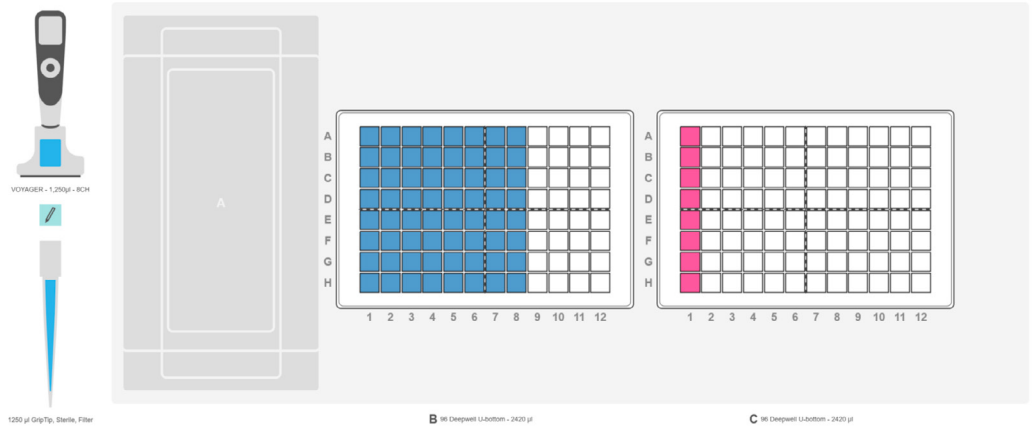


Figure 3: Deck set-up for the column pools. Position A: Empty. Position B: 96 deep well plate containing 64 samples (blue) in landscape orientation. Position C: Empty 96 deep well plate.

2. Column pools

Transfer the samples to create the column pools.

Place the sample plate with saliva samples in wells A1-H8 in landscape orientation on deck position B, and an empty 2 ml deep well plate in landscape orientation on to deck position C. Select the program 'Column_pools' on the VOYAGER pipette and press run. The ASSIST PLUS immediately starts to transfer the samples from columns 1-8, creating the pools in wells A1-H1 in the pooling plate. The pools are mixed well following the last sample addition. Once the mixing step is complete, the VIALAB program informs the user to turn the sample plate (deck position B) 90° clockwise to portrait orientation.

Tip:

The pooling programs are set to pool 100 µl of sample from the source plate. This volume may be adjusted to suit individual needs.

Experimental set-up: Program 3

Deck position A:	Empty
Deck position B:	96 deep well V-bottom plate containing 64 samples (**Figure 4**, blue) in portrait orientation
Deck position C:	96 deep well V-bottom plate

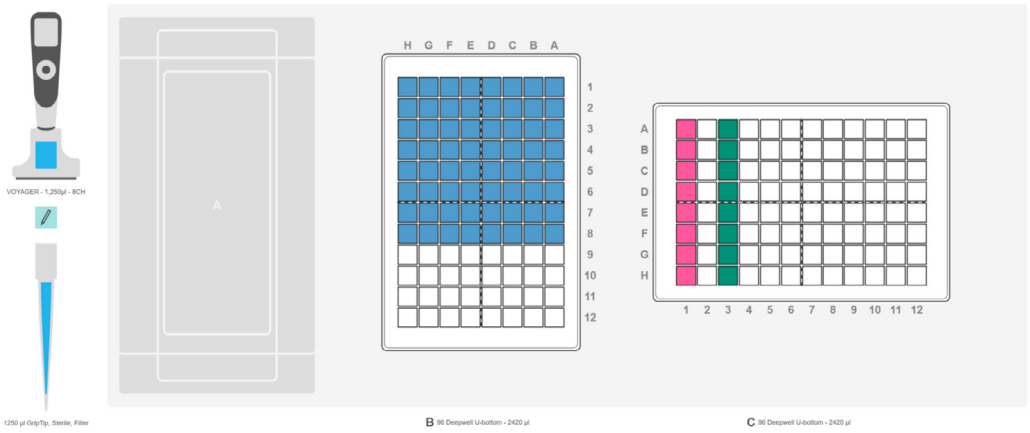


Figure 4: Set-up for the row pools. Position A: Empty. Position B: 96 deep well plate with 64 samples (blue) in portrait format. Position C: 96 deep well plate with the first set of samples pooled (pink).

3. Row pools

Transfer the samples to construct the row pools.

Select and run the program 'Row_pools' on the VOYAGER pipette. The ASSIST PLUS immediately starts to transfer the samples from columns A-H, creating the pools in wells A3-H3 in the pooling plate **(Figure 5)**. The pools are mixed well following the last sample addition.

An example for the result of 2-way pooling can be seen in the Appendix **(Figure 6)**.

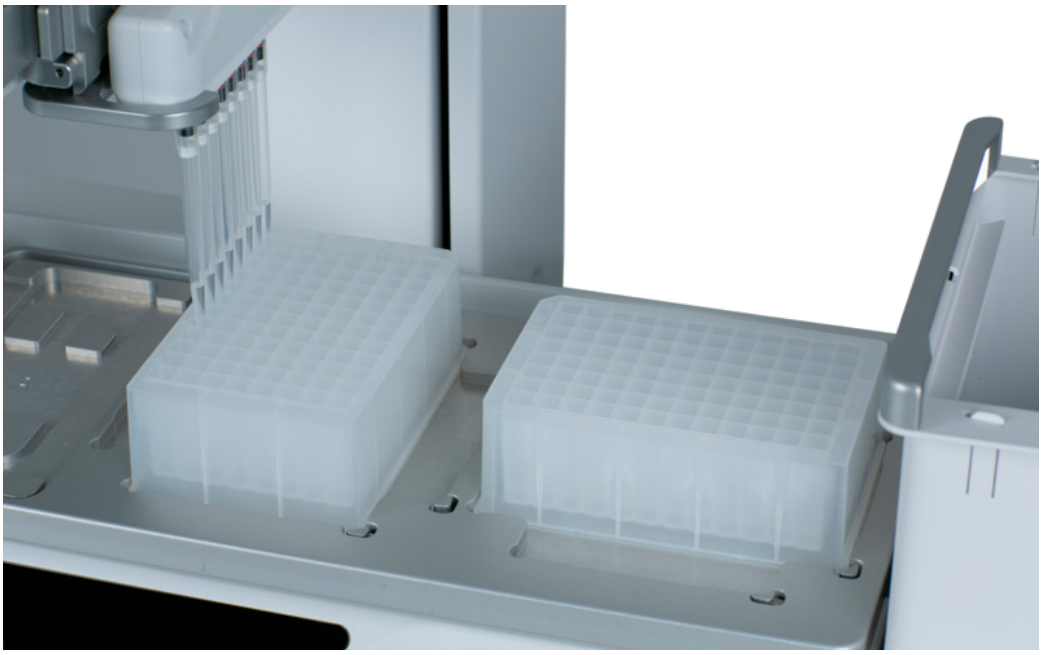


Figure 5: The ASSIST PLUS transfers the samples from columns A-H to create the row pools.

Remarks

- Sample pools may immediately be used for downstream methods, such as nucleic acid extraction or ELISA.
- The deep well sample plate may be sealed with a sealer, and stored for confirmatory testing.

VIALAB software

The VIALAB programs can be easily adapted to your specific labware and protocols, for instance when partial plates are needed.

Appendix

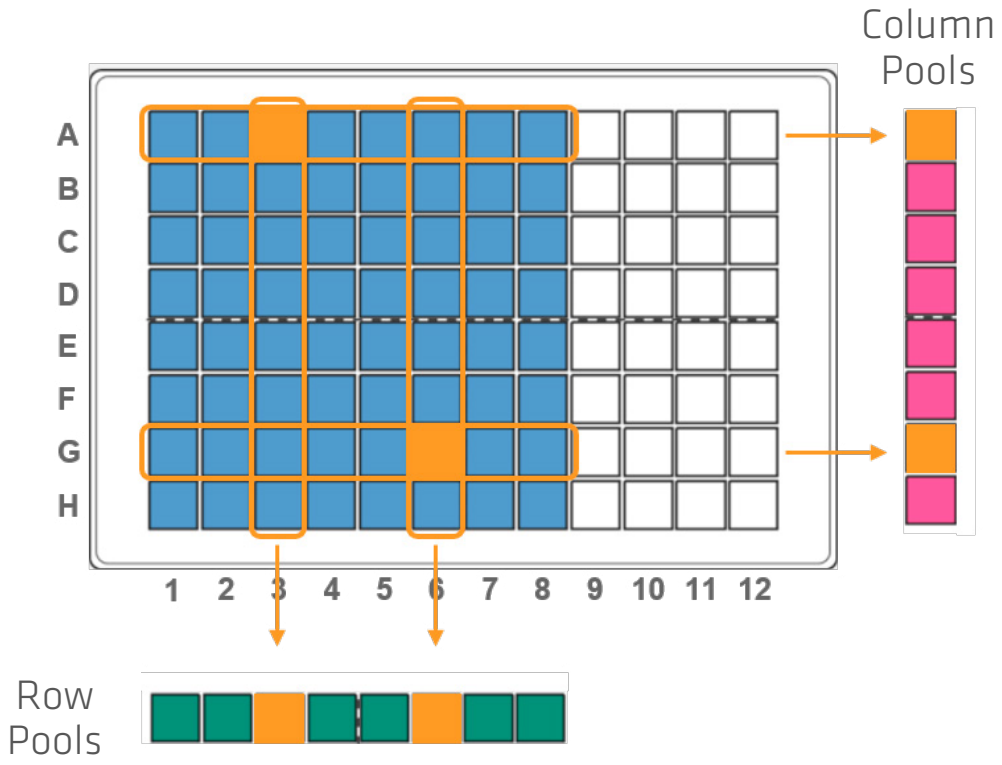


Figure 6: Hypothetical result of the 2-way pooling. Automated pooling with the ASSIST PLUS allows unique identification of the positive pools (labelled with orange on the picture). The confirmatory test needs to be done only with the 4 samples.

Summary

- The 2-way pooling strategy, performed on the ASSIST PLUS pipetting robot in combination with the VOYAGER pipette, reduces time and reagent resources needed to confirm positive pooled sample results.
- The easy implementation of the ASSIST PLUS in transferring samples from tubes to plates and sample pool construction eliminates pipetting errors and increases reproducibility of results.
- The ability to process different tube types and labware formats makes the ASSIST PLUS an invaluable tool to increase productivity in every lab, and it will never become obsolete.
- The ASSIST PLUS supports pipetting from plates in portrait and landscape orientations on the deck, allowing matrix pooling or other matrix pipetting schemes.

For more information, a list of materials used and VIALAB programs, please refer to our website.

5.5 Measuring dead volume in INTEGRA's multichannel reagent reservoirs

Save on expensive reagents and precious samples by using low dead volume multichannel and divided reagent reservoirs

As miniaturization and high throughput processing increase in popularity, the required reagent volumes for lab assays decrease while their value increases. This is particularly true of expensive master mix, enzymes, self-expressed antibodies and new compounds. Any loss of reagent due to a high dead volume is unacceptable, yet this is a reality that many researchers face on a daily basis when using a multichannel pipette to transfer a reagent from a reservoir into a multiwell plate. Most reagent reservoirs feature a V-shaped bottom to minimize dead volumes. However, as the liquid level lowers, the reagent starts to pool unevenly. As soon as a single pipette tip aspirates air, liquid transfer has to stop and the reagent remaining cannot be used for the defined application. This is known as the dead volume. INTEGRA developed a novel 25 ml divided reservoir to overcome this issue, which combines 2 innovative features: the SureFlo™ anti-sealing array and a unique surface treatment that spreads liquid evenly to offer even lower dead volumes. INTEGRA's 2-compartment reservoir enables the pipette tips to sit on the bottom and prevents liquids from pooling, offering the lowest dead volume on the market. This technical note provides experimental data to demonstrate this, and guidance to support users in setting up their experiments.

The problem – dead volume

Dead volume refers to the volume of liquid that cannot be used for an application without the risk of aspirating air into the pipette tips. The dead volume depends on several factors: the material and geometry of the container; the nature and initial volume of the liquid; the number of pipetting channels and the tip spacing; and pipetting techniques and atmospheric conditions, for instance the air temperature and humidity, which impact surface tension. High reagent dead volume is a serious issue in laboratories, especially when pipetting precious liquids – such as master mix, enzymes or antibody solutions – into well plates using a multichannel pipette.

Traditional reservoirs have a V-shaped bottom to minimize dead volumes. However, as the liquid level lowers, the reagent starts to pool in multiple locations. As a result, a number of pipette tips may aspirate air.

To ensure there is adequate liquid to transfer, surplus reagent should be placed in the reservoir when setting up an experiment. The use of optimal reservoirs is a considerable asset for users, in order to reduce loss of precious reagents.

INTEGRA's multichannel reagent reservoirs

INTEGRA's multichannel reagent reservoirs consist of disposable inserts that fit into reusable bases with clearly visible volume markings. Inserts are available as crystal clear polystyrene (PS) reservoirs, or polypropylene (PP) for improved chemical compatibility. For maximum fluid recovery and minimal waste, INTEGRA reagent reservoirs feature a full length, extra deep trough that is more easily accessible for pipette tips.

INTEGRA also developed a novel 25 ml divided multichannel reagent reservoir with a SureFlo anti-sealing array on the bottom and a unique surface treatment to further reduce dead volumes. The anti-sealing pattern consists of a series of small channels that allow the liquid to flow evenly across the bottom of the reservoir and prevent pipette tips from sealing. Pooling of liquid is prevented by a specially formulated, hydrophilic surface treatment. Additionally, the reservoir is divided, offering 5 and 10 ml volume compartments side by side, resulting in the lowest possible dead volume.

INTEGRA's divided reservoirs are designed to be compatible with all VIAFLO fixed spacing and VOYAGER adjustable tip spacing multichannel pipettes.

Divided reagent reservoir comprised of 5 (red) and 10 ml (blue) compartments.

Performance of INTEGRA's reagent reservoirs

The main purpose of this technical note is to support users in setting up their experiments, helping them to maximize precious reagents. In a series of tests, we measured and compared the dead volumes in each INTEGRA multichannel reagent reservoir. The properties of water are strongly influenced by environmental factors – air temperature and humidity – in an uncontrolled lab environment and so we used a foaming blocking buffer containing diverse salts and low surface tension liquids in order to get closer to real lab conditions.

RESERVOIRS	INITIAL VOLUME (µl)	DEAD VOLUME (µl)	% DEAD VOLUME / INITIAL VOLUME
PS 10 ml	3600	51 ±10	1.4
PS 25 ml	9000	80 ±12	0.9
PS 25 ml	9000	65 ±10	0.7
PS 100 ml	36000	120 ±14	0.3

Table 1: Measuring dead volumes with a blocking buffer in INTEGRA's PS and PP reservoirs: initial and dead volumes in µl and corresponding dead volume as a percentage of the initial volume.

Standard multichannel reagent reservoirs

We measured the dead volume of INTEGRA's standard polystyrene and 25 ml polypropylene reservoirs using a blocking buffer composed of 0.1 % Triton™ X-100 and 0.05 % Tween 20® in a 1x TBS (0.05 M TRIS.HCl and 0.15 M NaCl in water ISO 3696:1995, grade 3).

The majority of the buffer was aspirated using a VIAFLO 8 channel 1250 µl electronic pipette at speed 3. To determine the dead volume in the reservoir, we carefully aspirated the rest of the foaming buffer using a VOYAGER 12 channel 50 µl electronic pipette at speed 1 to ensure that no air entered the pipette tips. The tip spacing of the VOYAGER was set at 6.5 mm for the 10 ml reagent reservoirs and 9 mm for the 25 and 100 ml reservoirs. Results of the experiments are presented in **Table 1** and **Figure 2**.

Large reagent reservoirs (25 and 100 ml) show low dead volumes of less than 1 % of the initial volume, while 10 ml reservoirs display a dead volume of 51 ±10 µl, representing 1.4 % of the initial volume.

Divided reagent reservoirs

In a second series of tests, we measured the dead volumes of polystyrene and polypropylene 25 ml divided reservoirs in both the 5 ml and 10 ml compartments.

Tests were performed under the same conditions as those previously described using the blocking solution, and water ISO 3696:1995, grade 3, a 10 % Tween 20 solution in grade 3 water and an 80 % isopropanol (iPrOH) solution in grade 3 water. The aim of testing different solutions was to evaluate the influence of the liquid type on dead volumes, to provide more detailed insights.

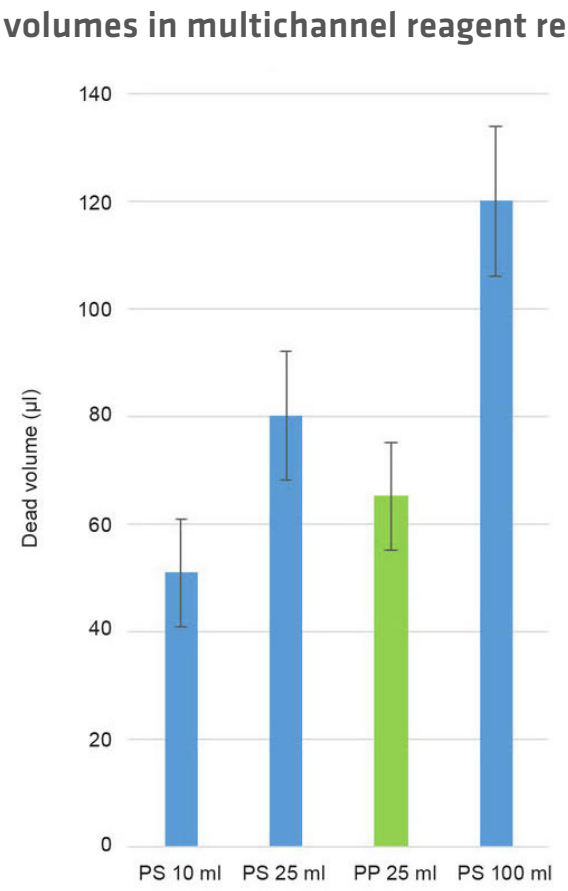


Figure 2: Averages and error bars of residual liquid amounts (µl) after dispensing solutions of 10 % Tween 20 (left), 10 % SDS (middle) and 80 % isopropanol solution (right) with 300 µl standard GRIPTIPS® (N) and low retention GRIPTIPS (LR).

Results were obtained by differential weighing of the reagent reservoirs using an analytical balance in the controlled environment of a calibration laboratory. Each measurement was repeated 10 times per reagent reservoir type to ensure result consistency.

For each reservoir size, we filled in the reservoir with an initial volume corresponding to 36 % of the nominal volume of the reservoir – a number easily divisible by the 8 and 12 channels of a multichannel pipette. Tips were pre-wetted 3 times to optimize pipetting performance and results.

To provide users with an ideal solution for small quantities of precious or rare reagents, INTEGRA designed 25 ml divided reservoirs with a SureFlo anti-sealing array on the bottom, a unique surface treatment and 2 compartments of 5 and 10 ml.

The main part of the liquid was aspirated with a VOYAGER 4 channel 300 µl electronic pipette at speed 5 for the 5 ml compartment and a VIAFLO 8 channel 1250 µl electronic pipette at speed 3 for the 10 ml compartment.

The remaining liquid was then carefully aspirated using a VOYAGER 8 channel 50 µl electronic pipette with 4.5 mm tip spacing for the 5 ml side and a VOYAGER 12 channel 50 µl electronic pipette with 6 mm tip spacing for the 10 ml side, at speed 1 to avoid any air entering the tips. The results of these tests are presented in **Figure 3** and **Table 2**.

The results of this series of tests demonstrate the performance of the 25 ml divided reagent reservoirs from INTEGRA. The novel design of the 10 ml compartment reduces the dead volume of a foaming blocking solution from 51 ±10 µl to 27 ±5 µl while keeping the same working procedure.

For smaller quantities of the precious reagents, the 5 ml side of the divided reservoirs reduces dead volumes to around 10 µl, ~0.5 % of the initial volume in the reservoir.

Polypropylene divided reagent reservoirs present similar performance while offering an improved chemical compatibility in comparison with polystyrene.

DIVIDED REAGENT RESERVOIRS	INITIAL VOLUME (µl)	WATER (µl) RATIO (%)	BLOCKING SOLUTION (µl) RATIO (%)	10 % TWEEN 20 (µl) RATIO (%)	80 % iPrOH (µl) RATIO (%)
Div. PS 5 ml	1800	6 ±3 0.3	9 ±2 0.5	12 ±1 0.7	14 ±1 0.8
Div. PP 5 ml	1800	6 ±3 0.3	10 ±4 0.6	10 ±2 0.6	17 ±3 0.9
Div. PS 10 ml	3600	27 ±6 0.8	27 ±5 0.8	27 ±5 0.8	38 ±6 1.1
Div. PP 10 ml	3600	29 ±10 0.8	30 ±10 0.8	43 ±6 1.2	40 ±8 1.1

Table 2: Dead volumes (µl) and dead volume percentage of the initial volume in divided reservoirs for the 4 tested reservoirs.

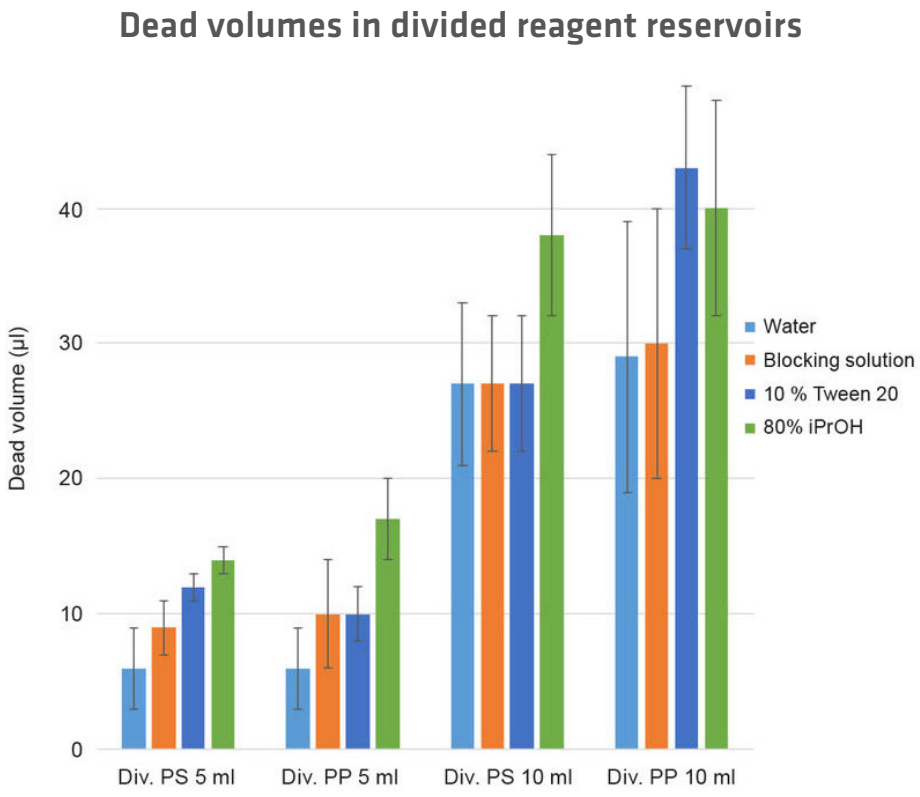


Figure 3: Average and error bars of dead volumes (µl) in INTEGRA's PS and PP divided reservoirs (Div.). Tested solutions: Water, foaming blocking solution, 10 % Tween 20 and 80 % isopropanol (iPrOH).

Summary

- Dead volume is a real problem when transferring reagents into multiwell plates using a multichannel pipette, especially when handling expensive or rare solutions.
- To offer users an appropriate solution for small quantities of precious or rare reagents, INTEGRA rethought the concept of reagent reservoirs by including 2 innovations in its 25 ml divided reservoirs. This led to highly reduced dead volumes, with an average of 0.5 % of the starting volume in the 5 ml compartment and 0.8 % of the initial volume in the 10 ml side.
- In this study, we measured the dead volumes of INTEGRA's multichannel reagent reservoirs using a foaming blocking solution to reflect realistic laboratory experimental conditions while trying to provide a guide for scientists when setting up their protocols.
- By combining INTEGRA's multichannel reagent reservoirs with best pipetting practices and appropriate pipetting systems in which tips and pipettes perfectly fit together, scientists have advanced tools at their disposal to minimize their dead volume and save precious reagents.
- INTEGRA's 25 and 100 ml multichannel reagent reservoirs showed excellent results with a dead volume of less than 1 % of the starting volume.

For more information and a list of materials used, please refer to our website.

CHAPTER 6: Conclusion

We trust that this eBook has given you the knowledge and know-how you need for effective and stress-free pipetting. If you'd still like to learn more about this extensive topic, we have plenty of additional resources and articles on our website, covering many different aspects of liquid handling more in depth. Whatever your specific pipetting requirements, we are always available to answer your questions and support you with the best workflow solutions.

CHAPTER 7: References

1.1 Everything you need to know about the different types of pipettes

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3.1 How to clean, decontaminate and store pipettes

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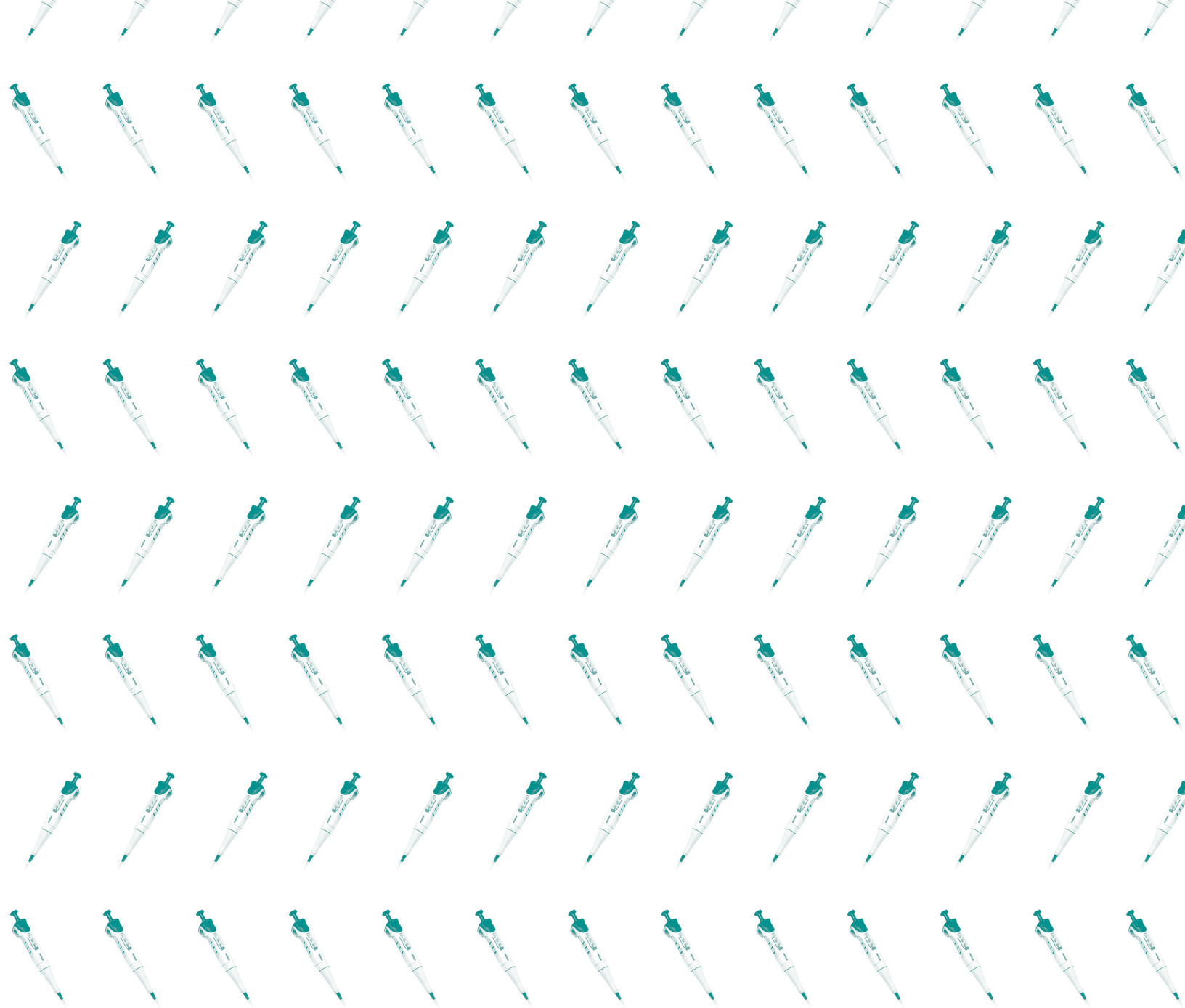

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