

Increasing the Reproducibility of Serial Dilutions

Michael Beier*

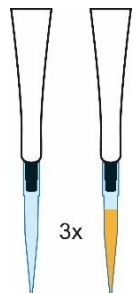
Abstract

Pipetting serial dilutions reproducibly can be difficult because it is a time consuming and consequently error prone methodology, especially in 384 well plates. Based upon the assumption that your pipettes are properly maintained and calibrated, the human influence has the largest impact on pipetting results. Observing good pipetting practice helps immensely to improve reproducibility and accuracy. In this article we focus on a selection of good pipetting techniques, which if maintained offer the best chance of increasing and maintaining the reproducibility of your serial dilutions. Mixing is a key component of dilution protocols and in this article we additionally discuss different mixing parameters to help you achieve a homogenous sample. To maximize consistency of dilution assays, automating the process offers many benefits. For ensuring highly reproducible serial dilutions we outline what features an automated pipetting system should have and introduce the VIAFLO ASSIST – a product purpose designed for this task.

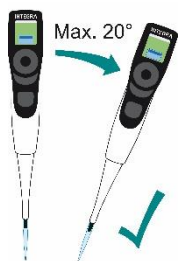
Introduction to good pipetting practice

Good pipetting practice describes guidelines covering the basic techniques to ensure that the pipettes are used in an optimal and reproducible manner.

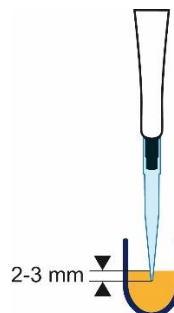
Before preparing your assay, you should perform a pre-wet of your pipette tips. Do this by aspirating and dispensing the full volume 2-3 times. This procedure not only equilibrates temperature differences between liquid and tip but also humidifies the dead air space inside the pipette and tip. Neglecting to perform a pre-wet can result in a smaller delivery volume in the first few dispenses, thereby introducing errors into your subsequent dilution steps.



The liquid volume in the tip is also influenced by the inclination angle of the pipette when drawing in liquid.



It is important to hold your pipette at a consistent angle, max. 20°, throughout the entire dilution series.



Immerse the pipette tip just below the liquid surface to allow the desired volume to be aspirated. If the tip is immersed too deep, the risk of carry-over is increased due to liquid drops clinging to the outer surface of the tip.

Unfortunately, in reality, the rules of good pipetting practice are not always followed even by the most conscientious laboratory personnel. Especially when tired or in a rush your technique might become inconsistent. In addition, the risk of translational errors increases. A row in a 96 or 384 well plate is easily skipped, especially when handling colorless liquids, which can ruin an entire plate and force you to start anew. It is unsurprising therefore that different users, using the same pipette, will get different pipetting results depending on their level of training, experience and for how long they have been working.

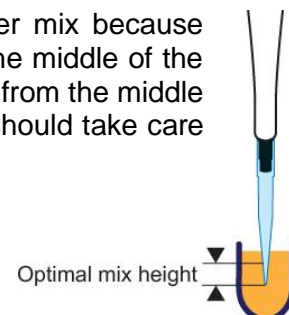
Optimizing mixing parameters

Mixing thoroughly the contents of each well in your microplate is essential when preparing a dilution series. An inhomogeneous mixture introduces a significant error which is then carried on to the subsequent dilution steps. The resulting concentration will be different from the assumed theoretical concentration across the plate. It is therefore important that you optimize the mixing parameters such as mixing height, number of required mixing steps and mixing speeds for a specific liquid.

Depending on the viscosity of the liquid it may be necessary to adapt the pipetting speed. It is best to pipette viscous liquids slowly in order to let the liquid flow through the small tip orifice.

Mixing with the tip end at the very bottom of a microplate well results in a poorer mix because insufficient turbulence is generated. Typically the most efficient mixing height is in the middle of the liquid column of a well or higher. Ideally, the liquid is dispensed on top and aspirated from the middle during the mixing process. However this can be tricky to perform by hand and you should take care not to remove the tips from the liquid during mixing to prevent drawing in air.

The number of mix cycles and the mix volume also significantly impact the mixing result. Increasing the number of mix cycles and having a larger mix volume result in a more homogenous mix but can substantially increase assay preparation time.



How automation can help

It is challenging to observe all these good pipetting and good mixing practice factors while performing series of serial dilutions with a handheld pipette. Undertaking a serial dilution in a 96 well plate with 5 mix cycles per row requires a total of 110 manual plunger movements, risks a potential strain on your thumb and wrist if done over extended periods of time. Using an electronic pipette can improve this process because you can define the number of mix cycles (including speeds) and it will then run through them automatically without having you to repeatedly press the plunger.

By automating your pipetting process you can eliminate pipetting practice and mixing issues thereby achieving an absolutely consistent workflow. An optimal automation system takes control of the entire dilution process and lets you simply define key parameters for serial dilutions such as mixing heights and speeds.



For many dilution applications the required automation does not necessitate the high investment and dedicated personnel required by a laboratory robot or fully automated liquid handling system. At a much lower price tag, Integra's VIAFLO ASSIST offers all the functionality, speed and automation needed. By clicking in a VIAFLO II electronic multichannel pipette, the VIAFLO ASSIST becomes a reliable pipetting assistant, which always observes good pipetting practice and never gets tired. It will carry out the serial dilution for you, from distributing diluent to diluting the sample.

In the words of Lauren Anderson Dring, research scientist and project manager at Pulmocide *"The VIAFLO ASSIST has become an integral part of our compound screening process. It is used on a daily basis to produce reproducibly accurate drug dilutions in a medium throughput manner."*

With VIAFLO ASSIST you can set all important parameters for an efficient mixing in serial dilutions: mixing height, pipetting speeds and number of mixing cycles. Ms. Dring added *"The ASSIST is*

particularly useful in that a multitude of parameters can be pre-defined, such as volume and pipette mixing speed, thus allowing us to set up and save a variety of dilution protocols to suit our needs."

In addition to setting a fixed height, the VIAFLO ASSIST allows tracking of liquid inside microplate wells, avoiding the need to immerse the tip too deep and hence minimizing potential carry over. To fully comply

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with good pipetting practice, a pre-wet can either be done manually before the program start or it can be incorporated into the ASSIST program.

Conclusion

Preparation of reproducible serial dilution assays is a challenging task because they require many pipetting actions and are highly dependent on an operator's technique and experience. Observing good pipetting practice helps to achieve better pipetting results. However, if your goal is to streamline the serial dilution process, and at the same time ensure maximum reproducibility, then an automated pipetting system is necessary.



Automating a serial dilution on a 96 well plate with VIAFLO ASSIST.

The VIAFLO ASSIST is an affordable automated pipetting platform for carrying out serial dilutions in microplates that is highly capable, yet easy-to-use. Important parameters, such as pipetting heights and speeds, can be precisely controlled which improves pipetting results. It also improves overall pipetting consistency by eliminating human errors and inconsistencies, resulting in more reproducible assays and time savings.

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