Andrew Alliance

APPLICATION NOTE
No. 5 20160913
version 2

Accurate and consistent Serial Dilutions made easy with Andrew

Serial dilution of substances is a routine procedure in life and other experimental sciences. Nevertheless, an accurate and reproducible process is of paramount importance for the generation of trustable results. The user-friendly software Andrew Lab, together with the pipetting robot Andrew, simplifies volume calculations and the experiment design, and it allows performing serial dilutions that are in average 5 times but up to 10 times more reproducible and more accurate than an average operator.

SERIAL DILUTIONS ENABLE TO PRODUCE SAMPLES WITH KNOWN CONCENTRATION

Changing the concentration of samples, reagents and buffers is a daily reality in science. When the needed reduction in concentration is large, it is much more accurate to make several smaller stepwise dilutions to reach the final concentration. Some experiments even require each intermediate dilution step to be available, either for testing different concentrations of a reagent or to be used as standard curves for quantitative assays. In other situations, serial dilutions are used to reduce the initial sample viscosity and to dilute out inhibitors or substances in the samples that can interfere with downstream analytics. In a classical serial dilution, regular stepwise dilutions are made in cascade, resulting in an exponential reduction of the concentration. The Andrew Lab software makes easy to design this kind of experiments by automatically calculating the required volumes and concentrations, while Andrew - the pipetting robot – accurately handles the actual pipetting.

Accurate pipetting during preparation of serial dilutions is thus clearly critical, since any deviation will propagate to all subsequent steps. The accuracy and precision of serial dilutions also depend on a thorough mixing at each step: this is essential to achieve a homogeneous mixture with a constant concentration over the sample volume. Without a proper mixing, any aliquot can have a higher or lower concentration than expected, randomly **(Figure 1)**. In this case, when the sampled aliquot is transferred to the next step, it can dramatically affect the downstream concentrations.

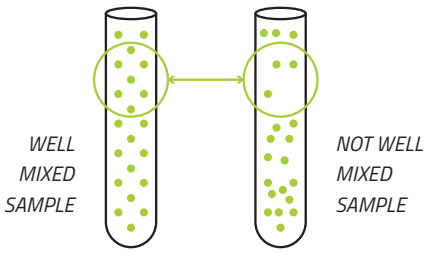

Figure 1: An example of what the distribution of a sample inside a tube could look like with and without good mixing.

ANDREW GUARANTEES HOMOGENEOUS AND REPRODUCIBLE MIXING AT EACH DILUTION STEP

Beyond the correct calculation and definition of serial dilution volumes, it is compulsory to include a precise and reproducible mixing method after each dilution step. Ideally, at least two thirds from the total volume of a solution should be used when mixing by up-and-down pipetting, especially when working with viscous solutions. However, this process often requires a continuous change of pipettes or volume settings at each step, dramatically increasing the number of repetitive motions and making such experiments cumbersome and prone to errors and variation. For example, in a 10-fold dilution with the targeted

final volume of 200 µl, the intermediate steps would have a buffer volume of 180 µl and an immediately upstream sample volume of 20 µl. In this case, since the final volume in each of the dilution is 200 µl, the minimal volume that should be used to ensure a proper mixing would be 100 µl. However, being the previous pipetting step done with a volume of 20 µl, a change of pipette and volume setting to at least 100 µl must be done to ensure proper mixing. With the Andrew Lab software, a proper and perfectly reproducible experimental workflow can be designed according to the user preference and excluding any ambiguity **(Figure 2)**. For example, the number of pipette aspirations and dispensing for mixing can be adapted, avoiding variations in the level of mixing at each step and thus experimental differences. The automatic execution of the experimental flow using Andrew will eliminate any human error and further ensure the reproducibility and accuracy of every experiment.

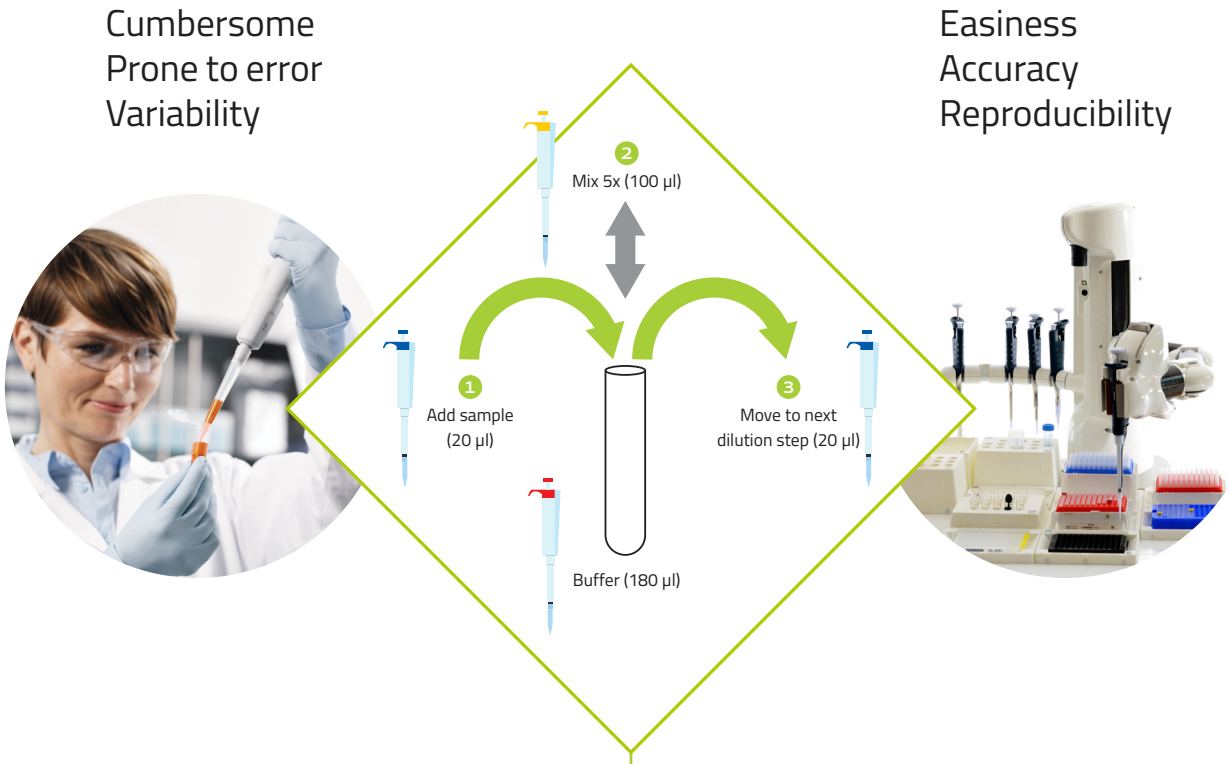

Figure 2: An example of a 1:10 serial dilution requiring the incorporation of a mixing step (2) with a higher volume for a proper mixing. Incorporation of this critical step in serial dilutions made by hand is cumbersome and renders the experiment prone to error and variation. The use of the Andrew pipetting robot not only facilitates the performance of serial dilutions but also allows more accurate and reproducible results.

TESTING QUALITY OF SERIAL DILUTIONS MADE BY HAND OR WITH ANDREW

Two-fold, eight-step serial dilutions of Ponceau S (700 mg/L) in water were done both manually and using Andrew. Of note, the same pipettes were used in both cases and distinct mixing steps were included at each dilution step. Since the total volume at the mixing step was 200 µl, we chose a mixing volume of 135 µl, corresponding to two-thirds of the total volume, for a final volume of 100 µl. The dye absorbance was measured using a standard high accuracy spectrophotometer and five of the eight serial dilution points (175, 87.5, 43.75, 21.9 and 10.9 mg/L) fall within the measurable dynamic range of the device. The accuracy and precision of these serial dilutions was tested over several days with a total of 12 replicates. As the absorbance of the first and last two dilution steps (350, 5.47 and 2.73 mg/L) were outside the range of the plate reader, they were not included in the analysis. To avoid any dependence on the initial sample concentration, the first readable concentration was defined as 100% and all other data points of the dilution series were compared relative to this reference point.

ANDREW CAN PERFORM REPRODUCIBLE SERIAL DILUTIONS

To assess the variability of serial dilutions performed by Andrew or by a highly trained human operator, we calculated the mean Optical Density (OD) of 12 independent serial dilutions for each method (Andrew and Manual) and the standard deviation of the mean. Then the mean ±3SD were plotted in a bar chart **(Figure3)**. As clearly evidenced by the smaller error bars, serial dilutions performed by Andrew are more consistent and reproducible. This was also shown by calculating the coefficient of variation of each dilution step **(Table 1)**. While the maximum co-efficient of variation (CV) observed for the Andrew-performed dilution steps was **1.29%**, this measure was consistently higher in all manually done dilution steps, reaching values from **1.56 %** and up to **9.54%**. This CV represents the random error encompassing the random error of the manual pipette (of 0.15% per maximum Gilson permissible errors), Andrew, and the sensitivity of the plate reader. It should be noted that 1% of this variability is contributed by the statistical error of the plate reader. This consistent performance through many replicates undoubtedly demonstrates that the pipetting robot Andrew can accomplish a serial dilution in a more accurate and reproducible way than a human operator.

Dilution step	CV observed with Andrew	CV observed with Manual
1 (175 mg/L)	0.48%	1.56%
2 (87.5 mg/L)	0.84%	3.08%
3 (43.75 mg/L)	0.98%	3.22%
4 (21.88 mg/L)	0.97%	9.12%
5 (10.94 mg/L)	1.29%	9.54%

Table 1: Concentrations measured at each dilution step as Percentage of the Initial 100% and calculated CVs (n=12).

SERIAL DILUTIONS WITH ANDREW ARE ALSO MORE ACCURATE

To assess the systematic error, a target dilution curve of dye concentrations at each and all dilution steps was calculated for the above serial dilutions, assuming that a perfect dilution was possible. This standard curve was then compared against the average serial dilution curves generated either by Andrew or manually to assess how closely the target concentrations were achieved with both methods at each dilution step. The final dilution step achieved by Andrew was only 0.05% of the target dilution, while all the intermediate steps were less than 0.2% of their target dilutions **(Figure 4)**. This difference from the target dilution, at every step, falls within the range of the CV, indicating that the systematic error observed in serial dilutions made by Andrew is negligible. In contrast, observed deviation in manually performed serial dilutions was consistently higher at every dilution step, being between 0.26 and 0.85% of the target dilution values **(Figure 4)**. These results strongly suggest that the pipetting robot Andrew can perform serial dilutions with a level of accuracy that goes beyond what can be achieved by hand. The main difference between manually and Andrew-made serial dilutions is the consistency of the separate mixing step, typically accomplished by a carefully controlled and consistent pipetting technique that can only be achieved with automated solutions like Andrew. Yet, other biases like variations in the pipette volume setting hysteresis, the pipette verticality, the tip insertion depth, the thumb speed, the tip insertion force and the first-stop detection force may also affect the precision of the serial dilution, all of which are eliminated by using Andrew.

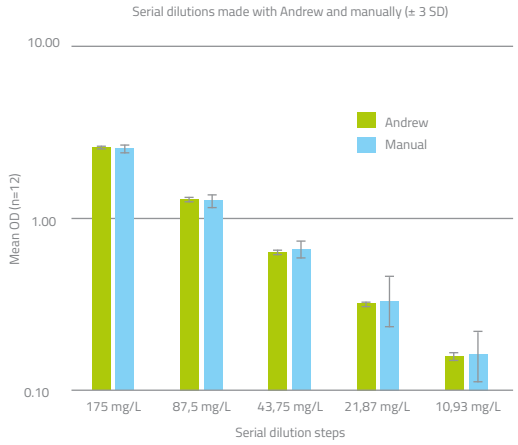

Figure 5: Serial dilutions made by Andrew are consistently more reproducible than their manually performed counterparts. Mean OD measured at each dilution step and ±3SD from the mean are plotted.

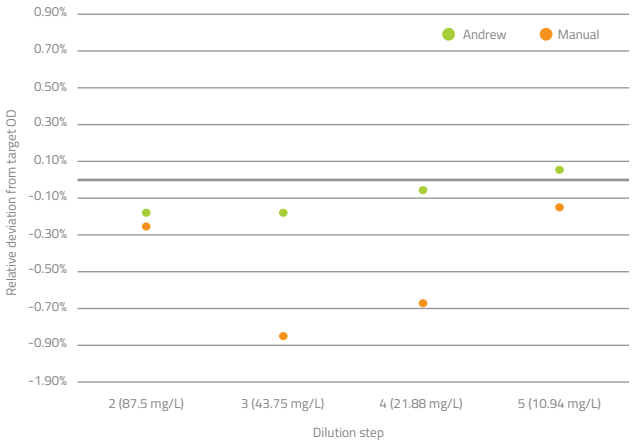

Figure 4: Serial dilutions made by Andrew are more accurate than their manually performed counterparts. A standard curve for the ideal target diluted concentration at each dilution point was calculated using the dilution factor of 2. This was compared with serial dilution curves produced either by hand or by Andrew. The relative deviation from the target OD value observed for both curves at each dilution step is depicted.

PROGRAMMING A SERIAL DILUTION IN ANDREW LAB

Andrew Lab includes an extremely user-friendly tool to make a serial dilution in few clicks, by simply indicating the amount and the concentration of the final sample or indicating the dilution factor. Andrew Lab will calculate the concentrations and volumes required at each dilution step and allows defining accurately the required mixing methods. To design a serial dilution, you can simply input the dilution factor as well as the buffer volume to be dispensed in each dilution step **(Figure 5a)**, or alternatively indicate the concentration of any of the serial dilution points. This will allow Andrew Lab to calculate the initial sample/reagent volume required, as well as the volume which will be moved from well to well during the dilution. Additionally, a different volume for the mixing step can be set up by simply introducing the numeric value in the entry box designated for that end in the mixing panel of options **(Figure 5b)**. For difficult to mix samples, at least two-thirds of the total volume should be used when **mixing** by up-and-down pipetting.

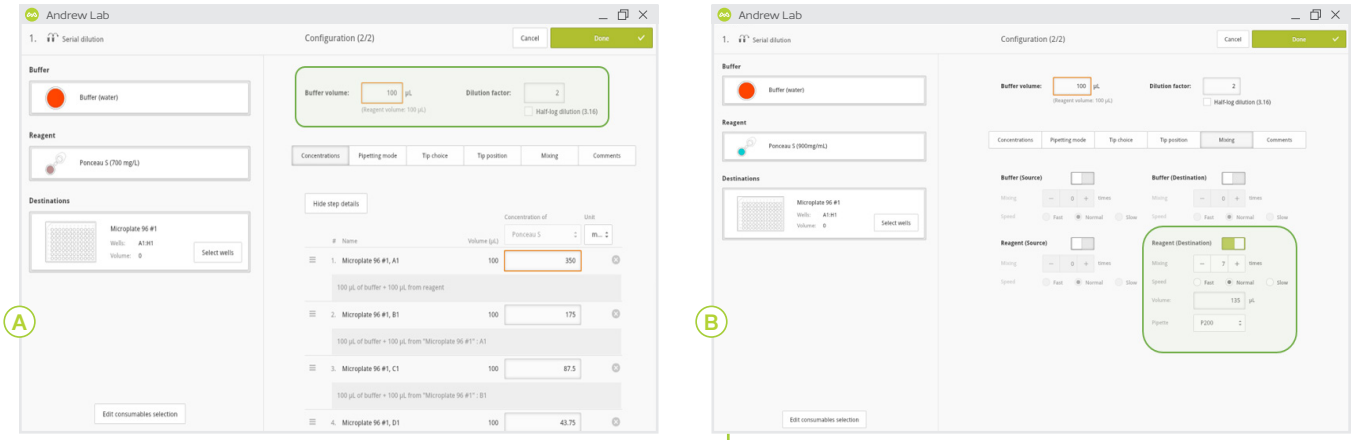

Figure 5: Example steps to easily create a serial dilution protocol in Andrew Lab 1.4. (A) Determine the Dilution Factor and Buffer volume to be dispensed in each dilution step. (B) Determine the optimal mixing volume in the designated entry box at the "reagent destination" conditions.

CONCLUSIONS

Precise pipetting and mixing is mandatory for accurate and reproducible serial dilution experiments. However, this is often overlooked when doing repetitively serial dilutions by hand, being this a repetitive and time-consuming operation. A serial dilution can be easily described in Andrew Lab in few seconds, and Andrew can deliver, unattended, results that are in average 5 times but up to 10 times more reproducible and 10 times more precise than an average operator.

Still have any questions about mixing with Andrew or mixing for serial dilutions? Get in touch!

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