Andrew Alliance

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Accurate repetitive dispensing by means of standard mechanical pipettes

The pipetting robot Andrew can perform a single aspiration followed by multiple dispensing actions with standard mechanical pipettes. Additionally, by dispensing on-the-fly, it enables contamination-free operations without tip replacement. Unattended, Andrew can fill up an arbitrary set of microplates, tubes or other consumables at a pace down to 4.5 seconds/destination. These performances allow an accurate, flexible and reproducible method for reagent distribution.

REPETITIVE PIPETTING: THE COMMON PRACTICE AND WELL KNOWN PROBLEMS

As one of the most common daily activities in life-science laboratories, pipetting consumes a major part of a scientist or technician's working day. In a typical workflow, samples, reagents, and solutions are pipetted in various volumes. In addition, there are procedures where the same volume needs to be transferred repeatedly from one source vessel to multiple destination vessels. Some common examples include filling 96-well microplates with master mixes or reagents to prepare PCR, qPCR, ELISA, or other clinical diagnostic and forensic tests, as well as aliquotting bodily fluid samples for biobanks. This type of work entails repeatedly refilling pipette tips from the source and dispensing the liquid to different destinations. Each such repetitive pipetting cycle requires the user's thumb to depress the pipette plunger twice and release it once with enough force and care to ensure accurate and precise liquid transfer. Together with other pipetting activities, this repeated pipetting increases the occurrence of Repetitive Stress Disorders (RSI) of users' thumb, hand, and shoulder, which are a well-known and costly medical problem **[1]**.

In addition to ergonomic issues, repetitive pipetting leads to lost efficiency and potential pipetting errors when executing lengthy protocols. Variations in user-to-user pipetting technique can lead to large deviations in the amount pipetted, causing significant downstream errors in data analysis and interpretation. Given the challenges to user health/safety, wasted time spent carrying out this menial yet essential task, and poor downstream data, many companies have focused on improving the process, until now, with limited beneficial results.

Multi-channel pipettes have been used to shorten the time needed to fill multi-well plates, with 8 or 12 well positions being filled in parallel. However, besides the degraded accuracy and precision, multi-channel pipettes are also limited in the usage of suitable consumables having a precise distance of 9 mm between the wells. Repeater pipettes, also called stepper pipettes, provide a different solution for transferring the same volumes to multiple destinations, allowing for more choice in consumables but with a significant hands-on time and narrow range of volumes that can be dispensed. Traditional robotic liquid handlers increase speed but limit consumable choice, are expensive, and require a specific IT skill set and high-level expertise to use them.

ANDREW ROBOT: A HANDS-OFF AND ROBUST SOLUTION TO REPETITIVE PIPETTING

At Andrew Alliance, we have developed one of the most effective solutions to the repetitive liquid handling challenges, which combines the positive features while eliminating the negative aspects of the repeater pipette and traditional automated liquid handling workstations. Both affordable and easily implementable to nearly any type of laboratory, this solution is comprised of the pipetting robot, Andrew, coupled with a graphical user-friendly software for designing pipetting protocols. Indeed, Andrew can execute the same functionality of a repeater pipette while using the standard conventional mechanical pipettes **(Figure 1)**, all automatically in an accurate and precise manner. Thus, Andrew provides a very competitive system for liquid distribution and aliquotting activities.

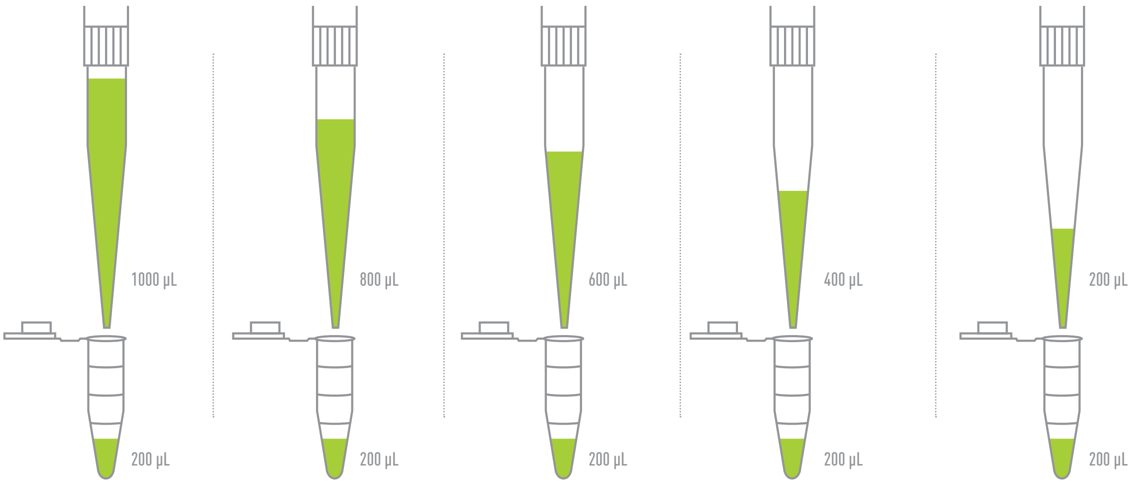

Figure 1: Repetitive pipetting by Andrew robot to dispense equal volume into multiple destinations from a single aspiration

As the most flexible pipetting system, Andrew can change a pipette volume, insert and eject tips, aspirate, mix, dispense liquid, reverse pipette, forward pipette, deal with viscous solutions, create air gaps for eliminating dripping or tip touch for accuracy at low volumes, and adapt to the consumables on deck with artificial intelligence, from any source vessel type to any destination vessel type pre-determined by the users.

ANDREW ACCURATELY AND PRECISELY DISPENSES EQUAL VOLUMES REPETITIVELY WITH STANDARD MANUAL PIPETTES

We tested the "repetitive dispensing" performance of Andrew using a photometric method by measuring the aliquot volumes of Ponceau S dye solution in water as described previously **[2]**. An Andrew model 1000G was used to repetitively dispense different dye volumes into clear flat-bottom 96-well microplates, and the maximum absorbance at 520 nm of the dye was measured with a spectrophotometer. We tested Andrew's repetitive dispensing mode using the 4 Gilson pipetman P20, P100, P200, and P1000 **(Table 1)**. The minimum tested volume was 2 µL, and the maximum was 100 µL. A pre-wet step was carried out for every tip by mixing the liquid at the source 10 times to equilibrate the vapor pressure in-side the tip, ensuring more accurate results. Eppendorf epTIP tips were used for the P1000 pipette, and Gilson Diamond tips for the other three pipettes. Each volume tested in Table 1 was repetitively dispensed as series of 8 aliquots in the "precise" mode **(Figure 2)** or 18 aliquots in the "fast" mode **(Figure 3)**, 10 – 12 series per tip, and 4 – 5 tips per aliquoted volume. All experiments were carried out at 21 – 24 °C with a relative humidity of 34 – 40%.

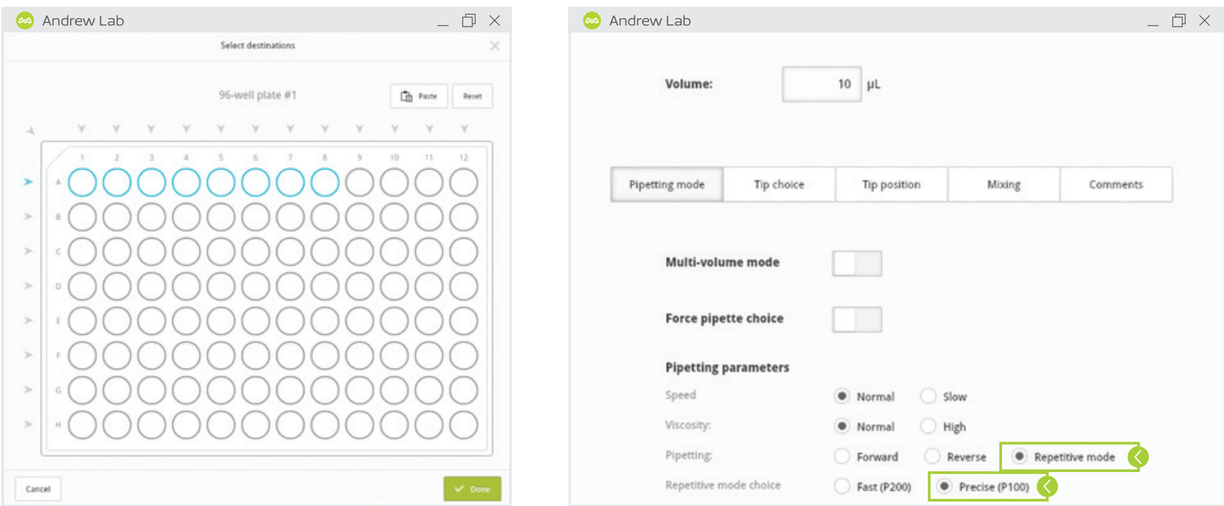

Figure 2: Repetitive pipetting 8 aliquots of 10 µL with the "precise" mode using the P100 pipette

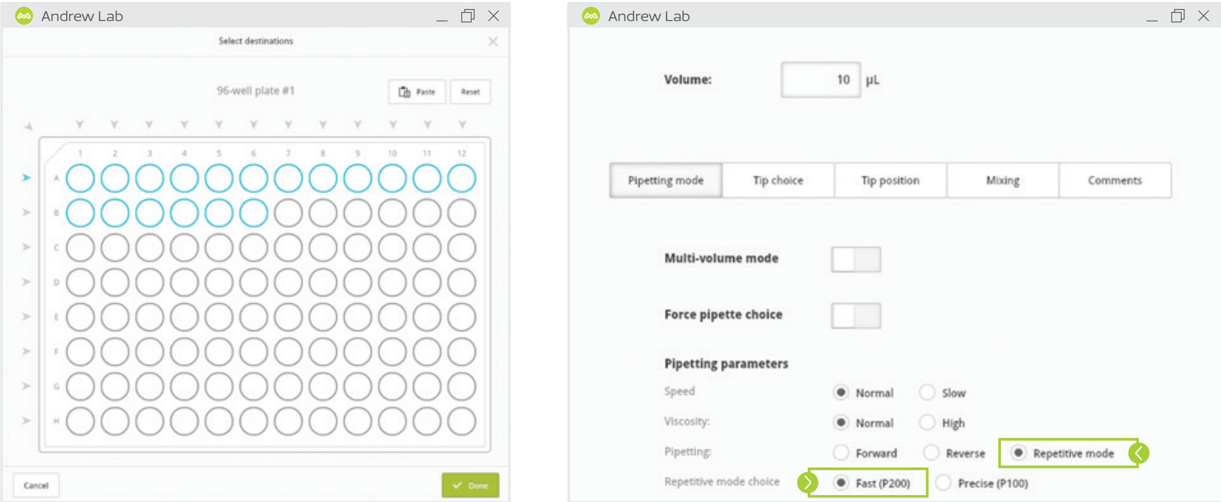

Figure 3: Repetitive pipetting 18 aliquots of 10 µL with the "fast" mode using the P100 pipette

The random error of the desired aliquoted volume that was repetitively dispensed in each series was calculated by multiplying the series standard deviation by the expected aliquoted volume. For calculating the systematic errors, we have previously provided the performance test results showing the superior accuracy of Andrew pipetting different volumes using single dispensing **[2]**. Therefore, the single-dispensed volumes by the pipette which is best fit for the volume (i.e. P2 for 2 µL; P20 for 5, 10 and 20 µL; P100 for 50 and 100 µL) were also obtained and used as the reference standard.

Pipette (operation mode)	**Aliquoted Volume** (µL)	**Number of Aliquots**	**Andrew Systematic Error (µL)**	**ISO8655 Systematic Error** (µL)		**Andrew Random Error (µL)**	**ISO8566 Random Error** (µL)		**Time to fill 96-well microplate** (min/sec)	
				Single-channel	**Multi-channel**		**Single-channel**	**Multi-channel**	**min**	**max**
P1000	100	8	**±0.75**	±8	±16	**<4.8**	<3	<12	10m23s	11m58s
(fast)	50	8	**±0.63**	±8	±16	**<4.5**	<3	<12	7m2s	9m40s
P200	20	8	**±0.69**	±1.6	±3.2	**<1**	<0.6	<1.2	9m51s	10m21s
(fast)	10	18	**±0.26**	±1.6	±3.2	**<0.7**	<0.6	<1.2	7m24s	10m10s
P100	10	8	**±0.42**	±0.8	±1.6	**<0.6**	<0.3	<0.6	9m30s	10m35s
(precise)	5	18	**±0.5**	±0.8	±1.6	**<0.6**	<0.3	<0.6	7m3s	8m42s
P20	2	8	**±0.29**	±0.2	±0.4	**<0.39**	<0.1	<0.2	9m9s	10m14s
(precise)										

Table 1: Andrew performance in "repetitive dispensing" mode

Table 1 shows Andrew's systematic and random errors of the tested aliquotted volumes for each pipette in comparison with the reference maximal permissible systematic and random errors recommended by the ISO 8655 standards for single- and multi-channel pipettes **[3]**. Note that in our test Andrew dispensed the liquid «on-the-fly» at ambient temperature and humidity of a normal lab in daily operation, while ISO 8655 standards are for dispensing the liquid into liquid at constant temperature and very high humidity to prevent evaporation. Thus, our data were obtained in strictly daily laboratory conditions as opposed to reference ISO 8655 data not normally seen in real-life laboratory settings. Despite the more demanding conditions, Andrew's systematic errors for the volume range of 5 – 100 µL are all below the ISO 8655 standards of the single channel pipettes. Even when dispensing volumes that are only 5% of nominal volumes of the pipettes, Andrew was still able to achieve an outstanding accuracy.

Andrew's precision of repetitive dispensing is generally as good as or better than the precision of the multi-channel pipette recommended by ISO 8655. Notably, the maximum random errors of 4.8 and 4.5 µL in 8- and 18-aliquot series of 100 and 50 µL, respectively, using the P1000 pipette, underscores that Andrew is able to maintain the excellent repetitive dispensing performance well within the ISO standard of 6 µL random error for the single dispense of the single channel pipette, and far below the 12 µL random error of the multi-channel pipette.

At lower volumes with the P200 (8 aliquots of 20 µL, 18 aliquots of 10 µL) and P100 pipettes (8 aliquots of 10 µL, 18 aliquots of 5 µL), the random errors fall at the maximal permissible error of the ISO 8655 standards for the multi-channel pipette. The observed difference in maximum random errors of multi-dispensing 10 µL from the P100 and P200 pipette was 0.1 µL, and the time to fill one 96-well microplate completely using the P200 pipette in "fast" mode for 18 aliquots was ~ 1.75 – 2.1 min shorter than the P100 pipette in "precise" mode. Hence, users can choose either pipetting mode option in Andrew Lab according to the tolerable level for random errors and the importance of processing time.

Designing the repetitive dispensing experiment in the software Andrew Lab for a 96-well plate (*www.AndrewAlliance.com/repetitive_pipetting_protocol_1*) takes less than a minute, and only another minute is needed to arrange the labware (microplate, tip boxes, liquid source) for Andrew. Thus, with small variable processing times for Andrew to completely fill out a 96-well microplate, the actual hands-on time needed by the human operator is only 2 minutes, a very significant time saving in comparison with using multi-channel pipettes or electronic repeater pipettes.

"REPETITIVE PIPETTING" MODE OF ANDREW – HOW DOES IT WORK?

When operating in the "repetitive pipetting" mode, Andrew aspirates the liquid samples from the source at the maximum allowed volume of the pipettes (i.e. "nominal volume"), moves the pipette to the correct destination, depresses the pipette plunger partially down to the appropriate level for dispensing the correct smaller volume of liquid, then releases the plunger and moves to the next destination to dispense the same volume in the same manner, in succession until the remaining volume in the tip is no longer sufficient for the next dispense. The left-over is dispensed back to the source, saving the precious reagents when applicable. As the tip end neither dips into the liquid inside the destination nor touches the bottom of the destination vessel, the liquid is dispensed **"on the fly"**, preventing contamination. This entire repetitive dispensing cycle is repeated until all destinations are filled.

A known issue with repetitive pipetting is the inaccurate dispense of the first and the last aliquot when handling liquids different from pure water and in difficult environmental conditions (such as quickly changed temperature and humidity). In order to circumvent this potential issue in "repetitive dispensing" operations, Andrew is programmed to discard the first and the last aliquots back to the source. Therefore, the number of aliquots per refill by Andrew is calculated as followed:

$$\textit{Number of aliquots per refill} = \frac{\textit{Maximum (Nominal) volume of pipette}}{\textit{Desired aliquoted volume}} - 2$$

The final result is the next smaller integer number. As pipettes perform better at volumes closer to their nominal volume (best at 35 – 100% nominal volume), pipette manufacturers generally recommend the 10% of the nominal volume as the minimum dispensed volume for single dispensing. However, Andrew is programmed to repetitively dispense the minimum volume at 5% the nominal volume. Therefore, in some cases, a desired aliquoted volume can be repetitively dispensed by two different pipettes. For example, 15 µL represents 15% of the P100 nominal volume and 7.5% of the P200 nominal volume. In this case, to design the pipetting protocol for Andrew, users can choose between the two pipettes when selecting a repetitive dispensing mode: the "precise" mode is designed to achieve higher accuracy with fewer aliquots **(Figure 2)** or the "fast" mode for more aliquots treated with a lower precision **(Figure 3)**.

In more details of the 15 µL example above, Andrew can use the P100 pipette in "precise" mode or a P200 pipette in "fast" mode. Respectively, Andrew will dispense (100/15 – 2) = 4.67 → 4 aliquots/refill in "precise" mode, while using a P200 pipette in "fast" mode it will dispense (200/15 – 2) = 11.33 → 11 aliquots/refill. Depending on the experiments and cost/benefit analysis, users therefore can select the best option according to their most important criteria.

SUMMARY

For the first time, we demonstrate the possibility and the performance of repetitive dispensing with standard mechanical single-channel pipettes. Excellent results emerge, showing Andrew as an effective and concrete solution to replace traditional liquid handlers and electronic pipettes for the purpose of repetitive pipetting. The Andrew robot enables effective automation by freeing your time and providing results in a fast and efficient way: designing a repetitive dispensing protocol to fill a microplate takes two minutes, and filling a 96-well plate can take down to 7 minutes, fully unattended. By using Andrew, laboratories can realize a very quick return on their investment while improving data reproducibility and preserving worker well-being without the need of altering existing workflows or the choice of consumables.

REFERENCES

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