Volatile Liquid Handling with Opentrons OT-2

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INTRODUCTION

Volatile liquids, liquids that evaporate readily at room temperature, are common reagents in various applications. For example, ethanol, isopropanol, acetone and methanol, notably for various methods of nucleic acid purification [1] . As the temperature increases, the volatility rate (or rate of evaporation) increases exponentially [2]. Even at room temperature, they are tricky to pipette whilst the most common problem is dripping out of the tip. Constant evaporation of the liquid presents challenges for pipetting volatile liquids. Higher vapor pressure results in faster evaporation of the liquid and faster ejection of liquid out of the tip. For volatile liquids, an increase in temperature increases the volatility of the liquid, and subsequently the evaporation rate and the vapor pressure. Conversely, diluting the liquid decreases the volatility and corresponding characteristics. Here we present liquid handling optimizations for commonly automated volatile liquids, including various dilutions of ethanol and isopropanol.

KEY VOLATILE LIQUID HANDLING TECHNIQUES

Pre-wetting tips `mix()`

Pre-wetting is a pipetting strategy to repeatedly aspirate and dispense a defined volume of the liquid which will be aspirated. The high vapor pressure of volatile liquids causes the air gap between the nozzle and the liquid surface to expand, forcing them to drip out of the tip. Pre-wetting saturates the vaporized solvents in the air gap between the nozzle and the liquid surface to help maintain a lower vapor pressure.

FIGURE 1

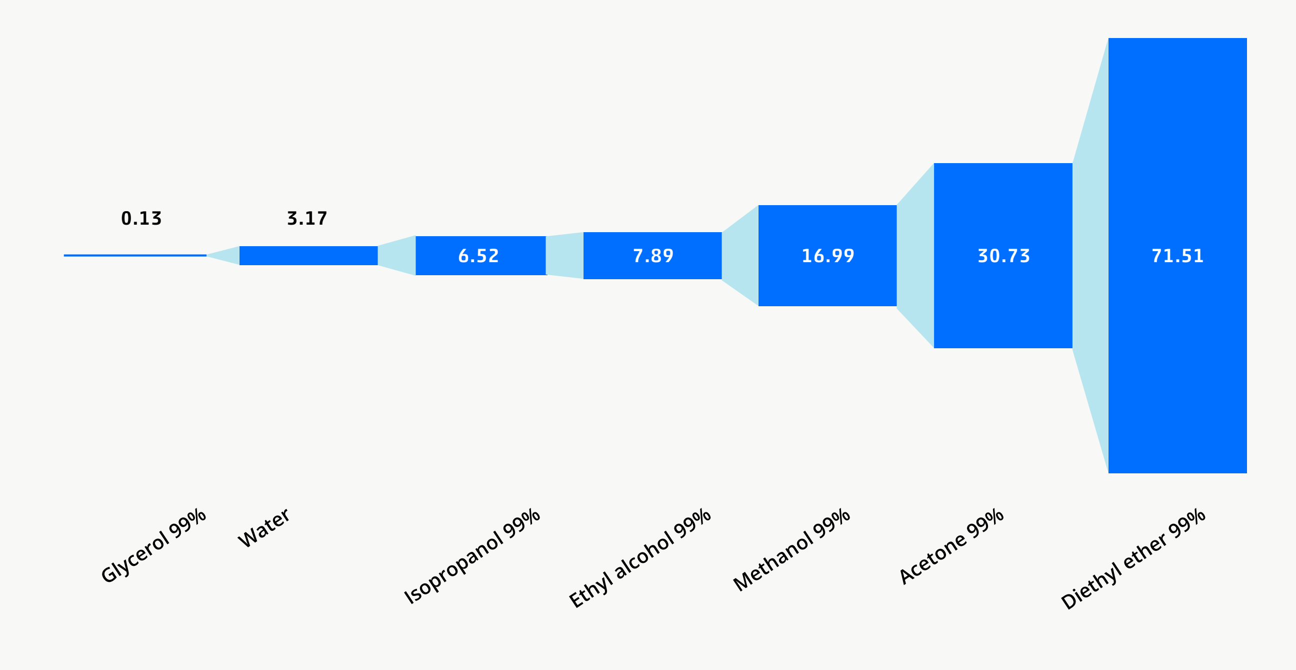

Figure 1. *TVapor pressure (kPa) of commonly used volatile liquids at 25˚C.*

Trailing air gap `aspirate()`

We can allow air to be present at the tip orifice to avoid the spilling. The air gap is dependent on the vapor pressure of the liquid, the volume of the liquid aspirated and most importantly the time taken by the tip to move from the aspirated deck position to dispense position. Most volatile liquids are less dense than water (<997 kg/m3). When air is aspirated following the volatile liquid aspiration, the liquid inside the tip accelerates upwards, which can cause clogging of the filter or pipette nozzle. To avoid such a problem, the trailing air gap flow rate speed must be maintained very low. Liquids with higher vapor pressure such as acetone, diethyl ether etc. we can see the 'tears of wine' effect [5], where rings of liquid are passed through the trailing air gap creating a vacant space between the aspirated liquid and liquid at the orifice further dripping off the tip.

Tip withdrawal speed `move_to()`

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Post-dispense delay `protocol.delay()`

Two factors are critical to accurately dispense volatile liquids. First, due to lower surface tension, the liquid sticks to the interior of the tip surface and slowly moves towards the tip orifice after the remaining liquid is dispensed. Second, the alcohol-rings or popularly known wine tears cause liquid sticking above the liquid surface to slide towards the tip orifice creating a dead volume. Adding a post-dispense delay reduces the dead volume.

Double Blowout `blow_out()`

An additional blowout can be added for complete dispensing. In order to perform a second blow out, 1 µL of air is aspirated by withdrawing the tip out of liquid and a second blow out is performed (in or out of liquid). Aspiration of 1 µL air allows the plunger to retain its original position with a small displacement.

OPTIMIZATION STRATEGY FOR VOLATILE LIQUIDS

Step 1

Hover to the top of the aspiration well and immerse the tip at a slower speed.

Step 2

Immerse the tip inside the liquid and perform prewetting. Choose the number of prewetting steps based on the volatility of the liquid. `mix()` in Python API, allows you to mix the liquid "n" times.

Step 3

`aspirate()` volatile liquid at water calibrated the flow rate.

Step 4

The tip withdrawal speed must be reduced to 50 mm/s for most of the volatile liquids. This avoids splashing of liquids on the labware walls. `move_to()` the top of labware at 50 mm/s.

Step 5

Depending on the vapor pressure, adjust the air gap volume. The `pipette.flow_rate.aspirate()` of the air gap must be kept 1/20th times the water flow rate for the respective pipette.

Step 6

`dispense()` volatile liquid with tip immersed <2.5 mm below the liquid at water calibrated flow rate. This prevents an increase in dead volume.

Step 7
Add `protocol.delay()` of 3 - 4 seconds depending on the vapor pressure of the liquid. Adding delay allows the liquid to settle at the tip before performing a blowout.

Step 8
Perform `pipette.blow_out()` at water calibrated blow out flow rate.

Step 9 (Optional)
If only, after the first blow out excess liquid is left inside the tip, withdraw the tip using `move_to(well.top())` and `aspirate()` 1 µL of air and immerse the tip `(move_to())` inside the liquid and perform a second blow out.

Step 9
If necessary, perform `touch_tip()` at a location depending on the necessary height inside the labware.

Step 10
Withdraw the tip, `move_to(well.top())` at a slower flow rate of 50 mm/s.

MATERIALS AND METHODS

Three dilutions of ethanol, 70%, 80% and 99% (Sigma Aldrich, Catalog no. 752X, E-030, 1.07017) were tested for volumetric quantification as representative volatile liquids and compared against water. Gravimetric measurements were performed with a microbalance (Radwag, XA 6/21.4Y.M.A.P PLUS Microbalance). Four Opentrons GEN2 P20, P300 and P1000 pipettes were tested at volumes. Opentrons P20 non-filter, P300 non-filter and P1000 non-filter tips were used for pipetting volatile liquids. All liquids were tested between 22˚C to 25˚C ambient temperature and 50% - 60% relative humidity. The maximum aspirated volume of each pipette was reduced to compare air gap effects. The optimized parameters listed in table 1 were used for volumetric testing on the gravimetric setup.

All parameters were programmed using the Opentrons Python API using internal pipette verification protocols. The tip remained outside of the liquid for approximately 15 seconds.Ten replicates per volume per pipette were collected with four different pipettes. The target volumes for the Gen2 P20 pipettes were 1 µL, 10 µL, 15 µL and 18 µL. The target volumes for Gen2 P300 were 20µL, 100µL and 200µL. The target volumes for Gen 2 P1000 were 100 µL, 500 µL and 950 µL.

RESULTS AND DISCUSSIONS

To measure the performance of the OT-2 pipettes' capability in accurately handling the most commonly used representative volatile liquids, experimentally relevant dilutions of ethanol, isopropanol, acetone and methanol were investigated. Ethanol and isopropanol have similar vapor pressure ranges, therefore we have employed similar pipetting parameters.

Pre-wetting increases the volume inside the pipette due to the volume adhering to the surface of the tip after the pre-wetting action is completed. The benefit of the pre-wetting can be seen as lowering the dripping of volatile liquid. Without pre-wetting, higher concentrations of ethanol, particularly 99% ethanol, were more prone to dripping rapidly even when trailing air gap aspiration was performed. This resulted in contamination around the deck and unable to reach target dispense volume.

Though there are limited use cases of aspiration of <10 µL volatile liquid, the aspiration and dispensing can present challenges. Pre-wetting for these low volumes can cause overaspiration. However, while dispensing with P20, ~1.5 µL of dead volume is consistently observed for all the tested volumes. The dead volume comprises the evaporated liquid in the tip and the remaining liquid is adhered to the tip.

Reverse pipetting, wherein liquid is pre-aspirated for full volume and dispensed with target volume, for liquids with high vapor pressure can also affect accuracy. A higher air gap volume can help reduce this effect. Since it remains unclear the amount of liquid to be dispensed to achieve the target volume due to loss in air gap volume, the reverse pipetting can result in additional inaccuracies.

CONCLUSION

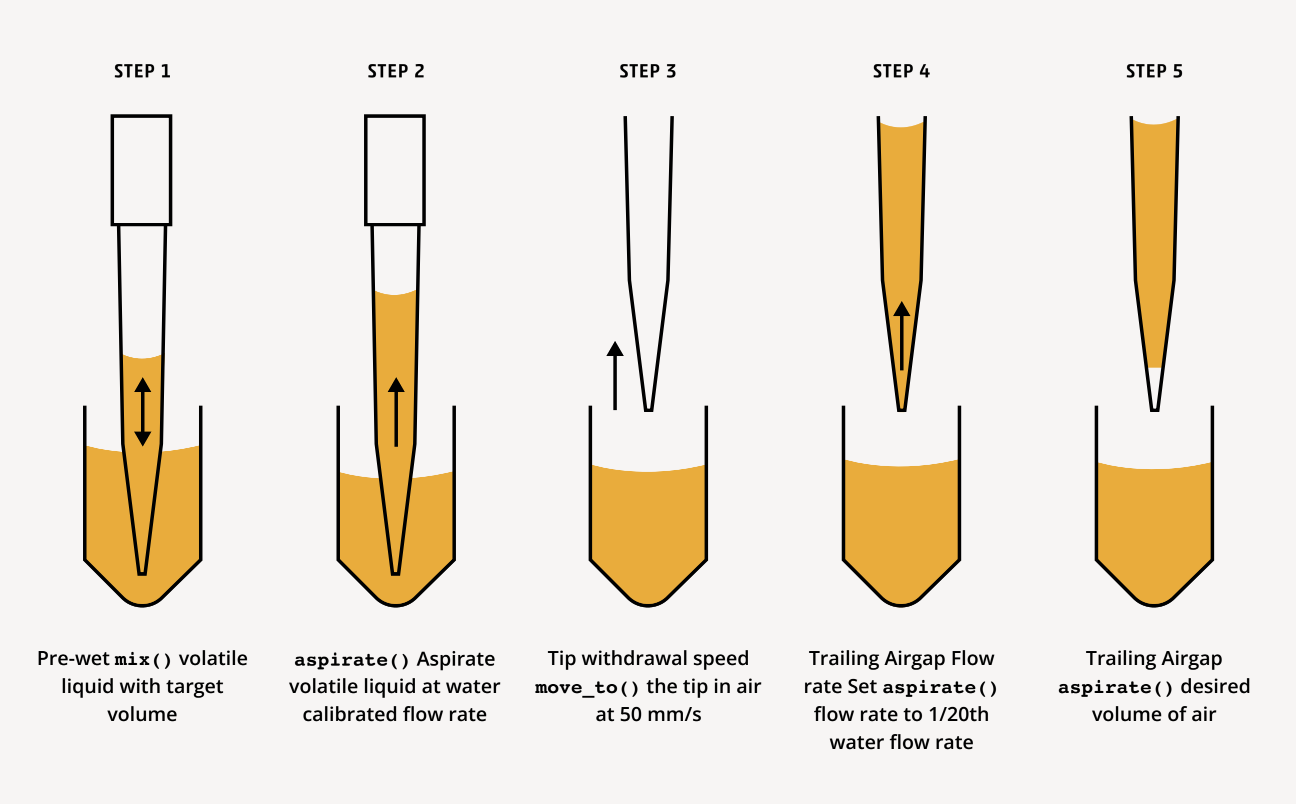
The Opentrons GEN2 P300 and P1000 show accuracy similar to the pipette performance for water. Influence of pre-wetting steps results in overaspiration of volumes in all the three tested pipettes. In case of P20 usage for volatile liquid handling, it is recommended to perform a pre-evaluation of the desired target volume for the protocol with consideration of 1 to 2 μL dead volume. Please, contact our support for appropriate recommendations based on your protocol. All the optimized parameters are specific for discussed environmental factors and any change in temperature or humidity may affect the discussed parameters.

While handling liquids with higher vapor pressure, greater than 30 kPa depending on the ambient temperature, we recommend increasing pre-wetting mixes (>2 times), adding appropriate air gap volume and decreasing flow rate for efficient liquid transfer.

Dead volume is dependent on the vapor pressure of volatile liquid, pipette volume (e.g. P20, P300 or P100), tip size and immersion of tip depth during dispensing. Immersing the tip <2.5 mm during dispense will result in lower dead volumes. Liquids with lower vapor pressure and higher density will have lower dead volume. If touch tip or blowout are not desired in the protocol, it is recommended to consider the target volume based on the dead volume.

The results present significant improvement in volatile liquid handling performance by implementing the optimized volatile liquid handling parameters . The users will be able to customize parameters depending on the specific volatile liquid employed in the protocol.

FIGURE 2a



STEP 1

Pre-wet `mix()` volatile liquid with target volume

STEP 2

`aspirate()` Aspirate volatile liquid at water calibrated flow rate

STEP 3

Tip withdrawal speed `move_to()` the tip in air at 50 mm/s

STEP 4

Trailing Airgap Flow rate Set `aspirate()` flow rate to 1/20th water flow rate

STEP 5

Trailing Airgap `aspirate()` desired volume of air

Figure 2 (a). *Optimized strategy for aspiration of volatile liquid.*

FIGURE 2b

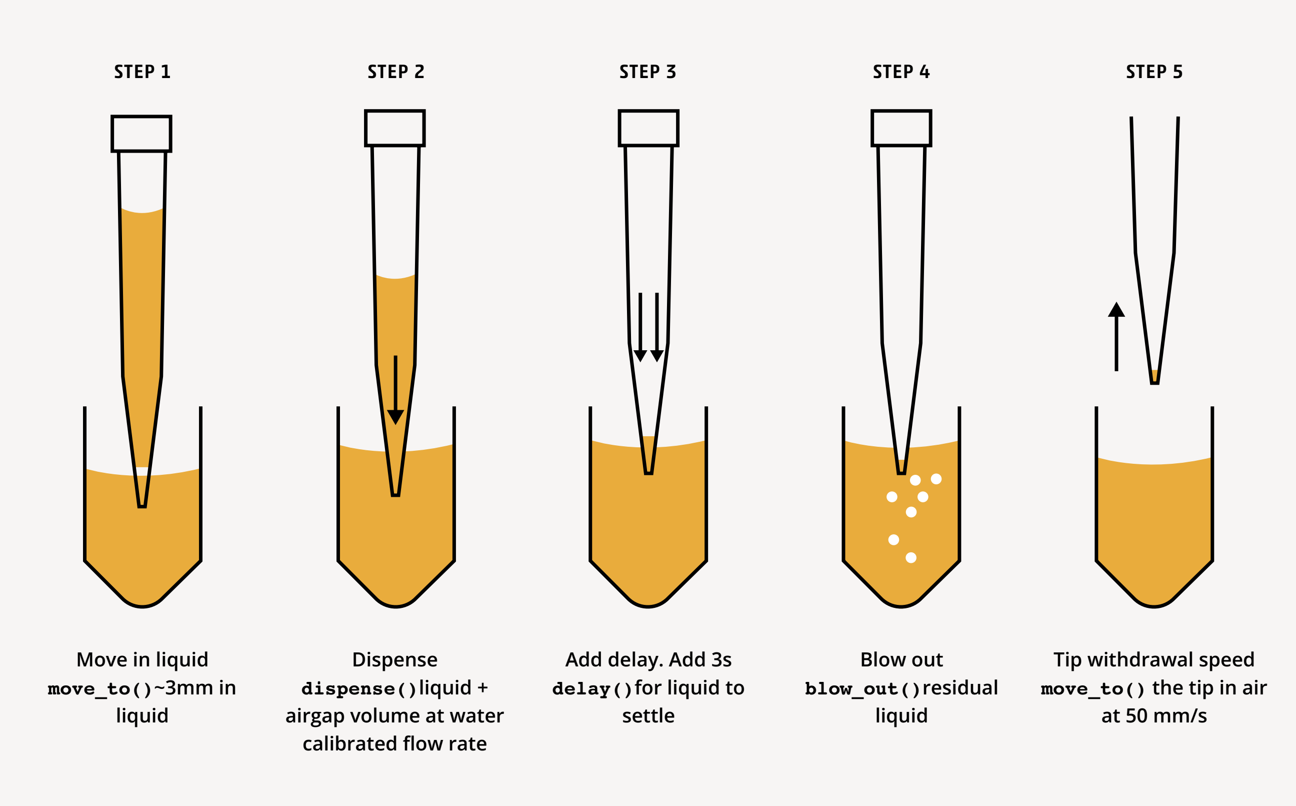
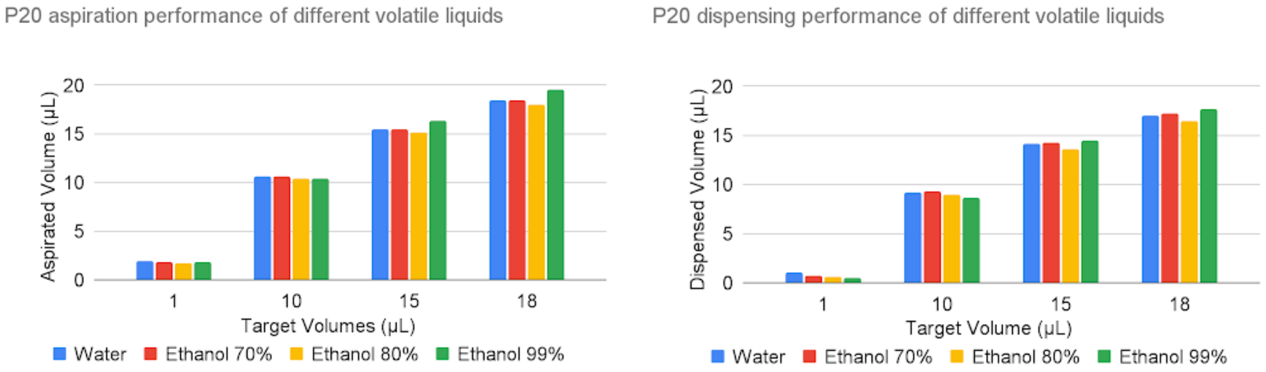


Figure 2 (b). *Optimized strategy for dispensing volatile liquids.*

FIGURE 3


P20 aspiration performance of different volatile liquids

P20 dispensing performance of different volatile liquids

Figure 3. *Comparison of different liquids aspirated (left) and dispensed (right) by P20.*

FIGURE 4

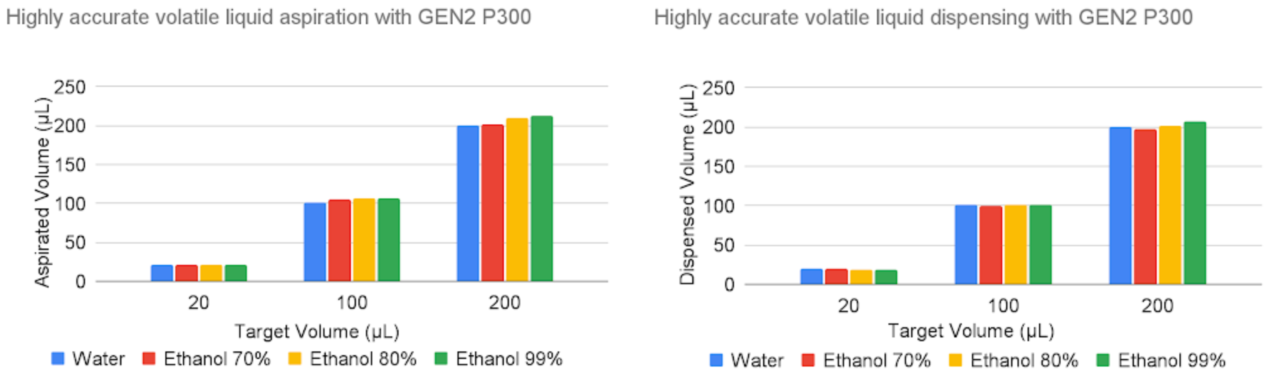


Figure 4. *Highly accurate volatile liquid aspiration (left) and dispensing (right) with Opentrons GEN2 P300 pipettes.*

FIGURE 5

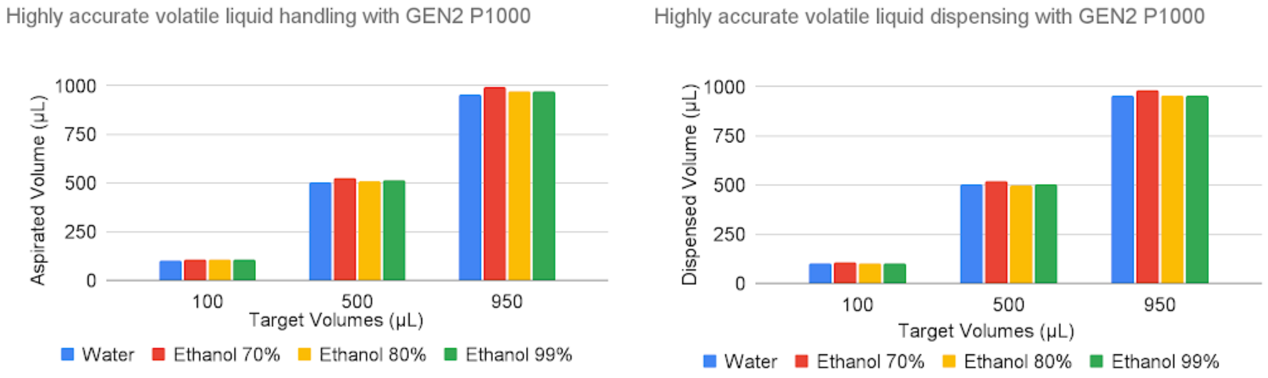


Figure 5. *Highly accurate volatile liquid aspiration (left) and dispensing (right) with Opentrons GEN2 P1000 pipettes.*

TABLE 1

		ETHANOL 70%	ETHANOL 80%	ETHANOL 90%
P20	**Vapor Pressure (kPa)**	5.95	6.43	7.869
	Pre-wet	1	1	1
	Pre-wet Volume	Liquid Volume	Liquid Volume	Liquid Volume
	Aspirate Rate (µL/s)	7.56	7.56	7.56
	Withdraw to Top Speed (mm/s)	50	50	50
	Aspirate Air Gap	2	2	2
	Aspirate Air Gap Flow Rate (µL/s)	0.504	0.504	0.504
	Dispense Volume	Desired Volume	Desired Volume	Desired Volume
	Dispense Additional Volume	Air Gap Volume	Air Gap Volume	Air Gap Volume
	Delay (s)	3	3	3
	Blow out (s)	2	2	2
	Touch Tip	Yes	Yes	Yes
	Blow Out at Wall	Yes	Yes	Yes
	Withdraw to Top Speed (mm/s)	50	50	50

TABLE 1

		ETHANOL 70%	ETHANOL 80%	ETHANOL 90%
P300	**Vapor Pressure (kPa)**	5.95	6.43	7.869
	Pre-wet	1	1	1
	Pre-wet Volume	Liquid Volume	Liquid Volume	Liquid Volume
	Aspirate Rate (µL/s)	92.5	92.5	92.5
	Withdraw to Top Speed (mm/s)	50	50	50
	Aspirate Air Gap	7	7	10
	Aspirate Air Gap Flow Rate (µL/s)	4.625	4.625	4.625
	Dispense Volume	Desired Volume	Desired Volume	Desired Volume
	Dispense Additional Volume	Air Gap Volume	Air Gap Volume	Air Gap Volume
	Delay (s)	3	3	3
	Blow out (s)	2	2	2
	Touch Tip	Yes	Yes	Yes
	Blow Out at Wall	Yes	Yes	Yes
	Withdraw to Top Speed (mm/s)	50	50	50

TABLE 1

		ETHANOL 70%	ETHANOL 80%	ETHANOL 90%
P1000	**Vapor Pressure (kPa)**	5.95	6.43	7.869
	Pre-wet	1	1	1
	Pre-wet Volume	Liquid Volume	Liquid Volume	Liquid Volume
	Aspirate Rate (μL/s)	274.5 uL/s	274.5 uL/s	274.5 uL/s
	Withdraw to Top Speed (mm/s)	50	50	50
	Aspirate Air Gap	20	20	20
	Aspirate Air Gap Flow Rate (μL/s)	5	7	10
	Dispense Volume	Desired Volume	Desired Volume	Desired Volume
	Dispense Additional Volume	Air Gap Volume	Air Gap Volume	Air Gap Volume
	Delay (s)	4	4	4
	Blow out (s)	2	2	2
	Touch Tip	Yes	Yes	Yes
	Blow Out at Wall	Yes	Yes	Yes
	Withdraw to Top Speed (mm/s)	50	50	50

REFERENCES

1. Dairawan, Mariyam, and Preetha J. Shetty. “The evolution of DNA extraction methods.” *American Journal of Biomedical Science & Research* 8.1 (2020): 39-45.
2. Olsen, Erik, and Frands Nielsen. “Predicting Vapour Pressures of Organic Compounds from Their Chemical Structure for Classification According to the VOCDirective and Risk Assessment in General.” Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry vol. 6,4 370–389. 31 Mar. 2001, doi:10.3390/60400370
3. Wolfram|Alpha: Making the World’s Knowledge Computable. https://www.wolframalpha.com. Accessed 15 Oct. 2021.
4. Opentrons. “Opentrons Electronic Pipettes.”, 15 Oct. 2021, https://opentrons.com/publications/Opentrons-Master-Pipette-White-Paper.pdf,Accessed 15 Oct. 2021.
5. Gugliotti, Marcos and Todd Silverstein. “Tears of Wine.” Journal of Chemical Education, vol. 81, no. 1, Jan. 2004, p. 67. DOI.org (Crossref), https://doi.org/10.1021/ed081p67.