



Tips and Tricks for Automating Viscous Liquid Handling

November 10, 2021

Webinar Overview

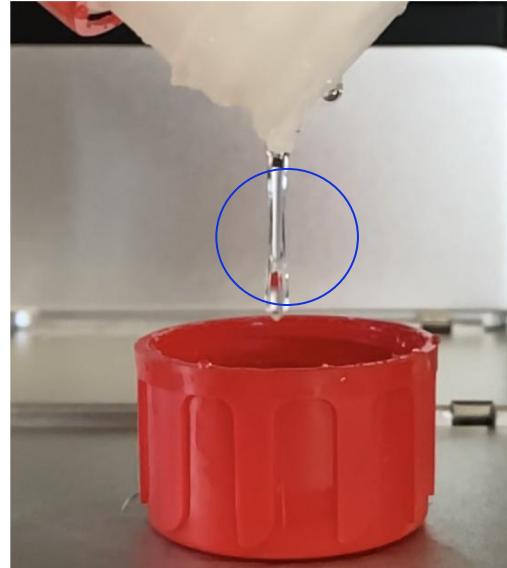
- Understand the properties of different viscous liquids and optimize liquid handling parameters
- Accurately handle viscous liquids on the OT-2
- Reduce reagent waste and avoid cross contamination
- Improve the reliability of protocols running viscous liquids

Issues pipetting viscous liquids



Adhesion

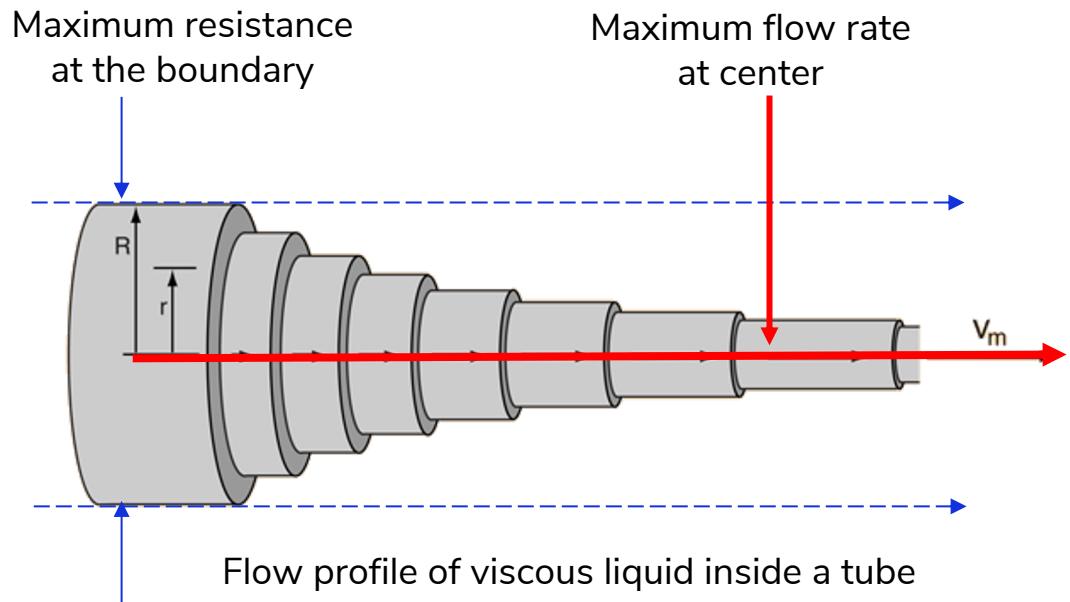
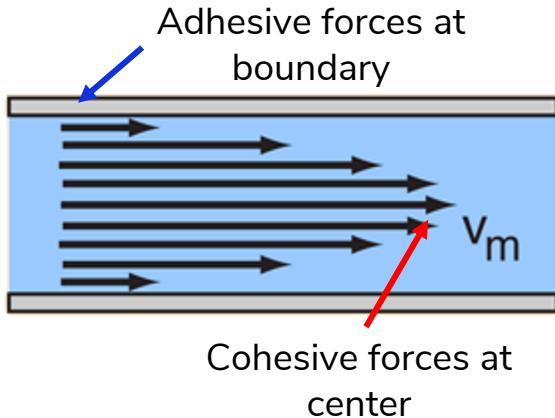
Forces between labware and liquid



Cohesion

Forces between liquid

Laminar flow inside a tip

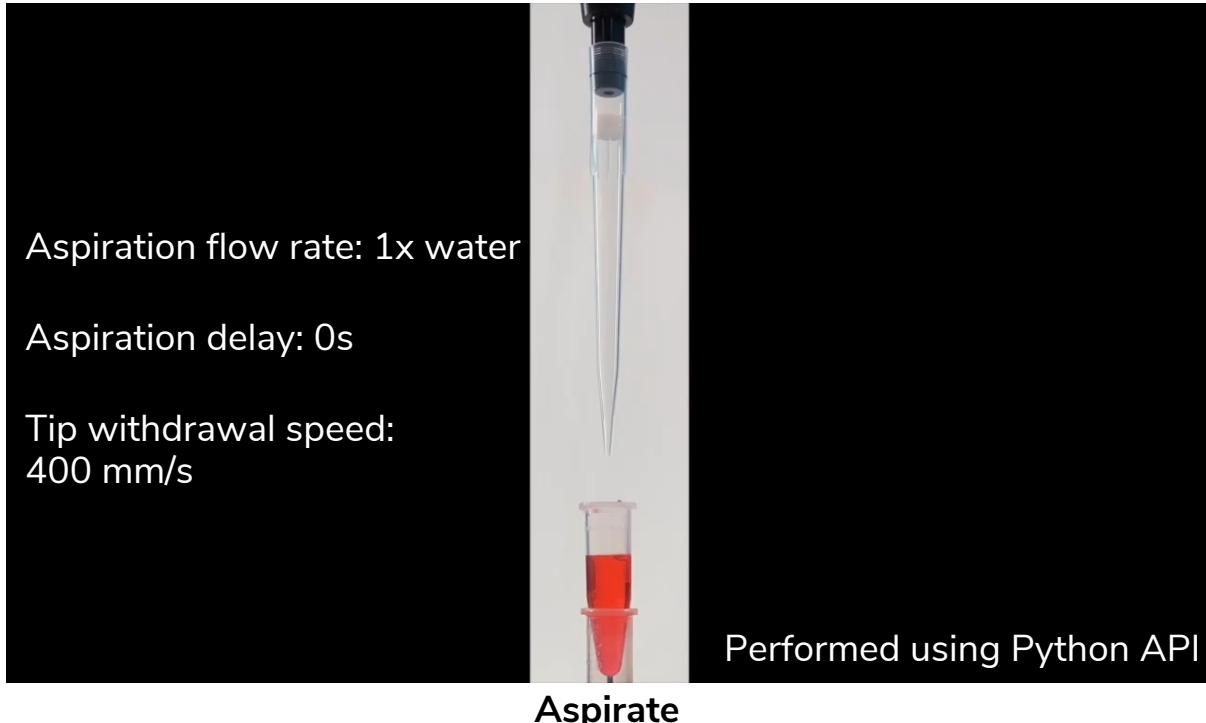


Based on the relative ratio of the adhesive and cohesive forces, which dominate inside a tip for a given liquid, we characterize viscous liquid classes

Viscous liquid characterization

Glycated Liquids	Volatile Viscous Liquids	Surfactant Viscous Liquids	Oils
Honey, Glycerol, PEGs etc.	Beads, hydrophobic coatings, hand sanitizer etc.	Liquid soaps, Tween® 20, Triton® X – 100 etc.	Mineral oil, engine oil etc.
 High adhesion  High cohesion	 Very High adhesion  Neutral cohesion	 Very high adhesion  Very low cohesion	 High adhesion  High cohesion

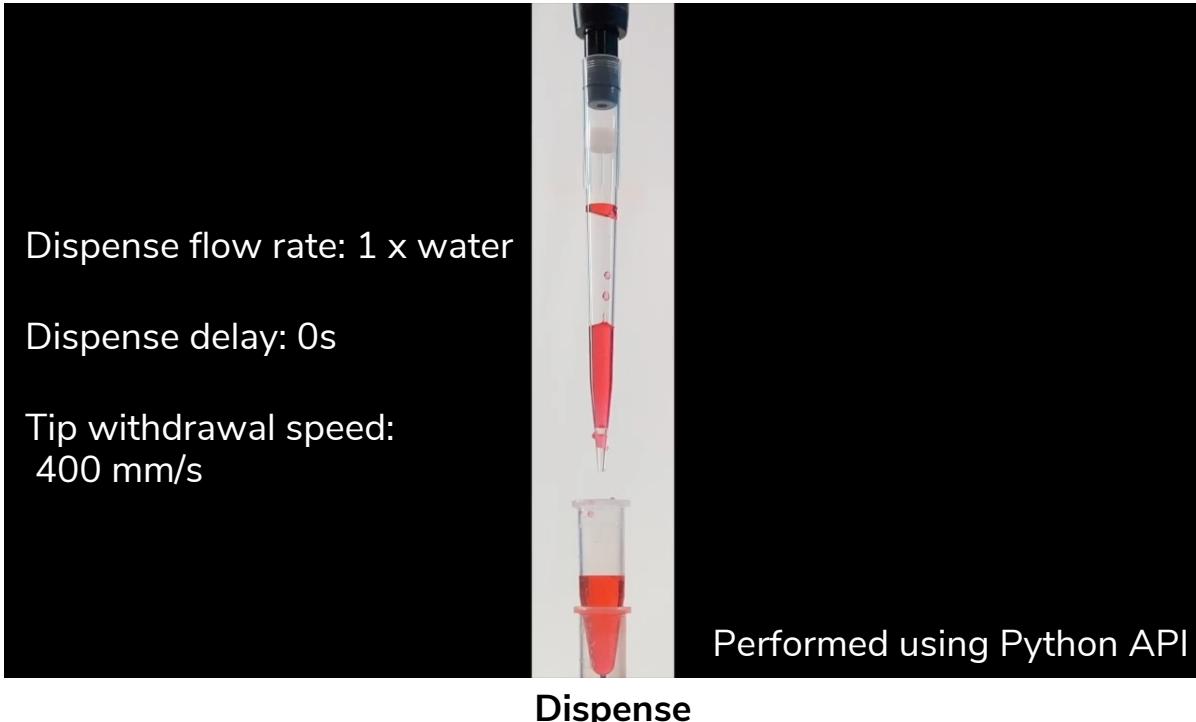
Pipetting viscous liquid with water parameters on OT-2



Common Problems

- Incomplete aspiration
- Droplets formed around the tip
- Spilling of reagent
- Incomplete Dispense
- Reagent waste
- Overflow of reagent
- Non-reproducible volumes

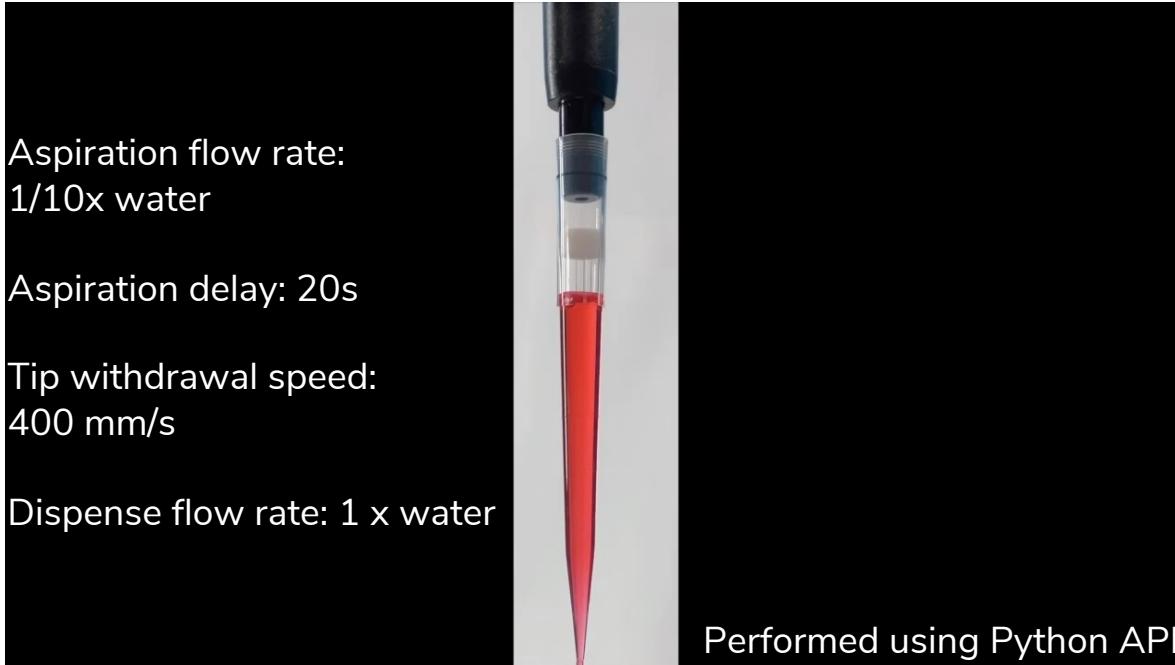
Pipetting viscous liquid with water parameters on OT-2



Common Problems

- Incomplete aspiration
- Droplets formed around the tip
- Spilling of reagent
- Incomplete Dispense
- Reagent waste
- Overflow of reagent
- Non-reproducible volumes

How to optimize any viscous liquid parameters for smoother handling



Aspiration flow rate:
1/10x water

Aspiration delay: 20s

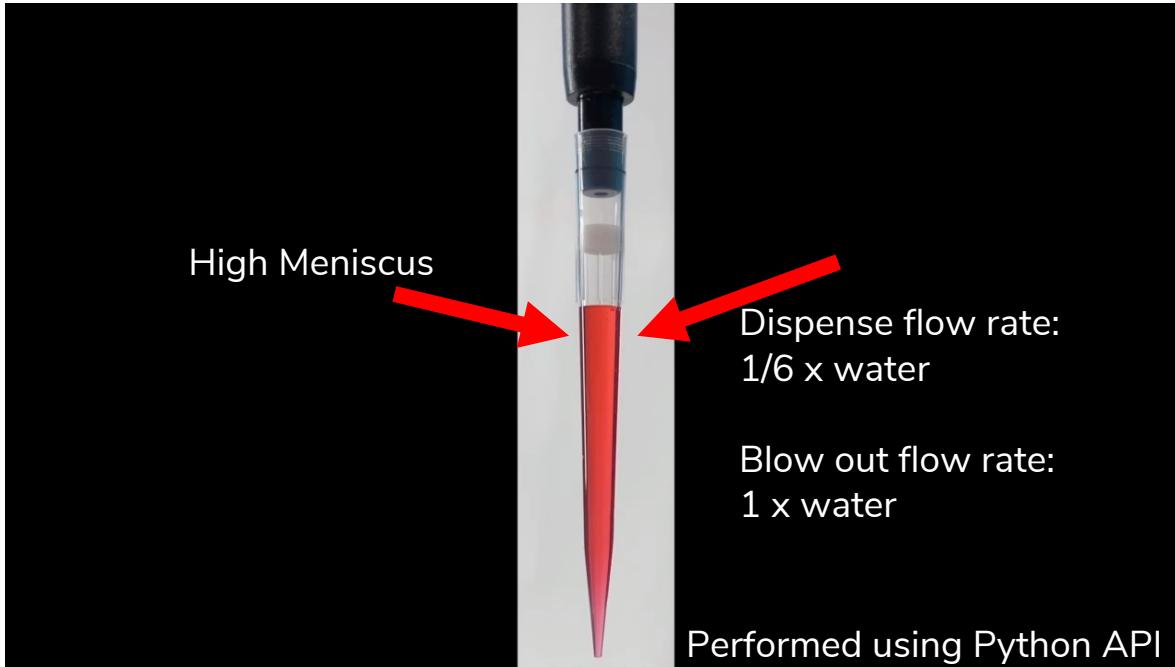
Tip withdrawal speed:
400 mm/s

Dispense flow rate: 1 x water

Performed using Python API

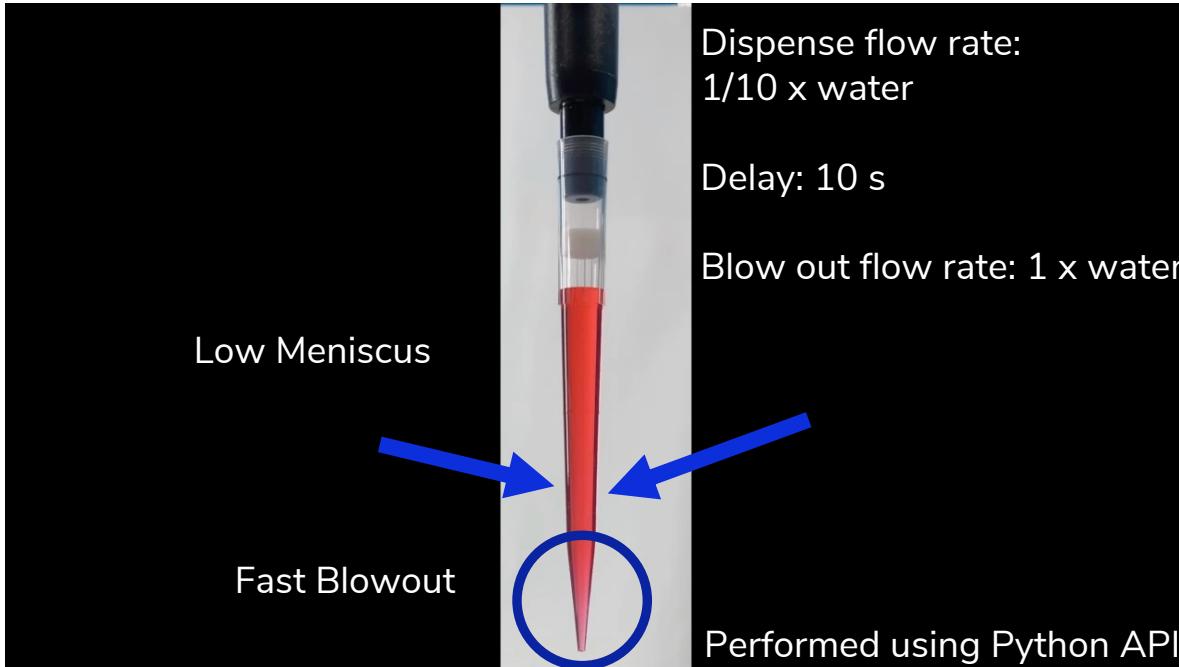
Step 1: Aspirate with slowest flow rate and start dispense with water flow rate

How to optimize any viscous liquid parameters for smoother handling



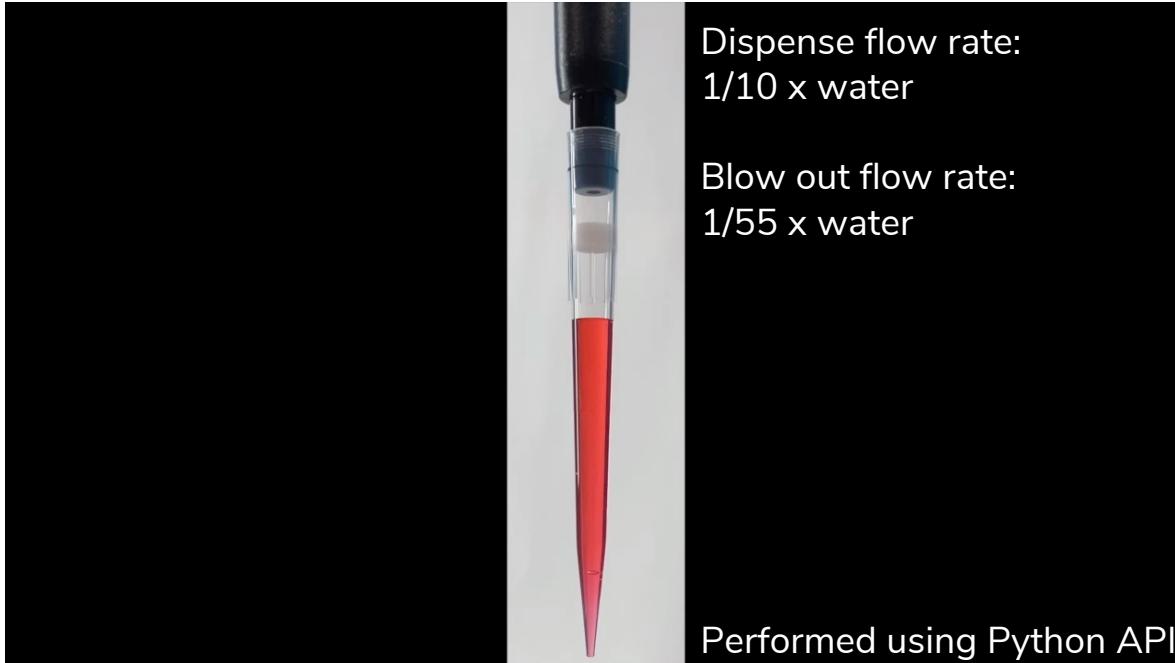
Step 2: Lower the flow rate to prevent breaking of meniscus at the top of tip

How to optimize any viscous liquid parameters for smoother handling



Step 3: Lower the blow out flow rate to remove any liquid retention

How to optimize any viscous liquid parameters for smoother handling



Step 4: Clean and complete dispense of glycerol

Optimized strategy for viscous liquid handling: aspirate



Step 1: Hover to Top

- `move_to()` to top of labware at default protocol speed



Step 2: Move to bottom

- `move_to()` to bottom of labware at 10 mm/s speed to aspirate



Step 3: Aspiration

- `aspirate()` at slower flowrate
- Add `delay()` after aspiration for the liquid to aspirate completely



Step 4: Tip Withdrawal

- `move_to()` from liquid to top of labware at 1 – 5 mm/s
- Use default speed to move to another well

Performed using Python API

Optimized strategy for viscous liquid handling: dispense



Step 1: Hover to Top



Step 2: Dispense



Step 3: Blowout



Step 4: Tip Withdrawal

- `move_to()` to top of labware at default protocol speed
- `move_to()` to bottom of labware at 10 mm/s speed to aspirate
- `dispense()` at slower flowrate
- Add delay() for the liquid to settle down and complete

- Residual liquid is removed with “slower” blowout
- `blowout()` at slower flowrate

- `move_to()` from liquid to top of labware at 1 – 5 mm/s
- Set the blowout flowrate to default pipette flowrate

Performed using Python API

Glycated viscous liquids

Properties

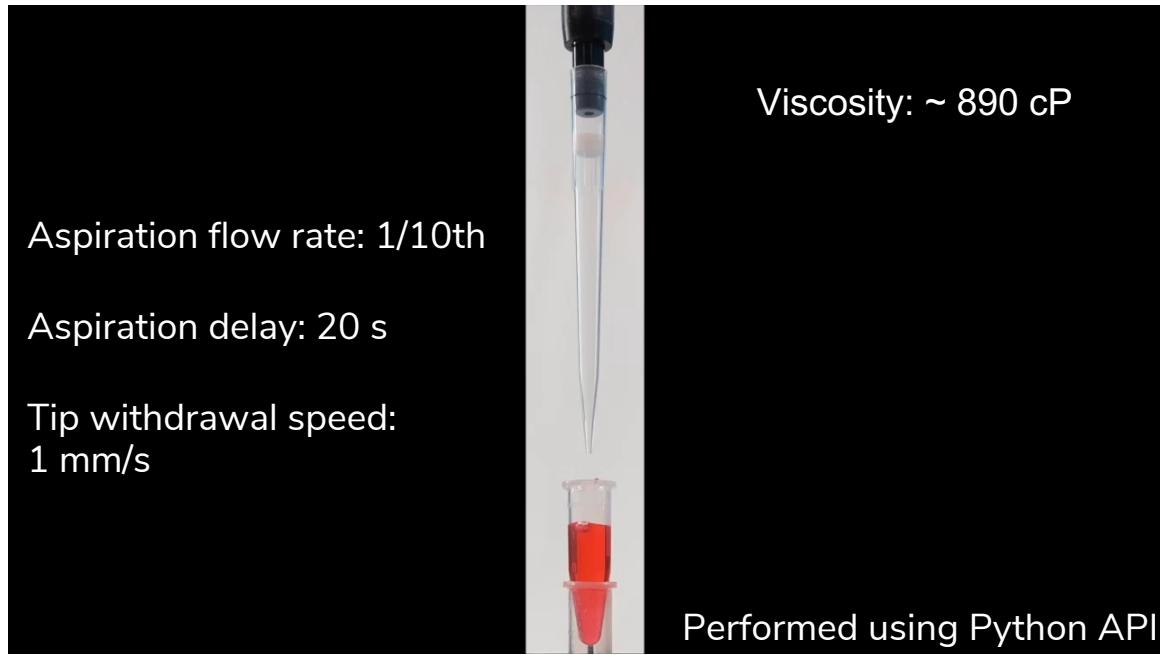
- Liquids with sugar or glycerol content
 - Examples: Glycerol, PEG etc.
- High adhesion to the pipette tip
- Highly cohesive

How do they move through the tip:

- Aspiration and dispense speeds are generally synchronized
- The liquid slowly slips off the tip's surface and accumulates to its bottom
- Small droplets may remain adhered to the tip

Optimized glycated liquid handling

Cohesion 
Adhesion 



Viscosity: ~ 890 cP

Aspiration flow rate: 1/10th
Aspiration delay: 20 s
Tip withdrawal speed:
1 mm/s

Benefits

- Highly accurate
- Clean Pipetting
- No spilling
- No droplets formed

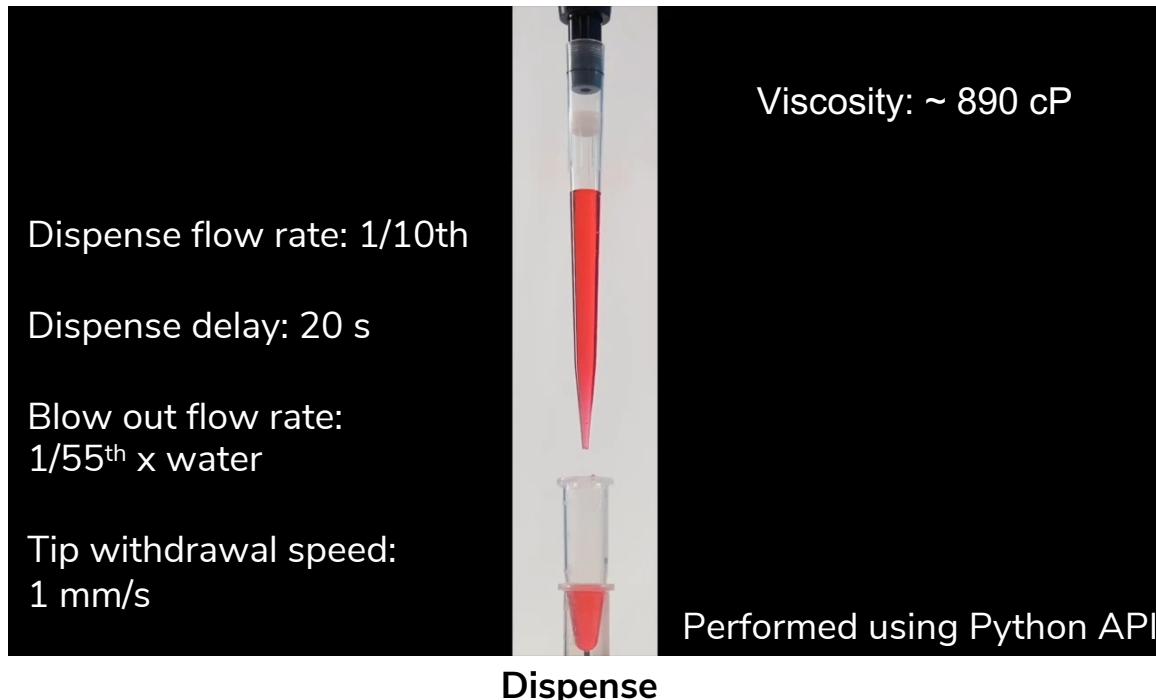
Limitations

- Longer Protocol runtime

Optimized glycated liquid handling

Cohesion 

Adhesion 



Benefits

- Highly accurate
- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime

Volatile viscous liquids

Properties

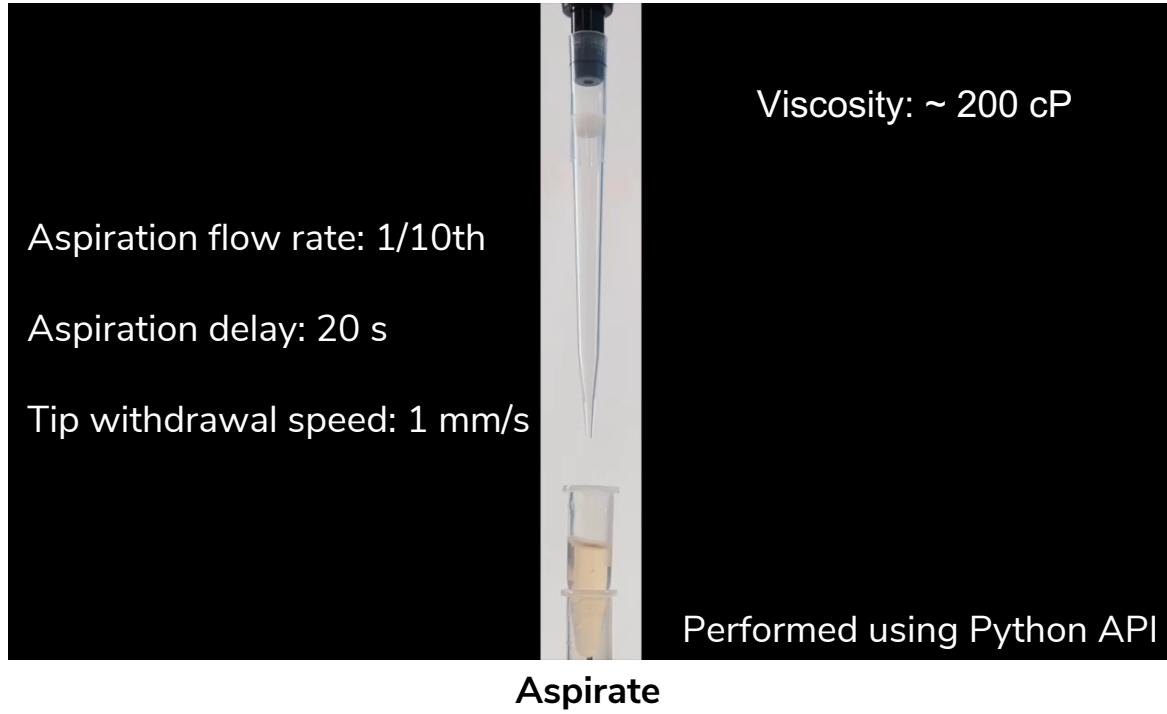
- Contains volatile solvent + viscous liquid such as beads or glycerol
 - Examples: Hydrophobic coating solvents, beads, hand sanitizer etc.
- High adhesion to the tip and very low cohesion to itself
- Variable volatility

How do they move through the tip:

- Sticks to the tip surface more than itself
- Forms an air jet if dispensed quickly
- May need air gap after aspiration to prevent spilling out from the tip

Optimized volatile viscous liquid handling

Cohesion
Adhesion 



Benefits

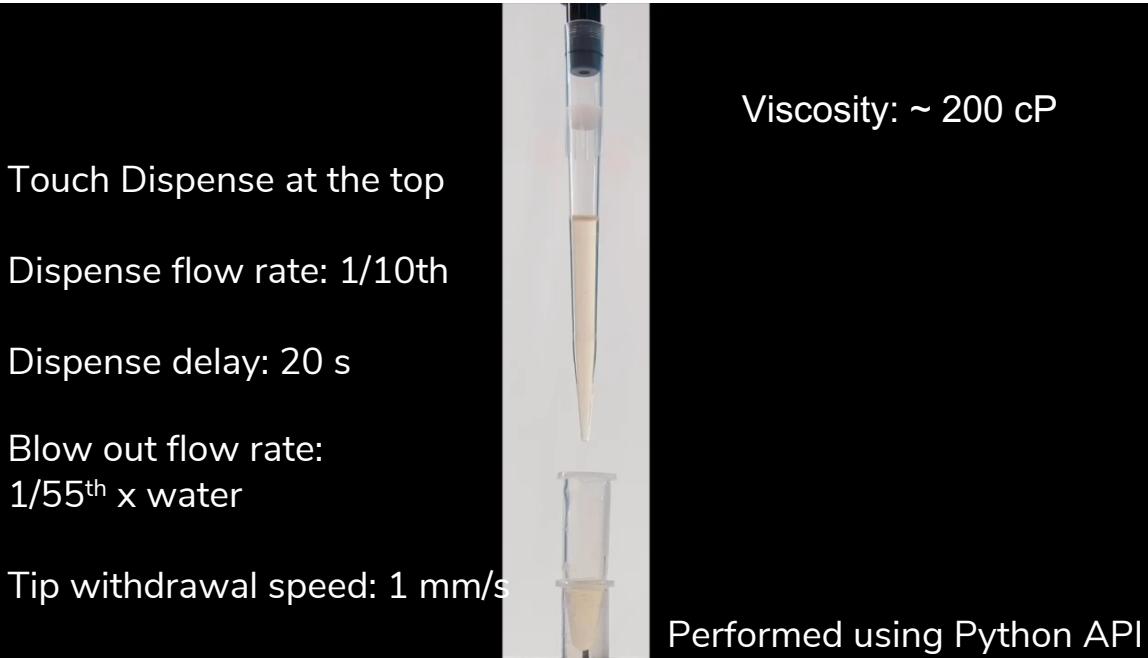
- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime
- Residual volume

Optimized volatile viscous liquid handling

Cohesion
Adhesion 



Dispense

Benefits

- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime
- Residual volume

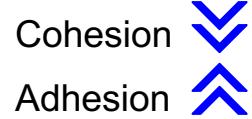
Surfactant viscous liquids

Properties

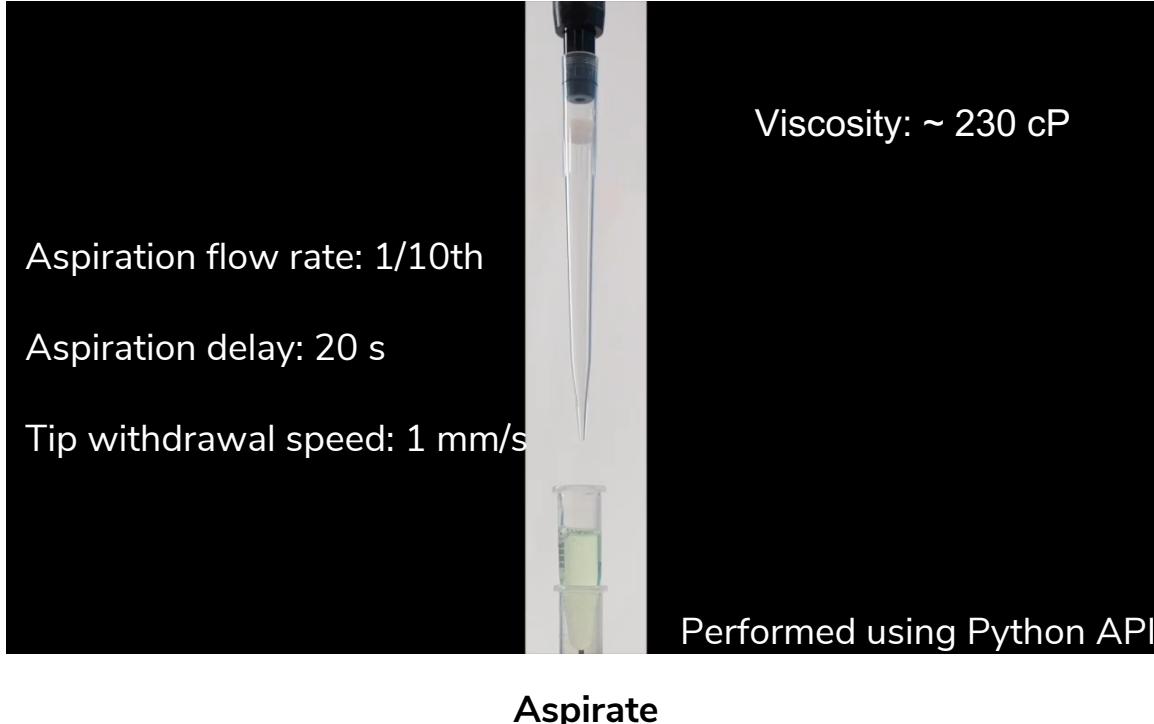
- Detergents with high viscosity
 - Examples: Tween 20, Triton X-100, liquid hand soap etc.
- Very high adhesion to the pipette tip
- Very low cohesion

How do they move through the tip:

- Dispensing may require multi-dispensing steps with longer delays for the liquid to completely dispense from the pipette tip
- With air trapped they form bubbles
- Due to excess liquid retention on the surface of the tip, dead volume consideration is essential



Optimized surfactant liquid handling

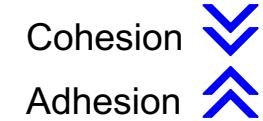


Benefits

- Clean Pipetting
- No bubbles formed
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime
- Longer Delays
- High dispense volume variability due to residual volume



Optimized surfactant liquid handling

Dispense at the top

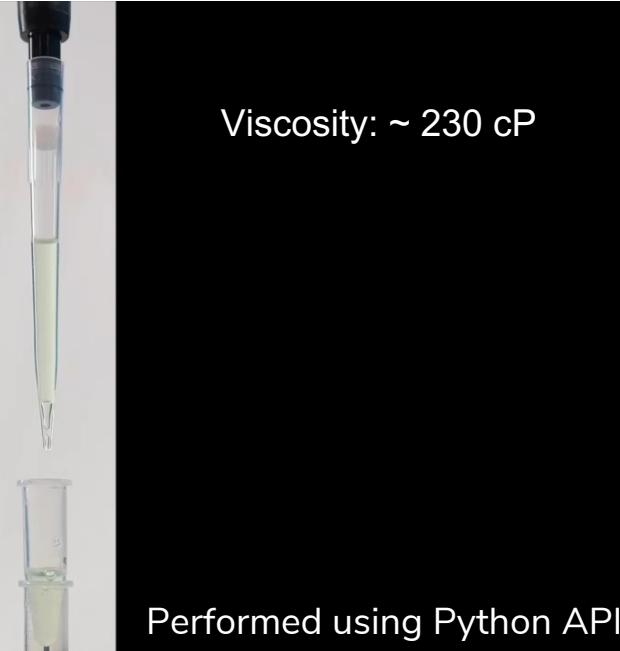
Dispense flow rate: 1/15th

Dispense delay: 20 s

Total dispenses: 2

Blow out flow rate:
1/55th x water

Tip withdrawal speed: 1 mm/s



Dispense

Benefits

- Clean Pipetting
- No bubbles formed
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime
- Longer Delays
- High dispense volume variability due to residual volume

Oils

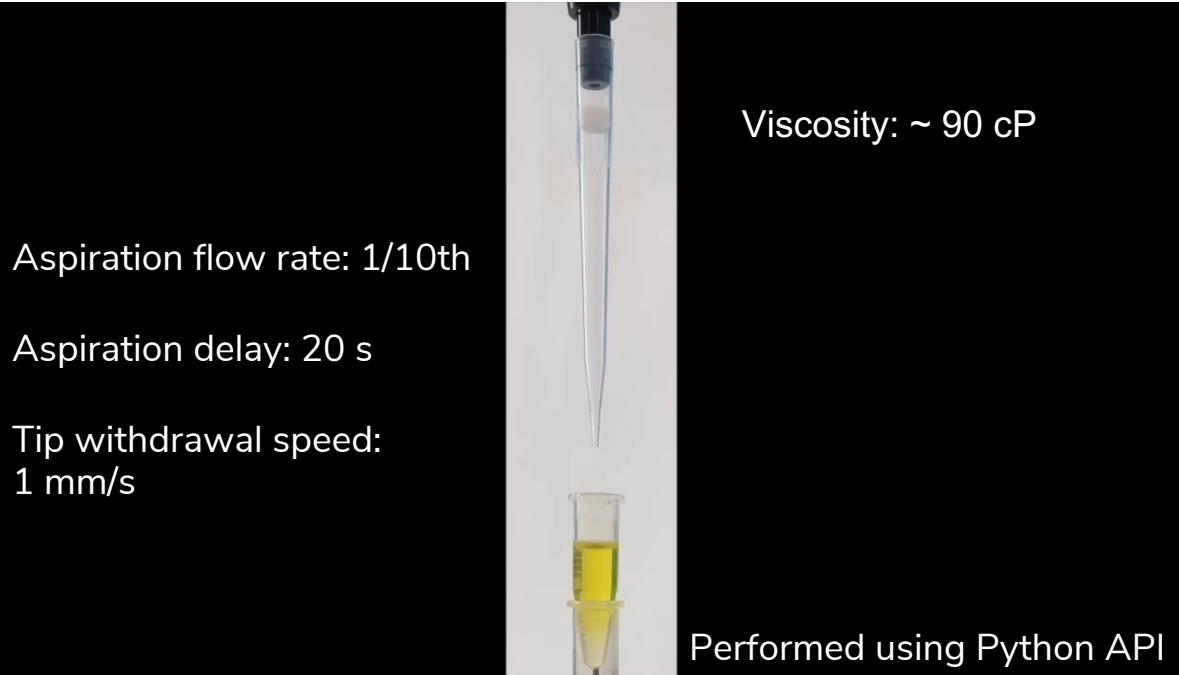
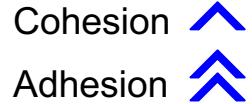
Properties

- Petroleum and plant oils
 - Examples: Mineral oil, engine oil etc.
- Very high adhesion to the pipette tip
- High cohesion

How do they move through the tip:

- Slower flow rates allow easy aspiration and dispensing of oils
- Dispensing requires increased delay for the liquid to completely dispense off the tip
- Oils retain inside tip after dispense

Optimized oil handling



Benefits

- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer protocol runtime
- Residual volume

Cohesion 
Adhesion 

Optimized oil handling

Dispense at the top

Dispense flow rate: 1/10th

Dispense delay: 20 s

Blow out flow rate:
 $1/55^{\text{th}}$ x water

Tip withdrawal speed: 1 mm/s

Viscosity: ~ 90 cP



Performed using Python API

Dispense

Benefits

- Clean Pipetting
- No spilling
- No droplets formed

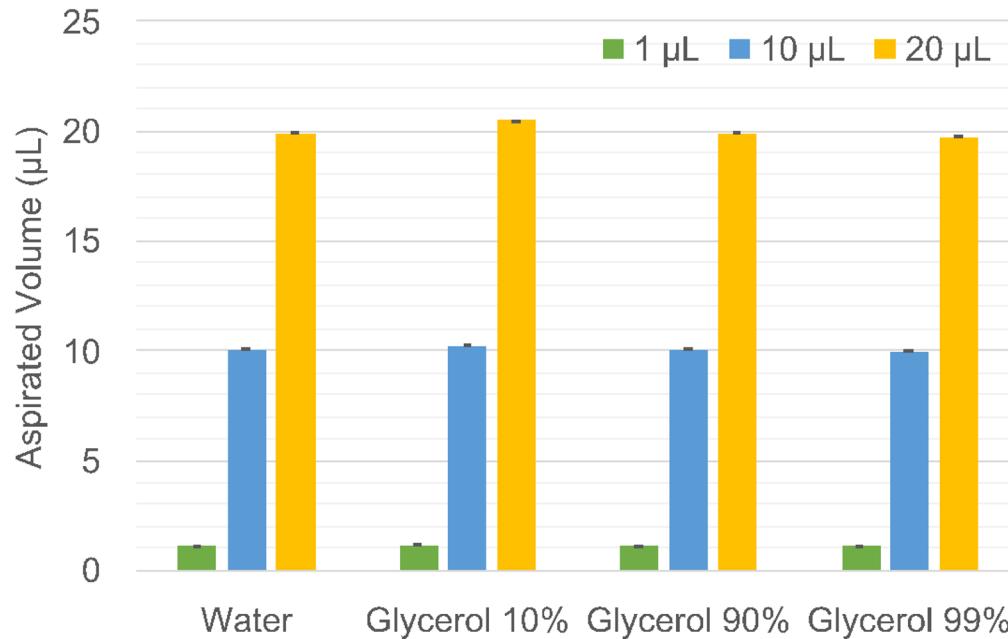
Limitations

- Longer protocol runtime
- Residual volume

Highly accurate viscous handling using Opentrons Gen2 pipettes

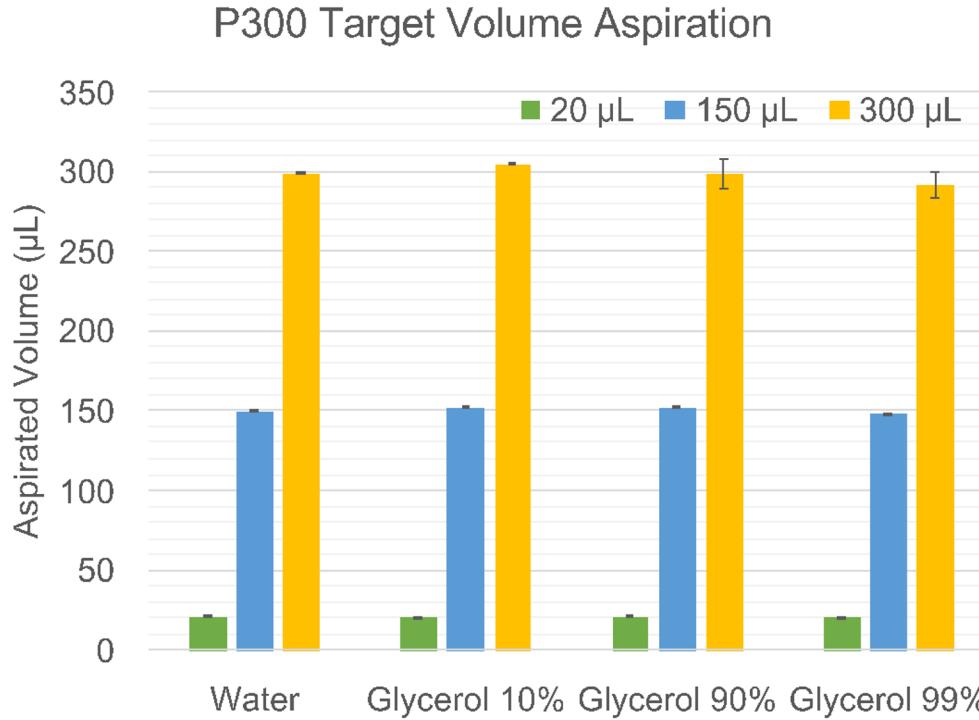
P20 Target Volume Aspiration

P20



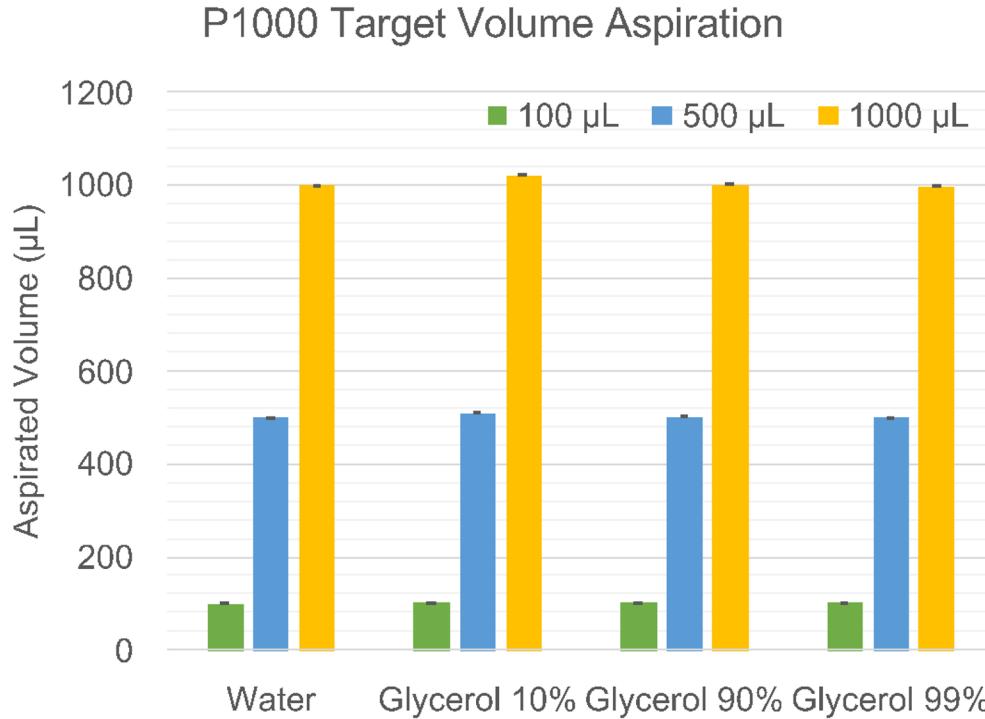
Highly accurate viscous handling using Opentrons Gen2 pipettes

P300



Highly accurate viscous handling using Opentrons Gen2 pipettes

P1000



Benefits of automating pipetting challenging viscous liquids

- Smooth and controlled flow rate
- Accurately aspirate and dispense to desired volume
- Increased reproducibility in results
- Reduced reagent waste
- Completely automated and zero human interference

Acknowledgements

Many thanks to our Testing team members



Carlos Fernandez



Ojas Patel

Questions?

Thanks for Coming!



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Please feel free to reach out to Mellisa for product inquiries, demo requests and sales related questions.

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Appendix: % Loss of Liquid after dispense

Pipette	Volume	Water	Glycerol 10%	Glycerol 90%	Glycerol 99%
P20	1 µL	0.099867	0.081619	0.015068	0.007467
	10 µL	0.12	0.09827	0.100163	0.000373
	20 µL	0.108667	0.128436	0.162276	0.001547
P300	20 µL	0.212867	0.085341	0.001734	0.137013
	150 µL	0.198133	0.173294	0.088238	0.140693
	300 µL	0.282	0.309958	0.757832	0.093333
P1000	100 µL	0.134467	0.304146	0.111545	0.05888
	500 µL	0.525067	0.793013	0.098266	0.122507
	1000 µL	0.490467	0.287692	0.066179	0.042667

Appendix: Example pipette parameters for different liquids and pipettes

Pipette	Liquid	Aspiration Rate ($\mu\text{L/s}$)	Aspiration Delay (s)	Aspiration Withdrawal Rate (mm/s)	Dispense Rate ($\mu\text{L/s}$)	Dispense Delay (s)	Blowout Rate ($\mu\text{L/s}$)	Touch tip
P20	Glycerol 99%	3.78	10	2	3.78	10	0.5	No
	Sanitizer 62% Alcohol	1	2	20	3.78	2	0.5	Yes
	Tween 20	5.292	7	2	3.024	7	0.5	Yes
	Engine oil	6.048	7	1	6.048	7	0.5	Yes

Performed using Python API

Appendix: Example pipette parameters for different liquids and pipettes

Pipette	Liquid	Aspiration Rate ($\mu\text{L/s}$)	Aspiration Delay (s)	Aspiration Withdrawal Rate (mm/s)	Dispense Rate ($\mu\text{L/s}$)	Dispense Delay (s)	Blowout Rate ($\mu\text{L/s}$)	Touch tip
P300	Glycerol 99%	55.5	10	1	55.5	10	4	No
	Sanitizer 62% Alcohol	92.5	2	20	92.5	2	4	Yes
	Tween 20	13.9	10	1	13.9	11	7	Yes
	Engine oil	74	3	2	46.25	7	10	Yes

Performed using Python API

Appendix: Example pipette parameters for different liquids and pipettes

Pipette	Liquid	Aspiration Rate ($\mu\text{L/s}$)	Aspiration Delay (s)	Aspiration Withdrawal Rate (mm/s)	Dispense Rate ($\mu\text{L/s}$)	Dispense Delay (s)	Blowout Rate ($\mu\text{L/s}$)	Touch tip
P1000	Glycerol 99%	41.175	20	1	19.215	20	5	No
	Sanitizer 62% Alcohol	41.175	20	1	19.215	20	5	No
	Tween 20	41.175	20	1	19.215	20	5	No
	Engine oil	41.175	20	1	19.215	20	5	No

Performed using Python API