Tips and Tricks for Automating Viscous Liquid Handling

Scientist-To-Scientist Webinar Series – September 09, 2021

PRLNYC Pandemic Response Lab

opentrons

Speakers

Presenter: Anurag Kanase

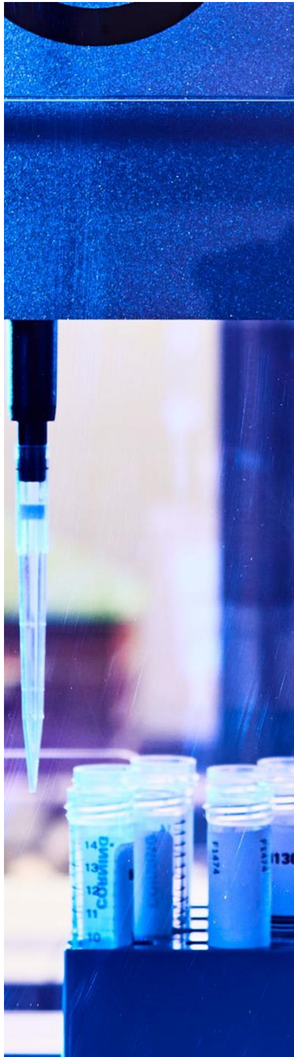
Process Development Scientist, Opentrons

Anurag joined Opentrons after working as a Test Engineer at DropGenie. Prior to that, he co-founded a rapid diagnostic company in India. Anurag earned a Bioengineering degree from Northeastern University, USA in 2020 and a Physics master's degree from the University of Stuttgart, Germany in 2016.

Moderator: Dr. Janeen Vanhooke

Automation Consulting Engineer, Opentrons

Janeen received her Ph.D. in Biochemistry in 1993 from the University of Wisconsin-Madison. She has a diverse background in biochemical and biophysical studies of protein structure, function and mechanism. Dr. Vanhooke joined Opentrons in 2021 as a Field Applications Scientist and provides consultation to assist researchers with automated workflow development.



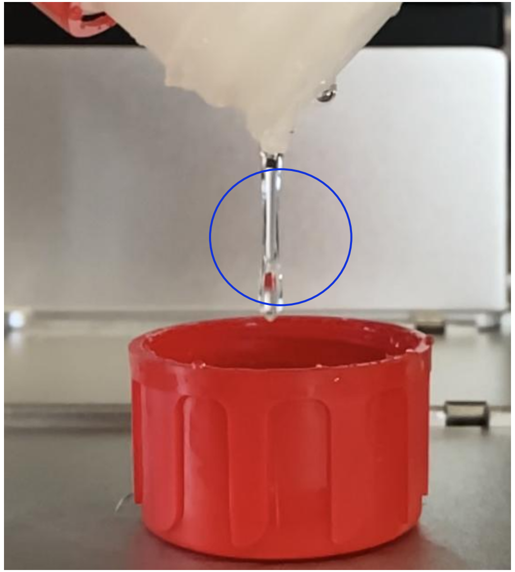
Webinar Overview

- 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

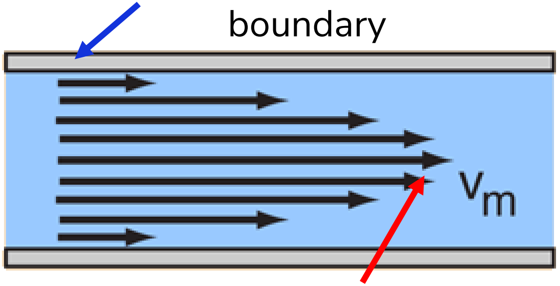
Forces between labware and liquid

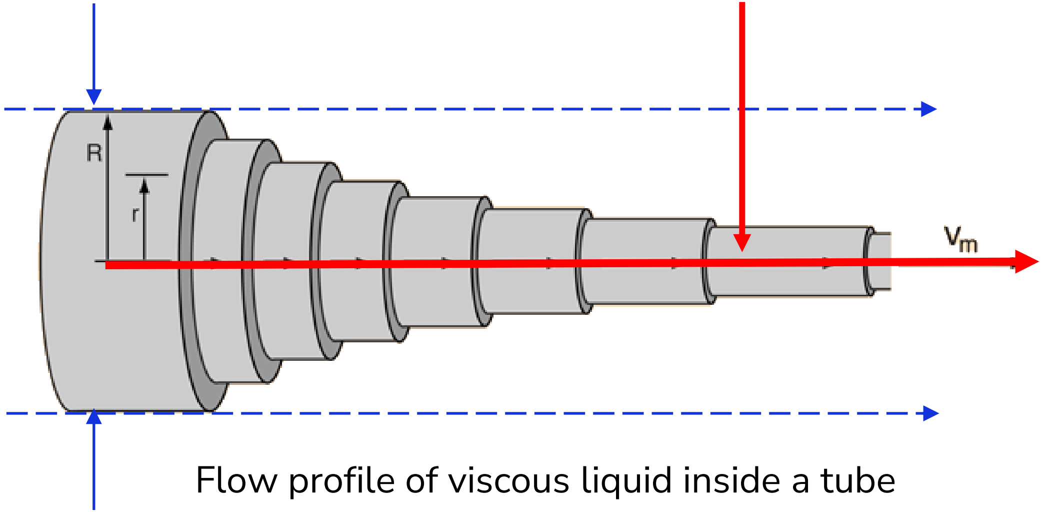


Cohesion

Forces between liquid

Laminar flow inside a tip





Flow profile of viscous liquid inside a tube

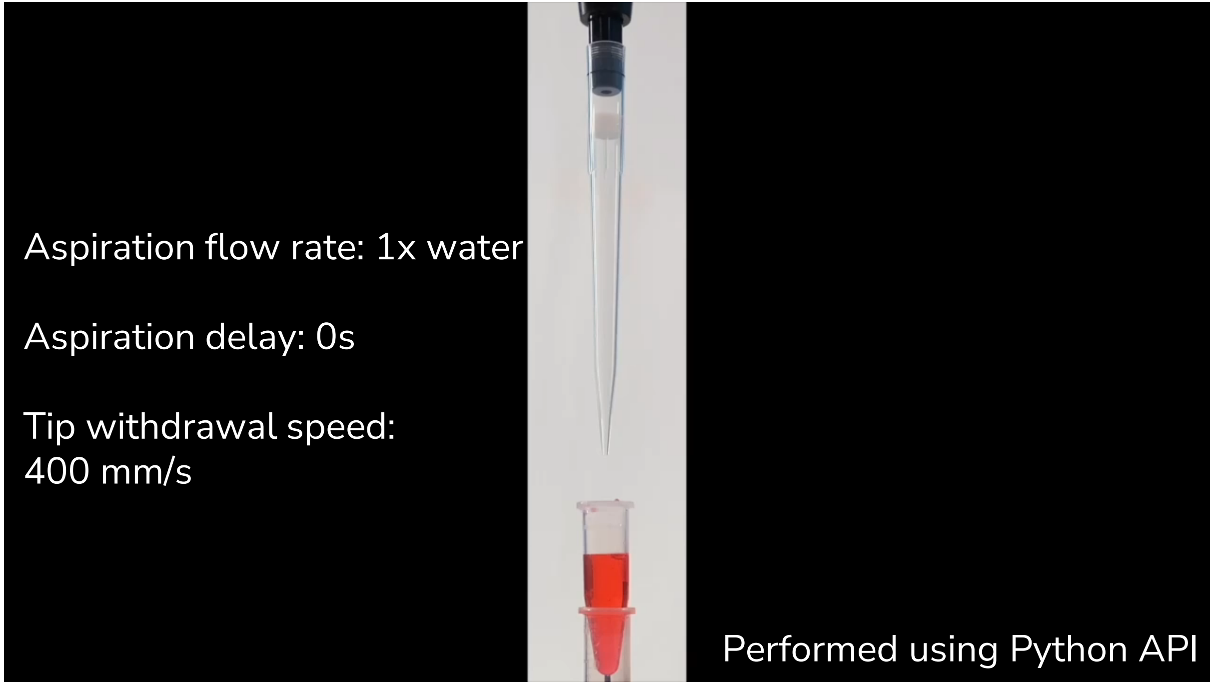
Based on the relative ratio of the adhesive and cohesive forces, which dominate inside a tip for a given liquid, we characterize viscous liquid classes

Source: Hyperphysics - GSU

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 vv Very low cohesion	^^ High adhesion ^ High cohesion

Pipetting viscous liquid with water parameters on OT-2



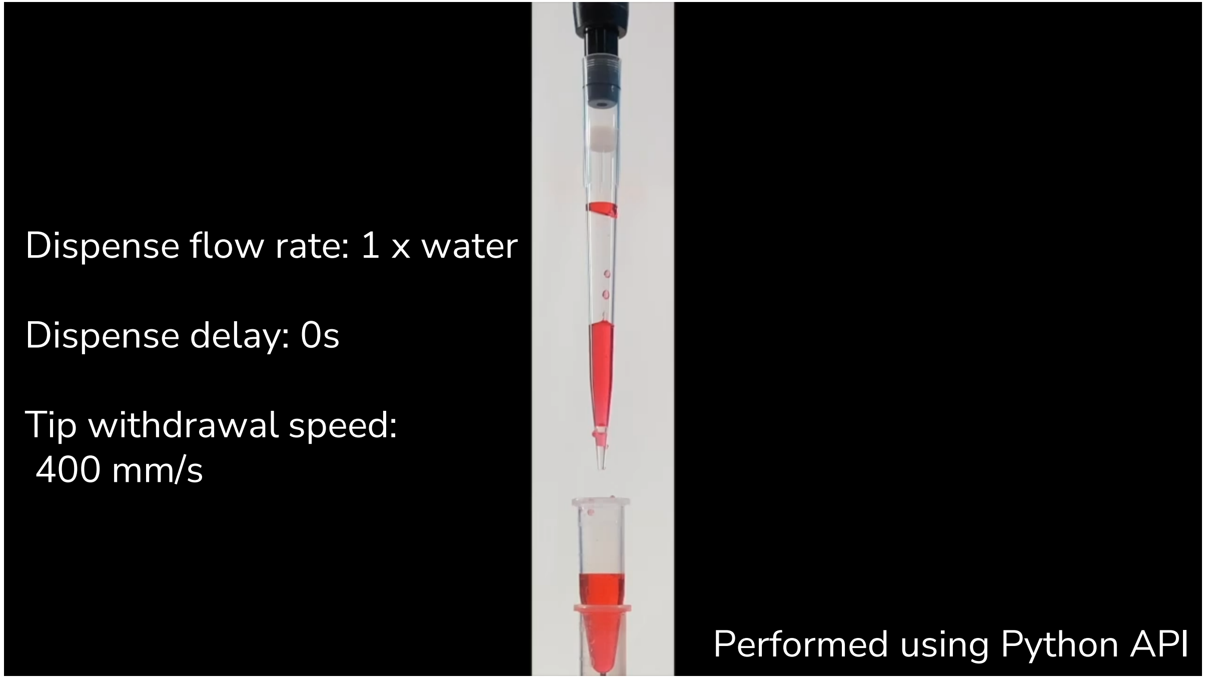
Aspirate

Common Problems

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

Test liquid: Glycerol 99% with 10 uL food coloring (video speed: 2x)

Pipetting viscous liquid with water parameters on OT-2



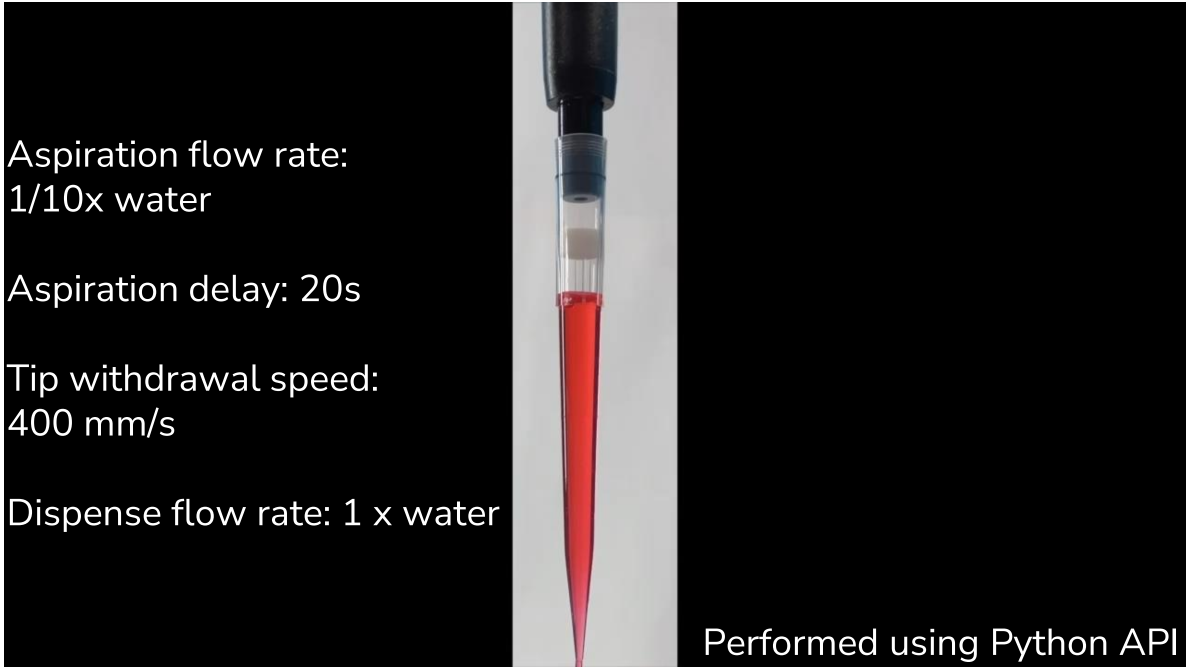
Dispense

Common Problems

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

 Test Liquid: Glycerol 99% with 10 uL food coloring (video speed: 2x)

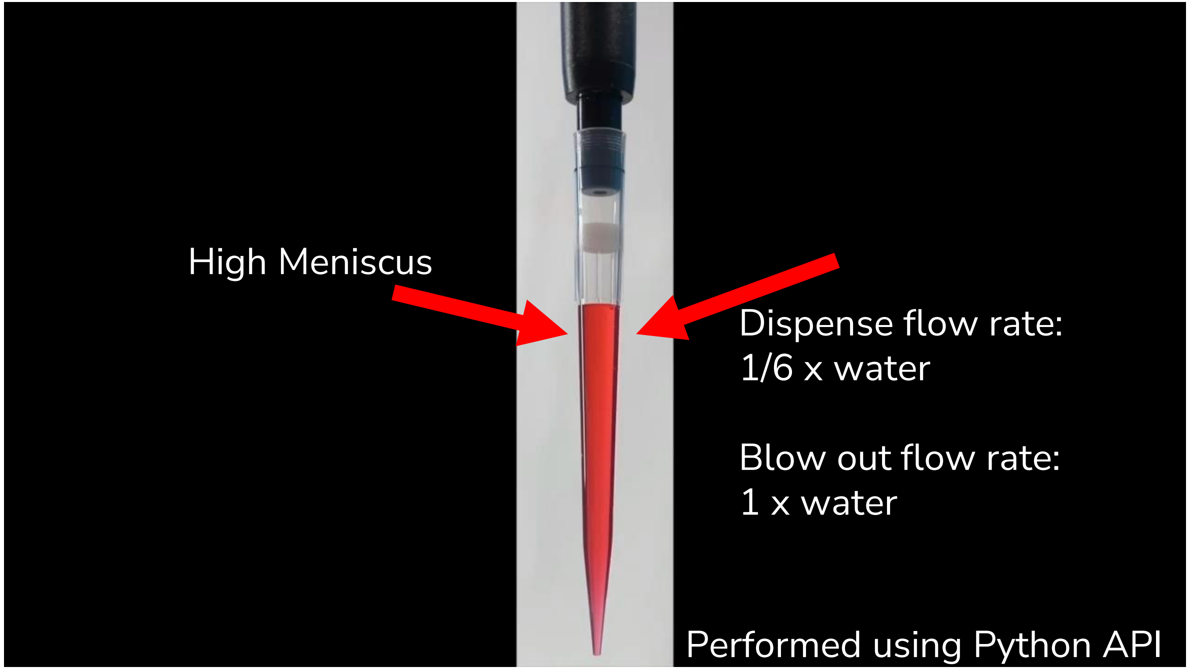
How to optimize any viscous liquid parameters for smoother handling



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

Test Liquid: Glycerol 99% with 10uL food coloring (video speed:8x)

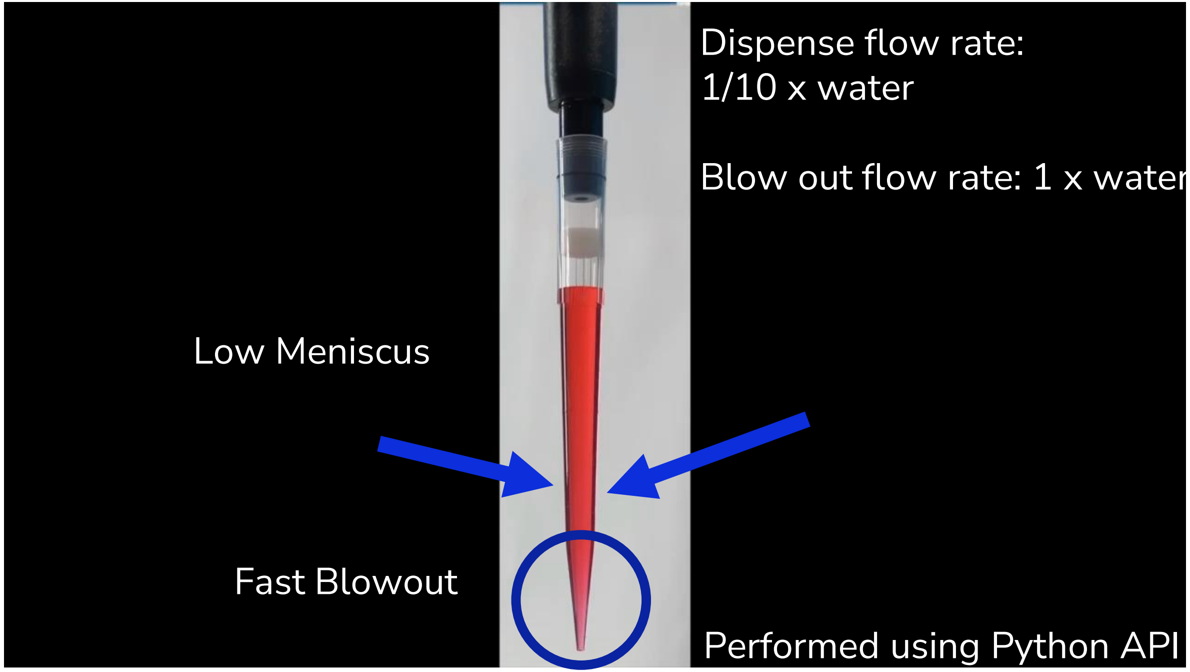
How to optimize any viscous liquid parameters for smoother handling



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

Test Liquid: Glycerol 99% with 10uL food coloring (video speed:8x)

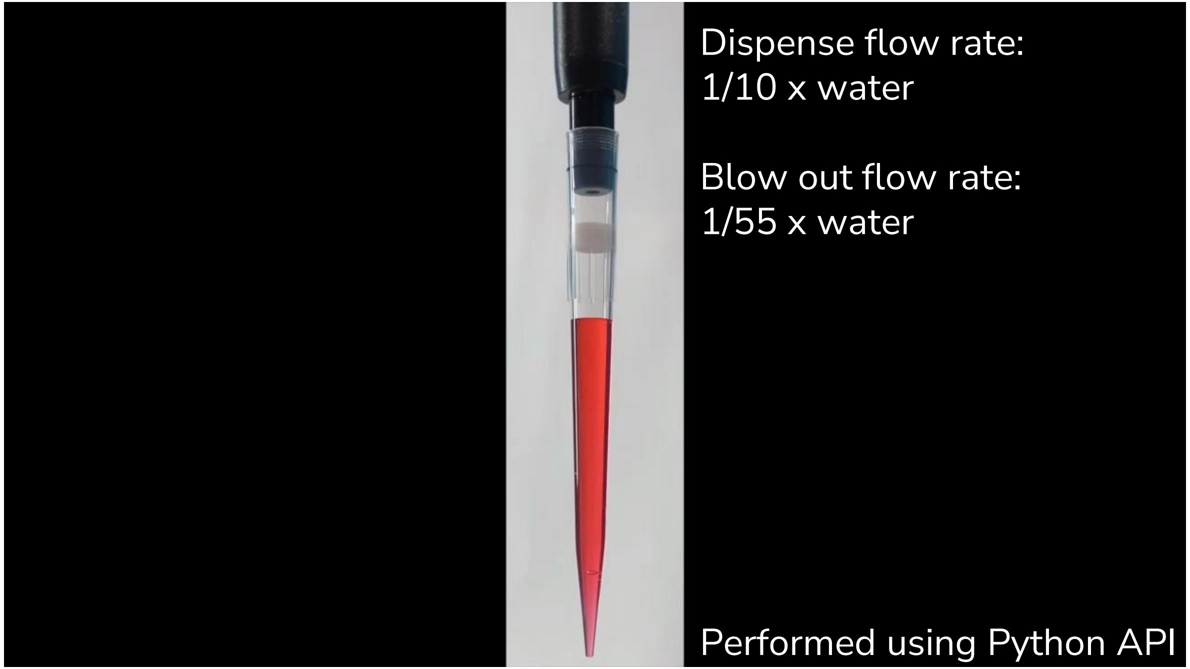
How to optimize any viscous liquid parameters for smoother handling



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

Test Liquid: Glycerol 99% with 10uL food coloring (video speed:8x)

How to optimize any viscous liquid parameters for smoother handling



Step 4: Clean and complete dispense of glycerol

Test Liquid: Glycerol 99% with 10uL food coloring (video speed:8x)

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

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

Step 2: Dispense

- `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

Step 3: Blowout

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

Step 4: Tip Withdrawal

- `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

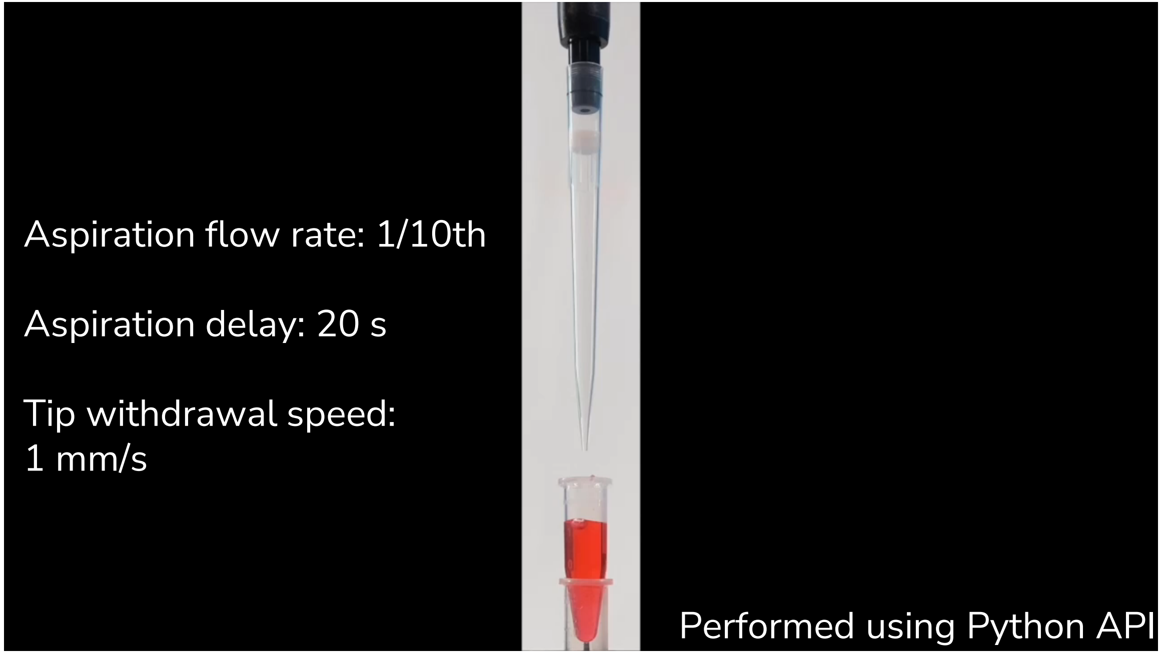
- Liquids with sugar or glycerol content
    - Examples: Glycerol, PEG etc.
- High adhesion to the pipette tip
- Highly cohesive

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 ^



Aspirate

Benefits

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

Limitations

- Longer Protocol runtime

PRLNYC opentrons Test Liquid: Glycerol 99% with 10 uL food coloring (video speed: 8x)

Optimized glycated liquid handling

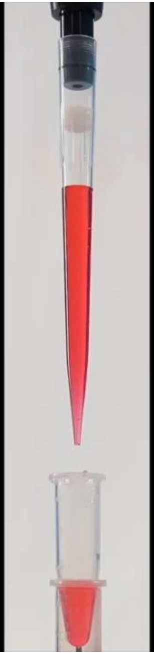
Cohesion ^
Adhesion ^

Dispense flow rate: 1/10th

Dispense delay: 20 s

Blow out flow rate:
1/55th x water

Tip withdrawal speed:
1 mm/s



Performed using Python API

Dispense

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

Limitations
- Longer Protocol runtime

Test Liquid: Glycerol 99% with 10 uL food coloring (video speed: 8x)

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

Aspiration flow rate: 1/10th

Aspiration delay: 20 s

Tip withdrawal speed: 1 mm/s

Performed using Python API

Aspirate

Benefits

- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime
- Residual volume

PRLNYC opentrons Test Liquid: Hand Sanitizer with 75% ethanol (video speed: 8x)

Optimized volatile viscous liquid handling

Cohesion
Adhesion

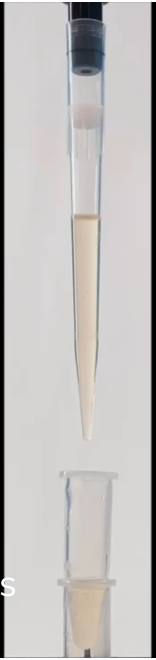
Touch Dispense at the top

Dispense flow rate: 1/10th

Dispense delay: 20 s

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

Tip withdrawal speed: 1 mm/s



Performed using Python API

Dispense

Benefits

- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer Protocol runtime
- Residual volume

 Test Liquid: Hand Sanitizer with 75% ethanol (video speed: 8x)

Surfactant viscous liquids

Properties

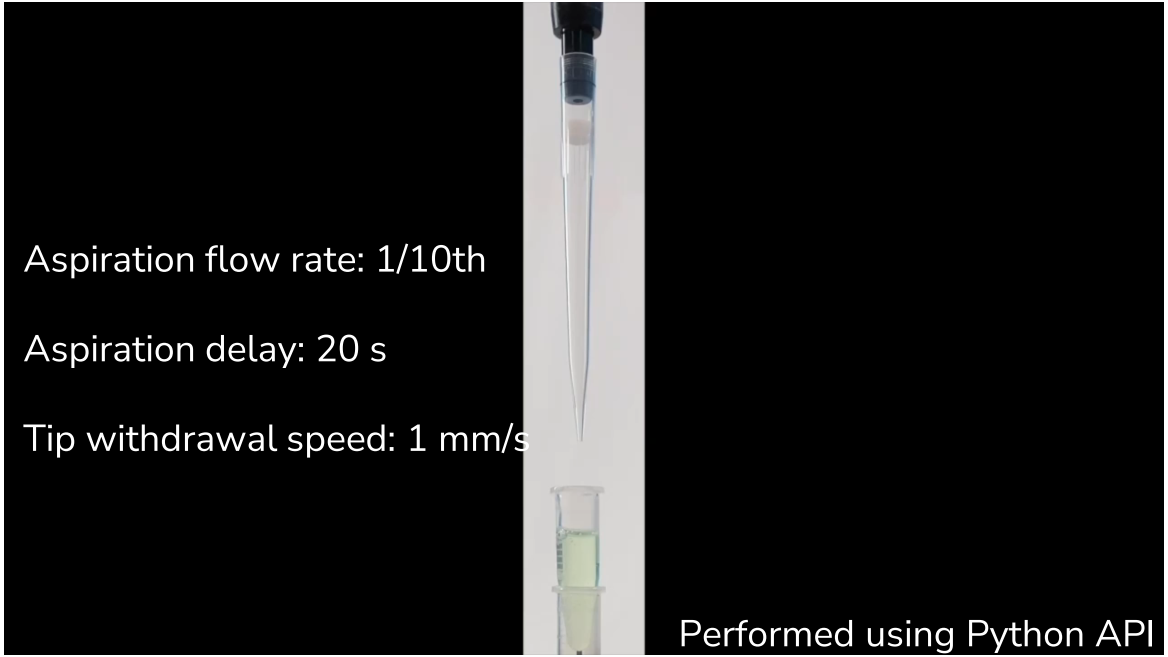
- Detergents with high viscosity
  - Examples: Tween 20, Triton X-100, liquid hand soap etc.
- Very high adhesion to the pipette tip
- Very low cohesion

How do they move through the tip:

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

Optimized surfactant liquid handling

Cohesion
Adhesion



Aspirate

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

 Test Liquid: Triton-X 100 with 1 uL green food color (video speed: 8x)

Cohesion

Adhesion

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

Performed using Python API

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

Test Liquid: Triton-X 100 with 1 uL green food color (video speed: 8x)

Oils

Properties

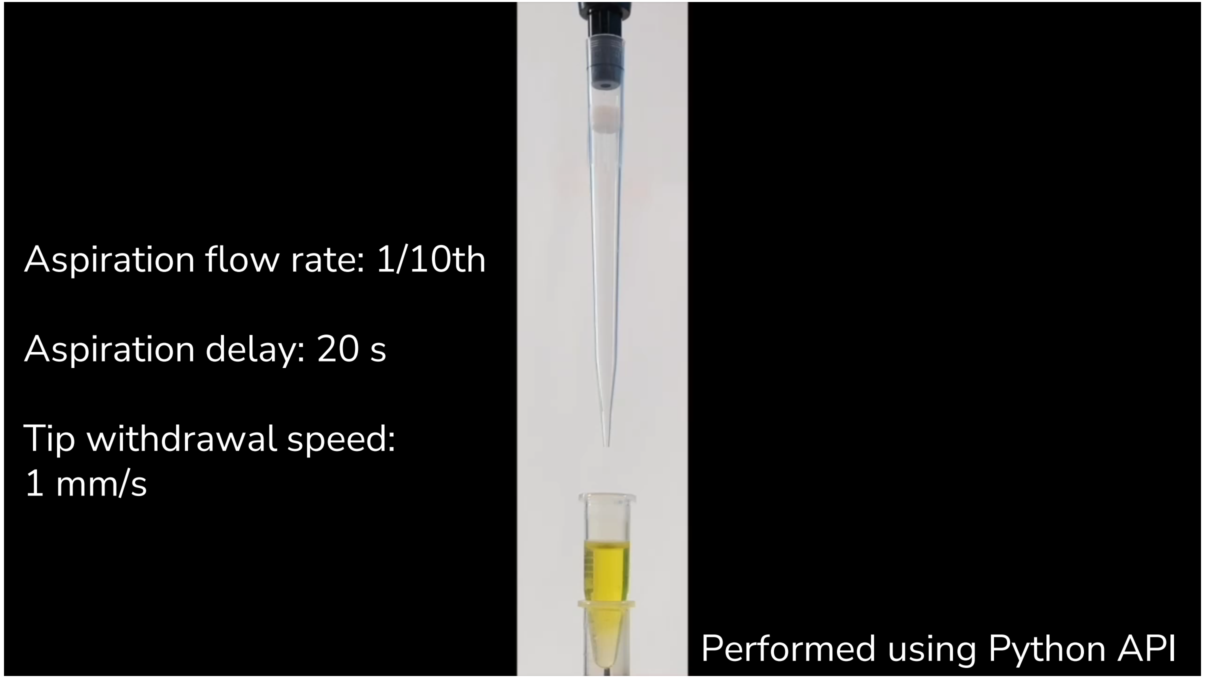
- Petroleum and plant oils
  - Examples: Mineral oil, engine oil etc.
- Very high adhesion to the pipette tip
- High cohesion

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
- Rarely tip comes off clean

Optimized oil handling

Cohesion
Adhesion



Aspirate

Benefits

- Clean Pipetting
- No spilling
- No droplets formed

Limitations

- Longer protocol runtime
- Residual volume

PRLNYC opentrons Test Liquid: Olive oil (video speed: 8x)

Optimized oil handling

Cohesion
Adhesion

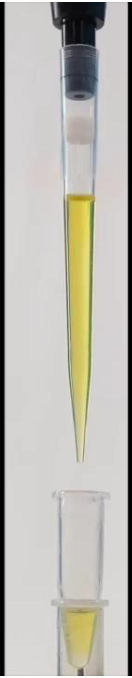
Dispense at the top

Dispense flow rate: 1/10th

Dispense delay: 20 s

Blow out flow rate:
1/55th x water

Tip withdrawal speed: 1 mm/s



Performed using Python API

Dispense

Benefits

- Clean Pipetting
- No spilling
- No droplets formed

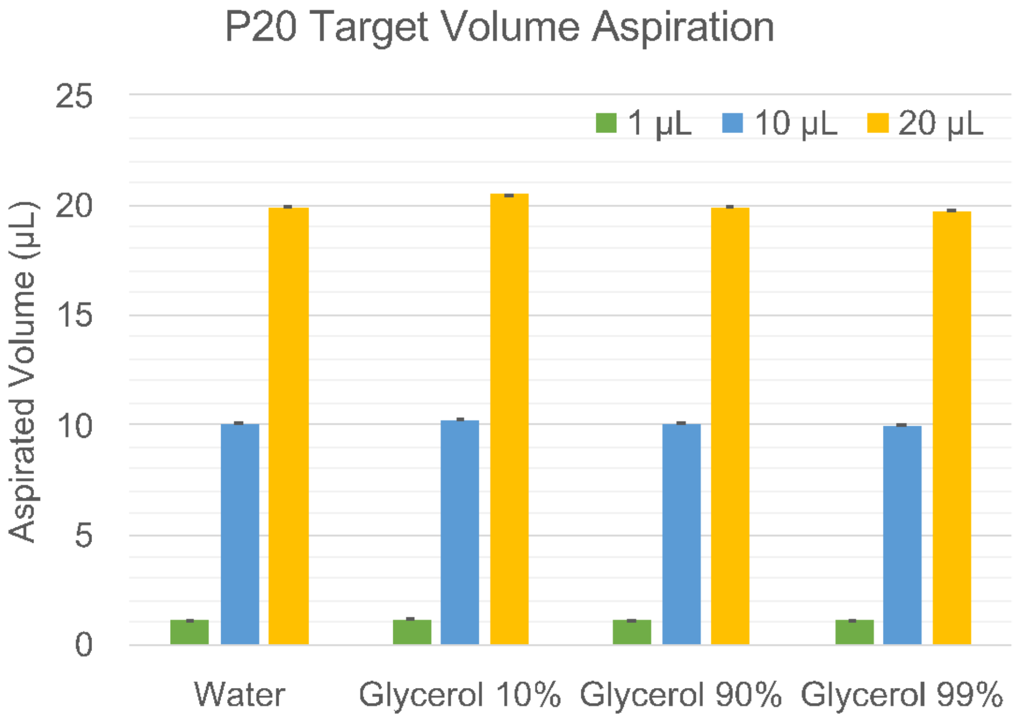
Limitations

- Longer protocol runtime
- Residual volume

Test Liquid: Olive oil (video speed: 1x)

Highly accurate viscous handling using Opentrons Gen2 pipettes

P20

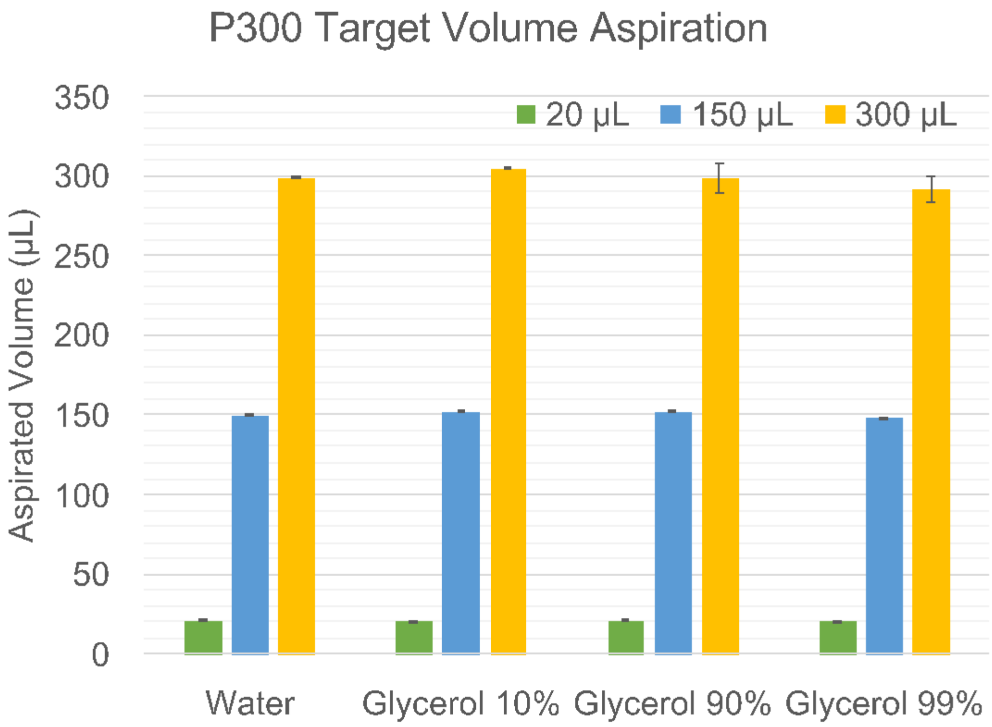


Gravimetric testing of Opentrons Gen 2 P20 with Python API

PRLNYC opentrons

Highly accurate viscous handling using Opentrons Gen2 pipettes

P300

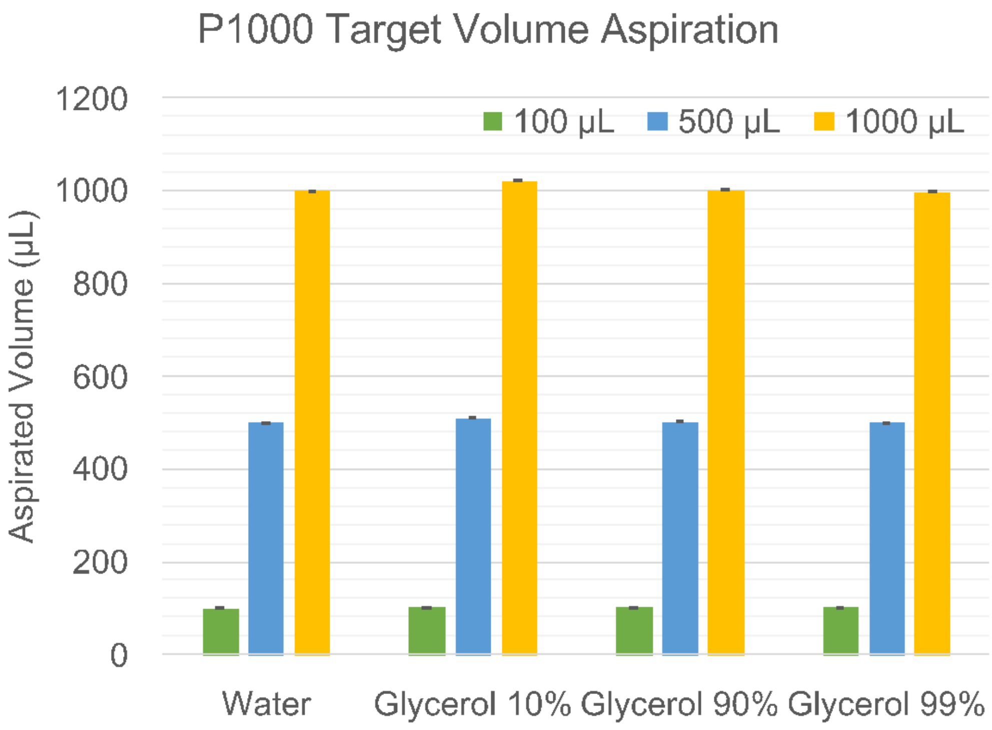


Gravimetric testing of Opentrons Gen 2 P300 with Python API

PRLNYC opentrons

Highly accurate viscous handling using Opentrons Gen2 pipettes

P1000



Gravimetric testing of Opentrons Gen 2 P1000 with Python API

PRLNYC opentrons

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!

anurag.kanase@opentrons.com

Janeen.vanhooke@opentrons.com

Check out more Opentrons webinars at https://blog.opentrons.com/webinars/

PRLNYC opentrons

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

Performed using Python API

Appendix: Example pipette parameters for different liquids and pipettes

Pipette	Liquid	Aspiration Rate (µL/s)	Aspiration Delay (s)	Aspiration Withdrawal Rate (mm/s)	Dispense Rate (µL/s)	Dispense Delay (s)	Blowout Rate (µL/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 (µL/s)	Aspiration Delay (s)	Aspiration Withdrawal Rate (mm/s)	Dispense Rate (µL/s)	Dispense Delay (s)	Blowout Rate (µL/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 (µL/s)	Aspiration Delay (s)	Aspiration Withdrawal Rate (mm/s)	Dispense Rate (µL/s)	Dispense Delay (s)	Blowout Rate (µL/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