



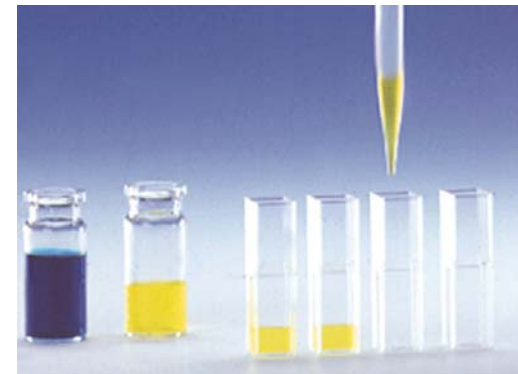
Innovating for Health

# Pipetting Techniques



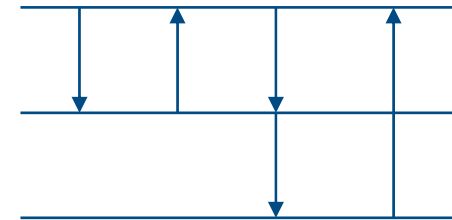
# Pipetting (P)

- The most common technique
- Large volumes
- Aqueous liquids
- Liquids containing small amounts of detergent or protein
- Solvents (pre-rinsing necessary)



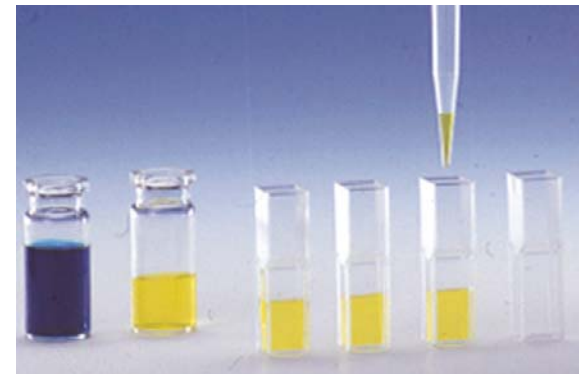
# Pipetting (P)

- Press the operating button to the first stop
- Place the tip just under the surface of the liquid (2-3 mm)
- Smoothly release the operating button
- Dispense the liquid by pressing the operating button to the first stop
- Press finally the operating button to the second stop



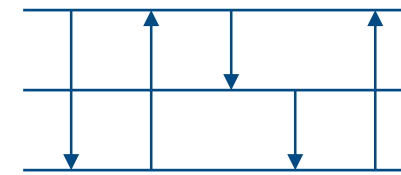
# Reverse Pipetting (rP)

- Biological fluids
- Viscous fluids
- Foaming (protein- or detergent-rich) fluids
- Small volumes
- Work demanding high accuracy and precision

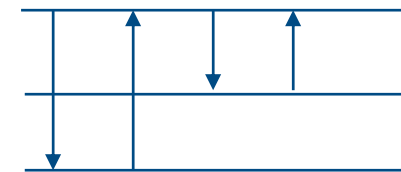


# Reverse Pipetting (rP)

- Press the operating button all the way to the second stop
- Place the tip just under the surface of the liquid (2-3 mm)
- Smoothly release the operating button
- Press the operating button to the first stop to deliver the liquid
- Discard the remaining liquid by pressing the operating button to the second stop



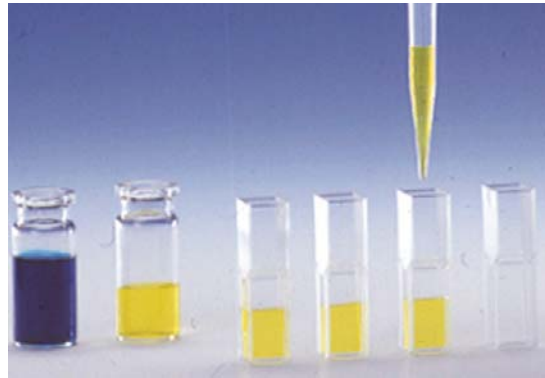
Reverse pipetting



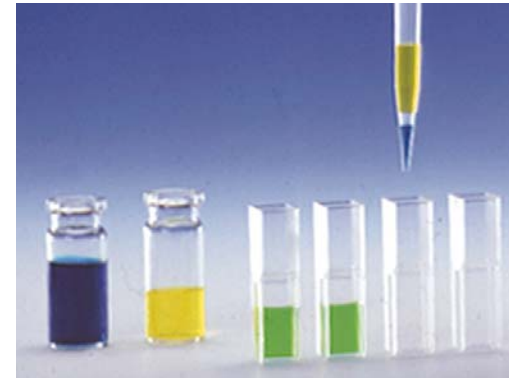
Repeated delivery



# Other Techniques



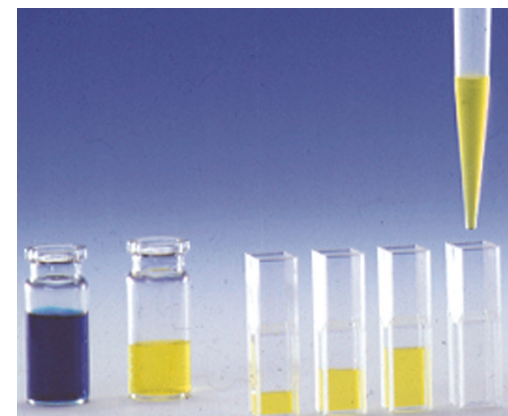
Dispensing (d)



Diluting (dd)



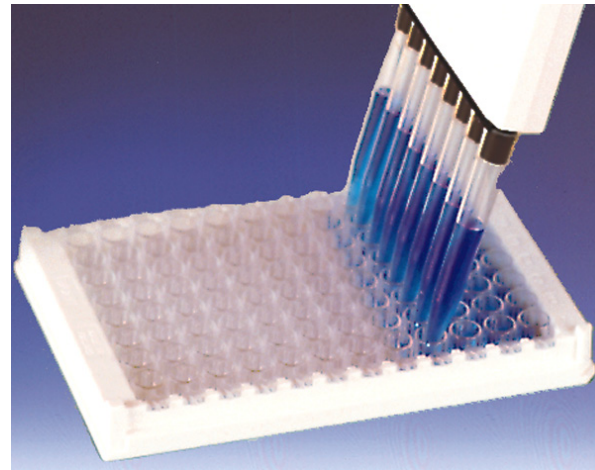
Mixing (\*)



Sequential  
dispensing (Sd)



# Other Techniques



Special Aspiration (SA)  
(Washing)



# Pipetting Recommendations

- Use manufacturer's original tips
- Use as small tips as possible (minimum air space)
- Hold the pipette vertically
- Immerse the tip only 2-3 mm when aspirating
- Pre-rinse 3-5 times before pipetting
- Keep the pipette, tip and liquid in same temperature
- Operate with smooth and consistent thumb action
- Pipette against the inside wall of the receiving vessel



# Factors Affecting the Pipetting Results

- Pipettor
- Tip
  - Quality
  - Shape/size
  - Material
  - Fitting to the pipette
- Environment
  - Temperature
  - Humidity
  - Air pressure



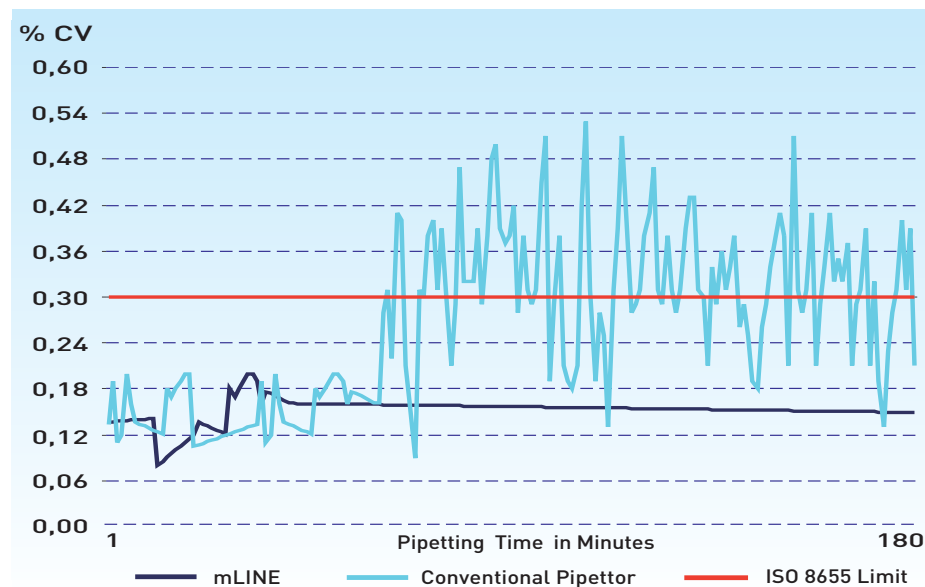
# Factors Affecting the Pipetting Results

- Human error
  - personality, skill
  - experience
  - strain (RSI)
- Pipetting technique
- Pipetting angle
- Pipetting rhythm
- Sample (type, viscosity)
- Application



# Factors Affecting the Pipetting Results

Bad precision caused by strain



Each pipettor was tested over a 5 hour period. Sets of 5 repeat weightings of 500  $\mu$ l were done 180 times into a calibrated balance. The same trained operator was used to perform the tests. The limit line for %CV is the maximum permissible as defined in ISO8655-2 permissible errors. The light pipetting action of *mLINE* delivers improved reproducibility over time.

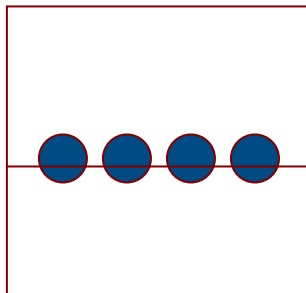


# Accuracy and Precision

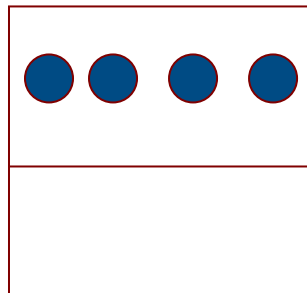
- Precision
  - Precision is an agreement between replicate measurements. Precision is quantified by imprecision
  - High precision, i.e. small imprecision means very little variation between repeated measurements
- Accuracy
  - It is possible to be very consistent, but consistently wrong
  - Inaccuracy is the numerical difference between the mean of a set of replicate measurements and the true value



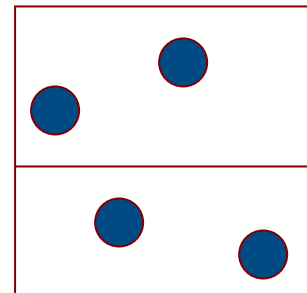
# Accuracy and Precision



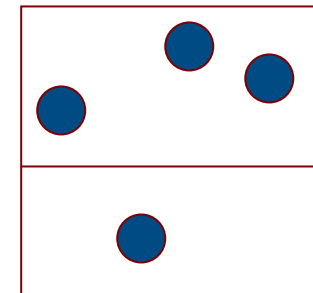
Accurate  
Precise



Precise  
Not Accurate



Accurate  
Not Precise

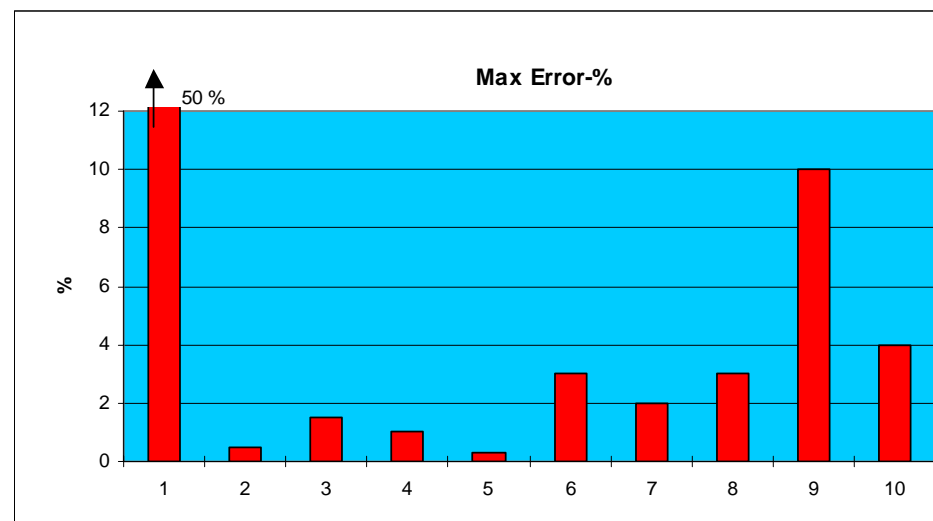


Not Precise  
Not Accurate

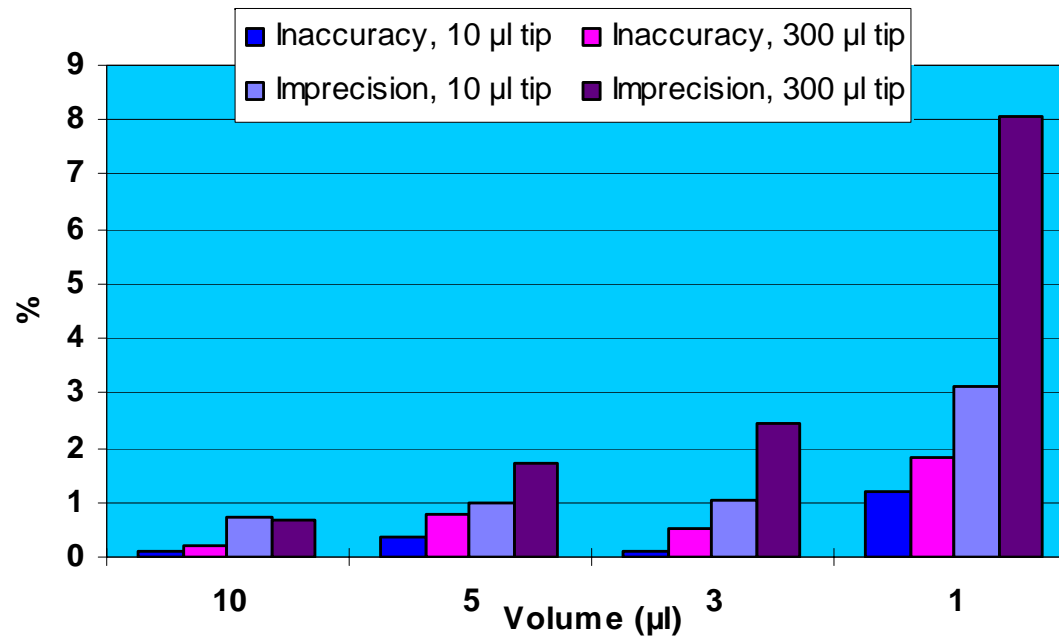


# Possible Sources of Error

1. Leaky piston/cylinder system
2. Uneven piston movement
3. Uneven rhythm and timing during pipetting
4. Depth of tip and angle of pipetting
5. Difference in temperature between pipette, liquid and room temperature
6. Difference in humidity
7. No pre-rinsing
8. Failure to wipe pipettor tip on vessel wall
9. Poor tip fitting
10. Reuse of tip

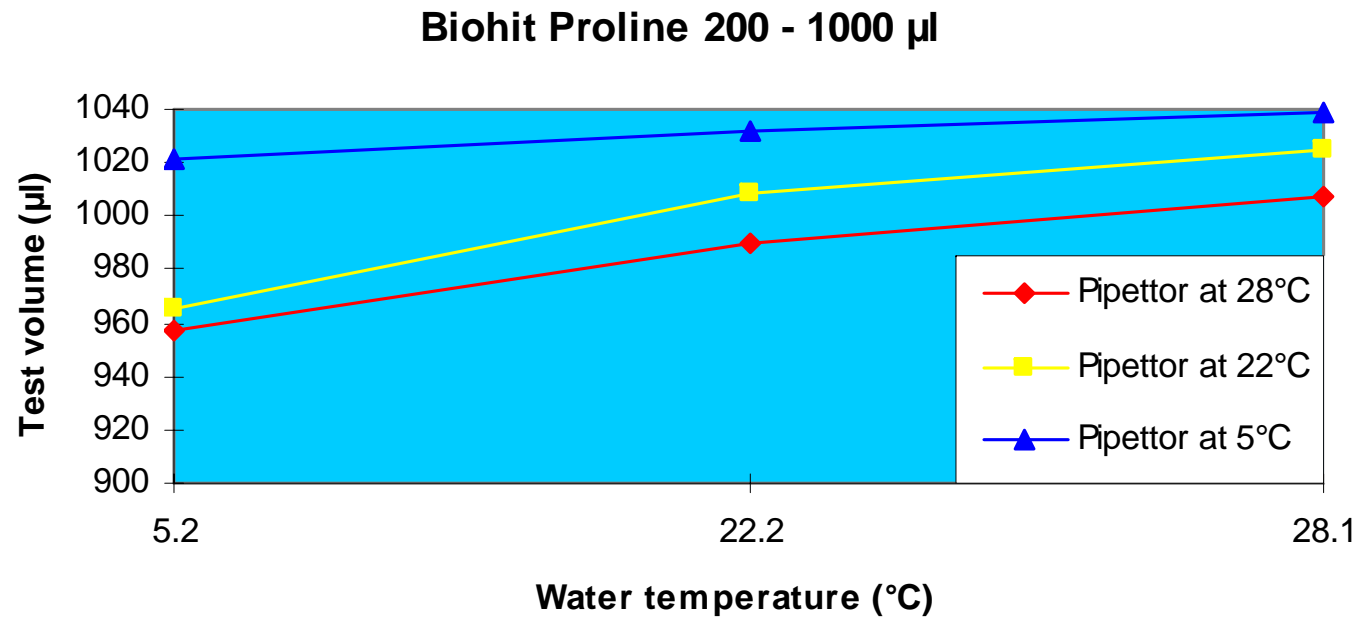


## Comparison of 10 $\mu\text{l}$ and 300 $\mu\text{l}$ tips (2-in-1 pipette)



Conclusion: Use as small tip as possible (minimise dead air volume) to get accurate results

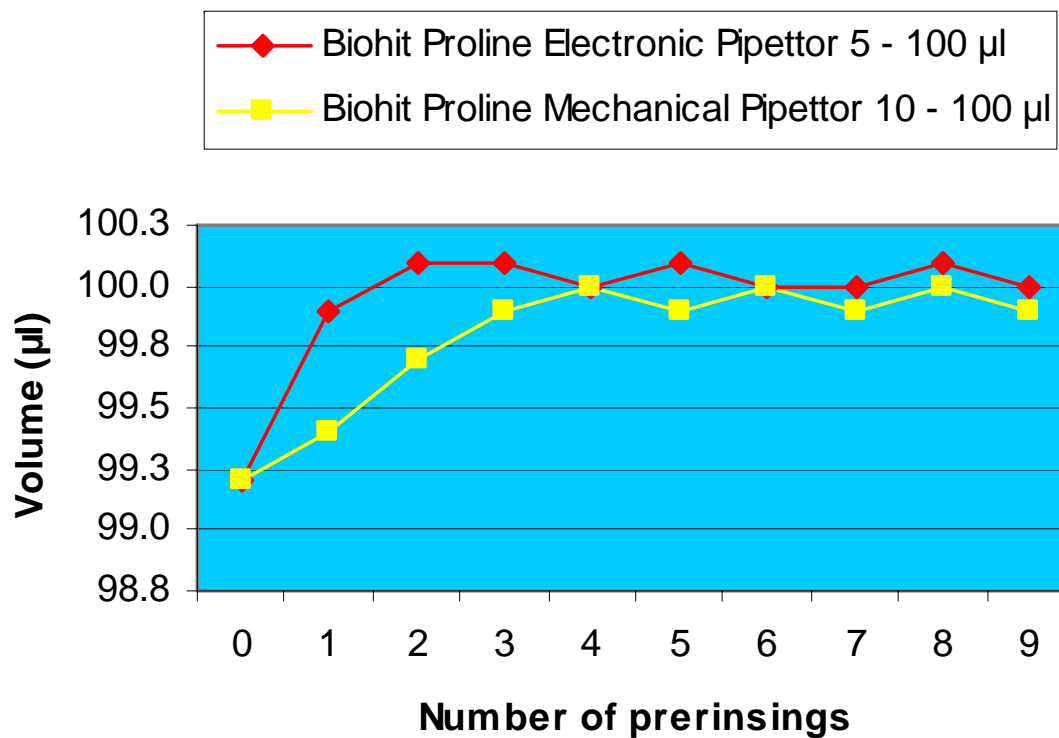
# Pipette Performance at Different Temperatures



Conclusion: Keep the pipette, tip and liquid at the same temperature, preferably room temperature



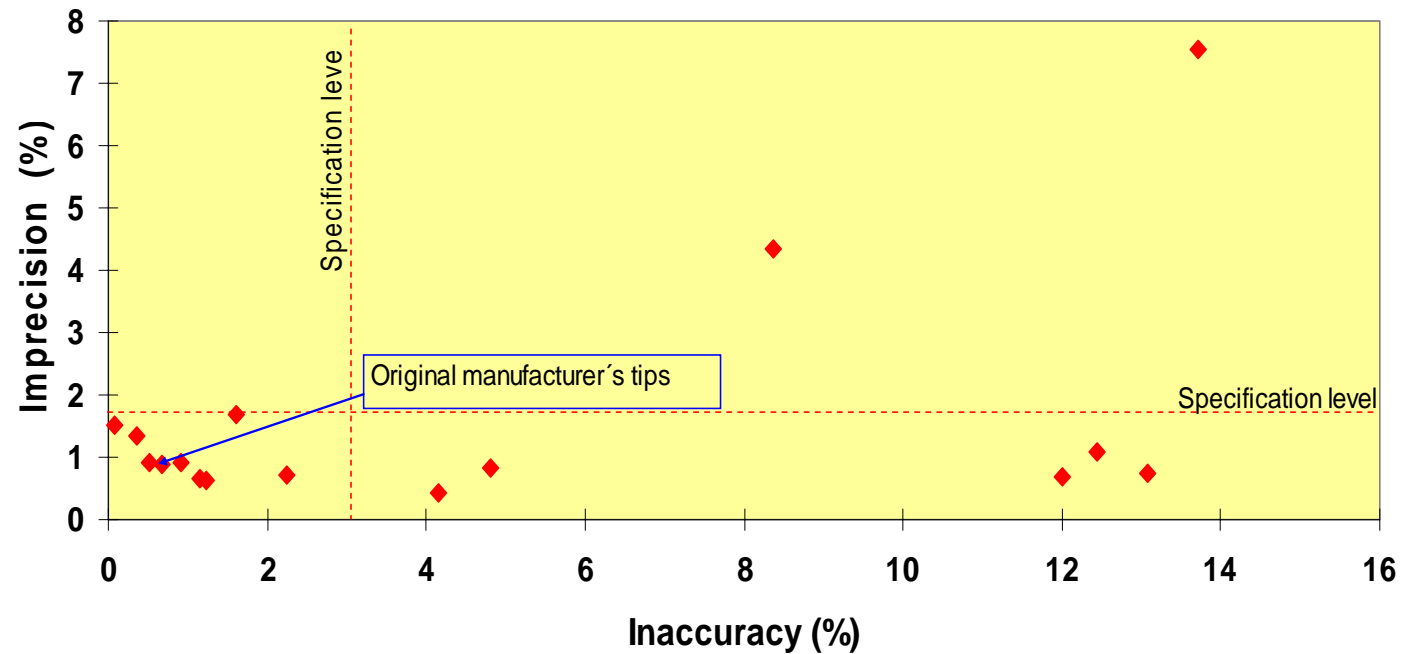
# The Effect of Pre-Rinsing



Conclusion: For best results, pre-rinse 3-5 times



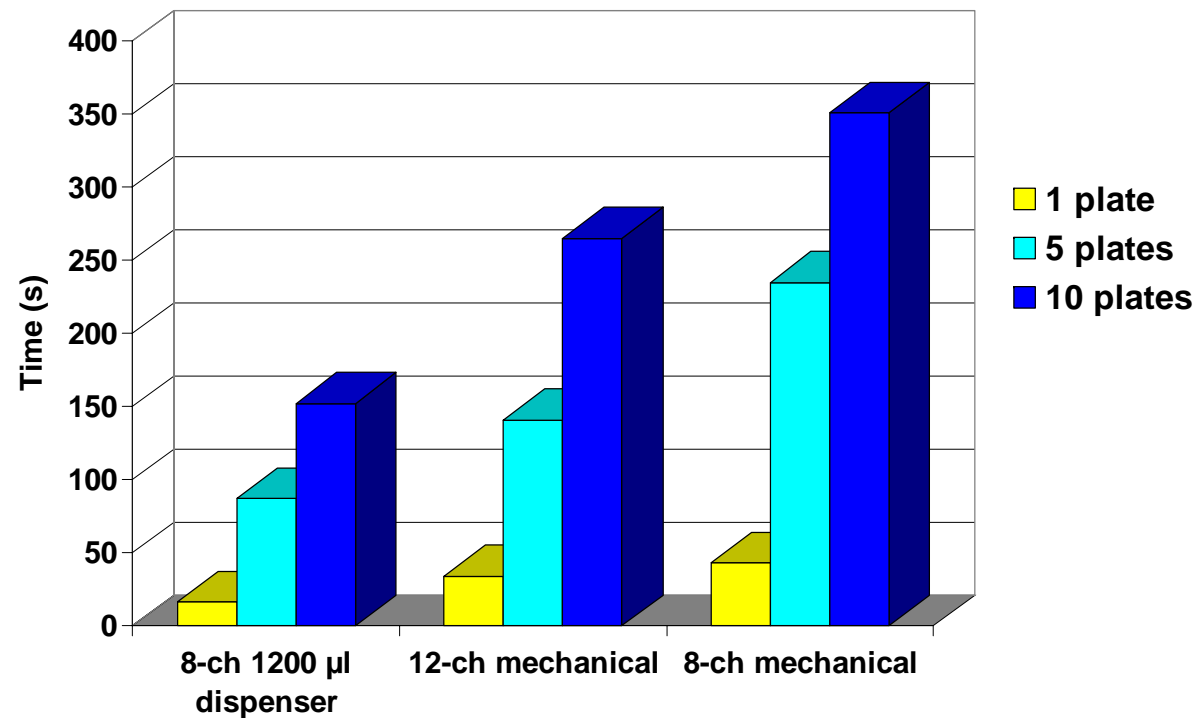
# Difference Between Different Manufacturers' Tips



Conclusion: Use tips specified by the pipette manufacturer

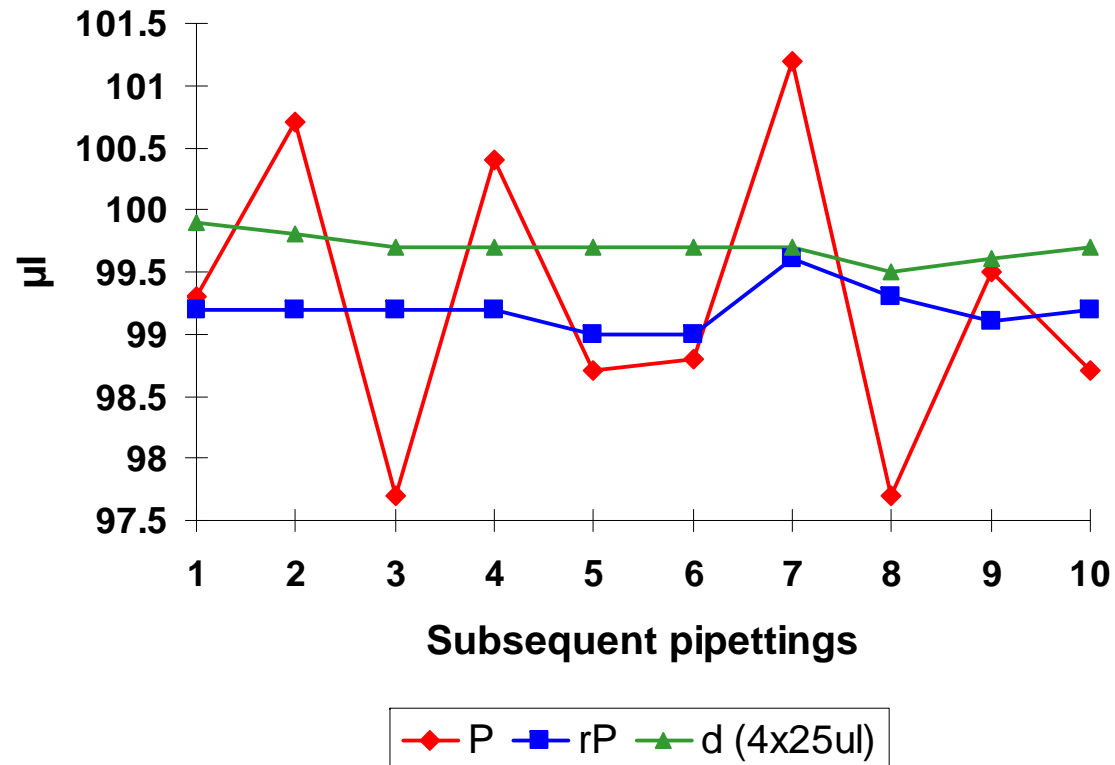


# Performance of Biohit Proline 1200 $\mu$ l Dispenser



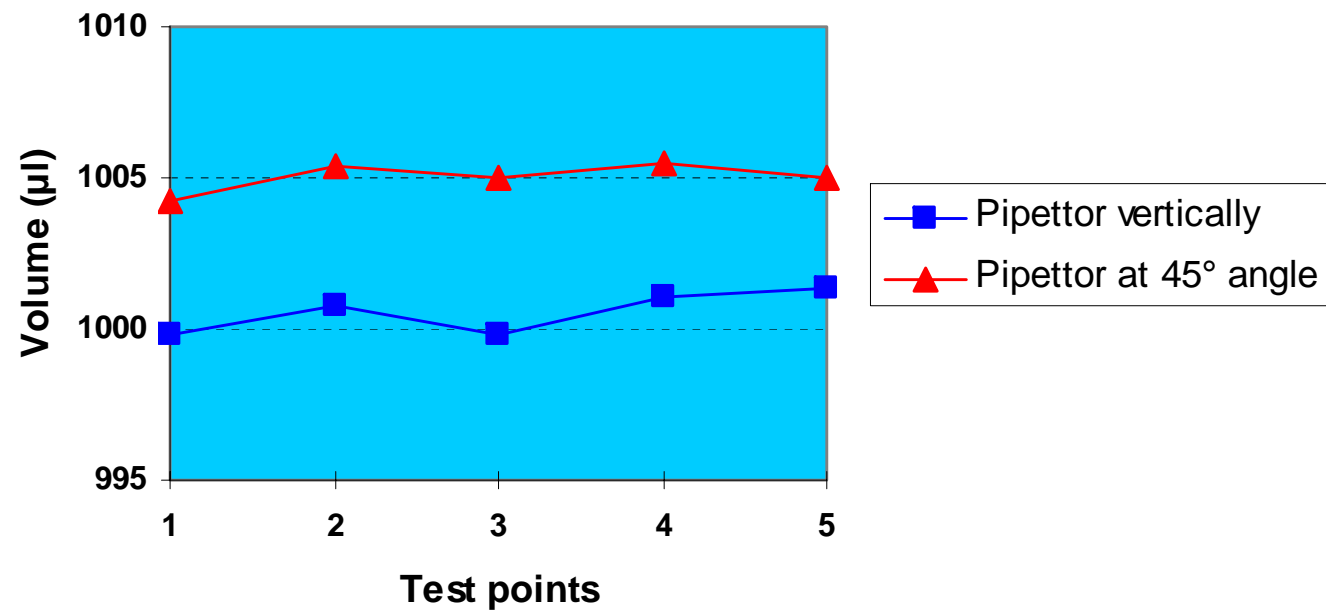
Conclusion: The 1200  $\mu$ l dispenser saves time, especially if working with multiple plates

# Pipetting Viscous Liquids (Glycerol)



Conclusion: Use the dispensing mode or at least the reverse pipetting technique when pipetting viscous solutions

# The Effect of Pipetting Angle on Pipetting Result

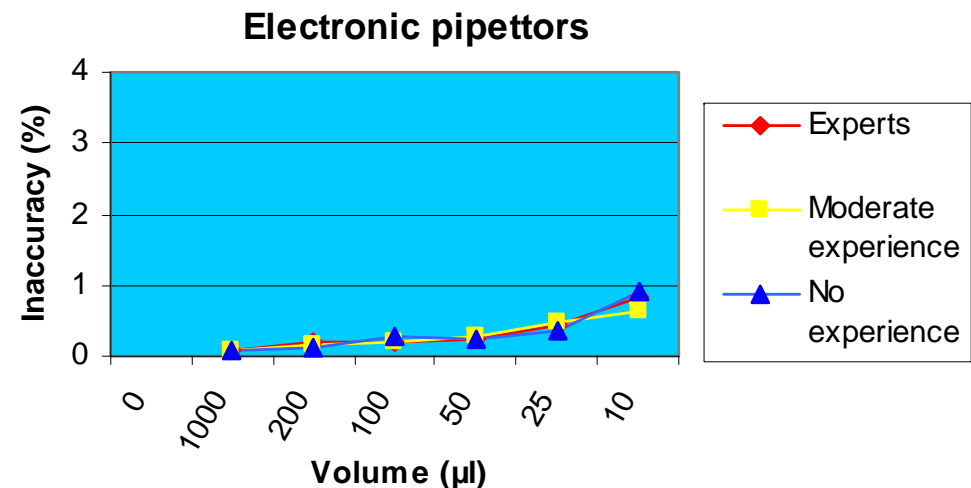
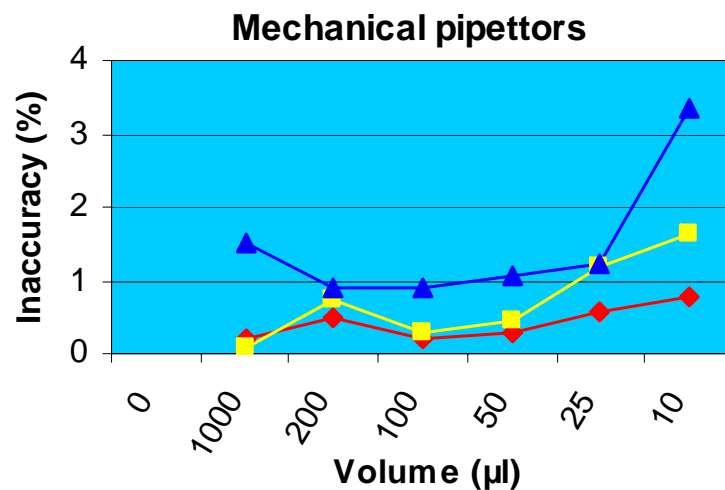


Conclusion: Keep the pipette as vertical as possible when pipetting



# The Effect of Experience on Pipetting

## *Electronic vs. mechanical pipette*



**Conclusion:** Mechanical pipettes require expertise to produce accurate results. With electronic pipettes everybody can get good results

