

Do you really know how much you're pipetting?

A comparison of different calibration methods to assure liquid handling quality

For life science researchers, accurately and precisely transferring small volumes of liquids is important for the success of most studies. Knowing you can trust that you've pipetted the correct volume is critical, but what is the best method for verifying your volume transfer? That depends on a number of different factors—use the information below to decide which methods is best for you.

The Methods

Gravimetry
A balance is used to weigh the transferred liquid, the weight of the liquid is converted to mass, and then the mass is converted to volume based on the density of the liquid.

Single Dye Photometry
A spectrophotometer measures absorbance of a known dye to determine the amount of dye present, which can be used to determine volume.

Fluorometry
Fluorescence intensity reports on the amount of dye present, and therefore the volume. Precision is measured by comparing relative fluorescence intensity between different samples.

Ratiometric Photometry
A spectrophotometer measures absorbance of two known dyes to determine the amount of each dye present and the ratio of one dye to another. The ratio measurement provides more robust accuracy determination.

How the methods compare

	Gravimetry	Fluorometry	Single Dye Photometry	Ratiometric Photometry
Usability				
Is the method suitable for automated liquid handlers? Can it be used to assess individual channels and individual wells?	⊘	●	●	●
Can you quickly perform volume verification with this method?	⊘	●	●	●
Can you quickly prepare reagents and QA them?	⊘	⊘	⊘	●
What are the total costs of this method (instrument, reagents, labor)?	\$	\$\$	\$\$	\$\$
Technical performance				
NIST traceable	●●●	●	●●	●●●
Precise	●●	●●●	●●	●●●
Accurate	●●	●	●	●●●
Volume ranges: ≥ 200 μL	●●●	●●●	●●●	●●●
50 - 200 μL	●●●	●●●	●●●	●●●
10 - 50 μL	●●	●●●	●●●	●●●
< 10 μL	●	●●●	●	●●●
Affected by additional factors				
Vibration	●			
Humidity	●			
Temperature	●	●		
Airflow	●			
Static electricity	●			
Chemical environment		●		
The need to know sample density	●			
Requires specific reagents		●	●	●

When to use each method

Gravimetry
Best for measuring the performance of single-channel devices when handling larger liquid volumes (~20 to 1000 μL).

Fluorometry
Best for demonstrating precision across nearly identical conditions when accuracy and traceability are not required.

Ratiometric Photometry
Best for measuring both accuracy and precision across a wide volume range.

Single Dye Photometry
Best for measuring precision. Accuracy measurements can also be made, although their robustness is limited due to the difficulty of ensuring that the method is properly standardized and that an uncertainty analysis yields acceptable performance.