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Technical Note

Understanding Measurement and Dilutions

Volumetric measurement is a common, repeated daily activity in most analytical laboratories. Many processes in the laboratory, from sample preparation to standards calculation, depend on accurate and contamination free volumetric measurements. Unfortunately, laboratory volumetric labware, syringes and pipettes are one of the most common sources of contamination, carryover and error in the laboratory.

The most cited sources for dilution and preparation errors stem from improper use and contamination. Improper use means that the volumetric is not used correctly either through mishandling or misunderstanding of the volumetric labware.

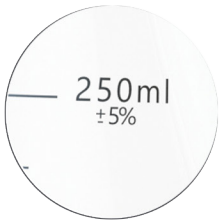
Looking at Labware

Many errors can be avoided by understanding the markings displayed on the volumetrics and choosing the proper tool for the job. There is a lot of information displayed on volumetric labware. Most labware, especially glassware, is designated as either Class A or Class B. Class A glassware is a higher quality analytical class of glassware, whereas Class B glassware is lower quality with a larger uncertainty and tolerance. If a critical measurement process is needed, then only Class A glassware should be used for that measurement. In recent years, similar markings and terminology have been applied to polymer based labware creating Class A equivalent plastic or polymer labware.

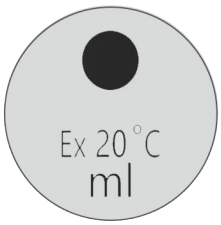
Standard information that can be found on labware are: name of the manufacturer, country of origin, tolerance, or uncertainty of the measurement of the labware, and a series of descriptors as to how the glassware should be used. Labware can be marked with letters which designate the purpose of the container. If a volumetric is designed to contain liquid, it will be marked by either the letters TC or IN. Labware which is designated to deliver liquid will be marked by either the letters TD or EX. Sometimes there are additional designations such as wait time or delivery time inscribed on the labware. The delivery time refers to the period of time required for the meniscus to flow from the upper volume mark to the reach the lower volume mark. The wait time refers to the time needed for the meniscus to come to rest after the residual liquid has finished flowing down from the wall of the pipette or vessel.

Manufacturer, Country, Certifications

Nominal Value



Tolerance



EX/IN To Deliver & To Contain

Precision Class
• Class A - Highest Quality
• Class B - Qualitative

Etchings, Graduations

The improper use and cleanliness of volumetrics can often be a significant source of error when performing dilutions or creating a calibration curve. One error or contamination issue in one of the first steps of the process can be amplified down the series of dilutions or curves.

Calibration curves are created by diluting standards into several target points along the dynamic range to cover the possible target results. Proper dilution of standards and samples is based on the understanding of basic dilution, volumetric procedures and dilution factors.

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Dilutions and Dilution Factors

The first dilution many labs make is a stock solution or starting solution. This type of working standard is made to create a higher concentration stock from raw materials or concentrated material from which other standards will be made. For this calculation, one needs the concentration of the target stock, the final weight or volume of the total stock and the purity of the raw material (or concentration of the concentrate being used).

Starting Material or Stock Starting Calculation:

X (mass or volume units of target)/final mass/volume * purity (concentration) of material * 106

Example: 90% pure compound with which you want to make a 10,000 ppm stock standard and you need to make 10 mL of standard.

Set up your equation as:

X/10 mL * 0.90 * 106 = 10,000 ppm (µg/mL)

Solve for X: 10,000 * 10/(0.9 * 106) = 0.111 µg starting material

Another type of dilution is a simple dilution using a dilution factor as seen in the equations below:

Simple Dilutions:

Dilution = volume or mass of sample / total volume or mass of (sample + diluent)

Dilution factor = total V of (sample + diluent) / V of sample
** or we can simply say the reciprocal of dilution

Example: You have a 1,000 ppm stock solution and you need to make a 10 mL of a 10 ppm for your other scientists, how much do you use?

X/10 mL x 1,000 = 10 ppm (µg/mL)

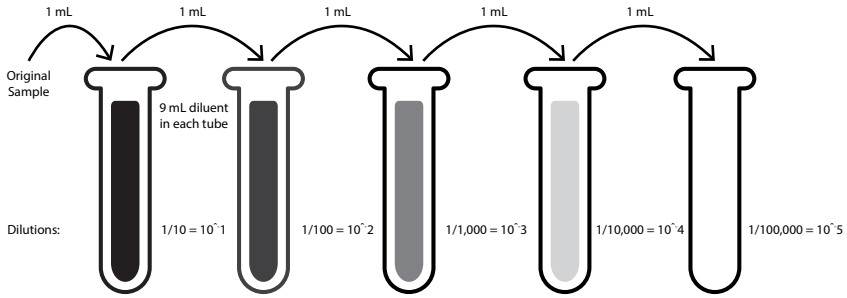
This would be a 100x dilution using 0.1 mL

In order to make calibration curves, most people employ a mixture of simple dilutions separate from each other or use a serial solution which is a series of dilutions, where each dilution is cumulative.

Serial Dilution - Series of Dilutions

(1/10) x (1/10) x (1/10) x (1/10) x (1/10) = 1/100,000 = 10-5 dilution

A typical series is shown below:



One of the best practices is to create stock solutions in a range that will need the least number of dilutions for all points in the calibration curve or can be used as the highest point of the calibration curve. Another practice to reduce error is to use larger rather than smaller volumes of the stock solutions since smaller volume measurements have larger uncertainties and can create more error. For example, one type of syringe (10 µL) Spex analyzed had about 5% error when filled to the maximum volume whereas a 1 mL syringe filled to its maximum had less than 1% error.

The first questions before dilution are: what is the dynamic range for your analysis and what is the detection range of your instrument? Ideally the detection range and the sample range should be on the same or similar magnitude. Then standards and calibration curves need to fit those ranges. For example, if one is analyzing pharmaceuticals by ICP-MS, then the target range is ppb or lower. The ideal standard stock solution for a ppb calibration range would fall in the low ppm level which then could be diluted a few times with larger volumes than if a higher ppm stock was used. The use of lower concentration standards reduces the number of overall dilutions and reduces error. The following tables can help convert your dilutions between different units of concentration.

Table 1. Weight to Weight Concentrations

Name	Symbol	Equivalence			
Parts per thousand*	ppt*	g/kg	mg/g	µg/mg	ng/µg
Parts per million	ppm	mg/kg	µg/g	ng/mg	pg/µg
Parts per billion	ppb	µg/kg	ng/g	pg/mg	fg/µg
Parts per trillion**	ppt**	ng/kg	pg/g	fg/mg	ag/µg

Table 2. Weight to Volume Concentrations

Name	Symbol	Equivalence			
Parts per thousand*	ppt*	g/L	mg/mL	µg/µL	ng/nL
Parts per million	ppm	mg/L	µg/mL	ng/µL	pg/nL
Parts per billion	ppb	µg/L	ng/mL	pg/µL	fg/nL
Parts per trillion**	ppt**	ng/L	pg/mL	fg/µL	ag/nL

Table 3. Concentration Conversions

Units	Symbol	ppt*	ppm	ppb	ppt**
1 part per thousand	ppt*	—	1 x 10^ 3	1 x 10^ 6	1 x 10^ 9
1 part per million	ppm	1 x 10^– 3	—	1 x 10^ 3	1 x 10^ 6
1 part per billion	ppb	1 x 10^– 6	1 x 10^– 3	—	1 x 10^ 3
1 part per trillion	ppt**	1 x 10^– 9	1 x 10^– 6	1 x 10^– 3	—

* ppt = parts per thousand
** ppt = parts per trillion

Tips for Dilutions

- Make sure you keep track of units (see tables provided)
- Unify units when possible - i.e. convert to grams, or µg, uniformly
- Don't forget purity when making a stock solution
- Don't forget to account for the weight or volume of internal standards or spiking solutions
- Use the dilut-u-lator on our website if needed
- Make sure your calibration points are within range of your analytical target and within the dynamic range of the instrument; if not, either the samples or standards will need to be diluted
- When in doubt, reach out to Spex for help!

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