QUALIFIED MEDICAL LABORATORY TECHNICIAN (QMLT)

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A Guide to Calculations

Prepared by the NZIMLS as a reference to calculations commonly used in Pathology

Welcome

There are many areas in Pathology where it is necessary to undertake calculations of some form e.g. making up solutions. This 'Guide' has been prepared to provide a basic knowledge of undertaking calculations in the laboratory. It is not comprehensive but it will cover the type of calculations that might be expected in the Qualified Medical Laboratory Technician (QMLT) examination. Many of the calculations may already be familiar and it is not intended to cover every type of calculation in all of the disciplines. However, the basic principles are covered in this document and it is hoped that this will help understand the use of calculations in the laboratory. The 'Guide' will work through simple symbols and terms and progress to some more detailed calculations.

Commonly Used Terminology in Calculations	
Common denominator	A common multiple of the denominators of two or more fractions (see below).
Concentrate	A substance, either liquid or solid, that is strong because it has had fluid removed from it.
Concentration factor	The strength of a chemical in a solution.
Conversion factor	A ratio that relates the same measure in the system of units; used to convert from one unit to another.
Decimal	Any number expressed in base 10, or a fraction in which the denominator is a power of 10.
Denominator	The part of a fraction that is at the bottom of a fraction. It functions as a divisor (see below).
Diluent	An agent that reduces the strength of a substance to which it is added.
Dilution	A solution that has been weakened by the addition of a diluent.
Dividend	A number to be divided.
Divisor	The number by which a dividend is divided.
Equation	A mathematical statement that expresses equality between two expressions on either side of the equals sign.
Equivalent fractions	Fractions that look different but have the same quantity.
Exponent	A symbol written above and to the right of a number. An exponent indicates how many times the number is multiplied by itself.
Formula	A rule written in mathematical symbols and numbers. A formula expresses the relationship between two or more quantities.
Fraction	A numerical representation of the quotient of two numbers (see below).
Gram (g)	The basic metric unit for weight or mass.
International System of Units (SI)	The worlds most widely used modern from of the metric system of units.
Inverse	Opposite or reverse. The inverse of a fraction is created by turning it upside down.
Kilogram (kg)	The metric unit of weight or mass that is equal to 1000 grams.
Litre (L)	The basic unit of volume in the metric system that is equal to 1000 millilitres.
Metre (m)	The basic unit of length in the metric system that is equal to 100 centimetres
Metric system	The system of measurement based on the metre, in which each unit is related to a basic unit of volume, length or mass to the power of 10.
Milligram (mg)	The metric unit of weight or mass obtained by dividing the gram by 1000.
Millimetre (mm)	The metric unit of length obtained by dividing the metre by 1000.
Numerator	In any fraction or ratio, the number at the top of the fraction.
Percent (%)	Out of one hundred; a fraction with 100 as the denominator.
Quotient:	The number resulting from the division of one number by another.
Ratio	The relationship in size or quantity between two things.
Reconstitute	To add liquid to a dried powder to return it to its original liquid form.
Simplify	To express a fraction as a ratio between smaller numbers
Solute	The substance dissolved in a liquid to form a solution.
Solution	The liquid containing a dissolved substance or substances.

Solvent	The liquid in which substances are dissolved to form a solution.
Total volume	The amount of a solution, including both solute and solvent.

Commonly Used Symbols in Calculations	
Symbol	**Meaning**
+	Plus or addition
-	Minus or subtraction
x	Times or multiplication
÷	Divide
>	Greater than
≥	Equal to or greater than
<	Less than
≤	Equal to or less than
=	Equals
≠	Not equal to
°	Degrees (temperature)
∞	Infinity
%	Percentage
#	Number
Δ	Delta, used to indicate change
√	Square root
Σ	Greek symbol; often used to indicate summation

SI Prefixes		
Multiple	**Prefix**	**Symbol**
10^{3}	kilo	k
10^{6}	mega	M
10^{9}	giga	G
10^{12}	tera	T
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p

Time

In Pathology and science in general, time is expressed as the 24-hour clock. This avoids confusion between am and pm. The 24-hour clock is based on a continuous time process throughout the day e.g.

- Morning is 0 to 12 hours (hr)
- Afternoon is 13 to 24 hours (hr)

The minutes are added after the hours but remain the same format using sixty minutes to the hour e.g.

- 0945 hours = 45 minutes past 0900 hours or 15 minutes to 1000 hours
- 1430 hours = 30 minutes past 1400 hours (2pm) or 30 minutes to 1500 hours (3pm)

Using time from the am/pm system would be converted as follows:

- 1015am =1015 hours
- 4-15pm = 1615 hours

Although the hours numerically change with the 24 hour clock, the conventional use of 60 seconds to the minute and 60 minutes to the hour remain.

Fractions, Decimals and Percentages.

All of these terms are different ways of expressing the same thing, which is a proportion or ratio. For example, half a centimetre can be expressed as a fraction ½, a decimal 0.5 or a percentage 50%.

Fractions

This is a way of expressing the ratio or relationship on either size or quantity of two things e.g. the ratio of 1 to 4 is the fraction ¼. The use of a — sign or a / sign indicates division so in reality; ¼ would be the same as 1 divided by 4 (0.25 or 25%). The number at the top of the fraction is known as the numerator (1) and the number at the bottom the denominator (4). In some circumstances the numerator may be larger than the denominator, which would mean that the ratio or outcome would be greater than 1 e.g. 5/4 (5 ÷ 4 = 1.25 or 125%).

Decimals

Decimal means based on the number 10 which means that units are divided into units of 10 or 100. This means that they are always multiples of 10 and related to the power (use) of 10. The position of the decimal point or place is of great importance when working with decimals. Examples of the relationship of the decimal point are shown below:

Fraction	Decimal Equivalent
1	1.0
1/10	0.1
1/100	0.01
1/1000	0.001

So the first position to the right of the decimal point is tenths, second position hundredths and so on. The more zeros to the right of the decimal point, the smaller the number. To avoid errors when using decimals of number less than 1, a zero should always be used immediately to the left of the decimal place e.g. .1 should be 0.1.

Rounding Numbers

This is often done to reduce the size of the answer when multiples are given and when a calculator is used e.g. 58 ÷ 65 = 0.892307692. This is clearly a longer number than is necessary and could be 'rounded' to 0.892. However if rounding numbers are used in laboratory practice, it is important that everyone rounds numbers in the same way and this need to be verified in individual laboratories e.g. 0.63856 may be rounded up to 0.639 or rounded down to 0.638. As a general rule when rounding, never include more digits than the numbers used initially e.g. 0.573 x 3.412 = 1.955076. As there are only three numbers to the right of the decimal point the answer would be 1.955. A second general rule is to round up if the next digit is 5 or greater and round down if it is 4 or less.

Percentages

This means out of 100 therefore, any number expressed, as a percent is a fraction of 100 e.g. 75% means 75 out of 100 or 75/100. In all situations with percentages the denominator is 100. Therefore decimal conversion of any per cent number is divided by 100 to get the decimal equivalent e.g. 60% is 0.6 and 6 per cent is 0.06.

Expressing Small or Large Numbers

When dealing with very small numbers or very large numbers, the method known as scientific notation is usually used. This uses exponents which are numbers written to the right and above a number e.g. 10^2 is the same as 10 x 10 or 100; 10^4 is the same as 10 x 10 x 10 x 10 or 10,000. This can also be used in reverse for decimal places e.g. 10^{-2} is the same as 1/100 or 0.01. Using this system large numbers with decimal places can be reduced to more manageable expressions e.g. 0.000002764 can be expressed as 2.764×10^{-6}.

Formulas or Formulae

These are rules that are written in mathematical symbols and numbers. They are used to express relationships between two or more quantities. Most formulas are written in the form of equations. These are mathematical statements that, in general, express equality (balance) on both sides of the equation. An example of this is shown below with the conversion of degrees Fahrenheit (°F) to degrees Celsius (°C). The formula for conversion is:

$$°C = 5/9\ (°F - 32)$$

Where:

°F = degrees Fahrenheit
°C = degrees Celsius
32 = the freezing point of water in °F
5/9 = conversion factor (see below)

The freezing point of pure water in °F is 32° F but in the Celsius scale it is 0°C, therefore 32 must be subtracted from the °F to give an equivalent zero point on both scales before starting the calculation.

The boiling of pure water the °F is 212°F and for °C it is 100°C. If the freezing point is first corrected (°F – 32) then the remainder is 180 giving two scale divisions as shown below:

°C	°F
0 to 100 scale divisions	0 to 180 scale divisions

To get the necessary conversion factor as seen in the overall conversion formula the fraction 100/180 is used, which works out at 5/9. Therefore if the conversion follows for °F -32 x5/9 the correct °C will be calculated as shown below.

Boiling point of water = 212°F. Convert to °C.

$$°C = 5/9 \times (212 - 32)$$
$$°C = 5/9 \times 180$$
$$= 100°C$$

There are three important rules to be remembered.

- When doing any calculation that has numbers in brackets, this must be done first.
- Although there is often a multiplication sign included, when there are brackets the sign may be left out e. g. 5/9(212-32) still means multiplication. It is a form of shorthand.
- The numerator of the fraction (in this case 5) is used to do the multiplication and the denominator (9) is used for the division. The concept of formula is used throughout Pathology.

Solutions

Solutions are liquids with a substance dissolved in them, known as a solute. The solute can added either as a solid or as a liquid. In Pathology all solutions have to be made-up to known concentrations, which will vary with the method or requirement for a particular solution. There are a number of ways a solution concentration can be expressed. Before preparing solutions always check for any safety requirements.

Percent composition (%w/w)

This is the solute mass (weight) per 100g solution, (w/w = weight for weight). It requires each component to be weighed but water can be taken as 100g = 100ml, therefore a measuring cylinder can be used and the weighed solute can be added.

Percent concentration (%w/v and %v/v)

For solutes added in solid from, this is the number of grams of solute per 100ml solution. This is more commonly used than Percent composition as the weighed solute can be made-up to a precise known volume using a volumetric flask (%w/v). If liquid solutes are used the same principle applies and would then be %v/v. How the solution was prepared should always be indicated using the abbreviations.

Molar solutions

This solution uses the formula or molecular weight (FW or MW both are the same) of a compound. The weight is usually given on the bottle. The weight in grams or milligrams is made up to one litre with solvent (usually water). The molecular (or formula) weight is the sum of all the constituent elements that make up the chemical. For example:

Sodium chloride has a molecular weight of 58.5, which is made up of sodium (atomic weight 23) and chlorine (atomic weight 35.5). These are added together to give the molecular weight of sodium chloride. To prepare a 1 Molar solution, 58.5 g of sodium chloride would be dissolved in water and made up to one litre using a volumetric flask. It follows that a 2 Molar (2M) solution would be 58.5 x 2 = 117g or a 0.5M solution would be 58.5 x 0.5 = 29.25g, all in one litre. Molar solutions are precise solutions.

For a 1M solution of sodium hydroxide (NaOH), the molecular weight is 40 (sodium 23; oxygen 16; hydrogen1) therefore 40g.L^{-1} would be used.

Solutions are rarely used in convenient concentrations however, the two key factors are the molecular weight and the concentration required e.g. a chemical has a molecular weight 0f 195.3 and a 0.15M solution is required.

Molecular weight = 194.3
0.15M solution required

194.3 x 0.15 = 29.14g.L^{-1}

If a smaller quantity/volume is required:

Molecular weight 194.3
0.15M solution required
25ml volume is required

First convert the volume required to a decimal so that all the units are the same, therefore 25ml = 0.025L

0.025 x 0.15 x194.3 = 0.728625g

This weight would be rounded as previously discussed, therefore:

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0.728625 = 0.729g made up to 25ml
Note the 0.729g is the same as 729mg)

Finding the Atomic Weights

All compounds are made up of elements and all elements have an Atomic Weight. These are fixed and can be found in the Periodic Table, often located in the back of textbooks. Each element has a unique weight e.g. sodium is 23. No other element has that Atomic Weight. When a chemical is presented look at what the formula conveys. e.g. potassium sulphate K_2SO_4. This indicates that are two atoms of potassium (K), one of sulphur (S) and four of oxygen (O). If the three Atomic Weights are added together, potassium 39, sulphur 32.1 and oxygen 16 (total 87.1) nowhere near the stated molecular weight of 174.1. This is because there are 2 atoms of potassium, one of sulphur and four of oxygen required to make potassium sulphate (K2SO4). Therefore to get the correct molecular weight:

Potassium (K) = 39 x 2 = 78
Sulphur (S) = 32.1 x1 = 32.1
Oxygen (O) = 16 x 4 = 64
Molecular weight = 174.1

A 1M solution of potassium sulphate would require 174.1g made up to one litre.

The same applies for liquids e.g. acids:

Hydrochloric acid (HCl)
(Hydrogen 1+chlorine 35.5 = 36.5)

Sulphuric acid (H_2SO_4)
(Hydrogen 1 x 2; Sulphur 32.1; Oxygen 16 x 4 =98.1)

An important point to remember is that the Periodic Table uses chemical notation rather than word, e.g. sodium is Na after Natrium but Sulphur is S. The naming was dependent on who discovered them and how they were discovered.

Dilutions

Frequently samples or reagents are required to be diluted in the laboratory. It is important to remember that the process of dilution makes a solution weaker than the original (stronger) solution. The amount or ratio of the concentrated or stock solution added to the total solution volume is known as the dilution factor. Therefore, as the dilution factor increases, the concentration will decrease. As the dilution factor is a simple ratio it can be calculated as follows e.g. 100 parts of a sodium solution is needed from 2500 parts of a stock solution:

$$\frac{100}{2500} = 0.04 = 1 \text{ in } 40 \text{ dilution}$$

Often dilutions are made from serum or plasma e.g. a 1 in 4 dilution would mean 1 part of serum or plasma to 3 parts of diluent and can be written as 1/4. This is very different to making a 1 **to** 4 dilution, where 1_part is added to 4 parts therefore the dilution factor would be 1/5. Remember for diagnostic analysis the diluent is very important and should always be verified if not routine.

If the dilution is from a concentrated stock solution and only a relatively small volume is required, then the dilution factor is used in the calculation. A 1 in 8 dilution is required from a concentrated stock solution to a volume of 400ml with distilled water.

The unit volume is 400ml ÷ 8 = 50ml
400ml – 50ml =350ml

Dilute 50ml of concentrated stock with 350ml of distilled water.

Similarly a 1: 5 dilution of liquid bleach to a total volume of 1.5L

Convert 1.5L to ml =1500ml
1500ml – 300ml = 1200ml

Dilute 300ml of bleach with 1200ml distilled water.

An alternative formula can be used if three of the variables are known e.g.

Stock solution New solution
$V_1 \times C_1 = V_2 \times C_2$

Where V_1 = Stock solution volume
C_1 = Concentration of stock solution
V_2 = Volume required of new solution
C_2 = Concentration required of new solution

All must be in the same units e.g. Dilute 200ml of a 0.5mol.L^{-1} sodium chloride solution to give a final molarity of 0.1mol.L^{-1}.

$$V_2 = \frac{200\ (V_1) \times 0.5\ (C_1)}{0.1\ (C_2)}$$

$$= V_2 = 1000ml$$

To obtain a 0.1mol.L^{-1} solution water would have to be added to the 200ml of 0.5mol.L^{-1} to a volume of 1000ml.

Another example might be: How much 100% bleach is needed to make 500ml of a 10% beach solution?

V_1 = unknown
C_1 = 100%
V_2 = 500ml
C_2 = 10%

$V_1 \times C_1 = V_2 \times C_2$

This time we need to know V_1

$$V_1 = \frac{C_2}{C_1} \times V_2$$

$$V_1 = \frac{10}{100} \times 500$$

Simplify the equation: $\frac{10}{100} = 0.1$

$V_1 = 0.1 \times 500ml = 50ml$

50ml of 100% bleach would be needed to make 500ml of a 10% solution.

Serial Dilutions

This is a multiple progression of diluting a sample in a known ratio. It progresses from a concentrated solution through a progressively decreasing concentration or activity. Before starting, the volume should be considered and also the availability of the sample to be diluted. This will influence the dilution factor.

If a 1 in 8 dilution is required, three tubes should be set up with one ml in each (the diluent). The initial sample dilution is 1 in 2 (1 part of sample to I part diluent). One part of the 1 in 2 is then added to the second tube containing diluent making a 1 in 4 dilution. This is then added to the third tube containing one ml of diluent giving a 1 in 8 dilution. The volumes can change e.g. only 100μL may be used but the important consideration is to keep everything constant i.e. with 100μL of sample each of the tubes would contain 100μl of diluent.

Another method of serial dilution is to consider what the final dilution factor of the sample is needed e.g. a 1 in 100 dilution is required. The initial dilution factor here is 1 in 10 (1 part sample to 9 parts diluent). Next, one part of the 1 in 10 is added to two parts of diluent making it a 1 in 20 dilution. To prepare a 1 in 100 dilution from the 1 in 20 dilution a 1 in 5 dilution is made (1 part of 1 in 20 to 4 parts of diluent), giving a 1 in 100 dilution.

Centrifugation

This is a process in which centrifugal force is used to separate solid material in a liquid suspension e.g. blood cells from whole blood to provide serum or plasma. There are many types of centrifuges used in Pathology but they all have the same basic components, these being a rotor and carriers (or buckets) attached to a vertical shaft. Centrifugation works on the principle of centrifugal force and this depends upon three factors: mass, speed and radius. The speed is expressed as revolutions per minute or rpm and the relative centrifugal force or RCF (may also referred to as relative centrifugal field or RCF also) or in some situation as gravities (G). Units are expressed as the number of times greater than gravity e.g. 500G.

The RCF is calculated using the formula shown below:

$$RCF = 1.118 \times 10^{-5} \times r \times rpm$$

Where: 1.118×10^{-5} is a constant determined from the angular velocity;
r is the radius, usually in centimetres (cm), from the centre of rotation to the bottom of the tube in the cavity or bucket during centrifugation;
rpm is the speed of the rotor in revolutions per minute.

Although this is the frequently stated formula, an easier alternative version to work with is:

$$RCF = 1.118 \times r \times (rpm/1000)^2$$

Using this formula, r = radius in mm. An example calculation is shown below:

Calculate the RCF from 4000rpm with a rotor radius 120mm. (*Note the brackets are always calculated first*).

$$RCF = 1.118 \times 120 \times (4000/1000)^2$$
$$= 1.118 \times 120 \times (4)^2$$
$$= 1.118 \times 120 \times 16$$
$$RCF = 2147G$$

Statistics

Statistics is a numerical approach to information, which enables an evidence-based understanding of the information. In Pathology, statistics are used to establish reference ranges and aid in quality control of assays to ensure a given method is working correctly. In some disciplines e.g. Histology, numerical information is not always used however, controls can be used. These may be known staining reactions for a particular tissue section so that a positive and a negative staining reaction can be assessed when staining patient tissue. This gives quality assurance that the method is working correctly.

When using numerical data, there are set terms and calculations that allow the classification of the text results and assessment of the accuracy of the methods. Some of the basic terms used in statistics are defined below.

Measures of location

Mode	this is the result that occurs most often from a series of number or results e.g. six numbers: 3, 4, 5, 5, 6, 7. The number that occurs most often is 5, therefore the mode is 5.
Median	this is the result that is in the middle of all the values. Using the numbers above, it is also 5.
Mean	this is the arithmetic average of all the individual results. All the numbers are added together and divided by the total number of the numbers added. Using the same numbers as above: 3+4+5+5+6+7 = 30 Total number = 6 Mean = 30/6 = 5

Note: The mode, median and the mean are not always the same e.g. take the following numbers:

60, 90, 90, 91, 92, 93, 94

Here the mode is 90, the median is 91 and the mean is 87.

Measures of dispersion

To reliably assess test results, the mean will not indicate how the range of results are spread. This is illustrated in the example below with two samples.

Sample 1: 87, 88, 89, 90, 90, 92, 93
Sample 2: 80, 80, 81, 85, 95, 99, 100, 100

Both will have the same mean of 90 but the values have very different distributions (spread). A measure of dispersion will reflect the difference in dispersion.

Range

The simplest measure of dispersion is range, which is simply the difference between the highest and the lowest numbers e.g.

Range = Highest value – Lowest value

The problem is that this depends on the highest and lowest values and extreme values will bias the results. A better measure of dispersion is the standard deviation.

Standard Deviation

The standard deviation has a number of abbreviations. SD is the most frequently used in Pathology but it may also be represented as σ (lower case sigma). The standard deviation is calculated as the average difference of each of the observed (known) values from the mean value. In general, the greater the standard deviation indicates the greater the spread of the values around the mean. This indicates the greater the sample's variability e.g. a mean of 5.5 with an SD of 2.3 has greater variability than a mean of 5.5 with an SD of 1.2. Most quality control systems will automatically calculate the standard deviation and it is not necessary to manually calculate this statistic as a routine measure. Overall the result would indicate the mean plus or minus the standard deviation e.g.

$$\text{Mean} \pm \text{SD} = 5.5 \pm 1.2$$

Normally for most results 2 x SD is taken, which covers 95% of all results therefore:

Mean = 5.5
2 x SD = 2.4
the 95% limits (reference range) = 3.1 to 7.9

Variance

Both the mean and the standard deviation are measured in units e.g. mmol.L^{-1}. If it is necessary to compare results that are measured in different units or have very different means, a measure known as coefficient of variation (CV) can be used. Here the standard deviation is divided by the mean and multiplied by 100 to give a percentage e.g.

$$CV = (SD/mean) \times 100$$

Because this has been standardized, any two coefficients of variation can be compared. In general the larger the coefficient of variation, the poorer the precision e.g.

Method 1: mean = 100μmol.L^{-1} SD =2.4
Method 2: mean = 91μmol.L^{-1} SD = 2.9

Method 1: CV = (2.4/100) x 100
= 2.4%

Method 2: CV = (2.9/91) x 100
= 3.1%

Method 1 with a lower coefficient of variation is more precise as it has less variation.

Quality Control

The full scale of quality control in a Pathology laboratory is outside the consideration of this Guide. However, there are three important concepts - precision, accuracy and random.

- Precision is considered to be the closeness of repeated measurements to each other.
- Accuracy is the closeness of a measured or derived result to the true value.
- Random as the name implies is neither precise nor accurate
- In Pathology it is expected that results would be both Precise and Accurate.

For normal quality control there are a number of techniques used to monitor both quality control over various 'run' numbers and possible changes from day to day. One technique is to use a known value quality control sample and plot the analytical values against the known mean and standard deviation (this sets the control limits). This is known as a Levy-Jennings chart (or plot) and it is a method of rapid quality control for a given method. Normally the limits are set at the mean $\pm$ 2 standard deviations.

Graphs

Many result and quality control results may be represented as a graph. The basic values of preparing a graph are considered here. Normally graphs have two axes, which are mutually perpendicular. It is conventional to refer to the horizontal axis as the x-axis (or abscissa) and the vertical axis as the y-axis (or ordinate). The point where the x and y axis intersect is known as the origin. As a general rule in Pathology, only positive values are shown but it is possible to use graphs for negative values also. Normally the x-axis is used for the independent variable (treatment or test) and the y-axis for the dependent variable (response). Each axis must have a descriptive label with the units of measurement or response. Both the x and y-axis must have a scale (units) with reference points on each axis to show how numbers are located.