How many measurements should we make in order to be confident of our reported results?

We know that the spread of analytical data in a population after reasonable large n measurements is shown by the bell-shaped curve under a normal probability distribution, as measured by the standard deviation or its square, the variance. However it is more informative if we could give a range of values (i.e. confidence interval) that would encompass some proportion (say 95% or 99%) of repeated data, and possibly the *true value* of the measurand.

For a normal distribution with population mean μ and standard deviation σ, the symmetric interval about the mean containing a confidence fraction (1-α) of the results is given by

$$\mu \pm z_{\alpha/2}\sigma \qquad (1)$$

where $z_{\alpha/2}$ value is obtained from the normal distribution tables and α is less than 1. In other words, $z_{\alpha/2}$ is the number of standard deviations on either side of the mean containing a confidence fraction (1-α) of the distribution. For example, a 95% confidence interval, α = 0.05.

When we have obtained n means due to repeated measurements, the interval containing (1-α) of the means is given by the standard deviation of the mean, σ_n as below:

$$\mu \pm z_{\alpha/2}\sigma_n = \mu \pm \frac{z_{\alpha/2}\sigma}{\sqrt{n}} \qquad (2)$$

$\sigma/\sqrt{n}$ is called the "**standard error of the mean**". It is interesting to note that means of even a small number of repeats tend to be close to normally distributed. This is an outcome of the Central Limit Theorem. In the case of 95% confidence interval, $\alpha/2$ = 0.025, $z_{0.025}$ = 1.96, and thus

$$\mu \pm z_{0.025}\sigma_n = \mu \pm \frac{z_{0.025}\sigma}{\sqrt{n}} = \mu \pm \frac{1.96\sigma}{\sqrt{n}} \qquad (3)$$

In other words, the 100(1-α)% confidence interval on our experimental mean $\bar{x}$ is given by

$$\mu - \frac{z_{\alpha/2}\sigma}{\sqrt{n}} < \bar{x} < \mu + \frac{z_{\alpha/2}\sigma}{\sqrt{n}} \qquad (4)$$

By rearranging the above equation, we have a confidence interval on μ given $\bar{x}$

$$\bar{x} - \frac{z_{\alpha/2}\sigma}{\sqrt{n}} < \mu < \bar{x} + \frac{z_{\alpha/2}\sigma}{\sqrt{n}} \tag{5}$$

In the case of 95% confidence interval, the probability (Pr) of a mean falling outside the range $\mu \pm 1.96\sigma/\sqrt{n}$ is 0.05. From here we can deduce that

$$\Pr\left[\frac{|\bar{x} - \mu|}{\sigma/\sqrt{n}} > 1.96\right] = 0.05 \tag{6}$$

or, in general:

$$\Pr\left[\frac{|\bar{x} - \mu|}{\sigma/\sqrt{n}} > k\right] = p \tag{7}$$

where k is the appropriate coverage factor and p, the probability.

In practice, we can only analyze and obtain a small number of data which are not enough to define them as μ and σ. When we have a series of data ($x_1, x_2, \ldots, x_i, \ldots, x_n$), we can only calculate the corresponding sample statistics' $\bar{x}$ and s. The "sample mean" $\bar{x}$ is the ordinary arithmetic mean given by equation (8):

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \tag{8}$$

and the "sample standard deviation" s is

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \tag{9}$$

The statistics $\bar{x}$ and s are estimates of the unknown parameter μ and σ, and usually approach them more closely when n increases. As $\bar{x}$ and s are variables and would be different each time we repeat the experiment, they cannot be used directly to replace the parameters $\bar{x}$ and s in the probability equations above. Instead, we can substitute z with a variable t (also called Student's t) , giving

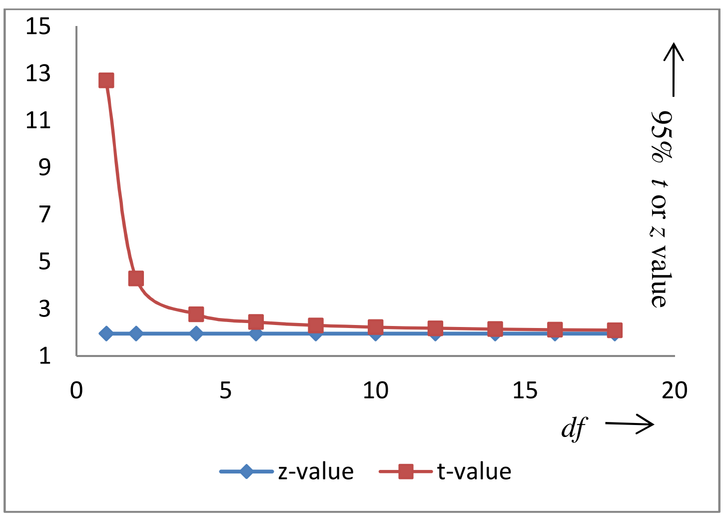
$$\bar{x} - \frac{t_{\alpha/2}s}{\sqrt{n}} < \mu < \bar{x} + \frac{t_{\alpha/2}s}{\sqrt{n}} \tag{10}$$

from which we obtain for two-tailed probabilities

$$\Pr\left[\frac{|\bar{x} - \mu|}{s/\sqrt{n}} > t\right] = p \tag{11}$$

The equation $\Pr\left[\bar{x} - \frac{ts}{\sqrt{n}} < \mu < \bar{x} + \frac{ts}{\sqrt{n}}\right] = 1 - p$ tells us the range in which the unknown population mean μ falls with a probability of (1-p) and is known as the $100(1\text{-}p)\%$ confidence interval. Therefore, if p=0.05, we have a 95% confidence interval.

Figure 1 below shows a plot of the Student's t-value for calculation of a 95% confidence interval with increasing degrees of freedom (df). The corresponding z-value from the normal distribution is also shown here ($z_{\alpha=0.025}$ = 1.96).



We can rewrite the Student's t equation (10) as below:

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}} \tag{12}$$

or, $$|\mu - \bar{x}| = \frac{ts}{\sqrt{n}}$$

or, $$t = \frac{|\mu - \bar{x}|\sqrt{n}}{s} \quad (12A)$$

or, $$n = \left(\frac{ts}{|\mu - \bar{x}|}\right)^2 \quad (12B)$$

Now, the difference of μ and $\bar{x}$, *i.e.* $|\mu - \bar{x}|$ is the measurement error. We can cast the equation (12B) in terms of the relative standard deviation (RSD in %) and ε as the relative error (%), as below:

$$n = \left(\frac{tRSD}{\varepsilon}\right)^2 \quad (12C)$$

Note : Relative error, % $\varepsilon = \dfrac{100|\mu - \bar{x}|}{\mu}$

The question on how many n measurements we should make from a population is answered by how precise we want the result to be. This is particularly important form the sampling statistic point of view when the number of samples to be randomly taken is to be determined with confidence.

As we normally do not know the population statistics μ and σ but can only have some preliminary experiments done, we are in a dilemma on how many measurements that we have to carry out in order to be meaningful.

However, we do notice from the equation (12) that at n=7 and α=0.05, t-value at 6 degree of freedom = 2.45 and √7 = 2.65. It is apparent therefore that the equation (12) would almost cancel off the t-value and √7, leaving s as about the 95% confidence interval of the mean.

By applying equation (12C), the **Table 1** below shows an interesting comparison between the degree of freedom (n-1) on one hand and the relative error % and RSD on another.

Table 1: The relationships amongst relative error, relative standard deviation and n measurements

Rel Error %	5	Rel Error %	5	Rel Error %	10
RSD %	15	RSD %	10	RSD %	15
t-value @ α=0.05, df=6	2.45	t-value @ α=0.05, df=6	2.45	t-value @ α=0.05, df=6	2.45
$df=n-1$	n	$df=n-1$	n	$df=n-1$	n
6	54	6	24	6	13
53	36	23	17	12	11
35	37	16	18	10	11
36	37	17	18	10	11
36	37	17	18	10	11
36	37	17	18	10	11

The conclusion of Table 1 is that in order to make lesser number of measurements, we need either to raise the expectation of small relative error ε% or to reduce the RSD, indicating that the precision of the experiment has to be improved further. How many measurements to be carried out therefore call for your professional judgement and past scientific experience on this particular analysis.