MERIDIAN BIOTECHNOLOGIES Ltd
Vials & Cocktails for Liquid Scintillation

LSC Counting - "Do's and Don'ts"

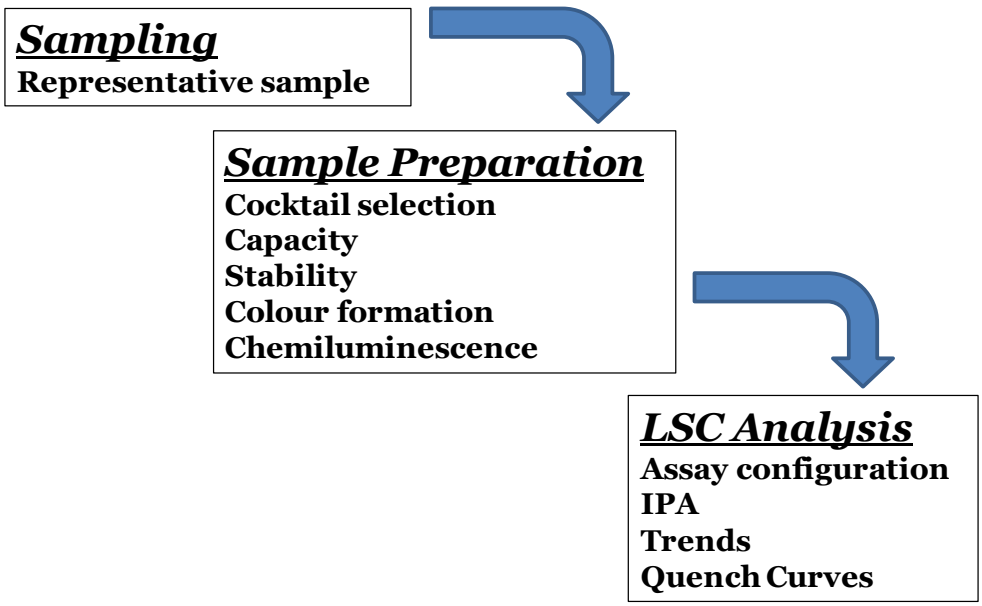
Introduction

The art of LSC counting is littered with pitfalls for the unwary and without careful attention to detail the reported results can end up being almost meaningless.

This is a somewhat frightening statement but it is derived from many years experience of providing customer application support and guiding customers into methods that produce substantive results. To many users the combination of an LSC cocktail and an LSC counter is simply a vehicle that converts their sample into a number. However, by taking a little time to consider all aspects of sample preparation and counting the chances of producing erroneous results may be almost eliminated.

The Analysis Path

The process that converts a sample into results is shown in Figure 1 below and shows the important aspects of each step that require careful consideration.



Sampling

It may seem strange to start at "sampling" but remember that everything after this point is totally dependent upon the integrity and representativeness of the sample. It is critical that a truly representative sample is taken. Everything after this step can be ruined if the sampling process is flawed in any way. A badly taken sample can only give incorrect results, irrespective of the sophistication of the LSC counter.

Sample Preparation

LSC Cocktail selection

Selecting the correct LSC cocktail for the sample is an essential part of the process and should be considered carefully. The usual temptation is to try "whatever is in the cupboard" and see if it works.

This approach is not recommended.

There is a possibility the LSC cocktail will actually work. The LSC cocktail may appear to work, but if the added sample is close to the maximum capacity of the LSC cocktail subsequent lots may not be suitable and form an unstable mixture. The first step is to look in the supplier's catalogue, or online, to see if the LSC cocktail can accommodate the type and volume of the sample on test. If that information is not shown then

"Contact the supplier and ask for advice"

Capacity & Stability

Whenever either a new LSC cocktail is being used or a different sample is being analysed it is highly recommended that the cocktail/sample mixture be evaluated in glass vials. Simply add the sample to the cocktail and shake thoroughly. If a clear microemulsion is formed than the first part is complete. Allow this mixture to stand for at least 15 minutes and if there is no change in appearance then it can be confidently assumed that the mixture will remain stable. It is critical to consider that the counting temperature may be significantly different to the preparation temperature e.g. some mixtures are prepared at 20°C but are counted at 14°C. If there is any difference between preparation temperature and counting temperature simply place the prepared mixture in the counter and leave for at least 30 minutes but preferably for the count time. If the mixture remains stable then it is suitable for counting. After this evaluation is complete it is possible to change from glass vials to plastic vials safe in the knowledge that the mixture is stable.

"Always carry out first evaluation in glass vials to observe stability"

"Always confirm stability at counting temperature"

Colour Formation & Chemiluminescence

It is important to be aware that certain samples can induce colour formation when added to an LSC cocktail. Such colour formation is almost always associated with the generation of spurious counts, known as chemiluminescence. Chemiluminescence is light produced by a chemical reaction and as such will have a finite lifetime i.e. it decays with time as the reactants are consumed. The addition of both acids and alkalis can produce colour and chemiluminescence in many LSC cocktails and their potential effects must be carefully considered and monitored.

Alkaline materials, especially strong alkalis, are particularly prone to inducing chemiluminescence. This chemically induced light is formed when alkaline materials react with free peroxides which originate from the ethoxylate detergents present in the LSC cocktail. Over time the ethoxylate linkage will degrade and produce free peroxides. Some LSC cocktails contain free radical scavengers which help while others are treated to remove all peroxides at the point of manufacture. Prior to bottling they are purged with nitrogen or argon to reduce the potential for peroxide formation.

"Do not use cocktails which have been stored past their expiry date without checking"

There are a variety of ways to overcome this potential problem:-

1. Add a small amount of acid (e.g. acetic acid) which is usually sufficient to alter the pH back near neutrality. If the alkali is neutralised there is no reaction and hence no chemiluminescence.
2. If adding acid is not an option then simply allow the sample/cocktail mixture to stand for 30 to 60 minutes to allow the reaction to go to completion. Once the reactants are used up there will be no more light

produced. Do not heat the mixture to speed up the reaction as this has the potential to degrade the entire cocktail.

3. If appropriate reduce the energy region of counting to "mask" the chemiluminescence. Almost all the chemiluminescence is confined to the 0 to 4keV window and therefore for Tritium it is possible to use 4 to 18keV and for Carbon-14 use 4 to 156keV. If DPM mode is being used then remember that for some older LSC counters it might be necessary to reinstall the quench curve using this reduced energy region setting.

4. Ideally it is better to use an LSC cocktail that has guaranteed chemiluminescence resistance and to check with the supplier to confirm suitability with strong alkalis.

5. Finally, when using a strong alkali it is always better to run a blank (no activity present) with the cocktail to confirm that there is no chemiluminescence problem.

"**Take care and check when working with strong alkali samples"**

It is difficult to imagine that an acid can also induce chemiluminescence, especially since we recommend adding an acid to subdue or overcome the problem. However, there is one particular acid that can induce chemiluminescence and it is TCA (tri-chloroacetic acid). The process by which this produces chemiluminescence is currently not know but be aware of the potential problem.

In general the only problem that can result from adding an acid sample is colour formation but this is restricted to very strong or concentrated mineral acids. Concentrated hydrochloric will produce a yellow colour; concentrated nitric acid will produce a brown colour and concentrated sulphuric acid will produce a variety of colours. Simply dilute the acid prior to adding to the cocktail.

"Avoid adding concentrated mineral acid samples"

LSC Analysis

Liquid Scintillation Counter Settings

When designing and making a protocol or assay for counting the sample it is important to ensure that the energy regions chosen are appropriate for the isotope in use. The count time should be relevant to the level of activity in the sample. The count time should be such that there is a high level of confidence in the result. Using a measure like %2s value will give an indication of the level of accuracy that may be recorded. Ensure that the correct type of quench correction curve is used if the analysis is to be reported in DPM or Bqs.

Ensure your instrument settings are appropriate for your sample

IPA

Most LSC counters in use today have some form of standardisation and calibration and with the Perkin Elmer Tri-Carbs it is called IPA (Instrument Performance Assessment). It involves running a set of three sealed standards (Carbon-14, Tritium and Background) to verify instrument performance. It is recommended that the IPA is run at least once a week and the results should be viewed to ensure acceptable instrument performance. If the standards are continually left in the LSC counter then an IPA will be run daily.

"Run the IPA at least once a week"

IPA Evaluation

It is important that not only do you run an IPA but that you look at the historical results to look for trends and these can be seen in the diagnostics section. Finding that the background is rising and/or the Tritium efficiency is dropping almost always indicates that there is some form of contamination or that the PMT's (photomultiplier tubes) are dirty. At this point it is highly recommended that the instrument be serviced to return it to its optimal performance condition.

"Check for trends regularly"

Quench Curves

Most users work with commercially available quenched sets which are manufactured using NIST (National Institute for Science and Technology) traceable radioactivity. It is commonplace to accept that the purchased set of quenched standard are correct but it is good practice to confirm correct and accurate performance. This can be completed by simply running the sealed standards as samples in DPM mode, and the results should be within ± 4% of the expected DPM.

"Run the quenched sealed standards as samples to confirm accurate DPM recovery"

For best instrument performance the quench curve should be reinstalled at least every 6 months but preferably every 3 months. In addition, all quench curves should also be reinstalled after an instrument service or repair.

"Reinstall quench curves at least every 6 months and after servicing"

Once purchased it is important to consider storage of the quenched set. Ideally it best to store the quenched set in a refrigerator or in a cooled LSC counter. During storage do not expose the set to extremes of heat or light as these can potentially degrade the LSC cocktail carrier used in the quenched set.

"Store the quenched standards away from heat and light"

Overall the above produces a few statements that are pertinent to good LSC sample preparation and counting:-

" Any LSC cocktail will work with any LSC - The trick is to get the best combination"

" Poor sampling can only produce a bad sample"

" A badly prepared sample can only produce bad results"

" No amount of instrument sophistication can ever produce good results from a badly prepared sample"