In LSC counting the measured substance is dissolved into a scintillation cocktail i.e. liquid consisting of a solvent and fluors which produce light when excited by radiation. Because the sample is mixed with the scintillator its chemical and physical properties may affect the scintillation process resulting in variable light production. This phenomenon is called quench and its main forms are chemical quench, in which light production is affected by the chemical composition of the sample and colour quench in which transmission of light from scintillating atoms to the PMT varies due to the absorption in the sample. Colour quench varies also depending on the sample volume, while the chemical quench does not. In addition to these optical quench occurs if dirty scintillation vials are used.

**Constant quench**

Quench correction should be applied if the quench varies from sample to sample. If it is constant the correction is not needed and it is enough that the result value is multiplied by a factor. The results are then to be expressed as DPM rather than CPM.

**Variable quench**

You can measure and compensate for the variable quench in LSC samples with the Triathler. There are two methods available:

1. **Internal standard method**

   In this method additional samples, with known activity are used. An unknown sample is first measured and its CPM value is recorded. A known amount of the nuclide (internal standard) is then added to the cocktail and the sample is recounted.

   The efficiency value is:

   \[
   \text{Eff} = \frac{\text{CPM} (sd + x) - \text{CPM} (x)}{\text{DPM} (sd)}
   \]

   The DPM value of the unknown sample can be now calculated:

   \[
   \text{DPM} (x) = \frac{\text{CPM} (x)}{\text{Eff}}
   \]

   where

   \[
   \text{Eff} = \text{counting efficiency}
   \]

   \[
   \text{CPM} (x) = \text{the recorded CPM value of the sample}
   \]

   \[
   \text{CPM} (sd + x) = \text{the recorded CPM value of the sample mixed with the internal standard}
   \]

   \[
   \text{DPM} (sd) = \text{the activity of the internal standard}
   \]

   Internal standards for H-3 and C-14 are available from Wallac. This is the most reliable method available to determine the true activity of a quenched sample. This method is more convenient with manual liquid scintillation counter than with the automatic ones.

2. **The quench curve method**

   This method relies on the use of a quench curve. The curve establishes the relationship between a quench parameter and the counting efficiency. First the quench curve must be determined by counting a set of samples with the same activity but variable quench. When unknown samples are counted, the quench parameter is measured for each sample. Corresponding efficiency for the measured (or
interpolated) quench parameter is obtained from the curve and the corresponding DPM value is calculated.

Quench parameter indicates a relative light production from the sample and there are many methods to calculate it from the sample spectrum. In the Triathler the quench parameter (Qp) is, in mathematical terms, the centre of the gravity of the spectrum in the counting window.

If the quench increases the light production decreases and the spectrum and its centre of gravity decreases as well. Qp and counting efficiency have seldom a linear relationship therefore the quench correction curve is not usually linear either. The Triathler uses parabolic regression to form the curve.

**Calibrating the DPM standard curve**

Prepare a suitable quench set, i.e. a number of samples having the same activity but variable quench level.

Select the nuclide e.g. H-3, C14 or P-32. Go to '4'.

reset (0)

Take the first sample of the quench series (the least quenched) and count it. After the counting has terminated change the sample. Count all samples in the quench series. After the last one press stop

MaxDPM 55749

sd DPM: 18.0

Give the activity (as DPMs) of the quench standards or just press Enter. In the latter case the CPM value of the first sample is taken as the true DPM. The Triathler now prints the quench parameters and the corresponding efficiencies of the standards. It also indicates the efficiency taken from the curve and an error percentage (err%), which is the difference of efficiencies (difference between measured eff. and the one taken from the quench curve). An example of the printout follows:

<table>
<thead>
<tr>
<th>Sample Time &lt;s&gt;</th>
<th>Counts</th>
<th>CPM</th>
<th>QP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>278743</td>
<td>55749</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>265425</td>
<td>53085</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>208079</td>
<td>41616</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>153357</td>
<td>3067</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>69876</td>
<td>13975</td>
</tr>
</tbody>
</table>

*** DPM standards ***

Std DPM: 55749

\[
Eff = Qp^2 * (-0.0008) + Qp * (0.0881) + (-0.7777)
\]

<table>
<thead>
<tr>
<th>Eff</th>
<th>qp</th>
<th>Eff</th>
<th>err%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>26.44</td>
<td>1.001</td>
<td>0.13%</td>
</tr>
<tr>
<td>0.952</td>
<td>25.37</td>
<td>0.951</td>
<td>-0.15%</td>
</tr>
<tr>
<td>0.746</td>
<td>21.38</td>
<td>0.746</td>
<td>-0.07%</td>
</tr>
<tr>
<td>0.550</td>
<td>17.96</td>
<td>0.551</td>
<td>0.16%</td>
</tr>
<tr>
<td>0.251</td>
<td>13.23</td>
<td>0.250</td>
<td>-0.25%</td>
</tr>
</tbody>
</table>

Value saved

End Time  2: 36 : 4

Select Yes to save the standardisation. Triathler accepts minimum of 2 or maximum of 10 standards. If the number of standards is 2 or 3 the err% is zero because the parabolic curve can always fit to 3 points.

**Counting samples with DPM correction**

To enable the DPM counting select edit and the following part in the protocol:

Y<YES>DPM corr.

yes exit no

Selecting YES enables DPM counting. However the quench parameter is calculated and printed regardless of whether the DPM calculation is enabled or not.
The counting procedure is otherwise exactly the same as when counting normal LSC samples. The DPM value is calculated as follows (the actual numbers naturally vary and the following values are only for the DPM calibration values shown in the previous example):

\[ \text{Eff} = -0.0008 \times Qp^2 + 0.0881 \times Qp + 0.7777 \]

and

\[ \text{DPM} = \frac{\text{CPM}}{\text{Eff}} \]

This method is more economical and faster than the use of internal standard. The quench curve is made once and it could be used for a long time without it being necessary to remake it. It should be remembered, however, that the quench parameter is influenced by the same random variation than the radioactive decay in general. The variation in the DPM results is therefore larger than the corresponding variation in CPM results. In order to produce reliable DPM results a high enough number of counts should be collected. Therefore this method is inferior in low count rates when compared to its internal standard method. It is also important to realise that a quench curve applies to only one set of measurement conditions - one nuclide, scintillation cocktail, counting window and sample volume.

### Setting counting windows

The idea behind the use of counting windows is to register only the counts, which originate from the sample. There are other counts (background), which arise from cosmic rays or radioactivity from the environment. Solely the counts with certain energy, i.e. the ones within the counting windows are recorded, cutting part of the background. In the H-3 and C-14 counting the number of photons generated is low and therefore it is important also to separate multiphoton (at least two) events from the single photon events such as chemiluminescence in the sample and thermal emission in the PM tube.

In the ideal case the MCA scale should be calibrated (by setting PMT voltage) to keVs and this is the case also in the Triathler, when gamma counting is performed. One keV corresponds to one channel and as a consequence the setting of counting windows is straightforward too. But this is not applicable to the LSC counting because the spectrum position and shape changes depending of the quench. Each cocktail and sample has a unique quench level and the optimum window is not in the theoretical place. As a consequence there is no one and only “correct” window. The practical way to set counting windows is as follows:

- **The lower limit is set so that the single photon peak does not cause elevated counts in the counting window (see background counts from the calibration certificate).**

- **The upper window is set so that the whole spectrum falls below the upper level (the least quenched sample is the most critical).**

The factory set value of 35 - 200 is suitable for an unquenched sample (Wallac H-3 standard which is nitrogen purged). However in practice a lower upper limit is needed. The lower limit does not depend on the samples quench. The narrower counting window the lower the background counts.

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The most accurate way to set windows is to send a spectrum to an external PC (minimum Excel 5.0 in Windows 3.11) and set the windows based on printed spectra.

Typical spectra of the unquenched H-3, C-14 and Blank samples are described in the appendix 1 on the following page. There you can see that the spectra of a blank sample goes down at about channel number 35. This would be
suitable lower limit of discrimination for both H-3 and C-14 when a new PM-tube is used (type EMI). H-3 spectrum ends at about channel 200 and C-14 spectra at about channel 1000.

Typical spectra for H-3 and C-14 samples (unquenched sealed standards). H-3 windows from channel 35 to 200. C-14 windows from 100 to 1000.

Fig 1 Discrimination lower and higher windows