What are the Practical Limitations on Multilabel Counting?

One of the most useful and most exploited applications of a liquid scintillation (LS) counter is its ability to accurately determine two (or three!) radioisotopes in the same sample simultaneously. However, as this application becomes more popular we are receiving more questions on how to set up the LS counter to perform these types of experiments, how the calculations are performed and what are the basic sources of counting error in multilabel experiments. In order to answer these questions we will start with an explanation as to why multilabel counting experiments are “difficult” and proceed to answer questions dealing with the practical limitations and sources of error in multilabel studies.

Beta Decay and the Concept of “Spill”

Radioisotopes which emit beta particles actually emit two particles simultaneously, these being an electron and an antineutrino. In the process of electron emission the atomic number \( Z \) of the atom is increased by one — the atomic mass \( A \) remaining unchanged.

Beta Emission:

\[
\begin{align*}
\begin{array}{c}
A \\
Z
\end{array}
\rightarrow
\begin{array}{c}
A \\
Z+1
\end{array}
\begin{array}{c}
\beta^- \\
+ \bar{\nu}
\end{array}
\end{align*}
\]

(where \( \beta^- \) is the electron and \( \bar{\nu} \) is the antineutrino). The energy released in this decay is SHARED between the beta particle and the antineutrino. That is, sometimes the beta particle will get all of the energy, while other times the antineutrino will get all of the energy. Generally, however, the beta particle and the antineutrino will both get a portion of the available energy. Since the antineutrino is NOT detectable in the LS counter, the energy spectrum of the beta particle varies from zero to some maximum energy \( E_{\text{max}} \). This \( E_{\text{max}} \) value is unique for every beta emitting isotope; for example, tritium has an \( E_{\text{max}} \) of 18 KeV; carbon-14 is 156 KeV and phosphorous-32 is 1710 KeV. Figure 1 details the energy spectrum of a tritium sample.

![Energy Spectrum of a Tritium Sample](image1)

**Figure 1.** “Energy Spectrum” of unquenched tritium sample in an LS counter.

All beta energy spectra vary from zero to their unique \( E_{max} \). Figure 2 shows spectra of tritium and carbon.

![Beta Energy Spectra](image2)

**Figure 2.** LS Spectrum of a dual label tritium/carbon sample detailing the “spill” of the carbon into the tritium window (Shaded area).
Consideration of Figure 2 illustrates two important aspects of multilabel counting. First, the higher energy isotope (carbon-14 in this case) can be counted without interference by the lower energy isotope (tritium). Second, the lower energy isotope cannot be counted without also counting some of the higher energy isotope (the shaded region in Figure 2). The interference of the higher energy isotope with the lower energy isotope is referred to as "spill".

The Effect of Quench:

The major problem in multilabel isotope counting is dealing with varying quench levels in a series of samples. The reason for this is that as quench increases, the beta energy spectrum shifts to the left. This results in an increase in the amount of carbon-14 spill in the tritium window as demonstrated in Figure 3. (Compare the amount of "spill" in Figure 2, an unquenched spectrum, with that in Figure 3, a quenched spectrum.)

![Diagram of Counts vs Channel (log ENERGY)](image)

FIG. 3. The effect of quench on the carbon-14 "spill" into the tritium window.

When analyzing multilabel samples, the instrument must be capable of correcting for the counts in the lower energy isotope window due to this spill of the higher energy isotope at any quench level.

Dual Label DPM Calculations

From the above figures one can see that CPM in the tritium window is a combination of both tritium and carbon-14. Likewise, the CPM in the carbon-14 window may be a combination of carbon-14 and tritium (albeit, only a small amount). Thus,

\[
CPM_1 = \text{EFF}_{11} DPM_1 + \text{EFF}_{12} DPM_2 \\
CPM_2 = \text{EFF}_{21} DPM_1 + \text{EFF}_{22} DPM_2
\]

CPM<sub>1</sub>: Counts per minute in Channel 1 (tritium)
CPM<sub>2</sub>: Counts per minute in Channel 2 (carbon-14)
\text{EFF}_{11}: Efficiency of tritium (isotope 1) in channel 1
\text{EFF}_{12}: Efficiency of carbon (isotope 2) in channel 1
\text{EFF}_{21}: Efficiency of carbon (isotope 2) in channel 2
\text{EFF}_{22}: Efficiency of tritium (isotope 1) in channel 2

DPM<sub>1</sub>: Actual tritium (isotope 1) activity
DPM<sub>2</sub>: Actual carbon (isotope 2) activity

Solving the above equations for DPM<sub>1</sub> and DPM<sub>2</sub> (the relevant quantities) one gets:

\[
DPM_1 = \frac{CPM_1 \text{EFF}_{22} - CPM_2 \text{EFF}_{21}}{\text{EFF}_{11} \text{EFF}_{22} - \text{EFF}_{12} \text{EFF}_{21}} \quad \text{Eq}
\]

\[
DPM_2 = \frac{CPM_2 \text{EFF}_{11} - CPM_1 \text{EFF}_{12}}{\text{EFF}_{11} \text{EFF}_{22} - \text{EFF}_{12} \text{EFF}_{21}} \quad \text{Eq}
\]

CPM<sub>1</sub> and CPM<sub>2</sub> are determined by the instrument and \text{EFF}_{11}, \text{EFF}_{22}, \text{EFF}_{12}, and \text{EF} are calculated by the instrument from previously stored quench curves (see: YOU WANTED TO KNOW... Volume 1 Number 1, page 6).

Quench and Automatic Quench Compensation (AQG)

Four quench curves must be set up in the LS counter in order to perform dual label experiments:

A. Tritium efficiency in the tritium window (EF<sub>11</sub>)
B. Tritium efficiency in the carbon window (EF<sub>12</sub>)
C. Carbon efficiency in the tritium window (EF<sub>21</sub>)
D. Carbon efficiency in the carbon window (EF<sub>22</sub>)

These four quench curves are generated from two quench sets, in case, one for tritium and one for carbon. As quench increases, tritium efficiency in the tritium window decreases and the spill (or efficiency) of the carbon in the tritium window increases (see Figure 2). Representative quench curves for the tritium window detailed in Figure 4.