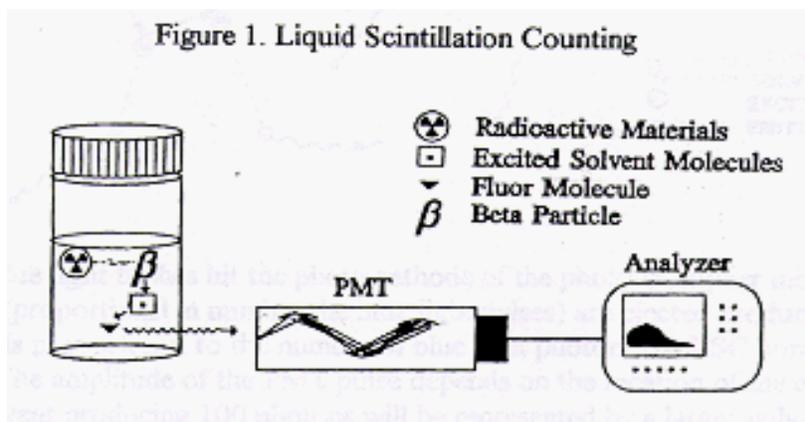


LIQUID SCINTILLATION COUNTING

Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting (β and α) radioactive samples, it can also detect the Auger electrons emitted from ^{51}Cr and ^{125}I samples.

LIQUID SCINTILLATION PRINCIPLES

Figure 1 provides a graphic illustration of the way the emitted radiation interacts with the cocktail (a mixture of a solvent and a solute) leading to a count being recorded by the system.



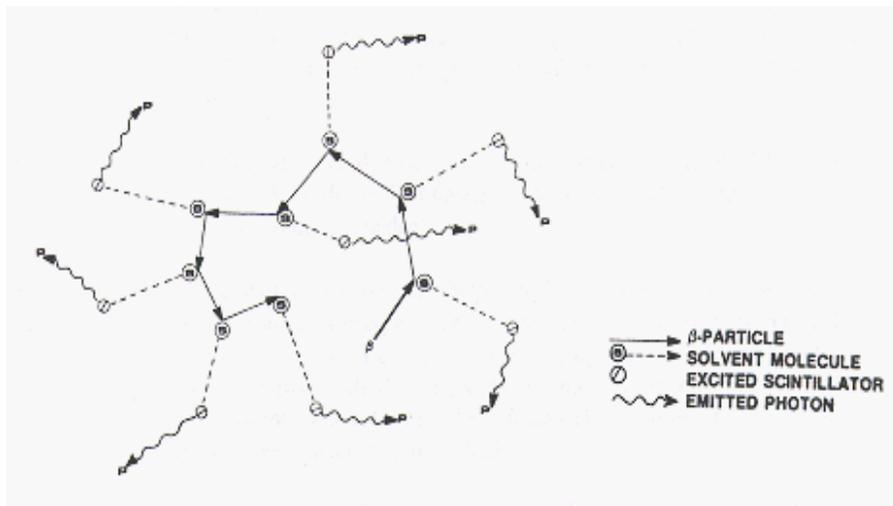
Step 1. Beta particle is emitted in a radioactive decay. To assure efficient transfer of energy between the beta particle and the solution, the solution is a solvent for the sample material.

Step 2. In the relatively dense liquid, the beta particle travels only short distances before all of its kinetic energy is dissipated. Typically a beta particle will take only a few nanoseconds to dissipate all its kinetic energy. The energy is absorbed by the medium in 3 forms: heat, ionization and excitation. Some of the beta energy is absorbed by solvent molecules making them excited (not ionized).

Step 3. Energy of the excited solvent is emitted as UV light and the solvent molecule returns to ground state. The excited solvent molecules can transfer energy to each other and to the solute (Figure 2). The solute is a fluor. An excited solvent molecule which passes its energy to a solute molecule disturbs the orbital electron cloud of the solute raising it to a state of excitation. As the excited orbital electrons of the solute molecule return to the ground state, a radiation results, in this case a photon of UV light. The UV

light is absorbed by fluor molecules which emit blue light flashes upon return to ground state. Nuclear decay events produce approximately 10 photons per keV of energy. The energy is dissipated in a period of time on the order of 5 nanoseconds. The total number of photons from the excited fluor molecules constitutes the **scintillation**. The intensity of the light is proportional to the beta particle's initial energy.

Figure 2. Illustration of the Collision Process



Step 4. Blue light flashes hit the photo cathode of the photo multiplier tube (PMT). Electrons (proportional in number the blue light pulses) are ejected producing an electrical pulse that is proportional to the number of blue light photons. A LSC normally has two PMT's. The amplitude of the PMT pulse depends on the location of the event within the vial. An event producing 100 photons will be represented by a larger pulse if the event is closer to the PMT than if the event is more remote. The signal from each PMT is fed into a circuit which produces an output only if the 2 signals occur together, that is within the resolving time of the circuit, approximately 20 nanoseconds (coincidence circuit). By summing the amplitude of the pulses from each PMT, an output is obtained which is proportional to the total intensity of the scintillation. This analog pulse rises to its maximum amplitude and falls to zero.

Step 5. The amplitude of the electrical pulse is converted into a digital value and the digital value, which represents the beta particle energy, passes into the analyzer where it is compared to digital values for each of the LSC's channels. Each channel is the address of a memory slot in a multi-channel analyzer which consists of many storage slots or channels concerting the energy range from 0-2000 keV.

Step 6. The number of pulses in each channel is printed out or displayed on a CRT. In this manner, the sample is analyzed and the spectrum can be plotted to provide information about the energy of the radiation or the amount of radioactive material dissolved in the cocktail.

LSC TERMINOLOGY

Chemi-luminescence	Random single photon events which are generated as a result of the chemical interaction of the sample components. Except at high rates, most chemiluminescence events are excluded by the coincidence circuit.
Chemical Quenching	A reduction in the scintillation intensity seen by the photomultiplier tubes due to materials present in the scintillation solution that interfere with the processes leading to the production of light. The result is fewer photons per keV of beta particle energy and usually a reduction in counting efficiency.
Cocktail	The scintillation fluid; a mixture of 3 chemicals (solvent, emulsifier, and fluor) which produces light flashes when it absorbs the energy of particulate radioactive decay.
Compton Scattering	Elastic scattering of photons (x/γ -rays) by electrons. In each such process the electron gains energy and recoils and the photon loses energy. This is one of the three ways photons lose energy upon interacting with matter, and is the usual method with photons of intermediate energy and materials of low atomic number. Named for Arthur H. Compton, the American physicist who discovered it in 1923.
CPM	Counts per minute. This is the number of light flashes or counts the LSC registered per minute. The number of decays produced by the radioactivity is usually more than the number of counts registered.
Discriminator	An electronic circuit which distinguishes signal pulses according to their pulse height or voltage. It is often used to exclude noise or background radiation counts.
DPM	Disintegration per minute. The sample's activity in units of nuclear decays per minute.
Efficiency	The ratio, CPM/DPM, of measured counts to the number of decays which occurred during the measurement time.
Fluor	A chemical component of the liquid scintillation cocktail that absorbs the UV light emitted by the solvent and emits a flash of blue light.
Fluorescence	The emission of light resulting from the absorption of incident radiation and persisting only as long as the stimulating radiation is continued.
Luminescence	A general term applied to the emission of light by causes other than high temperature.

Optical Quenching	A reduction in the scintillation intensity seen by the photomultiplier tubes due to absorption of the scintillation light either by materials present in scintillation solution or deposited on the particle energy and usually a reduction in counting efficiency.
PMT	The Photo-Multiplier Tube is an electron tube that detects the blue light flashes from the fluor and converts them into an electrical pulse.
Phosphor	A luminescent substance or material capable of emitting light when stimulated by radiation.
Photo-luminescence	Delayed and persistent emission of single photons of light following activation by radiation such as ultraviolet.
Pulse	Electrical signal of the PMT; its size is proportional to the radiation energy absorbed by the cocktail.
Quenching	Anything which interferes with the conversion of decay energy emitted from the sample vial into blue light photons. This usually results in reduction in counting efficiency.
QIP	The Quenching Index Parameter is a value that indicates the sample's level of quenching. Another parameter that describes the amount of quenching present is the transformed Spectral Index of External Standard (tSIE) or "H" number.
Secondary Scintillator	Material in the scintillation cocktail which absorbs the emitted light of the primary scintillator and re-emits it at a longer wavelength, nearer the maximum spectral sensitivity of the photomultiplier tubes. It is added to improve the counting efficiency of the sample.
Solvent	A chemical component of the liquid scintillation cocktail that dissolves the sample, absorbs excitation energy and emits UV light which is absorbed by the fluors.

LSC EXTERNAL SETTINGS

LSC's come in a variety of shapes and types and manufacturers may use different terminology, however, the following basic external controls are commonly found on most systems.

Gain	A control used to adjust the height of the signal received by the detecting system. The gain control for newer LSC's is often automatically set for the particular radionuclide selected.
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LLD The lower level discriminator setting is used to discriminate against (i.e., not count) betas with energy below that setting. This setting is also used to decrease system noise which often occurs in the region below 3 keV.

ULD The upper level discriminator setting is used to discriminate against any beta energy higher than that setting.

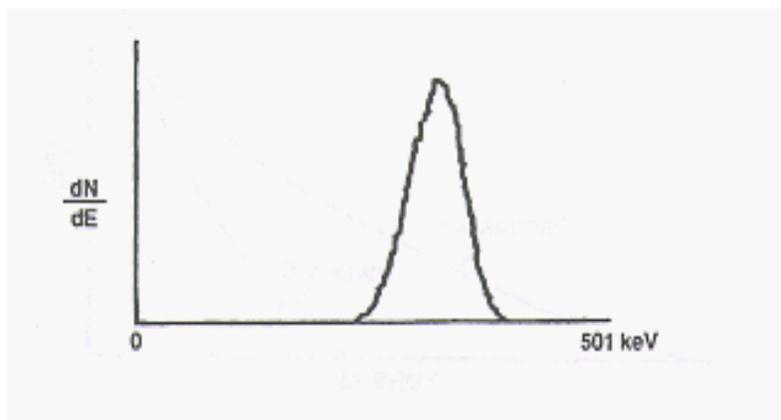
A particular LSC may have other external controls depending on the counter type and model. Read the instruments operating manual to gain familiarity with the controls and operating characteristics.

CONSIDERATIONS IN ISOTOPIC ANALYSIS

The beta particle must have sufficient energy to produce at least 2 photons in the cocktail and one must interact with each PMT. Below a few keV of energy the yield of photons, under ideal conditions, is 7-8 photons per keV. The photo cathode of the PMT is not 100% efficient. The conversion efficiency from a photon to a photoelectron is only about 30%. The coincidence threshold occurs below 1 keV.

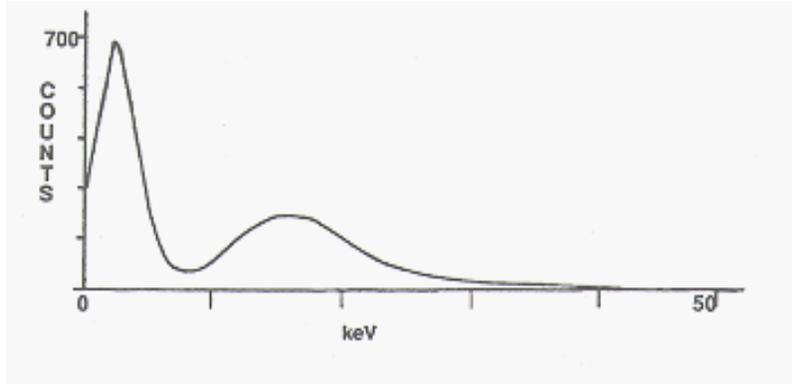
The use of LSC for alpha emitters is attractive because it offers counting efficiencies of 100% and simplicity of sample preparation. Most alpha radionuclides emit high-energy particles in the range of 4 - 6 MeV. A characteristic property of alpha particle interaction with liquid scintillation media is a low scintillation or photon yield as compared to beta or even gamma emitters (light yield is about a factor of 10 lower). Almost all the kinetic energy associated with an alpha emission is given up to the media in a relatively short distance. The relative scintillation yield from this depends upon specific ionization potentials. The higher the specific ionization potentials, the lower the relative photon yield. Result is poor alpha radionuclide energy resolution. Even though alpha emission is monoenergetic, the pulse height distributions are broad (Figure 3).

Figure 3. Alpha Spectrum of ²⁴¹Americium



The organic scintillators used in LSC's have a lower gamma ray absorption coefficient than inorganic (NaI) scintillators. The photoelectric effect is small when $E_\gamma > 300$ keV and Compton scattering becomes the main absorption process. The pulse thus depends upon gamma energy. For $E_\gamma < 20$ keV, the photoelectric effect in which all gamma energy transferred to a single electron, predominates. For $20 \text{ keV} < E_\gamma < 100$ keV, both the photoelectric and Compton effects contribute. And, for $100 \text{ keV} < E_\gamma < 3000$ keV, the Compton effects predominates. But for ^{125}I , counting efficiency can be as high as 76% in a typical emulsifier type LSC (Figure 4).

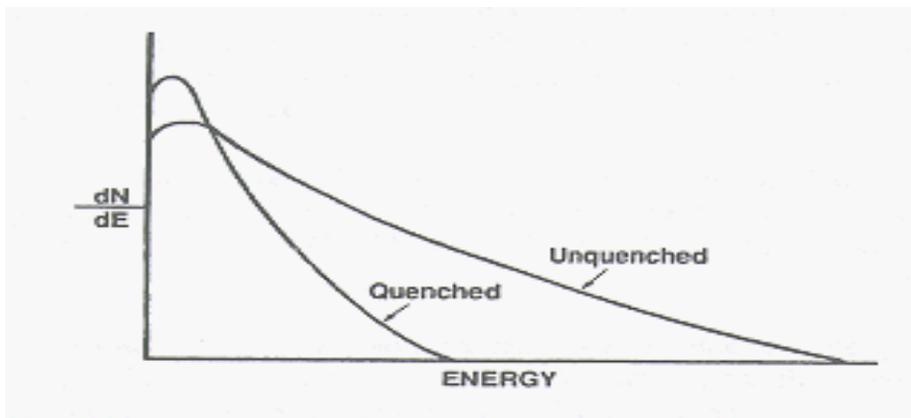
Figure 4. Spectrum Plot of ^{125}I Using LSC



QUENCH

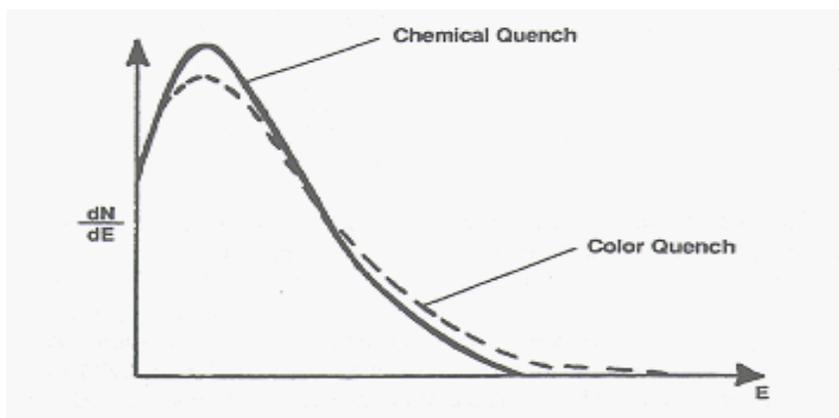
Quench is a reduction in system efficiency as a result of energy loss in the liquid scintillation solution. Because of quench, the energy spectrum detected from the radionuclide appears to shift toward a lower energy (Figure 5). The three major types of quench encountered are photon, chemical, and optical quench. **Photon** quenching occurs with the incomplete transfer of beta particle energy to solvent molecules. **Chemical**, sometimes called impurity, quenching causes energy losses in the transfer from solvent to solute. **Optical** or **color** quenching causes the attenuation of photons produced in solute.

Figure 5. Effect of Quenching on an Energy Spectrum



Chemical quenching absorbs beta energy before it is converted to photons while color quenching results from the passage of photons through the medium. Color quenching depends on the color of the interfering chemical and path length that the photon must travel (Figure 6). In a chemically quenched sample, all energy radiations appear to be equally affected whereas, for a colored sample, events that take place close to one PMT will give rise to a large pulse and a smaller pulse in the other PMT. By summation, the pulses are added so the resultant pulse height may be as large as from unquenched, only the # of events will be significantly reduced. Thus, at equal quench levels, the pulse height of colored samples are spread over a wider range than chemical quench samples.

Figure 6. Chemical vs Color Quench

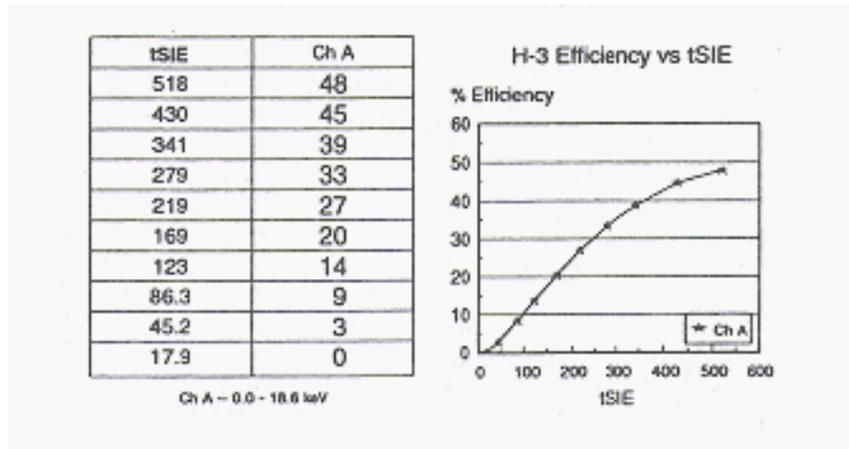


Because quench affects the efficiency of sample detection, quench could have a significant impact on your LSC results. To better understand the importance of quench on your work note these three different quench curves and the resulting efficiencies. These quench standards were counted on a Packard 1900 LSC at the University of Wisconsin - Madison. On a different system it is likely that the quench numbers and resultant efficiencies will be a little different, but not the general effects of quench. The Packard allows the user to select keV regions of interest. For this demonstration the three channels selected were: Channel A, 0.0 - 18.7 keV, Channel B 18.6 - 156 keV, and Channel C 0.0 - 2000 keV.

Two of the standards counted were ^3H (Figure 7) and ^{14}C (Figure 8). Channel A encompassed the entire energy region for ^3H . Channel B was selected as a region from the top of Channel A to the maximum possible energy of ^{14}C , 156 keV. Channel C was selected as the entire region (up to 2000 keV).

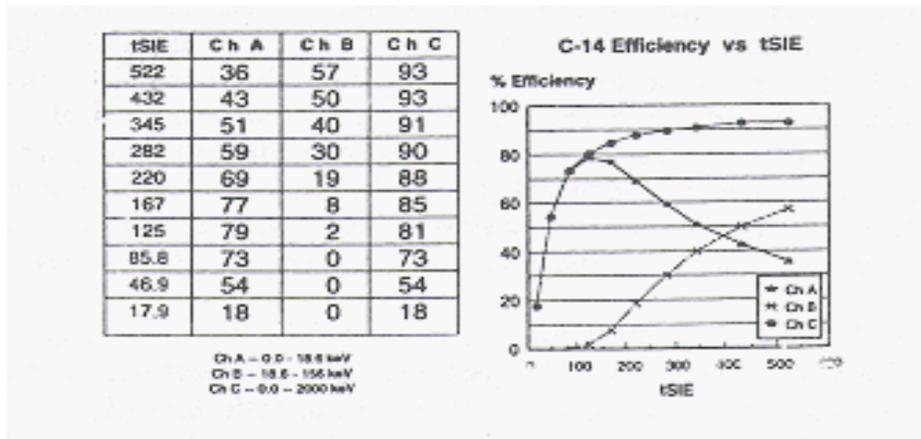
The ^3H results are shown in Figure 7. Note the extremes of values for the quenching parameter (tSIE). A maximum efficiency of approximately 48% is achieved with a quench parameter (tSIE) of 518. The minimum efficiency of 0.33% is obtained with a quench of 17.9. Thus a quench of 45 or below would result in essentially background counts (efficiency ~ 3%).

Figure 7. ³H Quench Curve



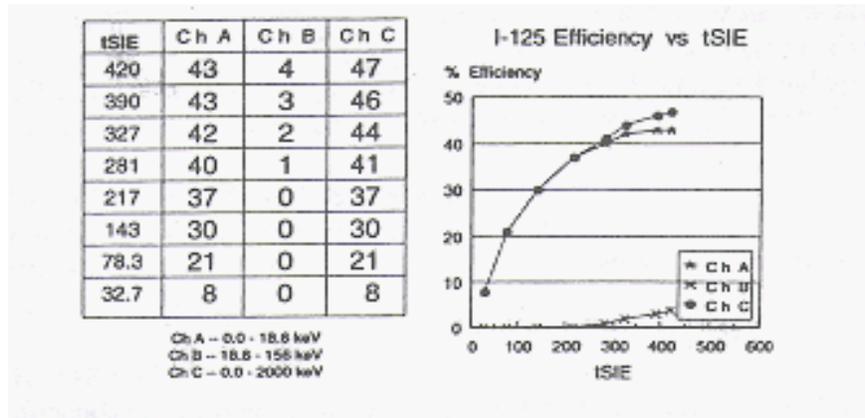
The ¹⁴C was deliberately counted in two different channels to demonstrate the effect quench has on where the count is produced in the LSC. Figure 7 depicts the Channel A, Channel B, and Channel C (A + B) results. Again, the extremes from quench are evident. Looking only at Channel C, a maximum efficiency of approximately 92% is achieved with a quench parameter (tSIE) of 522. A minimum efficiency of about 18% is obtained with a quench of 17.9. The higher energy beta (¹⁴C) sample means it is not as severely affected by quench as a low energy (³H) beta source. But, notice that as quench increases (tSIE decreases) the counts begin shifting from Channel B to Channel A even while the total efficiency remains above 80% (at tSIE = 167) as depicted in Channel C, the majority of counts are now occurring in Channel A as opposed to Channel B.

Figure 8. ¹⁴C Quench Curves



A quenched set of ¹²⁵I standard was also counted. The decay of ¹²⁵I is by internal conversion and the decay energy interact with K- and L-shell electrons ejecting monoenergetic **Auger** electrons at 3.6 keV and, at a much lower abundance, 22 keV. This is reflected in Figures 4 and 9 by the small number of counts in Channel B (18-156 keV). Quench rapidly shifts all counts from Channel B to Channel A. An efficiency of 47% is achieved with a quench parameter (tSIE) of 420. The minimum efficiency of 8% is seen with a quench of 32.7.

Figure 9. ^{125}I Quench Curves



The point of this discussion: **Quench is important.** You must understand the impact of quench and how the system you are using represents it if you want to obtain viable results. Quench calibration delimits the valid ranges for quantifying a sample. Samples with quench numbers outside the calibration range will raise a **flag** which indicates the value is out of range. The conversion to dpm will be made, but will be an extrapolation from the highest/lowest recorded quench value.

CHEMILUMINESCENCE/PHOTOLUMINESCENCE

Luminescence is a single photon event and is registered as a count due to the probability of having coincidence events at high luminescence activity. Although LSC's employ a coincidence circuit, luminescence events stimulate each PMT within the resolving time of the coincidence circuits.

Chemiluminescence is the production of light as a result of a chemical reaction between components of the scintillation sample in the absence of radioactive material. This most typically occurs when samples of alkaline pH and/or samples containing peroxides are mixed with emulsifier-type scintillation cocktails, when alkaline tissue solubilizers are added to emulsifier type scintillation cocktails, or when oxidizing agents are present in the sample. Reactions are usually exothermic and result in the production of a large number of single photons. **Photoluminescence** results in the excitation of the cocktail and/or vial by UV light (e.g., exposure to sunlight or UV lights). Chemiluminescence has a relatively slow decay time (from 0.5 hr to > 1 day depending on the temperature) while photoluminescence decays more rapidly (usually < 0.1 hr).

The luminescence spectrum has a pulse height distribution which overlaps the ^3H spectrum. The maximum pulse height corresponds to approximately 6 keV and the spectrum is (chemical) quench independent. The equivalent of a few keV of beta particle energy, the maximum number of events will occur between 0 and 2 keV and remain there independent of quenching. Contrary to popular belief, cooling the luminescent scintillation samples reduces the photon intensity to low levels, but interference is still present and provides false indication of luminescence control.

STATIC ELECTRICITY

Static electricity on liquid scintillation vials is a single photon event with pulse height limited to about 10 keV. Many items used in the liquid scintillation counter environment are conducive to the development of static charges. In general, glass vials have less problems with static than plastic vials; small vials in adapters are particularly prone to static charge build up. Most systems offer an option which employs a static charge device or an electrostatic controller.

SAMPLE VOLUME/DUAL PHASE SAMPLES

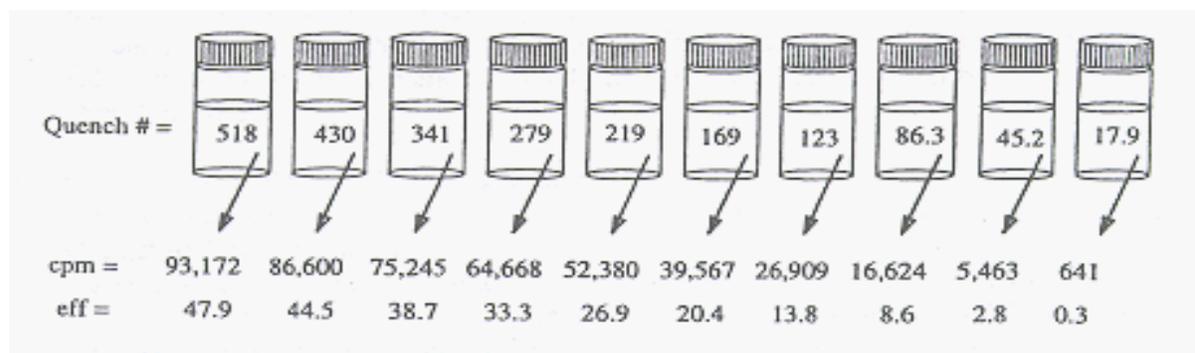
As the sample volume decreases, light output falls on less efficient areas of the PMT, so energy detection becomes less efficient with low volumes. When 2 phases are present, each phase will have its own counting efficiency.

CALIBRATION OF LSC

LSC efficiency depends on the degree of quenching, the nature of the sample, the scintillator used and the preparation method. Not all particulate (α, β) radiations are detected equally well by the LSC. Usually the higher the beta particle energy the higher the efficiency of the counter. To use the system's efficiencies for dpm calculation, a particular LSC counter must first be calibrated for the type of sample that it will analyze. Although there are several methods by which LSCs can be calibrated, the external standard method described below is the most widely used method of efficiency calibration.

- C A 10 vial standard set (Figure 10) each containing the same amount of radioactivity (i.e., dpm) but mixed with increasing amounts of a quenching agent (e.g., nitromethane, $C-Cl_4$) is used. Quenching agents absorb the radiation energy and, instead of emitting a pulse of UV light, they radiate infrared so the fluors do not get excited. Thus, the more quenched the sample, the fewer the counts detected in the desired channel. The reduced amount of light emitted per radiation energy absorbed usually results in a shifting of the spectrum to lower channels.

Figure 10. Set of 3H Quenched Standards



Each vial contains 194,433 dpm -- 3H

Table 1. Typical LLD, ULD and Gain Values

Isotope	LLD (keV)		ULD (keV)		Beckman # ¹	Gain ²
	Max Energy	Optimum	Max Energy	Optimum		
³ H	0	0	18.6	12	400	50%
¹⁴ C	0	12	156	156	670	6%
³² P	0	5	1700	1700	1000	2%

¹The LLD and ULD can be set using keV or channel controls. The configuration of Beckman LSC's uses channel controls and the corresponding channel numbers are listed.

²The gain control for newer LSC's is automatically set

- C The quenched standards are placed into a LSC tray which is then placed into the LSC. Set the LLD, ULD (or channel # for Beckman LSCs) and Gain as appropriate (Table 1), and initiate counting. Note that for statistical reasons, the ULD for ³H is usually set to a lower value than the endpoint of the ³H spectrum.
- C The number of counts registered (cpm) for each standard vial and the amount of sample quench (QIP) are determined by the counter and printed out.
- C Because all standards contain the same amount of activity, the efficiency, the ratio **cpm/dpm**, of the counter for each of the various levels of quenching can be plotted as in the graph in Figure 6.

SAMPLE ACTIVITY "DPM" DETERMINATION

- C Count your samples, the **counts per minute** and the **quench** level are printed out for each sample.
- C Look up the efficiency for each sample at its quench level from the calibration curve (Figure 6).
- C To determine the activity (dpm) from the reported counts per minute (cpm), divide the number of counts by the efficiency (i.e., $dpm = cpm/eff$). Most new counters, if set up properly, will perform this calculation for you for 1 or 2 isotopes. If dual label dpm calculation is required a quenched standard set for each isotope must be run and that data stored in the counter.

OPERATING PROCEDURES FOR LS COUNTERS

- C Read the instruments operating manual to gain familiarity with the controls and operating characteristics of the machine.
- C Place samples into LSC vials and add the correct amount of liquid scintillation cocktail (e.g., 5, 10, 15 or 20 ml, as appropriate). Include a background vial which contains scintillation cocktail and a non-radioactive sample similar in make-up (e.g., geometry) to your radioactive samples.

- C Place your sample vials with background vial into the LSC tray (or belt) and place into the LSC.
- C Set count time, noting that shorter count times give poor counting statistics.
- C Set your LLD, ULD, and Gain as appropriate and begin your counting.
- C Unless the counter is programmed to calculate **dpm** you will need to calculate the true radioactivity of the sample in units of **dpm** by dividing the sample **cpm** by the counter efficiency for that energy of the sample (i.e., $dpm = cpm/eff$). As previously discussed, the counter efficiency may be different for different vials depending on the amount of quenching present.

BECKMAN LSC CONSIDERATIONS

All LSCs operate in the same manner, but different manufacturers may use different terminology or offer more options than others. Some systems allow the user to select the regions of interest by selecting a keV range of interest. Others offer several options (channel or keV). Most of the comments made so far apply to Packard style counting systems, where the user selects the energy regions. The channels correspond to energy in 0.5 keV increments; that is, the 4000 channels are each ½keV wide, so the system can detect energies from 1-2000 keV.

If you are using a Beckman counting system, usually the channel option is the default option for the window setting. Beckman counting systems have 1000 channels and the energy is related to the equation:

$$\text{Channel \#} = 72 + 280 \log_{10} (E_{\max})$$

where E_{\max} is in keV. Thus the ULD channel settings on a Beckman LSC to detect the maximum possible beta energy for ^3H , $^{14}\text{C}/^{35}\text{S}$ and ^{32}P would be approximately 400, 670 and 1000 respectively.

CERENKOV COUNTING

Some beta emitting isotopes can be analyzed on an LSC without using any cocktail. The literature of several manufacturer's discusses counting high energy ($E_{\max} > 800$ keV) beta emitters without cocktail or with only a little water, using a technique called **Cerenkov** counting.

When high energy beta particles travel faster than the speed of light relative to the medium they are traversing (e.g., water, etc.) Cerenkov radiation (i.e., light) is produced. Cerenkov radiation is the blue light that you see when you look into a reactor pool. Cerenkov radiation allow some beta emitting radionuclides to be analyzed with a liquid scintillation counter without using any cocktail. For Cerenkov radiation to be produced, the beta particle must exceed a minimum threshold energy (E_{th}) which is calculated by:

$$E_{\text{th}} = \frac{511 n}{\%n^2 - 1} - 511$$

In this equation, **511** is the rest mass of an electron in keV and **n** is the refractive index of the medium (i.e., $n_{\text{glass}} = 1.5$, $n_{\text{water}} = 1.33$). Consider, for example, using water instead of cocktail.

Then, for water, $E_{th} = 263$ keV. If you were counting filter papers in glass vials, then $E_{th} = 175$ keV.

Given these energy constraints, ^{32}P , ^{36}Cl and $^{90}\text{Sr}/^{90}\text{Y}$ have sufficient energy to be analyzed using Cerenkov counting. From a practical point of view, the only beta emitting radionuclide likely to be analyzed by Cerenkov counting is ^{32}P which emits a beta particle with $E_{max} = 1710$ keV. Because beta particles are emitted in a spectrum of energies, approximately 86% of the ^{32}P beta particles have energies exceeding $E_{th} = 263$ keV for counting in water. With proper LSC adapters, researchers could directly analyze their samples in 0.5 and 1.5 ml microfuge tubes.

Consider an example of Cerenkov counting of a ^{32}P labeled compound that a laboratory counted. The lab used 5 mCi in a 10 ml aliquot of [alpha-P-32]UTP. The radioactive sample was placed in a 20 ml glass vial and counted with various quantities of water added (Table 2). The samples were counted at ambient temperature using a Packard system with the counting window/region set at 5 - 1700 keV. The activity used was estimated by counting an identical sample in LSC cocktail and assuming 90% efficiency. As seen from Table 2, counting ^{32}P in a 20 ml glass vial, with 4 - 12 ml of added water gives optimum efficiency. However, note that relatively good efficiencies were obtained for all samples. Typically the counting efficiency of ^{32}P in 4 - 12 ml of water is expected to be approximately 40 - 50% compared to the efficiency obtained by using LSC cocktail for the same ^{32}P sample of nearly 100%.

Table 2. Cerenkov Efficiencies

mm water	% efficiency
0	30.8
1	42.2
2	44.1
4	48
8	46.8
12.8	46.9
16	46.3

As with any counting method, Cerenkov counting has advantages and disadvantages. Advantages include simple sample preparation, (i.e., only add water, the volume is not too critical), less expensive (i.e., no LSC cocktail employed), and waste can be treated as solid if no water was used or as aqueous if water was used. Disadvantages include lower efficiency, higher color quench, volume dependence (particularly if using less than 2 ml of water), and medium dependence (e.g., glass/plastic vials, water, air, etc.)

The biggest factor preventing universal use of Cerenkov counting is beta energy. In order to achieve adequate efficiency, the average beta energy ($E_{ave} \cdot \alpha E_{max}$) must be greater than the required threshold energy, E_{th} . Thus, from a practical point of view, this criteria limits Cerenkov counting to beta emitters with maximum energies greater than 1 MeV. The only commonly used radionuclide fitting this criteria is ^{32}P .

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