

Columbia University Environmental Health & Safety Radiation Safety Program August 25, 2021

Liquid Scintillation Counter User Manual

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Introduction to Liquid Scintillation Counting

Liquid scintillation counting (LSC) is widely used for detecting non-penetrating radiation such as beta radiation of weak (low) energy, alpha radiation and penetrating radiation such as gamma radiation of very low energy (energy <40 keV). For this type of radiation, the detection efficiency of LSC is very high as compared to other detectors. However, any radioactive material can be detected by LSC. The common isotopes used in LSC are:

Isotope	Half-Life	Decay
¹²⁵ I (Iodine)	60 days	γ
¹³¹ I (Iodine)	8 days	β and γ
³ H (Hydrogen also called Tritium)	12.3 years	β
¹⁴ C (Carbon)	5730 years	β
³⁵ S (Sulphur)	87.4 days	β
³² P (Phosphorus)	14.3 days	β

Notice that all except I-125 and I-131 are pure beta emitters. Beta emissions are often low or very low energy and although the emission can sometimes be detected by gamma radiation detection equipment such as a Geiger-Mueller Counter, the energy can easily be absorbed by the compound itself, by the surroundings and covers on detection equipment.

LSC is a well-established measurement method. The goal of this manual is to provide the user with basic information and knowledge to get the most out of this instrument.

Scintillation Detection

Liquid scintillation is the detection of ionizing radiation in a condensed medium known as a scintillation cocktail. Particles or photons emitted during decay of radioactive materials travel through the cocktail with substantial kinetic energy. As the particle or photon interacts with molecules in the cocktail, their kinetic energy is reduced when they excites or ionizes the target molecules. Excited molecules return to the ground state by emitting light. This light is captured by other molecules in the cocktail that transform it into light that can be collected and amplified by a detector (**Figure 1**). Electronic circuitry then analyzes the acquired signals and translates the data into decay rate of the radioactive material and energy of the emitted particle or photon.

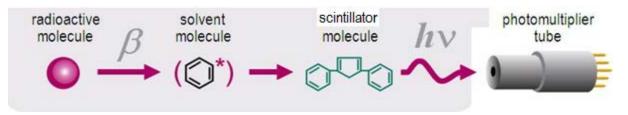


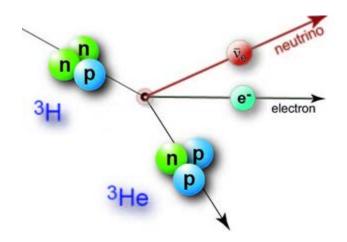
Figure 1. Liquid scintillation detection. (www.perkinelmer.com)

Roles of Liquid Scintillation Cocktail

The scintillation cocktail is composed of three main components. The overall function of the cocktail is two-fold: (i) solubilize the sample and maintain a uniform suspension of the radioactive material and; (ii) capture the kinetic energy of the radiation and transforming it into light that can be collected by the detector (Figure 3). The components are as follows:

1. Radioactive substance: This is the radioactive sample to be measured. The radioactive atom undergoes radioactive decay by emitting particles (α or β) or photon (γ or X-ray).

For example, tritium decays by emission of low energy beta particle (**Equation 1** and **Figure 2**).



 $^{3}_{1}H \rightarrow ^{3}_{2}He + ^{0}_{-1}\beta$ Equation 1

Figure 2. Beta decay of ³H (www.katrin.kit.edu)

2. Solvent: The sample is dissolved or suspended in the solvent. The solvent molecules capture energy from the incident particles or photons emitted by the sample, and pass the energy back and forth among the solvent molecules until the energy gets absorbed by scintillator molecules. Aromatic organics such as DIPN, PXE and LAB are commonly used chemically-safe solvents. Surfactants can be added to emulsify any aqueous sample into the organic solvent.

There are many commercially available liquid scintillations cocktails that are environmentally friendly and contain low hazard. At Columbia University, toluene or xylene-based cocktails are not allowed.

3. Scintillator: Primary scintillator molecules absorb the energy released by the solvent and emit light. Secondary scintillators absorb the light emitted by the primary scintillators and re-emit the light at a longer wavelength, which can improve detection and counting efficiency.

Selection of Liquid Scintillation Cocktail

Liquid scintillation cocktail are available for purchase from vendors such as PerkinElmer that are designed for various research purposes. It is recommended to use liquid scintillation cocktails that have high flash point to avoid generation of mixed waste.

Liquid Scintillation Counting

The light from the scintillators is collected by the detector. The light is transformed into electrons in the photomultiplier tube (PMT or PM-tube). These electrons are multiplied more than 100,000-fold by the PMT. As such, each radioactive decay of the sample induces an electrical pulse. The intensity of a pulse is proportional to the kinetic energy of the particles or photons emitted from the sample; and the pulse rate is proportional to the amount of radioactivity in the sample. Hence, one can find out some characteristics and the amount of the radioisotope in the sample by performing a liquid scintillation count.

Emission of light in the cocktail is isotropic. Two PMTs are employed to reduce the background noise by rejecting single photon events which are not created during decay. The PMT output pulses travel through a coincidence gate which helps to filter out single photon events (**Figure 3**).

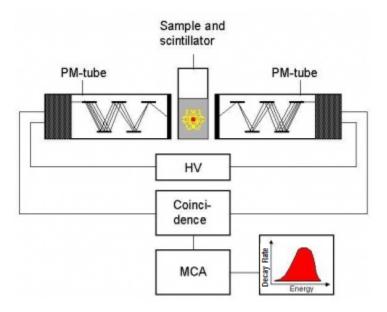


Figure 3. Electronic circuitry of a liquid scintillation counter. (<u>www.wiki.uio.no</u>)

Pulses that pass the coincidence gate are converted to digital values and are assigned to slots in the multi-channel analyzer (MCA), based on the intensity of the pulse. Each memory address corresponds to a channel on the LSC spectrum, so each channel number corresponds to an energy level of the incident particle or photon emitted by the sample (**Figure 4**).

REGION	LL	UL	CPM	25%	
A	2.0	18.6	25844.3	0.41	
в	18.6	156.0	103281.8		0.20
C	156.0	2000.0	747.0	2.40	

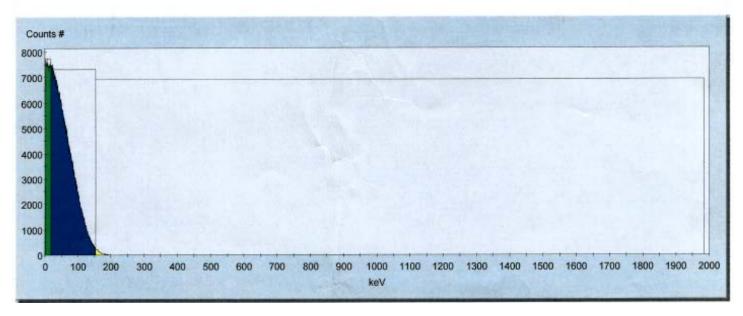


Figure 4. Liquid scintillation counting spectrum view of a sample analysis

The higher the channel number, the more energetic the particle or photon. The pulse rate corresponds to the count rate for each channel, and hence the radioactive decay rate of the sample. The final report (**Figure 5**) which contains the CPM, DPM, tSIE provide the most relevant and useful information for LSC users.

1/16/2015	11:56:25 AM	QuantaSmart	(TM)	- 4.00 -	Serial#	117815	Page # 1
1. In the second second second	The second se						

Protocol# 9 - NEW_ROUTINE_WIPES_2D+C.lsa

5mCi Cs-137 Sealed Source

User: AM

Assay Definition

Assay Description: DPM (DUAL) Assay Type: DPM (Dual) Report Name: Report1 Output Data Path: C:\Packard\Tricarb\Results\AM\NEW_ROUTINE_WIPES_2D+C\20150116_1153 Raw Results Path: C:\Packard\Tricarb\Results\AM\NEW_ROUTINE_WIPES_2D+C\20150116_1153 \20150116_1153.results RTF File Name: C:\Packard\Tricarb\Results\AM\NEW_ROUTINE_WIPES_2D+C\20150116_1153 \Report1.rtf Comma-Delimited File Name: C:\Packard\Tricarb\Results\AM\NEW_ROUTINE_WIPES_2D+C\20150116_ 1153\Report1.txt Assay File Name: C:\packard\TriCarb\Assays\NEW_ROUTINE_WIPES_2D+C.1sa

Count Conditions

Nuclide: 3H-14C+3rd	
Quench Indicator: tSIE/AEC	
External Std Terminator (se	ec): 0.5 2s%
Pre-Count Delay (min): 0.0	D
Quench Sets:	
Low Energy: 3H-UG	
Mid Energy: 14C-UG	
Count Time (min): 1.00	
Count Mode: Normal	
Assay Count Cycles: 1	Repeat Sample Count: 1
#Vials/Sample: 1	Calculate % Reference: Off

Background Subtract

Background Subtract: On - IPA Low CPM Threshold: Off 2 Sigma % Terminator: Off

Regions	LL	ÜΓ	Bkg	Subtract
A	2.0	18.6		9.45
В	18.6	156.0		6.55
С	156.0	2000.0		11.73

Count Corrections

Static Controller: On	Luminescence Correction: n/a
Colored Samples: Off	Heterogeneity Monitor: n/a
Coincidence Time (nsec): 18	Delay Before Burst (nsec): 75

Cycle	1 Results									
S#	Count Time	CPMA	DPM1	CPMB	DPM2	CPMC	SIS	tSIE	MESSAGES	LUM
1	1.00	0	0	0	0	0	0.00	583.40		5

Figure 5. Sample LSC final report

Quench

Quench is the reduction of counting efficiency.

- Chemical quench
- Color quench occurs when other substance in the cocktail absorb the light emitted by the scintillator molecules

All of these processes reduce the number of photons available for collection by the PMT. The total CPM is reduced by the number of pulses of light that were prevented from being collected by the PMT. This leads to an underestimation of energy and amount of radioactivity present in the sample (**Figure 6**).

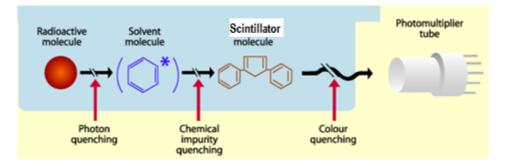
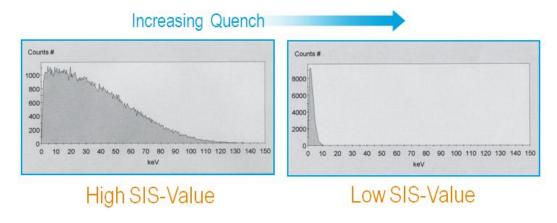


Figure 6. Quenching (www.perkinelmer.com)

Quench correction

Packard TriCarb scintillation counters calculate two quench indication parameters (QIPs) that quantitate quenching. The Spectral Index of the Sample (SIS) uses the sample isotope spectrum to track quenching. The SIS is most accurate with high-count rate samples. SIS decreases as quench increases (**Figure 7**).



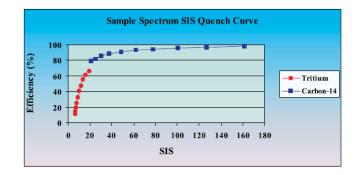


Figure 7. Quench and SIS values (www.perkinelmer.com)

The Transformed Spectral Index of the External Standard (t-SIE) is calculated from the Compton spectrum induced in the scintillation cocktail by an external radiation source (usually 133 Ba). The source is placed under the sample vial, causing a Compton spectrum to be produced in the cocktail. The t-SIE is a relative value, on a scale of 0 (most quenched) to 1000 (unquenched) (**Figure 8**).

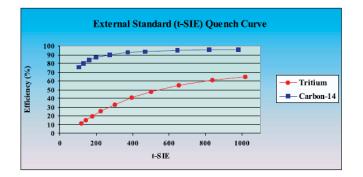


Figure 8. t-SIE vs. counting efficiency (www.perkinelmer.com)

If the sample is cloudy or colored or appears to have suspended solids, you should expect quenching. Also, if the SIS is less than 20 for the ³H channel or less than 100 for the ¹⁴C channel , then quench is probably present.

A set of ³H and ¹⁴C standards are available from the manufacturer of the liquid scintillation counter. These standards should be counted as directed in the operator's manual. Once the quench curve is stored in the instrument computer, it can be used for automatic disintegrations per minute (DPM) calculations.

For example:

A ³H sample is analyzed and has Count rate = 10,000 CPM t-SIE = 400

From the data presented in Figure 8 above, the counting efficiency is 40%. The DPM is calculated

DPM = CPM/Efficiency = 10,000/.4 = 25,000 DPM

Best practice: run the quench correction standards at least once per year]

Counting Efficiency

In addition to quench, other factors such as sample geometry less than 4π , heterogeneity of the sample, insufficient volume of liquid scintillation cocktail could also reduce the counting efficiency.

Counting Statistics and Counting Time

Radioactive decay is a random process and therefore fluctuations are expected in the radio-activity measurement. That's why measurement of radioactivity must be treated by statistical methods.

All normal distributions are symmetric and have bell-shaped density curves with a single peak. In order to speak specifically of any normal distribution, two quantities have to be specified: the mean μ , where the peak of the density occurs, and the standard deviation (SD) σ , which indicates the spread or girth of the bell curve (**Figure 9**). Different values of μ and σ yield different normal density curves and hence different normal distributions. Although there are many normal curves, they all share an important property that allows us to treat them in a uniform fashion.

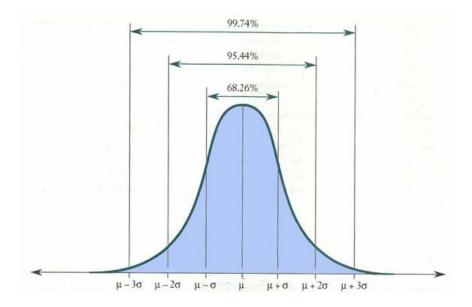


Figure 9. Bell curve and standard deviation

The 68-95-99.7% rule

All normal density curves satisfy the following property which is often referred to as the Empirical Rule.

68% of the observations fall within 1 standard deviation of the mean, that is, between μ - σ and μ + σ .

95% of the observations fall within 2 standard deviations of the mean, that is, between μ -2 σ and μ +2 σ .

99.7% of the observations fall within 3 standard deviations of the mean, that is, between μ -3 σ and μ +3 σ .

For a normal distribution, almost all values lie within 3 standard deviations of the mean.

Radiation events, unlike the value of many other non-radiation variables, are distributed in a very special way. The value of the standard deviation, expressed in number of counts, is related to the actual number of photons counted during the measurement. Theoretically, the value of the standard deviation is the square root of the mean of a large number of measurements. In practice, we never know what the true count value of a sample is. In most cases, our measurement value will be sufficiently close to the true value so that we can use it to estimate the value of the standard deviation as follows:

Standard deviation (σ) = $\sqrt{Total Gross Counts}$

 $2SD(2\sigma) = 2\sqrt{Total Gross Counts}$ Equation 2

Example: if 9500 counts are collected in 1 minute, then $2SD = 2\sqrt{9500} = 2(97.5) = \pm 195$ counts variation

2SD expressed as a Percent:

%2SD = (100)2SD/Total Gross Counts = Total Gross Counts = 2.05%

Total Counts = $9500 \pm 2.05\%$ error

Count Rate Statistics

The standard deviation for any count rate is calculated as

$$2SD (2\sigma) = \pm 2 \sqrt{\frac{CPM}{TIME}}$$
 Equation 3

Example: To calculate 2SD a 9500 CPM count over a 3 minute counting time is

$$2SD (2\sigma) = \pm 2\sqrt{\frac{9500}{3}} = \pm 113 \text{ CPM or } 2SD\% = \pm 2x100 / \sqrt{\frac{9500}{3}} = \pm 1.18\%$$

Counting the sample for 3 minutes compared to 1 minute reduced the error form 2.05% to 1.18% Determining a count time to reach a %2SD value can be found as follow:

Time =
$$\frac{1}{CPM} \left[\frac{200}{\% 2SD \ desired}\right]^2$$
 Equation 4

Example: The time required to achieve a %2SD of 0.5% for a 9500 CPM sample

Time
$$= \frac{1}{9500} \left[\frac{200}{0.5}\right]^2 = 16.84 \ minutes$$

However, 1min counting time for each sample is generally sufficient.

General Sample Preparation

- 1. Sample should be prepared to avoid the static. A user should avoid handling plastic vials with latex gloves. Latex gloves cause static buildup and erroneous counting results. Use vinyl (nitrile) or polyethylene gloves if possible, and wipe sample vials with an anti-static cloth (i.e. laundry drier sheet) before analysis.
- 2. Cassettes are the plastic racks which hold sample vials and allow them to be moved on the instrument's sample changer deck. The individual cassettes are identified by unique numbers (cassette IDs) located at the end of each cassette. The protocol flag and cassette ID can be used as a means of providing Positive Sample Identification (PID) when utilizing the Worklist feature. The instrument moves the cassettes in a counterclockwise (forward) direction during sample analysis. (**Figure 10**)

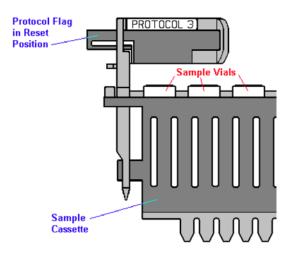


Figure 10. Setup of LSC cassette for sample analysis

- 3. You must attach the correct protocol flag (the protocol flag number to which you have associated the assay) to the first cassette to be counted. Be sure the protocol flag is in the reset position (the flag is all the way to the left when the flag is on the left end of the cassette). You should RESET the protocol flag each time that you put new samples into the cassette(s).
- 4. It is generally advised to keep a control sample (i.e. radiation-free sample) as the first vial

Maintenance

The LSC is a sophisticated piece of analytical equipment with moving parts and delicate detection equipment. Before any samples are counted, you should calibrate, normalize and assess the performance of the instrument.

If you are not sure how to set Calibration, Normalization, or IPA (Instrument Performance Assessment), please contact LSC manufacturer or Radiation Safety Office.

The calibration, normalization and IPA procedures occur automatically by leaving the Self-Normalization and Calibration (SNC) and IPA cassette (containing the Carbon-14 calibration standard, unquenched Tritium standard and background standard) on the instrument counting deck. (**Figure 11**)

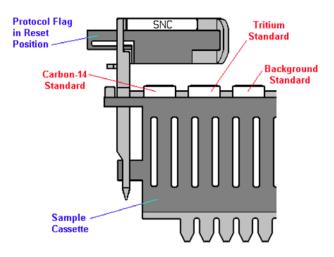


Figure 11. Setup of LSC cassette for SNC test

Running the SNC Protocol routinely is very important to maintain LSC and count sample correctly. The RSO recommends that the SNC Protocol should be run daily or before use. The counting efficiencies are calculated automatically in the SNC report (**Figure 12**).

8/25/2021 11:10:51 AM QuantaSmart (TM) - 4.00 - Serial# DG05117815

SNC Protocol

Calibration Information Software Version IC: 3.00 Software Version EC: 4.00 Instrument Model: Tri-Carb 2910TR Instrument Serial Number: DG05117815 3H Chi Square: Not Processed 14C Chi Square: Not Processed 3H E^2/B (1-18.6 keV): 321.55 Date Processed: 8/25/2021 11:10:50 AM 14C E^2/B (4-156 keV): 638.94 Date Processed: 8/25/2021 11:10:50 AM 3H Efficiency (1-18.6 keV): 60.15 Date Processed: 8/25/2021 11:10:50 AM 14C Efficiency (4-156 keV): 92.99 Date Processed: 8/25/2021 11:10:50 AM IPA Background Date Processed: 8/25/2021 11:10:50 AM 3H Background CPM (1-18.6 keV): 11.25 Date Processed: 8/25/2021 11:10:50 AM 14C Background CPM (4-156 keV): 13.53 Date Processed: 8/25/2021 11:10:50 AM 3H Calibration DPM: 288000 3H Reference Date: 8/22/2016 14C Calibration DPM: 113600

Figure 12. Sample SNC Report

General Counting Procedure (PerkinElmer TriCarb Model)

When you are ready to begin a counting procedure, you will need to perform the following tasks:

- 1. Calibrate and normalize the instrument. Run the SNC protocol with ¹⁴C and ³H standards.
- 2. Select an assay type: Alpha Beta, Alpha Beta Standards, CPM Assay, DPM Single, DPM Dual, DPM Triple, FS DPM, Direct DPM, Quench Standards or Single Photon Counting. The choice of assay types will differ depending on the model you process.
- 3. For any DPM Assay except Direct DPM, create quench data by acquiring quench standard data through quench standard assay.
- 4. Define and save the new assay parameters.
- 5. Associate (link) the assay parameters to a protocol.
- 6. Attach the correct protocol flag to the first cassette to be counted and load the cassette(s) with samples.
- 7. Start the running measurement.

Report

Liquid scintillation counters can be set up to automatically read out in DPM (Disintegrations per minute) using calibrated standards. Otherwise, the following information can be used to conservatively convert CPM to DPM:

$$DPM = \frac{CPM}{Efficiency}$$

Efficiency can be found SNC report (**Figure 12**). If you are not sure about efficiency of your LSC, please contact the Radiation Safety Office. All liquid scintillation counting results shall be reported in DPM per Article 175 of NYCDOH Health Codes. At Columbia University, the action level for decontamination is 200 DPM.

False high readings could be contributed by a few factors, including chemical luminescence and excitation of scintillator by sun light, electrostatic discharge buildup on the exterior of the liquid scintillation vials in dry and cold room condition, random electric noise in the LSC. If high readings are noted in the liquid scintillation results, let the sample sit for a while and rerun the sample may help to reduce the false high readings. If readings are persistently high upon a repeat analysis of a wipe test, consider decontaminating the area where the wipe was taken from and document the post-decontamination wipe test results.

Disposal of Liquid Scintillation Vial

Used liquid scintillation vials with medium and sample can be disposed of as radioactive liquid scintillation vials and collected in a properly labeled radioactive waste container. Record estimated amount of radioactivity on the waste label and write down the brand of the liquid scintillation cocktail solution that was used in the content description. Some of the old SNC standards contain tolune which is toxic and should be disposed of as mixed waste.

Disposal of Liquid Scintillation Counter

LSC contains a radioactive sealed source, lead shielding and electronic equipment containing lead and other hazardous metals. It also may be contaminated with low levels of radioactive material. For these reasons it is important to ensure the LSC is free of Contamination and that the source has been removed prior to disposal. The policy on *Disposal of specific articles containing radioactive materials* outlines the steps necessary to allow for appropriate decommissioning and disposal of a LSC.