Quench & Quench Curves

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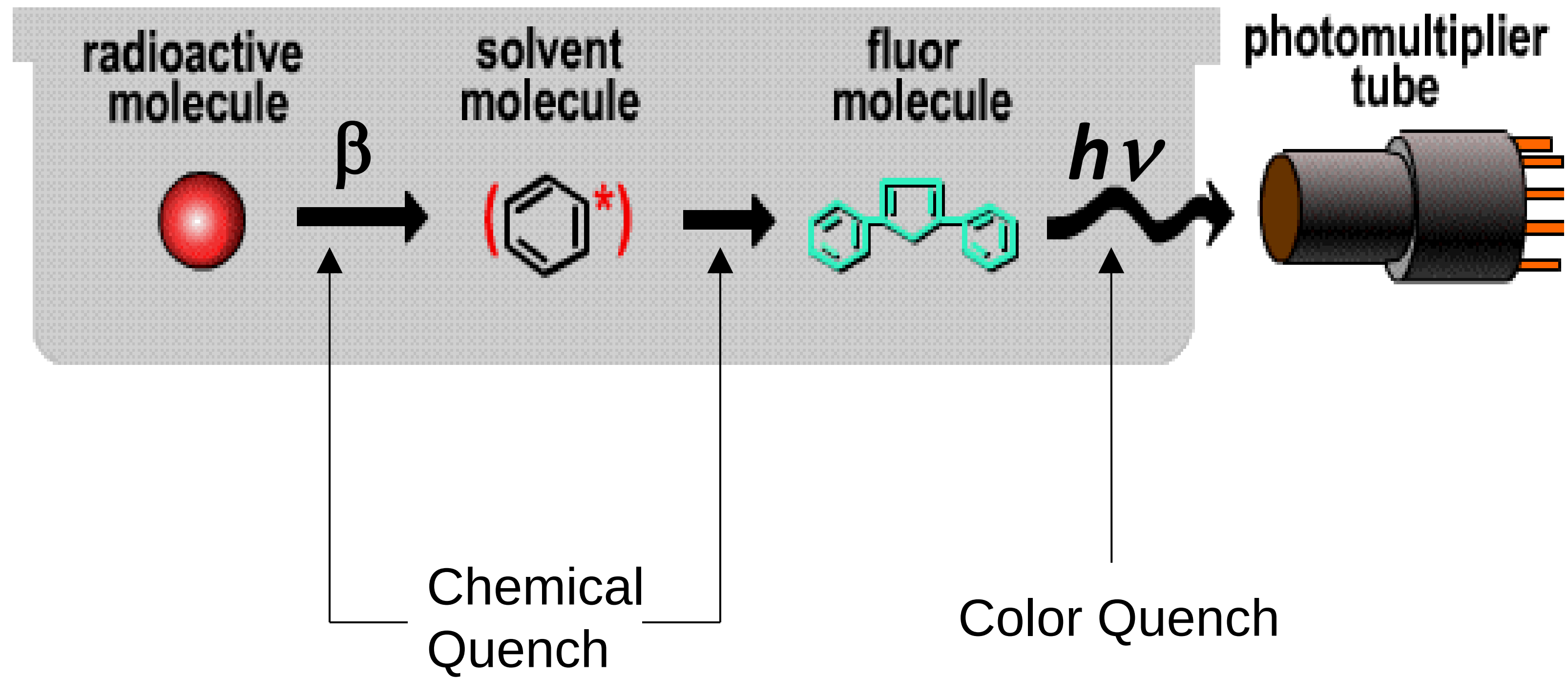
What is Quench?

- Basically, the liquid scintillation process is the conversion of the energy of a radioactive decay event into photons of light.
- Photomultiplier tubes (PMT's) are used to detect and convert the photons into electrical pulses. Both the sample and the scintillator are dissolved in an aromatic solvent, which allows energy to be transferred.
- **Any factor, which reduces the efficiency of the energy transfer or causes the absorption of photons (light), results in quenching in the sample.**

The Scintillation Process



Types of Quench

- There are two main types of quench:
  - ***Chemical quench and Color quench.***
- **Chemical quench** occurs during the transfer of energy from the solvent to the scintillator. Any chemical species that is electronegative (electron capturing) will affect the energy transfer process by capturing or stealing the π electrons associated with the aromatic solvent and thus reduce the availability of π electrons necessary for efficient energy transfer.
- **Color quench** is an attenuation of the photons of light. The photons produced are absorbed or scattered by the color in the solution, resulting in reduced light output available for measurement by the PMT's

Quench

- The collective effect of quench is a reduction in the number of photons produced and therefore detected CPM (i.e. counts per minute).
- Counting efficiency is affected by the degree of quenching in the sample.
- Thus, to determine absolute sample activity (DPM), it is necessary to measure the level of quench of the samples first, then make the corrections for the measured reduction in counting efficiencies.

What is a Quench Curve?

- A quench standard curve is a series of standards in which the absolute radioactivity (DPM) per vial is constant and the amount of quench increases from vial to vial.

- A quench curve uses the relationship between counting efficiency and QIP to correct the measured CPM (counts per minute) to DPM (disintegrations per minute or absolute activity).

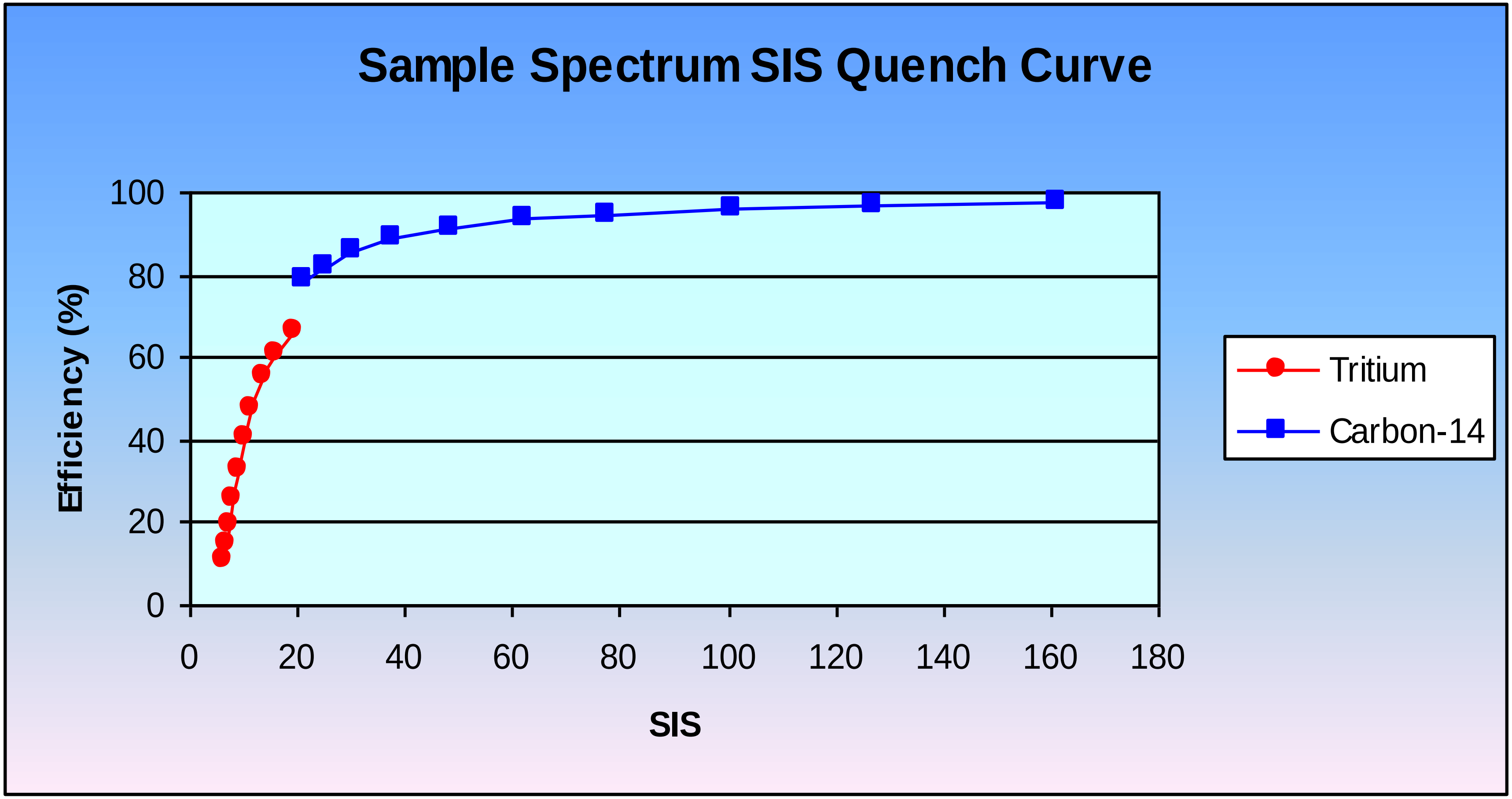
What is a Quench Curve?

When a quench curve is made, the DPM value in each standard is known. Each standard is counted and the CPM is measured. The counting efficiency is calculated using the following relationship:

$$\frac{CPM \times 100}{DPM} = \%\ \text{Counting Efficiency}$$

At the same time, the QIP is measured for each standard. A correlation is made using the QIP on one axis (X) and the % efficiency on the other axis (Y). A curve is fitted t0 the standard points.

What is a Quench Curve?



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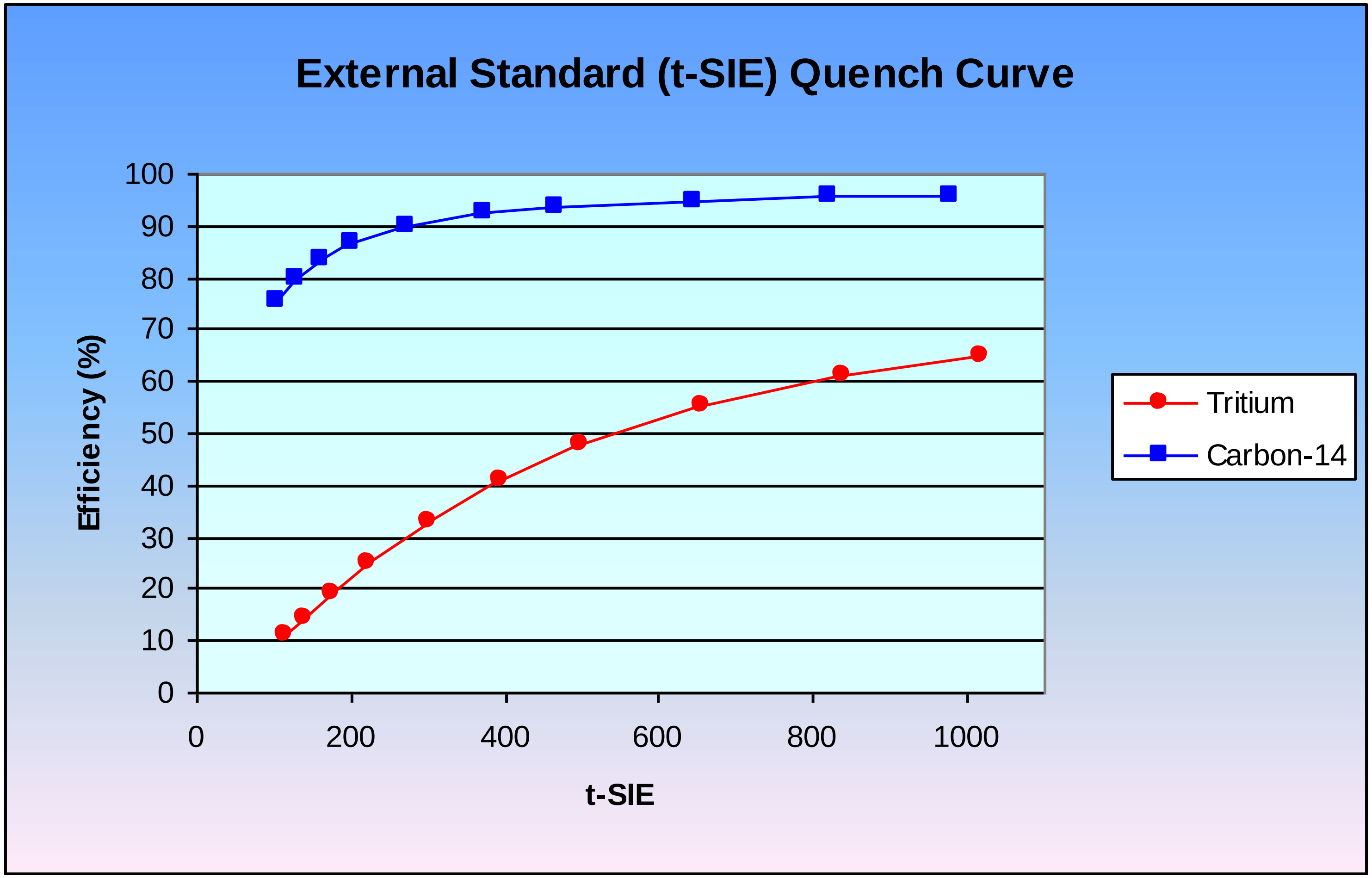
What is a Quench Curve?



Notes on Preparing a Quench Curve

- **The standards and unknowns must be counted with the same energy regions.**
- Perkin Elmer LSC's with spectra based libraries (2500 series, 2700 series and the new 2900 and 3100 series with QuantaSmart) allow the curve to be stored in a 0-E_{max} window and allow the curve to be recalculated for the windows used in the protocol.
- For other LSC's (1600,1900, 2100, 2200 and 2300) the windows used to acquire the quench curve must be used in the actual dpm determination.

Preparing a Quench Curve

- It is necessary to obtain a calibrated source of radioactivity to use as the source of the activity (DPM). It is essential that a known amount of activity be added per vial.
- The standard material must be compatible with the cocktail chosen.
- A suitable quenching agent must be chosen. It is desirable to closely approximate the chemical environment in the samples. If samples contain water with various other constituents, add the same material in increasing amounts to the standards. Additional quenching agents that are most often used include nitromethane (CH_3NO_2), carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and acetone (CH_3CH_3CO).

Preparing a Quench Curve

Method 1

Accurately add the radioactive solution to the chosen cocktail such that the desired DPM are transferred to each individual vial when dispensing the cocktail.

- Use 50K to 200K dpm in order to be able to count the standards with good statistics in a short time.
- Count the individual unquenched standards for at least five minutes to check for constant activity (CPM).
- Any sample that deviates more than 2% from the mean should be discarded.
- Add incremental amounts of the quenching agent to vials 2 ... n (quenching agent is not added to vial 1) to obtain the desired quench range.

Preparing a Quench Curve

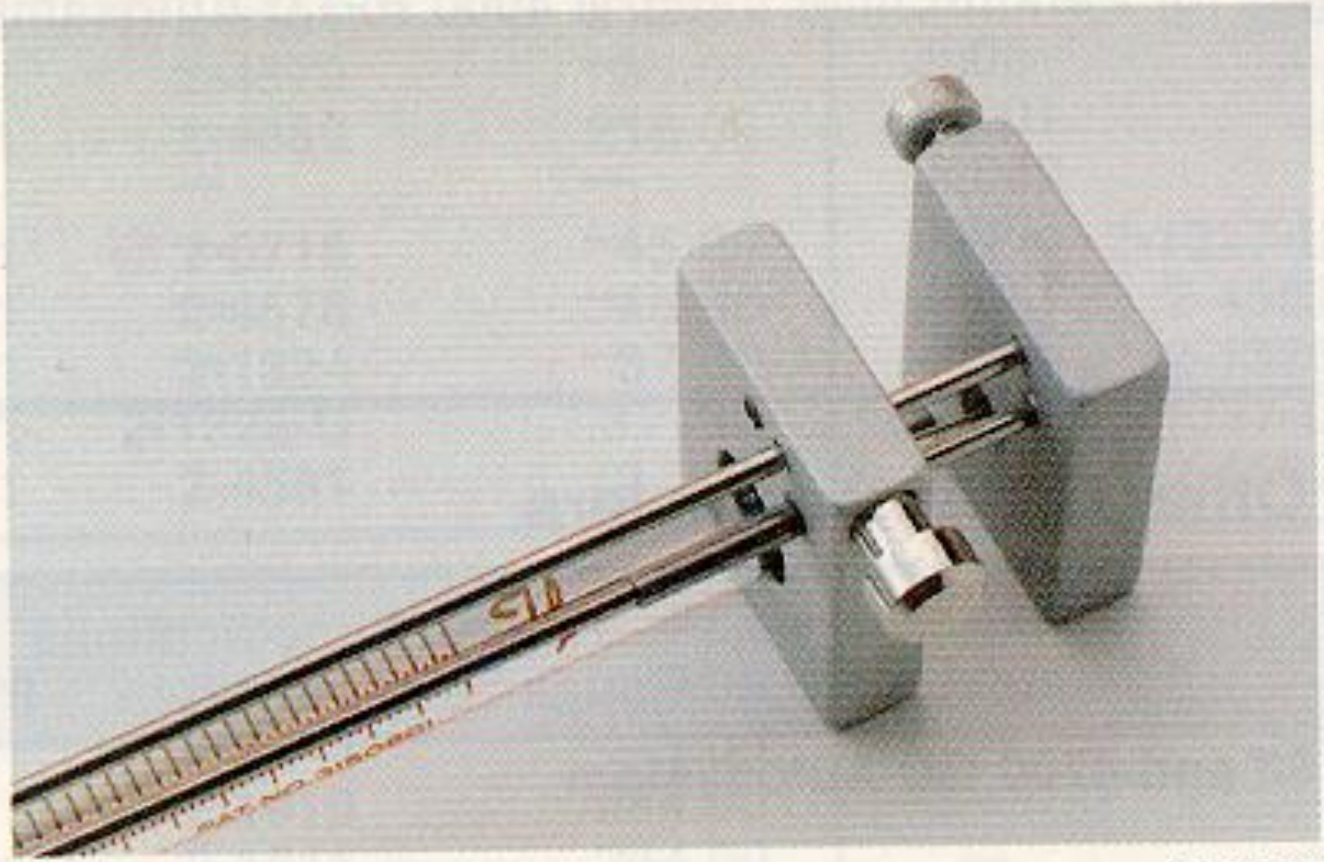
Method 2

- Dispense 10.0 mL or 15.0 mL of LSC cocktail into ten high performance glass vials, preferably by weight.
- Add activity to each vial (200K DPM for Tritium or 100K DPM for Carbon-14).
- Count the individual unquenched standards for at least five minutes to check for constant activity (CPM).
- Number the vials 1 to10 or A to J and add the suggested amounts of Nitromethane based on the information given in Table 1, or other quenching agent.

Preparing a Quench Curve

- **Notes: -**
- For dispensing the activity I suggest the use of a glass barreled microliter syringe fitted with a Chaney adapter. Such an adapter ensures reproducible dispensing of activity.
- Cheney type adapters are available from e.g. Hamilton.
- After preparation the standards should be stored in the dark preferably at 5°C to 10°C for best stability (domestic type refrigerator is recommended).
- Ultima Gold type standards stored in this way should be stable for about 2 years.

Chaney Adapter



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Preparing a Quench Curve

- Count the complete set under the conditions described in the instrument operation manual for storing a quench curve.
- Practically we suggest that the standards are counted to a pre-selected level of statistical accuracy (generally 0.5% 2s).
- Once the quench curve(s) are counted and stored, count unknown samples using the stored quench curve to determine the DPM value for each sample.

Preparing a Quench Curve

How much Nitromethane should I add?

Quench Level	Toluene standards (15mL)	Ultima Gold standards (15mL)
A (1)	0µL	0µL
B (2)	1µL	5µL
C (3)	5µL	10µL
D (4)	11µL	15µL
E (5)	17µL	26µL
F (6)	25µL	45µL
G (7)	35µL	70µL
H (8)	45µL	110µL
I (9)	55µL	150µL
J (10)	66µL	230µL

How is a Quench Curve used?

Once the quench curve is stored in the instrument computer it can be used for automatic DPM calculations. When unknowns are counted, the sample CPM and the QIP are measured.

Using the QIP, the counting efficiency is determined from the quench curve.

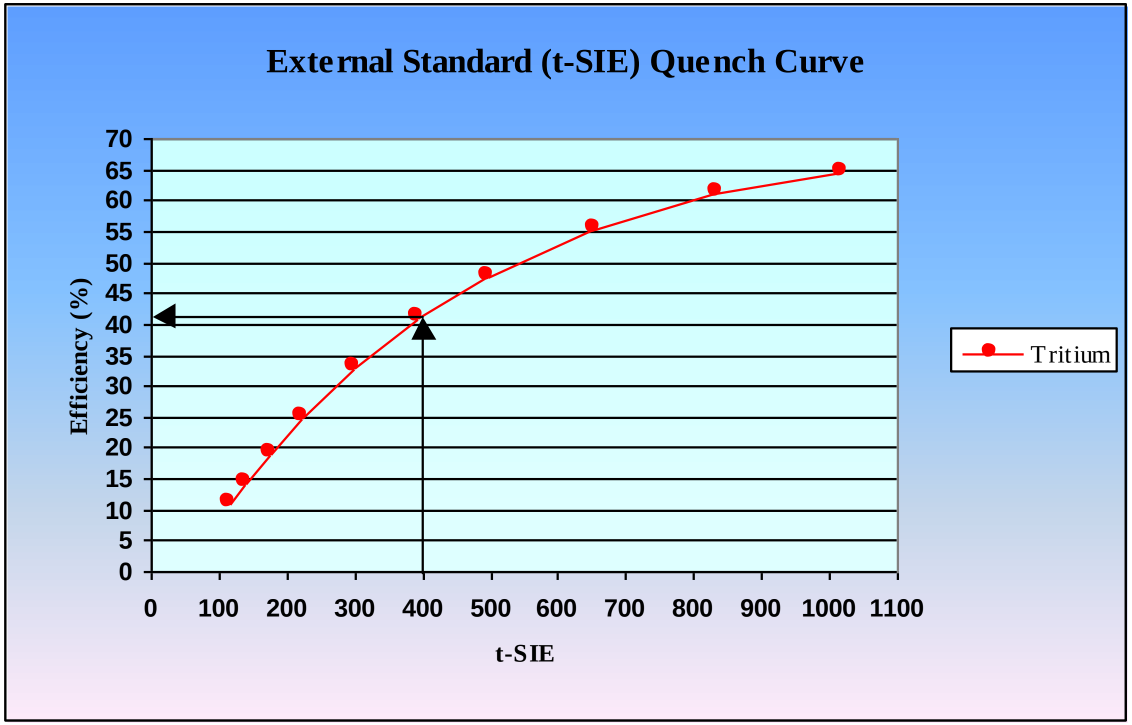
Sample DPM are calculated by applying the appropriate efficiency to the CPM of the sample :-

$$\text{DPM} = \frac{\text{CPM}}{\text{Efficiency (expressed as a decimal)}}$$

How is a Quench Curve used?

1. t-SIE is **400**
2. CPM = **10,000**
3. From Curve
Efficiency = 42%
4. Using $DPM = \frac{CPM}{Efficiency}$

$DPM = \frac{10,000}{0.42} = \mathbf{23,809}$



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Using the Quench Curve

- ✓ t-SIE is independent of the sample isotope and of the activity in the vial, and has a large dynamic range. This makes it a very reproducible means of tracking the quench of the cocktail.

- ✓ SIS should only be used when there is **at least 500 CPM** activity in the sample. Remember that SIS uses the sample isotope spectrum to track quench; it is most accurate with high-count rate samples. For an accurate SIS a good sample spectrum needs to be acquired.

- ✓ SIS must not be used for low activity samples since an accurate sample spectrum cannot be acquired.

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Using the Quench Curve

- ✓ Many customers prefer to purchase pre-prepared and certified quench standards.
- ✓ For cocktails using toluene, xylene, pseudocumene or LAB (Linear Alkyl Benzene) as the solvent, **Toluene Quench Standards should be used**.
- ✓ For cocktails using DIN (Di-isopropylnaphthalene) or PXE (Phenylxylylethane) as the solvent, **Ultima Gold Quench Standards should be used.**
- ✓ ***If the wrong quench standard is used there can be an error in DPM. This error is most pronounced with low energy isotopes such as Tritium.***

Tritium DPM Errors – Ultima Gold Quench Curve

	% error in Tritium DPM recovery				
	Ultima Gold (DIN)	Toluene	Opti-Fluor (LAB)	Insta-Gel Plus (pseudocumene)	Pico-Fluor 15 (pseudocumene)
No quench	- 0.12%	- 1.04%	+ 6.00%	+ 2.70%	+ 4.89%
Low quench	- 0.46%	+ 4.24%	+ 7.06%	+ 5.14%	+ 6.45%
Medium quench	+ 0.04%	+ 5.87%	+ 8.43%	+ 5.82%	+ 6.91%
High quench	- 0.14%	+10.10%	+ 14.41%	+ 10.02%	+ 11.89%
Highest quench	- 0.20%	+13.42%	+ 18.01%	+ 13.36%	+ 13.43%

Various Cocktails vs. Ultima Gold Quench Curve (Tritium)

Tritium DPM Errors – Toluene Quench Curve

	% error in Tritium DPM recovery				
	Ultima Gold (DIN)	Toluene	Opti-Fluor (LAB)	Insta-Gel Plus (pseudocumene)	Pico-Fluor 15 (pseudocumene)
No quench	- 4.10%	- 0.49%	- 0.57%	- 1.58%	- 0.32%
Low quench	- 5.33%	- 0.27%	- 0.13%	- 1.22%	+ 0.23%
Medium quench	- 6.51%	+ 0.01%	+ 0.45%	- 0.19%	+ 0.60%
High quench	- 10.39%	- 0.01%	+ 1.21%	- 0.79%	- 0.49%
Highest quench	- 16.16%	- 0.70%	+ 0.11%	+ 0.56%	- 0.21%

Various Cocktails vs. Toluene Quench Curve (Tritium)

Tritium DPM Errors

- These data show that the Ultima Gold Tritium quench curve should only be used with cocktails based on DIN solvent for Tritium DPM measurements.

- Additionally they show that LAB based cocktails such as Opti-Fluor, Emulsifier Safe, and Formula- 989 **should not be used with the Ultima Gold quench curves for Tritium.**

Carbon-14 DPM Errors – Ultima Gold Quench Curve

	% error in Carbon-14 DPM recovery				
	Ultima Gold (DIN)	Toluene	Opti-Fluor (LAB)	Insta-Gel Plus (pseudocumene)	Pico-Fluor 15 (pseudocumene)
No quench	+ 0.06%	- 1.37%	+ 2.51%	+ 2.25%	+ 1.96%
Low quench	+ 0.03%	+0.12%	+ 2.04%	+ 0.78%	+ 0.80%
Medium quench	- 0.13%	+ 0.84%	+ 1.72%	+ 1.15%	+ 1.20%
High quench	+ 0.02%	+ 1.30%	+ 2.51%	+ 3.11%	+ 1.71%
Highest quench	- 0.63%	+ 4.52%	+ 3.81%	+ 3.59%	+ 2.77%

Various Cocktails vs. Ultima Gold Quench Curve (Carbon-14)

Carbon-14 DPM Errors – Toluene Quench Curve

	% error in Carbon-14 DPM recovery				
	Ultima Gold (DIN)	Toluene	Opti-Fluor (LAB)	Insta-Gel Plus (pseudocumene)	Pico-Fluor 15 (pseudocumene)
No quench	+ 0.45%	+ 0.42%	+ 1.71%	+ 1.90%	+ 1.96%
Low quench	+ 0.27%	- 0.03%	+ 0.89%	+ 1.20%	+ 0.43%
Medium quench	- 0.54%	- 0.37%	+ 1.28%	+ 0.31%	+ 0.83%
High quench	- 0.81%	+ 0.01%	+ 1.86%	+ 0.77%	+ 0.81%
Highest quench	- 6.51%	+ 0.33%	+ 1.04%	+ 0.49%	+ 0.88%

Various Cocktails vs. Toluene Quench Curve (Carbon-14)

Carbon-14 DPM Errors

With ^{14}C it can be seen that the two sets of quench curves can be intermixed except at high quench levels where the errors are increased.

Which Quench Curve to use?

Supplier	LSC Cocktail	Solvent	^{3}H & ^{14}C quench curves
Perkin Elmer	Ultima Gold, Ultima Gold XR, AB, LLT, uLLT, MV & F	DIN	Ultima Gold
	Optiphase Hisafe 2, 3 & Betaplate Scint	DIN	Ultima Gold
	StarScint, Lumasafe Plus, Irgasafe Plus	PXE	Ultima Gold
Meridian	Gold Star, Gold Star Quanta & Gold Star LT[2]	DIN	Ultima Gold
	ProSafe+, HC+ & FC+	DIN	Ultima Gold
	ProFlow G+, P+ & Gold Flow	DIN	Ultima Gold
Zinsser	Aquasafe 300+, 500+ & 800	DIN	Ultima Gold
	Quicksafe A, 400 & N, Quicksafe Flow 2 & Filtersafe	DIN	Ultima Gold
	Irgasafe Plus	PXE	Ultima Gold
National Diagnostics	Ecoscint XR, A, H, Flow & Bioscint	PXE	Ultima Gold
	Ecoscint Ultra	DIN	Ultima Gold
Roth	Rotiszint Eco Plus & Rotiszint Eco	DIN	Ultima Gold

Which Quench Curve to use?

Supplier	LSC Cocktail	Solvent	^3H & ^{14}C quench curves
Perkin Elmer	Insta-Gel Plus, Pico-Fluor Plus, Bio-Fluor Plus	PC	Toluene
	Insta-Fluor Plus, Hionic-Fluor & Filter-Count	PC	Toluene
	Flo-Scint II & FloScint III	PC	Toluene
	Permafluor E+, Monophase-S	PC	Toluene
	Opti-Fluor, Opti-Fluor O, Emulsifier Safe	LAB	Toluene
Meridian	CarbonCount & TritiumCount	PC	Toluene
	MicroFlow G	PC	Toluene
Zinsser	Quickszint 1, 212	Xylene	Toluene
	Quickszint 2000	PC	Toluene
	Quickszint 501	Toluene	Toluene
	Oxysolve-T & Oxysolve C-400	PC	Toluene
National Diagnostics	Uniscint BD & Ecoscint Original	LAB	Toluene
	Monoflow 1, 2, 3 & 4 & Soluscint XR	C9-arom	Toluene
	Monoflow 5	LAB	Toluene
	Hydrofluor, Liquiscint, Betafluor, Monofluor, Filtron-X	C9-arom	Toluene
	Oxosol 306	Xylene	Toluene
	Oxosol C-14	Toluene	Toluene

C9-arom is a fraction containing predominantly C-9 aromatics (similar to pseudocumene)

Which Quench Curve to use?

Supplier	LSC Cocktail	Solvent	^3H & ^{14}C quench curves
Perkin Elmer	Ultima-Flo M & AF	PXE/LAB	???????
	Ultima-Flo AP	Mixture*	???????

* Mixture of phenylethylbenzene and benzyltoluene

Which Quench Curve to use?

Low Level Count Mode

Different quench agents produce slightly different quench characteristics.
This affects the shape of the quench curve.
Curves prepared with different quench agents do not overlay exactly – not identically.
This will cause slight errors in DPM recovery if samples are chemically different than the curves used to calculate DPM.
This error can be more noticeable when counting low level samples using electronic background discrimination techniques like PSA or Time Resolved techniques.
These techniques rely on pulse shape analysis and that is influenced by sample chemistry.

Statement contributed by Mr. C. Passo (Perkin Elmer).

Quench Curves - Closing remarks

Make sure you have the correct quench curve for your LSC cocktail !!!

When in doubt - prepare your own !!!