BCHEM 365
Lecture 11
November 27, 2018

The channels ratio method cont'd

Multichannel scintillation counters operate on the same principle as the two-channel approach, however, instead of manual calculation of SCR, a graph is plotted (relative number of disintegrations against energy of the emission) from the electronic signals within the equipment and the **whole shape and position of the spectrum** is analyzed. Parameters obtained from the graph may be:

1. Area under the curves for unquenched and quenched sample
2. Height of the curves

The graph parameter is given a **digital value** that relates to counting efficiency. Manufacturers have developed their own titles for such parameters. For example, LKB Instruments use Automatic Quench Compensation and Hewlett Packard uses Packard's Automatic Efficiency Control as the digital value terminology. These systems have greater precision than the two-channel approach, as the whole spectrum of the graph is used for analysis.

Advantages of channels ratio method over internal and external standardization methods are:

1. The channels ratio method is suitable for all types of quenching.
2. Counting in more than one channel is simultaneous, less time consuming than either internal or external standardization.
3. Accurate method for determining counting efficiency, provided care is taken in preparation of the calibration curve.

Demerits of the channels ratio method

1. It is inaccurate at low count rates, because error on the counts per minute is high, (refer to statistic of counting decay events)
2. Method is inaccurate for very highly quenched samples

Channels ratio efficiency calculation

Counting efficiency is positively related to the sample channel ratio (SCR). To get a conversion factor between SCR and efficiency, equal amounts of the isotope (sample) are placed in a series of vials. Increasing amounts of a quenching agent, such as carbon tetrachloride, are added to each vial. The vials are counted and the c.p.m. of each is divided by known d.p.m. to get the counting efficiency. For example, if a vial with 20,000 d.p.m. of ^{3}H yields 5,000 c.p.m., the

counting efficiency would be 5,000/20,000 = 0.25, that is, only 25% of the atomic disintegrations were detected. The counting efficiency is plotted versus channels ratio to yield a quench curve.

The instrument prints out c.p.m. and sample channels ratio (SCR) for each sample, therefore to get actual d.p.m. in a sample the investigator must (1) subtract background or blank (i.e., without sample) c.p.m. from the c.p.m. for the sample, (2) determine counting efficiency from the SCR for the sample, and (3) divide net c.p.m. by the counting efficiency.

Remember, $\text{counting efficiency} = \frac{\text{c.p.m}}{\text{d.p.m}}$

Worked example

The efficiency of counting 100,000 d.p.m. of a [^{35}S]methionine was estimated in a scintillation counter using two channels, A and B, in a scintillation fluid containing increasing amounts of chloroform as the quencher. The following data were obtained:

Chloroform (ml)	c.p.m. A	c.p.m. B
0	48,100	54,050
1	31,612	42,150
2	17,608	28,400
3	7,400	15,000

An unknown sample of [^{35}S]methionine gave the following data:
Channel A 1,890 c.p.m.
Channel B 2,700 c.p.m.
How much radioactivity is present in the unknown sample?

Solution

Calculate efficiency for channels A and B. Plot counting efficiency in channel A or in channel B against channels ratio. Calculate the sample channel ratio $\frac{\text{c.p.m. A}}{\text{c.p.m.B}}$

Steps

Calculate counting efficiency for channel A
Calculate counting efficiency for channel B
Compute the channels ratio for the standard at each level of quenching
Plot a graph of efficiency of counting on the Y axis against channels ratio of standard on X axis
Compute the channels ratio for the sample
Read off efficiency of counting sample from the graph

Calculation of efficiency for channel A

100,000 d.p.m. = 100%

So 48,100 c.p.m. in channel A = $\frac{48{,}100 \text{ c.p.m.}}{100{,}000 \text{ d.p.m}} x\ 100 = 48.1\%$

Now calculate efficiency for the rest of the data. This is shown in Table 11.1.

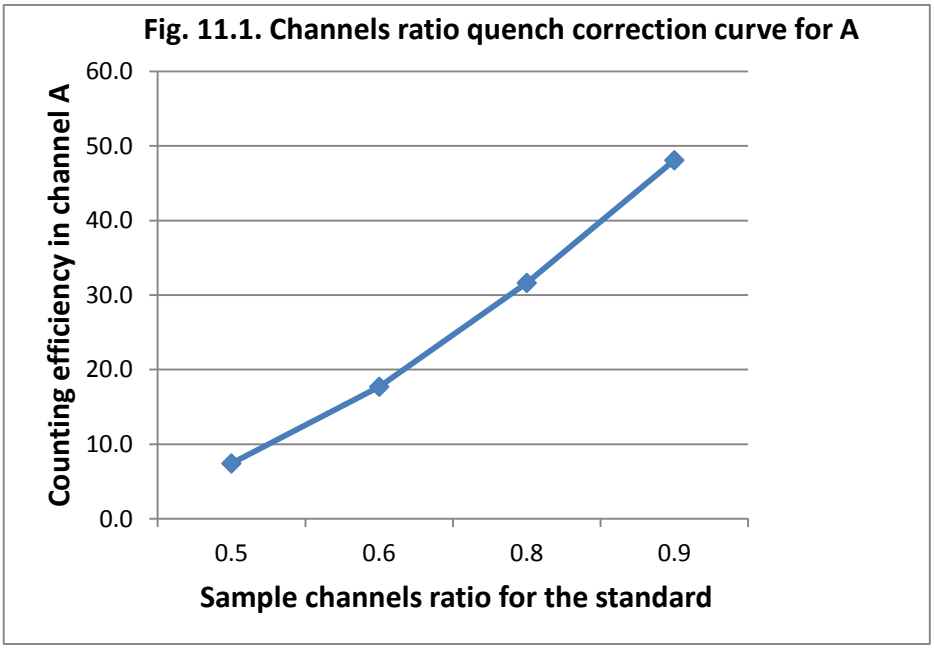
Table 11.1. Calculation of counting efficiency

Channel A	Counting eff. in A	Channel B	Counting eff. in B	Sample channel ratio for the standard $\text{c.p.m. A}/\text{c.p.m. B}$
$\frac{48{,}100 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	48.1	$\frac{54{,}050 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	54.05	$48{,}100/54{,}050 = 0.89$
$\frac{31{,}612 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	31.612	$\frac{42{,}150 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	42.15	$31{,}612/42{,}150 = 0.75$
$\frac{17{,}608 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	17.68	$\frac{28{,}400 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	28.4	$17{,}608/28{,}400 = 0.62$
$\frac{7{,}400 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	7.4	$\frac{15{,}000 \text{ c.p.m.}}{100{,}000 \text{ d.p.m,}} x\ 100$	15.0	$7{,}400/15{,}000 = 0.49$

Calculate c.p.m. A/c.p.m. B for the experimental sample.

$$\text{S.C.R}_{\text{experimental}} = 1890/2700 = 0.7$$

Read off the efficiency of counting the unknown sample from the graph.



Now, convert the c.p.m. for the experimental sample in channel A or B (depending on which one you choose to calculate the efficiency). If you use channel A, the corresponding efficiency from the graph is 26.5 %. So the corrected c.p.m. is

26.5% gives 1,890 c.p.m in channel A
Therefore 100% = ?

1890 × 100/26.5 = 7,130 d.p.m.

Note: try plotting the two graphs (efficiency in A or B versus c.p.m A/c.p.m B) and work out the answer using both in turn; you should get the same answer of 7,132 d. p.m. if you use channel B.

External standardization

This method is based on the principle that, the spectrum for an external standard varies according to the degree of quench in the sample vial and therefore, the efficiency of counting of the internal experimental sample also varies.

It is used to estimate counting efficiency. In this method, a **γ-emitting** external standard is built into the scintillation counter. Under the control of the counter, each radioactive sample to be counted is exposed to this external source. In addition, a quenching substance is added at increasing concentrations as explained before. The γ-radiation penetrates the sample vial and excites the scintillation fluid, and causes the ejection of an electron, called Compton electron) from the solvent molecules. This electron behaves like a beta particle beta particles, and will induce the same scintillation effects. The resulting spectrum is unique to the sample and is significantly different from that produced by the sample's radioactivity alone in the vial. the difference in spectrum is quantified and is assigned a value, such as transformed spectral index of External Standard, tSIE, based on the pulse height, or area under the curve. The γ-source commonly used are ^{137}Cs, ^{133}Ba, or ^{226}Ra and these may be varied with the make of the equipment. The spectrum obtained by ^{226}Ra is shown in Fig. 11.2.

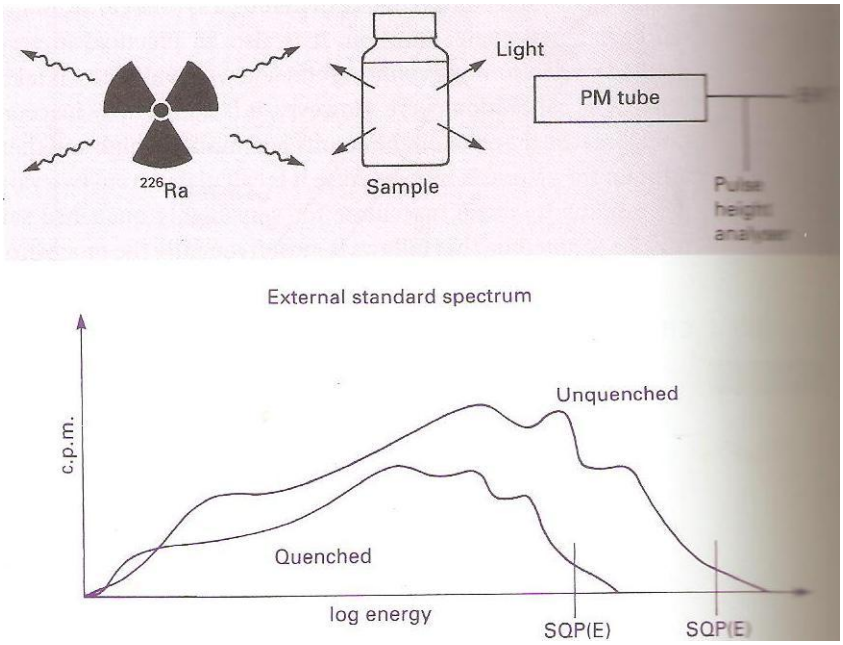


Fig. 11.2. The external standard for estimating counting efficiency. The external source of γ-emitter, ^{226}Ra, irradiates the sample. The counter analyzes the sample spectrum, which shifts to lower energies if the sample is quenched. The SQP(E), i.e., standard quench parameter (external), expressed without units, is derived from the log energy axis and relates to the extent of quench. The greater the quench in the sample, the lower the counting efficiency (Table 11.2)

Quenching agents present in the scintillation fluid will significantly affect the spectrum obtained. The instrument analyses this quenched spectrum and assigns a **quench parameter** to it. A standard quench parameter used by Perkin Elmer is the **transformed Spectral Index of the Sample (tSIS)**. Quench parameter is a numerical value obtained by **quantifying the change in spectral shape** caused by quenching and can be estimated by **calculating the mean pulse height**. The quench parameter then **correlates** changes in counting efficiency with changes in the shape (i.e., pulse height) of the sample spectrum; LKB Instruments refer to a standard quench parameter for the external source as (SQP(E)). Other manufacturers use slightly different approaches but the principle is the same, i.e.,

"the spectrum for an external standard varies by the degree of quench in the sample vial and therefore, the efficiency of counting of the internal experimental sample also varies."

Similar to the channels ratio method, a standard curve is required i.e., a range of quenched standards is counted and the external standard spectrum analyzed in each case. As an example, the resulting data (Table 11.2) are used to prepare a standard curve that is saved in the counter's computer. Unknown samples are then counted in the same way, the efficiency read from the standard curve and the sample counts corrected from c.p.m. to d.p.m.

Table 11.2. Recorded radioactivity from a ^{14}C standard sample with increasing quench detected by an external standard

Sample	c.p.m.	External quench parameter	Counting efficiency (%)
^{14}C standard (203,600 d.p.m.) unquenched	194,930	810	95.7
^{14}C standard (203,600 d.p.m.) with increasing quench	146,141	422	93.5
	181,171	207	89.0
	167,731	126	82.4
	145,879	76	71.6
	126,913	55	62.3
	108,641	42	53.3
	96,103	37	47.2

The external standard approach is now a routine in most labs. The main advantage it has over the channels ratio method is that it is **suited to samples with low count rates.**

Disadvantage: it requires a standard curve for each set of circumstances, as is seen with the channels ratio.

In all cases where an automated procedure for calculating counting efficiencies is used, it is important to count a few prepared samples in which the true amount of radioactivity is known.

Worked example: External standard efficiency calculation
Question
The efficiency of detecting ^{14}C in a scintillation counter was determined by counting a standard sample containing 105,071 d.p.m. at different degrees of quench analyzed by the external standard approach.

c.p.m	SQP
87,451	0.90
62,361	0.64
45,220	0.46
21,014	0.21

SQP = standard quench parameter

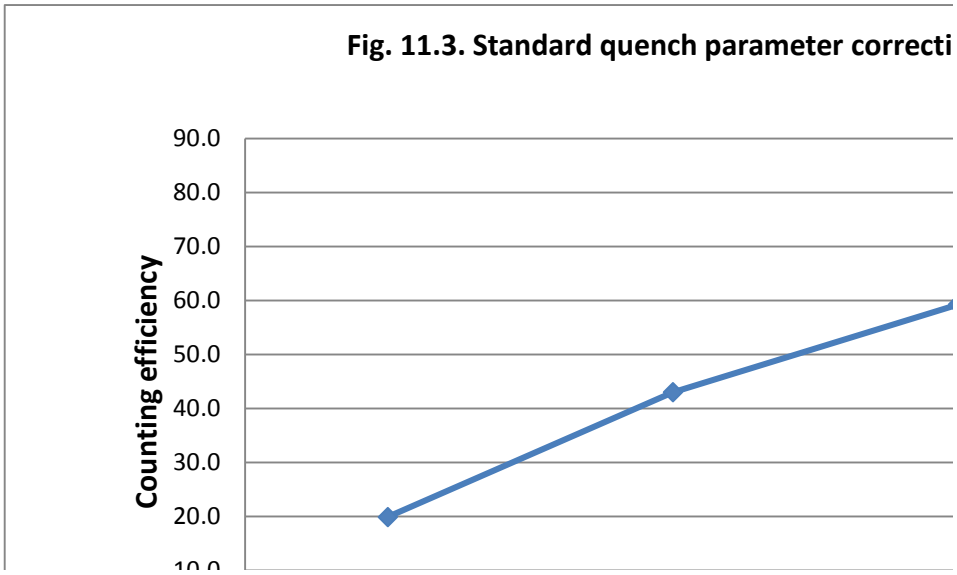
An experimental sample gave 2,026 c.p.m. at an SQP of 0.52. What is the true count rate?

Solution

Calculate efficiency for the counts at the various quench parameters. Note, standard sample contains 105,071 d.p.m

c.p.m	Counting efficiency calculation	Counting eff. (%)
87,451	$\frac{87{,}451 \text{ c.p.m.}}{105{,}071 \text{ d.p.m}} x\ 100$	83.2
62,361	$\frac{62{,}361 \text{ c.p.m.}}{105{,}071 \text{ d.p.m}} x\ 100$	59.3
45,220	$\frac{45{,}220 \text{ c.p.m.}}{105{,}071 \text{ d.p.m}} x\ 100$	43.0
21,014	$\frac{21{,}014 \text{ c.p.m.}}{105{,}071 \text{ d.p.m}} x\ 100$	19.9

Plot the SQP values against counting efficiency. Read off the graph the corresponding counting efficiency for the experimental sample at SQP of 0.52. This gives ~48% efficiency.
Now, correct the 2,026 c.p.m to d.p.m. by:
48% = 2,026 c.p.m
Therefore 100% = ?
2,026 × 100/48 = 4,221 d.p.m.



Sample preparation for scintillation counting

Major considerations should be taken when carrying out scintillation counting. These are:

1. Sample vials
2. The scintillation cocktail

3. Volume of cocktail
4. Method of overcoming quenching
5. Tissue solubilizers used
6. Combustion methods.

SAMPLE VIALS

In solid scintillation counting, sample preparation is easy and only involves transfer of sample to a glass or plastic vial compatible with the counter. In liquid scintillation counting, sample preparation is more complex and starts with a decision on the type of sample vial to use. Sample vials may be glass, specifically low potassium glass, with low levels of ^{40}K that reduce background count. Vials may also be polyethylene.

Polyethylene is cheap, but not suitable for cleaning and reuse. Glass vials can be reused many times provided they can be thoroughly cleaned. Polyethylene vials give better light transfer and result in slightly higher counting efficiencies but are inclined to exhibit more phosphorescence than do glass vials. Presently, mini-vials, which use far smaller volumes of expensive scintillation cocktails, are in frequent use. Modern counters are able to accept many kinds of vials. Smallest sizes are chosen to save cost and give consideration to environmental issues, as scintillation fluids are toxic. Some counters are designed to accept very small samples in special polyethylene bags split into an array of many compartments; these are particularly useful to pharmaceutical industries which work with large numbers of receptor binding assays.

Scintillation cocktails

Toluene-based cocktails are the most efficient, but will not accept aqueous samples, because toluene and water are immiscible and massive quenching results. Cocktails based on 1,4-dioxane and naphthalene that can accommodate up to 20 % (v/v) water can be used, but because of toxicity, they are not in use.

Emulsifier-based cocktails are the most frequently used for counting aqueous samples. They contain the emulsifier, Triton X-100 and can accept up to 50% water (v/v), however, phase transitions occur from single phase to two-phase or gel formation as the water content increases. Accurate counting cannot be done if samples are in the two-phase state. Many ready-made cocktails are sold with precise instructions regarding sample preparation.

Volume of cocktail

The efficiency of scintillation counting varies with sample volume. Therefore care should be taken that sample vials in a given series of counts contain the same volume of sample and that all instrument calibration is done using the same volume as for experimental sample.

Overcoming major color quenching

Color quenching can be removed by bleaching samples before counting. Bleaching agents such as H_2O_2 can give rise to chemiluminescence in some scintillation cocktails. Other color removal agents are activated charcoal and Fuller's earth.

Tissue solubilizers

Solid samples such as plant and animal tissue may be best counted after solubilization by quaternary amines such as NCS solubilizer or Soluene (a mixture of quaternary ammonium bases in toluene). These solutions are highly toxic and great care is required. The sample is added to the counting vial containing a small amount of solubilizer and allowed to digest. When digestion is complete, scintillation cocktail is added and the sample counted. Chemiluminescence is a problem with tissue solubilizers.

Combustion methods

An alternative to bleaching of colored samples or digestion of tissues is the use of combustion techniques. Samples are combusted in oxygen contained in a combustion apparatus. Samples containing ^{14}C would be combusted to $^{14}CO_2$ which is collected in a trapping agent such as NaOH and then counted; ^{3}H-containing samples are converted to $3H_2O$ for counting.

Methods based on exposure of photographic emulsions- Autoradiography

Autoradiography is a means of detecting location of radioactive compounds with a photographic emulsion. Ionizing radiation acts on a photographic emulsion to produce a latent image just as photography with visible light. To produce a photograph, one needs a radiation source, an object to be imaged, and a photographic emulsion. the sample containing the radioactive material is placed in close contact with the photographic emulsion.

The emulsion may consist of a large number of silver halide crystals embedded in a solid phase, such as gelatin. As energy from the radioactive material is dissipated in the emulsion, electrons are produced and the silver halide is reduced to metallic silver (Ag^0) which appears as black deposits of silver grains. The metallic silver acts as a catalyst for further reduction of Ag halide in its vicinity. Upon development to remove any remaining silver halide, a permanent image of the location of the original radioactive event is obtained.

Autoradiography is very sensitive and has been used in a wide variety of biological experiments, such as to locate the distribution of radioactivity in biological specimens of different types. The site of localization of a radiolabeled drug throughout the body of an experimental animal can be determined by placing whole body sections of the animal in close contact with a sensitive emulsion such as X-ray plate. After a period of exposure, the plate upon development will show an image of the sections in tissues and organs in which radioactivity was present.

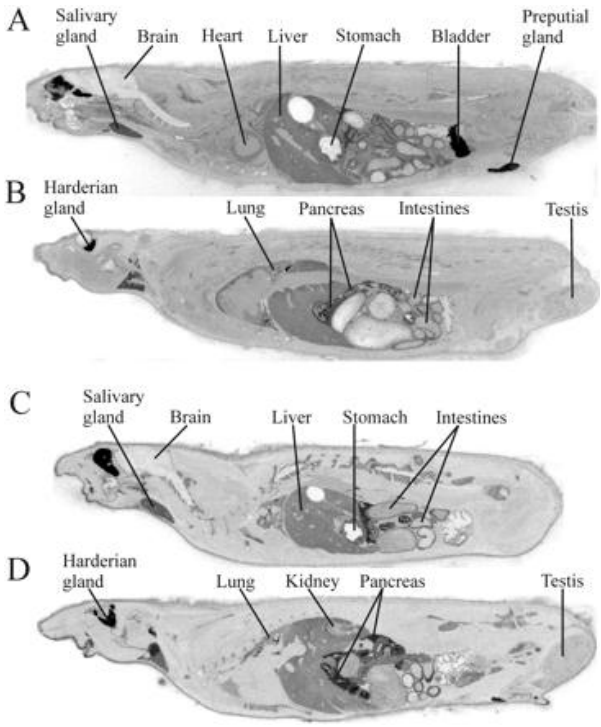
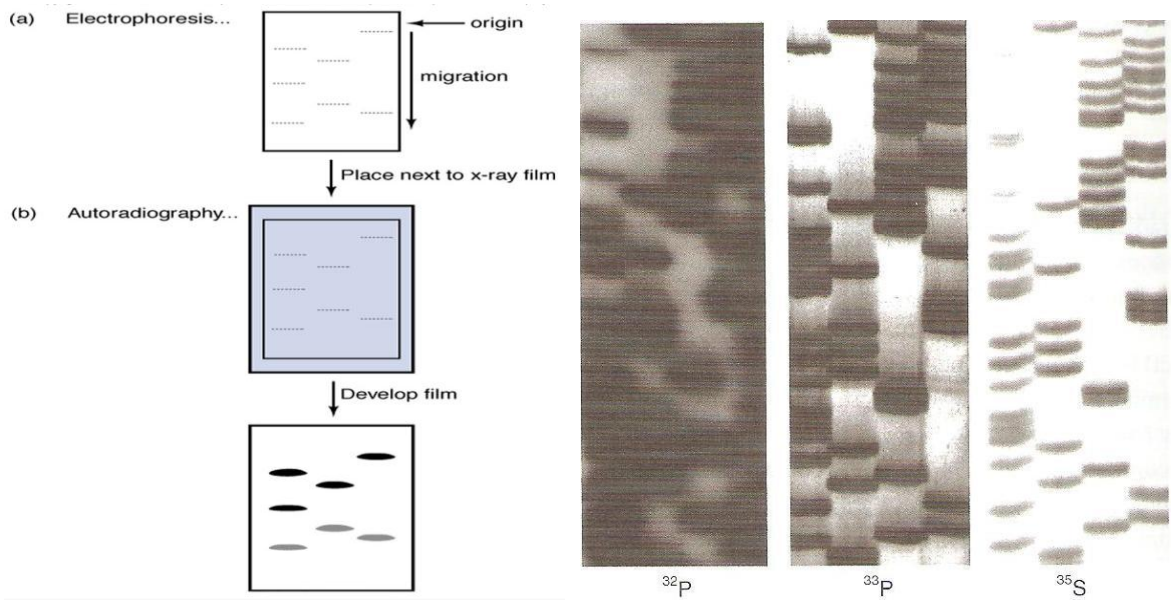


Fig. 11.4. Whole body autoradiography in a rat sacrificed at 15 min (A and B) and 60 min (C and D) after I.V. administration of [^{14}C] Ethanol. Dark spots indicate sites of radioactivity

Radioactive metabolites isolated and separated by chromatography or electrophoresis can be located on the chromatograph or the electrophoregram and the radioactive spots can subsequently be recovered for counting and identification.

Molecular biologists use X-ray film for autoradiography. In the Fig. 11.5, the researcher electrophoresed some radioactive DNA fragments on a gel and then placed the gel in contact with X-ray film in the dark for few hours or even days. The radioactive emissions from the bands of DNA reduced the film to produce dark bands. In effect, the DNA bands took a picture of themselves which is why the technique is called **autoradiography**.



(A) (B)

Fig. 11.5. (A) Steps in autoradiography. (B) Three autoradiographs showing the use of different isotopes in DNA sequencing. The isotope with the highest energy (^{32}P) leads to the poorest resolution because the radiation spreads out further, making DNA bands appear thicker. The lowest energy radiation (^{35}S) gives the best resolution (Source: Cunningham et al. 2002)

Important aspects of autoradiography

In general, weak β-emitters (^{3}H, ^{14}C and ^{35}S) are most suitable for autoradiography, particularly for cell and tissue localization experiments. This is because, as a result of the low energy of the negatrons, the ionizing track of the isotope will be short and a discrete image will result. This is particularly important when radioactivity associated with subcellular organelles is being located. For this reason, ^{3}H is the best radioisotope since its energy will all be quickly dissipated within the emulsion.

Electron microscopy can then be used to locate the image in the developed film. For location within whole organisms or tissues, either ^{14}C or ^{3}H is suitable; more energetic isotopes, such as ^{32}P are less suitable because their higher energy negatrons produce much longer track lengths and result in less discrete images that are not sufficiently discriminatory for microscopic location.

Conversely, for location of DNA bands in an electrophoretic gel, ^{32}P is useful. In this case, low energy ^{3}H negatrons would largely dissipate their energy within the gel before they reach the film, thereby reducing sensitivity to a low level. However, the more energetic ^{32}P will

leave the gel and produce a strong image on the film. If very thin gels are prepared, then ^{35}S or ^{14}C can be detected with high resolution, for example in DNA sequencing gels where ^{35}S is used as the label as an aid to rapid DNA sequencing.

Choice of emulsion and film

A variety of emulsions is available with different packaging densities of silver halide crystals. Care must be taken to choose an emulsion suitable for the purpose of the experiment, since the sensitivity of the emulsion will affect the resolution obtained. Manufacturer's literature must be consulted if one is in doubt.

X-ray film is generally suitable for macroscopic samples such as whole-body sections of small mammals, chromatographs or electrophoregraphs. When light or electron microscopic detection of the location of the image in the emulsion is required, (cellular and subcelllar localization of radioactivity), very sensitive films are necessary, as a very close apposition of sample and film is needed. In this case, a **stripping film technique** can be used in which the film is supplied already attached to a support. It is stripped from this and applied directly to the sample. Alternatively, liquid emulsions are prepared by melting strips of emulsion at 60^oC. Then, either the emulsion is poured onto the sample or the sample, attached to a support, is dipped into the emulsion. The emulsion is then allowed to set before drying it. This method is often referred to as **dipping-film** method and is preferred when very thin films are required.

Background

Accidental exposure to light, chemicals in the sample, natural background radioactivity (particularly ^{40}K in glass) and even pressure applied during handling and storage of films will cause a background fog i.e., latent image on the developed film. This can be problematic, especially in high resolution work such as in microscopy, and care must be taken at all times to minimize this effect. Background fog will always increase during exposure time, which should therefore always be kept to a minimum. A trial and error is often involved in arriving at the most suitable time of exposure.

Direct autoradiography

In direct autoradiography, the X-ray film or emulsion is placed as close as possible to the sample and exposed at any convenient temperature. Quantitative images are produced until saturation is reached. The approach provides high resolution but limited sensitivity; isotopes of energy equal to, or higher than ^{14}C (E_{max} =0.156 MeV) are required.

Fluorography

Many of the currently popular methods in molecular biology involve separation of macromolecules or fractions of molecules by gel electrophoresis. The separated

macromolecules or fractions form bands in the electrophoregraph that must be located. This is often achieved by radiolabeling the macromolecules with ^{3}H or ^{14}C and subjecting the gel to autoradiography. Because these are weak β-emitters, much of their energy is lost in the gel and long exposure times are necessary even when very high specific activity sources are used. However, if a fluor (e.g., PPO or sodium silicate) is infiltrated into the gel, and the gel dried and then placed in contact with a preflashed film, sensitivity can be increased by several orders of magnitude. This is because the negatrons emitted from the isotope will cause the flour to become excited and emit light, which will react with the film. In this case, the use of both ionizing radiation and excitation effects of radioactivity in fluorography become relevant.

Intensifying screens

When ^{32}P-labeled or γ-isotope-labeled samples such as ^{32}P-DNA or ^{125}I-labeled protein fractions in gels are to be located, the opposite problem to that presented by low-energy isotopes prevails. These much more penetrating particles and rays cause little reaction with the film as they penetrate right through it, producing a poor image. The image can be greatly improved by placing, on the other side of the film, a **thick intensifying screen** consisting of a solid phosphor. Negatrons penetrating the film cause the phosphor to fluoresce and emit light which superimposes its image on the film. There is therefore an increase in sensitivity but a parallel reduction in resolution due to the spread of light emanating from the screen.

Preflashing

The response of photographic emulsion to radiation is not linear and usually involves a slow initial lag phase followed by a linear phase. Sensitivity of films may be increased by preflashing. This involves a millisecond light flash prior to bringing the sample into juxtaposition with the film and is often used where high sensitivity is required or if results are to be quantified.

Quantification

Typically, autoradiography is usually used to locate rather than to quantify radioactivity. However, it is possible to obtain quantitative data directly from autoradiographs by using a densitometer, which records the intensity of the image. This in turn is related to the amount of radioactivity in the original sample. There are many varieties of densitometers available and the choice depends on the objective of the experiment. Quantification is not reliable at low or high levels of exposure because of the lag phase or saturation, respectively. However, preflashing combined with fluorography or intensifying screens obviated the problem for small amounts of radioactivity from previous experience. In this case, all photons contribute equally to the image of the pre-exposed film.

Practical aspects of counting radioactivity

A) Counter characteristics

BACKGROUND COUNT

Radiation counters of all types register a count event every few seconds, even in the absence of radioactive material in the equipment. Where do these come from? Radioactivity is all around us – air, ground, and ocean. The reading is not constant but keeps going up and down because decay process is random. Source of background radiation in counters

- Cosmic rays
- Radon decay products
- Nearby X-ray generators
- Circuit noises

Cosmic rays are fast-moving particles from space that enter the earth's atmosphere, along with their decay products. Since the atmosphere absorbs some of these particles, the rate of detection of cosmic rays increases with increasing altitude. If you were to take a Geiger counter on a jet flight, you would observe a marked increase in count rate while at high altitude.

Another radiation source comes not from above us, but from below. The earth's crust contains radioactive elements, such as uranium-238 (^{238}U). ^{238}U has a long half-life, but its decay products do not. One of these products is radon gas (^{222}Rn). As a result of the long uranium half-life, there is a nearly steady production of radon, which itself decays with a short half-life of 3.8 days. Since radon is a gas, it diffuses out of the soil into the air, and can collect in low-level enclosed areas, such as basements. Radon decays to a series of products including polonium, lead, and bismuth, which precipitate out of air onto dust particles since they are solids unlike gaseous radon.

Background radiation may be considerably reduced by lead shielding but its value must always be recorded and accounted for in all experiments. Some commercial instruments have automatic background subtraction facility.

DEAD TIME

At very high count rates in G-M counting, counts are lost due to dead time of the G-M tube. Correction tables are available to correct for lost counts. Dead time is not a problem in scintillation counting.

Geometry

When samples with end-window ionization counter, such as G-M tube, are compared, it is important to standardize the position of the sample in relation to the tube, otherwise, the fraction of the emitted radiation entering the tube may vary and hence the observed count will also vary.

B) Sample and isotope characteristics

SELF ABSORPTION

Self-absorption is primarily a problem with low energy β-emitters, where radiation is absorbed by the sample itself. It is often encountered with particulate samples, or samples stuck to a membrane filter. It can be reduced by digesting or solubilizing the particulate samples prior to counting. Self absorption is a major problem with G-M counting and significantly reduces sensitivity and reliability.

Statistics of Counting

Emission of radioactivity is a random process. This can be demonstrated readily by making repeated measurements of the activity of a long-lived isotope, each, for an identical period of time. The resulting individual counts will not be the same but will vary over a range of values, with clustering near the center of the range. If a sufficiently large number of such measurements are made and the data are plotted, a normal distribution curve will be obtained.

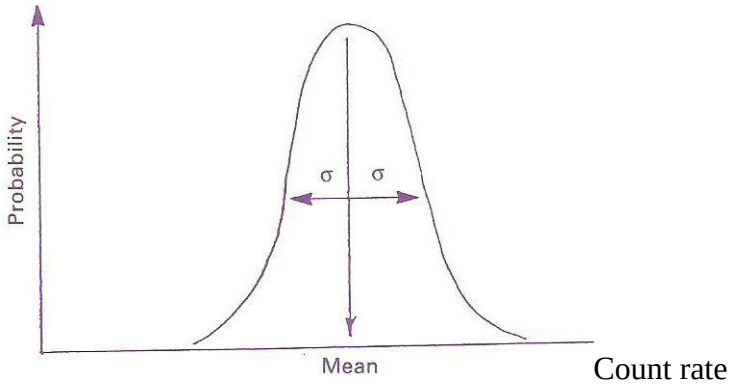


Fig. 11.6. The distribution of count rates around a mean, showing standard deviation, σ.

Because of this, a large number of counts, that is, counting more than a few times, are needed to establish a mean. The mean is then compared to the expectation, that is, the true count. However, the accuracy of this mean will depend on the spread or standard deviation (σ) for that data. The Empirical Rule states that, for a normal distribution such as that shown in Fig.

11.6, 68.2% of values obtained would lie ±1σ from the mean $\bar{x}$; and 95.5% of the data would lie ± 2σ from the mean, $\bar{x}$; and 99.5% of the data would lie ± 3σ from the mean $\bar{x}$, in other words, nearly all of the data will cover 3σ from the mean.

To compare if samples contain different amounts of radioactivity, we need to consider the counting statistics based on Poisson's distribution which describes the probability of a given number of events occurring in a fixed interval of time or space if these events occur with a known constant rate and they are independent, i.e., the occurrence of one event does not depend on the occurrence of another event,

From Poisson's distribution, the standard deviation of decay events is given by:

$$\sigma = \sqrt{\text{total counts taken}} \quad \ldots (1) \quad \text{or} \quad \sigma = \sqrt{\text{count rate} \times \text{time}} \quad \ldots. (2)$$

Therefore, to quote a figure with 95.5% certainty, that is, 2σ, we state a standard deviation of

$$\text{total counts} \pm 2\sqrt{\text{total counts}} \quad \ldots(3)$$

For example, if 1,600 counts are recorded, this can be expressed as 1,600 ± 80. There is therefore a 95.5 % chance that the true figure lies between 1,520 and 1,680 c.p.m.

When data are expressed as d.p.m. or c.p.m., again using 95.5% certainty, then

$$\text{error on count rate} = 2\sqrt{\text{count rate} \times \text{time}} \quad ..(4)$$

Using the same example, if the 1,600 counts were obtained in 1 min:

$$\text{error on count rate} = 2\sqrt{1{,}600 \times 1} = 80$$

and therefore, 1,600 c.p.m. ± 80 c.p.m.

If the 1,600 c.p.m. were obtained in 10 mins:

$$\text{error on count rate} = 2\sqrt{1{,}600 \times 10}$$

and therefore 1,600 ± 252 c.p.m.

Using the same example, if the 160 counts were obtained in 1 min

$$\text{error on count rate} = 2\sqrt{160 \times 1}$$

and therefore 160 c.p.m. ± 25 c.p.m.

Let's work these other simple examples also for a series of 1 min counts at a confidence level of 68.2%:

Counts = 100, $\sigma = \sqrt{100}$ = 10 therefore ± 10% ($^{10}/_{100}$) error at 68.2% certainty

Counts = 1,000, $\sigma = \sqrt{1000}$ = 31 therefore ± 3% ($^{31}/_{1000}$) error at 68.2% certainty

Counts = 10,000 $\sigma = \sqrt{10000}$ = 100 therefore ± 1% ($^{100}/_{10,000}$) error at 68.2% certainty

In summary, the counts per minute data become more accurate (i.e., error reduces) for higher counts and less accurate for longer counting times. It is common practice to count to 10,000 counts or to count for shorter times, whichever is quicker.

Worked example: Accuracy of counting

Question: A sample recording 564 c.p.m. was counted over 10 mins. What is the accuracy of the measurement for 95.5% confidence?

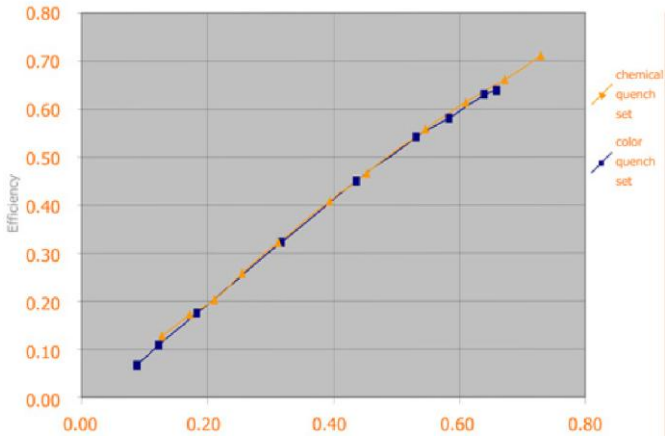
Solution

We apply equation 4: $\text{error on count rate} = 2\sqrt{\text{count rate} \times \text{time}}$

$$\text{error on count rate} = 2\sqrt{564 \times 10} = 150$$

So, the range in which the counts should fall within 95.5% confidence is 564 ±150 c.p.m.

Automatic Liquid Scintillation Beta Counter



SQP