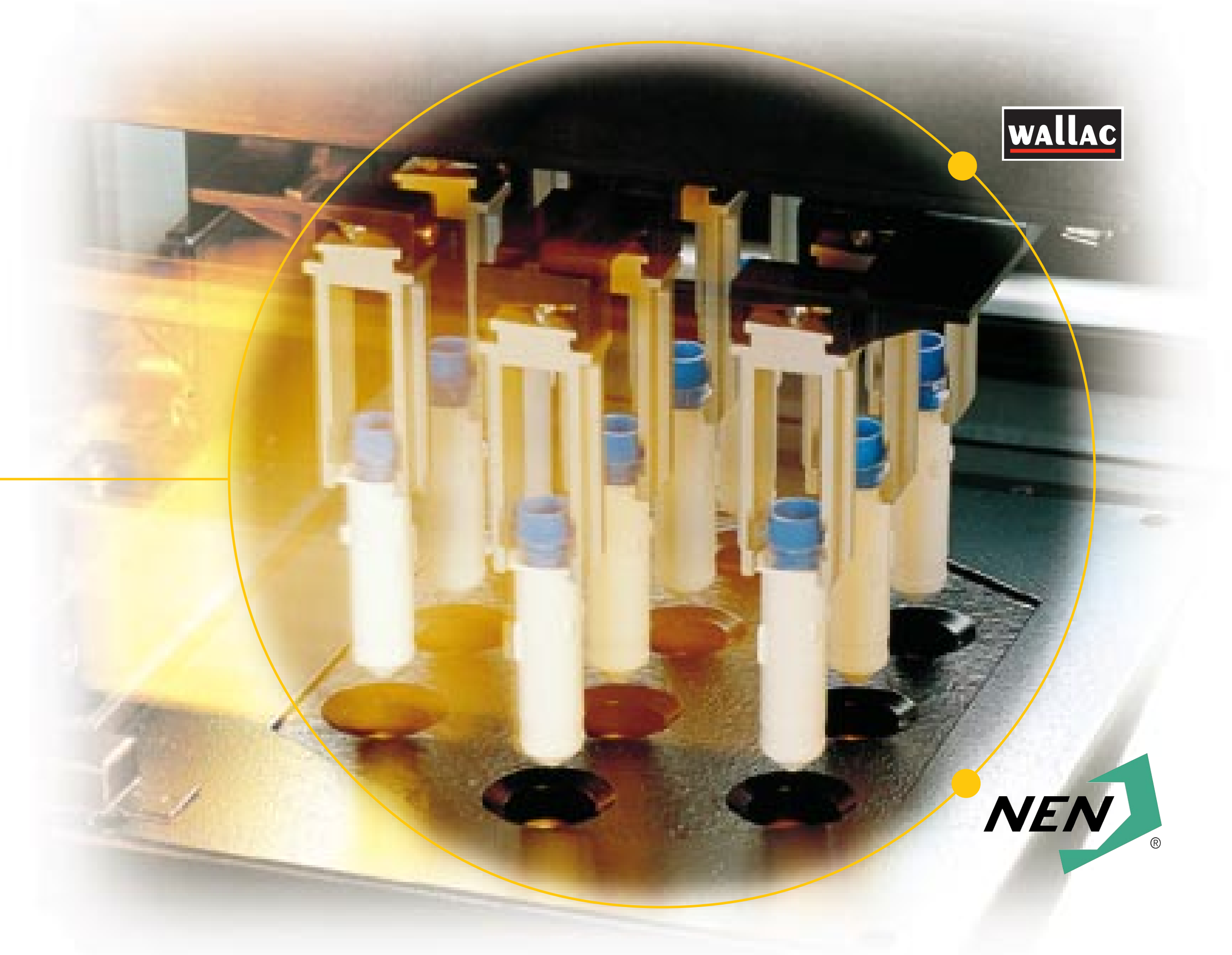
The gamma counting handbook



A guide to the state of art of gamma counting

PerkinElmer™
life sciences.

INDEX

1 Introduction

Gamma counting instrumentation has been in routine use in life science laboratories around 30 years. While users are well aware of test procedures they may be unaware of the instrumentation and related topics, particularly the ones concerning the rapidly changing field of data handling.

This publication is intended to help the user understand the various aspects of gamma counting and the technological improvements included in designs of the 1990's. It includes the information which is needed in order to be sure that acquired instrumentation will result in the best productivity and be optimally suited to the application.

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The 4th edition of the gamma counting handbook includes minor corrections. A new section has been added on counting radon and radium.

June 1997
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Measurements were made with program version 2.1 or 3.0 (1470) and 2.0 or 3.0 (1480).

Features described are available with program version 3.0 and above.

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2 Historical review

Wallac entered the field of gamma counting in 1962. The first automatic counter, GTL-500, featured a serpentine type conveyor and a fixed gripping unit, which replaced the “cherry picker” type of sample changer, commonly utilized in those days. This model, of which some units were still in regular use after 30 years of operation, proved to be extremely reliable. When LKB AB purchased Wallac in 1969, the model name was changed to 80000. The next generation, 1280 Ultrogamma was introduced in 1974 and it included a proprietary computer which enabled it to calculate directly CPM results as well as to carry out dual label counting, dead time correction etc. These counters were employed exclusively in nuclear medicine or research laboratories.

Two researchers, Dr. R. Yalow and S. Berson, MD were among the many who used this counter model extensively in their research work in the late sixties. In 1960 they introduced a novel technique, Radioimmunoassay, abbreviated RIA, an invention which earned Yalow the Nobel prize in medicine in 1977. Another contributor to the RIA method, Prof. R. Ekins in the UK, also used an 80000 gamma counter.

The RIA method caused a tremendous boost in the field of gamma counting, the demand for counters tripled in a few years. Originally universal counters were used, which constituted a degree of overkill because RIA usually utilized only the measurement of ^{125}I, a low energy nuclide demanding a relatively small detector and shielding. The calculations which were needed to determine concentration values were usually done manually or occasionally in a mainframe or minicomputer.

In 1975 Wallac, or LKB Wallac in those days, introduced the 1270 RackGamma, a revolutionary instrument designed particularly for RIA. It was a small bench top instrument which made use of the LKB rack format and it also included something unique in 1975 instrumentation: the microcomputer. The actual microprocessor chip, the Intel 8080, was introduced only a year earlier. The microcomputer provided the capability for the 1270 to produce concentration results directly, a demanding task for a computer having only 4 kb of total memory. However, mass memory was also required; perforated paper tape, read in through an ASR 33 teletype offered an unlimited, albeit noisy way to save assay protocols.

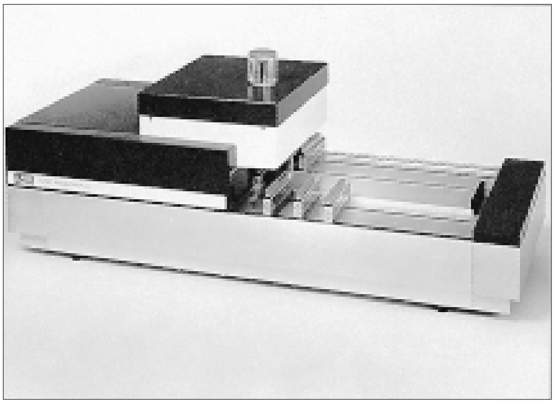


Fig. 2.1 1270 RackGamma was a workhorse in the RIA laboratories in the seventies.

Two years later Wallac introduced the spline smoothing method which greatly improved the evaluation accuracy, at the same time paper tape was replaced with semiconductor memory.

The next evolution took place in 1978, in England, when Nuclear Enterprise Ltd. introduced a new concept, a manual multidetector gamma counter having no less than 16 detectors. Wallac’s answer, the microcomputer based 1260 MultiGamma, was launched a year later, in 1979.

This was the era when RIA had its heyday, the peak in the RIA market was reached in about in 1982. There were almost 30 gamma counter manufacturers, producing around 3000 instruments annually. New instruments appeared regularly and sales were substantial. Wallac introduced two new instruments, the 1282 CompuGamma was a universal instrument and the multidetector (up to 4) version of RackGamma, the 1274 CliniGamma.

At the beginning of the eighties two instrument types dominated the market, manual multi-well counters and automatic counters having up to four detectors. It was time to combine these two.

This step was carried out by two companies, Micromedic Inc. in US and Wallac. The 1277 GammaMaster, which was introduced in 1984, was an automatic instrument having 10 detectors. It featured a staggered array of 1.5 inch through-hole detectors, a technique which was actually a multidetector concept of the RackGamma. But it also included something totally unique at that time, powerful laboratory management software, RiaCalc running on a separate Personal Computer. In those days the few microcomputers which were found in laboratories were usually 8 bit Apple II or Z80 based ones running programs under CP/M operating system, PCs were a rarity, and software which needed 512 kb of RAM was rarer still. The RiaCalc features, such as curve overlays, precision profiles, population histograms etc. were unknown in all other software packages at the time.

The RiaCalc development started in 1981 and the introduction took place in 1984. The development aim was to offer the customer unprecedentedly powerful software to take care of all aspects of RIA, quality controls etc. RiaCalc, and its successor, MultiCalc, have had a tremendous impact on the RIA market and the total number of more than 3000 installations have made MultiCalc unquestionably the industry standard.

1277 GammaMaster was a good instrument for the RIA work it was designed for and the first copy of it was introduced to the market just two years after the original one.

Since these introductions took place the RIA market has come under attack. The pressure against using radioactive methods has resulted in a number of competing, non-radioactive alternatives, including enzyme and fluorescence assays as well as luminescence methods. These took their share of the market and even though the whole immunoassay field was still strongly growing, and partially offsetting the decline of RIA, the demand for gamma counters started to go down. Another trend was toward automatic instrumentation and data handling. The small companies, which specialized in producing manual counters only, had to leave the market.

Fig. 2.2 Gamma counting in the eighties.
1277 GammaMaster featured a staggered array of
1.5 inch through-hole detectors and RiaCalc software.

In the late eighties the clinical RIA market declined while the area of research applications using gamma labels was more or less stable.
The RIA technology changed too, coated assays and very sensitive IRMA kits set new demands for the counters. The GammaMaster with its 1.5 inch through hole detectors was a good instrument for conventional RIA but these new demands coupled with those from gamma researchers were difficult to cope with. The position sensitivity, an intrinsic weakness in through hole detectors due to the unsymmetrical design, caused problems with coated tubes. Chromium release was hampered by high crosstalk, and contamination of the cassette, elevator etc. was a constant risk.

Wallac had used well type detectors for a long time in the most demanding applications, e.g. nuclear medicine. The 80000 Gamma, UltroGamma, and CompuGamma were the instruments widely favoured in this field. The question which naturally arose was simple: because the use of well type detectors will solve all these problems how could we use the same technology in a ten detector instrument as in a single detector one? At first the answer seemed difficult. It demanded innovative robotic techniques as well as three years of hard work before the new generation, WIZARD was born.

After 18 years and four different instrument families Wallac has now abandoned through hole detectors. Although other companies still promote the through-hole technology, as far as Wallac is concerned it belongs to the past.

The future

There seems to be no immediate alternative for gamma emitters in most research applications. However chromium release is increasingly being carried out in microtitration plates and measured using liquid scintillation counting. Wallac MicroBeta is the alternative to WIZARD for this application.

If we take a longer perspective of the use of gamma labels in life-sciences the trend will be as in clinical routine, toward non-isotopic labelling techniques. DELFIA® research system, which is based on time-resolved fluorometry (TRF), offers already extraordinary sensitivity which no isotopic method can match. Because there is virtually no overlap between the emission, the technology is well suited to multilabel assay designs, even quadruple assays have already been developed for research use.

It is difficult to see how WIZARD can be improved upon as a mechanical design and there are no candidates to replace the NaI(Tl) detector type in the near future.

MultiCalc offers the basic features, such as QC plots, Cusum plots, Westgard Multi-rules, retrospective and real time QC, population plots, etc. have been present for years as well as the possibility to connect the software with any counter system, pipetting station and mainframe. The future trend is not to add many more features (it is already difficult to find something that the MultiCalc cannot do) but to make it still easier to use.

As MultiCalc works equally well in techniques involving radioactive and non-radioactive labels it provides a familiar platform ensuring smooth transition to new labels.

Most of the MultiCalc features have been implemented in the optional internal instrument software package, RiaCalc WIZ. It supports creation of special assay protocols such as:

- Hepatitis screening
- RAST screenings
- Ratio assays such as T3 uptake or ETR
- Dual label Schilling test
- Combined assays like FTI
- Chromium Release studies
- RIA assays with variable NSB, eg. Renin

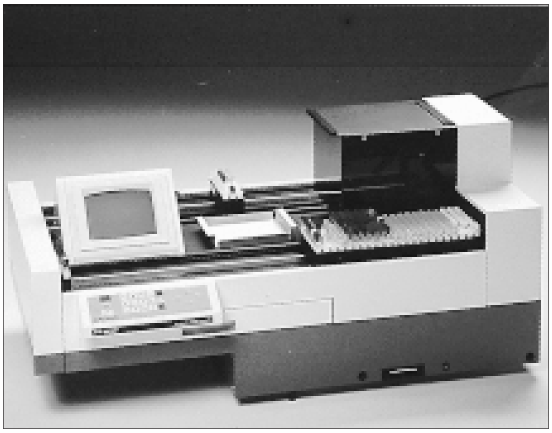


Fig. 2.3 Gamma counting in the nineties. 1470 WIZARD features well type detectors, robotic sample changer and MultiCalc and RiaCalc WIZ software.

Up to 20 radionuclides can be counted simultaneously. Raw count results as well as spillover corrected CPM results are reported in up to 20 discrete energy regions.

All individual nuclides are corrected for background and decay.

3 General considerations

3.1 Gamma radiation and detection

Gamma rays as well as X-rays are electromagnetic (EM) radiation similar to light but with much shorter wavelengths. Both have well defined energies, like the light produced by a laser, since they result from transitions between discrete energy levels. Gamma rays are produced in energy transitions of the atomic nuclei and X-rays outside in the electron shells.

EM radiation has dual wave-particle nature and therefore gamma rays are often regarded as discrete "packets" or quanta of energy emitted from the nuclide during nuclear disintegration. In this context they are called photons. The energy of the gamma photon is much higher than that of the light photon, around three to six orders of magnitude. The energies measured in WIZARD are from 20,000 electron volts to 2,000,000 electron volts (20 keV to 2 MeV) while light photons are in the region of three electron volts.

Even though light and gamma rays are analogous forms of electromagnetic radiation, we normally have different terms to describe them. The light term "colour" corresponds to the gamma "energy" and light "intensity" corresponds to gamma "activity".

The detector type used in Wallac gamma counters is a single crystal of thallium-activated sodium iodide, NaI(Tl). In the energies below 2 MeV interaction of gamma rays with the crystal may take place by two principal mechanisms.

In the photoelectric effect a gamma photon disappears and a (photo)electron is ejected from one of the atomic electron shells with a kinetic energy, which is the difference between the gamma photon and the orbital electron binding energy. The photoelectric absorption is an ideal process in measurement of the energy of a gamma photon.

In the Compton effect the gamma photon collides elastically with a free electron and after sharing part of its energy with the recoil electron, the photon scatters at an angle to its original direction with a lower energy. The electron acquires

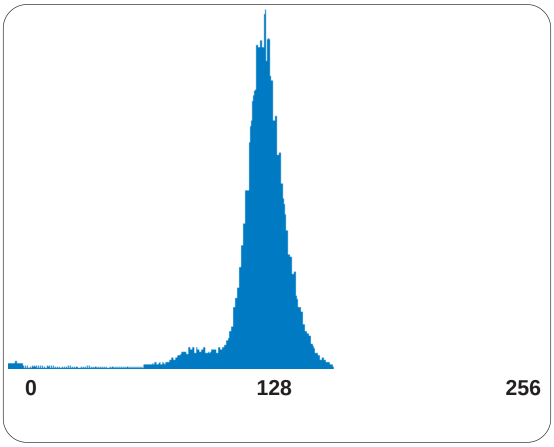


Fig. 3.1.1 shows measurement of ^{57}Co in 1470 WIZARD (peak energy at 122 keV). The total energy peak is high compared with the Compton peak. X-rays produced are insignificant. The spectrum obtained with 1480 WIZARD 3" is practically identical.

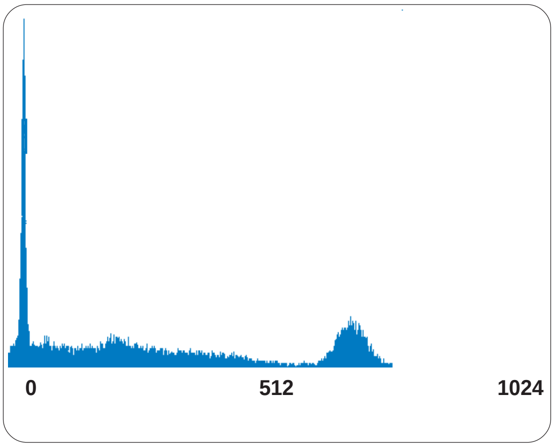


Fig. 3.1.2 shows measurement of ^{137}Cs in 1470 WIZARD (peak energy at 662 keV). The main photopeak efficiency is now much lower compared with the secondary peak. The secondary peaks consist of a Compton edge (at 478 keV), a backscatter peak (at 184 keV) and large X-ray peak at 37 keV.

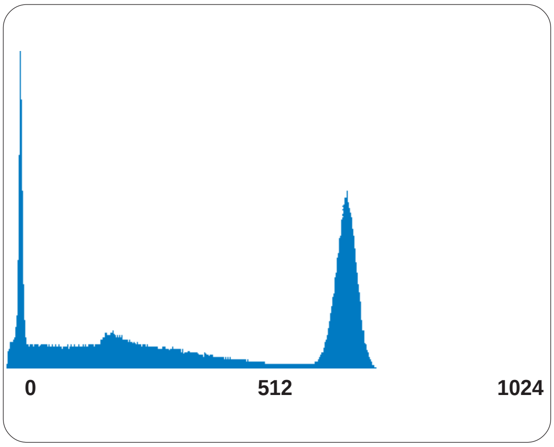


Fig. 3.1.3 shows measurement of ^{137}Cs in 1480 WIZARD (with 3" detector) The main photopeak efficiency is higher than in Fig. 3.1.2, due to larger crystal size.

no energy when the photon is scattered at a zero angle with respect to its original direction. When the photon backscatters, the electron acquires its maximum energy $E_{el} = (2E/511)/(1+2E/511)$ and the scattered photon energy is $E' = E/(1+2E/511)$, when its incident energy is E keV. Backscattering may take place outside the crystal, in the protective casing for instance and the scattered photon then enters the crystal and is recorded as low backscatter energy.

The ejected electrons are absorbed in the crystal and a fraction of the absorbed energy creates light by fluorescence. The light is collected and converted to an electric pulse by the photoelectric effect in the photomultiplier tube cathode in optical contact with the crystal (see Ch. 3.2). The height of the electric pulse is proportional to the number of light photons generated in the crystal.

The main gamma energy peak recorded in a detector is also called the full-energy peak which results in full absorption of the gamma photon energy in the crystal. Sometimes this peak is also called the photopeak, although photoabsorption may not be the only process involved. If the gamma photon energy is high there may be Compton scattering, but the scattered photon escapes from the crystal without absorption. This causes a pulse which corresponds to the difference in energy between the original gamma ray and the Compton scattered (escaped) gamma photon. The Compton edge and backscatter peak occur below the full-energy peak and their magnitudes depend on the crystal size and nuclide energy.

There are also other mechanisms which produce secondary peaks, such as the photoelectric interaction with iodine atoms in the detector or in lead shield, producing K X-rays. The more energetic the radioactive nuclide is, the more X-rays are produced.

The gamma photons are mono-energetic but the detection process produces a peak with a broad distribution. The main reason for the broad peak at low gamma photon energies is the small number of photoelectrons emitted from the photocathode. The small number of them per event leads to large relative statistical variation in their number and consecutively in the amplitude of the electric pulse, the photopeak width being proportional to the square root of the incident gamma radiation.

3.2 Practical detector construction

The light photons produced in the crystal are guided to a photomultiplier tube (PMT), which is optically coupled to the crystal. The photons encounter the photocathode, a glass plate covered by a thin layer of (alkali) metal, which can emit an electron when a photon is absorbed. These electrons are accelerated by an electrical field to the first of a series of electrodes called dynodes. A voltage difference, about 100 volts, is applied between each dynode. The electrons emitted by the first dynode are accelerated to a more positive second dynode. The multiplication process is repeated until the electrons are collected at the anode (last dynode) as an electrical pulse.
The height of the pulse at the anode is proportional to the gamma ray energy absorbed in the crystal. The pulse is further amplified and changed to digital form in the multichannel analyzer.

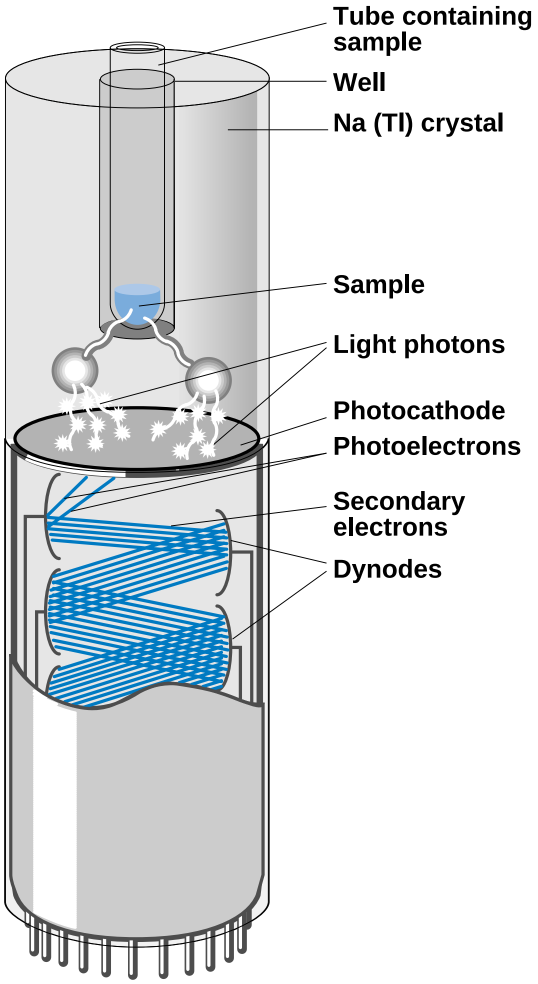


Fig. 3.2.1 The principle of a gamma detector.

For maximum possible efficiency the sample should be situated in the middle of the crystal. There are two different detector constructions for this, well-type and through hole type, see Fig. 3.2.2.

In well-type detectors gamma photons heading upwards are either lost or cause insufficient absorption, in through hole type, not just photons going upwards, but also photons going downwards are lost. Incomplete absorption causes secondary peaks and reduces counting efficiency. Because of this, well-type detectors give better efficiency than through hole type detectors of comparable size.

Another drawback of the through hole detector construction is that the output is very dependent on the sample position, particularly if the detector size is small with respect to the hole diameter. The light collection of the crystal part facing towards the PMT is much better than the one shadowed by the hole, resulting in different efficiency when the radioactive material is not evenly distributed in the sample tube. This phenomenon may occur when working with coated tubes.

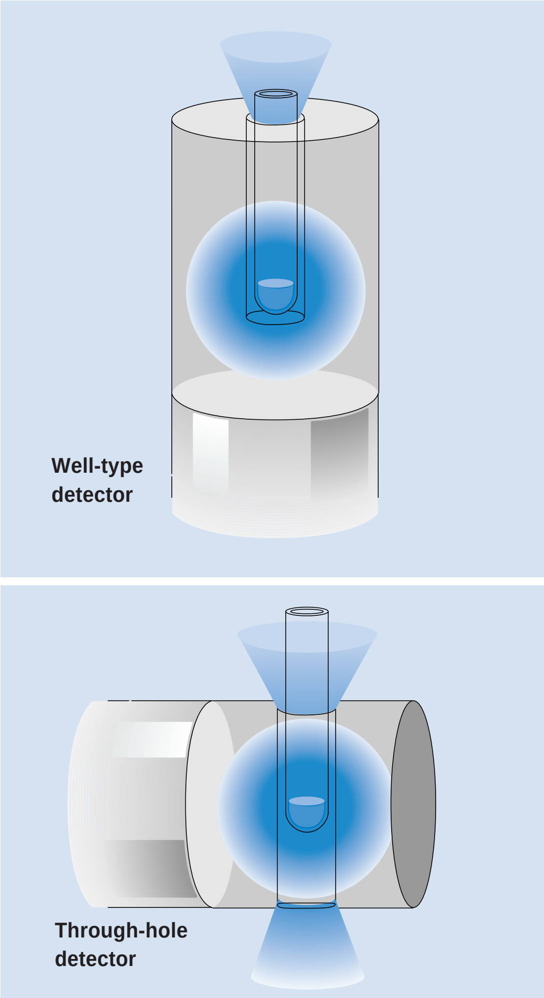


Fig. 3.2.2 Practical detector constructions.

Through hole detectors are also volume dependent which means that separate volume normalization is often recommended whenever they are used.

To move the sample into a through hole detector, a counterweight is required to keep the tube vertical. The counterweight rests on the top of the tubes and should the rims be contaminated with radioactivity (a common problem when decanting), the counterweight is also likely to become contaminated.

3.3 Multichannel analyzer

The output pulses from the detector are typically as shown in Fig. 3.3.1. The number of pulses in a given time is related to the CPM value (activity × counting efficiency) and the height with the isotope energy. The pulses with suitable energy (in the counting window) are registered.

It is often more informative to show the number of pulses as a function of isotope energy, as a form of isotope energy spectrum. The word "spectrum" comes from the corresponding way to show light intensity as a function of light photon energy (colour spectrum). The energy spectrum is analyzed in circuitry called a multichannel analyzer (MCA), the process is often called pulse-height analysis (PHA).

In principle the multichannel analyzer can be imagined to be a group of single channel analyzers. In picture 3.3.1 there are 8 single channel analyzers, each registers a pulse if (and only if) the incoming pulse height exceeds the lower limit but not the higher limit. For example, analyzer 1 (channel 1) registers 1 count, analyzer 2 (channel 2) 3 counts and so on.

This kind of circuitry is also known as an analogue-to-digital converter, often called ADC, because it converts the analogue signal into discrete numbers (in the example, 8 numbers). In addition to the ADC the MCA must include a memory which stores the registered counts. Each channel must have a corresponding memory address the contents of which are incremented each time a conversion takes place.

In the example we would have collected the following data:

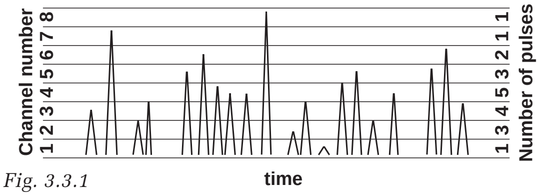


Fig. 3.3.1

It is more convenient to show the result in the form of a histogram, or spectrum, see Fig. 3.3.2

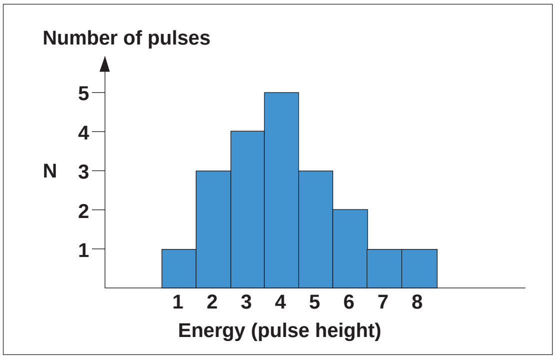


Fig. 3.3.2

In the example the MCA has 8 channels. In practice however, the number of channels is much higher, from 64 upward. Commercially available ADCs have channel numbers as a power of 2, e.g. 8 bit ADCs are used in 256 channel MCA's ($2^8 = 256$). Other common sizes are 10 bit (1024 channel) and 12 bit (4096 channel) ADCs. As the number of channels increases the conversion speed decreases.

The MCA can reveal useful information from the energy spectra, such as peak shape and position, but it cannot create new information. Therefore the number of channels should not exceed the information that is revealed from the NaI(Tl) crystal spectrum or actually needed, this only makes the system slower. A 256 channel MCA is usually sufficient for single isotope work. Semiconductor detectors, which have a resolution around 1 %, may benefit by having ten times as much.

WIZARD employs a 12 bit (4096) channel ADC, calibrated for 1 keV/channel (10 bits are used, equivalent to 1024 channels). 1480 WIZARD has also a range with 2 keV/Ch and 1470 WIZARD has 1.5 keV/Ch. The 1480 WIZARD MCA is sufficient to separate up to eight simultaneous nuclides.

3.4 Counting Efficiency

Counting efficiency is defined as the ratio of the number of events detected to the actual number of disintegration events. It is calculated using the formula:

%E = CPM/DPM * 100%

It depends on 4 principal factors:

1) Abundance
2) Detector efficiency to register gamma photons
3) Counting window
4) Geometrical factor

Abundance is defined as the probability ratio of the number of events which produce gamma photon to the actual number of disintegration events. Abundance varies greatly, some disintegrations may produce more than one gamma photon (or X-ray, this is detected in the same way as a gamma photon). As an illustration of this, ^{125}I decays in two consecutive stages which may produce two detectable gamma or X-rays. Another reason why the terms differ is that only a fraction of the disintegrations may produce gamma photons. An example of this is ^{51}Cr, with only an average of 9.85 % of disintegrations producing gamma-photons, the remainder are beta particles which are not detected in a gamma counter. The abundance of ^{51}Cr is therefore 9.85 %. Because abundance may be more than 100 % the counting efficiency may also be bigger than 100 %.

The detector efficiency to register gamma photons depends primarily on the gamma photon energy, detector crystal size and shape. In the region up to about 150 keV it is around 90% but as the energy increases the gamma detection efficiency decreases. Below 50 keV it is also decreased because a fraction of the radiation (up to 10 % in the case of ^{125}I) is absorbed in the aluminium liner of the detector. (Because of this the detectors used in very low energy nuclides may employ liners made of beryllium).

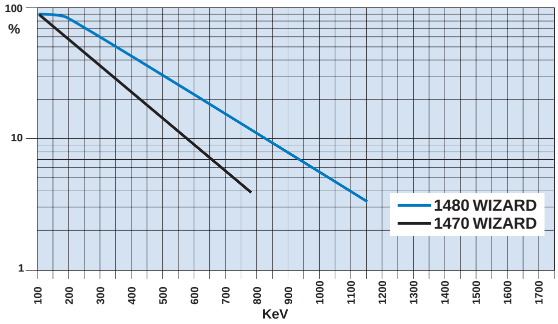


Fig. 3.4.1 Detector efficiency to register gamma photons when only the photopeak is counted. From this value the counting efficiency can be calculated if the sample abundance is known.

1470 WIZARD has a 1.3" × 2" (32 mm × 50 mm) end well-type crystal and it detects gamma photons well up to 1 MeV (up to the energy of the ^{58}Co nuclide). The 1480 WIZARD has a 3" x 3" (80 mm x 75 mm) size crystal and it has good performance up to 2 MeV.

The third decisive factor is the fraction of the spectrum collected, i.e. the counting energy "window", particularly with high energy nuclides. Often only the total energy peak is counted e.g. in MIA or environmental control samples, this minimizes the background value as well as spillover. If the counting efficiency is more important than the background, the scattered rays may be counted as well.

At higher energy the difference in counting efficiency due to the difference in counting window is dramatic. A typical example is ^{58}Co when counted in 1470 WIZARD. Its counting efficiency, when measured in a wide window ("A" in fig. 3.4.2) is typically 23 %. The 1.5" through hole detector has an efficiency of 17 % measured under the same conditions. However, the principal test for ^{58}Co is the Schilling test for which the optimal window setting may be the one in which there is only the main photopeak (window "B" in fig. 3.4.2), 15% of the total counts. This is the default setting for ^{58}Co in 1470 WIZARD giving a single photopeak efficiency of 3.5 % (23 % * .15).

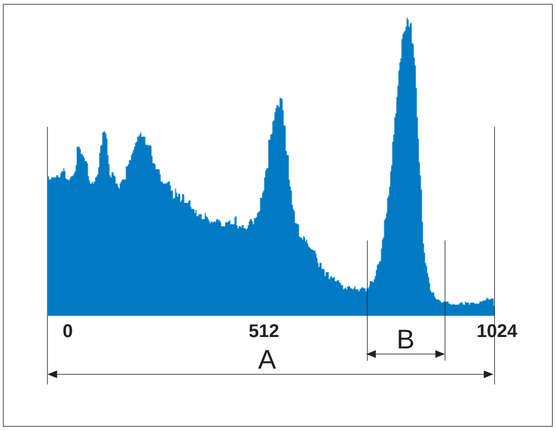


Fig. 3.4.2 ^{58}Co counting efficiency as a function of counting window in 1470 WIZARD. Counting efficiency is related to the window width.

If one compares 3.5% with 17% a strange conclusion can be reached; air in the second hole of a through-hole detector is more efficient in detecting gamma radiation than NaI(Tl) in the well type detector. Comparing 23 % with 17 % gives the right perspective. The difference, 6 %, is lost in the second hole.

The above phenomenon is related with the geometrical factor which is buried in the gross efficiency figure and discussed already in section 3.2. A geometrical factor = 1 would require the sample to be totally inside the detector (as in a liquid scintillation counter). A fraction of the gamma radiation will escape undetected because of the hole in the crystal and this fact makes a through hole detector less efficient than a well detector.

Even for the expert, some of the efficiency specifications for a gamma counter are confusing. This is because there are many factors which affect the efficiency figure in addition to the actual detector. The situation becomes worse if the expert intentionally tries to confuse the layman, for example, by using tricks like the one shown above.

In order to be on the safe side the following questions must be considered when comparing efficiencies of two instruments:

1. Which isotope is in question (abundance, gamma photon energy) ?
2. What is the counting window (wide, photopeak(s) only, something else) ?
3. What is the geometrical factor (well or through hole crystal) ?

In order to make efficiency comparisons between instruments as unambiguous as possible the counting efficiencies should be determined using wide windows, this eliminates one factor which may otherwise make results impossible to compare.

A list of common radionuclides are listed on pages 38…39 of this booklet with a typical efficiency value when counted in WIZARD. Note that the efficiency values are for the wide window which also consists of scattered peaks. The window in the WIZARD isotope library setting may be different.

^{125}I or ^{57}Co are isotopes whose counting efficiencies are virtually independent of detector size or construction, a well type detector is only fractionally better than the through-hole type. In the case of ^{125}I the smaller 1470 WIZARD detector has an even better efficiency value than the 3 inch detector used in 1480 WIZARD. This is because ^{125}I has such a low energy that the detection actually takes place in a few millimeter layer of NaI(Tl). Compton scattering is so small that the counting window to cover the photopeak only gives practically the same efficiency as the open window. Note also that counting the ^{125}I in a glass vial may lower the efficiency 3…4 % compared with counting it in a plastic vial due to higher absorption by glass.

Efficiency determination using the coincidence method

In order to determine counting efficiency, one must obtain the count rate (CPM) and know the absolute disintegration rate of the sample (DPM), in other words one must have a calibrated source. While most slowly decaying isotopes, such as ^{137}Cs or ^{129}I are commercially available as calibrated sources the most common gamma label, ^{125}I, is not easy to maintain as a calibrator because its half-life is only 60 days.

Due to the ^{125}I decay pathways it is possible to determine the sample's absolute decay rate by employing the coincidence method.

To illustrate the method let us suppose that we have an isotope which decays in such a way that it emits 2 gamma photons of identical energy in each disintegration. A NaI(Tl) detector cannot resolve the photon energy but instead detects one pulse with double energy. If the gamma counting efficiency is 100 % only a single peak (coincidence peak) appears. But suppose that the counting efficiency is less than 100 %. The probability of detecting one gamma in coincidence is p * p where p is the detection probability. The probability of detecting one gamma alone is 1 minus the probability that both or none are detected

$= 1 - p^2 - (1 - p)^2$

$= (2p - 2p^2)$. We obtain:

$$N_c = S\,p^2 \qquad (1)$$

$$N_s = S\,(2p - 2p^2) \qquad (2)$$

Nc = coincidence photopeak (CPM)

Ns = singles photopeak (CPM)

S = source activity (DPM)

The ratio R is defined $R = N_c/N_s$ (3)

$$R = p/(2 - 2p) \text{ and:} \qquad (4)$$

$$p = 2R/(1 + 2R) \qquad (5)$$

$$S = Nc/p^2$$

$$S = Nc\,\frac{(1 + 2\,R)^2}{(2R)^2} \tag{6}$$

This says that it is possible to estimate the sample strength by measuring the coincidence photopeak and singles photopeak counts.

^{125}I does not emit two gamma photons with identical energy, neither is the probability for gamma emission 1 as required in the example. It does, however, emit X-ray and gamma photons at 27.5 or 35.5 keV (Ns peak) which coincide with 55 or 63 keV (Nc), therefore the Nc/Ns must change as a function of counting efficiency.

Using rigorous mathematical analysis it can be shown (Ref. 2) that equation (6) is still accurate (within 1 %) in the case of ^{125}I.

The coincidence method

$$\%Eff = A/S * 100\ \% \tag{7}$$

$$A = Ns + Nc \tag{8}$$

$$\%Eff = \frac{Ns + Nc}{S} * 100\ \% \tag{9}$$

A = measured activity (CPM)

Nc = coincidence photopeak (CPM)

Ns = singles photopeak (CPM)

S = source activity (DPM), see formula 6.

This method is known as the Eldridge equation (Ref.1), Horrocks method (Ref. 2) or the coincidence method. Strictly speaking, there is no such concept as "Horrocks" efficiency, it is only the method used to estimate the DPM value of the source. The efficiency is always calculated using the equation Eff. = CPM/DPM * 100 % independent of the method by which the DPM value is obtained. In daily parlance, however, we often talk about the Horrocks method indicating that the efficiency is calculated using formula (9).

As the source DPM value can be determined simultaneously with counting the CPM value, the efficiency can be determined using uncalibrated sources such as waste or used total samples. The WIZARD utilizes this method when making GLP reports. The efficiency obtained is immune to the sample decay and in fact the sample being followed may change from day to day since its activity is irrelevant when the efficiency is determined.

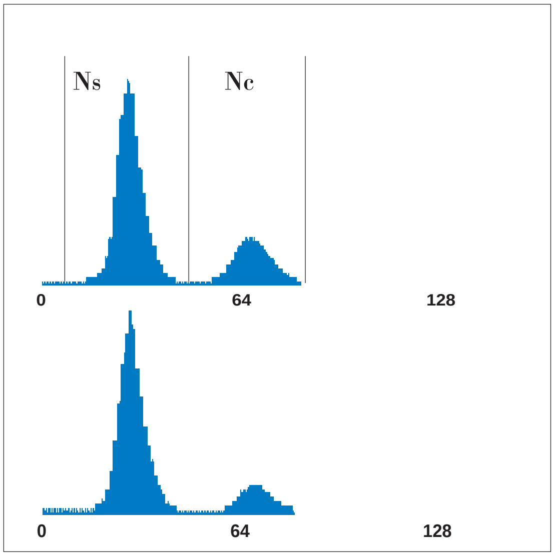


Fig. 3.4.3 Visual illustration of the coincidence peak height as a function of counting efficiency. High efficiency (top) and low (bottom).

The word "irrelevant" does not mean that the sample activity can be arbitrarily low, the accumulated counts (both Ns and Nc separately) are subject to the same statistical variation as any radioactivity.

The obvious limitation of the coincidence method is that it requires that 2 gamma photons are produced simultaneously by a decay event; only relatively few isotopes fulfil this requirement and in the case of WIZARD it is only used with ^{125}I. In principle it can be applied also to positron sources (PET studies) because the positron electron annihilation always results in two gamma photons with the energy of 511 keV. The positron sources used (such as ^{15}O or ^{18}F) result in a main peak at 511 keV and a coincidence peak at 1022 keV.

Reference

1) J. S. Eldridge and P. Crowther, "Absolute determination of ^{125}I in clinical applications". Nucleonics 22 (6),56 (1964).

2) D. L. Horrocks and P.R. Klein, "Theoretical considerations for standardisation of ^{125}I by the coincidence method." Nuclear Instruments and Methods 124, 585-589 (1975)

3.5 Throughput

Throughput determines the number of samples which can be measured in a given time, for a certain precision. The precision depends on the number of counts that have been accumulated during the counting period. Since the throughput depends on the sample activity and the needed precision, neither of which depends on the instrument, we can actually talk only about relative throughput, the throughput ratio between different counter types. There are three instrument related parameters which affect that:

- sample changing time
- counting efficiency
- the number of detectors

In normal RIA work, involving the use of ^{125}I and ^{57}Co nuclides only, counting efficiency is practically independent of the detector size. Having 1…2 minutes counting time, the speed of the sample changer mechanism does not affect throughput considerably. The relative throughput is therefore directly related to the number of detectors.

In chromium release work the situation is more complex. The 3" detector employed in 1480 WIZARD has considerably higher efficiency than the smaller ones used in 1470 WIZARD. The relative throughputs as compared with 1480 WIZARD 3" are as follows

1470, 1 detector	43%
1470, 2 detector	86%
1470, 5 detector	215%
1470, 10 detectors	430%

As a rule of thumb it can be said that in chromium work the two detector 1470 WIZARD is as fast as a gamma counter employing a 3" detector but in ^{125}I counting it is two times faster.

3.6 Background

Background is caused primarily by cosmic rays and radioactive decay in the ground or construction material. The latter depends very much on local conditions. In the counter construction, background is determined by the detector crystal volume, lead shield geometry and thickness. The effect of background as caused by the sources mentioned can be eliminated by simple subtraction. The only aspect which must be taken into consideration is the fact that the background value should not be large with respect to the measured activity because the statistical inaccuracy would increase. Compensating for this may lead to very long counting times.

The relatively compact lead shield means that 1470 WIZARD is also suitable for the counting of low activity samples, found e.g. in very sensitive IRMA assays.

1480 WIZARD is constructed to count very low activity samples as, for example, in some types of environmental studies. In addition to a massive (225 kg or 495 lbs) 4 pi shielding ensuring a low and stable background, it counts large volume (up to 30 mL solid) samples. This gives superior lower limit of detection.

Background depends on the counting window. The best efficiency/background ratio is normally obtained if the counting window is set to cover photopeak only, e.g. window “B” in Fig. 3.4.2.

Nuclide	1470 WIZARD	1480 WIZARD
I-125	40	30
Co-57	80	21
Cr-51	40	15
Cs-137	55	40

Fig. 3.6.1 Typical background values.

3.7 Energy range

From the instrument manufacturer's point of view, there is nothing easier than to extend the nominal energy range in a gamma counter; one simply reduces the pulse amplifier gain and the energy spectrum flows down. In fact all WIZARD models can be adjusted to have an energy range from 15 keV to 6 MeV. However, this does not change the basic counting performance criteria, such as counting efficiency or crosstalk, which are determined in the basic, mechanical design. To be safe as far as crosstalk is concerned the energy range of 1470 WIZARD is limited to 1 MeV and 1480 to 2 MeV. To allow counting of ^{59}Fe, 1470 WIZARD can also be delivered having the range 15 keV…1.5 MeV. This marginally affects the stability in ^{125}I counting (MCA resolution is changed from 1 keV channel to 1.5 keV/channel) but the practical effect is small. Most other manufacturers use 2 keV/channel (256 channel MCA and 15…500 keV range). The ^{59}Fe crosstalk is noticeable in 1470 WIZARD, and the recommended choice is 1480 WIZARD which is particularly designed to work with higher energy nuclides.

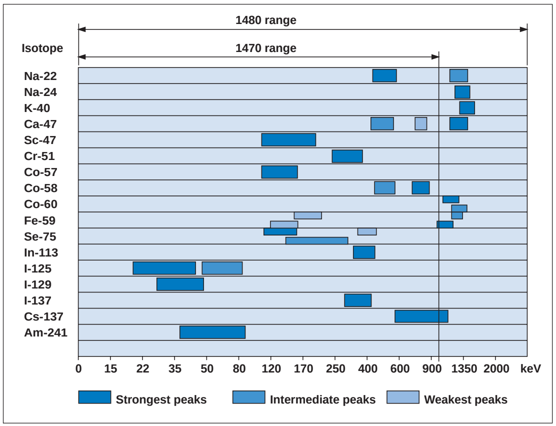


Fig. 3.7.1 The peak positions of some selected isotopes in the WIZARD energy range.

3.8 Linearity

For an ideal detector the "scintillation efficiency" or amount of light generated would be directly proportional to the detected gamma photon energy, and the response of the detector would be perfectly linear.

For photons in NaI(Tl), the scintillation efficiency does vary with photon energy, see fig. 3.8.1. This is an intrinsic property of the detection process of the NaI(Tl) itself and cannot be changed by the detector construction. The energy scale of WIZARD is calibrated at two points, about 30 keV (by ^{125}I) and 662 keV (^{137}Cs). This implies that some non-linearity should be expected in the 100 keV region. The typical non-linearity is + 7 % for the ^{57}Co isotope, for all other isotopes it is considerably smaller. The dynamic normalization process allows non-linearity of ± 20 %.

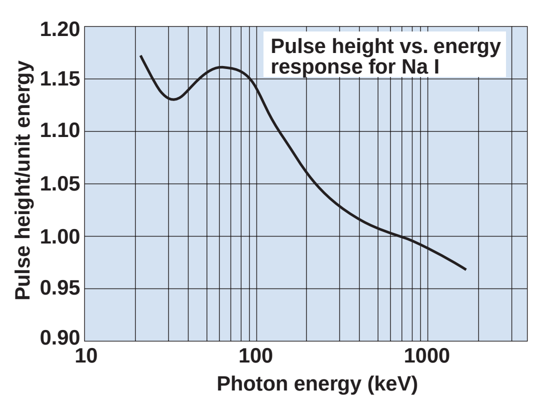


Fig. 3.8.1 Linearity of NaI(Tl).

A detector linearity table is programmed into WIZARD. The correction, based on this data is automatically applied in the spectrum display and other functions.

3.9 Lower limit of detection, LLD

The sample activity can be detected when it exceeds the variation of background counts by a given amount.

In the literature one can find numerous definitions of an LLD. This is because actually 3 different LLD figures have been defined:

- the count rate above which the CPM value can be recognized as “detected”
- the count rate above which the CPM value can be expected to lead to detection
- the count rate at which the measurement precision will be satisfactory for quantitative determination

We may also talk of critical level, detection level and determination limit.

Often simple definitions, such as background standard deviation (or 2 std, 3 std, 4 std etc.) are used. 10 percent of background or twice the background are “non-statistical” definitions which are also found in the literature.

It is in the instrument manufacturer’s interest to use a definition that gives the best LLD value, therefore if the LLD values of two instruments are compared, care must be taken that both LLD figures are calculated using the same equation. LLD depends on, in addition to the background level of the instrument, the counting efficiency. Sometimes the term minimum detectable activity (MDA) is also used for LLD.

It is calculated using the formula, where equal counting times T are devoted to sample and blank:

$$\mathrm{LLD} = \frac{4.65\, s_b + 3}{KT}$$

where s_b is the standard error of collected blank counts and K is a calibration constant, including counting efficiency and enrichment factor.

Minimum detectable concentration, MDC, is LLD per volume or mass, for example for ^{137}Cs

$$\mathrm{MDC} = \frac{4.65\sqrt{0.67/\mathrm{sec} * 3600\ \mathrm{sec}}}{0.18 * 3600\ \mathrm{sec} * 0.02\ \mathrm{L}} = 18\ \mathrm{Bq/L}\ ,$$

where

[B] = background 0.67 CPS (40 CPM)
[E] = counting efficiency 0.18 (18 %)
[V] = volume 0.02 L (20 mL)
[T] = counting time 3600 sec

It is also possible to measure solid samples directly in the holder, the max. volume is then up to 30 mL. This still lowers the MDC value as will a longer counting time.

References

Lloyd Currie, "Limits for Qualitative Detection and Quantitative Determination". Analytical Chemistry, 40(3), 586-593 (1968).

An American National Standard on Performance Criteria for Bioassay, HPSN13.30-1996, Health Physics Society, McLean 1996, 112 p.

3.10 Energy resolution

The energy resolution is expressed as the full width of the peak at half maximum (FWHM). It is customary to express FWHM as a percentage of the main photopeak energy. It depends on the crystal size, crystal quality, light collection

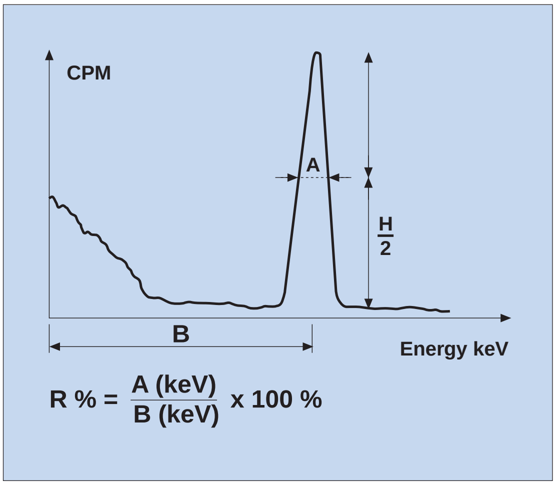


Fig. 3.10.1 Definition of detector energy resolution

efficiency, PMT cathode efficiency and gamma photon energy.

It is low (large percentage value) for a low energy radionuclide such as ^{125}I, which has an energy of 30 keV and a resolution of typically 25 %. The average ^{137}Cs (662 keV) resolution is 8 %. In normal single label work, the energy resolution is not very important because low resolution can be compensated for by setting a wider counting window.

Resolution becomes important if multiple nuclides are used. If the resolution is bad the counts may “spread” to another counting region, i.e. there is “spillover”. It is also an important factor in environment studies because resolution determines the width of the counting window and this is directly related to the background.

The detector energy resolution is often determined by using the gamma emission of 662 keV of ^{137}Cs.

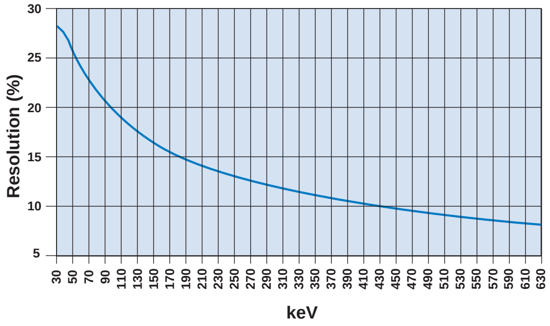


Fig. 3.10.2 Resolution in 1470 WIZARD

More reading on gamma counting:

Glenn F. Knoll, Radiation Detection and Measurement. John Wiley & Sons, New York, 1979.

Nicholas Tsoufaldinis, Measurement and Detection of Radiation. Hemisphere Publishing Co., Washington, 1983.

W.B. Mann, A. Rytz, A. Spernol and W.L. McLaughlin, Radioactivity Measurements. Principles and Practice. Appl. Radiat. Isot. 39(8), 717-937, 1988.

3.11 Instrument stability

The principal source for instability has been spectral “drift”, this is caused by a gain variation in the photomultiplier tube. There are two kinds of variation. Short term variation is due to the temperature change, activity change (high activity sample may cause the spectrum to drift downwards) and voltage change e.g. immediately after the instrument is switched on. Long term variation is caused by PM tube ageing, this drift takes place over a long period of time, usually months or years.

Both problems are solved by following the photopeak position constantly and moving the window accordingly. The technique is based on the use of an MCA and a built-in computer which allow constant analyses of the shape and position of the various spectra. It works as follows:

- The expected peak position and optimized windows are included in the library. (Window settings are empirically determined at the Wallac factory)

- During normalization the expected peak is compared to the measured one. The window is set to cover a given percentage of the total spectrum. This technique ensures that the window setting covers the same area, regardless of the detector resolution. In the multidetector models this ensures the same counting efficiency between the detectors.

- During counting the expected peak position (memorized in the normalization) is compared to the measured one and the ratio of the two peak positions is calculated. The window position (determined in the normalization) is moved by the same ratio. This window adjustment is done in each sample which has enough activity so that the peak can be reliably determined.

Because the instrument program knows where to expect the peak, and whether there should be one or many peaks, an incorrect peak position, caused e.g. by contamination, can be detected.

There is an option to set the window by giving its low and high boundary values in keV.

3.12 Efficiency normalization

Normalization is a process in which a single source is counted in all detectors in turn to determine the relative counting efficiencies.
In the actual measurement this factor is used to calculate corrected CPM values. An automatic normalization routine is included in WIZARD.

Because of the dynamic window setting system used, in which counting windows are adjusted according to the resolution, separate normalization is actually a little redundant for most gamma applications. A variation of ± 0.5 % is normal for ^{125}I and ^{57}Co nuclides without normalization.
With a normalization procedure, variation can be made still smaller; it is actually limited only by the statistical nature of the gamma decay.

The efficiency normalization is naturally not needed in a single detector instrument. However, the normalization counting is still included because the actual normalization also carries out other operations, such as window setting or spillover correction in dual label counting.

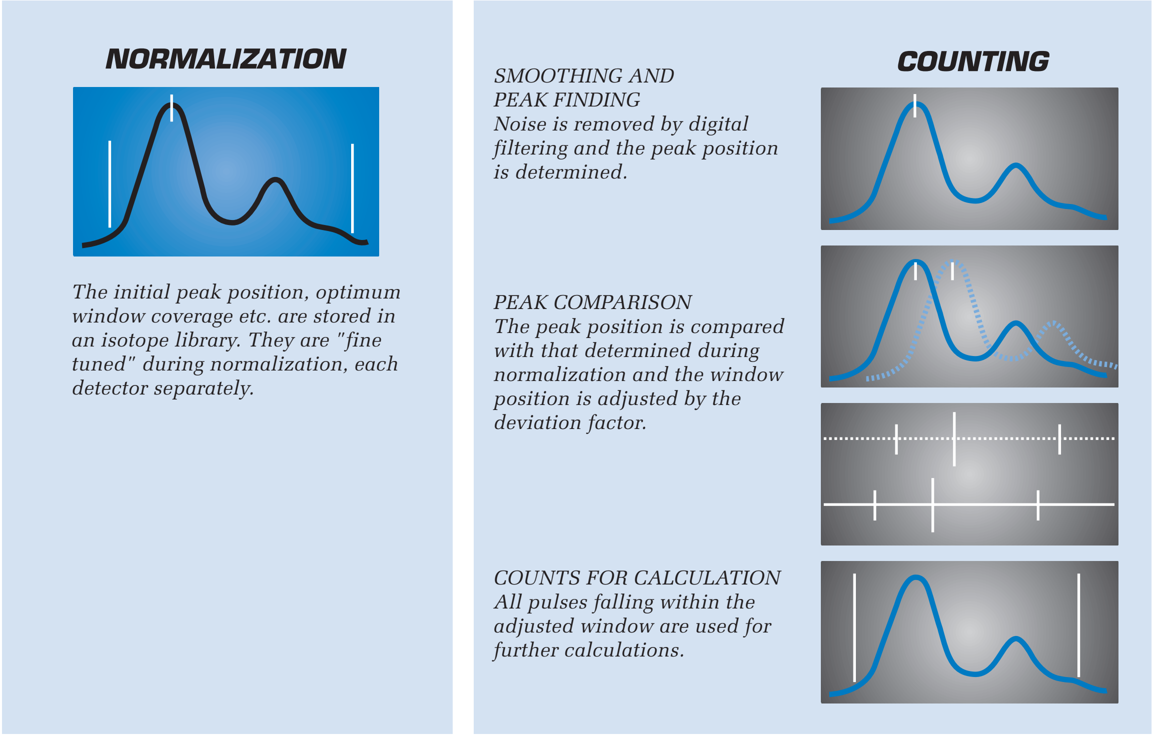
3.13 Detector to Detector Crosstalk

Detector to detector crosstalk occurs when a sample in one detector also causes counts to be registered in another detector. This sort of crosstalk does not exist in the single detector WIZARD.

The crosstalk is the percentage ratio of the count rate of an empty detector with the sample in an adjacent detector to the count rate when the sample is in it. Background correction is applied in the calculation.

Crosstalk depends on the penetration of the gamma rays in the lead and is therefore a function of the isotope energy. In 1470 WIZARD, in the energy range up to 200 keV the crosstalk factor is negligible. It becomes noticeable above 300 keV, however the WIZARD patented crosstalk correction method eliminates this so that the useful range is extended up to 1 MeV.

Dynamic normalization adjusts windows each time it measures a sample.



The initial peak position, optimum window coverage etc. are stored in an isotope library. They are "fine tuned" during normalization, each detector separately.

SMOOTHING AND PEAK FINDING
Noise is removed by digital filtering and the peak position is determined.

PEAK COMPARISON
The peak position is compared with that determined during normalization and the window position is adjusted by the deviation factor.

COUNTS FOR CALCULATION
All pulses falling within the adjusted window are used for further calculations.

Fig. 3.12.1 Dynamic normalization principle.

Crosstalk correction is possible because the number of crosstalk sources (other detectors) is limited and does not change during the assay. Knowing the activity of the crosstalk source, and the constant crosstalk factor, allows crosstalk elimination. The method itself is explained in US patent 4,348,588.

This kind of crosstalk is dangerous until corrected for. Wallac is the only company legally entitled to use this patented crosstalk method in its counters.

3.14 Conveyor to detector crosstalk

This is caused by high energy samples in the vicinity of the detector system e.g. the samples on the conveyor table.

The crosstalk value is the total events measured by an empty detector when the sample is in a sample cassette, closest to the detector, expressed as a percentage of the total events measured by the detector when it measures the sample.

It is hard to correct by mathematical means in the single detector counter and virtually impossible in the multidetector counter. The best elimination is achieved by careful design. In the previous generation of automatic counters, such as the now discontinued 1277 GammaMaster, detectors were of the through hole type, as a staggered array, above the conveyor belts. There had to be a hole in the shielding lead to allow the sample to be transported into the detector. Crosstalk occurred mainly via this hole. The design of WIZARD is different and the detectors are shielded from the conveyor by a solid lead shield which is 30 mm thick in 1470 WIZARD and 77 mm in 1480 WIZARD. This provides effective shielding up to 1 MeV and 2 MeV, respectively.

Crosstalk figures for WIZARD are included in section 5 of this book.

Conveyor to detector crosstalk is very deceptive. This is because the individual crosstalk figure may be very low, let's say 0.05%, which is a practical value for multidetector counters of the conventional design, used in chromium counting. This may seem insignificant when compared with other sources of errors, encountered in gamma counting.

In practice, however, the user is not counting one sample in his/her chromium assay but hundreds. They ALL contribute crosstalk. Suppose, for example that the typical assay size is 200 tubes, each having an average activity of 5000 CPM, the total is then 1,000,000 CPM. The crosstalk figure is 1,000,000 CPM * 0.05 % = 500 CPM. There may even be more samples but with over 200 samples crosstalk starts to go down because the sample distance increases.

But there is still another point. The samples in the conveyor represent the AVERAGE activity, while the sample in the measurement position may represent MINIMUM activity, i . e. a sample close to the spontaneous release.

Suppose that the sample activity is 250 CPM, a realistic figure in practical assays. The user is not getting the correct answer of 250 CPM, but 250 CPM + 500 CPM = 750 CPM. There is 200 % error in the reported answer.

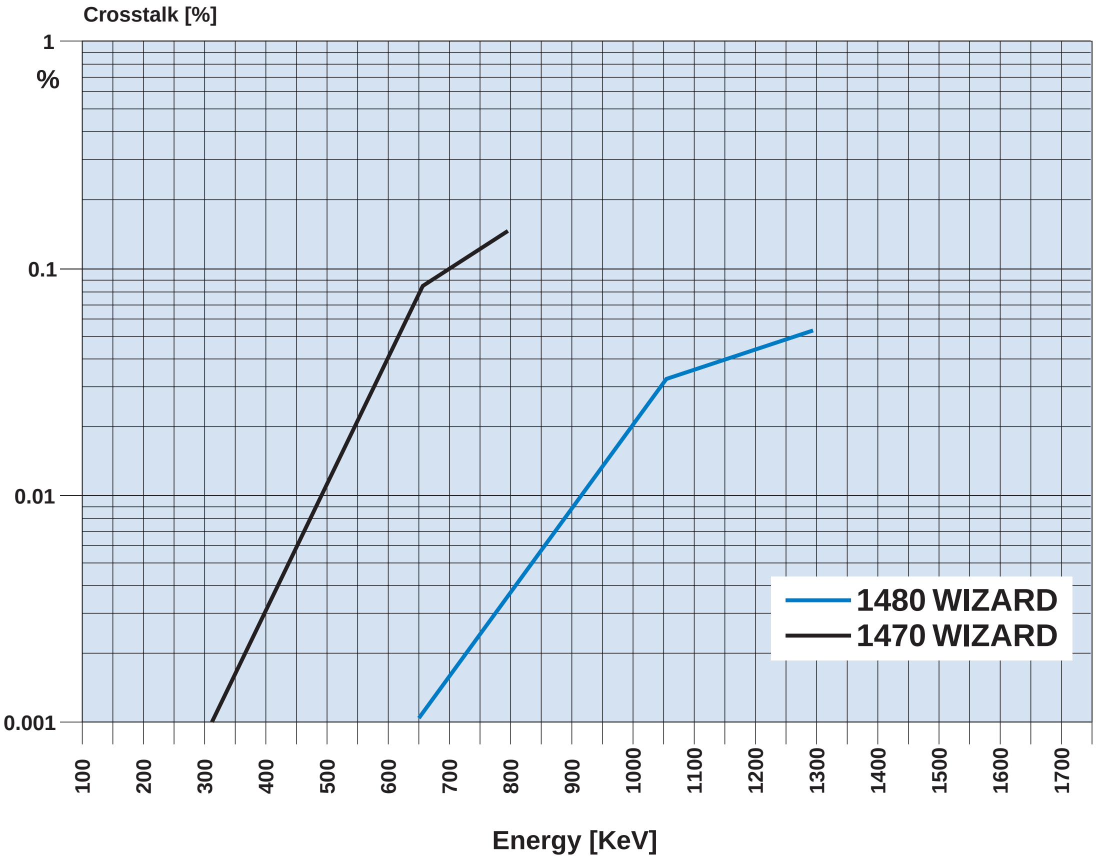
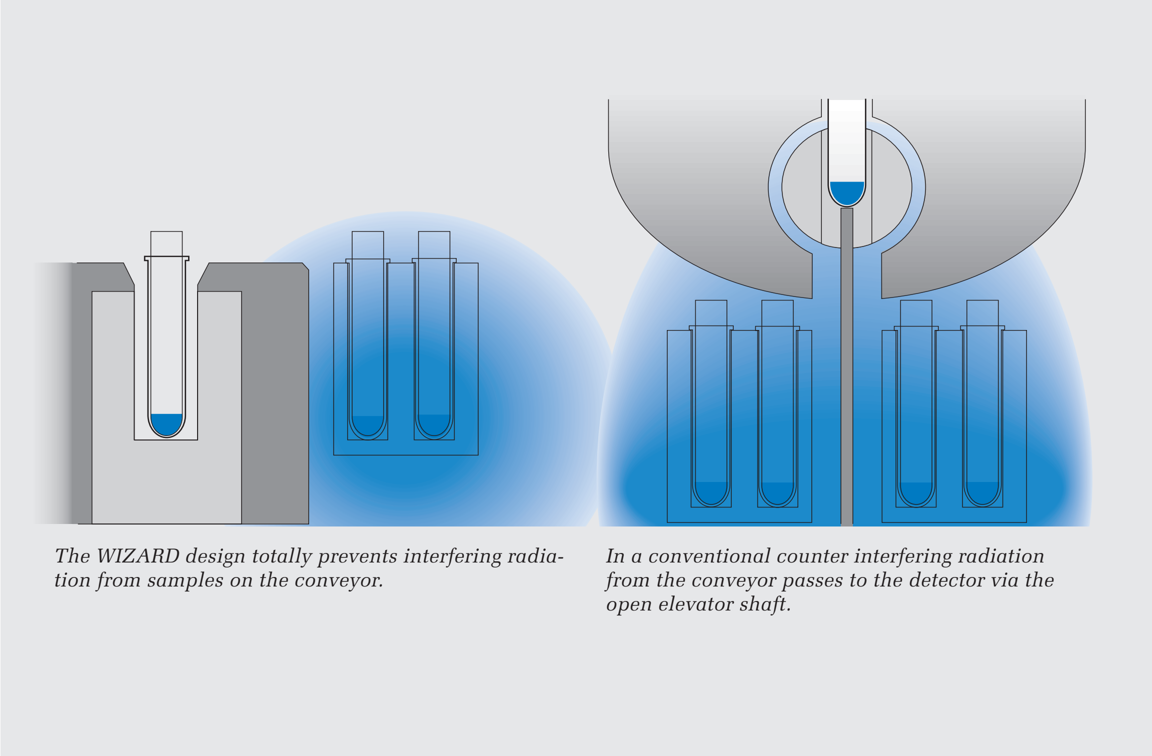


Fig. 3.14.1 Conveyor to detector crosstalk values.



The WIZARD design totally prevents interfering radiation from samples on the conveyor.

In a conventional counter interfering radiation from the conveyor passes to the detector via the open elevator shaft.

Fig. 3.14.2 Conveyor to detector crosstalk mechanism in different counter designs.

3.15 Spillover

Spillover (also known as "crossover") is another form of crosstalk in which counts in one counting window are registered in a neighbouring window. The problem occurs only if more than one isotope is measured at the same time i.e. multiple labelled assays.

The usual reason for spillover is that either counting windows are actually overlapping or that scattered peaks of the higher energy isotope cause counts in the lower energy isotope window.

The most common dual label application in RIA is the Vitamin B12 (^{57}Co)/Folate (^{125}I) assay. The Compton scattering of ^{57}Co causes a small spillover for the ^{125}I window, the uncorrected spillover is typically 1 .. 2 %.

Another example is the dual label Shilling test (^{57}Co/^{58}Co) in which the ^{58}Co secondary peaks in the ^{57}Co window are considerable.

An example of the case where windows are actually overlapping is ^{137}Cs and ^{134}Cs. The ^{137}Cs window is partially "inside" the ^{134}Cs window.

There are applications where ^{125}I is counted simultaneously with ^{131}I. In this case the windows are not overlapping but the spillover from the ^{131}I to the ^{125}I window is very big while the opposite spillover is practically zero.

WIZARD software compensates for the spillover effect in counting; the spillover factors are determined in the normalization. The matrix method, used in spillover correction, is purely a mathematical formula which corrects the spillover regardless of whether the windows are non-overlapping, overlapping or one is inside the other. However, it must be remembered that the correction is a mathematical method by which interfering counts can be cancelled but the statistical uncertainty remains. Suppose that the actual sample count rate is 1000 CPM (1 minute counting time) and the spillover from other nuclide(s) is 9000 CPM. WIZARD would report count rate 1000 CPM (average of a large number of repeat measurements) but we must realize that the standard deviation of the result is now 100 CPM, not 31.6 CPM as it would have been without the spillover. The more spillover increases, the less precise the answer becomes. The situation is critical if the activity ratio is large too, for example if the activity of nuclide "B" is 100 times that of nuclide "A" and spillover to "A" 20 %, the accuracy of the result is questionable.

There are some practical ways to minimize the spillover effect should it not be possible to use nuclides which do not cause much spillover to other windows.

– Do not use overlapping counting windows.

– As spill "down" is much more common than spill "up", select the activity ratios so that the low energy nuclides are more active than the high energy ones.

The spillover correction for dual label is mathematically analogous to the crosstalk correction in the case of a two detector instrument, using single label. <u>Because of this analogy simultaneous crosstalk/spillover correction can also be done</u>. (The matrix equation to be solved in 10 detector WIZARD, using dual label, consists of 400 factors i.e. 2*10 * 2*10 matrix). Spillover correction is not restricted to two isotopes; the multi-isotope software option for 1480 WIZARD is able to handle 20 simultaneous isotopes.

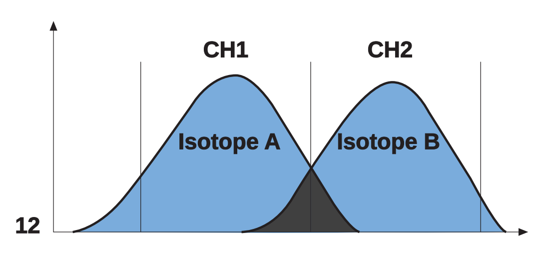


Fig. 3.15.1 Spillover in double label counting

3.16 High activity counting

The 1470 WIZARD counter is designed to work primarily in the clinical and research fields, which make use of moderate sample activities, up to 2,000,000 DPM. Higher activities can be measured but then we must keep in mind that the counting efficiency may start to decrease. This is due to two principal reasons:

– the “dead time” effect

– random coincidence

Dead time correction in 1480 WIZARD

Each pulse counting system possesses a certain “dead time” during which it is insensitive to the incoming pulse. The dead time becomes effective immediately after each detected event and it remains effective over a certain period. The dead time effect is corrected by software, the correction is based on the standard formula for extendable (paralysable) dead time:

$R_o = R_i (1 - e^{-R_iT_d})$,where:

R_i = mean input count rate [1/s]

T_d = dead time [$8.35*10^{-6}$ s] (including random coincidence of $0.3 * 10^{-6}$ s)

R_o = mean output count rate [1/s]

From this equation the input count rate (i.e. corrected count rate), Ri, can not be obtained without iterative computational procedures.

1480 WIZARD uses the formula:

$$R_i = R_o/(1 - R_oT_d - (R_oT_d)^2/2)$$

The accuracy of the formula is about 0.1 % for dead time losses of 10 % or less.

Random coincidence

If the counting pulses come very rapidly, the possibility that they cannot be distinguished increases. In this case there are not two separate counts but one in the spectrum position with double energy. If the counting window is narrow both pulses may be lost, in the case of a wide window only one pulse is lost. This effect is discussed more in section 3.21, it has practical effect only in multi-isotope work, in single label work the compensation is included in the dead time correction formula.

“Pile up”

If the count rates increase very much, the preamplifier starts to saturate, this results in a shift in the spectrum, see Fig. 3.21.1.

High activity mode

1480 WIZARD has a special mode for very high activity samples. When the mode is activated the counting efficiency is automatically reduced to as much as 25 % of normal. This allows counting of samples up to 30,000,000 DPM instead of the normal limit which is 8,000,000 CPM (10,000,000 DPM).

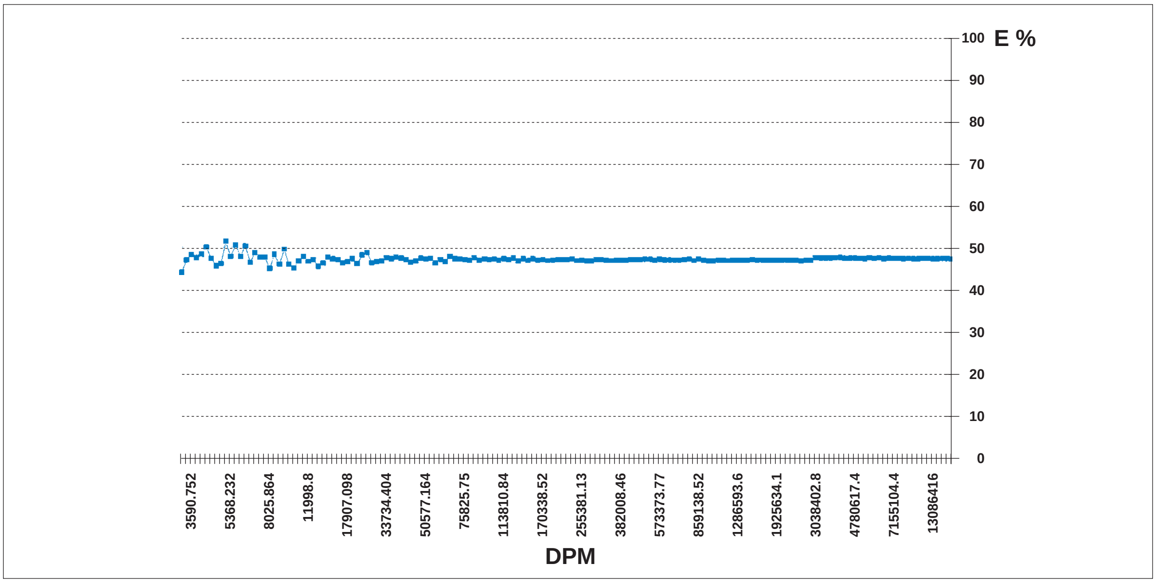


Fig. 3.16.2 Counting efficiency versus sample activity in 1480 WIZARD. ^{11}C positron source is measured in the time period of several half-lives, the original 13,000,000 DPM activity decayed to 3590 DPM. The "noise" in the left is statistical variation due to short counting time (30 sec).

3.17 Sample volume dependency

The volume dependency refers to the phenomenon that counting efficiency depends on the volume despite the fact that the activity of the sample stays constant. There are three principal causes of volume dependency:

- radiation is absorbed in the medium of the sample
- detection efficiency (light production) varies in different locations in the crystal (vertical sample position dependency)
- light collection from the different locations of the crystal into the PM tube photocathode varies (lateral sample position dependency & geometrical factor)

Self-absorption

If the sample volume is large the radiation is attenuated before it actually leaves the sample medium, this is known as self-absorption.The attenuation is caused by the photoelectric effect and as such varies approximately as a function of the atomic number of the element(s) in the medium. Self-absorption is a noticeable factor in the low energy region (particularly in ^{125}I) or also higher energy if the sample volume is very large.

Self-absorption has no effect in gamma counting in which the sample volume is small e.g. RIA or chromium release. It should be noticed that coated tubes do not suffer from self-absorption. The volume of coated surface is too small to attenuate gamma radiation. The Schilling test uses large volume (up to 20 mL) samples and the self-absorption is noticeable but because the standard volume is the same as the unknown volume the ratio is independent of the volume used.

Self-absorption must often be taken into consideration if one measures low energy nuclides in large volume environmental samples, particularly soil samples because these consist of minerals which may strongly attenuate the radiation. The effect is also noticeable if whole tissues are measured e.g. in MIA studies.

Self-absorption does not depend on the detector construction because the radiation is absorbed before it leaves the sample vial. There are some commercial papers claiming that some NaI(Tl) detector types are not volume dependent, e.g. “constant geometry counting”. If the claims are true these detectors detect the radiation which never enters the detector.

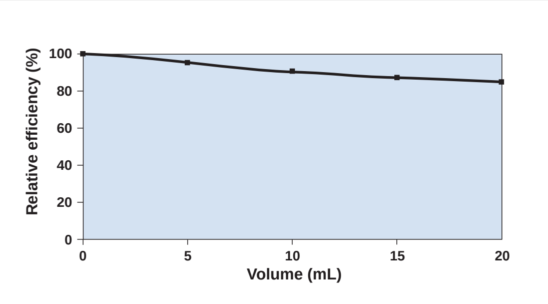


Fig. 3.17.1 Self-absorption of ^{125}I radiation in water. Measured in 20 mL plastic LSC vial.

Vol (mL)	Rel. eff. %
0	100.0
5	94.1
10	90.1
15	87.7
20	83.5

Vertical sample position dependency

Light production varies in different positions in the crystal, it is maximum in the center and lower in the corner areas, in which a larger proportion of gamma photons may escape the crystal before total absorption, as a form of Compton scattering. The effect depends on the crystal size and gamma photon energy and it may also result in change in the resolution, therefore the magnitude of the variation also depends on the window settings, a “wide” window is less sensitive than the one which only covers the photopeak.

Within a reasonable range (e.g. 0...20 mm in 1480 WIZARD) the position dependency is almost non-measurable in low energy nuclides, e.g. ^{125}I and ^{57}Co, due to the fact that the radiation is absorbed in a thin layer of the NaI(Tl), regardless the position. On the other hand self-absorption is large.

Both detector types, well-type and through-type suffer from small vertical position dependency when high energy samples are counted. Volume dependency depends on detector size, the longer the crystal, the more a sample can expand. For this reason 1470 WIZARD uses a detector which is 50 mm (2 inch) high. Conventional multidetector counters usually employ 37.5 mm high (1.5 in) through-hole detectors.

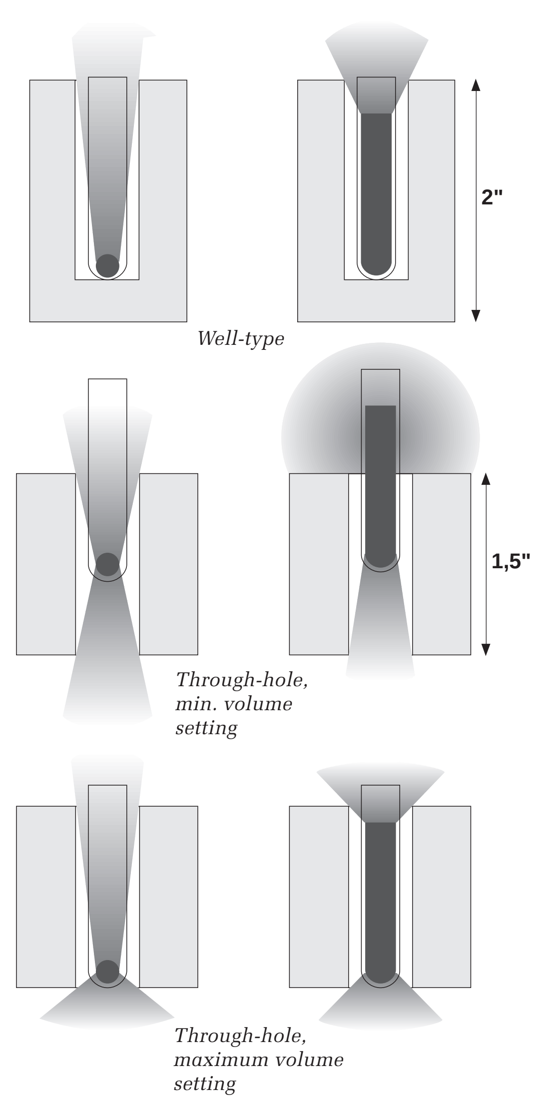


Fig. 3.17.2 Vertical position dependency mechanism in different detector types.

As a point of interest, some effort was used to make the Wallac 1277 GammaMaster volume dependence as small as possible e.g. as shown in Fig. 3.17.3. Because of the small 1.5 inch through detectors used the volume dependence is noticeable. The elevator had 4 positions so the sample could be centered in the detector.

The system compensated for the effect quite well if all the sample volumes were the same within the assay because the position could be set in advance. But in practice this feature was not really needed because under such a condition the unknown/standard ratio is constant anyway. The situation is totally different if there is a sample volume difference in the same assay. In this situation the centring system did not work in

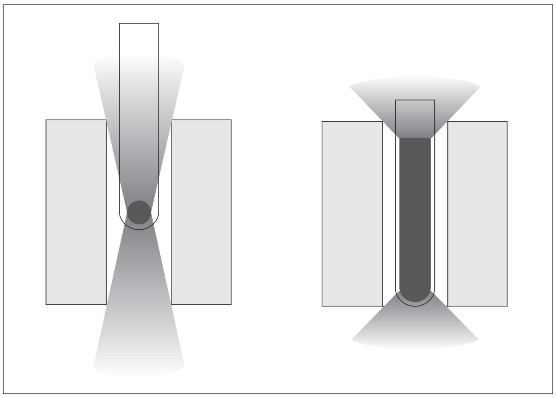


Fig. 3.17.3 Centered detector arrangement.

practice because:

– in order to centre the sample automatically the volume must be known (typed in before counting or measured somehow)

– each elevator must have an individual motor to set the sample at the optimum height within the same assay. This would require ten motors in a ten detector instrument.

If it were possible to build this kind instrument, it still could not give the constant counting geometry that Fig. 3.17.3 illustrates.

So the only solution to achieve constant geometry would have been the following one shown in Fig. 3.17.4:

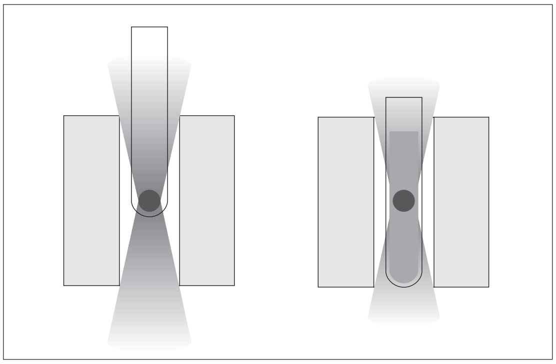


Fig. 3.17.4 Optical illusion of constant "geometry counting".

This is an impractical solution. When the volume is increased the radioactive substance does not stay as a point-like a source suspended in the middle of the increased liquid but is dissolved in the rest of the volume causing radiation as shown in Fig. 3.17.3.

The whole question is a little academic because in practice the position dependence is hardly measurable in low energy isotopes.

Fig. 3.17.5 shows the efficiency variation in 1480 WIZARD while a point like ^{125}I sample is measured at different heights. The sample height, 20 mm, corresponds to 10 mL when counted in a 20 mL LSC vial. The max. efficiency loss, 2 %, is about one tenth of the corresponding self-absorption.

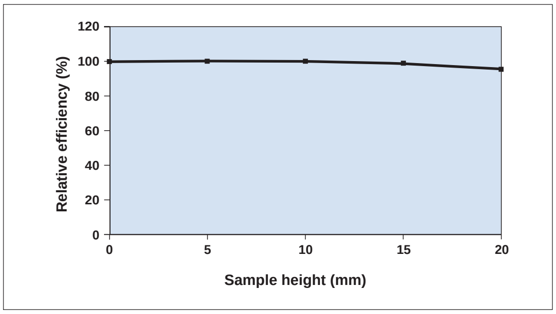


Fig. 3.17.5 Vertical position dependency in 1480 WIZARD 3". The isotope is ^{125}I.

Height (mm)	**Rel. eff. %**
0	**100.0**
5	**100.8**
10	**100.6**
15	**99.5**
20	**97.0**

Lateral sample position dependency

The detector may also be sensitive to the lateral sample position, efficiency is different depending on the side of the crystal (the side facing the crystal is better than the side opposite to it). This phenomenon may cause variations when measuring coated tubes and its severity is related to the crystal size. In the 1.5 * 1.5 inch through-hole detector, the light passage beside the hole is very narrow and the light generated in the side opposite the PM tube is not effectively collected; the lateral sample position dependency may be severe but manifest itself only in the case when (imperfectly) coated tubes are used.

This effect is not noticeable in well-type crystals due to the symmetrical detector design.

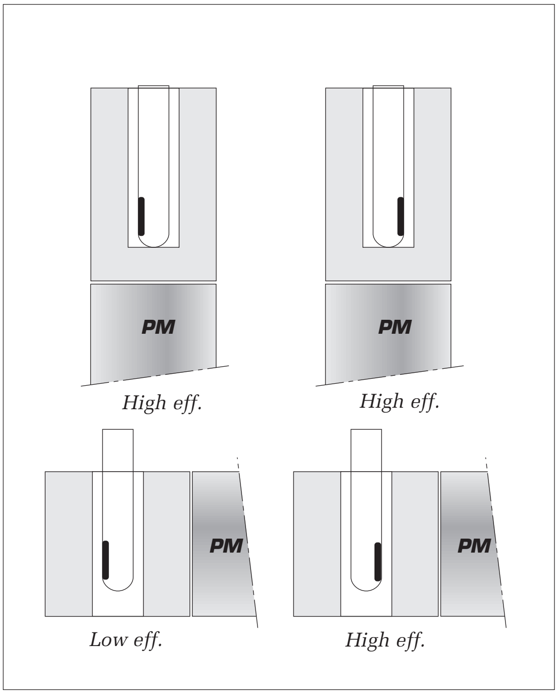


Fig. 3.17.6 Lateral position dependency.

As a general rule, when the isotope energy increases the self-absorption decreases and the position dependency increases, the sum effect, volume dependence, is rather constant. The total effect for ^{125}I and ^{51}Cr are shown in Fig. 3.17.7 and 3.17.8. Measurements are done in 1480 WIZARD 3" using a 20 ml LSC plastic vial. The counting windows are 15 keV ... 150 keV and 15 keV...450 keV, respectively. Volume dependency is 0.95 %/mL for ^{125}I and 0.82%/mL for ^{51}Cr.

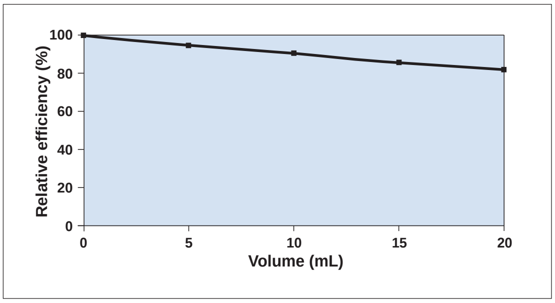


Fig. 3.17.7 Volume dependence of ^{125}I in 1480 WIZARD.

Vol (mL)	Rel. eff. %
0	100.0
5	94.9
10	90.6
15	87.3
20	81.0

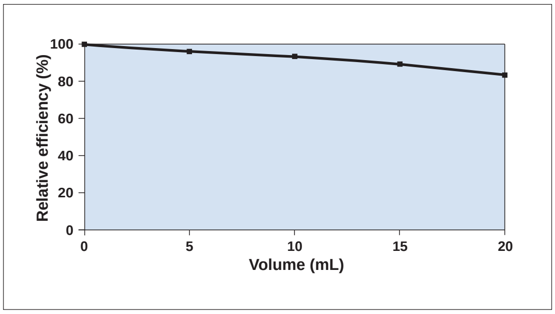


Fig. 3.17.8 Volume dependence of ^{51}Cr in 1480 WIZARD

Vol (mL)	Rel. eff. %
0	100.00
5	96.18
10	93.38
15	89.79
20	83.60

Volume dependency for ^{51}Cr is shown in Fig. 3.17.9. Measurements are done in 1470 WIZARD using a 13 mm plastic vial. The counting window is 15 keV..450 keV.

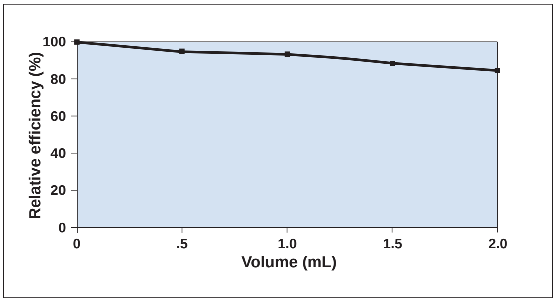


Fig. 3.17.9 Volume dependence of ^{51}Cr in 1470 WIZARD.

Vol (mL)	Rel. eff. %
0	100.0
.5	95.0
1.0	91.7
1.5	88.4
2.0	84.5

Heterogeneous samples

It may happen that the radioactive substance is not evenly dissolved in the buffer solution. Counting efficiency may vary because of any combination of the previously explained reasons. If possible, heterogeneous samples should be avoided in gamma counting because the effect is not correctable.

Reference

M.O. Oresegun, K. M. Decker and C.G. Sanderson, Determination of Self-Absorption Corrections by Computation in Routine Gamma-Ray Spectrometry for Typical Environmental Samples. Journal of Radioactivity & Radiochemistry 4(1), 38-45 (1993).

General reading on scintillation detectors

P. Schotanus, G. Stam, E. Gerritse, B. Utts, B. Briaux, Scintillation Detectors - Harshaw/QS, Saint-Gobain, 1992.

Patents

Tapio Yrjönen, Urpo Pietilä and Tim Rawlins, Method for Compensating Measuring Values when Mesuring the Radiation from a Number of Radioactive Samples in an Automatic Radiation Detecting Instrument. US Patent 4,348,588. Sep. 7, 1982.

Juhani Aalto and Seppo Wallenius, Changer Mechanism for Individual Measurement of Radioactive Samples in a Gamma Counter. US Patent 5,185,525. Feb. 9, 1993.

Juhani Aalto, Multipurpose Gamma Counter and Method for Sample Handling in Gamma Measurement. US Patent 5,194,733. Mar. 16, 1993.

Juhani Aalto and Olli Hakala, Transfer Device for Cassettes Containing Radioactive Samples in a Gamma Counter and Cassette System. US Patent 5,268,574. Dec. 7, 1993.

3.18 Decay correction

The activity of any radionuclide decreases at a known rate. If the decay rate is short compared with the assay counting rate a correction must be applied. The WIZARD correction routine eliminates the effect and prints the results as if all the results were counted at the same time. In order to do this the half-life value of the isotope must be in the instrument memory. If the isotope is already in the WIZARD library, the half-life is already set in the instrument memory. If the half-life is short, e.g. 6 hours as with ^{99m}Tc the sample activity may decay during the normalization process, causing a systematic error between detectors. This is also corrected in 1470 WIZARD.

The traditional formula to calculate a CPM decay corrected to the value at the reference time is given in formula (1). However, in order to calculate the CPM value one must measure the activity in a certain period of time. The result is imprecise if the sample decays considerably during the measurement period. This does not usually take place (when counting times are in minutes and half-lives in days) but in specific isotopes (e.g. ^{15}O, half-life 120 seconds) the phenomenon becomes noticeable. Because of this WIZARD uses a more precise formula (2). Note that these give the same result when $\Delta t \ll (t1 - t0)$.

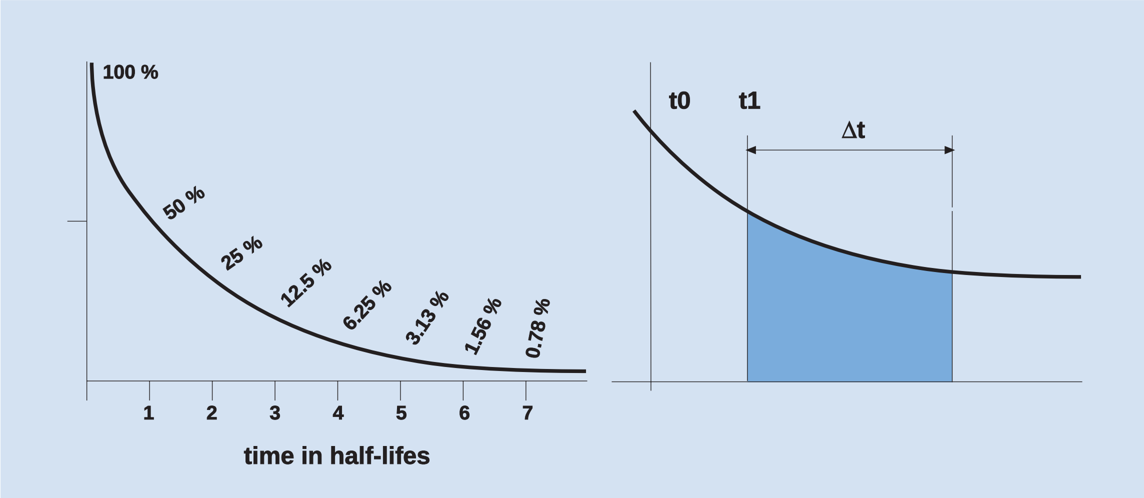


Fig. 3.18.1 Relation between half-life and radioactivity.

Decay correction

(formula 1)

$$\mathrm{CPM}\ (t0) = \mathrm{CPM} \cdot e^{(t1-t0)\lambda}$$

(formula 2)

$$\mathrm{CPM}\ (t0) = \mathrm{CPM} \cdot \frac{\lambda \cdot \Delta t \cdot e^{\lambda (t1-t0)}}{1 - e^{-\lambda \Delta t}}$$

t1 = the time when measurement started
Δt = the measurement time
λ = decay constant
t0 = reference time (given time or assay start)

$$\lambda = \frac{\ln 2}{T_{\frac{1}{2}}}$$

$T_{\frac{1}{2}}$ = half life

3.19 Calculation methods.

The registered counts in a counting region (window) are printed out as “COUNTS”.
These values do not include background subtraction or any other correction. The corrections, as explained in the preceding sections, are applied and the final counts per minute values are printed out as “CPM”. The following corrections are applied in WIZARD:

1) dead time correction

2) matrix correction, including:

- background correction (if background normalization done)
- crosstalk correction (selected in 1470 if energy > 200 keV)
- spillover correction (dual label assays)
- efficiency correction (not needed in single detector models)

3) decay correction (if selected)

The corrections are made in the order given above.

The CPM values shown in the live display are uncorrected.

The matrix correction may be quite complicated. A ten detector instrument, performing simultaneous spillover and crosstalk correction may need to solve an equation of 400 elements (20 * 20). Because the scope is too wide for this presentation a we refer you to “Calculation methods in WIZARD” included in the Instrument Manual.

In addition to these two outputs (four in dual label mode) a number of other parameters can be selected, the total number of fixed output columns is 46 in the built-in RiaCalc WIZ program. Because new output fields are freely programmable the number of possible outputs is without a set limit.

3.20 Contamination

Contamination is a serious matter in any laboratory measuring radioactive material. A contaminated instrument is a useless instrument. Essentially, contamination may occur for two reasons:

1) A sample tube is contaminated on the outside by a radioactive substance in the pipetting process

2) A glass sample tube is broken during sample transfer, maybe due to the bad quality of the tube.

The consequences in a conventional through-hole counter may be serious; an elevator tip, counter weight, rack or a conveyor table or any combination of these may became contaminated. If the decontamination procedure fails the instrument is useless.

The unique design of WIZARD prevents the sample from touching anything in the counter, except the disposable holder in the rack. If the holder is revealed to be radioactive in the test run, it can be changed in a few seconds.
Since a sample vial does not touch other parts, except the holder, the counter parts do not get contaminated.

Because the sample does not touch the sample changer mechanism during transfer, the quality of the tube does not affect the reliability of the sample changer in any way and there is no danger of breaking glass vials.

It is good laboratory practice to count all racks for contamination. The process is fully automatic and can be carried out overnight, contaminated holders are flagged. To screen 100 racks (1000 holders) takes half an hour, when counted in a 10 detector WIZARD. A separate “open” isotope setting is provided for this purpose.

3.21 Random coincidence

As the radioactive decay is a random process there is always the possibility that two or more decays occur so close in time that the detection process cannot separate them. The result is a single pulse with height being the sum of the individual pulses. This is consequently registered in the wrong counting window, which leads to errors in the measurement. WIZARD coincidence probability can be calculated using the formula:

$R_c = R_o\,(1 - e^{-R_oT_d})$,where:
R_o = count rate [1/s]
Td = coincidence time [~ $3*10^{-7}$ s]
R_c = coincidence count rate [1/s]

This phenomenon must be kept in mind when working with multi-isotope assays (MIA), and count rates must be kept to a moderate level. The maximum count rate (total of all nuclides) should not exceed 1,000,000 CPM.

Note that random (chance) coincidence is not the same as "true" coincidence caused by the same nuclear decay event which emits multiple cascade gamma photons. True coincidence is discussed in section 3.4. The practical consequence is that random coincidence depends on the count rate but true coincidence does not.

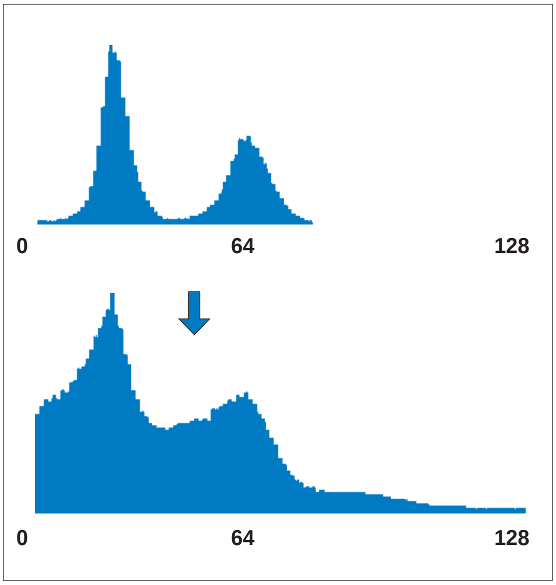


Fig. 3.21.1 Random coincidence effect in 1480 WIZARD. An additional 90 keV peak (30 keV + true coincidence peak in 60 keV) appears in high activity samples. The ^{125}I sample activity is 16,000,000 DPM, more than twice the recommended upper limit. The normal (1,000,000 DPM) sample is the upper spectrum.

3.22 Power failure recovery

In the case of power failure, the instrument is normally stopped and must be manually started. The samples measured in the uncompleted assays are lost. There are 3 principal ways to handle power failures, each have their relative merits and drawbacks.

They are:

– battery supplied CPU

– automatic restart

– uninterrupted power supply (UPS)

Battery supplied CPU

This supplies current to the volatile memory (RAM) during power failure. In the case of power failure the CPU is reset and the actual counter operations stop. When the power comes back, a special power failure routine starts the measurement again. However it is very difficult to write "foolproof" power failure software because a power failure may occur at any point in the program execution, including graphics printing etc. Because power failure also resets the printer, error free graphics printing is almost impossible, something must be done twice, which corrupts the printout. This technique was used in the previous generation of counters i.e. in LKB WALLAC 1272 CliniGamma.

Automatic restart

The microcomputer is not powered during power failure. When the power comes back the conveyor is rotated until the same ID is found which was being processed when the power failure occurred. There is a small battery powered memory in the instrument which keeps track of the instrument status, so the counting conditions are known by the power failure recovery program. This method is far more safe than the previous one but the drawback is that measurements take more time because the incomplete assay is recounted. This is hardly a problem at night because the whole process is automatic. WIZARD employs this design.

Uninterrupted power supply

The instrument is powered during power failure by an external device, so counting goes on as usual. This is the best method but it adds cost. It can be used with any gamma counter.

4 Typical radionuclides and applications

4.1 ^{125}I (RIA)

^{125}I is by far the most commonly used radionuclide today. It is favoured because of its low energy, good labelling characteristics and the length of its half-life. A half-life of 60 days allows reasonable shelf lives in reagents but, on the other hand, is short enough to ease waste disposal problems. Waste is practically non-radioactive after 2 years of storage.

The vast majority of commercial RIA and IRMA kits utilizes ^{125}I as a label. Rarely, kits for certain steroid hormones involve the use of a beta label i.e. tritium.

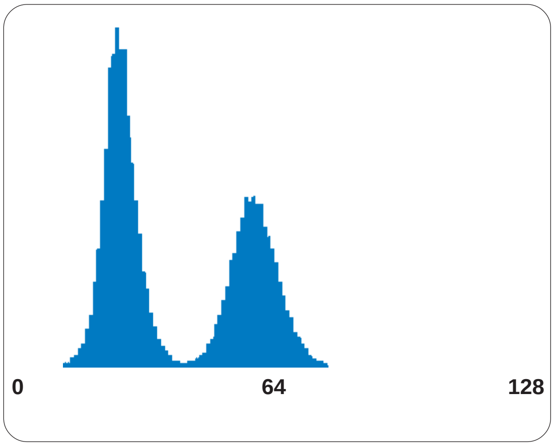


Fig. 4.1.1 Spectrum of the ^{125}I has the main photopeak at approx. 29 keV and the coincidence peak at 60 keV. Counted in 1470 WIZARD; the spectrum in 1480 is virtually identical.

^{125}I, having very low energy, does not cause any crosstalk. However, most commercial RIA kits have usually some traces of other nuclides in them, most often ^{131}I, which has a relatively high energy. The crosstalk from this may cause Compton scattering, which has such an energy that it falls into the ^{125}I window. In case of very large activity ratios (like those found in the very sensitive IRMA kits) it may cause noticeable effects.

4.2 Other iodine nuclides (RIA, etc.)

^{131}I is used sometimes in RIA, instead of ^{125}I. It is also used *in vivo* (in cancer treatment) and simultaneous determination of ^{125}I and ^{131}I is not uncommon. The ^{131}I spillover into the ^{125}I window is considerable; nevertheless it is easily corrected for. The ^{131}I principal energy is 364 keV but there are other peaks at 636 keV (7.2 % of total) and 722 keV (1.8 % of total).

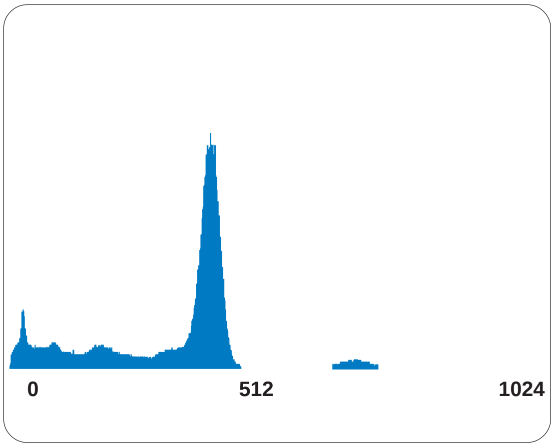


Fig. 4.2.1 Spectrum of the ^{131}I counted in 1470 WIZARD.

^{123}I is a short half-life (13.3 h) alternative to ^{125}I. It has principal energies at 27 keV and 159 keV, in addition there are coincidence peaks (27 keV + 27 keV and 159 keV + 27 keV).

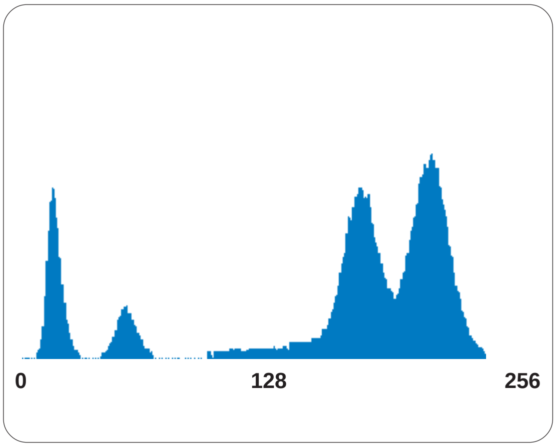


Fig. 4.2.2 Spectrum of the ^{123}I counted in 1480 WIZARD.

4.3 ^{57}Co

^{57}Co is used to investigate Vitamin B12 deficiency. A B12 molecule is prepared in which a normal cobalt atom is replaced by a radioactive ^{57}Co nuclide. The Vitamin B12 RIA assay is often combined with a Folate assay using ^{125}I. This is the most commonly used dual label RIA. ^{57}Co has a reasonably low energy, 122 keV. With this, no crosstalk problems will occur with any Wallac gamma counter.

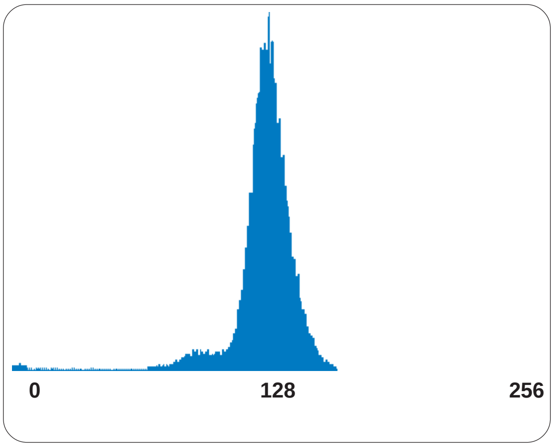


Fig. 4.3.1 Spectrum of ^{57}Co has the main photopeak at 122 keV. Counted in 1480 WIZARD; the spectrum in 1470 WIZARD is virtually identical.

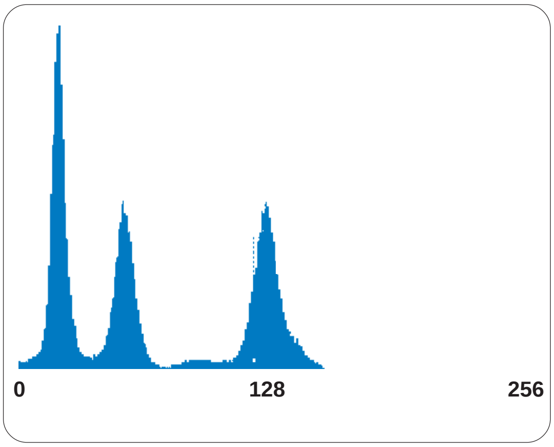


Fig. 4.3.2 Dual label ^{125}I/^{57}Co counted in 1480 WIZARD.

4.4 ^{51}Cr

^{51}Cr is another commonly used radionuclide in the laboratory. As the name implies, chromium release studies involve the measurement of radioactive ^{51}Cr which, after being artificially introduced into a cell, is released when the cell is ruptured as a result of an attack by a specialized cell of the immune system. ^{51}Cr is a beta emitter but 9.85 % of decay produce also a gamma photon with an energy of 322 keV. The CPM values involved are quite low, usually in the range of few hundred up to 5000 CPM but because the energy level is quite high background or crosstalk from other samples may disturb readings unless adequate lead shielding is provided in the counter. ^{51}Cr can be measured with all Wallac gamma counters employing well-type detectors.

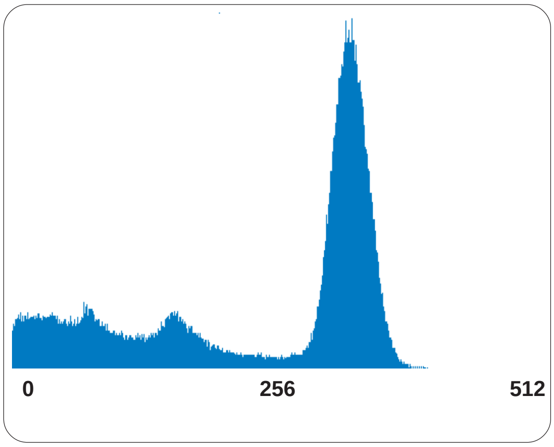


Fig. 4.4.1 ^{51}Cr has the main photopeak at 322 keV. The spectrum was measured in 1470 WIZARD.

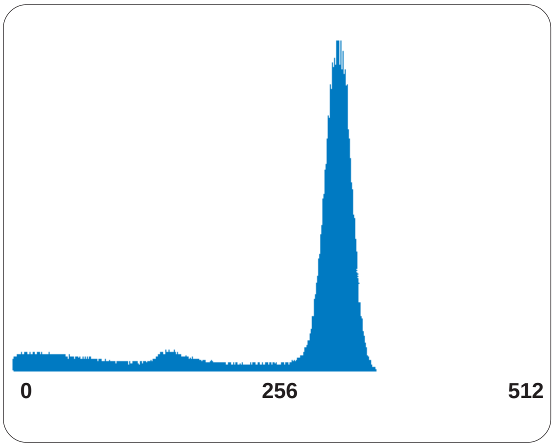


Fig. 4.4.2 ^{51}Cr measured in 1480 WIZARD.

4.5 ^{58}Co (The Amersham Dicopac test)

^{58}Co is used together with ^{57}Co to investigate Vitamin B12 malabsorption due to either pernicious anaemia or defective intestinal absorption. The test, called the Shilling test, relies on the use of the two different labels to achieve differential diagnosis based on the two forms of the vitamin B12 molecule. ^{58}Co has a reasonably high energy, 820 keV, which means that crosstalk can easily occur. 1470 WIZARD may very well be the only multidetector gamma counter which can perform the test. Multidetector counters with through hole detectors suffer crosstalk problems even more than in the case of ^{51}Cr, to such a decree that the manufacturers typically recommend that only one detector is used. In a ten detector model this reduces throughput more than 90 % when we remember that the counting efficiency is already low (17 %) compared with the 23 % of 1470 WIZARD. Even for 1470 WIZARD the test is demanding because of the low count activity and high energy. However, the simultaneous spillover/crosstalk correction and the 30 mm of lead between the conveyor and the detectors mean that here we have a multidetector counter which need not be changed to a single detector counter for this particular application. 1480 WIZARD 3" is a truly ideal counter for this test because, apart from being totally crosstalk free, it can count 20 mL of sample increasing the count rate therefore minimizing the statistical error.

References

The Amersham Dicopac test using 1470 WIZARD

The Amersham Dicopac test using 1480 WIZARD

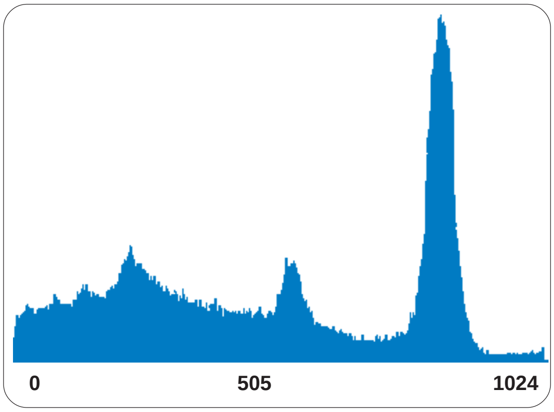


Fig. 4.5.1 ^{58}Co nuclide measured in 1480 WIZARD. It has the main photopeak at 820 keV. The spectrum in 1470 WIZARD is shown in chapter 3.4.

4.6 ^{59}Fe

^{59}Fe is used in haematology testing, the iron atom in a haemoglobin molecule is replaced by radioactive ^{59}Fe. This has a high energy, 1 – 1.2 MeV, which means that crosstalk problems inevitably occur in ordinary gamma counters, especially ones using through hole detectors. The specified, worst case, crosstalk figure in 1480 WIZARD is less than 0.05 % for a single sample.

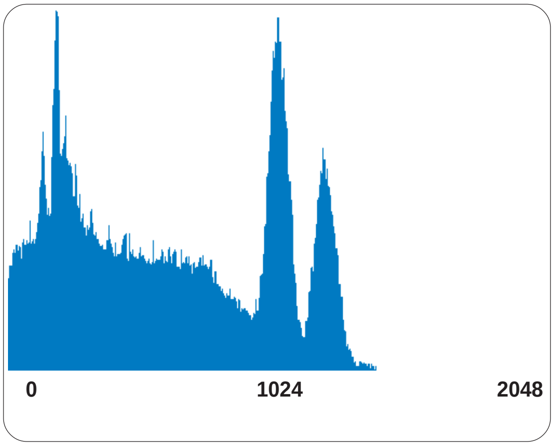


Fig. 4.6.1 ^{59}Fe measured in 1480 WIZARD has two main peaks at 1.1 MeV and 1.29 MeV.

4.7 ^{22}Na

^{22}Na shows gamma emission from a nuclear transition and positron annihilation.

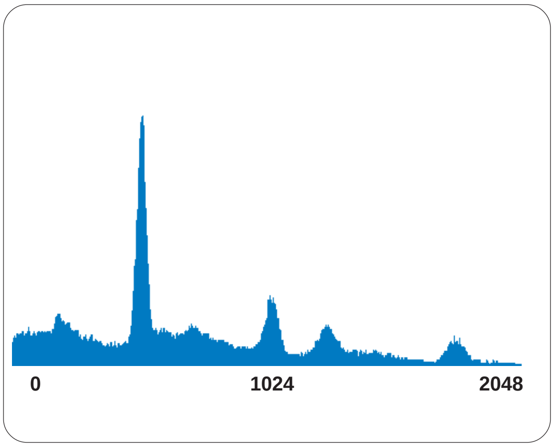


Fig. 4.7.1 ^{22}Na as measured in 1480. In addition to the principal peaks in 511 keV and 1.27 MeV there are coincidence peaks at 1.02 MeV and 1.78 MeV.

4.8 ^{111}In

^{111}In is used e.g. in the radiolabelling of a variety of separated blood cells. It has 3 principal peaks: 171 keV, 245 keV and 416 keV (coincidence peak is 171keV + 245 keV).

In addition to this there are 23 keV X-rays which sum with the other peaks in such a way that the result is as shown in the spectrum below.

The default library settings cover the range 150 keV ... 500 keV which includes the 171 keV and 245 keV principal and coincidence peaks.

Crosstalk is very low in both WIZARD models.

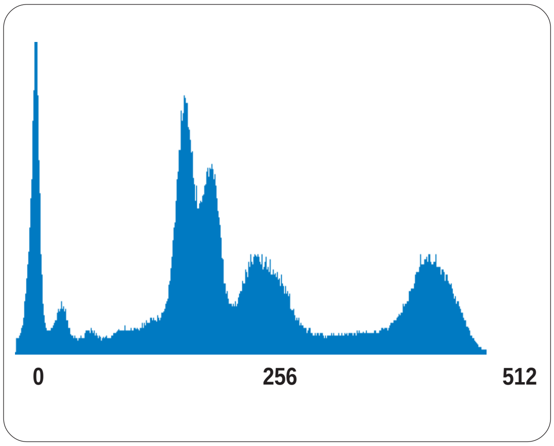


Fig. 4.8.1 In-111, measured in 1470 WIZARD.

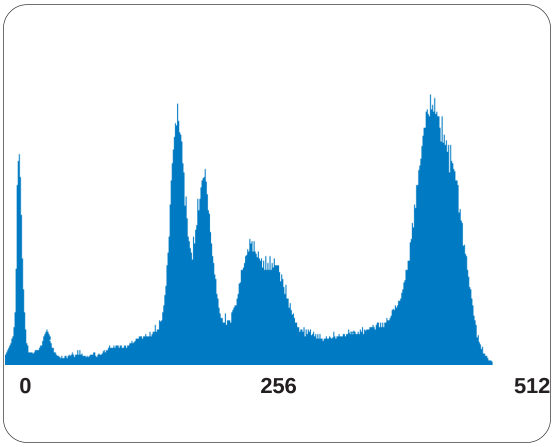


Fig. 4.8.2 In-111, measured in 1480 WIZARD.

4.9 ^{137}Cs and ^{134}Cs (Environmental studies)

^{137}Cs and ^{134}Cs levels are monitored in soil, plants (e.g. mushrooms) animals, fish, food products etc.. Activities are normally very low, therefore the low background figure and high volume are essential in making 1480 WIZARD the ideal choice for this application.

The ^{137}Cs principal energy is 662 keV while ^{134}Cs has two peaks, 604 keV and 795 keV. They have strongly overlapping counting windows; in order to separate the isotopes, the spillover correction program must be used.

Perhaps the most important criterion in this application is minimum detectable concentration, MDC). In 1480 WIZARD this figure is less than 20 Bq/L. The exact value depends on the environment, since the background varies locally.

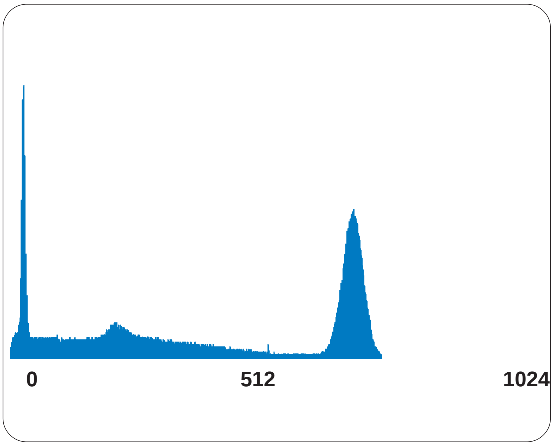


Fig. 4.9.1 ^{137}Cs, measured in 1480 WIZARD.

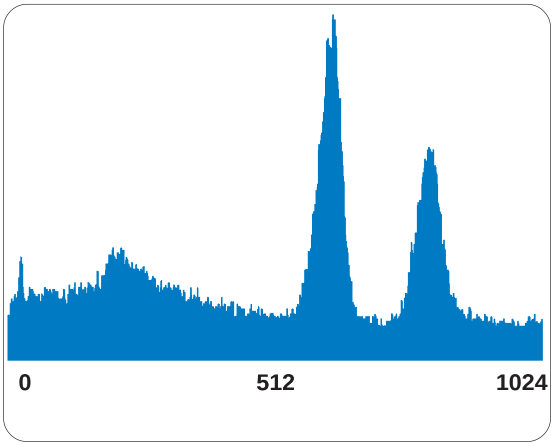


Fig. 4.9.2 ^{134}Cs, measured in 1480 WIZARD.

4.10 Positron sources (PET)

Positron Emission Tomography (PET) is used for investigating metabolism of various substances labelled with positron emitting nuclides. Positron sources are injected into the blood circulation of the animal or human body e.g. in the form of glucose solution. The emitted positrons almost immediately collide with the electrons. The particles annihilate each other and the excess energy is emitted as two gamma photons, having an energy of 511 keV.

The gamma photons are emitted in exactly opposite directions, and therefore any coincidence detection means that the positron source must be in the line from one detector element to another. By using a circular array of detectors a two dimensional picture can be obtained from the positron source.

It is important to know the actual activity over the time during which the PET picture is collected. For this purpose a series of blood samples (between 10 and 20) are taken, usually from the patient's artery. After measuring the exact sample volume, these are counted in a gamma counter. The positron sources, such as ^{15}O, ^{11}C or ^{18}F, decay fast and therefore accuracy of the decay correction is essential (see chapter "Decay correction"). The correction of the WIZARD is not always sufficient because a number of samples can be taken and the final data evaluation is carried out somewhere else (e.g. in LAN). For this purpose WIZARD can label each measurement with the absolute time (within a second). By keeping the WIZARD and LAN clock synchronized, the needed decay correction of the counting in WIZARD with respect to the PET measurement can be made.

Because the samples handled in a PET laboratory are typically very active (a subject may receive a dose of up to 40 mCi or 90,000,000,000 DPM), even as low crosstalk as 0.0001 % may cause totally false results. A good shielding is essential to ensure a stable background. 1480 WIZARD shielding is more than adequate for radiation at 511 keV. While the thickness itself, 75 mm, is a requirement in achieving the necessary attenuation, far more important is the shape. Any opening, such as the elevator hole required in the through hole detector systems, would easily break the shielding protection and cause crosstalk hundreds of times larger than acceptable. In this respect a radiation shield is roughly analogous with a kettle having water in it. The purpose of the former is keep the radiation out, the latter to keep water in. In the latter task the kettle made of thick cast iron is no better than the one made of thin aluminum if the former has a hole on the bottom.

All positron sources used in PET give similar spectra which consist of the main photopeak at 511 keV and the coincidence peak at 1022 keV. As was the case with ^{125}I the ratio of main peak height to coincidence peak height depends on the counting efficiency. ^{68}Ge is used as a calibrator because of its relatively long half life, 271 days. The library settings for the positron sources (^{15}O, ^{11}C, ^{18}F, ^{13}N and ^{68}Ge) in WIZARD differ only in their half live settings because otherwise the gamma spectra are identical.

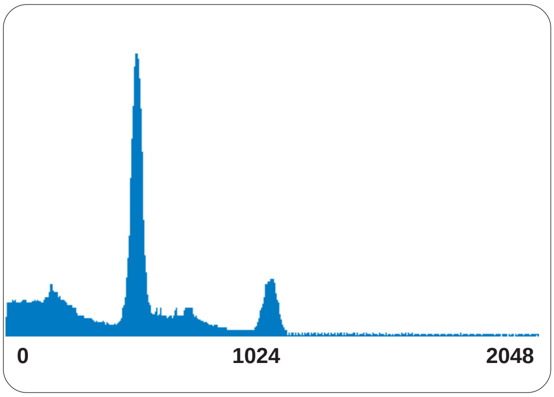


Fig. 4.10.1 ^{18}F measured in 1480 WIZARD.

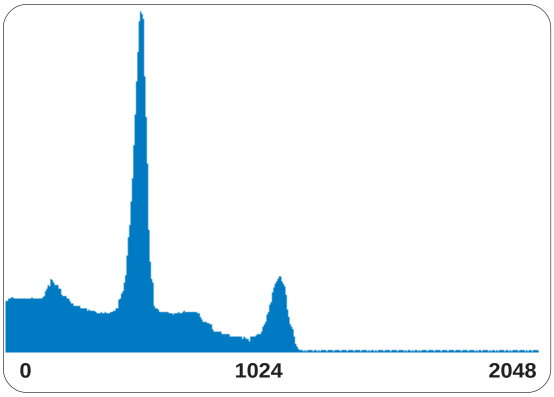


Fig. 4.10.2 ^{11}C measured in 1480 WIZARD. The spectra is similar to ^{18}F (above) and other positron sources.

4.11 Multinuclide studies

The usual reason to employ more than 2 nuclides simultaneously is to carry out blood flow measurements. In this technique, microspheres of different sizes are injected into the bloodstream of the test animal. Different sizes are labelled with different nuclides. The activity of the sample for a certain nuclide is related to the veins' ability to hold the microspheres of this particular size.

1480 WIZARD is particularly suitable for this kind of work because of the very good resolution of the well type detector (typically 8 % for ^{137}Cs) and its very low crosstalk from conveyor, which also allows the use of high energy nuclides thus making possible the use of a wide energy range.

The key factor in the work is good spillover correction. The MIA option software package for 1480 WIZARD performs spillover correction for up to 20 simultaneous nuclides as described in US patent 4,348,588.

Even though WIZARD software can distinguish between 20 nuclides, actually counting more than 2 is not always straightforward. The more nuclides used, the less sensitive the measurement becomes. There are two factors which limit the number of nuclides, or the precision of the reported result. These are spillover and random coincidence.

Spillover correction is discussed in section 3.15 and random coincidence in section 3.21

The practice has shown that WIZARD is well able to separate 8 simultaneous nuclides. Using more than 8, it becomes harder to find suitable nuclides which either do not decay too fast or cause too much spillover.

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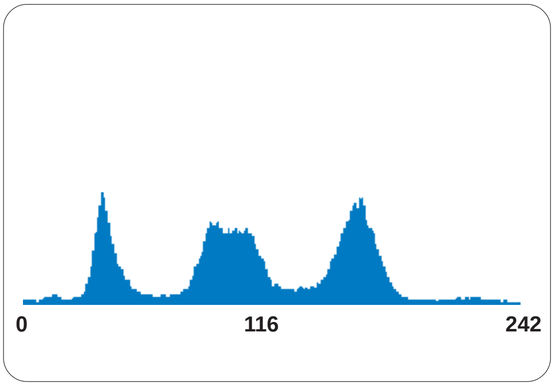
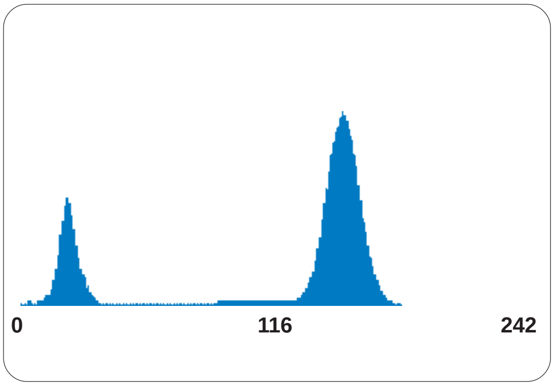
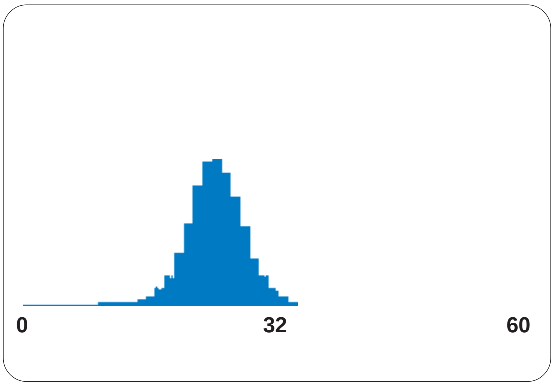
Fig. 4.11.1 Nuclides used as microspheres labels



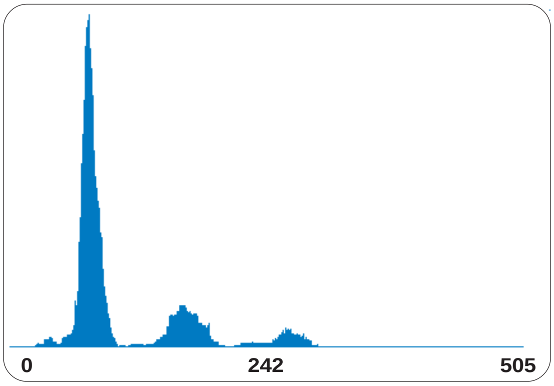
^{153}Gd



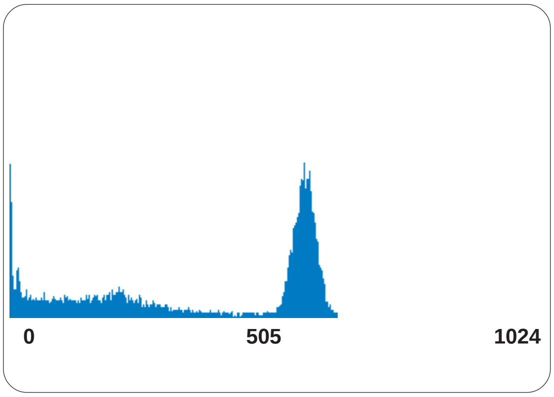
^{141}Ce



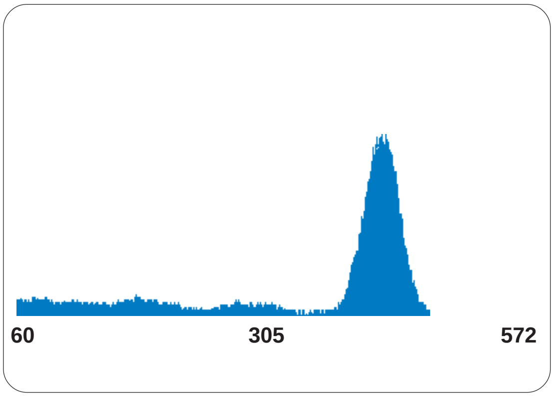
^{109}Cd



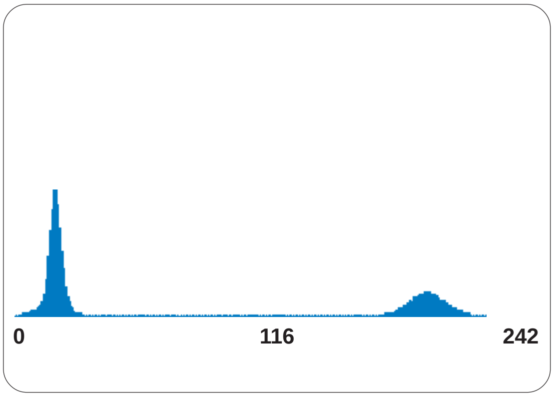
^{201}Tl



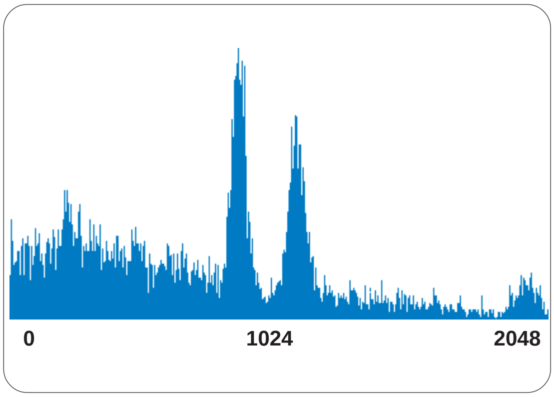
^{85}Sr



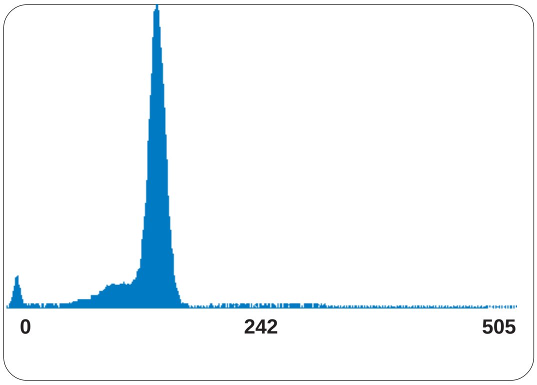
^{113}Sn



^{114}In



^{46}Sc



^{99m}Tc is also widely used in imaging in nuclear medicine

4.12 ^{222}Rn, ^{226}Ra

Radon in air can be measured with a gamma counter, because of its daughter products' emissions: ^{214}Pb at 242, 295 and 352 keV, ^{214}Bi at 609 keV and several at higher than 1 MeV. Collection of radon can take place using a charcoal canister, which is left open in the room for 4 days at the most and then sent to the laboratory. The method can be calibrated against a known radon concentration in a radon chamber. An open window can be used in the measurement.

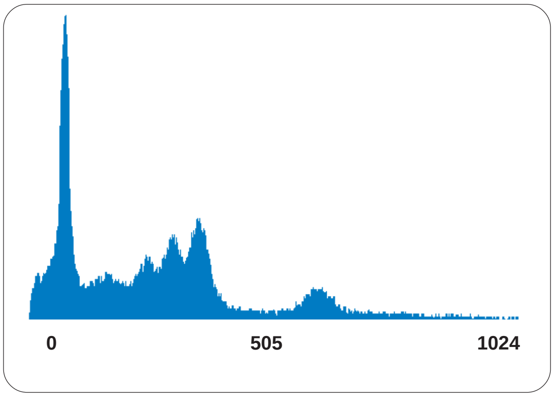


Fig. 4.12.1 ^{222}Rn spectrum from a charcoal canister exposed to air. Low energy peak is Pb Ka X-rays at 82 keV.

Recommendations specify that radon concentration in drinking water should not exceed 200 Bq/L. Measurement in 1480 WIZARD limits the sample size to 20 mL in direct counting of radon. Water samples can be concentrated for ^{226}Ra measurements by evaporation which allows correspondingly larger sample volume.

References
Radon Monitoring, Note 1, EG&G Ortec, Nr. 3809 0289
Radon Monitoring, Note 2, EG&G Ortec, Nr. 3874 5M 0288

5 The radionuclides

The radionuclides listed below can be measured with 1470 WIZARD.

ID	Nuclide	Energy (Kev)	Eff. (%)	Half-life (hours)	Cov. (%)	Low W. (Kev)	High W. (Kev)	Res (%)	Cx (%)	Cx (%)	Cx tot. (%)
			Note 1						Note 2	Note 3	Note 4
1	I-125	29	82	1445	97			24	0	0	0
2	Co-57	122	90	6480	92			12	0	0.02	0.002
3	Cr-51	320	3.7	667	80			9	0.001	0.35	0.03
4	I-129	31	65	1.49+11	96				0	0	0
5	As-76	559	7	26.4	31				0.08	1.8	0.24
6	Au-195	99	75	4390	95				0	0	0
7	Au-198	412	11	64.7	47				0.04	0.8	0.11
8	Ba-133	356	16	6.30E+4	54				0.002	0.5	0.05
9	Ba-139	166	76	1.38	87				0.1	0.01	0.02
10	Br-77	245	11	57	74				0.2	0.02	0.04
11	Ca-47	1297	38	109		1000	1500				
12	Cd-109	22	71	11136		16	32				
13	Ce-141	145	56	780		125	167				
14	Co-58	810	23	1711		180	950		0.16	4.1	0.53
15	Co-60	1332	14	4.62E+4		1060	1450				
16	Cs-134	795		18063		500	890				
17	Cs-137	662	26	2.63E+5	25			8	0.09	3.6	0.41
18	Er-171	308	13	7.52	62			9	0.3	0.03	0.06
19	F-18	511	28	1.83		200	1500	9	0.06	1.5	0.19
20	Fe-59	1292	14	1071		1020	1400				
21	Ga-67	185	70	78	85			10	0.15	0.015	0.03
22	Gd-153	147	100	5808		26	167				
23	Hg-203	279	31	1126	68			10	0.3	0.03	
24	I-123	159	80	13.3	88			11	0.1	0.01	0.02
25	I-131	360	15	193	54			9	0.03	0.7	0.09
26	In-111	245	52	67.7	74			10	0.3	0.02	
27	In-114m	190	30	1188	88	166	210				
28	K-42	1525	7	12.4		1200	1800				
29	K-43	373	14	22.6	52			9	0.02	0.5	0.06
30	Na-22	511	51	2.27E+4	37			9	0.14	3.7	0.47
31	Nb-95	766	15	841		686	846				
32	Pb-203	279	31	52.1	68			10	0.3	0.03	
33	Rb-86	1077	6	448		800	1300				
34	Ru-103	497	15	944		400	600				
35	Sb-125	428	10	2.37E+4	45				0.04	0.9	0.12
36	Sc-46	1098	10	2011.2		990	1200				
37	Sc-47	160	80	82.1	88			8	0.1	0.01	
38	Se-75	265	31	2880	75			10			
39	Sm-153	103	86	47	93			14	0		
40	Sn-113	392	22	2760		350	430				
41	Sr-85	514	8	1530	36			9	0.06	1.5	
42	Sr-87m	388	12	2.8	50			9	0.03	0.6	0.06
43	Tc-99m	140	86	6	90			12	0.07	0.01	0.08
44	Open					0	1024				
46	Ge-68	511	28	6504		20	1800		0.06	1.5	0.19
47	C-11	511		3.41E-1		20	1800		0.06	1.5	0.19
48	O-15	511		3.40E-2		20	1800		0.06	1.5	0.19
49	N-13	511		1.655E-1		20	1800				
50	Tl-201	70		73.06		60	90				

Note 1 Eff = CPM/DPM *100 %, typical values, open window. Efficiency inludes transition probability

Note 2 Crosstalk from conveyor

Note 3 Crostalk from detector (uncorrected)

Note 4 Total, corrected crosstalk from single sample

The radionuclides listed below can be measured with 1480 WIZARD.

ID	Nuclide	Energy [keV]	Eff. [%}	Half live [hours]	Coverage [%]	Low. W [keV]	High W. [keV]	Res. [%]	Crosstalk [%]
1	I-125	29	82	1445	97			25	<0.0001
2	Co-57	122	90	6480	92			13	<0.0001
3	Cr-51	320	7	667	80			9	<0.0001
4	I-129	31	65	1.49E+11	96			24	<0.0001
5	As-76	559	21	26.4	31				
6	Au-195	99	100	4390	95				
7	Au-198	412	39	64.7	47				
8	Ba-133	356	55	6.30E+04	54				
9	Ba-139	166	89	1.38	87				
10	Br-77	245	21	57	74				
11	Ca-47	1297	76	109		1000	1500		
12	Cd-109	22	71	11136		16	32		
13	Ce-141	145	56	780		125	167		
14	Co-58	810	65	1711		737	883		
15	Co-60	1332	28	4.621E4.		1060	1450	13	0.06
16	Cs-134	795	30	18063					
17	Cs-137	662	47	2.63E+05	25			8	0.001
18	Er-171	308	26	7.52	62				
19	F-18	511	48	1.83		453	567		0.0002
20	Fe-59	1292	28	1071		1020	1400	13	0.035
21	Ga-67	185	89	78	85				
22	Gd-153	147	100	5808		26	167		
23	Hg-203	279	70	1126	68				
24	I-123	159	89	13.3	88				
25	I-131	360	43	193	54			10	<0.0001
26	In-111	245	83	67.7	74				<0.0001
27	In-114m	190	42	1188		166	210		
28	K-42	1525	15	12.4		1200	1800		
29	K-43	373	42	22.6	52				
30	Na-22	511	89	2.27E+04	37				
31	Nb-95	766	30	841		686	846		
32	Pb-203	279	69	52.1	68				
33	Rb-86	1077	11	448		800	1300		
34	Ru-103	497	30	944		400	600		
35	Sb-125	428	37	2.37E+04	45				
36	Sc-46	1098	20	2011.2		990	1200		0.06
37	Sc-47	160	89	82.1	88				
38	Se-75	265	72	2880	75				
39	Sm-153	103	86	47	93				
40	Sn-113	392	43	2760		350	430		
41	Sr-85	514	25	1530		445	580		
42	Sr-87m	388	40	2.8		345	431		
43	Tc-99m	140	89	6	90				<0.0001
44	Open					0	1024		
45	Open					0	2048		
46	Ge-68	511	48	6504		20	1800		
47	C-11	511		3.41E-1		20	1800		
48	O-15	511		3.40E-2		20	1800		
49	N-13	511		1.655E-1		20	1800		
50	Tl-201	70		73.06		60	90		

Note Eff = CPM/DPM *100 %, typical values, open window.
Efficiency inludes transition probability

6 Data handling

6.1 Data reduction

Most gamma counters on the market have some sort of data reduction feature, which means that they are able to compute at least CPM values from registered counts. Since the most common gamma application has traditionally been radioimmunoassay, RIA, the data reduction is frequently RIA oriented. The data reduction facility implies that a counter can calculate RIA concentration automatically. This feature was introduced to gamma counting in the LKB Wallac RackGamma in 1976. Today data reduction software is more sophisticated and may include various quality assurance programs as well.

There are 3 principal approaches to data reduction:

- a counter produces CPM results.
- a counter has built-in RIA software.
- a counter produces only raw or intermediate data (CPM values or counts) and RIA evaluation, QC etc. is carried out in a separate computer.

WIZARD provides the possibility to use any of these three.

A CPM instrument

Evaluation, if needed, is carried out manually or on a separate computer using third party software, very often based on commercial spreadsheet software such as Excel or Lotus. This is the most simple solution, only a printer is needed in addition to the counter.

RIA program in built-in PC

A RIA program can also be installed in a PC located inside an instrument. However the use of a RIA program, including quality control as well, requires a lot of interactive operation, i.e. the use of display and keyboard. Conventional counters available are ergonomically very awkward to use; the long distance between a keyboard (under the counter) and a VDU (above the counter which is quite high) makes normal workstation type operation impossible.

In this configuration it is not possible to connect different kinds of instruments (LSCs, luminometers, fluorometers etc.), on line, to allow the same evaluation and QC system for all.

An advantage of the RiaCalc WIZ option for an internal PC is that it saves costs when compared with an external PC and MultiCalc. If a laboratory only needs concentration results on paper or transferred to mainframe on line, including perhaps the need for quality control, the logical choice is RiaCalc WIZ.

This configuration consists of a printer and RiaCalc WIZ option in addition to WIZARD.

Data evaluation in an external PC (MultiCalc)

The MultiCalc software in a separate workstation is more powerful and flexible and can be upgraded easily as new PC models became available. It is open to the use of other software packages, such as spreadsheet programs (e.g. in research applications) and patient data bases (e.g. in clinical routine). It also supports LAN functions and mainframe connection. One MultiCalc PC can control up to 9 devices in addition to connection to a LAN or a mainframe. It is the ideal choice for a large clinical lab in which data handling is automated as far as possible.

6.2 Data output

WIZARD outputs counting data in 5 independent ways:

- To the VDU
- To a printer
- To the PC port
- To the MF (mainframe) port
- To a floppy disk

The outputs, which are standard features of WIZARD, not options, are independent of each other. Printer, PC and MF ports are RS 232C compatible and the data format is according to the ASCII standard. Transmission parameters can be selected.

If MultiCalc is used, it communicates with WIZARD using the PC port, other ports are not needed. However it is still possible to use these and the system may e.g. have two printers connected, one for normal results, the other for STAT results.

6.3 Data transfer

It is more and more common that the results produced by any laboratory instrument need to be transferred to various data systems directly, without the medium of paper. There are many ways to do this but two systems dominate, using a floppy disk or "on-line" connection.

Data transfer by a data disk

This technique is common in research applications. WIZARD saves the results on a floppy disk, which is consequently read in by a suitable program, such as Excel or Lotus, in a separate PC. The software to further process the data is invariably made by the researcher who carried out the experiments. Spreadsheet, illustration and desktop publishing software may be used on the same PC so that the results may end up as a publisher- ready manuscript, without any need for paper printout, except in the final publication.

WIZARD produces results in .TXT type ASCII files, which all modern spreadsheet programs areable to import. Spectra can be imported into a separate spectrum analysis program, as the one from Wallac, 1411-301 or 1411-302. Spectrum output in a format is also available which is compatible with EG&G MicroMCB gamma spectroscopy software in Windows.

The typical configuration is shown in Fig. 6.3.1

Data transfer with a mainframe computer on-line

The term "mainframe" in this text is actually a general term which may refer to a number of data systems, but it is normally a central computer to which a number of data terminals are connected.

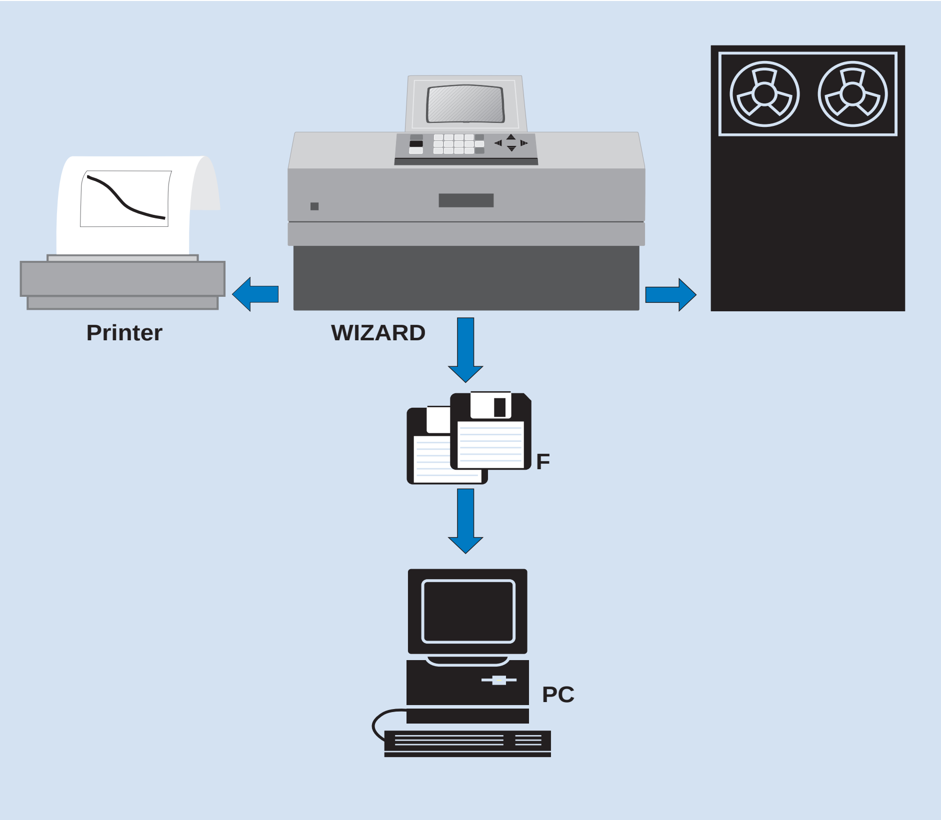


Fig. 6.3.1 Data transfer by a data disk.

Data transfer needs can be divided into two categories:

- where output data, CPM results or concentrations, are sent to a mainframe but the mainframe does not send instructions to the counter; this can be called "one direction data transfer"

- where output data, CPM results or concentrations are sent to a mainframe and the mainframe sends instructions (a worklist) to the counter; this can be called "bidirectional data transfer"

As far as a counter is concerned the requirements of these two options are quite different.

One way data transfer with a mainframe

This is a classical way and was a general requirement in the early days of MultiCalc. If a mainframe is connected with a LAN, the connection can be done via this, otherwise the RS-232 line is a suitable solution. The mainframe can be connected straight into the WIZARD MF port and WIZARD sends the results automatically to the mainframe as ASCII data with fixed format. This necessitates the MF having suitable communication and interpretation software.

A mainframe can also be connected using MultiCalc. MultiCalc offers more sophisticated data transfer facilities, such as error checking, re-sending and programmable format of the results file (decimals, calculation, flagging etc.).

Bidirectional data transfer with a mainframe

This feature is available only via MultiCalc. Data transfer is based on the exchanging of files between a MultiCalc PC and a mainframe via the RS-232 line.

There are alternative communication modes which are used, a typical one is the "Kermit" type of communication. Kermit is a public domain software which is often used in data transfer and is available in most mainframe computers. Other forms of communication can be used, the requirements in practice are that error checking and re-sending features are provided.

The usual arrangement is such that a mainframe sends a file which is essentially a patient list with information about the needed tests and how these should be carried out. This file is called a worklist and it includes e.g. the following:

- patient name and/or code
- test type
- sample structure (replication, dilution etc.)
- patient specific data (age, sex, weight, etc.).

MultiCalc sends the required counting protocols to WIZARD and prints the tube map which shows how test tubes must be loaded on the counter. It may also guide a pipetting station.

After the assay is counted, MultiCalc appends the concentration results to the worklist and gives it a new name, the "results file". This results file MultiCalc sends back to the MF, either automatically or after a supervisor's acceptance.

If the mainframe is connected to a LAN it is faster and safer to arrange data transfer using LAN services, see the next section.

6.4 Local area networks (LANs)

Networking, in which data is moved to an another computer of the laboratory or into a local area network (LAN) "on line" is a preferable choice in a modern clinical lab. As the subject is large and often complex, the following is a brief introduction.

Understanding the LAN

In essence a local area network, LAN for short, has only one basic function: to move information among shared computers on the network (servers) and the PC workstations of the network users. Usually this information is in the form of files. In addition to this the servers typically share various hardware, e.g. printers among the users.

The components of a network can be divided into two categories: hardware (computers, communication boards, cables etc.) and software i.e. the network operating system.

Fig 6.4.1. illustrates the hardware components of a basic network. The hardware can be categorized into three major groups: servers, workstations and the cables and boards that handle communication between them.

Servers are computers on a network that can be accessed by network users. By far the most common type is the file server, every LAN using a LAN operating system has at least one. A file server is an ordinary computer with one or more hard disks, LAN interface card(s) and a network operating system. Because multiple users access the file server it is typically of large capacity. The server computer has typically a higher performance than the workstations.

Network users can store and retrieve files from file servers as if the users were using their own disk drives. Sharing programs and data becomes possible and the network operating systems also provide means to set up private areas for individual users.

In addition to file servers, other shared resources can be called servers. A PC that houses a modem is called a modem server. A PC that has a data terminal emulator program and is connected to a mainframe is a server; it is sometimes called a gateway and it gives every workstation in the LAN access to the mainframe.

Servers can be dedicated, in this case a PC is used only as a (file) server and nothing else. A non-dedicated server can be used as a server and a workstation at the same time. Non-dedicated servers are used only in small LANs, they are not particularly stable because the individual user can interfere with the LAN operation and other users' work.

Connecting a PC to a LAN does not much alter the way the PC is used; a LAN connected PC merely has more places from which it can retrieve files. The PC still uses its normal operating system but in addition to this the LAN uses a special operating system, such as NetWare.

When a computer is connected to a LAN, a special board is installed in each PC and the board is connected to the server PC with a cable. The board, which enables high speed data transfer between computers, is called a network adapter or network interface board. Most LANs are capable of communication speeds from 1 million to 16 million bits per second. In comparison, an RS-232 line can only transmit data up to 9,600 bits per second under most circumstances.

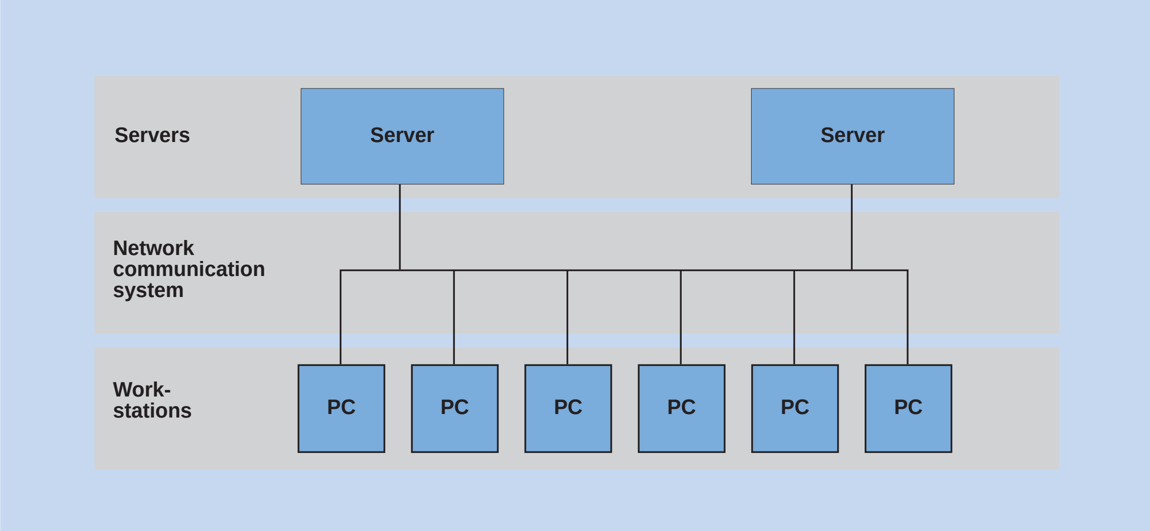


Fig. 6.4.1. The components of a network.

There are many different types of LAN cabling systems and interface cards: EtherNet, Token Ring and ARCNet are commonly used types of cabling in a network. Even these are divided: there are twisted pair and coaxial (both IBM type and Apple Macintosh) type Ethernet cards available.

Just as a PC needs an operating system (MS-DOS, OS/2) to work, the LAN needs an operating system too. NetWare is one of the common operating systems for PC networks.

Files are sent between the servers and workstations as data packages, defined in various communication protocols. These vary too; TCP/IP, IPX/SPX, Appletalk are the ones used. As a LAN operating system is useless without a communication protocol it typically includes at least one, e.g. NetWare includes IPX/SPX even though it can use other protocols too.

As can be seen, LANs vary in terms of the type of cable used, operating system and communicating protocol but as far as the workstation user is concern they are used in an identical way.

Because a cable must connect every computer on a network, there is a limit to the distance a network can span (the “local area network”). However, two networks can be joined; a router is a PC that has two or more network interface boards inside, one for each network that is being joined. A modem server in a LAN can bridge a network to any other LAN having a modem server too.

6.5 Connecting WIZARD to a LAN

Almost all MS-DOS, Windows and OS/2 programs can be loaded from LAN and the files can be saved on a server. Any PC software, whether designed for a LAN or not, can exchange files with a LAN, one simply uses the commands COPY *.* F: (LAN directories typically start with F:) or use the equivalent Windows command.

Most software packages that are run in a LAN are actually adaptations of single user programs. However, certain programs are designed to be used specifically in a LAN environment, a typical example is electronic mail.

WIZARD is connected to a LAN using a workstation PC and suitable software. The software may be MultiCalc, because it offers other functions as well, such as data evaluation, QC and GLP. MultiCalc runs in a PC (386, 486, or Pentium) and data transfer between MultiCalc and a LAN is more or less automatic when the PC has a LAN interface.

As far as MultiCalc is concerned the type of LAN is irrelevant. If the LAN operating system fully supports MS-DOS system calls (and all LANs in a PC environment do) the operation of MultiCalc in different LANs is identical.

MultiCalc is also designed so that certain LAN features can be utilized. The most common usage is that two or more MultiCalc users can share the same files (QC, standard curves, etc.), assay protocols and related data as well as worklists and result files.

Reference

Using Novell NetWare, 1990 by Que Corporation L.C.C. No.:89-62439, ISBN 0-88022-466-5

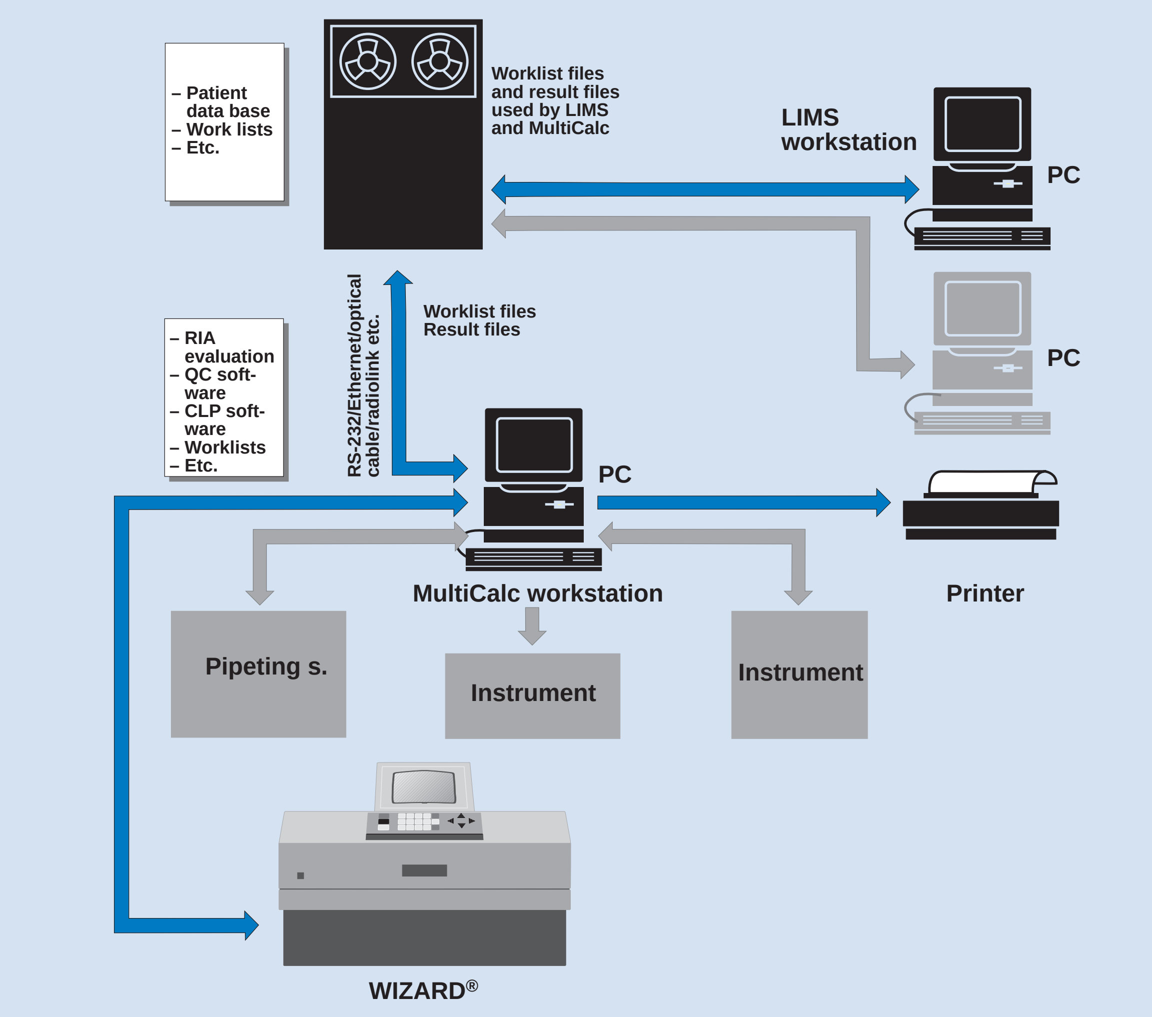


Fig. 6.5.1. WIZARD connected to a mainframe. Connection to LAN is similar, instead of a mainframe there is a LAN server

7 Quality assurance and GLP

7.1 General Guidelines

Good Laboratory Practice, GLP, is not a law but a way to operate, its exact interpretation depends on the country in question and it may vary from laboratory to laboratory. In some countries there are statutes for GLP, in others they do not exist or have not been enacted yet. For the reason that GLP requirements may vary so much from place to place and because they are not exactly defined in all countries, no detailed guidelines are given here. What follows is simply a description of the methods and features which WIZARD offers and how these can be best utilized. We leave it to the reader to judge how well our current design serves in achieving compliance with GLP regulations. An example of a country where statutes exist is the U.S.A. GLP is guided there by the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). Wallac has specifically taken care that the WIZARD works in accordance with CLIA'88 as a minimum standard. In fact, CLIA'88 requires only a small part of the total QC features WIZARD offers.

A modern gamma counter must offer as comprehensive a performance check as possible and, at the same time, the labour required must be kept to a minimum. And, in order to prove that results are correct and meet GLP requirements, the instrument must provide documentation. In order to provide documentation it must be decided what parameters to monitor and document. Obviously these parameters which may effect results are a focus of primary interest. The typical error sources as well as the best way to follow the parameters which detect the errors must be identified. Better still, we should establish parameters to predict the error or reduction in accuracy, if this occurs over a long period.

The error sources

A scientist working in the life sciences utilizes gamma isotopes as labels. The primary focus of interest is not the radioactivity itself but the determination of an analyte concentration or the scale of a physical phenomenon (e.g. blood flow in microsphere studies) it manifests. The relation linking radioactivity and analyte concentration is often non-linear and errors may originate from many sources. The most probable ones are:

- Experimental errors, associated with sample chemistry or preparation, perhaps because of incorrect incubation time or temperature, leading to low accuracy. The errors are already in a sample being measured.

- Measuring error are generated in the counting process. These errors are already in a given CPM value employed in RIA evaluation.

- Calculation error, generated in the RIA evaluation process, often a result of an inaccurate representation between the activity and the concentration.

The errors may be systematic (accuracy is low) or random (precision is low).

Modern GLP software should be able to detect an error, identify the source and even predict the likely future problems. How different errors are monitored in WIZARD and MultiCalc is explained in the next section.

7.2 Retrospective and real-time assay QC

Quality control can be divided into the following main categories
- within sample QC
- within assay QC
- between assay QC
- between laboratory QC
- between different methods QC

The task of a comprehensive QC program is to assist in making the following types of decisions:

- Reject/accept single standard measurement
- Reject/accept standardization
- Reject/accept single unknown measurement
- Reject/accept one unknown sample
- Reject/accept a part of the assay
- Reject/accept the whole assay

As can be seen the task is not easy and a good QC program may save a lot of labour in automating the drudgery of collecting, compiling, processing and presenting QC data.

Control plots

The basic tool for following assay accuracy is to utilize control samples among patient samples. MultiCalc allows practically unlimited numbers of control samples in up to 6 levels, and these can be followed on a day to day basis. In a routine lab, the controls may have the same structure (e.g. number of replicates) as patient samples but MultiCalc does not prevent the use of other options. All structures are allowed and the quantity to follow need not be concentration.

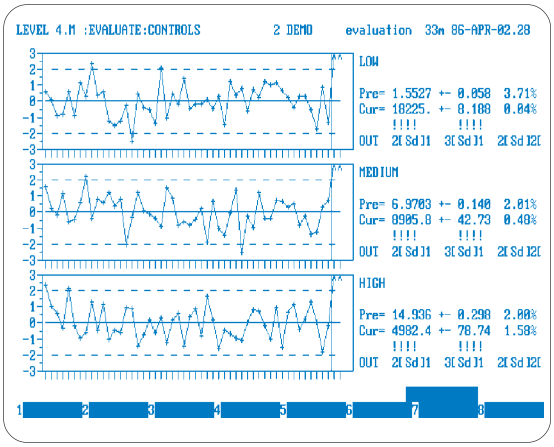
The classical method of QC employs the Levy-Jennings and Cusum charts to assist the user in finding out-of-control situations. In order to decide whether there is actually an out-of-control situation, some kind of rule must be used. Simple rules, such as, a control is out of limit if 2.5 standard deviations is exceeded, are sometimes practised. The disadvantage of a single rule is that either the warning levels are too wide, allowing bad results to slip through, or they are too tight, resulting in too frequent out-of-control situations.

MultiCalc utilizes a programmable multi-rule system. This allows the user to write practically any rule, using various individual criteria connected with logical operations. An example is the Westgard rule.

This rule is commonly used and a default setting in MultiCalc but nothing prevents the user from modifying the rule to better suit his needs. The Westgard Multi-Rule written in MultiCalc "language" is as follows:

10: 2 [Sd]1 & 3[Sd]1 2[Sd]2 4[Ra] | 1[Sd]4 | 10 [Me]

The Westgard Multi-Rule is a good, labour saving system but it does require that the software be sufficiently flexible. The control sample identification in an assay and appending to a database must be completely automatic. The software must accept control samples in any order among other samples even when the order changes daily. It is also an added benefit if the software can create QC plots in out-of-control situations. This gives the user an immediate visual indication of what may be wrong, in addition to the printed violation of the rule(s).



```
LEVEL 4.M :EVALUATE:CONTROLS          2 DEMO     evaluation  33m 86-APR-02.28

LOW
Pre= 1.5527 +- 0.058  3.71%
Cur= 18225. +- 8.188  0.04%
      !!!!      !!!!
OUT  2[Sd]1   3[Sd]1   2[Sd]2[

MEDIUM
Pre= 6.9703 +- 0.140  2.01%
Cur= 8905.8 +- 42.73  0.48%
      !!!!      !!!!
OUT  2[Sd]1   3[Sd]1   2[Sd]2[

HIGH
Pre= 14.936 +- 0.298  2.00%
Cur= 4982.4 +- 78.74  1.58%
      !!!!      !!!!
OUT  2[Sd]1   3[Sd]1   2[Sd]2[
```

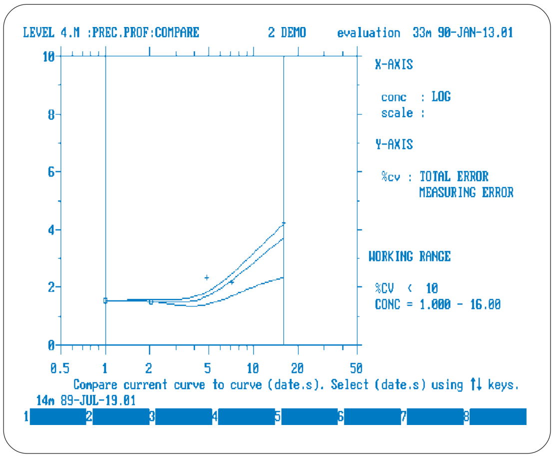
Fig. 7.2.1 Control plots and QC Multi-Rule violations

In addition to control samples, a similar analysis can be applied to the various parameters of the standard (dose-response) curve. However, the assay related parameters must be treated in a different way to the control samples. This is because changes in the kit (due to ageing, for example) will require the user to change target and range values frequently. If a parameter changes, the opportunity to compare the current standard curve with a previous one (curve overlay) may give valuable clues as to what may be wrong.

Precision profile analysis

Sample preparation (pipetting) may result in low precision. When the average volume is exact, the variation may be large. Sample preparation can be monitored by using replicate samples and a precision profile. This concept, which was first suggested by Dr. Roger Ekins, has proved to be a very valuable tool when monitoring assay precision. The precision profile follows all random errors, including also counting error which depends on the sample activity and the counting time.

The precision profile was included in the LKB Wallac 1270 RackGamma in the seventies but became more useful with the introduction of RiaCalc which offered precision profile overlays to compare assay precision and sensitivity on a day to day basis. Precision profiles are particularly useful in determining, and perhaps extending the analytical range.



```
LEVEL 4.M :PREC.PROF:COMPARE          2 DEMO     evaluation  33m 90-JAN-13.01
X-AXIS
conc  : LOG
scale :
Y-AXIS
%cv : TOTAL ERROR
      MEASURING ERROR
WORKING RANGE
%CV  <  10
CONC = 1.000 - 16.00
Compare current curve to curve (date.s). Select (date.s) using ↑↓ keys.
14m 89-JUL-19.01
```

Fig. 7.2.2 Precision profile showing measuring and total random error as a function of concentration. Curve overlay with a reference precision profile is also shown.

Useful as it is, we must remember that precision profile only follows assay precision (mainly activity difference between replicates). It is not an efficient means to follow systematic errors, these can be monitored by using control samples, population histograms and other such tools. Therefore precision profiles complement rather than replace the method of using control samples.

Sample population analysis

A population histogram is a tool for verification of an assay's normal range. An "abnormal" value may not be "abnormal" for the local (age, sex, hospital, region) population. Performing this data collection is time consuming and most modern QC programs do it automatically. A resource to compare a histogram with another is an added bonus because it gives evidence of the change of normal value which may indicate error in assay performance.

Mean of Normal Analysis

One way to detect assay drift is to follow the mean values of the normal sample population. Also in this respect the program must be flexible, there should be a possibility to select a time window and obtain the histogram using the samples in the chosen assays.

7.3 Measuring error

These errors are caused by the random nature of radioactive decay (statistical error) or generated in the counting process (counting error).

Statistical error

Radioactive decay is a process subject to inherent random variation. It is not possible to measure true decay rate, it is possible only to establish an interval around the mean value N in which the true value may be assigned. The theoretical standard deviation of counts can be calculated from the following formula:

1) $STD(N) = \sqrt{N}$

where

N = number of accumulated counts

STD = the standard deviation of accumulated counts

The way of collecting the count data does not alter standard deviation, only the number of total counts effect that. For example, if we register counts for one minute and get an average of 10000 counts it is likely that if we count in two periods of 30 seconds and add the results we will also get an average of 10000 counts. In both cases, 1 sigma variation is still 100 counts.

Combining the measurements:

$$STD\,(n1 + n2) = \sqrt{n1 + n2}$$

$$STD\,(n1 - n2) = \sqrt{n1 + n2}$$

$$STD\,(n1*n2) = \sqrt{(1/n1 + 1/n2) * (n1*n2)^2}$$

$$STD\,(n1/n2) = \sqrt{(1/n1 + 1/n2) * (n1/n2)^2}$$

It is customary to deal with CPM values (CPM=N/t), rather than counts because CPM values are related directly to the activity. If the counting time is given in minutes, the formula is:

2) $\mathrm{STD(CPM)} = \sqrt{\mathrm{CPM}/t[\mathrm{min}]}$

3) Standard error % = $100 * \mathrm{STD(CPM)}/\mathrm{CPM} = 100/\sqrt{N}$

Example 1: You measure background sample and get 43 CPM in 10 minutes.

You then measure the actual sample for 2 minutes, getting 89.4 CPM. What is the standard error of the background corrected result?

$$\mathrm{STD(CPM)} = \sqrt{43/10 + 89.4/2}$$
$$= \sqrt{4.3 + 44.7} = 7 \text{ CPM}$$

$$\text{Error }\% = \mathrm{STD(CPM)}/\mathrm{CPM} * 100\ \%$$
$$= 7 / (89.4 - 43) * 100\ \% = 15.1\ \%$$

Example 2: Binding ratio in RIA assay is

$$R = \frac{B - \mathrm{Blank}}{T - \mathrm{Blank}}$$

Blank and T are counted before B and are same for all B values. They are considered not to cause statistical variation inside an assay for R, ie. their errors are set = 0:

$$\text{error}\%\ R = \Delta R/R * 100\ \%$$

$$= \frac{\Delta B}{B - \mathrm{Blank}} * 100\ \%$$

where $\Delta B = \sqrt{B[\mathrm{CPM}]/t[\mathrm{min.}]}$

Note that the formula is different if each unknown (B) has individual blank or total (T)

Counting error

An ideal gamma counter produces results that are related to the DPM value using the following formula:

$R = A * E$ where:

R = reported activity

A = sample activity

E = counting efficiency

A counter which produces results following the formula is considered to be free of measuring error. The measuring errors may occur if, and only if:

- Counting efficiency varies over time or, in the case of multidetector systems, between detectors.

- Additional counts are added to the reported results due to the incorrect background, variable background crosstalk or spillover correction, contamination or other reasons.

Counting efficiency.

In the past, when counters made use of single channel analyzers counting efficiency errors were not unusual. The employment of multichannel analyzers and sophisticated spectrum shape analyses have now made these variations extremely rare. If they occur, they are easily detected. In practice the counting efficiency only decreases because the detector is deteriorating, mainly because the aluminum case is leaking and the moisture slowly destroys the hygroscopic sodium iodide crystal. The process is slow and may take years, the result is a shifted spectrum and decrease in the energy resolution. WIZARD adjusts the counting windows according to these parameters, therefore the counting efficiency is not affected. However, if the deterioration continues there comes a point when further compensation is not possible and the detector must be replaced. By following the energy resolution a detector failure can be predicted before it happens. There have been cases in which the crystal is partially cracked because of internal tensions, this also affects resolution strongly even though the counting efficiency may be quite unaffected.

In summary it can be said that if the resolution of a detector is good, the counting efficiency is almost certainly good too, but the opposite is not always true.

Other counting errors

The following are further possible causes of errors:

- background
- spillover
- crosstalk
- contamination

Their effect on results varies from case to case. These parameters which are stable (background and crosstalk between detectors) are relatively easy to correct by mathematical means. Statistical errors may increase if background level reaches the unknown sample activity.

Spillover errors may occur in double label work. The ^{125}I/^{57}Co pair is rather error free because of the good spillover correction and low uncorrected spillover value (typical values are 1 % before correction, and around 0.1 % after it).

Contamination of a detector is difficult, if not impossible to compensate even though its detection is relatively easy. It is seen as elevated background. The best policy is, of course, not to let the instrument become contaminated. WIZARD's protective holders strongly reduce the possibility of detector contamination.

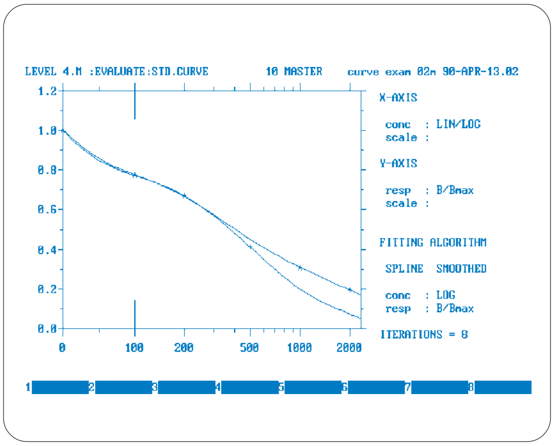
Conveyor to detector crosstalk is impossible to eliminate by mathematical means because it changes each time a sample in the conveyor is moved. WIZARD relies on a heavy, solid lead shield to prevent it with ^{51}Cr, ^{58}Co or other high energy isotopes.

In a single label (^{125}I) the measuring error is practically the same as statistical error. This affects concentration in a non-linear way, which is shown in a precision profile.

7.4 Calculation error

Standardization in immunoassay involves constructing a standard curve which shows the relationship between the measured response and the corresponding analytical concentration of the analyte of interest. The curve is constructed using samples of known concentration (standards) and used to interpolate the values of the unknown samples. Errors occur if the standard curve does not represent the exact concentration to response relationship. There are very many ways to construct a curve, from manual drawing (still one of the best methods, but too laborious for everyday practice) to sophisticated, smoothed spline functions which are restricted to those shapes originating from RIA. To select the appropriate method is often a compromise, the more freedom (parameters which may be changed), the better the fit with empirical data, but, at the same time, the less "rigid" (insensitive to the erroneous standards i.e. "outliers") it becomes.

The ultimate in curve fitting is perhaps the "Master Curve" the unique method which offers the flexibility of spline functions and simultaneously the rigidness of model-based methods. During an initial phase it is smoothed spline which is visually verified by a skilled operator. After that it is "frozen", meaning that only few parameters are allowed to change, these parameters which may change as the kit ages. The Master Curve follows when the kit get older but it does not follow aberrant points caused by outliers. A new Master Curve can be created using only one or a few standards. This also saves costs.


LEVEL 4.M :EVALUATE:STD.CURVE 10 MASTER curve exam 02m 90-APR-13.02

X-AXIS

conc : LIN/LOG
scale :

Y-AXIS

resp : B/Bmax
scale :

FITTING ALGORITHM

SPLINE SMOOTHED

conc : LOG
resp : B/Bmax

ITERATIONS = 8

Fig. 7.4.1. A Master Curve with the original as overlay. Individual replicates are close together indicating good precision in sample preparation.

The evaluation errors are easy to detect and rectify using modern software. Automatic outlier screening and curve overlays assist in the process to keep this error source in check. In this light the software ability to show the standard curve in a sharp plot is invaluable. It is also vital that the software is capable of showing individual replicates, not only mean values as older programs do. Showing only mean values partially wastes the information gained by using duplicate samples. In addition to this the software should provide means to visually modify response points on the screen; outliers can be further eliminated and various curve fitting methods investigated without actually measuring new samples.

7.5 Instrument performance verification

Introduction

QC assurance tools, control plots, precision profiles, etc. form the basis for instrument performance verification. Also the dynamic normalization system inspects the isotope spectrum each time it measures a sample. In addition to these, WIZARD allows automatic monitoring of a number of parameters for each detector. By following the parameters, existing or impending problems are detected. WIZARD also provides GLP compliance documentation on all parameters relating to instrument performance.

To monitor all these parameters in a routine lab may be problematic. Plotting 9 parameters for 10 detectors results in 90 plots. Even though the whole process is automatic this amount of data, in addition to some tens of control plots, precision profiles, histograms etc. per assay, forms a vast pile of GLP reports. No routine lab likes to deal with this amount of paper every morning. The instrument must, however, check each parameter frequently, so what is the solution? The answer is that the system checks each parameter and produces a plot only if a set rule is violated. QC rules are generally Westgard Multi-Rules to minimize false alarms. Detector-related rules (e.g. energy resolution, efficiency and background) can rely on single rules because the variation is small and no false alarms occur anyway. There are other tests which may be used in addition to the ones mentioned above.

The GLP parameters

WIZARD documents nine parameters each time the GLP test is performed. It is the users' responsibility to run the GLP rack (including one uncalibrated ^{125}I sample in it) once a day, WIZARD takes care of the rest automatically.

One may wonder why it is not possible to use a system in which the software detects the first ^{125}I sample each day and use this as a GLP source, eliminating the need for a special test sample. There are two reasons why random samples cannot be used as GLP sources.

– Whenever there are many users, there is the possibility that the sample which is used as the GLP source may belong to a different assay. If one is based on glass vials and the other on plastic vials, the efficiency differs considerably from day to day.

– There is always a chance that the instrument is not used for any ^{125}I assays during a day, but it is used for other labels. On a day like this no GLP data is available and the GLP criteria are not necessarily met.

As the design principle of WIZARD is to assure that every feature is 100 % reliable a separate sample is the only real possibility.

WIZARD follows all parameters but normally produces a complete report only if there is a reason to suspect instrument malfunction.

It is sufficient to follow detector energy resolution, background and detector efficiency on a daily basis. In addition it may be useful, should the above parameters indicate abnormal performance, to acquire a long term plot of the other parameters, too. The parameters and their meanings are as follows.

Detector energy resolution

This is the most reliable measurement of detector condition. Resolution, for a given isotope, depends essentially on the light production and collection efficiency, the more light photons produced, the less statistical variation there is and the better the energy resolution is. The role of energy resolution is that it can predict detector failure long before this actually happens. The mechanism is explained in more detail in section 7.3.

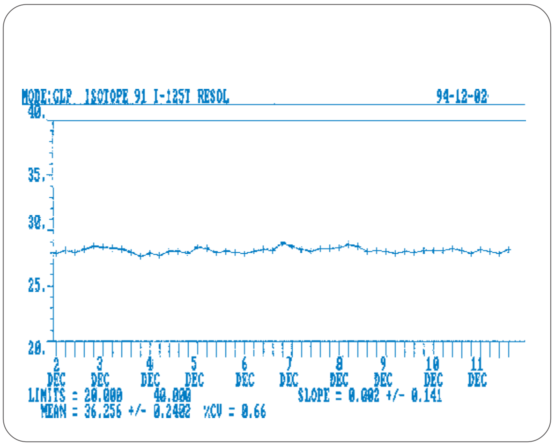


Fig. 7.5.1. Resolution history of a detector.

Absolute detector efficiency

This is determined for ^{125}I using the coincidence method (also known as the Horrocks method or Eldridge equation, see section 3.4). The method does not require calibrated sources (having a known DPM value).

Detector stability probability (Chi-squared test)

Statistically the radioactive decay obeys a Poisson distribution, the Chi-squared test compares the observed standard deviation with the theoretical one and provides a probability number indicating how well these compare.

Background

GLP regulations require background to be recorded because an elevated value indicates that the instrument is contaminated, a common phenomenon in counters where sample tube can physically come into contact with the sample changer mechanism or a detector. This is not possible in WIZARD because of the protective holder system. The background database is still provided to comply with regulations.

Calibration

Calibration follows the peak position of the isotope. Because counting windows are adjusted according to this, the calibration drift does not indicate error in itself; rather it indicates the wear of the PM tube. If the calibration becomes excessive the PMT gain must be adjusted.

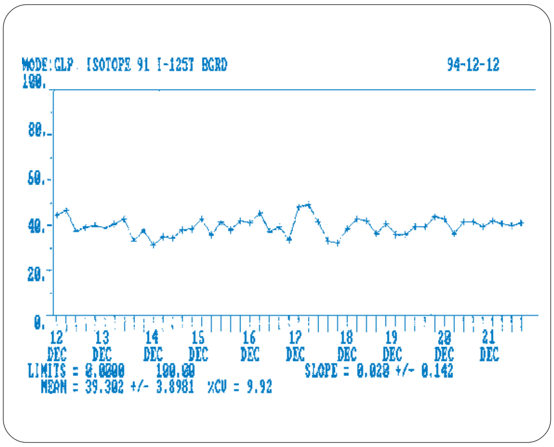


Fig. 7.5.2 Background history of a detector

Relative detector efficiency, i.e. normalization

The relative detector efficiency test indicates the integrity of the normalization. Because this is already reported at the end of normalization and the changes are reported in the detector efficiency test, the relative efficiency test offers a double check.

Other GLP parameters

It is also possible to follow:

* CPM values in a given window

* CPM values in total energy range

* Window settings if dynamic window setting are used.

These parameters may contribute auxiliary diagnostic data in case of malfunction.

8 Meeting Regulatory Standards

8.1 The standard types

The standards of gamma counters belong to three classes:

- Safety standards
- Electromagnetic compatibility (EMC) standards
- Good laboratory practice (GLP) standards

The following standards were followed when WIZARD was designed:

- IEC 1010-1 Safety requirements for electrical equipment for measurements, control and laboratory use. Part 1: General requirements.
- UL 1262 Safety Standard, Laboratory Equipment, USA
- CSA Standards C 22.2 No.0-M91, No.04-1092 and No. 151-1986 Laboratory Equipment, Canada
- JIS T 1001, JIS T 1002, JIS T 1003, JIS T 1004 -1983, Safety of Medical Electrical Equipment, Japan

The principal standard regulating radiated emissions (EMC) is CISPR 22 (EN 50081-1). Immunity test methods are described in IEE 801 series and test levels in EN 50082.

Although in principle every country may have its own nationalregulatory standards, in practice the situation is not so confusing. There are just a few groups of countries which have united as to the standards that they work with. The most important groups are presented below.

8.2 The European Union (EU)

EU Member States will eventually harmonize most standards, including the ones related to laboratory devices. In the transition period, which ended in 1995, a producer had the option to introduce/take into use :

- a device conforming with the EU standard.
- a device conforming with the national standard which was valid on 1 January 1992. The latter option means that a producer should know more than ten national standards, in practice however, most Member States lack any rigorous guidelines or authorities to certify laboratory instruments. The situation varies depending on the standard type, some Member States e.g. UK and Germany, have safety standards, but practically none has a standard for EMC.

According to the Cassis de Dijon principle, a product legally produced and marketed in an EU Member State must be allowed to be sold and used in any other EU Member State. Because WIZARD is produced and legally marketed in a Member State it automatically fulfils this requirement.

Safety standard

WIZARD is certified against the IEC 1010 laboratory equipment safety standard, effective on 1 January 1996.

EMC standard

EMC (Electromagnetic compatibility) regulates the EM interference a device must tolerate and, on the other hand, how much it is allowed to interfere with other devices (particularly the ones used in telecommunication). It is essentially defined in the EU Directive 89/336/EEC and is based on the following standards:

IEC801-2 The basic standard to define the electrostatic discharge immunity test method EN 50082-1 sets acceptance limits

IEC801-3 The basic standard to define the radiated electromagnetic field immunity test method EN 50082-1 sets acceptance limits

IEC801-4 The basic standard to define the electrical fast transient/burst immunity test method EN 50082-1 sets acceptance limits

EN55022 The basic standard to define the conducted emissions immunity test method EN 50081-1 sets acceptance limits

EN55022 The basic standard to define the radiated emissions immunity test method EN 50081-1 sets acceptance limits

European standard EN55022 is identical to CISPR22 (class B).

WIZARD meets the above mentioned standards. However, some instruments delivered in the past may not fulfill the EN 55022 or the corresponding CISPR22 (Special Committee on Radio Interference of the International Electrotechnical Commission). The radiation emissions may be exceeded, in earlier models (till 1993) up to 20dB. The construction was modified during 1994 to conform fully with all the EU Directive effective 1 January 1996.

One may wonder if this kind of field may interfere with other laboratory devices. The answer is no. The radiated emission limit under CISPR22 B for 10m distance is 30 µV/m (30 microvolts/meter) and 20 dB above this limit is 300 µV/m. In a typical FM radio broadcast, the field strength, at the distance of a few km from a station, is 3mV/m. Immunity level defined by EN50082-1 is 3V/m which is 10,000 times higher than emission from WIZARD. Cellular phones usually create 1V/m field strength for 10m distance, so there would have to be thousands of WIZARDs to interfere with as much as a single phone (which may even be outside the building).

There is large tolerance in the set radiation and immunity levels in the EMC standards because they are intended to protect telecommunication devices (which are presumed to detect EM radiation) rather than laboratory devices (which are presumed not to).

GLP standards

No Directive which regulates GLP in the EU has been adopted by the European Council. Member States may have their own legislation. In Germany the most relevant standard covering the instrument is DIN 6855, “Quality Control of Nuclear Medicine Instruments”.

Other considerations

The In Vitro Diagnostic Directive (IVD) is still in preparation. A transition period will be allowed to comply with its requirements. The Electromagnetic Compatibility (EMC) Directive will form part of IVD. The CE mark has been used on Wallac gamma counters from the beginning of 1997.

8.3 US and Canada.

Safety standard

In Canada the principal authority is CSA (Canadian Standards Association). Practically all Wallac products, including naturally WIZARD, are officially certified by CSA, the representative of a CSA performs follow-up audits twice a year. The cabinet of WIZARD will bear the label required by CSA.

In the US the principal authority is OSHA, Occupational Safety and Health Authority. This requires that all equipment used in the work environment in the US has an NRTL (Nationally Recognized Testing Laboratory) approval; the NRTL approved labs are registered by Federal Register.

UL and CSA are on the approved list.

WIZARD is certified by the OSHA approved lab. CSA, NUMBR 052500X0000 April 15, 1993, first acceptance November 1, 1991.

EMC

In the US, the FCC, Federal Communication Commission sets the standard. It regulates the amount of radiated emissions given off that can interfere with telecommunications equipment, other EMC standards are not defined.

Personal computers and accessories must be submitted to FCC for certification. Other products must be tested and shown to fulfill regulations, this is done by the user, (verification). The main standard to follow is CISPR22 (class B) which is similar to EN55022, which will become the standard in the EU in 1996. The preceding text concerning EN55022 is therefore also valid for FCC rules.

However, FCC rules (see reference, 15.103 (c) A) imply that the following devices are exempted: “digital device used exclusively as industrial, commercial or medical test equipment”. This actually exempts WIZARD from following the class B rule; but regardless of this it will be verified for the CISPR22 (class B) standard in 1994.

GLP standards

The Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) is a US federal law that provides standards for all clinical laboratories that perform patient testing. CLIA'88 has a much broader concept of quality that applied in the past. Formerly it may have referred, for instance, to the accuracy that one might expect from an analyzer. In future it will deal with all aspects of the laboratory, from the theoretical knowledge of the operator to the ways reports are given. The approach is fairly similar to the ISO 9001 and Total Quality Management being used in industry today. An individual analytical instrument can reduce the cost of fulfilling the CLIA'88 regulations by providing automatic CLIA'88 compliance report documentation as shown in section 7.5 "Instrument performance verification".

8.4 Japan

The principal standards are JIS T 1001, JIS T 10021, JIS T 10031 and JIS T 1004 -1983, Safety of Medical Electrical Equipment, Japan. The standards are based on the corresponding international standard IEC-601-1 without major deviations.

8.5 Others

Most European countries that are not EU members still follow EU Directives, particularly the countries in the E.E.A. (European Economic Area). It is probable that some non-European countries, e.g. Australia will accept CE marked devices without further controls. In a similar way most Latin American countries follow the US. The IEC 1010 is widely followed as well.

8.6 ISO 9001

The ISO 9001 is an international standard which is applied to companies' quality assurance systems. The standard does not apply directly to products, but does provide laboratories with a guarantee that correct and fully audited procedures are employed by their supplier. All Wallac operations are accredited to the ISO 9001 standard.

References

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5. Reference US: Code of Federal regulations 47, parts 0 to 19, October 1 1992.

6. EU.: Council directive of 3 May 1989, on the approximation of the laws of the Member States relating to electromagnetic compatibility. (89/336/EEC)

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9 Selecting a gamma counter

The following are general guidelines worth considering when choosing a new gamma counter.

9.1 Examining the specifications and features.

If the performance or a feature is not actually specified, but ignored or implied the reason may be inferior performance in this particular section. If the instrument performance is specified only for ^{125}I, this “easiest” nuclide is all that is actually guaranteed. Specification of an energy range is vague at best. A counter with energy range specified as 0 – 1 MeV may not actually permit you to work with all nuclides within this energy range; the instrument may supply a count result, but for various reasons the result may not be usable. If you are about to buy a ten-detector counter, specified for up to 1MeV you may reasonably assume that you can count the Amersham Dicopac Kit (^{58}Co, 810 keV) in it. It may be worth checking whether the crosstalk is so high that you are required to use only a single detector even though you have actually paid for ten!

The Ten-detector WIZARD is specified for up to 1MeV, optionally to 1.5 MeV. The range can be achieved with all detectors the instrument has.

Instruments using 3" through-hole detectors suffer from crosstalk to such a degree that manufactures often offer a special Crosstalk Reduction Option. While reducing the crosstalk to a level close to the 1480 WIZARD performance this sacrifices the sample size specifications allowing only very small sample tubes indeed. The vial specifications (given without Crosstalk Reduction option) and crosstalk figures (with the option) provide a nice-looking counter on paper but the practice is very different; what happens to the crosstalk when you want to count normal-sized vials?

In fact the specifications may be so limited that often the only way to make sure that the instrument would fulfill the intended use is to try to get it for testing for a day or two.

Obviously it is impractical to specify all possible nuclides the counter can measure, but it is important that the highest energy nuclide’s performance is specified, the performance with the lower energy nuclide is automatically better.

You either must take the information from a salesperson or you must make an inspection test yourself. For those who do not wish to test their IQ (and luck) but would like to test the counter themselves, a simple test is explained on the next page. Carrying out the test takes a few hours, but it provides you with a very good picture of what the instrument can do and what it cannot.

The natural question which often arises is, “What happens if the system goes out of calibration on the first run after the calibration was performed?” However, before actually going so far, perhaps the following one is more relevant: “Is the counter able to produce error free results in the first hand?”. The following test will answer this question.

9.2 The performance test for a multidetector counter.

* Take the lowest energy nuclide source which will be used. It is almost certain that this is ^{125}I if you are working in a clinical laboratory.

* Take the highest energy nuclide source which is likely to be used in the instrument's lifetime. If you do not know or are unsure take the one which is close to the specified energy range of the instrument. If the energy range is up to 1 MeV (as with WIZARD) you may take ^{58}Co, which is close to the upper limit.

* Suitable source activity is 0.1 µCi or 220,000 DPM, this is high enough so that the statistical variation is practically zero and you may presume that all errors in results are due to the counter performance.

* Normalize the counter for these nuclides using the recommended normalization procedure. Make background normalization using the recommended normalization procedure.

* Make a dual label protocol using the nuclides. WIZARD, does not require special dual label normalization for this.

* Take two racks and put one of the samples into the first rack, fill the rest of the positions with empty tubes. Put the second sample in the second rack, fill the rest of the positions with empty tubes.

* Count the racks as normal samples. Calculate the mean of all CPM (any empty tube). The mean should be close to zero and because of decay statistics there should be roughly equal numbers of negative and positive values.

* Calculate the ratio of CPM (any empty tube)/CPM (^{58}Co sample). If any ratio is > 1 % the instrument may be suffering crosstalk or background correction problems.

* Change the positions of the samples in the racks (e.g. put them into the successive positions) and count them a number of times in various locations. For a comprehensive test count both samples in all detectors.

* Calculate the ratio of CPM(^{125}I)/CPM(^{58}Co) for ^{58}Co sample. An error-free counter would give 0 % because there is no ^{125}I present. A value > 1% indicates spillover correction failure.

* Calculate the mean of all recorded CPM (^{125}I) and CPM (^{58}Co). The variation > 1 % indicates bad normalization, stability or other problems.

Passing this test does not guarantee that the actual results are always correct, (there is a comprehensive GLP documentation for this) but failure indicates that they very likely are not.

Unit	Abbreviation	Definition
Counts per minute	CPM	The recorded rate of decay
Counts per second	CPS	The recorded rate of decay
Disintegrations per minute	DPM	The actual rate of decay
Becquerel (SI unit)	Bq	1 DPS
Curie	Ci	3.7×10^{10} Bq
Microcurie	µCi	37000 Bq or 2,220,000 DPM
Picocurie	pCi	37 mBq or 2.2. DPM
Electron volt	eV	1.602×10^{-19} Joules (energy received in a 1 Volt potential)
Electron rest mass	9.1×10^{-31} kg	511 keV (by $E = m_{el}c^2$, c = velocity light)

10 Wallac gamma counters

1470 WIZARD

1480 WIZARD

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