INSTRUMENT MANUAL

1470-931-23
January 2002

wallac 1470 WIZARD®

Gamma Counter

PerkinElmer™
life sciences.

Wallac 1470

WIZARD®

Gamma counter

For instruments with software version 3.6

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Website: www.perkinelmer.com/lifesciences

Warning

This equipment must be installed and used in accordance with the manufacturer's recommendations. Installation and service must be performed by personnel properly trained and authorized by PerkinElmer Life Sciences.

Failure to follow these instructions may invalidate your warranty and/or impair the safe functioning of your equipment.

PerkinElmer™
life sciences.

Contents

Trademarks

1 Introduction

1 Introduction

1.1 Introduction to PerkinElmer Life Sciences, Wallac Oy

PerkinElmer Life Sciences, Wallac Oy is the world's leading manufacturer of automatic gamma counters and has been a pioneer in this field for many years. PerkinElmer Life Sciences, Wallac Oy has a well-founded reputation for technological innovation and excellence in quality both in products and service.

1.2 Introduction to 1470 WIZARD

WIZARD is the latest and most advanced gamma counter from PerkinElmer Life Sciences, Wallac Oy. It comes in different shapes and sizes but each is capable of performing similar magic in your laboratory. Your WIZARD may have 1, 2, 5 or 10 detectors and be able to take 550 or 1000 samples at a time depending on the model you have. Every WIZARD has its own built-in display and keyboard for full communication with its users.

WIZARD can operate as an automatic standalone CPM counter, or it can be used to do extensive data evaluation with its own internal RiaCalc WIZ program or it can be linked up to an external PC and use the power of a MultiCalc program. RiaCalc WIZ includes the built-in hard disk.

WIZARD can also be used as a manual counter if you require.

1.3 Introduction to this manual

1.3.1 Step-by-step instruction sheets

There are three sheets following this page which give a brief step-by-step outline of how to use WIZARD in each of the three operating modes:

1 WIZARD is used as a **stand-alone CPM** counter. Counting parameters are set in the counter. CPM results are sent to the built-in display and a printer.

2 WIZARD is run with **RiaCalc WIZ**. All counting protocols are set in the counter itself. Results are sent to the built-in display and a printer.

3 WIZARD is connected to a personal computer running **MultiCalc software**. All counting protocols are set in the personal computer. Results are automatically returned to the PC for final evaluation and output. More information about MultiCalc will be found in the MultiCalc User Manuals.

You should find enough information on the appropriate one of these sheets for normal operation in any of these modes.

1.3.2 WIZARD controls

If you are a first time user of WIZARD you should read **Part 2 "WIZARD controls"** before starting to operate the counter. This part explains the basic techniques involved in using the built-in display and keyboard(s). It also explains the barcode ID system.

Once you are familiar with the techniques of using WIZARD you can proceed to actually operate it.

1.3.3 Using WIZARD to get results

WIZARD counts samples and if necessary evaluates the results following instructions given in the form of protocols (lists of parameters). In normal operation these protocols are already set-up so all you need to do is follow the instructions on one of the step-by-step sheets. If however it is your responsibility to create or edit protocols then you will find the information you need to help you in the appropriate one of parts described below:

CPM counting requires only three parameters to be set as described in **Part 3 CPM operation**.

RiaCalc WIZ operation is described in **Part 4**. Each feature of RiaCalc WIZ is described and guidelines are given to help you use these features to achieve the results you want.

If you are going to be working with **MultiCalc** running on an external PC then turn to **Part 5**. This describes those things you need to know in order to use WIZARD and MultiCalc together. The use of MultiCalc is described in a separate User Manual which comes with the software.

Part 6 tells about Isotope and background **Normalization**. This is an operation which has to be done before WIZARD is used to count samples with a particular isotope. When it has been done once, it should be repeated occasionally e.g. after half a year. Part 6 also tells about how to do performance testing with **GLP test normalization**.

Part 7 describes a number of functions which are available in addition to the three main ways of using WIZARD described in parts 3, 4 and 5.

Part 8 of this manual gives you a **description of how WIZARD works**. You do not need this information for normal operation but it will help you to have confidence in your results when you know how WIZARD has been designed to give you the very best.

Detailed **specifications** are described in **Part 9** giving numerical values for e.g. efficiency, background etc.

In **Part 10** there is also a description of the **calculation methods** used in WIZARD.

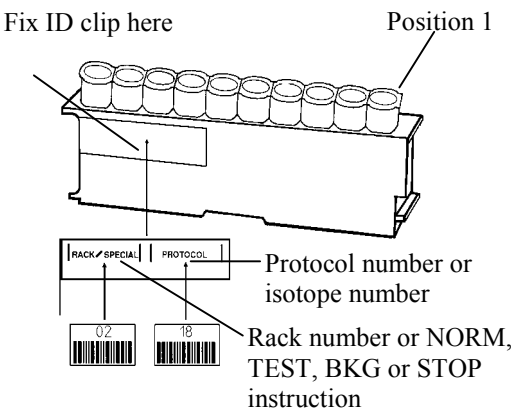
Part 11 contains the information you need when **installing WIZARD** for the first time. Normally this will be done by a service engineer so you will not need this information.

Part 12 contains the **alphabetical index** to this manual.

CPM operation of WIZARD®

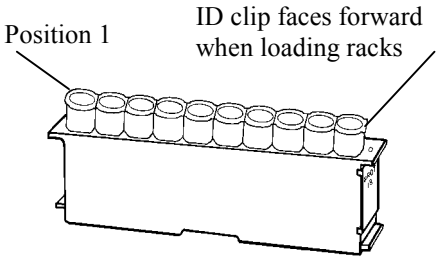
1 Fix ID clips to racks

ID labels (barcodes) are stuck to an ID clip which fits onto a rack to tell WIZARD the function of the rack.
A **counting protocol** is a set of three parameters time, max. counts limit and isotope, which control counting.
Rack number is optional and allows each rack to have its own number.
Normalization ensures that the counting **efficiency** of each detector is the same.
Background ensures that the effect of the background is removed from the measured counts.
Test initiates a **GLP performance** test normalization.
Isotope number shows the isotope to be used in normalization.
Stop tells WIZARD that no more racks are to be counted.



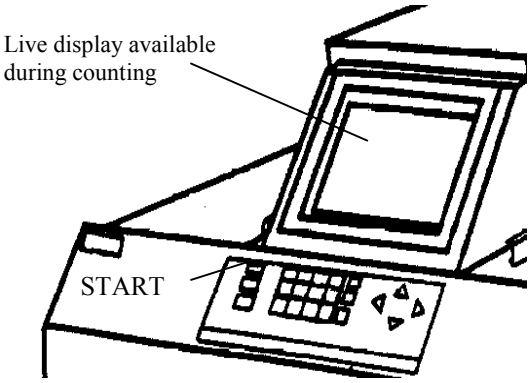
2 Load racks onto WIZARD

Make sure racks are loaded the correct way round with the ID clips facing away from you as shown in the figure. Start by loading the right-hand side of the conveyor.



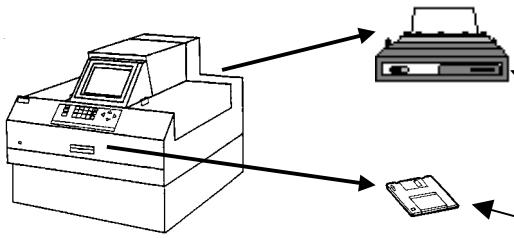
3 Press START to count

Live display during counting is obtained by selecting from the main menu on the WIZARD built-in display "Operate" and then "Show cpm results". Available live displays are: counting parameters, Counts, CPM, CPS and Spectrum.



4 Results are printed out

The built-in program allows counting and normalization protocols to be created and edited. The built-in display and keyboard is used. Results are sent to the display and a printer.



For **System setting** info. e.g. detector deactivation or clock setting select SYSTEM in the main menu

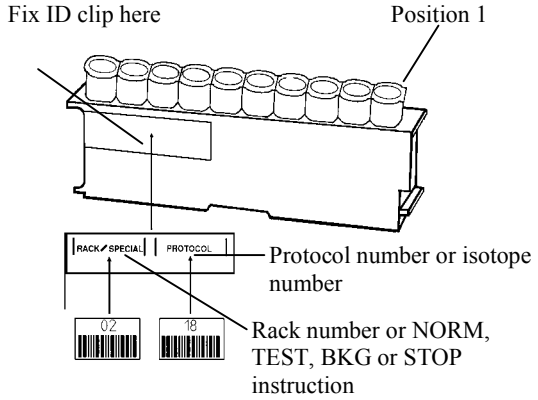
The **printer** connected to WIZARD port 1 is used for printing corrected CPM results directly from WIZARD

A **disk** can be used for transferring results from WIZARD

RiaCalc WIZ operation of WIZARD®

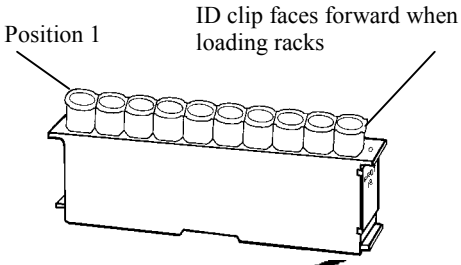
1 Fix ID clips to racks

ID labels (barcodes) are stuck to an ID clip which fits onto a rack to tell WIZARD the function of the rack.
A **counting protocol** is a set of three parameters time, max. counts limit and isotope, which control counting.
Rack number is optional and allows each rack to have its own number.
Normalization ensures that the counting **efficiency** of each detector is the same.
Background ensures that the effect of the background is removed from the measured counts.
Test initiates a **GLP performance** test normalization.
Isotope number shows the isotope to be used in normalization.
Stop tells WIZARD that no more racks are to be counted.



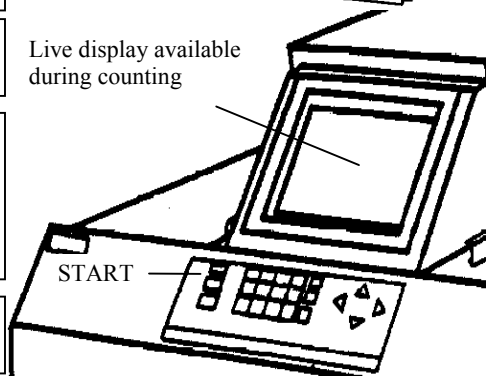
2 Load racks onto WIZARD

Make sure racks are loaded the correct way round with the ID clips facing away from you as shown in the figure. Start by loading the right-hand side of the conveyor.



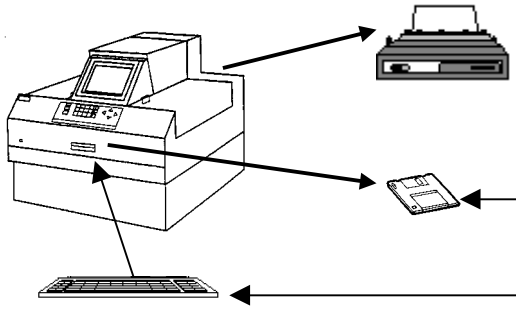
3 Press START to count

Live display during counting is obtained by selecting from the main menu on the WIZARD built-in display "Operate" and then "Show cpm results". Available live displays are: counting parameters, Counts, CPM, CPS and Spectrum. RiaCalc WIZ output can also seen by choosing the “Show evaluation results” function.



4 Results are printed out

The built-in program allows counting and normalization protocols to be created and edited. The built-in display and keyboard is used. Results are sent to the display and a printer.



For **System setting** info. e.g. detector deactivation or clock setting select SYSTEM in the main menu

The **printer** connected to WIZARD port 1 is used for printing corrected CPM results directly from WIZARD

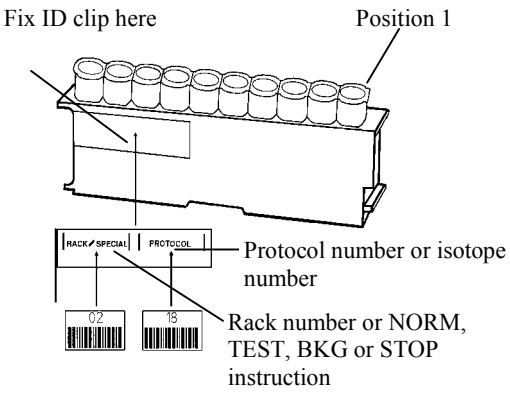
A disk can be used for transferring results from WIZARD

External keyboard in built-in drawer for extended protcol editing

MultiCalc™ Operation of WIZARD®

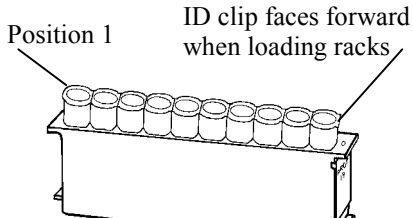
1 Fix ID clips to racks

ID labels (barcodes) are stuck to an ID clip which fits onto a rack to tell WIZARD the function of the rack.
A **counting protocol** is a set of three parameters time, max. counts limit and isotope, which control counting.
Rack number is optional and allows each rack to have its own number.
Normalization ensures that the counting **efficiency** of each detector is the same.
Background ensures that the effect of the background is removed from the measured counts.
Test initiates a **GLP performance** test normalization.
Isotope number shows the isotope to be used in normalization.
Stop tells WIZARD that no more racks are to be counted.



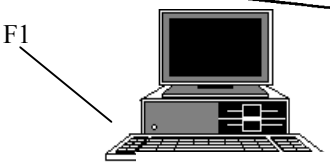
2 Load racks onto WIZARD

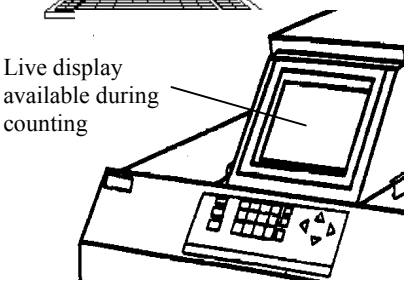
Make sure racks are loaded the correct way round with the ID clips facing away from you as shown in the figure. Start by loading the right-hand side of the conveyor.



3 Press F1 (=COUNTER) select 1470 and press Enter

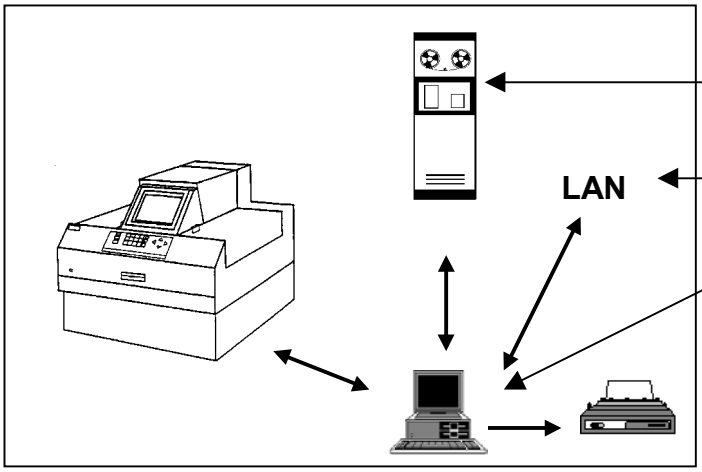
Live display during counting is obtained by selecting from the main menu on the WIZARD built-in display "Operate" and then "Show cpm results". Available live displays are: counting parameters, Counts, CPM, CPS and Spectrum. These appear on the built-in display.





4 Results go to MultiCalc

MultiCalc is a very versatile data handling program which runs on an external computer. You can use it to make counting protocols. These will be transferred to WIZARD to control counting. Results are returned to MultiCalc for evaluation. They can also be sent, via MultiCalc, to a local area network (LAN) or mainframe computer.



Output to external computer e.g. a **mainframe**. Connection is from the mainframe to the PC

Local area network connection (LAN) using the PC

Results go to the PC connected to port 2. Printout goes to the printer connected to the PC

For System setting info. e.g. detector de-activation or clock setting select SYSTEM in the main menu

2 WIZARD controls

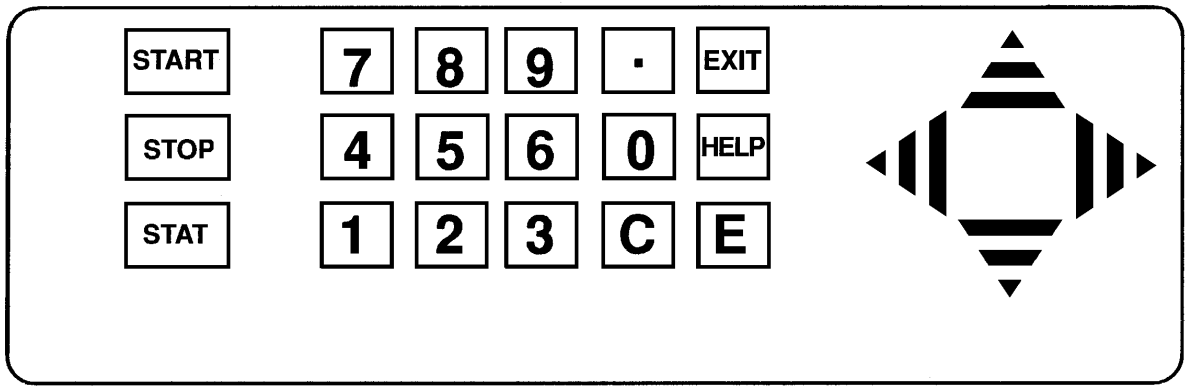
2 WIZARD controls

2.1 Introduction

This chapter describes features you need to use to control WIZARD. These features are: the one or two keyboards, the display, the ID system and the HELP function. When you understand how to use these features then you can proceed to the following chapters to see how to use WIZARD to get the results you want.

2.2 Keyboard

WIZARD can have two keyboards, a simple membrane type on the front of the instrument (see the figure below) and a second, a complete PC keyboard, which is in a separate compartment. The instrument is normally operated using the small keyboard whereas the larger keyboard is used for extended protocol editing because the small keyboard does not include letters and certain other characters which are needed in some editing operations.



The keyboards are connected in parallel with each other and each key on the built-in keyboard has its equivalent on the external one. The keys on the external keyboard which correspond to those on the built-in keyboard are as follows:

Built-in keyboard	External keyboard
START	F3
STOP	F4
STAT	F5
HELP	F1
EXIT	ESC
C (Clear)	Backspace
E	Enter

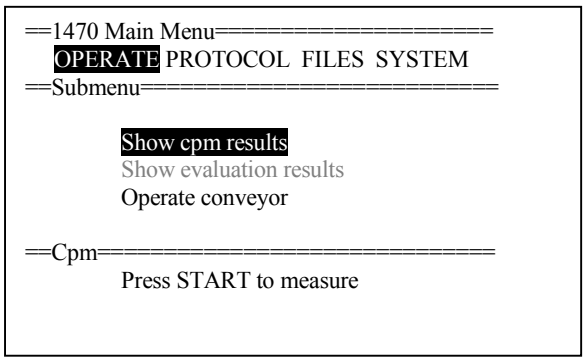
Use the EXIT (ESC) to escape from the operating level the program is on and go back to the previous one.

If you are using a PC running MultiCalc all commands involving MultiCalc are given via the PC keyboard.

2.3 Display

2.3.1 Main menu display

When you start to work with the instrument you will see on the built-in display something like:



2.3.2 Function selection

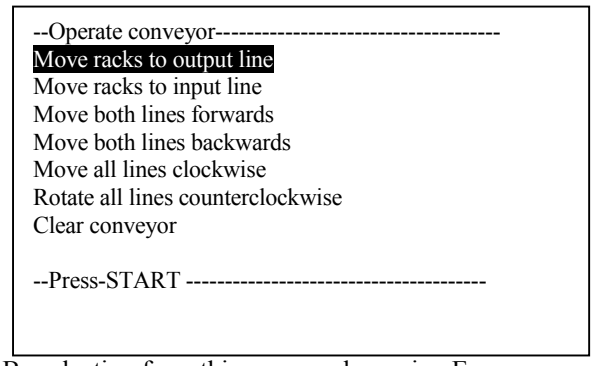
The line in capital letters shows four major functions of which the first, OPERATE, is currently selected. The following lines show the commands available as part of that function. Each function has its own set of commands. You can select the function with the left and right arrow keys. The individual command is selected with the up and down arrow keys. To give the selected command to WIZARD press the E key

2.3.3 Enabled and disabled functions

On the example screen shown on the previous page the first two command lines are in subdued colour. This indicates that the counter is not measuring and hence these functions are not enabled. The third item on the menu "Operate conveyor" is however available.

2.3.4 Operating the conveyor

If you select this and press E the display changes to:



By selecting from this menu and pressing E you can control the movement of racks on the conveyor. To return to the main display, press EXIT.

2.3.5 Selecting the mode

WIZARD can be used in one of three modes CPM, MultiCalc or RiaCalc WIZ. The current mode is shown on the lower part of the main menu display.

2.3.6 Status line

At the bottom of the screen there is a status line. This shows what is being measured. In the display example above the status line is "Press START to measure". The status line can have the following texts:

```
Press START to measure
Measuring an assay
Measuring background
Normalizing
Clearing the conveyor
Seeking assay
GLP test
```

2.4 Live display

When samples are being counted the display can show actual counting values, either collected counts, collected counts per minute (CPM values) or a complete isotope spectrum. The word "Live" indicates that the display is working in real time; values are updated at the same pace as counts are accumulated.

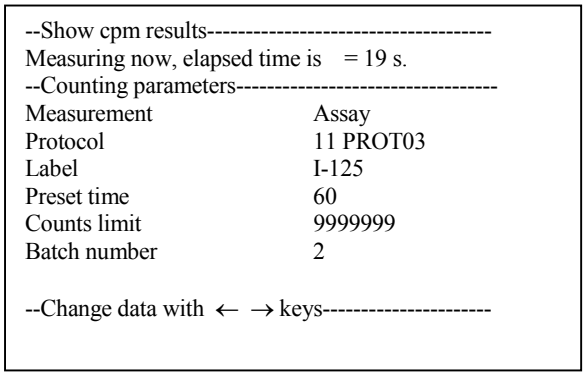
2.4.1 How to use the Live display

In order that the Live display works the counting must be actually happening.

Select the OPERATE menu. The status line must show "Measuring assay" or a corresponding text which indicates that counting is active. Select "Show cpm results" and press the "E" key. Counting parameters are shown (see the figure below).

2.4.2 Display modes.

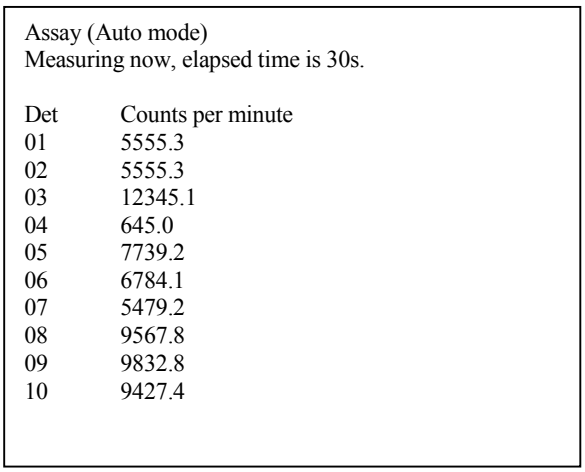
There are five display modes (Note: there are two counting windows in dual channel counting):



Counting parameters (as shown above)

COUNTS Accumulated counts in counting window
CPM Counts per minute in the counting window
CPS Counts per second in the counting window
Spectrum display

Select the appropriate one using the "right" and "left" arrow keys. Pressing the right key twice initiates the CPM display:

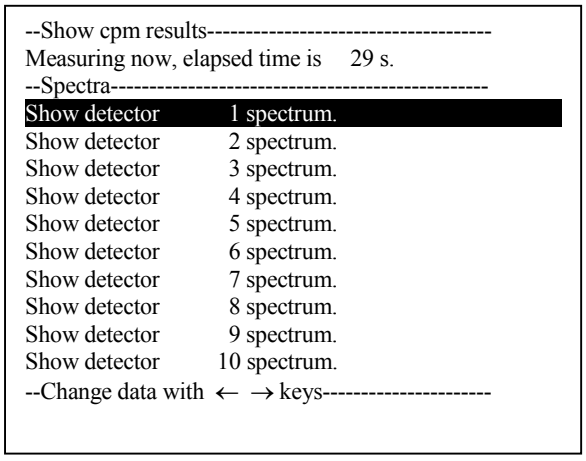


All detectors are shown simultaneously. The example is from CPM counts; the display for COUNTS and for CPS are analogous with the CPM display.

Note: the CPM values are not corrected for dead time, background, spillover, crosstalk or detector efficiency but are direct values of accumulated counts divided by measured time.

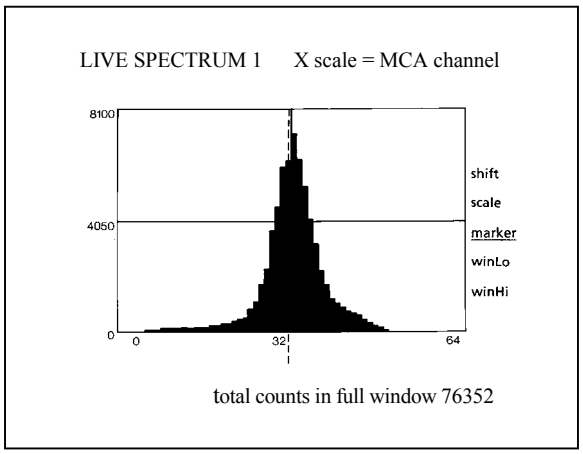
2.4.3 Displaying the isotope spectrum

Press the left/right arrow keys four times.



Select the detector with the up/down arrow keys, then press "E"; a spectrum is displayed. (For singe detector instruments there is no Spectra selection in Show cpm result, instead just press "E" to get the spectrum.) The spectrum display is a graphical representation of isotope activity with respect to isotope energy.

The X-axis shows either MCA channel number or keV (up to 1024). To toggle between these press Enter or E. The selected unit is shown at the top of the spectrum). The Y-axis shows the accumulated counts in each individual channel. The analyzer is initially adjusted so that each channel corresponds to 1 keV, therefore the X-axis is approximately from 0 to 1 MeV.



On the right hand side of the spectrum five functions are displayed. By using the up and down arrow keys you can select the function you want. A function is shown to be selected by the underline mark appearing under the function name. The functions are as follows:

Scale - This allows you to select the scale for the spectrum. The default is 0..1024. By pressing the left arrow you can change the scale to 0..512, 0..256, 0..128, or 0..64. To return to fuller scales, press the right arrow. The figure shows the 0..64 scale. See the next function "Shift" for how to move through the scale segments.

Shift - If the scale used is less than full scale (0..1024) e.g. 0.. 256), you can use the "shift" function to move the spectrum to see other parts which otherwise would not be displayed e.g. 256.. 512 etc. Just press the right or left arrow to move in the direction you want.

Marker - If you have the basic scale (0..1024) selected you can use this function to select a marker which you can then move with the arrow keys to mark any position in the spectrum. In this way you can find out the exact position of any peak.

WinLo and **WinHi** - These allow you to set an upper and lower window limit marker so that you can obtain the counts within the window.

You can also print out the spectrum if you press "**Print screen**" on the external keyboard provided CAPS LOCK is not on and there is a printer connected.

Spectra can also be printed via MultiCalc to the printer that is connected to the PC running MultiCalc.

To do this, the following conditions must be met: If the instrument is in "MultiCalc" mode, then MultiCalc itself must be in online mode when the Print Screen key is pressed. If the instrument is in "Cpm" or "RiaCalc WIZ" mode, then the instrument parameter "SYSTEM | Printout options | Without buffering PC" must be "YES" and MultiCalc must be receiving data from the counter.

Spectra are printed correctly only if in the WIZARD communication protocol the Terminal parameter is VT-52.

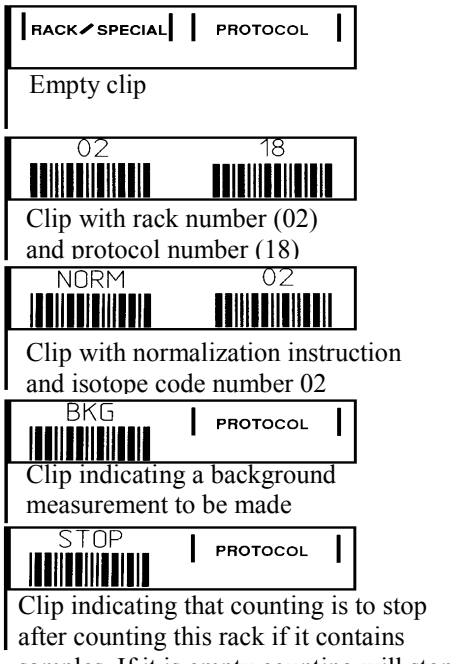
Note: to change quickly from the spectrum of one detector to another (1-10), just press the respective number key (1-0) on the keyboard. You can also use the + and - keys to scroll through the numbers.

2.5 Principle of the ID system

WIZARD is an automatic gamma counter. This means that samples from several assays can be loaded onto the conveyor and WIZARD can be left to count them by itself. To do this it needs to be able to identify each batch of samples. The WIZARD ID system uses a plastic clip onto which one or two barcode labels are stuck. The clip is then clipped onto the rack to be identified. The clip can easily be removed and a new one fitted when required.

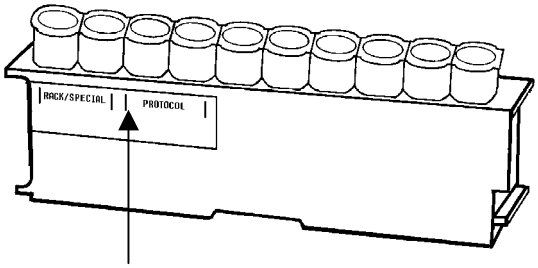
The barcodes give information to WIZARD about e.g. the counting protocol to be used for the samples, what the rack number is etc. Only the first rack in an assay needs to have a protocol label. Barcodes are to be found in a booklet supplied with the counter. There are two classes of barcodes, numerical ones (0 ... 99) and instruction ones. The figure below shows an empty clip and then several examples of clips with labels on them. Some have two labels and some only one.

In addition there is a third area of the clip which comes on the end of the rack. This can be used for you to write your own information on. The ID label booklet includes empty labels for you to write on and stick to the clip. There are also barcodes marked PCURVE and CONTROL which are not used in this program.



2.6 Fitting ID labels

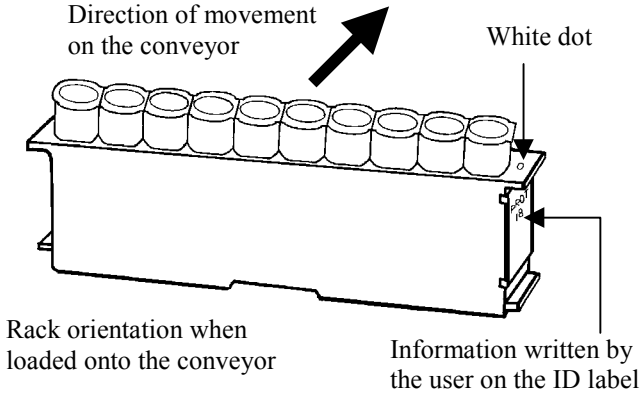
The figure below shows a rack with an empty ID clip fitted on it. There is a special recess on one side of each rack. This is where the clip fits. It will not fit to the other end of the rack. This is important because it defines which way round the rack should be on the conveyor. Make sure the ID clip is fitted properly so that it does not slip off when the rack is on the conveyor.



Empty clip fitted onto rack

2.7 Loading racks the right way round

When you load racks on the conveyor the ID labels must face away from you with the white dot on the right side as shown in the next figure.



The handwritten information on the label at the end of the rack is then clearly visible from the side of the conveyor.

All racks, even those without labels, must be put the same way round with the recess for the label (and white spot) on the right hand side when you load the racks. If it is not the right way round a warning is displayed and counting is stopped until the rack has been turned the right way round.

2.8 Instruction labels

Use instruction labels to select special functions; attach the label in the area marked RACK/SPECIAL. The instrument recognizes the following codes:

2.8.1 STOP

This stops counter operation after the counting of the rack with this label. An alternative is to use an empty rack as a STOP rack.

2.8.2 NORM (Normalization)

This label is used in conjunction with the isotope code label (see 2.9.2 below) to identify a rack as a normalization rack. The isotope must be in position 10 (the last position in a rack). Other positions must be empty and without holders. See chapter 6 for detailed instructions.

2.8.3 BKG (Background)

A rack with this label is used to make background normalization. No sample tubes should be in the rack, see chapter 6.

2.8.4 TEST (GLP test)

This label is used for coding the GLP (Good Laboratory Practice) test normalization. The isotope must be in position 10 (the last position in a rack). Other positions must be empty and without holders. See chapter 6 for detailed instructions.

Note: Make sure you remove NORM, BKG and TEST racks after use to avoid redoing a normalization unintentionally.

2.9 Numerical labels

2.9.1 Protocol number label

Protocols (1.. 99) are called into use by numerical barcodes, the number refers directly to protocol number. The label should be attached to the area marked PROTOCOL.

Although the ID system is used, not all racks need have ID labels on them. In automatic counting the racks are counted according to the last protocol number label until the next protocol number label or a special code is read.

2.9.2 Isotope number code label

The number of the isotope to be used in normalization must be shown by attaching an appropriate numerical label to the PROTOCOL area when the rack is being used as a normalization rack.

2.9.3 Rack number label

Use numerical labels 1 .. 99 to select the rack number, attach the label in the area marked RACK/SPECIAL. This is optional and need not be used at all.

2.10 Barcode errors

If the ID system fails to read the barcode successfully the rack is handled as if there was no barcode. If the code is read successfully but that particular protocol does not exist the message "Protocol not found" appears on the display and printer and the counter stops.

If there are two numerical codes on the same ID clip, the one in the RACK/SPECIAL area is taken as the rack number and the one in the PROTOCOL area as the protocol number.

2.11 Help

The WIZARD software includes an extensive context sensitive Help function. If at any point while operating WIZARD, you are not sure what to do, or what a particular function is, just press the HELP key on the built-in keyboard (or F1 on the external keyboard). An explanation of the currently selected feature will appear on the display.

When you have read the help text you can go back to the function about which you requested help by pressing the EXIT key.

2.12 Short cut keys

Two short cut keys Alt-I and Alt-E are available on the main menus level:

Alt-I (the I meaning "internal") sets the mode to "RiaCalc WIZ" and the SYSTEM parameter Printout selections | Write results to file" to "Yes".

Alt-E (the E meaning "external") sets the mode to "MultiCalc" and SYSTEM parameter Printout selections | Write results to file" to "No".

These short cut keys are available only when SYSTEM parameters can be edited (that is, when measurement is not occurring).

3 CPM Operation

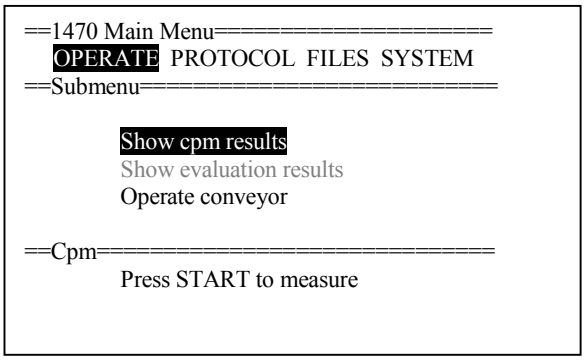
3 CPM operation

3.1 Introduction

The instructions here describe the routine operation of WIZARD when it is running in CPM mode.

3.2 Start up

1. Switch on the printer; this should already be connected to the counter.
2. You can put the data disk named 1470 Datadisk into the WIZARD disk drive. The name label should be facing upwards.
3. Switch on WIZARD. After about 3 min. the display will show:



4. Check the time and date by selecting SYSTEM mode and DATE. If it is not correct then give the correct value (See "System | Time & Date setting"). Return to the main display by pressing EXIT.

5. Make sure that the counter is in the right mode i.e. that the text CPM appears near the bottom of the screen as shown in the example above. If it does not then select "System" and then the operation mode. The mode must be CPM.

3.3 Normalization

Make sure that WIZARD has been normalized for the isotope you are going to use in your measurement. See chapter 6 "Normalization".

3.4 Protocol editing

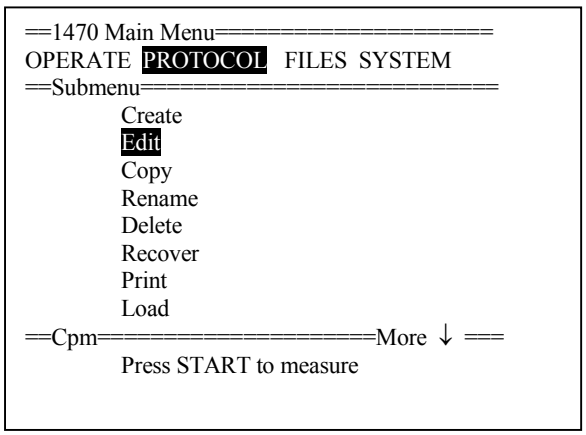
3.4.1 What is a protocol?

A CPM protocol contains three parameters for controlling counting conditions.

Before counting can be done, a protocol must exist. Each protocol must have a name and an ID number. This number allows the protocol to be called into use by means of a barcode label. To access protocol file handling you must first select the PROTOCOL option on the display.

3.4.2 Protocol file handling

There are several commands available to use with a protocol, the most common ones are Create (making a new protocol) and Edit (change the contents of the existing protocol). The list that appears after you select PROTOCOL is as follows.

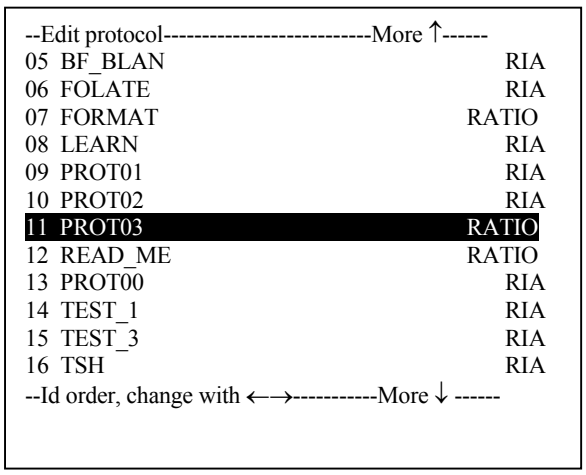


Create

Select Create and press E. Give the protocol name. There is a default name of the form "Protnn" where "nn" is the protocol number. If you want to change this name you must use the external keyboard unless you only use numbers for the name. A maximum of eight characters can be given. Select an unused ID number (between 1 to 99). Press E. The protocol parameters can be set in a similar way to those in editing.

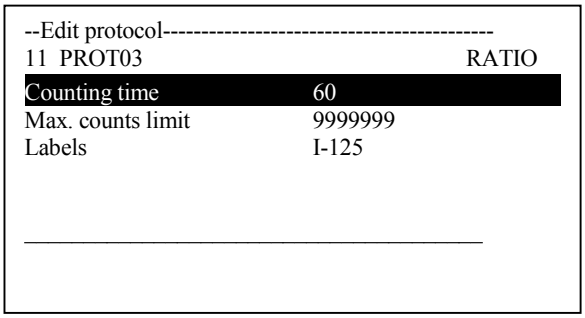
Edit

Select Edit and press E. Select the protocol by name from the list of protocols available and press "E".



You may now change the parameters. You can move up and down in the parameter list by means of the UP and DOWN arrow keys. See section 3.5 for details of parameters available in a protocol.

Note: In the example above the protocol types include both RIA and RATIO. It is not possible to create RIA protocols in CPM mode (protocols are automatically labelled RATIO) but if such a protocol has been created in another mode then it will still appear on the list. You can select any protocol from the list for editing in CPM mode but only the three parameters shown in the example below will appear.



Copy

An existing protocol can be copied so as to create another protocol with the same contents.

Select Copy and press E. Select the protocol to be copied. Give the name the new protocol is to have. You may also give an ID number. Select "Do copy" and press "E".

Rename

This option allows you to give a new name to a protocol. Select Rename and press E. Select the protocol to be renamed from the list of protocols. Give it a new name and/or ID number. Select "Do rename" and press "E".

Delete

Select Delete and press E. Select the first protocol to be deleted. Then select "Do delete" and press "E". The protocol and any associated data will then be deleted.

Recover

Deleted protocols can in most cases be recovered by selecting this option. They are saved in the protocol index area. There is room on the system disk for 100 deleted and active protocols. The larger the number of active protocols the fewer the number of deleted protocols that can be saved. If there is no room, the oldest deleted protocol will be permanently removed to make room for a newly created one. No protocol with the same name should have been deleted later than the one you want to recover.

Print

With this function you can print the contents of a single protocol or the protocol index according to the selection you make.

Isotope names are included in the protocol printout. For dual label protocols the code numbers for both isotopes are printed separately, e.g. 1; I-125 + 2; Co-57.

Load

You can load a single protocol or all protocols from an external microfloppy disk. Such protocols will have been saved there using the Save function (see below). Note: if you load a new protocol when a previous one with the same name or ID number exists in the instrument, you have the choice of either renaming the protocol to be loaded or deleting the previous protocol.

Normal Load will give you:

1 Protocol files
2 Controls files
3 Trends files

Extended Load will in addition give you:

4 Standard curve files
5 Data files
6 Results files

Note: if you load a new protocol when a previous one with the same name or ID number exists in the instrument, you have the choice of either renaming the protocol to be loaded or deleting the previous protocol.

Save

This function allows you to transfer files from WIZARD to an external microfloppy disk for storage. Subsequently these files can either be loaded back into WIZARD.

Purge

Deleted protocols and associated data files can be permanently erased from the instrument hard disk with this function.

Password

When a protocol is created, you can give a two-character password. All characters are allowed in the password and the password is case sensitive. If you do not give a password at this point, then the protocol can be edited, renamed, overwritten when a new protocol with the same name or ID number is loaded and deleted without giving any password. Otherwise the password is needed to do these operations. A protocol can always be copied, restored, saved and purged without giving the password. With rename it is possible to change or remove the password.

Passwords are retained even if power is turned off. The password feature can be disabled by removing the file C:\PASSWORD from the instrument hard disk. To enable it again, type ECHO aa > C:\PASSWORD at the DOS prompt to recreate a password file and boot the instrument. (You can enter DOS by installing the installation disk to the disk drive and by restarting the instrument.)

3.5 Parameters available

3.5.1 Counting time

Give the counting time in seconds. All samples are counted for this time. The default value is 60 and the maximum value is 65000 seconds.

3.5.2 Max.counts limit

If you want to terminate counting on the basis of the number of counts accumulated, enter the counts value for this parameter. Provided this number of counts is reached in each detector before the counting time expires, this parameter will terminate counting. If however the counting time expires first it will terminate counting even though the max. counts limit has not been reached. The maximum and default value for this parameter is 99999999.

3.5.3 Labels

If the isotope you are using is I-125 just press the down arrow key. If you use another isotope change the isotope with the "left" arrow key or by pressing E. The isotope list is shown in chapter 9 Specifications.

To change the contents of this isotope list go to the SYSTEM mode and the Isotopes parameter, see charpter 7.3 in this manual.

If you want to do dual label counting select the second isotope with the right arrow key and press E or press E to get the label selection menu.

3.6 Output

3.6.1 Default output

In CPM mode there is a default format for output and this cannot be changed. The items displayed and printed are:

Sample position, rack number, detector number, batch number, counting time, counts, CPM and CPM error.
In addition there are the following:

3.6.2 Start time

The exact time (to the nearest 1/10 second) when measurement of each sample was started can be included in CPM printouts.

If this starting time output has been selected, a field named "CLOCK" appears at the right end of the CPM printout.

The measurement start time field can be enabled and disabled in the following way.

Set the SYSTEM parameter Diagnostic output | Print meas. start time" to "Yes" to enable the field and to "No" to disable it.

3.6.3 Run ID

Each time a batch of samples is run it is given a run ID. This will be printed at the beginning of the CPM results. The run ID is specific for each protocol. This enables you to distinguish multiple runs of the samples with the same protocol ID.

3.6.4 Dead time factor

The Dead time factor can be included in the CPM printout. To do this, set the SYSTEM parameter "Diagnostic output | Print dead time factor" to "Yes". Dead time factor is explained in 10.1.3.

3.6.5 Bad spectrum results

Note: the output values for counts and CPM are 0 if the spectrum is bad e.g. if the coincidence peak is missing or too small for I-125.

3.7 Leaving the editor

Press "EXIT" to leave the editor A choice of three possibilities is shown:

Save changes and exit. Saves the parameter setting on file and leaves the editor.

Quit and ignore changes. Leaves the editor without saving the changes.

Edit. You return to the editor to do further editing.

3.8 Running an assay

Make sure that the first cassette has its correct protocol selection ID and that the appropriate protocol is stored in the instrument. For more information about the "ID system" see section 2.1.5. Put a "STOP" ID on the last sample rack to be counted or use a STOP rack or a totally empty rack in order to stop the instrument automatically.

Load racks, starting with the right-hand conveyor lane.

Start counting by pressing the START key.

The instrument will now count all the loaded samples and the WIZARD CPM software will print out the CPM results.

During counting you can see the live results on the live display as described in section 2.4.1 by selecting the menu item "Show CPM results" to see the output from WIZARD on the display.

The counting will stop automatically when a "STOP" rack is found. You can also press the STOP key on the WIZARD keyboard. In that case the following text will appear:

```
Continue
End assay, continue
End assay, clear conveyor
```

Depending on whether you want the next assay to be counted or all counting to stop, select "End assay, continue" or End assay, clear conveyor" respectively. To override the stop instruction and continue counting select "Continue".

3.9 The FILES function

Most FILES functions are not available in CPM mode, (they are in grey), they are only available in RiaCalc WIZ, however the following three are:

3.9.1 Spectra

Store spectra - this is for information only. It tells if spectra can be saved or not and if they are to be sent to an output device or not. The actual settings can be made in SYSTEM | Operation mode | Store assay spectra. See there for more details.

Operation - this allows you to handle the files of spectrum data. The options are:

Delete - delete the spectrum file data

Save to disk - save the spectrum information on the program disk in WIZARD

Send to PC - send the spectrum information to a PC that is connected to WIZARD

3.9.2 GLP data

This allows you to handle the GLP data obtained in GLP test normalization. There are four options, some of which lead to other options:

View - allows you to view the GLP data

Delete - delete GLP data

Criteria - allows you to select the warning limits for different types of GLP data. You can select:

Isotope - you can select the isotope type from those for which a GLP test normalization has been enabled

Item - many items appear from which you can select the one which you want the GLP data: PEAK, BGRD, EFFIC, RESOL, EFFICIENCY, COVERAGE, CHI-PROB, WIN-CPM, TOTAL CPM

Print criteria - select the isotope for which you want the criteria to be printed out.

3.9.3 Waste log file

A waste log file that contains the total CPS and DPS values of all measured assays, isotope normalizations and GLP TEST measurements can be printed or stored on a datalogger disk.

The file can also be deleted or sent via the PC port to an external PC.

The waste log can contain approximately 700 entries, after this the older half of the entries is deleted and the log starts growing again.

For isotope normalization and GLP test, TOTAL CPS is the average corrected sample activity in the isotope counting window in all detectors used. It is corrected for dead time, background activity and isotope decay. TOTAL DPS is the average corrected sample activity in the open window divided by Efficiency% which is a SYSTEM parameter that appears after you select Isotope and then the isotope name.

For assay measurement TOTAL CPS is the sum of all printed corrected CPM values of measured tubes in the assay converted to CPS. The DPS value is obtained by dividing the printed corrected CPM (converted to CPS) by the actual coverage of the isotope counting window and by the parameter Efficiency% refered to above. TOTAL DPS is thus the sum of all DPS values of measured tubes in the assay.

4 Operation with internal RiaCalc WIZ

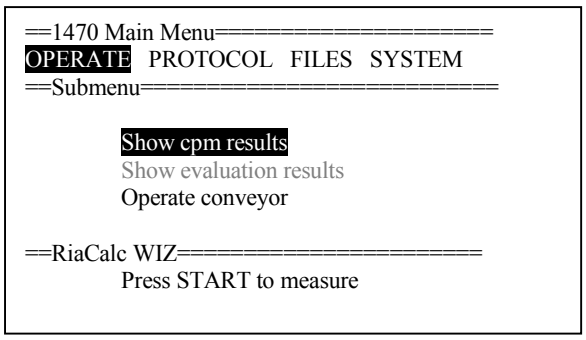
4 Operation with internal RiaCalc WIZ

4.1 Introduction

The instructions here describe the routine operation of WIZARD when it is running the internal RiaCalc WIZ software.

4.2 Start up

1. Switch on the printer; this should have already been connected to the counter.
2. You can put the data disk named 1470 Datadisk into the WIZARD disk drive. The name label should be facing upwards.
3. Switch on WIZARD. After about 3 min. the display will show:



4. Check the time and date by selecting SYSTEM mode and DATE. If it is not correct then give the correct value (See "System | Time & Date setting"). Return to the main display by pressing EXIT.

5. Make sure that the counter is in the right mode i.e. that the text RiaCalc WIZ appears near the bottom of the screen, see the figure above. If it does not then select SYSTEM and then Operation mode. The evaluation must be "RiaCalc WIZ".

4.3 Normalization

Make sure that WIZARD has been normalized for the isotope you are going to use in your measurement. See chapter 6 "Normalization".

4.4 Protocol editing

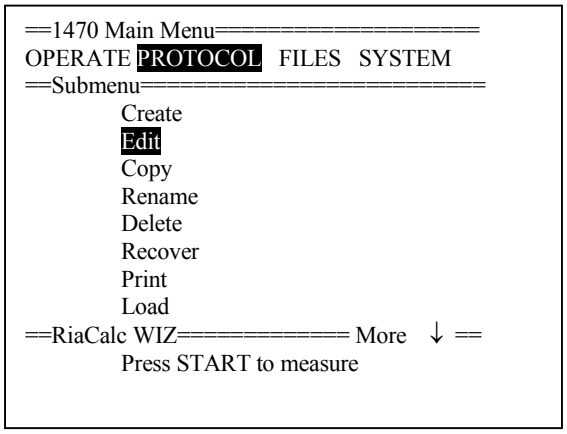
4.4.1 What is a protocol?

The conditions controlling samples e.g. counting time, curve fitting method etc. are stored in a protocol.

Before counting can be done, a protocol must exist. Each protocol must have a name and an ID number. This number allows the protocol to be called into use by means of a barcode label. To access protocol file handling you must first select the PROTOCOL option on the display.

4.4.2 Protocol operations

There are several commands available to use with a protocol, the most common ones are CREATE (making a new protocol) and EDIT (change the contents of an existing protocol). The complete list that appears after you select PROTOCOL is as follows.

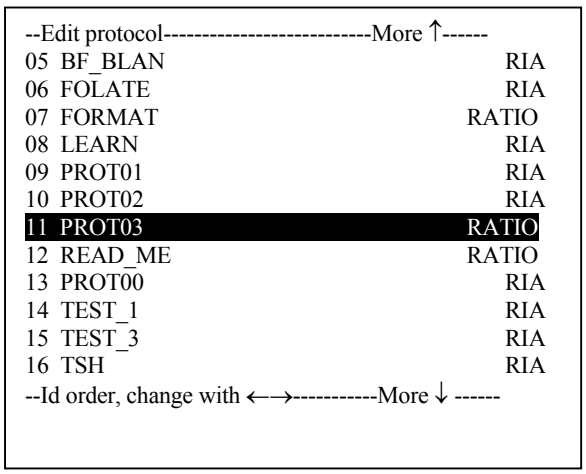


Create

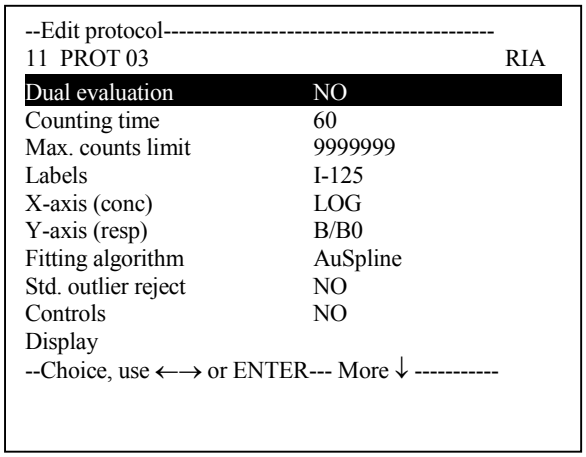
Select CREATE and press E. Give the protocol name. There is a default name of the form "Protnn" where "nn" is the protocol number. If you want to change this name you must use the external keyboard unless you only use numbers for the name. A maximum of eight characters can be given. Select an unused ID number (between 1 to 99). Give the assay type, RIA or IRMA. If you just want to get CPM results or you do screening, select RATIO. Press E. The protocol parameters can be set in a similar way to those in editing, see below.

Edit

Select Edit and press E (Enter). Select the protocol by name from the list of protocols available and press "E".



You may now change the parameters. You can move up and down in the parameter list by means of the UP and DOWN arrow keys. See section 4.5 for details of parameters available in a protocol.



Copy

An existing protocol can be copied so as to create another protocol with the same contents.

Select Copy and press E. Select the protocol to be copied. Give the name the new protocol is to have. You may also give an ID number. Select "do copy" and press "E".

Rename

Select this option to give a new name to a protocol. Select the protocol to be renamed from the list of protocols. Give it a new name and/or ID number. Select "do rename" and press "E".

Delete

Select Delete and press E. Then select the first protocol to be deleted. Next select "do delete" and press "E". The protocol and any associated data will then be deleted.

Recover

Deleted protocols can in most cases be recovered by selecting this option. They are saved in the protocol index area. There is room on the system disk for 100 deleted and active protocols. The larger the number of active protocols the fewer deleted protocols can be saved. If there is no room, the oldest deleted protocol will be permanently removed to make room for a newly created one. No protocol with the same name should have been deleted later than the one you want to recover.

Print

With this function you can print the contents of a single protocol or the protocol index according to the selection you make.

Isotope names are included in the protocol printout. For dual label protocols the code numbers for both isotopes are printed separately, e.g. 1; I-125 + 2; Co-57.

Load

You can load a single protocol or all protocols from an external microfloppy disk. Such protocols will have either been saved there using the Save function (see below) or will be from MultiCalc. In addition to the protocols themselves any associated data will also be loaded according to what you select as follows:

Normal Load will give you:

1 Protocol files
2 Controls files
3 Trends files

Extended Load will in addition give you:

4 Standard curve files
5 Data files
6 Results files

Note: if you load a new protocol when a previous one with the same name or ID number exists in the instrument, you have the choice of either renaming the protocol to be loaded or deleting the previous protocol.

Save

This function allows you to transfer files from WIZARD to an external microfloppy disk for storage. Subsequently these files can either be loaded back into WIZARD or into MultiCalc. As with Load there are two options Save or Extended Save. In the former case only protocols, controls and trends will be saved. In the latter case standard curves, data, precision profiles, input files and results files will be saved also.

Purge

Deleted protocols and associated data files can be permanently erased from the instrument hard disk with this function.

Password

When a protocol is created, you can give a two-character password. All characters are allowed in the password and the password is case sensitive. If you do not give a password at this point, then the protocol can be edited, renamed, overwritten when a new protocol with the same name or ID number is loaded, and deleted without giving any password. Otherwise the password is needed to do these operations. A protocol can always be copied, restored, saved and purged without giving the password. With rename it is possible to change or remove the password.

Passwords are retained even if power is turned off. The password feature can be disabled by removing the file C:\PASSWORD from the instrument hard disk. To enable it again, type ECHO aa > C:\PASSWORD at the DOS

prompt to recreate a password file and boot the instrument. (You can enter DOS by installing the installation disk to the disk drive and by restarting the instrument.)

4.5 Parameters available

4.5.1 Dual evaluation

If you do normal single isotope RIA/IRMA select NO. If you would like to measure samples labelled with two isotopes in the same vial select YES. See "Dual label counting" for more details.

4.5.2 Counting time

Give the counting time in seconds. All samples are counted for this time.

4.5.3 Max. counts limit

If you want to terminate counting on the basis of the number of counts accumulated, enter the counts value for this parameter. Provided this number of counts is reached in each detector before the counting time expires, this parameter will terminate counting. If however the counting time expires first it will terminate counting even though the max. counts limit has not been reached. The maximum and default value for this parameter is 99999999.

4.5.4 Labels

The default selection is single label I-125. If you want a different isotope, press E, then specify if you want dual label or not then select the isotope(s) from the list of isotopes, see chapter 8.2 Specifications.

To change the contents of this isotope list go to the SYSTEM mode and the Isotopes parameter, see charpter 7.3 in this manual.

If you want to do dual label counting you must also select the second isotope with the right arrow key or press E to get the label selection menu.

4.5.5 X-axis (conc)

Choose the scale of the X-axis of the standard curve. The choices are linear, logarithmic or linear/logarithmic, i.e. linear fitting and logarithmic plotting. Logarithmic scales are commonly used.

4.5.6 Y-axis (resp)

Choose the scale of the Y-axis from the list:

```
CPM          counts per minute
B(bound)     CPM - B(blank)
B/B0         (CPM-BLANK)/(BLANK-REFER)
LOGIT        ln (r/(1-r)), r=B/B0
LOG_B        lg (CPM-BLANK)
B/T          (CPM-BLANK)/(TOTAL-BLANK)
B0/B         inverse of B/B0
T/B          inverse of B/T
B/F          r/(TOTAL-r), r=(CPM-BLANK)
F/B          inverse of B/F
PROGR        customized response
PROG/LOG     PROG with logarithmic coordinates
```

In this list B(bound) is the response value, B0 is the reference sample's response value, with a BLANK (BLANK = NSB = Non Specific Binding). The commonly used responses are B/B0 and LOGIT. T is the total sample response value. In RIA it is presumed that T >B0 >X >BLANK where X is the response of any unknown or control value.

4.5.7 Fitting algorithm

Choose the fitting algorithm for the standard curve from the list:

```
LinInt      linear interpolation
LinUwReg    linear unweighted regression
ParUwReg    parabolic unweighted regr
CubUwReg    cubic unweighted regression
LinWgReg    linear weighted regression
ParWgReg    parabolic weighted regr
CubWgReg    cubic weighted regression
InSpline    interpolated spline
AuSpline    auto. smoothed spline
SmSpline    manually smoothed spline
```

4.5.8 Std outlier reject

Select NO if there is to be no check for outliers but if you select CONDITION then two more parameter lines appear:

```
Diff. from mean 20(%) and 400
Diff. from curve 10(%)
```

The first specifies that an outlier will be rejected if its percentage difference or the absolute difference from the mean of the replicates equals or exceeds the value given here. The second uses a standard curve as a reference instead of the mean. You can also specify whether the larger or smaller difference of two replicates is to be the deciding factor in the outlier rejection.

4.5.9 Curve edit halt

Choices are:

```
YES         halt for curve edit
NO          do not halt for curve edit
REF.CURVE   evaluate using reference curve
```

HALT = YES causes halt, meaning that the evaluation is suspended after the standard curve until accepted by the user. The third choice does not cause halt but uses the stored reference curve for evaluation.

4.5.10 Controls

If you do not want to use control samples select NO. If you select YES then the following parameters appear:

Field For control samples normally the concentration of each control is selected, however it is possible to select a different quantity and to specify whether it applies to individual controls, average values or averages of dilution series averages.

The next three lines allow target values to be set for the three types of controls: **Low, Medium** and **High**. followed by the additional controls **Control4, Control5** and **Control6**. In each case you can give a target value for the control, the upper or lower 2SD limit or both plus and minus 2SD limits.

List This parameter lets you specify a list of controls and the number of patients between controls, e.g.

```
<start of assay>
LOW CONTROL
10 SAMPLES
MEDIUM CONTROL
10 SAMPLES
```

You can set up to 12 controls of one kind (e.g. LOW) in the list. The list should be long enough to allow the evaluation of assays of varying lengths. If you want the assay to end with controls you must adjust the number of patients in the list before each assay. If you edit this protocol in an external MultiCalc, you can put your own codes in the list, but they cannot be stored.

Replicates Lets you specify a replicate value for controls (1..99), but no dilution. Alternatively, you can specify that the patient replicate and dilution from the coding part be used.

4.5.11 Saved files

You can specify which types of files are required. These protocols are generated automatically during evaluation. The choices are:

Input data	The data used for analysis. See Ria evaluation from file
Standard curve	Standard curve
Trend data	Results for trend profiles
Control samples data	Concentration results of control samples
Results data	Results for output to an external computer. This file is an ASCII file and its contents are selected on the line Result file

The default for each file type is NO (not selected).

4.5.12 Display

This parameter line allows you to specify which results are to be displayed on the screen.

4.5.13 Printer

This allows you to specifiy the results for printout. The list of items which from which selection can be made are the same as the list for the display but the actual selection for the printer may be different from that for the display.

4.5.14 Results files

This allows you to specify the contents of the Results (ASCII) file. The possibilities are the same as those for the display but the actual selection for the results files may be different from that for the display.

See the section entitled "Output editing" for a description of the types of output options available and how to select and edit them.

4.5.15 Coding

The program must know in which order RIA standards are loaded and what their nominal concentrations are. This information is supplied in the CODING. Other special samples such as Blank and Reference can also be specified.

When you are creating a new protocol there is a default coding available as follows:

```
<Start of assay>
2 BLANK
2 REFER
2 TOTAL
2 STD1 = 1
2 STD2 = 2
2 STD3 = 4
2 STD4 = 8
2 STD5 = 16
2 STD6 = 32
2 STD7 = 64
2 UNKN = 1.
```

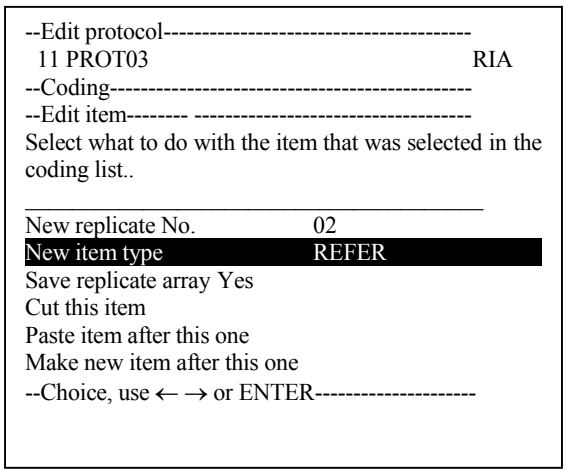
The number on the left is the replicate number and the number in the right is the nominal concentration. The replicate number preceding UNKN1 means that all unknowns have this number of replicates. In the example above all samples are duplicates.

To change a replicate number use the +/- keys to increase or decrease respectively the number of replicates.

Use the numerical keys to change concentration values.

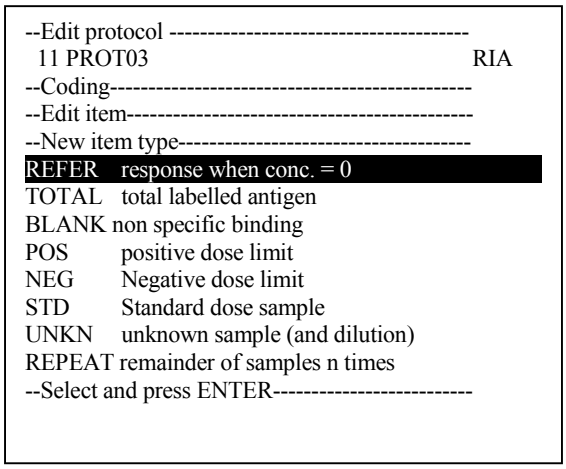
Use the delete key to remove a value.

If you want to edit these parameters or any other already created set of parameters use the cursor control keys to select the item to be edited and press Enter. This will cause a display like the following to appear:



In the example REFER was the item selected to be edited.

If you want to change the coding item type select it and press E. You will then get a list of all possible item types. Select the one you want and press EXIT twice.



The main control list will then show the new selection you have made.

The recommended order for sample tubes is:

blanks (NSB)
totals
references (zero sample)
standard samples
unknowns.

Unknowns are either patient samples or control samples in arbitrary order, the controls must be further specified on the line called Controls.

There is also a parameter "Repeat remainder of samples n times" where n can have the values 1 to 32767 or infinite. This allows you to repeat count those samples which follow the point in the list at which this parameter is placed.

4.5.16 Options

Unkn.%CV flag limit A flag can be set for unknowns by typing the %CV of the concentration above which the flag should appear. The flag is " %CV !". If you select "Not used" then the flag is disabled.

Unkn. multipl. factor The value you enter for this option will be used to multiply the unknown response values. The default value is 1. You can select "Not used" for this.

Unkn. outlier limit Give a %CV value. Any unknown which has a %CV exceeding this value is not included in the precision profile. You can select "Not used" for this.

Trends If you want trends to be printed they should be selected here. The possibilities are:

```
Slope at ed-50
Y intercept
Estimated concentration at 20% binding
Estimated concentration at 50% binding
Estimated concentration at 80% binding
Blank over Total ratio
Reference over Total ratio
Blank over Reference ratio
Minimum detectable concentration
Parallelism factor
Difference slope
Histogram
```

The default setting for each is NO.

4.5.17 Factors

You can set the values for the factors UNIT and UNIT_B to show what the units of the results are.

4.6 Output editing

4.6.1 Output media

The protocol lines Printer, Display and Results allow you to specify which results are to be printed on the paper, or displayed on the screen or saved as an ASCII file respectively. Output selection is specific for each counting protocol. The options are the same for each output medium, only the medium itself (printout device, built-in display or disk) is different. In what follows only Printer selection is explained because Display and Result selections are identical to it.

Note: 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 following corrections are applied to get the final CPM value:

- background correction (if background normalization done)
- dead time correction
- decay correction (if selected)
- spillover correction (dual label assays)

The CPM values shown in the live display are uncorrected.

4.6.2 Default or customized outputs

The program has a default set of output items (shown in the sections following) which it prints out if further instructions are not given. Therefore when you create a new protocol it is not necessary to change the printout format. However, you can choose a new type of output from a wide range of the possibilities. To alter the printout form first select Printer in the protocol and press "E". A menu will appear on the screen:

```
Printout options
Printout fields
Printout switches
Copy settings from a template.
```

Each of these selections is described below.

4.6.3 Printout options

Printout options allow certain types of output to be selected. Most of these options are plots but it is also possible to have the protocol listed as well as the protocol identifier. When you select this option, a list appears with the default selection as follows:

```
Protocol id number                  YES
Protocol                            NO
Standards in table format           YES
Std.curve in graphical format       YES
Comparison curve                    NO
Controls in table format            NO
Controls in graphical format        NO
Response error relationship         NO
Prec. prof. in table format         NO
Precision prof. in graphicalformat  NO
Trends                              NO
Histogram                           NO
```

"Protocol id number" prints the protocol name and date and "Protocol" prints the protocol contents.

Results for standards can be output as curves and/or tables of values. Results for controls can be output as plots or tables of values where the most recent control value is the first item in the table. The age of the results increases as you go down the items in the table.

Trend values are output as plots.

Precision profile output can be as tables or plots.

A plot of the response error relationship and a histogram of results are also selectable.

Select YES or NO by pressing the left or right arrow keys. Press "EXIT" when ready.

4.6.4 Printout fields

Printout fields comprises numeric values and flags. These are printed for individual samples unlike printout options which are printed for a complete assay.

Select this option and you will see a list of the default settings:

```
<Left margin of paper or display>
SEQ   sequence or tube number
PAT   patient number for unknwns.
CODE  type in coding or contr. list
TIME  counting time in seconds
CPM   counts per minute (chn.A)
CONC  concentration (chn.A)
%CV   coefficient of variation
FLAG  concentration flag (chn.A)
```

The items are printed from left to right, a single row of results for each sample. If replicate samples are employed then the average results are printed, on a separate line, after individual sample values. You can delete an item from the list or add new items to the list.

4.6.5 Deleting a printout field

Assuming for example that you want to delete the field FLAG from the list.

Select FLAG and press "E"

A menu will appear on the screen:

```
Change item type FLAG
Cut this item
Paste item after this one
Make new item after this one
```

Note: the "paste item" line will only appear if an item has previously been deleted.

Select "Cut this item" and press "E". The program will then show the new list; FLAG is not available any longer.

4.6.6 Inserting a printout field

Lets assume that you want to insert the field ETIME to the previous list, between "CPM" and "CONC".

Select "CPM" and press "EDIT"

A menu will appear on the screen:

```
Change item type CPM
Cut this item
Paste item after this one
Make new item after this one
```

Select "Make new item after this one" and press "E" twice. A list output items will appear on the screen. See the list on the previous page.

Use the arrow keys to move through the list to the item you want. e.g. in this case "ETIME" is the sixth item on the list.

Note: You can also use the following keys on the external keyboard to speed up accessing of items: Home, End, Page Up and Page Down.

Press Exit twice.

Notice that the part of the original list:

```
CPM   counts per minute (chn A)
CONC  concentration (chn A)
```

has changed to a new list:

```
CPM   counts per minute (chn A)
ETIME elapsed time in decimal hours
CONC  concentration (chn A)
```

4.6.7 Pasting a cut item

The item most recently deleted with the "cut item" instruction can then be pasted back by selecting "Paste item after this one".

4.6.8 Tabulation of output fields.

Each printout field has a maximum field length, for example "time" has a length of 5 characters. It takes this space even if the number of digits in the actual printout is less. A field is separated from the previous one by one space from the previous value. You can also define the position of the output field by giving a number called a tabulation number.

There are two approaches to tabulation: either giving an absolute position on a line in terms of the number of characters from the beginning of the line to the beginning of the field, or the number of characters between the beginning of the field and the end of the previous one. The former is called absolute tabulation and the latter relative tabulation.

The default setting is one space from the previous value.

Example: There is the selection:

```
SEQ   sequence number
PAT   patient number
TIME  counting time in seconds
CONC  concentration value
```

This is printed as follows:

```
SEQ   PAT  TIME      CONC
#### #### ##### ####.###
```

where the # mark is used here to define the field length.

Default field lengths are listed in section 4.10.

Assume now that you want the SEQ printout field to start from position 15 and that there should be 5 spaces between "TIME" and "CONC". Proceed as follows:

Select sample result output. Then select

```
<Left margin of paper or display>
```

and press "E". Select

```
"Make new item after this one"
```

and press "E" two times. Select

```
"MOVE TO"
```

A menu will appear on the screen:

```
Change item type  MOVE TO
Change item value       10
```

Use the numeric part of the keyboard to change the item value to "15".

Press "EXIT". You will see a new print selection:

```
<Left margin of paper or display>
15 <move to this column position>
SEQ sequence or tube number
```

Select

```
TIME counting time in seconds
```

and press "E". Select

```
"Make new item after this one"
```

and press "E" two times. Select

```
MOVE RIGHT move to the right
```

and press EXIT. A menu will appear on the screen:

```
Change item type  MOVE RIGHT
Change item value 10
```

Use the numeric part of the keyboard to change the item value to 5, then press EXIT.

4.6.9 Printout switches

This selection offers you further choices to modify the printout format. In its initial setting both individual results and average results are printed out. You may switch off or on values shown in the list by selecting NO or YES respectively:

```
Individual standard values    YES
Replicate standard averages   YES
Dilution standard averages    YES
2-fields after standards      YES
Individual unknown values     YES
Replicate unknown averages    YES
Dilution unknown averages     YES
2-fields after unknowns       YES
Individual control values     YES
Replicate control averages    YES
Dilution control averages     YES
```

`Dilution averages` effects only if samples are divided into separate dilution groups. 2-fields are those which would be the same for every item. By means of a switch you can tell the program to only output such fields once after standards or unknowns respectively.

4.6.10 Copy settings from template

This parameter is to allow you to reuse a particular set of sample result outputs without having to enter each one individually. You have the choice of selecting the Display sample result output or the output saved in one of three templates:

`Single label RIA/IRMA output`

```
i.e. SEQ PAT CODE TIME CPM CONC UNIT %CV FLAG
```

`Dual label RIA/IRMA output`

```
i.e. SEQ PAT CODE TIME CPM CPM_B CONC CONC_B UNIT %CV %CV_B FLAG
```

```
Single label RATIO
i.e SEQ PAT CODE TIME CPM
```

4.7 Leaving the editor

Press "EXIT" to leave the editor A choice of three possibilities is shown:

Save changes and exit. Saves the parameter setting on file and leaves the editor.

Quit and ignore changes. Leaves the editor without saving the changes.

Edit. You return to the editor to do further editing.

4.8 Running an assay

Make sure that the first cassette has its correct protocol selection ID and that the appropriate protocol is stored in the instrument. For more information about the "ID system" see section 2.5. Put a "STOP" ID on the last sample rack to be counted or use a STOP rack or a totally empty rack in order to stop the instrument automatically.

Load racks, starting with the right-hand conveyor lane.

Start counting by pressing the START key.

The instrument will now count all the loaded samples and the RiaCalc WIZ software will evaluate the final results and output them as determined in the counting protocol.

During counting you can see the live results on the live display as described in section 2.4. Alternatively you can select the menu item "Show evaluation results" to see the output from RiaCalc WIZ on the display.

The counting will stop automatically when a "STOP" rack is found. You can also press the STOP key on the WIZARD keyboard. In that case the following text will appear:

```
Continue
End assay, continue
End assay, clear conveyor
```

Depending on whether you want the next assay to be counted or all counting to stop, select "End assay, continue" or End assay, clear conveyor" respectively. To override the stop instruction and continue counting select "Continue".

4.9 The FILES function

4.9.1 Introduction

RiaCalc WIZ can produce different types of files. This function in the main menu called FILES allows you to perform operations on and with these files. In CPM mode these files are not produced and in the external MultiCalc mode the same files are handled by MultiCalc on the external computer not on WIZARD.

There are five types of files which can be handled with this function:

```
Input files
Standard curves
Results files
Controls
Trends
```

In order for any of these files to be produced you must select the ones you want. You do this with the Saved files parameter in a protocol, see section 4.5.11.

4.9.2 File operations submenu

When you have selected a file a "file operations submenu" will appear. This includes the following items for each file type:

Operation This shows the currently selected operation. If you want a different operation then highlight it and press E. A list of alternative operations will appear. The actual files operation available for each file type are described in later sections.

The actual operation you select will determine what the remaining lines of the file operations submenu are. In every case at least the following lines will appear:

Protocol Selects the protocol to which the file to be operated on belongs.

Run id. Specifies the run id. number of the file that is to be operated on. This run id. is selected from a list of free ids and is not entered with the numeric keys. This is to prevent duplication of run ids.

Do operation The submenu for all operations end with this line. Selecting it and pressing E actually starts the operation defined in the previous lines. If this line is displayed in subdued colour, it means that the selected operation cannot be done.

You can also do the operation by first pressing the EXIT key and then selecting the menu item **'Do specified operation**' or alternatively by pressing the **Ctrl-S** key. In these cases the menu selection bar can be on any menu item.

To return to the main menu without doing the operation first press the EXIT key and then select the menu item **'Quit, do not operate'** or alternatively press the **Ctrl-C** key. You can return to the main menu directly by pressing only the EXIT key if you have invoked the 'Do operation' and the parameters that specify the operation have not changed after that.

4.9.3 Input file

This comprises data which can be evaluated by RiaCalc WIZ. This data can be either previous output from RiaCalc WIZ or data entered by the user. The main point is that it is data in a format acceptable to RiaCalc WIZ. The functions available for input files are

Create Creates a new file and lets you edit it.
Edit Lets you edit a file.
Copy Lets you copy a file.
Print Lets you print a file.
Evaluate Lets you evaluate a data file.
Delete Lets you delete a file.

If the selected operation is **Create** then the line **Counting time** will appear in the file operation submenu. In principle each measurement result in a data (input) file has its own measurement time. However, in practice the measurement times for all samples in an assay are often the same. When a new data file is created, the counting time given here is assigned to all measurements in that file. This makes the creation of data files easier.

Edit An input data file consists of a series of records, one for each stored measurement result. Each record contains the measurement time, measured counts, CPM's and their errors for one or two channels.

This editor shows only the CPM-values and the measurement time of the first measurement in the file. When the data file is saved, this measurement time is assigned to all records in the file and for each record the counts and error fields are calculated based on the measurement time and the CPM value(s).

You can move up or down one page by pressing the PgUp or PgDn keys respectively, and to the beginning or end of the file by pressing the Home or End keys respectively. To edit a measurement or to jump to a specified line number press the E key.

If you have created a new data file and have not added any CPM's to it yet, try the following shortcut. Use only the E key to move from one menu or menu item to the next appropriate one when you enter new CPM values.

The menu items which may appear during editing are:

Make new line after this one You can insert a new line after the currently selected one by invoking this menu item.

Chn A cpm Enter here the CPM value for channel A. If CPM fields for both channel A and channel B are empty, it means that the measured tube was missing.

Chn B cpm Enter here the CPM value for channel B if you want to use it.

Cut this line If you invoke this menu item, the line selected for editing is deleted to a "cut buffer". It can be later pasted by choosing the menu item 'Paste item after this one'.

Paste line after this one If you invoke this menu item, the previously deleted line is pasted from the cut buffer after the line that was selected for editing.

Jump to a line If you invoke this menu item, you are asked to give the number of the line to where you want to jump. Then you must press the EXIT key.

When you have finished editing press the EXIT key.

In the case of the **Copy** operation the file operations submenu includes the following additional lines:

Copy to protocol Specifies to which protocol the copy of the file is to be attached.

Copy to run id. Specifies the run id number that the copy of the file is to have. If the protocol is the same as for the copied file then the run id. must be different. When you access the list of possible run ids. then you will see that used run ids. do not appear on the list so you cannot duplicate them.

When data has been saved in an input file it can be **evaluated** again. In addition to editing the data before evaluation you can change the curve fitting method during evaluation if you have selected Halt for curve edit in protocol setting.

4.9.4 Results files

Results files comprise data but they are in ASCII format and are intended for output to an external PC or mainframe which is able to handle the widely accepted ASCII format.

View	Lets you view (but not edit) a file.
Print	Lets you print a file.
Send to PC	Lets you send a file to PC.
Delete	Lets you delete a file.
Save to disk	Lets you save result files in the \EVAL directory of a micro-floppy disk.

When you have a View a result file you can move up or down one page by pressing the PgUp or PgDn keys respectively and to the beginning or end of the file by pressing the Home or End keys respectively.

Note: the view mode only shows the first 40 characters of any line in the Result file and does not allow any editing of the values.

4.9.5 Standard curve files

Standard curves are produced in RIA and IRMA and can be viewed with this function.

Note: For standard curve files the run id. designation "REF" means the reference curve.

View	Lets you view a file.
Delete	Lets you delete a file.
Set reference curve protocol.	Lets you set a copy of the selected standard curve as the reference curve for this

Standard curves can be **viewed** and if required **edited**. First you must select the curve you want to view. When it is displayed on screen four functions appear at the bottom of the display:

Edit (1) Press key 1 to start editing.

Print (2) Press key 2 to print the curve.

Undo (C) Press Clear (backspace) to clear all the changes made in editing and to restore the original curve to the display.

Exit (EXIT) Press EXIT to quit from curve viewing.

The editing operations are as follows:

Standard selection. Use the left/right arrow keys to select a particular standard on the curve

Replicate selection Use the up/down arrow keys to select a particular replicate point for the currently selected standard.

Delete (1) Pressing 1 deletes the currently selected replicate point. The point which is in the form of a cross will be replaced by a square. Although this point is still shown on the screen it will not be taken into account in curve fitting. If an inserted point is deleted then it will be taken completely away.

Move (2) Pressing 2 followed by the up/down arrow keys allows you to change the vertical position i.e. response value of the currently selected replicate.

Insert (3) After pressing 3 put the cursor at the point where you want a new point to be and press E. The new point will be in the form of a cross and will be taken into account in curve fitting.

Undo (C) Pressing Clear (Backspace) will undo the effects of the previous editing.

Fit (E) When you press E the existing curve points will be fitted and the selection bar at the bottom of the display will revert to the Edit, Print, Undo, Exit options. You must use this option to exit from Editing.

4.9.6 Control files

These are produced as part of the quality control operations of RiaCalc WIZ. They can be viewed or deleted with this file function.

Control type This allows you to specify the control type that is to be operated on. In principle there are six types: LOW, MEDIUM, HIGH, CONTROL4, 5 and 6. However the actual types which appear for viewing are only those which were selected in protocol setting.

When you have selected the type and "do operation" the control plot will appear allowing you a number of functions for editing the plot. These functions are the same as for trend plots and are described below.

4.9.7 Trend files

Trend files can also be viewed or deleted after they have been produced.

Trend type Specifies the trend type that is to be operated on. This list is fixed because unlike controls it is not affected by the protocol settings. The options available are:

```
Slope at ed-50
Y intercept
Estimated concentration at 20% binding
Estimated concentration at 50% binding
Estimated concentration at 80% binding
Blank over Total ratio
Reference over Total ratio
Blank over Reference ratio
Minimum detectable concentration
Parallelism factor
Difference slope
Histogram
```

4.9.8 Control and trend plot editing

The functions available for editing control and trend plots are:

Left/right arrows for selecting the value to be edited.

Up/down arrows for defining how many steps to move each time the left/right arrow is pressed.

WinL(1) Press 1 to set the left limit of a window.

WinR(2) Press 2 to set the right limit of a window.

Calc(3) Press 3 to recalculate values after making changes to a plot.

Del/Undel(4) Press 4 to delete a point. The + mark will change to a square and the point will not be included in calculations. Pressing 4 when the cursor is on a deleted point will cause the point to be treated again as a normal point.

Era(5) Pressing 5 allows points within a window to be *permanently* removed. They cannot be returned.

Print(6) Press 6 to printout the current plot.

When you exit from plot editing (done by pressing E) the changes you have made will be saved.

4.9.9 Spectra

Store spectra - this is for information only. It tells if spectra can be saved or not and if they are to be sent to an output device or not. The actual settings can be made in SYSTEM | Operation mode | Store assay spectra. See there for more details.

Operation - this allows you to handle the files of spectrum data. The options are:

Delete - delete the spectrum file data

Save to disk - save the spectrum information on the program disk in WIZARD

Send to PC - send the spectrum information to a PC that is connected to WIZARD

4.9.10 GLP data

This allows you to handle the GLP data obtained in GLP test normalization. There are four options, some of which lead to other options:

View - allows you to view the GLP data

Delete - delete GLP data

Criteria - allows you to select the warning limits for different types of GLP data. You can select:

Isotope - you can select the isotope type from those for which a GLP normalization has been done

Item - many items appear from which you can select the one which you want the GLP data:

PEAK, BGRD, EFFIC, RESOL, EFFICIENCY, COVERAGE, CHI-PROB, WIN-CPM, TOTAL CPM

Print criteria - select the isotope for which you want the criteria to be printed out.

4.9.11 Waste log file

A waste log file that contains the total CPS and DPS values of all measured assays, isotope normalisations and GLP TEST measurements can be printed or stored on a datalogger disk.

The file can also be deleted or sent via the PC port to an external computer.

The waste log can contain approximately 700 entries, after this the older half of the entries is deleted and the log starts growing again.

For isotope normalization and GLP test, TOTAL CPS is the average corrected sample activity in the isotope counting window in all detectors used. It is corrected for dead time, background activity and isotope decay. TOTAL DPS is the average corrected sample activity in the open window divided by Efficiency% which is a SYSTEM parameter that appears after you select Isotope and then the isotope name.

For assay measurement TOTAL CPS is the sum of all printed corrected CPM values of measured tubes in the assay converted to CPS. The DPS value is obtained by dividing the printed corrected CPM (converted to CPS) by the actual coverage of the isotope counting window and by the parameter Efficiency% referred to above. TOTAL DPS is thus the sum of all DPS values of measured tubes in the assay.

4.10 Selectable outputs

```
MOVE TO                        Move to this column
MOVE LEFT                      Move to the left
MOVE RIGHT                     Move to the right
NEW LINE                       Move the rest of the text to a new line
Vert. Vectors                  Print sample output result items (e.g. POS, TIME) vertically
RACK ### 1                     Rack number 3 digits for both groups, no average
DET ## 1                       Detector number for both groups, no average
SEQ #### 111                   Sequence or tube number
PAT #### 0,111                 Patient number with both averages, unknowns only
TIME ##### 1                   Counting time in seconds
ETIME ####.## 1                Elapsed time in decimal hours
COUNT ####### 11 $             Total counts and first average
COUNT_B ####### 11 $           Total counts and first average on B-channel
CPM ######.# 11 $              Corrected CPM* and first average
CPM_B ######.# 11 $            Corrected CPM* and first average on B-channel
CONC ####.### 111 $            Concentration with both averages
CONC_B ####.### 111 $          As above for channel B
%CV ##.## 011                  Coefficient of variation as a percentage, only averages
%CV_B ##.## 011                As above for channel B
%CVE ##.## 0,01                %CV from reference precision profile, average for only unknowns
%CVE_B ##.## 0,01              As above for channel B
CODE 111                       Code text from coding and control list
FLAG "     " 0,111             Concentration flag, all values but only unknowns
FLAG_B "     " 0,111           As above for channel B
BLANK ######.# 111             BLANK cpm, valid after BLANK in coding, all values for both groups
BLANK_B ######.# 111           As above for channel B
REFER ######.# 111             As above for REFER
REFER_B ######.# 111           As above for channel B
TOTAL ######.# 111             As previously for TOTAL
TOTAL_B ######.# 111           As above for channel B
NEG ######.# 111               As above for negative controls
NEG_B ######.# 111             As above for channel B
POS ######.# 111               As above for positive controls
POS_B ######.# 111             As above for channel B
RESP ######.# 11 $             Programmable response
RESP_B ######.# 11 $           As above for channel B
STS "   " ## 0,111             Numerical flag number. These are:
                               1 = OUT, 2 = >STD, 3 = <STD, 4 = ?amb, 5 = >>STD, 6 = <<STD,
                               7 = %CV!, 8 = >%CV, 9 = >>%CV
 STS_B "   " ## 0,111          Numerical flag number channel B
 GROUP "GR" ## 0,11            Group number of multiple UNKN-coding
 REPL "RP" ## 0,1              Replicate of individual sample for unknowns
 SAMPLE "SPL" #### 0,111       Sample No. (includes controls) for all values but only unknowns
DRESP #####.# 11 $             Response error
DRESP_B #####.# 11 $           Response error for channel B
DILF ###.## 0,11               Dilution factor
REMARK ############### 0,111   Remark
SEQA #### 1                    Application sequence
ROW ### 111                    Row number 1 = individual, 2 = average, 3 = second average
DATE ######## 111              Date
CLOCK ######## 111             Time
CLASS ###.# 111 $              E.g. 1 if CONC = STD 1 or 2.5 if CONC = (STD 3 + STD 2)/2
CLASS_B ###.# 111 $            As above for channel B
UNIT                           (Defined by the user)
* Corrections in CPM are dead time, decay, background, crosstalk and spillover.
$ These fields are used in statistical calculations e.g. a mean or average is calculated.
# This shows the format of the fields. The numbers after fields are the default switch settings.
See section 4.6.9.
```

5 Operation with external MultiCalc

5 Operation with external MultiCalc

5.1 Introduction

The instructions here describe the routine operation of WIZARD when it is connected to a PC running MultiCalc software. Since there are many ways to connect the counter the following list should not be taken as the only possible. In the example a single WIZARD is connected to a PC running MultiCalc laboratory data management software. The operation of MultiCalc is explained in the MultiCalc User Manuals.

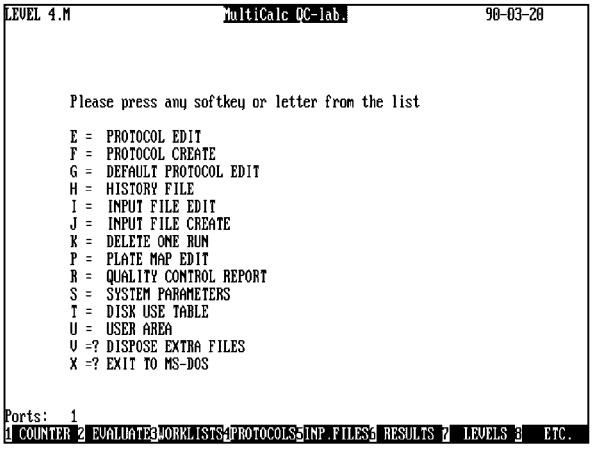
Note. the MultiCalc communication protocol should be the one designed to work with the version of WIZARD you are using. This will be found on the WIZARD program disk and should be copied from there during installation.

5.2 Start up

1. Switch on the printer. (Note: the printer must be connected to the PC).

2. Make sure that MultiCalc has been installed on your PC. If it has not then follow the instructions in the MultiCalc manual.

To start MultiCalc when the DOS prompt on your PC shows e.g. C: type in the command WIA and press Enter. After a short while the PC display will show the MultiCalc main menu.



At the bottom of the PC screen you will see eight softkeys labelled F1 to F8 corresponding to the function keys on your computer keyboard also labelled F1 to F8. A softkey changes its function according to the step in the program you have reached. The actual function of each key at any time is shown on the bottom of the PC screen. The eight softkeys which have functions in the main menu are:

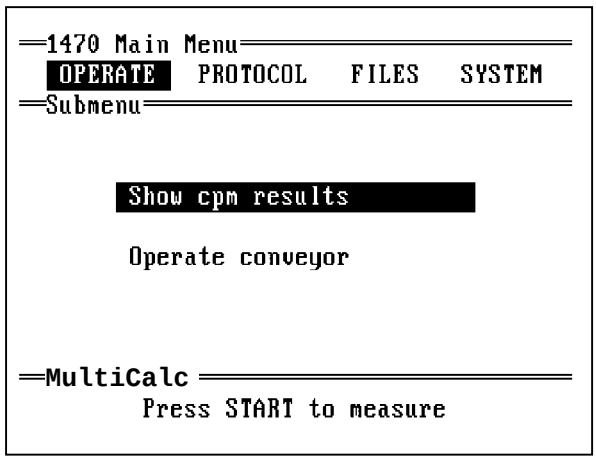
```
F1 COUNTER
F2 EVALUATE
F3 WORKLISTS
F4 PROTOCOLS
F5 INP.FILES
F6 RESULTS
F7 LEVELS
F8 ETC.
```

In addition to the eight softkeys the keys F9 and F10 have fixed functions. Function key F9 is always EXIT and F10 is HELP.

As an alternative to using softkeys there are quick commands listed on the MultiCalc main menu. By pressing the appropriate letter you can make the program go directly to a particular function instead of by pressing one or more softkeys.

MultiCalc supports different levels of operation e.g. learning or advanced, with automatic or optional helps etc. Choose the level that most suits you. You do this by pressing the softkey F7 (=LEVELS) and then the appropriate LEVEL softkey (F1-F5).

3. Check that microfloppy named 1470 Datadisk is in the WIZARD disk drive. If not, place it there so that the name label is upwards. Switch on WIZARD. After about 3 min. the display will show:



Check the time and date by selecting SYSTEM mode and DATE. If it is not correct then give the correct value (See "System | Time & Date setting"). Press EXIT to return to the main display.

Make sure that the counter is in the right mode, i.e. that the text MultiCalc is showing in the lower part of the screen, as in the example here. If it is not select "SYSTEM" and then the operation mode. The RIA evaluation must be "MultiCalc" .

Now you are ready to start "MultiCalc assay protocol operation" of WIZARD.

Note: The HELP command given to the instrument in MultiCalc mode from a terminal connected to the PC port (e.g. MultiCalc terminal) gives extensive information about communication with external MultiCalc. This information is also in the file COMMHELP.TXT in the instrument hard disk.

5.3 MultiCalc protocol

MultiCalc is controlled by "protocols". A "protocol" is a list of parameters which need to be set or given values e.g. protocol name or counting time.

A MultiCalc protocol is used mainly to define the way the data from the counter is to be handled e.g. items to be output, quality control curves to be plotted etc. These parameters are described in detail in the MultiCalc User manuals. The line 3 parameter is called "Measuring parameters". This is where you give the isotope number. See 5.4.3 Isotope selection and 9.8 Isotopes defined for 1470 WIZARD. You just select the number of a suitable isotope from the list of those available. Make sure that the one you select has been normalized. If it has not, you need to make one as described in Part 6 Normalization.

5.4 Editing a MultiCalc protocol

Starting from the MultiCalc main menu and depending on whether you want to edit an existing protocol or create a new one you can either press the letters E for protocol edit or F for protocol create or you can press softkey F4 (PROTOCOLS) followed by F1 (EDIT) or F2 (CREATE). You must then select a protocol number from the list of protocols. In the case of EDIT you must select an existing protocol but in the case of CREATE you select must give a new protocol and number. Usually this number will be the next one in the number sequence, but you can give a different number if you want. You must then select the Technology which in this case is Gamma.

If you create a protocol you must then specify the protocol type, RIA, IRMA or RATIO. This choice determines the type of parameter list you get.

The major parameters in the protocol are mentioned below. They are described in detail in the MultiCalc User Manual under "Protocol parameter setting".

5.4.1 Single/Dual label selection

01 DUAL LABEL Select YES if you want to count dual label samples otherwise select NO.

5.4.2 Length of counting

02 COUNTING TIME, MAX COUNTS Give the counting time in seconds and optionally the cut-off value for the number of counts accumulated. A larger count value than this number stops the counting even if the counting time limit has not been reached.

5.4.3 Isotope selection

03 MEASURING PARAMETERS This is where you select the isotope and the parameters which define measurement using that isotope. When you select parameter line 3, three softkeys appear:

```
F1 I-125 F2 Co-57 F3 I + Co
```

F1 and F2 correspond to isotope numbers 1 and 2. F3 is for dual label counting with iodine and cobalt. Any other isotope must be selected by typing the number, see the default list in chapter 9 Specifications but also the following section about limitations.

5.4.4 Available isotopes

The used isotope(s) are given in the MultiCalc assay protocol parameter “03 MEASUREMENT PARAMETERS”. We call this parameter here “isotope number”. Its range is 1..99 and it can be interpreted either as one isotope code number in the range 1..99 or as two concatenated isotope code numbers in the ranges 1..9 and 1..10.

If you set

```
DEFINE WIZARD = 1
```

in communication protocols WIZARD.C00 or WIZARDBG.C00 you can use isotope codes 1..99 in single evaluation assay protocols. In dual evaluation assays (having the assay protocol parameter 01 DUAL ASSAY = YES) you can only use isotopes 1..9; in this case you concatenate the two isotope codes to form a two-digit number. Number '3' is reserved to be equal to '12'. (How to measure the isotope number 3 in single label is explained in the next paragraph). If in a dual evaluation assay the second digit of the isotope number is 0, it means that the channel B isotope has code 10.

If you set

```
DEFINE WIZARD = 0
```

you can only use isotopes 1..9 in both single and dual evaluation assay protocols. However, even for single evaluation assays you can specify two isotopes by concatenating the two isotope codes to form a two-digit isotope number. In this case WIZARD uses two counting windows and the results can be retrieved during assay evaluation in variables COUNT, CPM and COUNT_B, CPM_B respectively. Single evaluation assays can only use one standard curve, even if two isotopes are used. As before, the number '3' is reserved to be equal to '12'. To measure the isotope number 3 in single label, use the number 33 and set the assay protocol parameter "01 DUAL ASSAY = NO".

The communication protocol WIZARD_T.C00 cannot be used to send measurement parameters to WIZARD, so the constant WIZARD does not appear in it. If you are using this communication protocol, set in WIZARD the parameter "SYSTEM | Operation mode | Evaluation" to "CPM" or "RiaCalc WIZ" and edit the assay protocol in WIZARD. You can save the MultiCalc assay protocol on a diskette and then load it into WIZARD. Check, however, that the specified isotope is the right one also after the protocol has been loaded into WIZARD.

5.4.5 Using the communication protocol WIZARD

As described above, this communication protocol is used when resutls are buffered in WIZARD. Each buffered assay is deleted only after MultiCalc has acknowledged that it has received it.

To use this communication protocol, set in WIZARD the parameters "SYSTEM | Operation mode | Evaluation" to "multiCalc" and "SYSTEM | Printout selections |Without buffering to PC" to "No". If you are not using a local printer that is connected directly to WIZARD, set "SYSTEM | Printout selections |Use printer port" to to "No".

5.4.6 Softkey "F5 INSTALL"

To see what isotopes are available, press in MultiCalc the softkeys "F1 COUNTER | F5 INSTALL". You get a list of available isotopes and short instructions on how to specify them in assay protocols. (If you have just turned on WIZARD you may get the "Framing error" message at this point. In this case, press ENTER and "F5 INSTALL" again.)

5.4.7 Curve plotting parameters

In the case of a RIA or IRMA the following parameters are available:

```
20 X-AXIS (concentration)
21 Y-AXIS (response)
22 FITTING ALGORITHM
23 STD OUTLIER REJECTION
24 HALT FOR CURVE EDIT
```

These are all to do with the standard curve used for determining concentration values. The built-in MultiCalc helps explain these parameter along with the part in the MultiCalc User Manual called Standard curves.

5.4.8 Quality control parameters

The next block of parameters are concerned with quality control. They are:

```
60 CONTROLS
61 HISTOGRAM
62 QC-ACCEPTANCE RULES
```

See the part on Quality Control in the MultiCalc manual.

5.4.9 Output parameters

The final block of parameters is concerned with output from MultiCalc:

80 STORED FILES These are the types of files stored e.g. data for further analysis with MultiCalc or other programs or computers, curves, QC information etc.

81 DISPLAY These are the items displayed

82 PRINTER These are the items printed

83 OUTPUT Here you specify what the actual items are in for storing in ASCII format.

84 RESULTS Here you specify what the actual items are in the files for external programs or computers.

5.4.10 Additional output information

The program must know in which order RIA standards are loaded and what their nominal concentrations are. This information is supplied in the CODING accessed by pressing F1. Other special samples such as Blank and Reference can also be specified.

The recommended order for sample tubes is:

blanks(NSB)
totals
references (zero sample)
standard samples
unknowns.

Unknowns are either patient samples or control samples in arbitrary order, the controls must be further specified on the line called CONTROLS.

```
2BLANK
2TOTAL
2STD=1
2STD1=2.5
2STD1=5
2STD1=20
2UNKN
```

The number on the left is the replicate number and the number on the right is the nominal concentration. The replicate number preceding UNKN means that all unknowns have this number of replicates. In the example above all samples are duplicates and no REFER samples are used.

Other output definition available with softkeys F2 to F7 and F8 F5 and F8 F7 are COMMENT, FACTORS, BEGIN, INPUT, OUTPUT OPTIONS, PATIENT and COUNTER.

For more details about MultiCalc assay protocol parameters see the MultiCalc User Manual "Protocol parameter setting".

A list of available output items for the MultiCalc assay protocol is shown in section 5.8 at the end of this chapter.

Entry of parameter values in a protocol is supported by various softkeys described in the MultiCalc User manual in the part referred to above.

5.4.11 Exiting assay protocol editing

When you have finished protocol editing you exit by pressing key F9 and then normally selecting F1 (=QUIT+SAVE).

The protocol then joins the list of available MultiCalc protocols.

To return to the main menu you must press F9.

5.4.12 MultiCalc protocol operations

If you want to manipulate MultiCalc protocols themselves (rather than just individual parameters in a protocol) e.g. copying a protocol or ordering the list alphabetically etc. you can do this by using the appropriate softkeys (see the User Manual).

5.5 Running an assay

5.5.1 Starting the run

Make sure that the first cassette has its correct protocol selection ID. For more information about this, see "ID system" in Part 2 of this manual. Put a "STOP" ID on the last sample rack to be counted or use a STOP rack or a totally empty rack in order to stop the instrument automatically.

Load racks, starting with the right-hand conveyor lane.

Select the MultiCalc main menu

Press "COUNTER", softkey (F1 key)

Select 1470 and press "ENTER"

MultiCalc will now store all protocols in the instrument automatically and the instrument will count all the loaded samples.

5.5.2 Stopping the run

The counting will stop automatically when a "STOP" rack or ID is found. You can also press the STOP key on the WIZARD keyboard. In this case the following text will appear on the built-in display:

```
Continue
End assay, continue
End assay, clear conveyor
```

Depending on whether you want the next assay to be counted or all counting to stop select "End assay, continue" or End assay, clear conveyor" respectively. To override the stop instruction and continue counting select "Continue".

5.5.3 Result handling

There are a number of options concerning the way the results are handled. These depend on the type of communication protocol you have. The normal situation is as follows:

Buffering of results is selected - the SYSTEM parameter "Printout selections | Without buffering to PC" is "NO". If counting is started by pressing the instrument START key or from MultiCalc by pressing F1 COUNTER and selecting WIZARD, measurement results are buffered in the instrument.

MultiCalc will evaluate the final results and print these out as determined in the counting protocol.

Buffering is not selected - "SYSTEM | Printout selections | Without buffering to PC" is "YES". If counting is started from MultiCalc or by pressing the instrument START key, measurement results are sent directly to MultiCalc, without buffering. This option is recommended only with the Resident filer installed, which case you should have the communication protocol WIZARD BG instead of the normal WIZARD.

The following special cases are also possible:

1. "SYSTEM | Operation mode | Evaluation" is "RiaCalc WIZ" or "Cpm" and "SYSTEM | Printout selections | Without buffering to PC" is "YES". In this case measurement results are sent directly to MultiCalc, without buffering.

2. "SYSTEM | Operation mode | Evaluation" is "RiaCalc WIZ" or "Cpm" and "SYSTEM | Printout selections | Without buffering to PC" is "NO". In this case measurement results are not sent at all to MultiCalc.

5.6 The FILES function

Most FILES functions are not available in CPM mode, (they are in grey), they are only available in RiaCalc WIZ, however the following three are:

5.6.1 Spectra

Store spectra - this is for information only. It tells if spectra can be saved or not and if they are to be sent to an output device or not. The actual settings can be made in SYSTEM | Operation mode | Store assay spectra. See there for more details.

Operation - this allows you to handle the files of spectrum data. The options are:

Delete - delete the spectrum file data

Save to disk - save the spectrum information on the program disk in WIZARD

Send to PC - send the spectrum information to a PC that is connected to WIZARD

Nr of channels - the number of channels that are included in the spectra. During assay measurement all spectra are stored using the full 1024 channels. This file is in binary format. If you are only interested in the lower part of spectra, you can set here the number of channels that are included when the file is converted to text form. The channels included always start from the first channel.

Format – When assay spectra are saved in the datalogger disk or are sent to PC or mainframe, you can specify with this parameter the format of the data. Available formats are ‘Wallac’, ‘Excel’ or ‘Ortec’.

- **Wallac format**: This is a text file that can be read by Wallac Spectrum Analysis Program
- **Excel format**: This text file format data belonging to the same spectrum is written in the same line and channel counts values are separated by tabulator characters. This makes it easier to read several spectra at the same time into a spreadsheet program.

- **Ortec format**: This binary file format is called 'Integer Data File' in Ortec documentation. The file extension is CHN. Each spectrum is stored in a file with the following path and name:

  A: \ EVAL \ <assay name>.E<run id number>\<assay position>.CHN

  <run id number> can take the values 00, 01, 02, … , 99 and <assay position> the values 000, 001, 002, …, 999.

  If "FILES | Spectra | Operation" is "Send to PC", then all spectra files are sent at the same time one after another and the receiving end must separate them apart.

5.6.2 GLP data

This allows you to handle the GLP data obtained in GLP normalization. There are four options, some of which lead to other options:

Operation:
- **View** - allows you to view the GLP data
- **Delete** - delete GLP data
- **Criteria** - allows you to select the warning limits for different types of GLP data. You can select:
- **Print criteria**
- **Item** - many items appear from which you can select the one which you want the GLP data: PEAK, BGRD, EFFIC, RESOL, EFFICIENCY, COVERAGE, CHI-PROB, WIN-CPM, TOTAL CPM

Isotope - you can select the isotope type from those for which a GLP normalization can be done

Detector

Do operation

5.6.3 Waste log file

A waste log file that contains the total CPS and DPS values of all measured assays, isotope normalisations and GLP TEST measurements can be printed or stored on a datalogger disk.

The file can also be deleted or sent via the PC port to an external PC.

The waste log can contain approximately 700 entries, after this the older half of the entries is deleted and the log starts growing again.

For isotope normalization and GLP test, TOTAL CPS is the average corrected sample activity in the isotope counting window in all detectors used. It is corrected for dead time, background activity and isotope decay. TOTAL DPS is the average corrected sample activity in the open window divided by Efficiency% which is a SYSTEM parameter that appears after you select Isotope and then the isotope name.

For assay measurement TOTAL CPS is the sum of all printed corrected CPM values of measured tubes in the assay. The DPS value is obtained by dividing the printed corrected CPM by the actual coverage of the isotope counting window and by the parameter Efficiency% refered to above. TOTAL DPS is thus the sum of all DPS values of measured tubes in the assay.

5.7 Real time clock

The real time clock of the counter is set from MultiCalc through a command included in the communcation protocol.

If for some reason you want to set the clock manually you can do it by giving one of the following commands when MultiCalc is in terminal mode:

CLOCK dd.mo.yy hh:mi:ss

This sets the data and time. To set only the date, send the command
CLOCK dd.mo.yy

To set only the time, send the comman
CLOCK hh:mi:ss

or
CLOCK hh:mi

The date is not accepted in any other formats than dd.mo.yy. The year must be a two-digit number. The other values must also be expressed as two-digit numbers, e.g. the month of May is 05.

Values from 80 to 99 refer to the 20th century and values below 80 to the 21st century. The time must be in 24-hour format.

After the date has been set, the counter responds with the string
DATE SET

After the time has been set, the counter responds with the string
TIME SET

If for some reason the date and/or time string could not be interpreted, the counter responds with
? 7 Bad date or time string.

If date or time is set, (as both normally are) the sending of time-of-day values with RIA/IRMA/RATIO assay results to MultiCalc is also enabled.

The exact time when measurement of each sample was started can also be sent to MultiCalc.

In MultiCalc mode the measurement start field can be enabled and disabled only from MultiCalc; the SYSTEM parameter Diagnostic output | Print meas. start time" has no effect in this case.

To enable the sending of measurement starting time with assay results, send from MultiCalc terminal the command
CLOCK ON
to the counter. To disable it, send the command
CLOCK OFF

The sending of this command can be made automatic if it is included in the WIZARD communication protocol by setting the parameter USECLK to 1. See the information that appears when you select F1 COUNTER F5 INSTALL.

The clock value will be assigned to the parameter COUNT_B. This is only available for this use in single label counting.

You can check the current setting by sending the command
CLOCK

If the measurement starting time has been enabled, the counter responds with the string
PRINTED

If it has been disabled, the counter responds with the message
NOT PRINTED

When the counter is turned on or after a power failure in MultiCalc mode, the measurement start time field is disabled until it is explicitly enabled again from MultiCalc.

5.8 Selectable outputs

```
RACK ### 1                    Rack number 3 digits for both groups, no average
DET ## 1                      Detector number for both groups, no average
SEQ #### 111                  Sequence or tube number
PAT #### 0,111                Patient number with both averages, unknowns only
TIME ##### 1                  Counting time in seconds
ETIME ####.## 1               Elapsed time in decimal hours
COUNT ####### 11 $            Total counts and first average
COUNT_B ####### 11 $          Total counts and first average on B-channel
CPM ######.# 11 $             Corrected CPM* and first average
CPM_B ######.# 11 $           Corrected CPM* and first average on B-channel
CONC ####.### 111 $           Concentration with both averages
CONC_B ####.### 111 $         As above for channel B
%CV ##.## 011                 Coefficient of variation as a percentage, only averages
%CV_B ##.## 011               As above for channel B
%CVE ##.## 0,01               %CV from reference precision profile, average for only unknowns

%CVE_B ##.## 0,01             As above for channel B
CODE 111                      Code text from coding and control list
FLAG "     " 0,111            Concentration flag, all values but only unknowns
FLAG_B "     " 0,111          As above for channel B
BLANK ######.# 111            BLANK cpm, valid after BLANK in coding, all values for both
groups
BLANK_B ######.# 111          As above for channel B
REFER ######.# 111            As above for REFER
REFER_B ######.# 111          As above for channel B
TOTAL ######.# 111            As previously for TOTAL
TOTAL_B ######.# 111          As above for channel B
NEG ######.# 111              As above for negative controls
NEG_B ######.# 111            As above for channel B
POS ######.# 111              As above for positive controls
POS_B ######.# 111            As above for channel B
RESP ######.# 11 $            Programmable response
RESP_B ######.# 11 $          As above for channel B
STS "   " ## 0,111            Numerical flag number. These are:
                              1 = OUT, 2 = >STD, 3 = <STD, 4 = ?amb, 5 = >>STD, 6 = <<STD,
                              7 = %CV!, 8 = >%CV, 9 = >>%CV
STS_B "   " ## 0,111          Numerical flag number channel B
 GROUP "GR" ## 0,11           Group number of multiple UNKN-coding
 REPL "RP" ## 0,1             Replicate of individual sample for unknowns
 SAMPLE "SPL" #### 0,111      Sample No. (includes controls) for all values but only unknowns
DRESP #####.# 11 $            Response error
DRESP_B #####.# 11 $          Response error for channel B
DILF ###.## 0,11              Dilution factor
REMARK ############### 0,111  Remark
SEQA #### 1                   Application sequence
ROW ### 111                   Row number 1 = individual, 2 = average, 3 = second average
DATE ######## 111             Date
CLOCK ######## 111            Time
CLASS ###.# 111 $             E.g. 1 if CONC = STD 1 or 2.5 if CONC = (STD 3 + STD 2)/2
CLASS_B ###.# 111 $           As above for channel B

* Corrections in CPM are dead time, decay, background, crosstalk and spillover.
$ These fields are used in statistical calculations e.g. a mean or average is calculated.
# This shows the format of the fields. The numbers after fields are the default switch
settings. See section 4.6.9.
```

6 Normalization

6.1 Normalization

6.2 GLP test normalization

6 Normalization

6.1 Normalization

6.1.1 Principle

The two, five or ten detectors of WIZARD allow several samples to be measured simultaneously. However, as each detector has a slightly different efficiency and background value it is necessary to correct the measured sample counts so that the final result is as if every sample had been measured in the same detector.

In dual label measurements one isotope being measured will often cause counts to be recorded in the second isotope window and vice versa. This effect is called "spillover" and must be corrected for.

For higher energy isotopes (over 200 keV) there will also be "crosstalk" between detectors. This means that a sample being measured in one detector will cause counts to be recorded in other adjacent detectors. The higher the isotope energy the greater the crosstalk. Thus crosstalk must be calculated and corrected for when it occurs.

The detector system gain and window must be periodically tested to ensure optimum counting conditions i.e. that the optimum efficiency and background ratio is achieved.

WIZARD makes all these corrections automatically based on the information obtained during "normalization". This procedure involves measuring a single sample in each detector in turn. The instrument must be normalized for each isotope used.

Normalization can be made infrequently. When you have installed WIZARD, normalize it for the isotopes you are going to be using. Unless the instrument gives a warning asking for a normalization, a time period of six months before you redo the normalization is appropriate.

Once you have made a normalization you can then create or edit a normal counting protocol where you can select the isotope you have normalized and use it to count samples.

If the instrument is not normalized for a particular isotope and at some time you try to run an assay with this unnormalized isotope selected, WIZARD will give an error message telling that the isotope is unnormalized and will terminate the assay.

The procedure for doing background and isotope normalization is explained in the following sections.

6.1.2 Background normalization

A background normalization is a method of determining the background for each detector. It is important that it has been made at least once. The background value is subsequently subtracted from count values during actual counting.

To make a background normalization stick the ID label "BKG" onto an ID clip in the area marked "RACK/SPECIAL". Fix the clip onto an otherwise empty rack.

Load the rack on the conveyor and press "START".

A complete background normalization report will be printed out and background values for the entire energy spectrum will be saved in the instrument memory. Since the whole spectrum is saved there is no need to make background measurements for each individual isotope.

6.1.3 Normalization for isotopes used

Information about the isotopes to be normalized (name, ID number, energy, window settings etc.) must be given to the instrument before the normalization starts. This information is factory set for the isotopes in the list in chapter 9 Specifications. See chapter 7.3 System mode for information about isotope parameters and how to change them should you need to do so. Be careful about changing isotope parameters because this will in many cases mean that you have to make a fresh normalization.

Remove the holders from positions 1 to 9 in a rack.

Place an isotope source in the last position of an otherwise empty rack. The isotope should have an activity of between 50 000 DPM and 200 000 DPM.

Stick an ID label "NORM" to the ID clip in the position marked "RACK/SPECIAL".

Stick an ID label with the appropriate isotope code number to the ID clip in the position marked "PROTOCOL". The isotope codes are the numbers given in the table above.

Fix the clip to the rack, load it onto the conveyor. Insert an empty rack after the last normalization rack to stop the conveyor.

When you have loaded your normalization rack(s) and stop rack, press "START".

Repeat this normalization procedure for each isotope to be used.

Wait until the complete report, for each isotope, has been printed out. Make sure that the efficiency is within the limits 0.9 ... 1.1.

Make sure you do not leave a background normalization or isotope normalization rack on the conveyor when you have finished with it otherwise you may start an unwanted new normalization and lose the previous results.

6.1.4 Printout columns

The printout column headings are:

DET, PEAK CHN*, PEAK DEV%*, RESOL%*, ACTIVITY, COUNTS, EFFICIENCY**, ERROR** (where ERROR = 100 /□(Counts)), WINDOW keV*, LOW, HIGH, DECAYED ACTIVITY, MEASURED COUNTS, DETECTOR EFFICIENCY, RELATIVE ERROR %, HORROCKS EFFICIENCY*, STANDARD CPM, SIGNIF. LEVEL %

* these only appear if extended normalization printout has been selected.
** these two items do not appear for single detector instruments). If the instrument has several detectors installed, but only one of them is active, these fields are printed, however. Then efficiency is 1.0 and the error 0.0.

See also section 10.2.17.

6.1.5 Normalization sequence

No special dual label normalization sequence is necessary for the following reasons:

During normalization each time an isotope is counted with one detector the spectrum obtained from each detector is saved. This means that in the case of a ten detector instrument, 10 x 10 spectra are obtained and saved.

After this the first time you start to count samples labelled with a particular isotope or combination of isotopes the appropriate crosstalk and spillover correction factors are calculated and saved and then used in the actual sample counting. When the same isotope or combination of isotopes is counted again the correction factors already saved are used.

If you do a normalization with a particular isotope, any already existing correction factors calculated using the previous normalization made with that isotope are deleted from the memory but the actual spectrum information for other isotopes is not affected so other normalizations do not need repeating. New correction factors are then calculated, when needed, taking account of the new normalization.

If a background normalization is made, or if the number of active detectors is changed, all saved correction factors will be deleted and new ones calculated when needed.

If you change the crosstalk selection (on or off) for a particular isotope any correction factors involving that isotope will be deleted so that new ones can be calculated when needed.

This system has the advantage that you do not need to do special dual label normalization because the instrument can calculate the necessary dual label information based on the single label information already stored. It also means that you can make a background normalization whenever you like without repeating the isotope normalization.

6.1.6 Manual normalization

See the chapter on Manual operation for how to do manual normalization and things to be aware of with it.

6.2 GLP test normalization

6.2.1 Introduction

Instrument performance can be monitored by running GLP test normalizations at regular intervals. These store data that can later be viewed in graphical format.

GLP means "Good Laboratory Practice". A GLP test normalization is similar to isotope normalization, only results are stored differently. Data obtained in a GLP test normalization are not used in assay measurements, but are tested against preset limits and then stored so that they can later be compared with other test normalizations using the same isotope. This comparison is done by presenting the values of some measured parameters as a function of time, so that any systematic trends or large random deviations can easily be discerned.

6.2.2 GLP test normalization rack

A GLP test normalization rack has only one holder and sample which is at the last position of the rack. The rack has a clip with the TEST instruction at the RACK/SPECIAL position and the isotope code at the PROTOCOL position. Counting time is set by the parameter Normalization time which is found in the SYSTEM menu under "Isotopes | <Isotope name>". The printout is similar to isotope normalization printout.

It is possible to do GLP test measurements by only using the isotope numbers 4, 91, 92 and 93. (If needed, it is also possible to make other isotopes available for GLP test measurements).

6.2.3 Saved GLP values

The following values are saved during GLP test normalization:

PEAK - Isotope main peak channel number

BGRD - Background CPM in counting window

EFFIC - Relative detector efficiency (if a 1470 counter has more than one detector installed)

RESOL - Detector resolution (%)

EFFICIENCY - Absolute detector efficiency. This is determined for I-125 using the Horrock's method. For other isotopes, the measured CPM in the counting window is divided by the absolute activity of the test sample, which is given by the SYSTEM parameter "Isotope | <Isotope name> GLP test sample DPM".

COVERAGE - Window coverage (%). This is the fraction of counts in the whole spectrum that falls to the isotope counting window.

CHI-PROB - Detector stability probability. This can be calculated if the SYSTEM parameter "Isotope | <Isotope name> Repeat times" is greater than 1. See section 6.2.7 "Repeat Counting" for more details.

WIN-CPM - Measured CPM in counting window

TOTAL CPM - Measured total CPM in the whole spectrum

6.2.4 GLP Criteria

For each of the above quantities you can set a low and a high limit by setting the FILES menu item "GLP data | Operation" to "Criteria" and pressing the ENTER key when the highlight bar is on the menu item "Do operation". The other items in the menu are used to select the isotope and one of the stored values mentioned above. In the same menu that is used to set the limit values you can specify that a warning message, a graph or both are printed if during GLP test measurement some quantity is not within limits.

6.2.5 Viewing GLP data

The stored GLP test normalization data can later be viewed graphically by setting the FILES menu item "GLP data | Operation" to "View" and pressing the Enter key when the highlight bar is on the menu item "Do operation". The other items in the menu are used to select the isotope, detector (if a 1470 instrument has more than one detector installed) and one of the stored values mentioned above.

6.2.6 Outputting GLP data

GLP plots can later be printed by pressing the digit key "6" while the plot is displayed. The plot is sent to the printer that is connected to the WIZARD printer port if the SYSTEM parameter "Printout selections | Use printer port" is "Yes".

The plot is also sent via the WIZARD PC port to MultiCalc if either "SYSTEM | Operation mode | Evaluation" is "MultiCalc" or "SYSTEM | Printout selections | Without buffering to PC" is "Yes".

When the plot is sent to MultiCalc, special code characters are added to the data, so that the plot can be printed by the printer that is connected to the PC running MultiCalc. In order for the printing to succeed, MultiCalc must be receiving data from WIZARD, and in the WIZARD communication protocol the Terminal parameter must be VT-52.

6.2.7 Repeat counting

During GLP test normalization each measurement can be repeated several times to test each detector for stability.

The parameter "Repeat times" in isotope editor in the SYSTEM menu sets the number of times each isotope normalization measurement is repeated. Thus the total time a sample is measured in each detector is this number multiplied with the normalization time that is set in the isotope editor.

The measured counts in repeat measurements are compared with each other and the program calculates the probability that differences between expected and observed counts in these measurements occured just because of statistical variation. This probability is called "Significance level" and its unit is %. If it is near zero or one, this means that there is systematic error in repeat measurements.

The number stored is transformed from the Chi-square probability that is shown in the printout ("SIGNIF. LEVEL") so that

5 corresponds to 50%, 4 to 10%, 3 to 1%, 2 to 0.1%, 1 to 0.01%, 0 to <=0.001%, 6 to 90%, 7 to 99%, 8 to 99.9%, 9 to 99.99%, and 10 to >=99.999%. This is to make very small and large probability values stand out more clearly.

Possible isotope decay is taken into account when Significance level is calculated. For each detector the counting window over which counts are summed is the same for all repeat measurements and is determined from the sum spectrum of the repeat measurements.

7 Additional WIZARD functions

7.1 Dual label counting

7.2 Manual operation

7.3 System mode

7.4 STAT counting

7.5 Power failure

7.6 Routine maintenance

7.7 Safety information

7.8 Large Eppendorf tubes

7 Additional WIZARD functions

7.1 Dual label counting

7.1.1 Introduction

Dual labelled samples have actually two independent analytes in the same vial. In order to separate the radioactive labels from each other the labels must have separate energies. The most common dual label assay in practical work is the B12/Folate assay in which labels are Co-57 and I-125.

Before running a dual label assay you must normalize the instrument for both isotopes as described in Part 6.

The analysis for dual labelled assays requires two protocols to be set, one for each isotope used. The two protocols are connected by specifying in protocol A the name of protocol B. Protocol "A" is a master protocol and "B" is the slave.

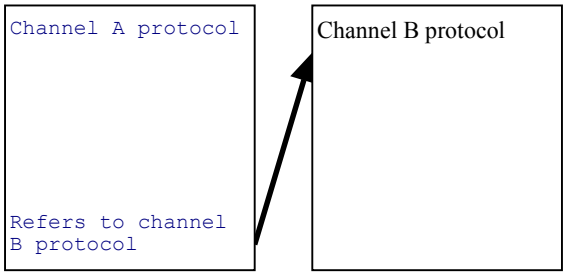


Figure showing relationship between protocols in dual label.

The program uses the word "channel" to describe the counting process controlled by a particular protocol. Channel A counting is controlled by protocol A and channel B by protocol B. Each channel may include counts from both labels so the dual label program has to "disentangle" the counts so as to arrive at a pure counts value for each label.

Protocol B must be defined before A otherwise you cannot select the B protocol when you edit the A protocol.

The explanation following applies to both the internal RiaCalc WIZ software and the external MultiCalc software.

Note: In dual label RIA/IRMA/RATIO assays a warning is printed if spill over cannot be eliminated from measured data or if the elimination would cause results to be inaccurate.

7.1.2 Setting dual label protocols

The following example shows setting of the protocol for a dual labelled B12/Folate assay

Create a protocol with the name B12 and ID 11.

Answer YES to the question DUAL LABEL. Two extra lines appear, e.g.:

```
Channel                 = A
Chn-B Protocol          = B12
```

Select:

```
Channel                 = B
```

The chn-B line will disappear. Give the coding for B12 standards and controls as in a single label assay protocol.

Select the label:

```
First Isotope           I-125
Second Isotope          Co-57
```

Give the rest of the parameters, including time and coding for the Folate standards and controls as in the case of a single labelled protocol.

Exit and Save the protocol.

Make a copy of this protocol with the name Folate and ID 10

Answer YES to question DUAL LABEL

Two more lines appear: Select:

```
Channel                 = A
Chn-B Protocol          = B12
```

Make other necessary changes to for example coding.

Note: The protocol types for A and B can be different, i.e. one can be RIA and the other IRMA.

Exit and Save the protocol.

Run the assay as for single label. The protocol ID, which in the example is 10, can be from 1 .. 99.

7.1.3 Parallel and successive evaluation

The actual evaluation may take place in two different forms, parallel and successive.

Parallel processing is when results from channel A and B are calculated simultaneously in real time.

Successive processing is when results from channel A are calculated and saved in real time. These results are then retrieved and the results for channel B are calculated as in later.

Parallel processing imposes strong constraints on the assay. Both analytes must have the same number of standards and controls and the number of replicates must be the same.

Successive processing has practically no constraints at all. The number of standards, controls, replicate etc. is freely selectable. The program selects, according to the protocol, whether the evaluation is parallel or successive.

7.1.4 Constraints on protocol setting

The following is a summary of protocol commands and how these are constrained by the requirements for dual label protocols.

DUAL LABEL, COUNTING TIME, LABELS
Only the channel A settings are valid. The channel B settings have no effect

X-AXIS, Y-AXIS, FITTING ALGORITHM, STD OUTLIER REJECT, CURVE EDIT HALT
May be set differently for channel A and channel B protocols

CONTROLS
Must be set identically for both protocols if parallel processing is to occur

PRINTER
Can be set differently if successive processing is to be done, but if parallel processing is done then the channel A settings dominate.

CODING
For parallel processing CODING must be identical, except for the numerical values for concentrations of standards. Unknowns must begin from the same position number and must have similar structure, number of replicates and dilutions (with the same dilution factors).

7.2 Manual operation

7.2.1 What is manual mode ?

In manual mode the automatic sample changer is not used but the samples must be loaded into the detector wells by the operator using a sample tray. The manual mode therefore only affects the way samples are moved to and from the detectors, the actual counting is exactly as in the "normal", i.e. automatic, mode.

7.2.2 When to use manual operation

You may wonder when to use manual mode since WIZARD is an instrument which offers automatic operation. The following are examples of occasions when manual mode should be used:

- in the case of conveyor contamination

- to count samples in large vials (over 13 mm in diameter or over 90 mm in height)

- in case of in conveyor malfunction until the service personel have cleared the problem.

- to count extremely active samples when additional attenuation is needed

7.2.3 How to set manual mode?

Select SYSTEM

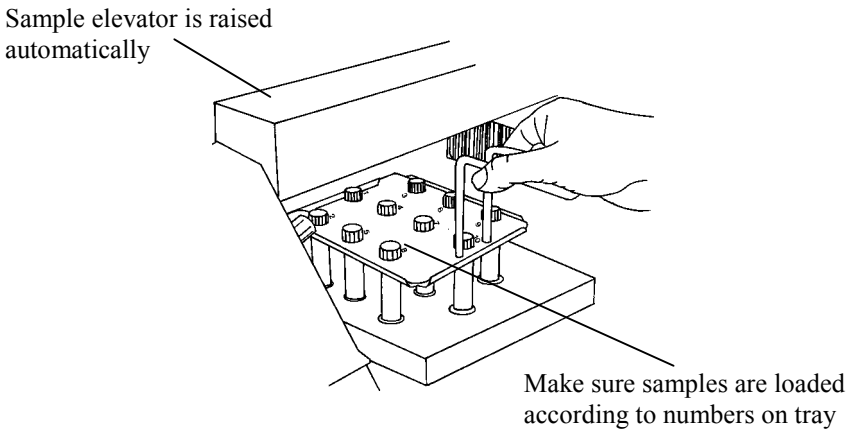
First the Evaluation mode selection appears. The three possible modes are:

CPM = WIZARD produces CPM results. These may be evaluated in another computer. Operation with this mode is described in Part 3 of this manual.

RiaCalc WIZ = Data evaluation is carried out in WIZARD with the RiaCalc WIZ software. See Part 4.

MultiCalc = Data evaluation is carried out in the PC using MultiCalc immunodiagnostic data management software. See Part 5.

You can operate WIZARD as an automatic or manual counter with any of these three evaluation modes (assuming the appropriate options are installed on your WIZARD). The change from automatic to manual is accomplished by selecting "Manual mode used" YES.



Note: the change to manual mode cannot happen while the cover over the detector block is open. This is because the change to manual mode causes the raising of the arm which transports samples from the conveyor to the detector block to make space for the sample tray to be loaded. For safety reasons this movement cannot happen without the cover being closed. Afterwards the cover must be opened for accessing the detector block.

Save the changes. Now you can exit the SYSTEM mode and go to the OPERATE mode.

7.2.4 Loading samples

Put samples into the wells in the sample tray. Each well is marked with the number of the detector which will be used to count it. Make sure you load samples according to these numbers so that output results are in the right order.

Move the sample tray to the detector block and slide the tubes protruding from the bottom of the tray into the detector wells as shown in the figure.

7.2.5 Counting samples

Press the START key. The display will show:

```
Measure (manual mode)

Select the type of measurement or press STOP to cancel measurement

Measure an assay
GLP test

Measure background
Normalize detectors

Press START to measure
```

Select "Measure an assay" and press E.

A list of available protocols will appear. Select the one you want and press E.

The display will then show

```
Ready to measure batch number 1.

To start insert samples and press START key. To end press STOP key.
```

When you have loaded the sample tray press START. Counting will start in the normal way and results will be displayed and output.

When the tray has been counted you will be requested to load the next tray with the following message:

```
Ready to measure batch number 2.

To start insert samples and press START key. To end press STOP key.
```

When the last batch has been counted, press the STOP key.

7.2.6 Returning to automatic counting

Remove the sample tray from the detector block and close the cover. Then go to System and select Operation mode and then change the Use manual mode to NO. The elevator arm will return to its normal position and WIZARD will be ready for automatic operation.

Note: In Manual mode the counting can be started from MultiCalc only in offline mode, not in online mode.

7.2.7 Manual normalization

All operations which are available in automatic operation are also available in manual mode; these include detector normalization, background normalization, GLP performance check-up and actual measurement and evaluation.

As the counting operations are identical in both modes results obtained in one mode can be used in the other. The instrument can, for example, be normalized in the manual mode and samples evaluated in automatic mode or vice versa.

Before starting to do normalization read chapter 6.1 "Normalization" to understand the principle of normalization.

Select manual mode if it is not already selected.

Press the START key.

The display will show:

```
Measure (manual mode)

Select the type of measurement or press STOP to cancel measurement

Measure assay
Normalize background
Normalize isotope
Do GLP test

Press START to measure
```

Select "Normalize detectors"

The list of isotopes is shown, see the list in chapter 9 Specifications. Select the isotope you want to normalize:

A display is shown telling you to put the normalization sample in detector number 1 and press the START key.

If you want to abort this normalization, press the STOP key.

When the display changes to:

```
Put the normalization sample in detector number 2 and press the START key.
```

Put the source to the indicated detector and press the START key.

Continue this way until the source has been in each detector.

Do the normalization for each isotope used. Wait until the report for each normalization has been printed out.

7.2.8 Manual background normalization

Select manual mode if that is not already selected.

Select the main menu:

```
Measure (manual mode)

Select the type of measurement or press STOP to cancel measurement
```

```
Measure an assay
GLPtest
Measure background
Normalize
Make a normalization tray

Press START to measure
```

Select "Measure Background"

The following display is shown:

```
Measure background (manual mode)

Make sure that there are no samples in the detectors and then press the
START key.

If you want to return to the previous menu, press the EXIT key. To cancel
the measurement, press the STOP key.

Press the START key.

The display shows the main menu. The text "Measuring background" in the
first status line indicates that the background measurement is active.
```

You can do other tasks while the background is being measured.

If you want to see counting results select "Show CPM results".

Wait until the complete normalization report is printed out.

7.2.9 Manual GLP normalization

See chapter 6.2 for the principles of GLP normalization and section 7.2.7 for details of the practice.

7.2.10 Detector usage

When some detectors are inactive, the conveyor can only use 1, 2 or 5 detectors of a 10-detector instrument. This can result in that there may be detectors that are active but nevertheless cannot be used in measurements when the conveyor is used. When measurements are made manually, all active detectors can be used in measurements. If an isotope has been normalized using the conveyor but an assay that uses this isotope is measured manually, there may be some active detectors that cannot be used in measurement, because they have not been normalized. All these detectors that cannot be used in manual assay measurement are indicated in the manual assay prompt screen.

If an isotope has been normalized manually and after this the background is normalized using the conveyor, the background values are not updated for those active detectors that cannot be used for measurement by conveyor. (This happens only if at least one detector has been set inactive.) If an assay using this isotope is subsequently measured manually the background values used for these detectors will be incorrect. Therefore it is recommended that you normalize the background manually whenever any isotope is normalized manually and at least one detector is set to be inactive.

7.3 System mode

7.3.1 System mode parameters

You may change the WIZARD program operation in a number of ways. Most of the parameters which affect the program operation are collected in the SYSTEM mode. The following settings can be found:

```
Date
Isotopes
Operation mode
Background
Printout selections
Active detectors
Active hardware
Diagnostic output
Communication parameters
Version
Disk operations
```

7.3.2 Date

The instrument has a built-in clock. The clock keeps time also when the power is switched off. If the power is off for an extended period of time (several months) it may be reset. After that you must set it again. Proceed as follows:

Select SYSTEM and DATE.

A display appears:

```
System date & time

The current date & time is:
Thursday, April 12, 1990 1:23 pm
To set new date & time, select the last line in the menu and press ENTER.
New year          1990
New month         April
New day           12
New hour          1 pm
New minute        23
Set new date & time
Choice, use <--> or Enter
```

To change a number, select the appropriate line with the "up" or the "down" arrow key and change the value with the "left" or "right" arrow key. To make time & date selection permanent:

Select "Set new date & time and press "E".

7.3.3 Isotopes

WIZARD has a built-in library which consists of the optimized counting conditions for a large number of number of isotopes, see chapter 9 Specifications. The list of isotopes also shows if an isotope is normalized or not. Remember that an isotope must be normalized before you can use it.

Each isotope is defined by many parameters (described below). In normal counting there is no need to change them because they are given optimum values in the factory. If for some reason you want to make changes you can

do so. *Note however that any changes will often invalidate the normalization and you must renormalize the isotope. Also the specifications are valid only with the factory settings.*

When you select "Isotopes" a list of existing isotopes will be shown. Select the isotope whose conditions you want to change. The following parameters can be changed:

Note: isotope parameter lines can be hidden with the Ctrl-K key and made again visible with the Ctrl-U key. The Ctrl-V makes all hidden parameter lines visible.

7.3.3.1 Name
This is the name of the isotope e.g. I-125. It is shown in the list of isotopes for normalization and assay measurement. The maximum length of the name is 8 characters.

7.3.3.2 Comment
This is shown in the list of isotopes for normalization and assay measurement after the isotope name. The maximum length of the comment is 13 characters e.g. Iodine.

7.3.3.3 Normalization time
Give the time for which the isotope is to be counted if it is used for normalization.

7.3.3.4 Repeat times
You can set the number of times each isotope normalization measurement is repeated. This means that the total time a sample is measured in each detector is this number multiplied by the normalization time that was set above.

The measured counts are compared with each other and the program calculates the probability that the differences between the expected and observed counts in these measurements occured. It then calculates the probability that the differences between expected and observed counts in these measurements occured just because of statistical variation. This probability is called significance level and its unit is %. If it is near zero or near one then it means there is a systematic error in the repeat measurements. See chapter 6.2 GLP normalization for more details.

7.3.3.5 Crosstalk correction
If you want crosstalk correction to be done when you count samples labelled with that isotope, then select Yes for this parameter. Yes is the default setting for isotopes with energy greater than 200 keV. If you select No then no crosstalk correction will be done.

7.3.3.6 Decay correction
If you select this then give the isotope half-life value if needed. The possible values are:

None: no decay correction is made

Start: results are corrected to the start of the normalization or assay

Explicit: the time to which the results are corrected is explicitly given. If the choice "Explicit" is selected, this keyword is not shown in the isotope editor menu, but instead the time itself is shown. The format in which the explicit time is shown depends on the country code specified in the CONFIG.SYS file of the instrument boot disk. Our sales representative or service person can set it to your preference.

The explicit isotope decay correction reference time can be edited with one second accuracy. Although the seconds value is not shown when the explicit time is displayed it is included when isotope parameters are printed.

See also section 10.2.8.

7.3.3.7 MCA high limit

This is given for information and cannot be changed. Its value depends on the value of “SYSTEM | Active hardware | Nominal gain”.

7.3.3.8 Counting window

Various parameters affecting the counting window can be set. These are:

Dynamic-% - The boundaries of the window are determined by the parameter "Window coverage (%)"; the window follows peak drifting during normalization and also during assay measurement if “SYSTEM | Operation mode | No dynamic normalization” is “No”.

Dynamic-keV - The boundaries of the window are determined by the user (in keV); the window follows peak drifting during normalization and also during assay measurement if “SYSTEM | Operation mode | No dynamic normalization” is “No”.

Fixed - The boundaries of the window are determined by the user (in keV); the window does not follow peak drifting.

Peak position (keV). This refers to the main photopeak energy.

Note: If the parameter "Counting window" selected for an isotope in the isotope editor is "Dynamic-%" or "Dynamic-keV" then in RiaCalc WIZ assays the window that has been determined during isotope normalization is shifted in accordance with deviation in peak position during normalization and also during assay measurement if “SYSTEM | Operation mode | No dynamic normalization” is “No”. If a sample is so weak that its peak position cannot be determined, the same counting window as in the previous batch is used.

If a weak sample is in the first batch of an assay, then either the same counting window is used as was used in the most recent assay run with that isotope or else this is determined by the parameter “SYSTEM | Operation mode | Default is norm window”.

Window coverage (%) Determines how wide the counting window is set in isotope normalization. It is the fraction of the counts of this isotope in the whole spectrum that must fall within its counting window. The window is first selected to be as small as possible but still to contain at least the specified fraction of counts. Then it is further widened five channels in both directions.

Low boundary (keV)
High boundary (keV).

These are the boundaries of the counting window. The unit is keV. Although the editor permits you to give values up to 6000 keV, in practice the hardware imposes a limit which is given by the menu line "MCA high limit".

Note: If you want to measure high energy photons, set the counting window to include the last MCA channel. This is because the last MCA channel collects all photons which have a higher energy than the high limit of the MCA. For example, to measure all photons registred by a detector, set the Counting window to Fixed, the Low boundary (keV) to 0 and the High boundary (keV) to e.g, 3000. For 1470 the MCA high limit is about 1024 keV assuming the gain set at the factory has not been changed. Since the "High boundary" setting exceeds this value, the last MCA channel is included in the counting window and so also photons with energies exceeding the MCA high limit are counted.

Threshold level % Specifies the height under which peaks other than the one given by the parameter "Peak pos. (keV)" are considered insignificant. This value is expressed as a fraction (%) of the height of this main photopeak.

Spectrum type There are three possibilities:

- **Single peak**, this applies to most isotopes, those which have only a single photopeak in the range of the multichannel analyzer.
- **I-125**, this is only for the I-125 isotope having a distinguished coincidence peak.
- **Many peaks**, for isotopes having several photopeaks, Compton or other secondary peaks.

Maximum coincidence deviation % This parameter gives the maximum allowed deviation of the I-125 coincidence peak with respect to its expected position, which is twice the channel number of the I-125 primary peak.

Minimum coincidence height % This parameter gives the minimum required height of the I-125 coincidence peak as a fraction of the height of the I-125 primary peak. If the coincidence peak is smaller than this limit, a 'Bad spectrum' message is printed.

Maximum assay deviation (%) This is the maximum allowed deviation of the isotope peak from its expected position in an assay measurement. If the deviation exceeds this value, the 'Bad spectrum' message is printed.

Maximum normalization deviation (%) This is the maximum allowed deviation of the isotope peak from its expected position in a normalization measurement. If the deviation of the peak position exceeds this value, normalization fails for that detector.

Warning assay deviation (%) This parameter gives the peak shift limit which, when exceeded, causes the warning 'Normalization recommended' to be printed in assay counting. Seeing this warning the user can do normalization(s) with the isotope(s) used in the assay and thus avoid any 'Bad spectrum' messages resulting from too large peak shifts.

Significant cpm per keV Defines the minimum acceptable height for the peak of this isotope in the smoothed spectrum. The unit is counts per minute per keV of the spectrum. If no peak height in the region defined by "Peak pos. (keV)" and "Max. assay dev. (%)" or "Max. norm. dev. (%)" in the smoothed spectrum reaches this value then the activity of this isotope in the assay sample is considered to be so weak that no peak search and window adjustment is made for this isotope. In isotope normalization this causes the normalization to fail for this detector.

Efficiency (%) This parameter lets you set the average absolute efficiency of the detector(s) in the counter. This enables you to calculate DPM results in addition to CPM results.

DPM is included in the Waste log data for isotope normalizations, GLP tests and assay measurements.

For I-125 when Counting window is either Dynamic.% or Dynamic-keV, the absolute detector efficiency is calculated during isotope and GLP test normalizations using the Horrock's method. However, you must set this parameter manually in this case.

This parameter can be changed without renormalizing the isotope.

GLP test sample DPM This value is used when absolute detector efficiency is calculated and stored in GLP data. The measured sample CPM is divided with this value to get the absolute efficiency.

This value is not needed for I-125, since in that case the absolute efficiency can be calculated directly with the Horrocks method.

Maximum detector efficiency deviation (%) In isotope normalization a check is made to ensure that the updated efficiency of each active detector differs from the average value 1.000 by, at most, the number of percentage points given by the value of this parameter. If the difference exceeds this value for one or several detectors, a corresponding warning message is printed in the normalization printout.

7.3.3.9 Restore default isotope

With this function you can copy the factory settings of any isotope to any isotope number.

Note: this function can be found at the end of the list of isotopes. To reach it quickly press End or PgDn.

To copy an isotope from the default list to the used isotope list, use the left/right arrow keys to select the isotope in the default list you want to copy. Then select the isotope in the used list you want to replace. Finally select "Do copy" and press E. The isotope in the used list will be replaced by the one from the default list and the display will revert to the SYSTEM function menu.

Note: When this parameter is selected for the first time after program installation, it takes a couple of minutes before the menu appears. This is because the program builds an index of the isotopes in the default list. To do this it must scan each protocol file and extract the isotope name and comment. Since there are over 50 isotopes, this takes some time. After the index has been built, the program writes it on the instrument hard disk. When the Copy isotope menu is entered subsequently, the program merely reads the index from the disk and therefore there is no apparent delay.

7.3.3.10 Print isotope and normalization

Note: this function can be found at the end of the list of isotopes. To reach it quickly press End or PgDn.

You can print out a list of isotopes, the data from all the isotopes, only normalized isotopes or one selected isotope. You can choose to print the isotope parameters, the measurement data or both.

7.3.4 Operation mode

7.3.4.1 Evaluation mode

The three possible choices are:

CPM = WIZARD produces CPM results. These may be evaluated in another computer. Operation with this mode is described in Part 3 of this manual.

RiaCalc WIZ = Data evaluation is carried out in WIZARD with the RiaCalc WIZ software. See Part 4.

MultiCalc = Data evaluation is carried out in the PC using MultiCalc immunodiagnostic data management software. See Part 5.

7.3.4.2 Manual mode

You can operate WIZARD as an automatic or manual counter with any of these three evaluation modes (assuming the appropriate options are installed on your WIZARD). The change from automatic to manual is accomplished by selecting "**Manual mode** Yes".

Note: the change to manual mode cannot happen while the cover over the detector block is open. This is because the change to manual mode causes the raising of the arm which transports samples from the conveyor to the detector block. For safety reasons this movement cannot happen without the cover being closed. Afterwards the cover must be opened for accessing the detector block. See the chapter on Manual mode for mode details.

7.3.4.3 Store assay spectra

Assay spectra can be stored in files and later retrieved in text form. They can be stored in files in the instrument hard disk. Each protocol and run id number 1..99 can have one assay spectra file that stores all spectra measured

during the assay. The spectra can later be saved to a datalogger disk or sent to a PC or a mainframe in text format. The files can also be deleted.

You can view or print the stored spectra in graphical format by importing the text file to a spreadsheet program such as Microsoft Excel.

To enable the storing of assay spectra set the parameter to "Yes". To send stored files to a PC, or to save them on a datalogger disk or to delete them, go to the FILES menu item "Spectra" and select the operation and file.

The assay spectra text file has the following format. (Entities in angle brackets are written as numbers in the file. Omitted lines are indicated with ----------.)

R <Number of spectra in the file> <Number of channels in each spectrum>0.00 0.00 0.00 0.00
SP# <Number of first spectrum, is equal to 1> <Counting time in seconds><Counts in first channel>

<Counts in last channel>SP# <Number of next spectrum, is equal to 2>< Counting time in seconds>

Note: When assay spectra are saved in text format, you can specify that all data belonging to the same spectrum is written in one line. In this case the channel counts values are separated by tabulator characters. This makes it easier to read several spectra at the same time into a spreadsheet program.

7.3.4.4 SYSTEM | Operation mode | Default is norm window

If a RIA/IRMA/RATIO assay has a sample with so small activity for some isotope that the main peak position cannot be determined, the previous,(possibly shifted) counting window for this isotope and detector is used.

If in the current assay there has not yet been (for this isotope and detector) a sufficiently active sample so that the main peak position could be determined, then if this parameter is “No”, the same counting window used in the most recent assay run using this isotope is used.

However, if the parameter is “Yes”, the counting window determined in the latest isotope normalization is used.

7.3.4.5 SYSTEM | Operation mode | No dynamic normalization

If this parameter is “Yes”, in RIA/IRMA/RATIO assays counting windows are not shifted.

If this parameter is “No”, in RIA/IRMA/RATIO assays where no isotope has been set to use a fixed counting window, the counting windows are shifted if peaks raise above background and possible spillover counts.

7.3.4.6 Ignore if no holder

If the parameter "SYSTEM | Operation mode | Ignore if no holder" is set to YES, then all sample positions without a holder are omitted in position counting. This means that replicate samples are considered to exist even if there is an uncounted position between and the mean value is calculated normally. If this parameter is set to NO then the replicate will be considered to have been in the uncounted position. This will then affect the result of the calculation of the mean value because the replicate value will be missing.

7.3.5 Background time

7.3.5.1 Background counting time

Background counting time can be changed with this parameter.

7.3.5.2 Print background

You can print out the background values.

7.3.6 Printout selections

7.3.6.1 Extended norm. printout

YES = Print complete report of normalization parameters. This printout will include the Horrock's efficiency for I-125 if that isotope is normalized.

7.3.6.2 Use serial printer port

Select “Yes” if you want results to be output to a printer connected to the serial printer port of the counter.

7.3.6.3 Use parallel printer port

Select YES if you want results to be output to a printer connected to the parallel printer port.

7.3.6.4 Without buffering to PC?

There is a result buffer on the hard disk. It enables you to disconnect the PC at any time without interrupting counting. It is also possible to start the counting from the keyboard of WIZARD by pressing the START key and save all assay printouts to the buffer and later connect the PC and evaluate the results.

If there is not enough space on the instrument hard disk the program stops. If 999 assays have already been saved in the buffer, then WIZARD starts writing over the oldest buffer files. You should evaluate the old assays from the buffer well before the hard disk becomes full or there are 999 unevaluated assays in the buffer. (The size of the instrument hard disk is about 30MBytes). The assay results in the buffer can also be deleted without sending them to the PC.

If you are using MultiCalc, then results are buffered when this parameter is "NO" if counting is started by pressing the instrument START key or from MultiCalc with the command B/S when the device parameter PC is not used. Results are sent directly to MultiCalc when this parameter is YES. If you are using the MultiCalc resident filer, set this parameter to YES.

If the RiaCalc WIZ or Cpm modes are used, results are never buffered. In this case, if this parameter is YES, results are sent to PC, otherwise not.

7.3.6.5 Write results to file

In addition to sending results to the serial ports described above WIZARD can write results to a floppy disk that is loaded into the drive in the instrument front panel. The disk should be a 3.5" high density 1.44 MB microfloppy disk and it must have been formatted before using. This SYSTEM parameter allows the datalogger feature to be switched on or off. If YES is selected then CPM results are saved on floppy disk and can be later evaluated in a PC. In particular you can use the MultiCalc program to evaluate WIZARD result files in the desired order direct from the floppy disk.

The results are saved in a subdirectory of the data disk called RESULT. All assays are put in their own files. The file names used are as follows:

Unknowns: AXX__YYY.T, where XX is the protocol number and YYY is the assay order

Background norm.: BKG__YYY.T, where YYY is the assay order

Isotope norm.: NZZ__YYY.T , where ZZ is the isotope number and YYY is the assay order

GLP test normalization: TZZ_YYY.T, where ZZ is the isotope number and YYY is the assay order

Examples: BKG__001.T, N01__002.T, N02__003.T A01__004.T, A01__005.T, A02__006.T

The assay order number starts again from one after 999, so it is possible that a file with same name already exists. In this case the old result files are overwritten. The extension of the already handled result files should be changed to .ZT to prevent this happening. If the data disk gets full, WIZARD automatically tries to delete all the files with the .ZT extension to make more space on the disk.

Error messages concerning the datalogger:

`DATA DISK NOT READY 1=RETRY 2=OMIT`
This could happen if no data disk was in place. Check this before selecting Retry.

`DATA DISK ERROR 1=RETRY`
This probably means that there is a fault on the disk itself or the write protect switch was on. Either change the disk or move the write protect switch as appropriate.

`DATA DISK FULL`
In this case you must either change the disk or delete some of the data saved on it.

Notes:

CPM output - see section 3.6 for information about CPM output. This information is also valid for the CPM output to the datalogger.

Datalogger file name printed - the name of the corresponding datalogger file is printed after each measurement for which data is saved in a file.

Run ID number is included also in the CPM output sent to the datalogger. This can be used to link printed assay results to stored assay spectra files.

7.3.6.6 Horizontal background printout

Background CPM values for different isotopes can either be printed vertically or horizontally. Specify the layout you want.

7.3.6.7 Printer type

The following two printer command languages ar supported: Epson FX and HP PCL3.

Epson FX
Most dot matrix printers support this.

HP PCL3
Many Hewlett-Packard printers support this.

7.3.6.8 Printout selections

Print page numbers?
If “Yes”, a header “PAGE n”, wwhere n is the page number, is printed on top of every page. Numbering starts from 1 when automatic counting using the conveyor starts or stops.

Printer paper length
WIZARD always issues a form feed when a page is full, so it is important to give paper hight here. Metric A4 sheet of paper is 11 inches long.
Valid range: 1 to 99

Top margin in lines
The number of empty lines at the top and the bottom of a page.
Valid range: 0 to 99

Paper width in inches
This information makes it possible for WIZARD to select a smaller font in order to fit a whole line to the page. Metric A4 sheet of paper is 8 inches wide.
Valid range: 1 to 99

Left margin in inches
Use this parameter to reserve space for left margin.
Note that for HP LaserJet printers a change in the value of this parameter becomes effective only after a new page has been printed. You can do this by printing e.g. the latest background measurement ("SYSTEM | Background | Print background")
Valid range: 0.0 to 10

Small text (8LPI)?
If "Yes", 8 lines of text per inch are printed. If "No", then 6 lines of text per inch are printed.

Small graphics?
If "Yes", graphical sections of the printout are printed smaller with greater resolution. This is convenient if the printer is able to print high resolution graphics.

Output negative CPM as 0?
If "Yes", negative CPM values are output as 0 CPM. In this case also negative CPM results to RiaCalc WIZ or MultiCalc are replaced with 0 values.

The statistical nature of radioactive decay and other errors in measurement can cause the CPM values that have been corrected for background, relative detector efficiency and crosstalk in multi-detector counters and spillover in multiple-labelled assays to be negative.

Max. assay run id.
When several assays having the same protocol id. number are measured one after another, this parameter determines how many RiaCalc WIZ data files (and assay spectra files) are saved before new ones overwrite the older. The assays are differentiated by the run id. number. The run id. number is increased by one for each new assay having the same protocol id. number. When the Max assay run id. has been reached, the next run id. number starts from 1 again.

The value for "Max. assay run id." can be from 2 to 99. Setting the value to a small number limits the number of RiaCalc WIZ data files (and assay spectra files) that are stored in the instrument hard disk. It also limits the number of RiaCalc WIZ data files that are loaded from diskette.

Note that the parameter "SYSTEM | Printout selections |Max. assay run id." affects only new run id. numbers that are being created during automatic measurement. You can still view, copy from and to and delete files with greater assay run id. numbers than "Max. assay run id." Also, when this parameter is made smaller, no files are deleted but you can always manually delete files with greater run id. numbers.

7.3.7 Active detectors

Select "SYSTEM" and "Active detectors". The following display will appear:

```
Active detectors

Detector  1=    ACTIVE
Detector  2=    ACTIVE
Detector  3=    ACTIVE
Detector  4=    ACTIVE
Detector  5=    ACTIVE
```

```
Detector  6=    ACTIVE
Detector  7=    ACTIVE
Detector  8=    ACTIVE
Detector  9=    ACTIVE
Detector 10=    ACTIVE

Choice, use <--> keys
```

Use the "up" or "down" arrow keys to select the detector you want to activate or deactivate.

Use the "right" arrow key to change the detector from ACTIVE to NOT ACTIVE or vice versa.

Press "Exit"

Select "Save changes and exit" when you leave the "Active detectors" mode.

After changing the "detectors active" list you must redo all the normalizations you are going to use.

Detectors that are inactive are excluded from all operations. Sample numbers are adjusted accordingly. There is no need to rearrange the samples in the racks, the program takes care that all samples are measured in the right order. The measuring speed, however, may be considerably slower because the program is not always able to use all active detectors but uses active groups of one, two, or five detectors.

7.3.8 Active hardware

7.3.8.1 Use rack ID reader

NO disables the reader function. ID information must be entered manually when the instrument is started.

If the rack ID. reader is not used, background, isotope and GLP normalizations can still be done using the conveyor after it has been started manually.

To do this, press the START key and select the appropriate item from the menu.

If an isotope or background is normalised or a GLP test measurement made while the rack id. reader is not in use, there is no need to add an empty rack after the measurement rack in order to stop the conveyor.

7.3.8.2 Key click

YES enables and NO disables the audible signal when the keyboard is used.

7.3.8.3 Nominal gain

This parameter lets you change the nominal gain value used by the program. At the factory the value is preset to 1.00 keV per channel. If this value is changed, a corresponding change must be made to the instrument hardware.

7.3.8.4 Counter number

This parameter gives the instrument identification number in printouts. This is needed when several counters are used. The value is printed only if it is greater than 0. See 7.3.9.8.

7.3.8.5 Use end transfer lane

When this is set to YES racks are free to circulate around the conveyor including moving from the end of the output lane to the beginning of the input lane. If you select this parameter to be NO the end transfer lane will be disabled and racks which have passed through the counting position will just stop in the output lane.

7.3.8.6 Recover from power failure
Recovering from power failure can be enabled or disabled by setting this parameter to YES or NO respectively.

7.3.9 Diagnostic output
This allows you to get a printout of diagnostic information for all detectors specified by your response to the Select detectors for info. parameter.

7.3.9.1 Print diagnostic info?
Diagnostic information is printed for all those detectors that have been selected with the parameter 'Select detectors for info'.

7.3.9.2 Select detectors for diagnostic information
This parameter lets you select those detectors for which special information is desired.

7.3.9.3 Assay repeat times
The value of this parameter specifies how many times each batch of an assay is measured before proceeding to the next batch. This function is used when the counter is tested for stability.

7.3.9.4 Spill determinant
The determinant is included in the output if this parameter YES. Normally the value of the determinant should be near 1.0. If the value is near zero, the corrected CPM values can be inaccurate. See also sections 10.3.1 and 10.4.1.

7.3.9.5 Print dead time factor
To do this, set the parameter "Yes". The CPM printout is sent to a printer that has been connected directly to the WIZARD printer port when the Operation mode is "CPM" or you are saving RiaCalc WIZ results on a datalogger. If you are using WIZARD with MultiCalc, set "Print dead time factor" to "No". See also section 10.1.3.

7.3.9.6 Print measurement start time
The exact time when measurement of each sample was started can be included in CPM printouts and datalogger files. The time is given to the nearest second.

If this starting time output has been selected, a field named "CLOCK" appears at the right end of the CPM printout and as the last item in datalogger files.

Set the parameter to YES to enable the field and to NO to disable it.

Measurement start time can also be sent to MultiCalc if the mode is MultiCalc. but the field can be enabled and disabled only from MultiCalc; the parameter "Print meas. start time" has no effect in this case. See chapter 5 Operation with MultiCalc for more details.

7.3.9.7 Instrument serial number
This parameter shows the instrument serial number that has been stored in read-only memory. You cannot edit this number.

The instrument serial number is included when the instrument model and program number are printed. See the next parameter for details.

7.3.9.8 Print model and version

The counter model, program version and instrument serial number are printed before normalizations and assay measurements. The printing can be disabled by setting this parameter to "No".

The string has following form:

1470, 10 detectors, RiaCalc WIZ, program 3.4, serial #667273, counter #2.

If the counter has only one detector installed, the number of detectors is not mentioned. "RiaCalc WIZ" means that RiaCalc WIZ is enabled. The Serial number is present only if it has been stored in the counter's read-only-memory. The counter number is printed only if it is >0.

If not disabled, this string is printed before background normalisation, isotope normalisation, GLP TEST normalisation and RIA/IRMA/RATIO assays. The string is not included in files that are stored to datalogger disk and it is sent to MultiCalc only if results are not buffered.

7.3.9.9 Read spectra from diskette

To allow convenient testing of the data reduction program in WIZARD, spectra can be read from a text file in the datalogger disk instead of from the multichannel analyser. Special commands make it easy to write artificial spectra that have just the properties you want. However, you can also use real assay spectra if you want e.g. to test how different isotope normalization settings affect assay results. The application note "Testing WIZARD with artificial spectra" describes the format of this text file so that you can write your own tests. When this parameter is “Yes”, the text “Reading spectra from diskette” appears in the main menu status area.

When measuring normal samples, be sure to set this parameter to "NO", otherwise all counts values will be zero!

7.3.9.10 Disable dead time correction

Dead time correction can be switched off for testing purposes by setting this parameter to YES. The default is NO. See also section 10.1.3.

7.3.9.11 Disable spillover correction

If this parameter is set to YES, spillover correction is not made in dual labelled RIA/IRMA/RATIO assays.

If spillover correction is disabled, then so is also crosstalk correction, even if isotope parameter "Crosstalk correction" is YES. See also section 10.3.1.

7.3.9.12 Disable background correction

If this parameter is set to YES, background correction is not made for single and dual labelled RIA/ IRMA/ RATIO assays and isotope normalizations.

Background correction is also not made made when spillover or crosstalk correction coefficients are calculated for single and dual labelled RIA/IRMA/ RATIO assays. When you set this parameter back to NO, you must redo all isotope normalizations that have been done after this parameter was set to YES, so that background correction is applied to spillover and crosstalk correction coefficients. (Crosstalk correction is done only in 1470 multi-detector models).

7.3.10 Communication parameters

7.3.10.1 Three output ports

At the back of the instrument there are two RS-232 serial ports and one parallel port for output devices:

```
PRINTER (serial ) (1)
PC                (2)
PRINTER (parallel)
```

The PRINTER ports are to be used with IBM/EPSON compatible dot matrix printers or HP DeskJet and LaserJet printers and the PC port with personal computers (actually it can be used with any computer having an RS-232C interface).

The PC port has a simple ASCII communication and to communicate with the 1470 at least a terminal emulator program is needed in the external computer. The main use of the PC port is to connect the MultiCalc to the WIZARD.

7.3.10.2 Communication parameters

For successful communication the communication parameters must be set to be the same at both ends. In "System mode | communication parameters" you must set the baud rate, number of data and stop bits, parity and handshake signal for each port.

Select the appropriate port, the choices are:

```
PC port
PRINTER port
```

Select the appropriate parameter:

```
Baud rate         300/1200/1800/2400/4800/9600
Stop bits         1/2 bit(s)
Word length       7/8 bits
Parity            None/Even/Odd
Protocol          None/DTR/RTS/XONXOFF
```

The default items are underlined.

7.3.10.3 Output device status

All output devices can be switched on or off. If all devices are off, no results are output but the counting continues normally. The user can override these settings from the PC and when on-line counting is started from the PC, the CPM results (input data to an evaluation program) are sent from the PC port to the PC in any case.

During counting the instrument program checks if the output device used is connected (break signal) and ready (off-line/on-line signal). If not, an error message is seen on the display. At this point the user can correct the error and retry or omit the device. No characters are lost in the first case and in the second case the device is omitted until the counting is stopped and another one started.

The same applies to the case when the output device is busy (ready/busy signal) and the instrument cannot send data out.

7.3.10.4 Error messages

The following error messages concerning output devices may appear on the built-in display:

```
PRINTER not connected (1=retry, 2=omit)
PRINTER not ready (1=retry, 2=omit)
PRINTER timeout (1=retry, 2=omit)
```

```
PC not connected (1=retry, 2=omit)
PC not ready (1=retry, 2=omit)
PC timeout (1=retry, 2=omit)
```

Either correct the fault and select 1 (=retry) or disable the expected connection with 2 (=omit).

Note: PC and mainframe port timeouts do not cause output to these ports to be turned off. If a timeout occurs, a message informing about this stays on the instrument display until you select whether to retry or terminate transmission to this port. The instrument waits until the selection is made and no data is lost.

7.3.11 Version

If you select this you can see what version of the software is being used to control WIZARD.

7.3.12 Disk operations

7.3.12.1 Format disk

To format a disk for use as a data disk put the disk in the drive and select the Format Disk option. If you confirm this operation the disk will be formatted. The formatted disk will hold 1.44 Mbytes of data in normal MS-DOS format.

Note: If the disk was not an unformatted empty disk then the format procedure will wipe clean all the data on the disk.

7.3.12.2 Verify disk

This function checks that the disk is properly formatted and readable.

7.4 STAT counting

STAT counting allows you to interrupt counting of the current batch, load new samples manually and count them. Results will be briefly displayed on the screen and will also be printed out. The STAT samples can then be removed and counting of the current batch resumed.

To initiate STAT counting during normal automatic counting, press the STAT key on the built-in keyboard (or F5 on the external keyboard). Any samples in the detectors will be removed and returned to the racks. The sample elevator arm will be raised (as in manual counting), then the display will prompt you to set three parameters. These are:

the isotope to be used
the counting time
the maximum counts limit.

Select "Do STAT" and press E.

When you have set these parameters, load your samples in the sample tray and place the tray in the detector wells. Press START.

Samples will be counted and results will appear on the display. The five display modes are described in section 2.4.2.

Results will be sent to the printer. First the heading STAT ASSAY will appear. Then the results will be printed out. The output items are:

DET, PEAK CHN*, PEAK DEV%*, RESOL%*, ACTIVITY, COUNTS, EFFICIENCY**, ERROR**, (where ERROR = 100 /□(Counts)), WINDOW keV*, LOW, HIGH, DECAYED ACTIVITY, MEASURED COUNTS, DETECTOR EFFICIENCY, RELATIVE ERROR %, HORROCKS EFFICIENCY*, SIGNIF. LEVEL %

* these only appear if extended normalization printout has been selected.

** these two items do not appear for single detector instruments). If the instrument has several detectors installed, but only one of them is active, these fields are printed, however. Then efficiency is 1.0 and the error 0.0.

At the end of the STAT printout the words END OF STAT ASSAY will be printed.

When you want to resume automatic counting press STOP and before confirming "Yes, end STAT" remove the STAT sample tray and close the cover over the detector block. The sample elevator will then be lowered to the normal position and counting will resume, starting with the samples which were in the counting position when STAT was pressed. Confirm "Yes, end STAT".

Note: A STAT assay can also be measured in the middle of an isotope normalization but it is not possible to measure a STAT assay in the middle of background normalization.

7.5 Power failure

7.5.1 Introduction

If power failure occurs (or the instrument is switched off) the microcomputer loses power, all operations are aborted and the normal working memory (RAM) is completely cleared. When power comes back the program is automatically reloaded. The instrument detects that a power failure has occurred and a message "Recovering from power failure" is displayed; a power failure message is also sent to the output devices. The power failure recovery operation begins immediately. If a further power failure should occur during the period of the first failure it will not prevent the recovery being fully completed once power returns.

Note: Recovering from power failure can be enabled or disabled by setting SYSTEM parameter Active hardware | Recover from power failure" to "Yes" or "No".

7.5.2 Interrupting power failure recovery

You can abort the power failure recovery operation by pressing the STOP key. A menu appears allowing you three choices:

1 "Continue" - the STOP instruction is cancelled.

2 "Stop conveyor" - the recovery operation is stopped and the instrument returns to the main menu.

3 "Clear conveyor" - the counting and transfer lanes of the conveyor are cleared of racks, the recovery operation is totally cancelled and the instrument returns to the main menu.

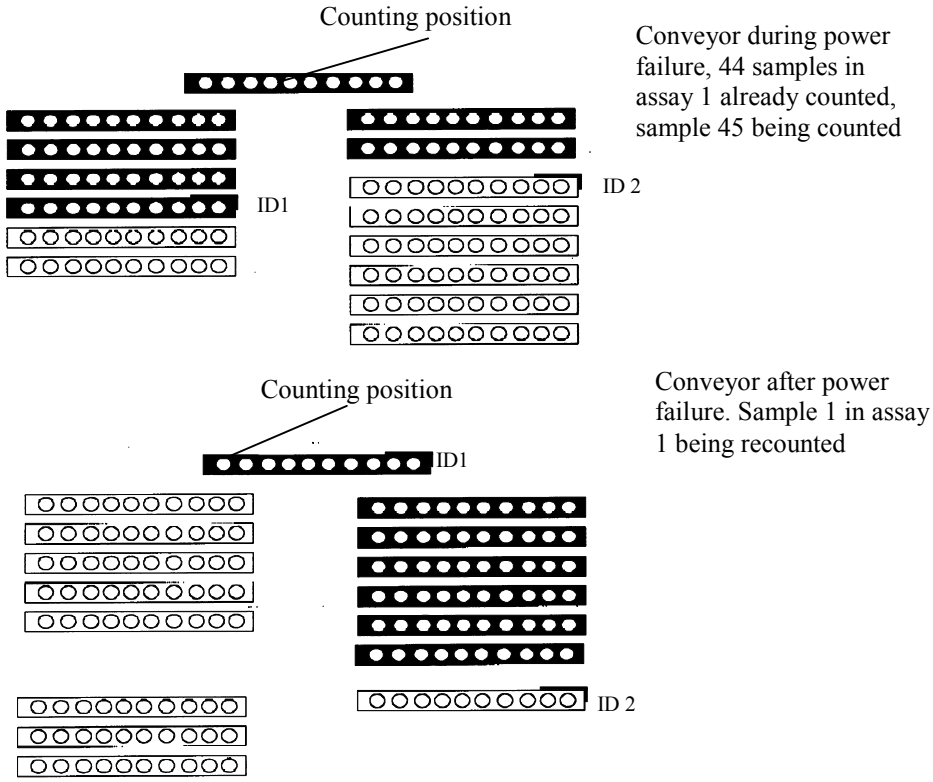
7.5.3 Power failure recovery

While recovery is occurring a text is displayed telling that power failure has occurred and that operation will begin again from the start of the assay. To remove this message press any key.

The program determines how many assays were uncompleted when the power failure occurred. "Uncompleted" means that although counting of an assay has been started (and may even have been ended) the results have not yet been either completely saved on disk or sent out to a printer or external computer (which of these is valid depends on the options selected). This information is stored in a special, battery powered memory. The conveyor will be then be run forward till the protocol ID cassette of the earliest of these uncompleted assays can be read again. The ID is read and counting starts in the normal manner for this assay followed by all the other uncompleted assays in turn.

The figure (see next page) shows how the power failure routine works. There are two assays on the conveyor, the one (dark colour) is being measured, the other assay (light colour) is waiting. Four racks have already been counted and the fifth position in the fifth rack (sample number 45 in the upper part of the figure) is being counted. Suppose that the power failure occurs at this moment. After power is restored the sample is taken from the detector and the conveyor is rotated anti-clockwise (the normal direction in counting) until the ID label of the first assay is encountered. This assay is then counted starting with sample 1. The previously obtained 44 results can be discarded.

Note: Do not remove any racks from the conveyor before the assay to wich they belong is completed because if a power failure occurs, wrong results would be obtained during power failure recovery.



7.6 Routine maintenance

7.6.1 Cleaning

The instrument and its environment should be cleaned regularly to avoid contamination and raised background. In particular the conveyor and elevator forks should be cleaned using a soft cloth and alcohol.

Note: The dust covers should be kept closed to reduce the amount of dust entering the detector wells.

7.6.2 Checking the background

If you want to check the background without actually saving the results, as will happen in background normalization, then you must count an empty rack which has holders but no sample tubes. Make sure the counting time is long enough to collect enough counts e.g. 10 min.

7.6.3 Decontamination

If the background does increase then you should carefully clean the detector wells using a soft cloth soaked in alcohol. *Avoid using any decontamination liquid which might be corrosive.* Beware of scratching the surface of the detector wells. Check that the background is back to normal after cleaning.

It can also be that the sample carriers become contaminated. These should be regularly checked and if necessary cleaned or replaced.

A mild decontamination solution can be used for this.

7.7 Safety and radioactive materials

The following comments about precautions and safety measures in handling radioactive materials are included as a guide and are not intended to be fully comprehensive. More complete details may be found elsewhere, for example in the booklet SAFE HANDLING OF RADIONUCLIDES, published by the International Atomic Energy Agency, Vienna; this may be recommended as a useful code of practice appropriate to radio-chemical laboratories.

Unless a specially designed radioisotope laboratory is used, limitations should be placed on the amount of active material in the laboratory area depending on toxicity and type of chemical operation. For high toxicity material and wet chemical operations involving the risk of spillage, the IAEA recommend a maximum activity of about 10 μCi.

Personnel should be properly trained in the safe handling of these materials, maximum levels of stored activities should be set, proper records should be kept, and a definite monitoring schedule maintained.

The areas where samples are handled should be kept clean and free of dust. This is most easily accomplished if all surfaces are as smooth as possible and if the minimum number of extra surfaces is introduced into the room. Lastly it is extremely important to store all radioactive materials in a separate room to which access is restricted.

7.8 Large Eppendorf tubes

7.8.1 Introduction

Note: this feature should be installed by a service engineer.

It is possible to measure large Eppendorf tubes. These have caps and can be inserted only at every other sample position in the rack, at odd sample positions (1, 3, .. 9). For measuring this type of tube, every second elevator fork must be removed. The instrument program must be informed of these changes as described in this chapter. The changes involve some SYSTEM parameters that are only used for this purpose.

7.8.2 SYSTEM setting

The parameter "SYSTEM | Active hardware | Forks only at odd positions" must be set to YES to tell the instrument program that the elevator forks are only at odd detector well positions. The leftmost fork must be at detector well position 1.

Note: "Forks only at odd positions" is visible only if the instrument has more than one detector installed.

The parameter "SYSTEM | Operation mode | Ignore even sample pos." must also be set to YES when you are measuring large Eppendorf tubes. The default setting NO is used when you are measuring normal vials that can occupy every sample position in the rack.

Note: "Ignore even sample pos." is visible only if the instrument has only 1 detector installed or if "SYSTEM | Active hardware | Forks only at odd positions" is set to YES.

If "Ignore even sample pos." is set to YES and a sample is detected at an even rack position, the conveyor stops and an error message appears. (A sample is allowed in an even rack position, however, if the rack is a background or an isotope normalization rack or a GLP TEST measurement rack).

Note: if the parameter "SYSTEM | Operation mode | Ignore if no holder" is set to YES, then all even or odd sample positions without a holder are omitted in position counting. If "Ignore even sample pos." is YES then only even sample positions are omitted in position counting and the rack is also checked so that there are no holders in even rack positions.

7.8.3 Optional detector removal

Since now only detectors at odd detector well positions can be used the detectors at even well positions can be removed although they need not be.

If they are removed, then set the parameter "SYSTEM | Active hardware | Dets ”Dets only at odd positions>only at odd positions" to "YES". This parameter is visible only if the number of detectors installed is 2 or 5. If the installed detectors are only at odd well positions, they can all be used, otherwise only those at odd well positions can be used. In the latter case detector numbering in assay printouts ignores detectors at even well positions.

If the value of either the parameter "Forks only at odd positions" or the parameter "Dets only at odd positions" is changed, all normalizations will be deleted. A warning is issued before this is done.

8 Instrument description

8 Instrument description

8.1 Introduction

In this chapter the main features of 1470 Wallac WIZARD are presented, showing what they are and what benefits they offer you as a user.

8.2 Self-contained

WIZARD has the possibility to be a completely self contained counter with its own built-in computer. This is a 16 bit computer having one 3.5" disk drive and a 30 Mbytes hard disk. A warning is issued when the instrument harddisk becomes full. If at the beginning of an assay or normalization there is less than 3 Mb free disk space in the instrument hard disk, an error message is displayed and printed and the conveyor is cleared. The computer contains 1 Mbyte of RAM memory, two serial RS232C ports, one parallel port and a built-in 9 " screen monitor. There is a built-in keyboard and an optional additional full PC keyboard.

8.3 Interactive control

The built-in display can be used in conjunction with the keyboard for interactive control of the counter. In addition it can display information about the state of counting, providing either live numerical or graphical information.

The built-in keyboard has pressure sensitive keys with tactile feedback. Most operations can be set up and controlled with this keyboard which means that you do not need to have a PC near the counter.

8.4 External communication

WIZARD can also be controlled via an external computer and can send results via a serial RS-232C interface to external devices such as a printer or PC or via MultiCalc to a LAN or mainframe.

8.5 Multi-technology data management software

WIZARD can be used to deal with all gamma counting as part of an integrated label measurement system by using the Wallac MultiCalc software package which runs on an external PC. This software not only supports gamma counting, but all other technologies too, so the system can also include measuring devices for almost all other label technologies.

8.6 Customization and upgradability

A number of versions of the instrument are available with 1, 2, 5 or 10 detectors, offering throughputs from 50 to 500 samples/hour. The smaller conveyor size can take 550 samples when fully loaded. The larger model can take 1000 samples.

This combination of operating systems and physical configurations offers you a wide range of possibilities to choose from. You can select the one which best suits your current needs and resources knowing that when necessary you can upgrade the system to meet new requirements.

8.7 Compactness

WIZARD has an exceptionally small footprint for a multi-detector automatic gamma counter: the 550 sample, 10-detector table top version of WIZARD is only 65 cm wide by 70 cm deep. This has been achieved by locating the

display and detector block between the lanes of the conveyor rather than adding them onto the end, thus increasing the instrument length.

8.8 High efficiency detectors

In the past automatic multi-detector counters have been based on the incomplete geometry of through-hole detectors, but this is not so in the case of WIZARD. PerkinElmer Life Sciences, Wallac Oy has successfully incorporated well type detectors into the design of a high throughput automatic gamma counter. These give WIZARD the best possible counting geometry and hence high counting efficiency, see the specifications for actual values. This design breakthrough has been made possible by means of a unique sample changer mechanism which takes the ten samples arranged linearly in a rack and moves them to the detector wells. These are arranged in a staggered configuration which maximizes the shielding between detectors while minimizing the size of the detector block.

8.9 Detector shielding

The detector assembly is surrounded by a minimum of 12 mm of lead shielding against radiation in the vertical plane. The shielding against radiation from samples on the conveyor is 30 mm (1 1/4 "). The shielding between detectors is 7 mm.

8.10 Flexible sample handling

Samples are carried in racks which are made of plastic with place for 10 samples/rack. They are provided with individual sample carriers which can be replaced in case of contamination. These racks and sample carriers can be used as they are in a centrifuge.

This sample carrier system enables WIZARD, in automatic mode, to count sample vials of any shape up to 13 mm in diameter, or microspheres, which need not even be in vials. In manual mode you can count sample vials up to 17 mm.

8.11 Multi-user ID system

WIZARD is designed for automatic multi-user operation. Each rack can be provided with two barcodes, each having capacity for 2 digits or a special code word. These barcodes are used to tell WIZARD how to count the samples. Each user can load samples with racks coded for the particular counting conditions he or she wants. Each time the conveyor presents the WIZARD ID reader with a set of samples coded with new ID, the appropriate counting protocol is selected and the samples counted according to it. These counting protocols can be either set up with the internal WIZARD computer or an external PC running RiaCalc or MultiCalc.

8.12 Multiple label, multi-detector counting

Dual label samples can be counted because WIZARD corrects for the spillover from one counting channel into another which often occurs with particular pairs of isotopes e.g. I-125 and Co-57. A process called "normalization" is used to calculate this correction. Once a normalization has been done for a pair of isotopes the results are stored and can be used any time that samples labelled with that pair of isotopes are to be counted.

The normalization process also makes possible multi-detector counting by eliminating the effect of gain variation in detectors. The normalization process ensure that counts for any detectors are within ±1 % of the mean counts of all detectors. Peak positions and window settings are also optimized and background corrected for.

Another problem to be faced with multidetector counters, especially with higher energy samples such as Cr-51 is that of crosstalk. Radiation from a sample in one detector or on the conveyor contributes to the counts recorded in another detector. Special software solves this problem for WIZARD.

8.13 Isotope selection

WIZARD has a list of isotopes, see the list in the next chapter 8.2 Specifications. All the isotopes in WIZARD have predefined settings.

The energy range of samples which WIZARD is set to cover is up to 1000 keV which means that WIZARD can handle Chromium Release tests as well as the usual single and dual labelled RIA's. The settings for these isotopes can be customized by the user in the range up to 1000 keV. The energy range can be extended by qualified service personnel.

8.14 Multichannel analyzer

There is a linear multichannel analyzer with 1024 channels, calibrated for the range 1 - 1024 keV. It has a 12 bit Analogue/Digital converter and automatic dead time compensation.

8.15 Automatic and manual operation

WIZARD is both an automatic and a manual counter. Manual counting allows you to use even bigger samples than in automatic mode. It also gives you security in case you should have problems with the conveyor; you can still count samples manually. If you just want to count a few samples without bothering to put them into a rack and load it onto the conveyor you can do it with manual mode.

8.16 GLP performance testing

Instrument performance can be monitored by running GLP TEST normalizations at regular intervals. These store data that can later be viewed in graphical format.

GLP means "Good ”Good Laboratory Practice”>Laboratory Practice". A GLP TEST normalisation is similar to isotope normalisation, only results are stored differently. Data obtained in a GLP TEST normalisation is not used in assay measurements, but is tested against preset limits and then stored so that it can later be compared with other TEST normalisations using the same isotope. This comparison is done by presenting the values of some measured parameters as a function of time, so that any systematic trends or large random deviations can easily be discerned.

9 Specifications

9 Specifications

9.1 Physical dimensions

Height: 560 mm (22.0")
Width: 650 mm (25.6") or 1170 mm (46.1")
Depth: 770 mm (30.3") or 650 mm (25.6")
Weight: 128 kg or 140 kg

The smaller dimensions are for the 550 sample version and the larger for the 1000 sample version.

9.2 Electrical requirements

230 V+/- 10% at 50/60 Hz
115 V+/- 10% at 50/60 Hz
200 VA maximum

9.3 Environmental requirements

Temperature range from +15 °C to +35 °C
Max. humidity 85 %

9.4 Sample handling

9.4.1 Sample vials

Max diam. 13 mm (17mm in manual mode without a tray)
Max. cap diam. 14 mm
Min. diam. no limit
Max. height 90 mm (including cap)
Vial shape no shape restrictions

9.4.2 Sample racks

Plastic racks, 10 samples/rack
Length: 164 mm
Width: 18 mm
Height: 57 mm
Max. centrifugation force: 2500 G
Max. temp: 40 °C

Racks are provided with individual sample carriers which can be replaced in the case of contamination.

9.4.3 Sample changer

Bi-directional automatic sample changer with a storage capacity of 55 racks (550 samples) or 100 racks (1000 samples).

9.4.4 Sample ID

Each rack can be provided with two barcodes, each having capacity for 2 digits or a special code word.

9.5 Detector system

Detectors are of the end-well type with a 50 mm x 32 mm (2.0 " x 1 1/4") thallium activated, sodium iodide crystal.

9.5.1 Shielding

The detector assembly is surrounded by a minimum of 12 mm of lead shielding against radiation in the vertical plane. The shielding against radiation from samples on the conveyor is 30 mm (1 1/4 "). The shielding between detectors is 7 mm.

9.5.2 Detector Matching

Within ±1 % of mean counts of all detectors after normalization.

9.5.3 Isotopes

The instrument is preset for the isotopes listed in the table at the end of this chapter. All isotope settings can be changed by the user.

9.5.4 Efficiency

I-125 > 78 %
Co-57 > 80 %
Cr-51 > 3 %
Cs-137: 26 % , (typical)
Co-58: 3.5 %, (typical)

9.5.5 Energy resolution

I-125 < 30 %
Co-57 < 16 %
Cr-51 < 14 %
Cs-137: 10 % (typical)
Co-58: 8 % (typical)

9.5.6 Spilldown

Spilldown of Co-57 into I-125 preset regions:
< 3% (uncorrected)
< 1% (corrected)

9.5.7 Crosstalk

Crosstalk detector to detector, uncorrected, worst case:
I-125 – negligible
Co-57 – negligible
Cr-51 < 0.5 %
Cs-137 < 4 %
Co-58 < 5 %

Crosstalk conveyor to detector, single source, worst case:
I-125 – negligible
Co-57 – negligible
Cr-51 – negligible

Cs-137 < 0.12 %
Co-58 < 0.2 %

The effect of crosstalk is corrected by patented crosstalk correction software.

9.5.8 Background
I-125 : 50 CPM
Co-57: 90 CPM
Typical values at the factory, Turku, Finland (background may be different elsewhere depending on local conditions).

9.5.9 Energy range
Default setting is up to 1024 keV

9.5.10 Gain stabilization
Optimised window setting for each isotope is based on the use of multichannel analyser techniques. The stability and reproducibility of the results are ensured by checking resolution, efficiency, spectrum drift and background.

9.5.11 MCA
Linear multichannel analyser with 1024 channels, calibrated for the range 1 - 1024 keV. 12 bits Analogue/ Digital converter. Dead time max. 25 µs, dead time effect is compensated by a program.

9.6 Instrument control

9.6.1 Hardware
The system is controlled by a 16 bit computer having a single 3.5" disk drive and a hard disk. The computer contains 1 Mbyte of RAM memory, two serial RS232C ports and one parallel port and a built-in 9" screen monitor. Built-in keyboard with pressure sensitive keys.

9.6.2 Software
Operating system: MS-DOS v. 3.21. Control by an external computer using MultiCalc immunodiagnostic data management software.

9.6.3 Connections
Serial ASCII interface RS-232, 3 output terminals:
Terminal 1 for printer
Terminal 2 for system PC (external MultiCalc)
Terminal 3 for parallel printer

9.7 Electrical safety requirements

The design of the instrument is based on the following electrical safety requirements:
EN 61010-1 1993 (IEC 1010-1)
CSA-C22.2 No. 151-1986
CSA-C22.2 No. 0-4-1982
CSA-C22.2 No. 0-M91
EN 50082-1:1992; EN 50081-1:1992

9.8 Isotopes defined for 1470 WIZARD

ID	Isotope	Name	Energy [keV]	Eff.* [%]	Halflife [hours]	Coverage [%]	Low W [keV]	High W [keV]	Res. [%]
1	I-125	Iodine	29	82	1445	97			25
2	Co-57	Cobalt	122	90	6480	92			13
3	Cr-51	Chromium	320	3.7	667	80			9
4	I-129	Iodine	31	65	1.49E+11	96			24
5	As-76	Arsenic	559	7	26.4	31			
6	Au-195	Gold	99	75	4390	95			
7	Au-198	Gold	412	11	64.7	47			
8	Ba-133	Barium	356	16	6.30E+04	54			
9	Ba-139	Barium	166	76	1.38	87			
10	Br-77	Bromine	245	11	57	74			
12	Cd-109	Cadmium	22	71	11136		16	32	
13	Ce-141	Cerium	145	56	780		125	167	
14	Co-58	Cobalt	810	4	1711		660	930	
15	In-111	Indium	416	42	67.7		150	500	
16	Cs-134	Caesium	795	30	18063	25	500	890	10
17	Cs-137	Caesium	662	26	2.63E+05	62			
18	Er-171	Erbium	308	26	7.52				
19	F-18	Fluorine	511	8	1.83	85	20	1800	
21	Ga-67	Gallium	185	70	78				
22	Gd-153	Gadolinium	147	100	5808	68	26	167	
23	Hg-203	Mercury	279	31	1126	88			
24	I-123	Iodine	159	80	13.3				10
25	I-131	Iodine	360	15	193		260	430	
26	In-111	Indium	416	42	67.7		320	541	
27	In-114m	Indium	190	42	1188	52	166	210	
29	K-43	Potassium	373	14	22.6	37			
30	Na-22	Sodium	511	8	2.27E+04				
31	Nb-95	Niobium	766	30	841	68	686	846	
32	Pb-203	Lead	279	31	52.1				
34	Ru-103	Ruthenium	497	30	944	45	400	600	
35	Sb-125	Antimony	428	10	2.37E+04	88			
37	Sc-47	Scandium	160	80	82.1	75			
38	Se-75	Selenium	265	31	2880	93			
39	Sm-153	Samarium	103	86	47				
40	Sn-113	Tin	392	43	2760	36	350	430	
41	Sr-85	Strontium	514	8	1530	50			
42	Sr-87m	Strontium	388	12	2.8	90			
43	Tc-99m	Technetium	140	86	6.02				
44	Open	15-1000 keV	513	100			0	1024	
46	Ge-68	Germanium	512	100	6504		20	1800	
47	C-11	Carbon	511	100	3.41E-1		20	1800	
48	O-15	Oxygen	511	100	3.40E-2		20	1800	
49	N-13	Nitrogen	511	8	1.655E-1		20	1800	
50	Tl-201	Thallium	70	100	73.06		60	90	
51	Cu-64	Copper	511	8	12.701		425	640	
52	Ti-45	Titanium	511	8	3.08		420	600	
53	Re-188	Rhenium	155	70	16.98	97	95	203	
91	I-125T	Iodine GLP	29	82	1445	25			
92	Cs-137T	Caesium GLP	662	5.6	2.63E+5				
93	Ge-68T	Germanium GLP	512	100	6912		20	1800	

* Eff. [%] = (CPM/DPM) x 100, typical values, open window. It includes transition probabilities.

10 CALCULATION METHODS

Applies to program version 3.5

August 1999

10 Calculation methods

10.1 Background normalization

10.1.1 How background is measured

When an empty rack having an ID clip with the "BKG" label sticked in the area marked "RACK/SPECIAL" is encountered during automatic measurement, counts are collected from all detectors for the time given by the parameter "SYSTEM | Background | Bgrd. counting time". The obtained spectra are then stored on the instrument hard disk. For the 1470 counter model the background measurement can also be started manually when the conveyor or rack id reader are not used. (The conveyor can be disabled for the 1470 counter model by setting "SYSTEM | Operation mode | Manual mode used" to "Yes" and the rack id reader can be disabled by setting "SYSTEM | Active hardware | Use rack id reader" to "No".)

For the 1480 counter model background is measured for both the normal and the extended energy range if there is no label stuck in the "PROTOCOL" area of the ID clip. If an additional odd number ID label is in the "PROTOCOL" area, the background is measured only for the normal energy range (15 - 1000 keV). An even number label causes background to be measured only for the extended energy range (15 - 2000 keV).

Total background CPM is calculated for all detectors that are in use. In addition for each normalized isotope the background CPM in the counting window of the isotope is determined.

If background normalization is done in 1470 using the conveyor, there may be detectors that are active but nevertheless are not used for measurement, since with the conveyor only active sets of adjacent 10, 5, 2 or 1 detectors can be used for measurement.

10.1.2 Total background CPM

This is calculated by summing counts from all multi channel analyser (MCA) channels except the first 10. This channel range is called the "open window". Since the last MCA channel includes high energy counts that have energies exceeding the range of the MCA, they are also reported in the total background CPM. The open window background counts value is corrected for detector dead time and then converted to CPM. The conversion from counts to CPM is done with the formula

$$CPM = \frac{60 * Counts}{CountingTimeInSeconds}$$

Sometimes isotope activity is expressed in CPS, counts per second. The conversion from counts to CPS is done with the formula

$$CPS = \frac{Counts}{CountingTimeInSeconds}$$

10.1.3 Dead time correction

Dead time correction compensates for counts lost due to the finite counting rates of the detector and the measuring circuit. For 1480 the following formulas are used.

$$DeadTimeCorrectedCPM = DeadTimeFactor * MeasuredCPM$$

$$\tau = 6.45 * 10^{-6}$$

$$DeadTimeFactor = \frac{1}{1 - CPS * \tau - \frac{1}{2}(CPS * \tau)^2}$$

Here CPS is the counts per second activity and τ is the dead time of the measuring circuit in seconds for each measured count. The formula takes into account that at high sample activities pulses pile up so that several pulses are detected as one pulse with higher energy.

A different formula for calculating the dead time factor is used in 1470. For this counter model up to five detectors can be connected to the same analogue-to-digital (A/D) converter. A 10 detector counter has two A/D converters. Because of this the dead time factor of one detector is affected by counts from other detectors that are connected to the same A/D converter. The dead time factor is calculated as shown by the following pseudo-code. The formula for Tmp2 was chosen heuristically to account for the influence of other detectors. CountsFromAllDets is the total counts from all detectors that have been connected to the same A/D converter as the current detector. CountsFromCurrentDet is the total counts from the detector for which we are calculating the dead time factor.

```
CountsFromOtherDets = CountsFromAllDets - CountsFromCurrentDet
CountCheckTime = 25.E-8
CountConvertTime = 475.E-8
DetectorDeadTime = 100.E-8
Tmp1 = (1. - CountConvertTime * CountsFromAllDets / CountingTimeInSecs) / (5. * CountCheckTime)
Tmp2 = (CountConvertTime * CountsFromOtherDets / CountingTimeInSecs) / Tmp1
CpmFromDet = 60. * CountsFromCurrentDet / CountingTimeInSecs
CorrFac1 = 60. / (60. - CpmFromDet * Tmp3)
Tmp3 = CountConvertTime + 5. * CountCheckTime / 2. + Tmp2 / 2.
Tmp4 = CpmFromDet * CorrFac1
CorrFac2 = 60. / (60. - Tmp4 * DetectorDeadTime)
DeadTimeFactor = Tmp4 * CorrFac2 / CpmFromDet
```

If a sample decays very quickly, the dead time factor also decreases during measurement. This effect has not been taken into account in the above formulas.

10.1.4 Converting isotope counting windows from keV's to MCA channels

In order to sum background counts over an isotope counting window, the window boundaries must be known in terms of MCA channels.

For each normalized isotope the isotope counting window boundaries in keV's are known. If "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-%", they are determined during isotope normalization, otherwise they are given by the parameters "SYSTEM | Isotopes | <isotope name> | Low boundary (keV)" and "High boundary (keV)". These can be converted to MCA channels if the gain (keV / channels) is known.

If "Counting window" is "Dynamic-%" or "Dynamic-keV", then the effective gain is calculated by dividing the energy of the main isotope peak in keV's by the actual position of the main isotope peak in MCA channels. The program has stored the position of the main isotope peak in MCA channels the last time an assay was measured using this isotope (or when the isotope was normalized, if no assays using this isotope have been measured since normalization). If "Counting window" is "Fixed", then nominal gain is used when this conversion is done.

For 1470 and 1480 normal energy range the nominal gain is equal by the parameter "SYSTEM | Active hardware | Nominal gain". The factory setting for it is 1.0 keV / channel. If the value of this parameter is changed, it affects only how keV's are converted to channels and vice versa by the software, not the effective gain of the electronics circuit. The latter must be set by a service technician. For the 1480 model the parameter "Nominal gain" is the normal energy range gain. The nominal gain for the 1480 extended energy range gain is 2.0 keV / channel and cannot be changed.

10.1.5 Calculation of background CPM in the isotope counting window

Counts are summed over the isotope counting window, the result is multiplied by the dead time correction factor and converted to CPM. In the printout these CPM values are further divided by the relative detector efficiency (1470 only). However, the background values stored internally do not have this correction. Therefore in assay counting, background activity is subtracted before correction for relative detector efficiency is made.

As far as assay counting is concerned, background and isotope normalization can be made in any order. Background normalization uses the latest effective gain value to calculate background CPM in all isotope counting windows and updates isotope normalization results with the measured background activity. During isotope normalization the latest background spectra are used to update background CPM to correspond to the latest counting windows. However, when crosstalk and spillover correction coefficients are calculated, the latest background normalization done prior to the isotope normalization is used. This is a reason to do background normalization before isotope normalization(s).

When the program determines peak positions in terms of MCA channels, it uses interpolation to estimate the peak position as a fraction of channel. Likewise counting window boundaries are determined to a fraction of channel. When counts are summed over a counting window, counts from the low and high boundary channels are added only in the proportion that these channels are included inside the counting window.

10.1.6 Diagnostic info

Diagnostic info is printed during background normalization if "SYSTEM | Diagnostic output | Print diagnostic info." is "Yes". For 1470 you can select the detectors for which diagnostic info. is printed in the menu "SYSTEM | Diagnostic output | Select detectors for info.". The following values are printed.

For all detectors that are in use:

- Open window counts
- Dead time factor

For all normalized isotopes and for all detectors that are in use:

- Effective gain (keV / channel)
- Counting window in MCA channels
- Measured counts in the counting window

```
BACKGROUND NORMALIZATION      22-Sep-1994 10:37:05

DIAGNOSTIC INFO
DETECTOR 2
Open window counts         413
Dead time factor           1.000044
ISOTOPE  1 I-125 Iodine
DETECTOR 2
Gain                       0.957
Window (channels)          17.5      71.9
Counts in window           32
ISOTOPE  2 Co-57 Cobalt
DETECTOR 2
Gain                       0.941
Window (channels)          94.7      145.5
Counts in window           49
ISOTOPE  3 Cr-51 Chromium
DETECTOR 2
Gain                       1.004
Window (channels)          83.1      347.9
Counts in window           201
ISOTOPE  4 I-129 Iodine
DETECTOR 2
Gain                       0.946
Window (channels)          19.6      49.2
Counts in window           7
END OF DIAGNOSTIC INFO

Counting time              60

Counts per minute:
DET           1      2      3      4      5      6      7      8      9     10
OPEN      409.0  413.0  382.0  374.0  382.0  388.0  412.0  411.0  395.0  368.0
I-125      38.0   32.4   24.7   25.4   32.1   20.5   34.5   23.4   24.4   23.6
Co-57      56.5   49.6   51.5   38.9   45.5   58.7   58.5   57.0   46.7   41.6
Cr-51     190.8  199.8  188.3  168.0  179.1  172.2  194.8  213.8  193.6  173.9
I-129      24.1    7.1    7.8   12.8   14.0   14.0   14.0   12.0   10.4    9.2

VALUES SAVED

END OF BACKGROUND NORMALIZATION
```

Figure 1. Background normalization printout when "SYSTEM | Diagnostic output | Print diagnostic info." is "Yes" and only detector 2 has been selected in the menu "SYSTEM | Diagnostic output | Select detectors for info." and "SYSTEM | Printout selections | Horizontal bgrd printout" is "No".

```
BACKGROUND PRINTOUT

Measured on                22-Sep-1994 10:37:06
Counting time              60

Counts per minute:
DET           OPEN          I-125          Co-57          Cr-51          I-129
  1          409.0           38.0           56.5          190.8           24.1
  2          413.0           32.4           49.6          199.8            7.1
  3          382.0           24.7           51.5          188.3            7.8
  4          374.0           25.4           38.9          168.0           12.8
  5          382.0           32.1           45.5          179.1           14.0
  6          388.0           20.5           58.7          172.2           14.0
  7          412.0           34.5           58.5          194.8           14.0
  8          411.0           23.4           57.0          213.8           12.0
  9          395.0           24.4           46.7          193.6           10.4
 10          368.0           23.6           41.6          173.9            9.2

END OF BACKGROUND PRINTOUT
```

Figure 2. The same background normalization is printed again using "SYSTEM | Backgroud | Print background". Now "SYSTEM | Printout selections | Horizontal bgrd printout" is "Yes". The width of this layout will not exceed 80 characters if there are at most 10 isotopes that are normalized.

10.2 Isotope normalization and GLP TEST measurement

When a rack having an ID clip with the "NORM" label stuck in the area marked "RACK/SPECIAL" is encountered during automatic measurement, WIZARD starts normalising the isotope that has the code number that is in the "PROTOCOL" area of the ID clip. The rack must have only one holder and sample in the last position of the rack.

The 1480 counter model can also normalize isotopes at the beginning of a multiple isotope assay (MIA). This is done if an isotope standard used in MIA has a replicate number greater than 0.

A GLP TEST measurement is otherwise similar to isotope normalization, only the obtained results are not used in assay counting but are stored separately so that they can be compared with the results obtained in previous GLP TEST measurements. GLP means "good laboratory practice".

In the 1470 counter model isotope normalization can also be started manually when the conveyor or rack id readers are not used.

10.2.1 What is done during isotope normalization

- Peak resolution is determined if "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-%" or "Dynamic-keV". It is the half-height width of the isotope main peak divided by the energy of the peak.
- • Detectors are tested for stability if "SYSTEM | Isotope | <isotope name> | Repeat times" is greater than 1. (This is not done in 1480 MIA isotope standard normalizations.)
- Background activity in the counting window is updated to correspond to the new counting window.
- The MCA channel number of the isotope main peak is determined if "Counting window" is "Dynamic-%" or "Dynamic-keV". This makes it possible to determine the effective gain (keV / channel) during normalization. This in turn is used when counting windows are converted between keV's and channels.
- Horrocks efficiency is determined if "SYSTEM | Isotope | <isotope name> | Spectrum type" is "I-125" and "Counting window" is "Dynamic-%" or "Dynamic-keV". This makes it possible to determine the absolute detector efficiency for I-125 without using an external standard.
- If "Counting window" is "Dynamic-%", the counting window in keV's is determined for each detector. This is done so that starting from the isotope main peak the window is widened until it includes at least the fraction given by "SYSTEM | Isotope | <isotope name> | Window coverage (%)" of all counts in the spectrum. If "Spectrum type" is "I-125", both the main peak and the coincidence peak are always included in this counting window.
- Relative detector efficiencies are determined for 1470 counters that have multiple detectors installed.
- Measured isotope normalization sample spectra are stored for all detectors. This makes it possible to eliminate spillover in dual label RIA/IRMA/RATIO assays and 1480 multiple isotope assays (MIA). If a 1470 counter has more than one detector installed and "SYSTEM | Isotope | <isotope name> | Crosstalk correction" is "Yes", then for each detector where the normalization sample is measured, the spectra of all other (empty) detectors are also stored in the instrument hard disk. These are used to eliminate crosstalk in single and dual label assay counting. In the latter case also the crosstalk of one isotope to an other isotope's counting window in another detector is corrected for.
- The corrected CPM activity of an isotope standard is determined if isotope normalization is done at the beginning of a 1480 multiple isotope assay (MIA). It is used to calculate the ratio of an unknown sample's activity to the standard sample's activity.

```
ISOTOPE 1

Name                    I-125
Comment                 Iodine
Normalization time      60
Repeat times            1
Crosstalk correction    NO
Decay correction        YES
¨Half-life (hours)      1445.
¨Norm. zero time        Start
¨Assay zero time        Start
Counting window         Dynamic-%
¨Peak pos. (keV)        29.
¨Window coverage (%)    97.
¨Threshold level (%)    20.
¨Spectrum type          I-125
¨¨Max.coinc. dev. (%)   25.
¨¨Min.coinc.height(%)   25.
¨Max. assay dev. (%)    30.
¨Max. norm. dev. (%)    50.
¨Warn. assay dev. (%)   20.
¨Signif. cpm per keV    10.
Efficiency (%)          82.
Max.det.eff.dev. (%)    5.
```

```
ISOTOPE 14

Name                    Co-58
Comment                 Cobalt
Normalization time      60
Repeat times            1
Decay correction        YES
¨Half-life (hours)      1711.
¨Norm. zero time        Start
¨Assay zero time        Start
Energy range            Normal
Counting window         Dynamic-keV
¨Peak pos. (keV)        810.
¨Low boundary (keV)     180.
¨High boundary (keV)    950.
¨Threshold level (%)    20.
¨Spectrum type          Many peaks
¨Max. assay dev. (%)    10.
¨Max. norm. dev. (%)    20.
¨Warn. assay dev. (%)   5.
¨Signif. cpm per keV    2.
Efficiency (%)          65.
GLP test sample DPM     10000
```

Figure 3. Default isotope parameters for I-125 (1470 counter) and Co-58 (1480 counter). They can be printed with the dialog screen "SYSTEM | Isotopes | Print isotope and normalization".

10.2.2 Summing spectra of repeat measurements

IF "SYSTEM | Isotopes | <isotope name> | Repeat times" is greater than 1, the sample is measured in each detector as many times as specified in Repeat times. The total counting time for the sample in each detector is in this case "Repeat times" multiplied by "SYSTEM | Isotopes | <isotope name> | Normalization time". Isotope normalizations that are done at the beginning of a 1480 multiple isotope assay (MIA) cannot have repeats and in this case the parameter "Repeat times" is ignored if it is greater than 1.

The purpose of repeat measurements is to check detectors for stability. In extended normalization printout the field "SIGNIF. LEVEL" gives the probability that differences in counts values in repeat measurements are just random variations. If "SIGNIF. LEVEL" is often very near zero or 100, there is reason to suspect that the counter does not operate properly.

The separate repeat measurement spectra are not needed to calculate any other data except "SIGNIF. LEVEL". Therefore the repeat spectra are summed together and all other normalization results are determined from this sum spectrum. This gives better accuracy than for example using only the first repeat measurement spectrum.

10.2.3 Summing spectra of MIA standard replicate measurements

If an isotope standard normalization is done at the beginning of a 1480 multiple isotope assay (MIA) the isotope standard sample can consist of several replicate tubes. These are all measured and their spectra are summed. When the isotope counting window has been determined from the sum spectrum, the individual replicate spectra are used to determine the counts, CPM and relative activity of each standard replicate tube. These are included in the isotope normalization printout. Otherwise all other normalization results are determined from the sum spectrum.

10.2.4 Isotope counting window

In order to sum counts over an isotope counting window its boundaries must be known in terms of MCA channels.

If "SYSTEM | Isotope | <isotope name> | Counting window" is "Fixed" or "Dynamic-keV", the keV-values of the boundaries are given by the parameters "SYSTEM | Isotope | <isotope name> | Low boundary (keV)" and "High boundary (keV)" and they only need to be converted to MCA channels.

If "Counting window" is "Dynamic-%", the counting window is first determined in terms of MCA channels and these are later converted to keV's. They are again converted to MCA channels during assay counting using the effective gain at assay counting time.

Counting windows in terms of MCA channels are needed during isotope normalization to calculate the following data:

- Relative detector efficiencies if a 1470 counter has more than one detector installed.
- Crosstalk correction coefficients that are used in single label RIA/IRMA/RATIO assay counting if a 1470 counter has more than one detector installed. (Spill and crosstalk correction coefficients that are used in dual label RIA/IRMA/RATIO assays are calculated at the beginning of the assay; so are MIA spillover correction coefficients).
- The isotope standard sample activity and standard replicate tube activities if isotope normalization is done at the beginning of a 1480 multiple isotope assay (MIA).
- To test detectors for stability if "SYSTEM | Isotope | <isotope name> | Repeat times" is greater than 1.

10.2.4.1 Fixed window

When the boundaries of a fixed window are converted from keV to MCA channels, the nominal gain (keV / channels) is used as the effective gain. Thus any drifting due e.g. to temperature variations is not taken into account.

Nominal gain is explained in Section 10.1.4 on page 132.

10.2.4.1.1 When fixed window should be used

Fixed counting window is handy in that there is no need to specify the isotope main peak. You can use it if you do not know what the isotope spectrum looks like or are otherwise unsure how various isotope parameters should be set for the isotope. Using a fixed window you can even measure samples that contain unknown isotopes. If you set "SYSTEM | Isotopes | <isotope name> | Low boundary (keV)" to 0 and "High boundary (keV)" to a value higher than the MCA high limit (e.g. to 3000 keV), you get an open counting window that includes all counts registered by the detector.

10.2.4.1.2 Converting keV's to MCA channels

If the ratio of keV and the corresponding MCA channel number were constant one could convert keV's to channels with the formula

$$ChannelNumber = \frac{keV}{EffectiveGain}$$

However, this is complicated by the fact that the energy scale of the detector is not linear between 31 and 662 keV. We can take this into account by introducing so called "absolute keV's" and "detector keV's". When an energy value is converted from keV's to channels, it is done in two steps: first it is converted from absolute keV's to detector keV's. Then it is divided by the effective gain to get a MCA channel number.

Conversion from a MCA channel number to keV's is done in the reverse manner: first the channel number is multiplied by the effective gain to get a detector keV value, this is in turn converted to an absolute keV value.

The function to convert from absolute keV's to detector keV's is the following. (We use pseudo code.)

```
IF Absolute_keV < 31.0 THEN
        Detector_keV = Absolute_keV
ELSE IF Absolute_keV < 122.0 THEN
        Detector_keV = (Absolute_keV - 3.875) / 0.875
ELSE IF Absolute_keV < 320 THEN
        Detector_keV = (Absolute_keV + 11.65) / 0.99
ELSE IF Absolute_keV < 662 THEN
        Detector_keV = (Absolute_keV + 30.367) / 1.045872
ELSE
        Detector_keV = Absolute_keV
END
Detector_keV = Detector_keV +
MCAoffsetForTheUsedEnergyRange
```

MCA offset is set with the 1480 service program separately for both energy ranges. For the 1470 counter model it is always zero. The function to convert from detector keV's to absolute keV's is the inverse of the one above.

As was said before, the effective gain value during isotope normalization using a fixed window is equal to the nominal gain.

10.2.4.2 Dynamic-keV and Dynamic-% window

These window types take into account that the effective gain is not always exactly the same as the nominal gain, but may vary according to e.g. temperature and measured activity. The effective gain is calculated by dividing the main peak energy in detector keV's by its observed MCA channel number. To do this the program must know the peak energy and be able to find the actual channel number of the peak. The peak energy is given by the parameter "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)". The next sections explain how the peak channel number is determined.

10.2.4.2.1 Finding the isotope main peak

Finding peaks in a spectrum is a pattern recognition problem. The analogue to digital (A/D) converter is not ideal, so in reality the MCA channels are not all equally wide. This, together with the statistical nature of isotope decay makes the spectrum ragged. In order to recognise a peak, we must have some idea where to seek it and how wide it is. Since we know the energy of the peak and the nominal gain, we can calculate the approximate channel number of the peak. To filter out random and systematic errors in channel count values, the spectrum is first smoothed.

10.2.4.2.1.1 Spectrum smoothing

The smoothing is such that peaks with half height widths between 10 and 100 channels get almost monotonous slopes. It consists of two parts. (We denote the counts in channel N with CTS[N].) In the first part the counts value of a channel N is replaced by

(CTS[N-2] + CTS[N-1] + 2 * CTS[N] + 2 * CTS[N+1] + CTS[N+2] + CTS[N+3]) / 8

If a channel number in the above formula would fall outside the range from 0 to 1023, the counts are taken to be 0.

In the second part the modified counts value of a channel N is further replaced by

(CTS[N-4] + CTS[N-3] + CTS[N-2] + CTS[N-1] + CTS[N] + CTS[N+1] + CTS[N+2] + CTS[N+3]) / 8

Note that all window counts and CPM values are later calculated by summing counts from the original unsmoothed spectrum. The smoothed spectrum is only used to determine the isotope main peak channel number and to make a qualitative assessment of the spectrum (e.g. whether the spectrum contains unexpected peaks).

10.2.4.2.1.2 Marking peaks

Peaks are sought in the smoothed spectrum by sliding a five channels wide window over the spectrum. The counts values in the two channels on both sides of the centre channel are compared to determine whether the centre channel is a local maximum or minimum. Later, whenever the program needs to find peaks within a certain channel interval, it only looks at those channels that are local maxima.

The conditions for a local minimum or maximum are the following. Denote with CS[N] smoothed spectrum counts at channel N. Each extremum type requires that both the listed conditions are true.

Extremum type	First condition	Second condition
Maximum at N	(CS[N-1] <= CS[N]) AND (CS[N] > CS[N+1])	(CS[N-2] <= CS[N-1]) AND (CS[N+1] > CS[N+2])
Maximum at N	(CS[N-1] <= CS[N]) AND (CS[N] > CS[N+1])	(CS[N-2] <= CS[N]) AND (CS[N] >= CS[N+2])
Small max. at N	(CS[N-1] <= CS[N]) AND (CS[N] > CS[N+1])	There is not a maximum at N
Minimum at N	(CS[N-1] > CS[N]) AND (CS[N] <= CS[N+1])	(CS[N-2] > CS[N-1]) AND (CS[N+1] <= CS[N+2])
Minimum at N	(CS[N-1] > CS[N]) AND (CS[N] <= CS[N+1])	(CS[N-2] >= CS[N]) AND (CS[N] <= CS[N+2])
Small min. at N	(CS[N-1] > CS[N]) AND (CS[N] <= CS[N+1])	There is not a minimum at N

10.2.4.2.1.3 The interval where the peak is expected to be

In order to find the isotope main peak, the program first defines an interval within which the main peak is assumed to be the highest (most active) peak. This is done with the parameters "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)" and "SYSTEM | Isotope | <isotope name> | Max. norm. dev. (%)". First "Peak pos. (keV)" is converted to MCA channels. This is done as explained in Section 10.2.4.1.2 on page 137. Here nominal gain is used as the effective gain. Let us call the result ExpectedPeakChannel. Then the interval boundaries are calculated with the following formulas.

IntervalLowBorder = ExpectedPeakChannel / (1.0 + "Max. norm. dev. (%)" / 100.0)
IntervalHighBorder = ExpectedPeakChannel * (1.0 + "Max. norm. dev. (%)" / 100.0)

10.2.4.2.1.4 Finding the peak in the expected interval

Of all the channels in the interval that have previously been marked as "Maximum", the one with most counts in the smoothed spectrum is taken to be as the isotope main peak. If no channels marked as "Maximum" exist in the interval, the whole spectrum is checked for peaks that exceed ThresholdChannelCounts (see Section 10.2.4.2.1.6 on page 140). If such a peak is found, the spectrum is classified as "bad", otherwise it is considered to have too low activity. In both cases the isotope normalization is aborted.

But if the isotope main peak is found, the program seeks the nearest channels to the left and right of the peak channel that has been marked either as "Maximum" or "Minimum". If either one is a "Maximum", the peak is considered abnormal. Otherwise they are considered to be the start and end point of the peak. They are named in diagnostic info printout as "Peak begin channel" and "Peak end channel". If there are between "Peak begin channel" and "Peak end channel" channels that have been marked either as "Small. min" or "Small max.", then the peak is considered to have "noise". This is also mentioned in the diagnostic info. printout.

10.2.4.2.1.5Finding the I-125 coincidence peak

If "SYSTEM | Isotopes | <isotope name> | Spectrum type" is "I-125", the program determines the position and height of the coincidence peak. The expected position of the coincidence peak is twice the channel number of the isotope main peak. (This should be twice the keV value, but detector unlinearity is not taken into account here. Both the main and the coincidence peak actually consist of two peaks that are near each other, but since the detector cannot resolve them from each other, the program can ignore this).

The interval where the coincidence peak is searched is

```
IntervalLowBorder = ExpectedCoincChannel / (1.0 + MaxCoincDev / 100.0)
IntervalHighBorder = ExpectedCoincChannel * (1.0 + MaxCoincDev / 100.0)
```

where MaxCoincDev = "SYSTEM | Isotopes | <isotope> | Max.coinc. dev. (%)"

Of all the channels in the interval that are marked as "Maximum", the one with most counts is taken as the coincidence peak. If no such channel exists in the interval, the spectrum is considered not to have a coincidence peak and in this case the normalization is aborted.

Reference:

Monidetektorinen gammalaskin (A multi-detector gamma counter), by Tapio Yrjönen. *Pro gradu work at Turku University, 1984. (Page 72.)*

10.2.4.2.1.6Checking whether the isotope main peak is high enough

First the program calculates a limit counts value that the smoothed spectrum height of the isotope main peak must exceed. This is done with the following formula.

```
ThresholdChannelCounts = SignifCpm * NominalGainForTheUsedEnergyRange * CountingTimeInSeconds / 60
```

where SignifCpm = "SYSTEM | Isotopes | <isotope> | Signif. cpm per keV."

NominalGainForTheUsedEnergyRange is explained in Section 10.1.4 on page 132.

If the smoothed spectrum height of the isotope main peak does not exceed ThresholdChannelCounts, the whole spectrum is checked for peaks that exceed ThresholdChannelCounts. If such a peak is found, the spectrum is classified as "bad", otherwise it is considered to have too low activity. In both cases the isotope normalization is aborted.

Sometimes a spectrum may be classified as "bad" although it in fact is just too weak. This happens if "SYSTEM | Isotopes | <isotope name> | Spectrum type" is "Many peaks" and there are peaks outside the interval given by "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)" and "SYSTEM | Isotope | <isotope name> | Max. norm. dev. (%)" that are higher than the isotope main peak. If "SYSTEM | Isotopes | <isotope> | Signif. cpm per keV." is so large that the height of the isotope main peak is smaller than ThresholdChannelCounts, but some other peak in the spectrum is higher than this, then the spectrum is classified as "bad" although actually it is too weak. In this case you have to decrease "Signif. cpm per keV." so that the main isotope peak exceeds ThresholdChannelCounts.

10.2.4.2.1.7 Checking if the coincidence peak is high enough

This checking is done only if the isotope main peak height is above ThresholdChannelCounts.

The minimum coincidence peak height in the smoothed spectrum is given by the formula

```
MinCoincPeakChannelCounts = MinCoincHeight * PeakChannelCounts / 100
```

where PeakChannelCounts is the smoothed spectrum height of the isotope main peak and MinCoincHeight = "SYSTEM | Isotope | <isotope name> | Min.coinc.height (%)".

If MinCoincPeakChannelCounts is less than ThresholdChannelCounts, the coincidence peak is not checked; it may be buried in the background and/or spillover counts.

Otherwise, if the smoothed spectrum height of the coincidence peak is less than MinCoincPeakChannelCounts, the normalization is aborted.

10.2.4.2.1.8 Checking for unexpected peaks

ThresholdChannelCounts is next increased with a counts number that is equal to the smoothed spectrum height of the isotope main peak multiplied by "SYSTEM | Isotopes | <isotope name> | Threshold level (%)". It is assumed that the isotope activity increases the background activity level outside the main peak area by this amount.

In the Diagnostic info printout the channel numbers and heights of those peaks whose smoothed spectrum height exceeds this new ThresholdChannelCounts are printed. See Section 10.3.8 on page 164.

If "SYSTEM | Isotopes | <isotope> | Spectrum type" is "Single peak" or "I-125" then the smoothed spectrum is scanned for unexpected peaks. If there are channels that are marked as "Maximum" and the heights of which exceed ThresholdChannelCounts and which are not the main peak or the coincidence peak, then these are marked as unexpected. If such peaks are found, the normalization is aborted. The first unexpected peak is included in the diagnostic info printout.

10.2.4.2.1.9 Improving the accuracy of the peak position

The peak channel number is estimated to a fraction of a channel by fitting the smoothed spectrum with a parabola through the isotope main peak and two adjacent channel count values.

10.2.4.2.1.10 Calculating the effective gain

The effective gain can be calculated by dividing the main peak energy expressed in detector keV's by the peak position expressed in MCA channels.

First the main peak energy in absolute keV's, which is given by "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)", is converted to detector keV's. Then it is divided by the observed MCA channel number of the peak. Absolute keV's and detector keV's are discussed in Section 10.1.4 on page 132.

10.2.4.2.2 Dynamic-keV window in MCA channels

If "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-keV" the counting window is determined by the parameters "SYSTEM | Isotope | <isotope name> | Low boundary (keV)" and "High boundary (keV)". First the effective gain is calculated as described in the previous section and then the window boundaries are converted to MCA channel numbers using this gain value.

10.2.4.2.3 Dynamic-% window in MCA channels

If "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-%", the counting window is determined by the parameter "SYSTEM | Isotope | <isotope name> | Window coverage (%)". The window is set around the observed isotope peak so that it is as small as possible but still contains at least the preset coverage fraction of all counts in the open window. Once this window has been determined, its boundary channel numbers are converted to keV's and stored with isotope normalization results.

If "SYSTEM | Isotope | <isotope name> | Spectrum type" is "I-125", the counting window always includes the area between the isotope main peak and coincidence peak.

Background activity is not taken into account when window coverage is calculated.

10.2.4.2.4 Calculation of Horrocks efficiency for I-125

Horrocks efficiency is calculated when "SYSTEM | Isotopes | <isotope name> | Spectrum type" is "I-125" and "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-%" or "Dynamic-keV". In this case in extended isotope normalization printout the column title "HORROCKS EFFICIENCY" appears. Horrocks efficiency is not calculated if "Counting window" is "Fixed".

Horrocks efficiency depends on the total number of counts under the isotope main peak and under the coincidence peak. The border between the peaks is taken to be the channel number between the peaks where the smoothed spectrum counts are the smallest. If "Counting window" is "Dynamic-%", the low end of the main peak is taken to be the low boundary of the counting window and the high end of the coincidence peak is taken to be the high boundary of the counting window. If "Counting window" is "Dynamic-keV", the low end of the main peak is the first channel to the left of the main peak that is marked as "Minimum" (this is named "Peak begin channel" in the diagnostic info printout). The high end of the coincidence peak is the first channel to the right of the coincidence peak that is marked as "Minimum", (this is not included in the diagnostic info printout); however, if such a channel is not found or the channel number would be greater than 170, the high end is taken to be 170.

Because the main peak and coincidence peak windows are not the same when "Counting window" is "Dynamic-%" or "Dynamic-keV", the calculated values for Horrocks efficiency may differ a little. Furthermore, in the "Dynamic-%" case the width of these areas depends on the value of "SYSTEM | Isotopes | <isotope name> | Window coverage (%)", so changing it may also affect calculated value for Horrocks efficiency.

The calculation uses the following formulas.

$$Ratio = \frac{MainPeakCounts}{MainPeakCounts + CoincidencePeakCounts}$$

$$HorrocksEfficiency = \frac{4(1 - Ratio)}{(2 - Ratio)^2}$$

In GLP TEST normalization Horrocks efficiency is stored as the absolute detector efficiency if it is calculated. Otherwise the stored absolute detector efficiency is calculated by dividing the measured CPM of the sample with the value of the parameter "SYSTEM | Isotopes | <isotope name> | GLP test sample DPM". The CPM value is corrected for dead time, background activity and isotope decay. The parameter "GLP test sample DPM" is visible whenever Horrocks efficiency is not calculated. See Section 10.2.15 on page 149.

Background activity is not taken into account when Horrocks efficiency is calculated; its effect is assumed to be negligible.

References:

Monidetektorinen gammalaskin (A multi-detector gamma counter), by Tapio Yrjönen. *Pro gradu work at Turku University, 1984. (Pages 72 to 74.)*

Standardising ^{125}I Sources and Determining ^{125}I Counting Efficiencies of Well-Type Gamma Counting Systems, by Donald. L. Horrocks. *Clinical Chemistry 21/3, 370-375 (1975).*

10.2.4.2.5 Calculation of isotope peak resolution

Resolution is defined as the ratio of the half-height width of the peak to the energy of the peak.

It is calculated by fitting a bell-shaped Gaussian curve over the peak using the least squares method and calculating the resolution of this curve. This is called Zimmermann's method.

A Gaussian curve with an area A and peak energy x_0 is represented by the function

$$y = \frac{A}{\sigma\sqrt{2\pi}} e^{\frac{-(x-x_0)^2}{2\sigma^2}}$$

where x is the energy and y is the activity or the number of counts in the measured spectrum. From this we can derive for a general Gaussian curve

$$\frac{y(x-1)}{y(x+1)} = Q(x) = e^{\frac{2(x-x_0)}{\sigma^2}}$$

from which follows

$$f(x) = \ln(Q(x)) = \frac{2}{\sigma^2}(x - x_0)$$

This is represented by a straight line.

The half height width of a Gaussian curve is

$$Hhw = 2\sigma\sqrt{2\ln 2}$$

If $\ln(Q(x))$ is written in the form

$$f(x) = \ln(Q(x)) = a_1 x + a_0$$

we can express the resolution of the Gaussian curve in terms of a_1 and a_0 as follows

$$Resolution\% = 100\frac{Hhw}{x_0} = -400\sqrt{\ln 2}\frac{\sqrt{a_1}}{a_0}$$

To determine a_1 and a_0 from a measured spectrum, a straight regression line is fitted through the points $(x_i, f(x_i))$, where the index i is used to number the channels in the interval over which the fitting is done. x_i is the energy at channel i. (The correspondence between energy and channel number is not entirely linear, see Section 10.2.4.1.2 on page 137).

Next we must decide what the interval is. It is taken to be the one where the smoothed spectrum channel count values are at least 67 % of the smoothed spectrum height of the isotope main peak. In any case the interval does not extend in either direction beyond "Peak begin channel" and "Peak end channel". If it would, the peak is marked as "abnormal" and resolution is set to 0. If a peak is "abnormal" this is mentioned in the diagnostic info printout, but isotope normalization is not aborted because of this. The channels named by "Resol start chn" and "Resol end chn" in the diagnostic info printout give the interval over which the curve is fitted during resolution calculation.

The values a_1 and a_0 of a least-squares regression line can be determined from the matrix equation

$$\begin{bmatrix} \sum W_i & \sum W_i x_i \\ \sum W_i x_i & \sum W_i x_i^2 \end{bmatrix} \begin{bmatrix} a_0 \\ a_1 \end{bmatrix} = \begin{bmatrix} \sum W_i f_i \\ \sum W_i f_i x_i \end{bmatrix}$$

where

x_i = is the number or the energy of the i^{th} channel in the interval

$f_i = \ln(Q(x_i))$

$W_i = \sqrt{y_i}$ is the value of the weight function for channel i

y_i = activity or counts in the i^{th} channel

The weight function was chosen to emphasise the importance of points that lie near the top of the peak.

Solving for a_1 and a_0 we get

$$a_0 = \frac{1}{D}\left((\sum W_i f_i)(\sum W_i x_i^2) - (\sum W_i f_i x_i)(\sum W_i x_i)\right)$$

$$a_1 = \frac{1}{D}\left((\sum W_i)(\sum W_i f_i x_i) - (\sum W_i x_i)(\sum W_i f_i)\right)$$

$$D = (\sum W_i)(\sum W_i x_i^2) - (\sum W_i x_i)^2$$

The resolution values calculated with this method are fairly large for the I-125 and I-129 isotopes. Therefore the calculated resolution values are multiplied with 0.83. Resolution is not calculated if "SYSTEM | Isotopes | <isotope name> | Counting window" is "Fixed".

References:

Monidetektorinen gammalaskin (A multi-detector gamma counter), by Tapio Yrjönen. *Pro gradu work at Turku University, 1984. (Pages 68-70).*

Evaluation of photopeaks in scintillation gamma-ray spectroscopy, by W. Zimmermann. *Rev. Sci. Instrum. 32, 1063 (1961).*

Fitting of Gaussian to peaks by non-iterative method, by T. Mukoyama. *Nuclear Instruments and Methods 125, 289 (1975).*

Determination of peak area, by L. Kokta. *Nuclear Instruments and Methods 112, 245 (1973).*

10.2.5 Summing counts over the counting window

If "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-%", the window is determined first in terms of MCA channels and then converted to keV's. During assay counting the boundary keV's are converted back to MCA channels using the effective gain at the assay counting time.

However, if "Counting window" is "Dynamic-keV" or "Fixed", the window is given in keV's and then converted to MCA channels. The converted MCA channel values are not necessarily integer values. When counts are summed over a window the boundaries of which are non-integer channel numbers, only a corresponding fraction of counts in the boundary MCA channels is taken to the sum. This ensures that slight changes in the position or width of the counting window change the counts sum only a little.

10.2.6 Background CPM in the counting window

Background CPM in the counting window is obtained by summing counts in the background spectrum over the counting window and correcting the result for dead time and converting it to CPM. The background dead time factor is determined when background is measured and it is stored with the background spectrum, as is the background measurement time used. The 1480 counter model has separate background spectra stored for both energy ranges.

10.2.7 MIA standard CPM

MIA standard CPM is obtained by summing counts in the measured spectrum over the isotope counting window and correcting the result for dead time and converting it to CPM, after that background activity is subtracted. Only the 1480 counter model includes this value in normalization printout. Its purpose is to provide a reference value for the calculation of MIA RATIO values.

Although not printed, the standard CPM is also calculated by the 1470 program. There it is used after a further isotope decay correction to determine relative detector efficiencies if the counter has more than one detector installed.

10.2.8 Correcting for isotope decay

If "SYSTEM | Isotopes | <isotope name> | Decay correction" is "Yes" and "SYSTEM | Isotopes | <isotope name> | Norm. zero time" is not "None", all normalization measurements are corrected for isotope decay. If "Norm. zero time" is "Start", decay correction is done to the start of the normalization. The starting time of a normalization is printed in the title line of the isotope normalization printout. If the value of "Norm. zero time" is an explicit time, decay correction is done to that time.

The formula used to calculate the decay correction factor is

$$\frac{\lambda \Delta t e^{\lambda(T_1 - T_0)}}{1 - e^{-\lambda \Delta t}}$$

where

T_1 = the time when the measurement was started

T_0 = a reference time (an explicitly given time or the start of isotope normalization)

Δt = the measurement time

$\lambda = \frac{\ln 2}{T_{1/2}}$, where

$T_{1/2}$ = half life of the isotope

When measured counts or CPM are multiplied with the above decay correction factor, we get the corresponding value at the reference time.

If the isotope half life $T_{1/2}$ is long compared to the measurement time Δt, both the numerator and the denominator of the formula above become almost 0. Therefore, if $\lambda \Delta t < 0.1$, the formula is transformed to

$$\frac{e^{\lambda(T_1 - T_0)}}{(\frac{e^{-\lambda \Delta t} - 1}{-\lambda \Delta t})}$$

where the denominator is calculated using the series expansion ($x = \lambda \Delta t$)

$$1 - \frac{x}{2!} + \frac{x^2}{3!} - \frac{x^3}{4!} + \frac{x^4}{5!} - \cdots$$

If an isotope normalization is done at the beginning of a 1480 multiple isotope assay (MIA) and the normalization has several standard replicate tubes, the tube spectra are summed and the counting window is determined from this sum spectrum. Likewise, if "SYSTEM | Isotopes | <isotope name> | Repeat times" is greater than 1, each normalization measurement is repeated as many times and the spectra are summed and the counting window is determined from this sum spectrum. What decay correction factor should be assigned to the sum spectrum? The value must be such that after decay correction the activity indicated by the sum spectrum is the same as the activities indicated by the individually decay corrected component spectra. The formula for DCF_{SUM}, the decay correction factor for the sum spectrum, can be derived by assuming that all decay corrected activities are the same

$$\frac{\sum_i CTIME_i}{DCF_{SUM}} = \sum_i \frac{CTIME_i}{DCF_i}$$

In the above formula

DCF_i = the factor that when multiplied with the measured counts gives isotope decay corrected counts in repeat measurement *i*

$CTIME_i$ = the counting time used in repeat measurement *i*

Actually the counting time is the same for all normalization repeat measurements and MIA standard replicate measurements.

Note that if "SYSTEM | Isotopes | <isotope name> | Norm. zero time" is "Start", the decay correction is done to the start of the normalization, even if the normalization is done at the beginning of a 1480 multiple isotope assay (MIA). If such an assay has several isotope normalizations all with "Norm. zero time" equal to "Start", the decay corrections are made to the start of each individual normalization, not to the start of the MIA.

10.2.9 Calculating results for individual standard replicate tubes in 1480 MIA isotope normalization

If an isotope normalization is done at the beginning of a 1480 multiple isotope assay (MIA), the standard may consist of several replicate tubes. The measured replicate tube spectra are summed and this sum spectrum is used to calculate normalization results. However, the individual replicate tube spectra are used to print a summary info about each replicate tube at the beginning of the isotope normalization printout. The following data are printed for each replicate tube

- replicate number
- counting time for that replicate (it is the same for all replicates)
- measured counts
- corrected CPM
- ratio of corrected CPM of the individual replicate tube to average CPM of all replicate tubes

The counting window used is the same for all replicates and is the one determined from the sum spectrum. The counts values are corrected for dead time, background and isotope decay to get corrected CPM.

10.2.10Checking detectors for stability if repeat normalization is used

The parameter "SYSTEM | Isotopes | <isotope name> | Repeat times" gives the number of times each isotope normalization measurement is repeated. The total time a sample is measured in each detector is this number multiplied with "SYSTEM | Isotopes | <isotope name> | Normalization time".

The measured counts in repeat measurements are compared with each other and the program calculates the probability that a Poisson-distributed random variable having the same mean would have had a greater variance than was observed in these repeat measurements.

This probability is called "Significance level" and its unit is %. If it is often near zero or near 100, this means that there is systematic error in repeat measurements. In the extended isotope normalization printout the column heading is "SIGNIF. LEVEL". Since the counting window is determined from the sum spectrum and thus is the same in all test repeat measurements, short term gain variations are detected by this measurement.

The formula used to calculate Significance level is as follows. Let

$CTIME_i$ = the counting time used in repeat measurement *i*

$COUNTS_i$ = the counts measured in the isotope counting window during repeat measurement *i*

DCF_i = the factor that when multiplied with the measured counts gives isotope decay corrected counts in repeat measurement *i*

Then define

$$ESTIMATE_i = \frac{(\sum_i COUNTS_i) * \frac{CTIME_i}{DCF_i}}{\sum_i \frac{CTIME_i}{DCF_i}}$$

$$CHI_i = \frac{(COUNTS_i - ESTIMATE_i)^2}{ESTIMATE_i}$$

The Significance level is the value of the Chi-square probability function with the argument equal to $\sum_i CHI_i$ and the degrees of freedom equal to one less than the number of repeats.

References:

Monidetektorinen gammalaskin (A multi-detector gamma counter), by Tapio Yrjönen. *Pro gradu work at Turku University, 1984. (Pages 74 to 78.)*

Numerical Recipes in C, by William H. Press, Brian P. Flannery, Saul A. Teukolsky, William T. Vetterling. *Cambridge: Cambridge University Press, copyright 1988. ISBN 0-521-35465-X.*

The algorithm for calculating the Chi-Square probability function is based on the one given in the book referred to above (pages 488 and 171 to 178).

10.2.11Relative detector efficiency

Relative detector efficiency values are determined by correcting standard sample CPM values for isotope decay and dividing the value for each detector with the average of all detectors that are in use. See Section 10.2.7 on page 144 and Section 10.2.9 on page 146.

10.2.12Storing normalization sample spectra to enable spillover correction

During isotope normalization the normalization sample spectrum is stored for each detector in the instrument hard disk. Latest background spectra are also copied and stored with normalization spectra. These are used to calculate spillover correction coefficients at the beginning of dual label RIA/IRMA/RATIO assays and 1480 multiple isotope assays after it has been determined what fraction of one isotope's counts falls into another isotope's counting window.

10.2.13Storing crosstalk spectra to enable crosstalk correction

If in the 1470 counter model "SYSTEM | Isotopes | <isotope name> | Crosstalk correction" is "Yes", then every time a normalization sample is measured in a detector, spectra from all used detectors in the counter are stored. This makes it possible to determine how much crosstalk there is between detectors. The crosstalk correction factors for single label RIA/IRMA/RATIO assays are calculated during isotope normalization. For dual label RIA/IRMA/RATIO assays the correction factors are calculated at the same time as spillover correction coefficients, that is the first time such an assay is measured after one of the isotopes has been normalized.

The single label crosstalk correction factors are calculated in the following way. The program has already determined the counting windows in terms of MCA channels and the relative detector efficiencies for all detectors. To determine the crosstalk from detector K to detector L, the spectrum measured in detector L when the normalization sample is in detector K is looked up. Counts are summed in this spectrum over the counting

window for detector L, this is then corrected for dead time, converted to CPM, background activity is subtracted and the result is corrected for the relative detector efficiency for detector L. Finally the value is decay corrected with the decay correction factor calculated for the batch having the normalization sample in detector K (if decay correction has been selected). The following N×N matrix is set up, where N is the number of detectors that are in use.

CPM in detector 1 using the counting window of detector 1 when the normalization sample is in detector 1.	CPM in detector 1 using the counting window of detector 1 when the normalization sample is in detector 2.	•••	CPM in detector 1 using the counting window of detector 1 when the normalization sample is in the last used detector.
CPM in detector 2 using the counting window of detector 2 when the normalization sample is in detector 1.	CPM in detector 2 using the counting window of detector 2 when the normalization sample is in detector 2.	•••	CPM in detector 2 using the counting window of detector 2 when the normalization sample is in the last used detector.
•••	•••	•••	•••
CPM in the last used detector using the counting window of the last used detector when the normalization sample is in detector 1.	CPM in the last used detector using the counting window of the last used detector when the normalization sample is in detector 2.	•••	CPM in the last used detector using the counting window of the last used detector when the normalization sample is in the last detector.

If some detectors are not in use, the corresponding rows and columns are missing in the matrix.

Then values in each column are divided by the diagonal value in that column. This converts measured activities to relative crosstalk values. Let us denote this new matrix with the letter **M**. If this matrix is multiplied with the following 1×N vector **B**

B =

Sample activity in detector 1.
Sample activity in detector 2.
•••
Sample activity in the last used detector.

the result is the following 1×N vector **A**

A =

Measured activity in detector 1.
Measured activity in detector 2.
•••
Measured activity in the last used detector.

Thus $\mathbf{A} = \mathbf{MB}$. Then the matrix $\mathbf{M}$ is inverted. Let us denote the inverted matrix with $\mathbf{M}^{-1}$. When this is multiplied during assay counting with a vector consisting of the measured CPM values in all used detectors, the result vector consists of crosstalk corrected sample activities in those detectors, that is $\mathbf{B} = \mathbf{M}^{-1}\mathbf{A}$.

If "SYSTEM | Isotopes | <isotope name> | Repeat times" is greater than 1, the repeat spectra are not stored after isotope normalization, only the sum spectra are.

After a GLP TEST normalization all temporarily stored spectra are deleted.

10.2.14"TOTAL CPS" and "TOTAL DPS"

These values are stored in the file WASTELOG.TXT in the root directory of the instrument hard disk. "TOTAL CPS" is the average corrected sample activity in the isotope counting window in all detectors used. It is corrected for dead time, background activity and isotope decay. "TOTAL DPS" is the average corrected sample activity in the open window divided by "SYSTEM | Isotope | <isotope name> | Efficiency (%)".

10.2.15Storing GLP TEST normalization results

Instrument performance can be monitored by running GLP TEST normalizations at regular intervals. These store data that can later be viewed in graphical format.

GLP means "Good Laboratory Practice". A GLP TEST normalization is similar to isotope normalization, only results are stored differently. Data obtained in a GLP TEST normalization is not used in assay measurements, but is stored so that it can later be compared with other GLP TEST normalizations using the same isotope. This comparison is done by presenting the values of some measured parameters as a function of time, so that any systematic trends or large random deviations can easily be discerned.

A GLP TEST measurement rack has only one holder and sample at the last position of the rack. The rack has a clip with the "TEST" instruction at the "RACK/SPECIAL" position and the isotope code at the "PROTOCOL" position. All isotopes that can be normalized can also be used for GLP TEST measurements. The counting time is set by the parameter "SYSTEM | Isotopes | <Isotope name> | Normalization time" and the printout is similar to the isotope normalization printout.

If "SYSTEM | Isotopes | <isotope name> | Repeat times" is greater than 1, the sample is measured in each detector as many times as specified in Repeat times. The total counting time for the sample in each detector is in this case "Repeat times" multiplied by "SYSTEM | Isotopes | <isotope name> | Normalization time".

The following values are saved during GLP TEST normalization:

- Isotope main peak channel number
- Background CPM in the counting window
- Relative detector efficiency (if a 1470 counter has more than one detector installed)
- Detector resolution (%)
- Absolute detector efficiency. This is determined for I-125 "Dynamic-%" and "Dynamic-keV" windows using the Horrocks method. For other isotopes and for I-125 "Fixed" window the efficiency is calculated by dividing the measured corrected CPM in the counting window by the absolute activity of the test sample, which is given by the parameter "SYSTEM | Isotope | <isotope name> | GLP test sample DPM". An isotope is considered to be I-125 if "SYSTEM | Isotope | <isotope name> | Spectrum type" is "I-125". See Section 10.2.4.2.4 on page 142. The corrected CPM above is corrected for dead time, background activity and isotope decay, but not for relative detector efficiency.

- Window coverage (%). This is the fraction of counts in the whole spectrum that falls in the isotope counting window. Background activity is ignored when this is calculated.
- Detector stability probability. This can be calculated if the parameter "SYSTEM | Isotope | <isotope name> | Repeat times" is greater than 1. The measured counts in repeat measurements are compared with each other and the program calculates the probability that a Poisson-distributed random variable having the same mean would have had a greater variance than was observed in these repeat measurements. This probability is called "Significance level" and its unit is %. If it is often near zero or near 100, this means that there is systematic error in repeat measurements. The number stored is transformed from the Chi-square probability that is shown in the printout ("SIGNIF. LEVEL") so that 5 corresponds to 50%, 4 to 10%, 3 to 1%, 2 to 0.1%, 1 to 0.01%, 0 to <=0.001%, 6 to 90%, 7 to 99%, 8 to 99.9%, 9 to 99.99% and 10 to >=99.999%. This is to make very small and large probability values stand out more clearly.
- Measured CPM in counting window. This is corrected for dead time and background activity, but not for relative detector efficiency or isotope decay.
- Measured total CPM in the whole spectrum. This is equal to measured CPM in the counting window which has been corrected for isotope decay and divided by window coverage.

For each of the above quantities one can set a low and a high limit by setting the menu item "Operation" to "Criteria" in the menu "FILES | GLP data" and by pressing the ENTER key when the highlight bar is on the menu item "Do operation". The other items in the menu are used to select the isotope and one of the stored values mentioned above. In the same menu that is used to set the limit values you can specify that a warning message, a graph or both are printed if during GLP TEST measurement some quantity is not within limits.

The stored GLP TEST normalization data can later be viewed graphically by setting in the menu "FILES | GLP data" the item "Operation" to "View" and by pressing the ENTER key when the highlight bar is on the menu item "Do operation". The other items in the menu are used to select isotope, detector (if a 1470 instrument has more than one detector installed) and one of the stored values mentioned above.

GLP graphs can later be printed by pressing the digit key "6" while the graph is displayed. The graph is sent to the printer that is connected to the WIZARD printer port if the parameter "SYSTEM | Printout selections | Use printer port" is "Yes". The graph is also sent via the WIZARD PC port to MultiCalc if either "SYSTEM | Operation mode | Evaluation" is "MultiCalc" or "SYSTEM | Printout selections | Without buffering to PC" is "Yes". When the graph is sent to MultiCalc, special code characters are added to the data, so that the graph can be printed through MultiCalc using the printer that is connected to MultiCalc. In order for the printing to succeed, MultiCalc must be receiving data from WIZARD, and in the WIZARD communication protocol the Terminal parameter must be "VT-52" and in the MultiCalc system parameters the Printer parameter must be set to "Epson-FX".

10.2.16 How the relative error of relative detector efficiency is calculated

In normalization printout this quantity has the title RELATIVE ERROR %. The following formulae are used:

```
Variance1 = SQUARE(DeadTimeFactor * (60 / CountingTimeInSeconds)) * CountsInWindow
RelativeErrorInBackgroundCpm = 1.0 / SQUAREROOT(BackgroundCountsInWindow)
Variance2 = Variance1 + SQUARE(BackgroundCpmInWindow * RelativeErrorInBackgroundCpm)
Variance3 = Variance2 * SQUARE(DecayCorrectionFactor)
```

Let CorrectedCpm be the CPM value in the counting window that is corrected for dead time, background activity and isotope decay. Then the relative error of CorrectedCpm is.

```
RelativeErrorOfCorrectedCpm = SQUAREROOT(Variance3) / CorrectedCpm
```

Let AverageCpm be the average of CorrectedCpm's for all used detectors. Then for each detector the relative efficiency is equal to its CorrectedCpm divided by AverageCpm. If the counter has only one detector in use, then AverageCpm = CorrectedCpm and from this it follows that in this case RelativeDetectorEfficiency = 1 and RelativeErrorInRelativeDetectorEfficiency = 0. Otherwise the following formulas are used to calculate RelativeErrorInRelativeDetectorEfficiency. The coefficients have been empirically determined using Poisson distributed random numbers.

2 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.7091 * RelativeErrorOfCorrectedCpm
3 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.8146 * RelativeErrorOfCorrectedCpm
4 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.8696 * RelativeErrorOfCorrectedCpm
5 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.8997 * RelativeErrorOfCorrectedCpm
6 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.9126 * RelativeErrorOfCorrectedCpm
7 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.9270 * RelativeErrorOfCorrectedCpm
8 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.9357 * RelativeErrorOfCorrectedCpm
9 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.9442 * RelativeErrorOfCorrectedCpm
10 detectors in use:	RelativeErrorInRelativeDetectorEfficiency = 0.9479 * RelativeErrorOfCorrectedCpm

Figure 4. Calculation of relative error in relative detector efficiency.

If an isotope is normalized at the beginning of a 1480 multiple isotope assay and this isotope has more than one standard replicate, the measured spectra of these replicate tubes are summed together and the sum spectrum is handled as if had resulted from the measurement of a single tube. Only when the decay correction factor for this sum spectrum is calculated it is taken into account that this spectrum has been obtained from several separate measurements. This does not affect the error calculation routines, however. The individual replicate tube CPM's that are printed at the beginning of normalization printout are determined from each replicate tube's own spectrum using the counting window that has been determined from the sum spectrum (see Section 10.2.9 on page 146).

If an isotope normalization measurement for each detector is repeated more than once in order to test the detector for stability, the resulting spectra are handled much in the same way as described in the preceding paragraph. The repeat measurement spectra are summed together and the sum spectrum is handled as if it had resulted from a single measurement. But when the decay correction factor for this sum spectrum is calculated, it is taken into account that this spectrum has been obtained from several separate measurements. The error calculation routines are not affected by this. The individual repeat measurement CPM's that are used to calculate the Chi-Square probability are determined from each repeat measurement's own spectrum using the counting window that has been determined from the sum spectrum. An isotope normalization cannot have repeat measurements if it is done at the beginning of a 1480 multiple isotope assay.

10.2.17Isotope normalization printout

Here are some printout examples.

```
NORMALIZATION OF  4 I-129        26-Aug-1994 09:59:14

Total counting time           45
Repeat times                  3
Nominal gain                  1.00 keV/channel
Main peak                     31.0 keV
Main peak at nominal gain     31.0 channels
Nominal window coverage       96.00 %

       PEAK   PEAK    RESOL     WINDOW keV    DECAYED    MEASURED    DETECTOR    RELATIVE SIGNIF.
DET    CHN    DEV %     %       LOW    HIGH   ACTIVITY    COUNTS    EFFICIENCY  ERROR %  LEVEL %
  1    32.2     3.8    21.0     16.9   46.5     1.0000     106501       1.0008      0.28   44.494
  2    32.5     5.0    19.2     18.6   46.0     1.0000     106565       1.0014      0.28   93.265
  3    32.6     5.1    21.3     17.6   45.9     1.0000     107351       1.0090      0.27    6.074
  4    32.5     4.8    22.0     17.7   46.1     1.0000     106443       1.0002      0.28   76.265
  5    32.1     3.6    21.9     16.9   46.5     1.0000     106904       1.0047      0.28   48.776
  6    32.8     5.8    20.4     17.5   45.6     1.0000     106199       0.9979      0.28   44.679
  7    32.2     3.8    20.6     17.8   45.6     1.0000     107295       1.0084      0.27   43.328
  8    32.7     5.5    21.1     17.5   46.6     1.0000     106034       0.9963      0.28   85.655
  9    31.9     2.8    21.9     17.0   46.9     1.0000     106420       0.9999      0.28   25.735
 10    32.4     4.6    21.2     17.7   46.1     1.0000     104479       0.9814      0.28    9.331
-------------------------------------------------------------------------------------------------
AVG                                                        106419       1.0000      0.28

VALUES SAVED

END OF ISOTOPE NORMALIZATION
```

Figure 5. 1470 normalization printout when "SYSTEM | Isotope | I-129 | Counting window" is "Dynamic-%" and "SYSTEM | Printout selections | Extended norm. printout" is "Yes".

```
NORMALIZATION OF  4 I-129        26-Aug-1994 10:11:26

Total counting time           45
Repeat times                  3
Nominal gain                  1.00 keV/channel

       WINDOW keV    DECAYED    MEASURED    DETECTOR    RELATIVE SIGNIF.
DET     LOW    HIGH   ACTIVITY    COUNTS    EFFICIENCY  ERROR %  LEVEL %
  1    26.0    34.0     1.0000      68311       1.0195      0.34   14.797
  2    26.0    34.0     1.0000      68444       1.0215      0.34   91.101
  3    26.0    34.0     1.0000      65691       0.9805      0.35   26.005
  4    26.0    34.0     1.0000      65559       0.9785      0.35   86.975
  5    26.0    34.0     1.0000      67558       1.0082      0.35   89.982
  6    26.0    34.0     1.0000      65592       0.9791      0.35   56.103
  7    26.0    34.0     1.0000      69318       1.0346      0.34   51.491
  8    26.0    34.0     1.0000      64563       0.9637      0.36   56.986
  9    26.0    34.0     1.0000      69154       1.0321      0.34   91.725
 10    26.0    34.0     1.0000      65850       0.9825      0.35    7.336
-------------------------------------------------------------------------
AVG                                 67004       1.0000      0.35

VALUES SAVED

END OF ISOTOPE NORMALIZATION
```

Figure 6. 1470 normalization printout when "SYSTEM | Isotope | I-129 | Counting window" is "Fixed" and "SYSTEM | Printout selections | Extended norm. printout" is "Yes".

```
NORMALIZATION OF  4 I-129        26-Aug-1994 10:33:07

Total counting time           15
Detectors not in use          6, 7, 8, 9, 10

      DECAYED   MEASURED   DETECTOR   RELATIVE
DET   ACTIVITY   COUNTS   EFFICIENCY  ERROR %
  1     1.0000     35254      0.9955      0.43
  2     1.0000     35478      1.0019      0.42
  3     1.0000     35377      0.9991      0.43
  4     1.0000     35356      0.9984      0.43
  5     1.0000     35585      1.0050      0.42
-------------------------------------------------
AVG                35410      1.0000      0.43

VALUES SAVED

END OF ISOTOPE NORMALIZATION
```

Figure 7. 1470 normalization printout when "SYSTEM | Isotope | I-129 | Counting window" is "Dynamic-%" and "SYSTEM | Printout selections | Extended norm. printout" is "No". Detector 6 has been set inactive; hence only detectors 1 to 5 can be used.

```
NORMALIZATION OF  3 Cr-51        22-Sep-1994 17:24:18

Total counting time           30
Repeat times                  2
Nominal gain                  1.00 keV/channel
Main peak                     320.0 keV
Main peak at nominal gain     335.0 channels
Nominal window coverage       80.00 %

      PEAK  PEAK   RESOL    WINDOW keV   DECAYED   MEASURED   DETECTOR   RELATIVE SIGNIF.
DET    CHN  DEV %    %      LOW   HIGH   ACTIVITY   COUNTS   EFFICIENCY  ERROR %  LEVEL %
  1  326.3   -2.6    8.8   80.7  347.6     0.9999     19255      0.0000      0.00   0.000
  2  319.0   -4.8    7.9   82.4  347.8     0.9999     19084      0.0000      0.00   0.000
  3  324.5   -3.1    8.9   82.0  350.7     0.9999     19040      0.0000      0.00   0.000
  4  336.5    0.5    8.9   82.5  351.0     0.9999     18878      0.0000      0.00   0.000
  5  337.0    0.6    8.2   84.0  349.5     0.9999     19003      0.0000      0.00   0.000
  6  327.7   -2.2    8.9   83.8  348.0     0.9999     18988      0.0000      0.00   0.000
  7  326.4   -2.6    8.4   80.7  349.6     1.0000     19075      0.0000      0.00   0.000
  8  343.5    2.5    8.3   83.3  346.2     1.0000     18846      0.0000      0.00   0.000
  9  Failed: unexpected peak.
 10  330.7   -1.3    8.1   83.9  346.6     1.0000     19178      0.0000      0.00   0.000
-------------------------------------------------------------------------------------------
AVG                                                   19039      0.0000      0.00

VALUES NOT SAVED

END OF ISOTOPE NORMALIZATION
```

Figure 8. A printout of a Cr-51 normalization that has failed. In detector 9 a peak due to Compton scattering was so high that is was regarded as an unexpected peak. The normalization succeeded when "SYSTEM | Isotopes | Cr-51 | Threshold level (%)" was raised from 20 to 30.

Total counting time is the time a tube is measured in one detector. If "SYSTEM | Isotope | <isotope name> | Repeat times" is greater than 1, the counting time printed is this number multiplied with "SYSTEM | Isotope | <isotope name> | Normalization time".

Repeat times is the number of repeat measurements given by "SYSTEM | Isotope" | <isotope name> | Repeat times". It is printed only if it is greater than 1.

Nominal gain is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes". For the 1470 and 1480 normal energy ranges it is equal to "SYSTEM | Active hardware | Nominal gain". The factory setting for

it is 1.0 keV / channel. If the value of this parameter is changed, it affects only how keV's are converted to channels and vice versa by the software, not the effective gain of the electronics circuit. The latter must be set by a service technician. For the 1480 model, the parameter "Nominal gain" is the normal energy range gain. The nominal gain for the 1480 extended energy range gain is 2.0 keV / channel and cannot be changed.

Main peak is the value of "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)". It is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Counting window" is not "Fixed".

Main peak at nominal gain is the 'Main peak' above converted to an MCA channel number assuming that effective gain is equal to nominal gain. If effective gain is different from nominal gain, then 'PEAK CHN' below is different from 'Main peak at nominal gain'. This item is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Counting window" is not "Fixed".

Nominal window coverage is the value of "SYSTEM | Isotope | <isotope name> | Window coverage (%)". It is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-%".

Detectors not in use lists those detectors that are not used for measurement because some detectors have been set to be inactive. If normalization is done in 1470 using the conveyor, there may be detectors that are active but nevertheless are not used for measurement, since with the conveyor only active sets of adjacent 10, 5, 2 or 1 detectors can be used for measurement.

PEAK CHN is the observed peak channel number. It is determined from the smoothed spectrum; the counts values of channels on both sides of the peak channel are used to interpolate the peak position to a fraction of channel. The peak channel is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Counting window" is not "Fixed".

PEAK DEV % is the relative difference of 'PEAK CHN' from 'Main peak at nominal gain'**.** It is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Counting window" is not "Fixed".

RESOL % is the ratio of the main peak half height width to the main peak energy. It is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Counting window" is not "Fixed".

WINDOW keV LOW HIGH are equal to "SYSTEM | Isotope | <isotope name> | Low boundary (keV)" and "High boundary (keV)" if "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-keV" or "Fixed". If "Counting window" is "Dynamic-%", window boundaries are determined during isotope normalization on the basis of the parameters "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)" and "SYSTEM | Isotope | <isotope name> | Window coverage (%)". Window boundaries are printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes".

DECAYED ACTIVITY is the relative activity of the sample compared to a reference time. It is printed only if "SYSTEM | Isotope | <isotope name> | Decay correction" is "Yes" and the parameter "SYSTEM | Isotope | <isotope name> | Norm. zero time" is not "None". The isotope half life used in decay correction is given by the parameter "SYSTEM | Isotope | <isotope name> | Half-life (hours)". The decay that occurs during measurement is taken into account. If the reference time given by "Norm. zero time" is "Start", decay correction is done with reference to the start of the normalization. This normalization start time is printed in the normalization printout title line.

MEASURED COUNTS is the number of counts in the counting window given by 'WINDOW keV LOW HIGH'. It is <u>not</u> corrected in any way, e.g. for detector dead time or background activity.

DETECTOR EFFICIENCY is the relative efficiency of a detector compared with other detectors. It is calculated from 'MEASURED COUNTS', which is corrected for dead time, background activity and isotope decay. It is printed only if a 1470 counter has more than one detector installed.

RELATIVE ERROR % is the relative error of relative detector efficiency. How it is calculated is explained in Section 10.2.16 on page 150. It is printed only if a 1470 counter has more than one detector installed.

HORROCKS EFFICIENCY is the absolute detector efficiency for I-125. Horrocks efficiency is printed only if "SYSTEM | Printout selections | Extended norm. printout?" is "Yes" and "SYSTEM | Isotope | <isotope name> | Spectrum type" is "I-125" and "SYSTEM | Isotope | <isotope name> | Counting window" is not "Fixed".

STANDARD CPM is calculated from 'MEASURED COUNTS', which is corrected for dead time and background activity and isotope decay. It is printed only for the 1480 counter model and is used in multiple isotope assays (MIA) as the activity of an isotope standard when the RATIO field is calculated. (RATIO is equal to an isotope's CCPM activity in an unknown sample divided by the corresponding standard sample's activity).

SIGNIF. LEVEL % indicates whether measurement results are repeatable. During isotope normalization each measurement can be repeated several times to test detector(s) for stability. The parameter "SYSTEM | Isotope | <isotope name> | Repeat times" sets the number of times each isotope normalization measurement is repeated. Thus the total time a sample is measured is this number multiplied with "SYSTEM | Isotope | <isotope name> | Normalization time". Repeats are not done for MIA standard normalizations. The measured counts in repeat measurements are compared with each other and the program calculates the probability that a Poisson-distributed random variable having the same mean would have had a greater variance than was observed in these repeat measurements. This probability is called "Significance level" and its unit is %. If it is often near zero or near 100, this means that there is systematic error in repeat measurements.

10.3 RIA/IRMA/RATIO assay counting

When a rack having an ID clip with a numeric label in the range from 1 to 99 stuck in the area marked "PROTOCOL" is encountered during automatic measurement, WIZARD starts measuring the assay that has this protocol number. An optional rack number can be given by placing a numeric code in the "RACK/SPECIAL" area of the ID clip; otherwise the first rack is assumed to have the number 1 and this increases by one for each subsequent rack.

The possible assay types are RIA, IRMA, RATIO and MIA. The assay type is determined when an assay is created. How RIA, IRMA and RATIO assays are evaluated once corrected CPM values are known is not handled in this manual; you may refer to MultiCalc documentation for that information. Multiple isotope assays are handled in chapter 10.4 on page 171.

For the 1470 counter model assay measurement can also be started manually when the conveyor or rack id readers are not used. (The conveyor can be disabled for the 1470 counter model by setting "SYSTEM | Operation mode | Manual mode used" to "Yes" and the rack id reader can be disabled by setting "SYSTEM | Active hardware | Use rack id reader" to "No").

10.3.1 Calculating crosstalk and spillover correction coefficients

If "SYSTEM | Isotopes | <isotope name> | Crosstalk correction" is "Yes", crosstalk is eliminated in RIA/IRMA/RATIO assay counting. Spillover is always eliminated in dual label RIA/IRMA/RATIO assay counting. In order to do this, correction coefficients must be known.

10.3.1.1 Single label counting

The correction coefficients are determined during isotope normalization as described in Section 10.2.13 on page 147.

10.3.1.2 Dual label counting

Correction coefficients have to be calculated for each isotope pair every time either one of the isotopes is normalized. Since it is probable that only a few isotope pairs are used in dual label counting, it is practical to calculate the coefficients the first time a dual label assay using such an isotope combination is used after normalization of either of its isotopes. The coefficients are then stored in the instrument hard disk.

ASSAY 22-Sep-1994 10:44:43

Protocol id 29 PROT01
Time limit 30
Count limit 99999999
Dual isotopes I-129 + Co-57
Protocol date 22-Sep-1994 10:43:52
Run id. 10

					I-129			Co-57		
POS	RACK	DET	BATCH	TIME	COUNTS	CPM	ERROR %	COUNTS	CPM	ERROR %
1	1	1	1	30	356	690.9	6.25	21	-14.3	82.86
2	1	2	1	30	3343	6602.8	2.48	30	10.7	121.55
3	1	3	1	30	3973	7917.0	2.33	22	-7.3	160.70
4	1	4	1	30	348	673.0	6.30	35	30.9	43.62
5	1	5	1	30	4184	8386.3	2.28	24	2.7	438.12
6	1	6	1	30	3485	6971.9	2.43	33	8.1	170.84
7	1	7	1	30	5730	11453.6	2.06	26	-6.5	196.25
8	1	8	1	30	8527	17077.2	1.82	37	16.3	88.06
9	1	9	1	30	13752	27522.9	1.59	34	21.1	64.43
10	1	10	1	30	278	23.6	6.72	80956	164663.4	0.67
11	2	1	2	30	0	0.0	0.00	0	0.0	0.00
!Error: Bad spectrum										
12	2	2	2	30	9985	19766.9	1.74	35	20.9	66.28
13	2	3	2	30	9883	19732.5	1.74	30	8.8	149.36
14	2	4	2	30	9962	19672.5	1.75	29	19.1	65.67
15	2	5	2	30	8502	17072.4	1.82	25	4.5	266.89
16	2	6	2	30	8550	17143.5	1.82	28	-2.7	483.22
17	2	7	2	30	7086	14172.5	1.92	24	-11.5	107.39
18	2	8	2	30	6920	13851.2	1.94	32	6.8	198.86
19	2	9	2	30	19139	38353.0	1.46	22	-2.7	424.70
20	2	10	2	30	21855	44981.5	1.41	27	20.5	105.55

END OF ASSAY

Figure 9. A dual label RIA/IRMA/RATIO assay printout when "SYSTEM | Operation mode | Evaluation" is "Cpm". In position 11 there was an I-125 tube.

10.3.1.2.1 Neither isotope has crosstalk correction

This means the for both isotopes "SYSTEM | Isotopes | <isotope name> | Crosstalk correction" is "No".

The spillover correction factors are calculated in the following way. During isotope normalization the spectrum of the normalization sample is stored for each detector in the instrument hard disk. Latest background spectra are also copied and stored with normalization spectra.

The program determines counting windows in terms of MCA channels. This is done by converting the stored keV values of counting window boundaries by using the effective gain during the isotope normalization that produced the spectrum on which the counting window is used.

If "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-%", the window boundary keV values are determined during isotope normalization on the basis of the parameters "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)" and "SYSTEM | Isotope | <isotope name> | Window coverage (%)". For window types "Dynamic-keV" and "Fixed", the boundaries are given by the isotope parameters "SYSTEM | Isotopes | <isotope name> | Low boundary (keV)" and "High boundary (keV). If "Counting window" is "Dynamic-%" or "Dynamic-keV", then the effective gain during isotope normalization is calculated by dividing the energy of the main isotope peak in keV's by the MCA channel number of the main peak. If "Counting window" is "Fixed", then the nominal gain is used as the effective gain.

Next the activities of both isotopes in both counting windows are determined in both normalizations. The following is repeated for each detector that is in use: For both isotope spectra, counts are summed over both isotope counting windows. Sums are corrected for dead time, converted to CPM, the background activity is subtracted and the result is divided by the relative detector efficiency and, if selected, corrected for isotope decay.

Dead time, background activity and possible isotope decay correction factor are determined by the detector and the isotope spectrum used; the relative detector efficiency by the detector and the isotope counting window used. Detector efficiency depends on the counting window used and may also change with time. We assume that the energy dependency of a detector's efficiency has not changed between the two isotope normalizations.

The following 2×2 matrix is set up for each detector that is in use.

CPM in isotope A spectrum using the counting window of isotope A.	CPM in isotope B spectrum using the counting window of isotope A.
CPM in isotope A spectrum using the counting window of isotope B.	CPM in isotope B spectrum using the counting window of isotope B.

Then values in each column are then divided by the diagonal value in that column. This converts measured activities to relative spillover values. Let us denote this new matrix with the letter **M**. If this matrix is multiplied with the following vector **B**

$$\mathbf{B} = \begin{array}{|l|}\hline \text{Isotope A activity in a sample.} \\ \hline \text{Isotope B activity in a sample.} \\ \hline \end{array}$$

the result is the following vector **A**

$$\mathbf{A} = \begin{array}{|l|}\hline \text{Measured activity in isotope A counting window.} \\ \hline \text{Measured activity in isotope B counting window.} \\ \hline \end{array}$$

Thus $\mathbf{A} = \mathbf{MB}$. Then for each detector the matrix **M** is inverted. Let us denote the inverted matrix with $\mathbf{M}^{-1}$. When this is multiplied during assay counting with a vector consisting of the measured CPM values in both counting windows, the resulting vector consists of spillover corrected isotope activities, that is $\mathbf{B} = \mathbf{M}^{-1}\mathbf{A}$.

If the matrix **M** cannot be inverted, the message "! The spill matrix could not be inverted. No spill correction is made." is printed. If the absolute value of the determinant of the matrix **M** is smaller than 0.1, the message "! Results for CPM may be inaccurate." is printed.

10.3.1.2.2 At least one of the isotopes has crosstalk correction (1470 only)

If at least one of the isotopes has "SYSTEM | Isotopes | <isotope name> | Crosstalk correction" set to "Yes", then crosstalk is corrected for in addition to spillover. In this case every time a normalization sample is measured in a detector, spectra from all detectors used in the counter are stored. (Possible repeat measurement spectra are summed). This makes it possible to determine how much crosstalk there is between detectors for this isotope.

To do this, the program determines counting windows in terms of MCA channels for both isotopes in all detectors used. This is done by converting the stored keV values of the counting window boundaries by using the effective gain during the isotope normalization that was used to obtain the spectrum in which counts are summed.

Next the activities in both counting windows for all detectors used and for all normalization sample measurements in both normalizations are determined. This is done by summing counts over counting windows. The results are corrected for dead time, converted to CPM, the background activity is subtracted and then they are divided by the relative detector efficiency and, if selected, corrected for isotope decay.

Dead time, background activity and possible isotope decay correction factor are determined by the detector and the isotope spectrum used; the relative detector efficiency by the detector and the isotope counting window used. Detector efficiency depends on the counting window and may also change with time. We assume that detector efficiencies have not changed between the two isotope normalizations.

The following (2*N)×(2*N) matrix of CPM activities in counting windows is set up, where N is the number of detectors that are in use.

Isotope A normalization sample is in detector 1. Spectrum is taken from detector 1. Counting window is for isotope A.	•••	Isotope A normalization sample is in the last used detector. Spectrum is taken from detector 1. Counting window is for isotope A.	Isotope B normalization sample is in detector 1. Spectrum is taken from detector 1. Counting window is for isotope A	•••	Isotope B normalization sample is in the last used detector. Spectrum is taken from detector 1. Counting window is for isotope A.
•••	•••	•••	•••	•••	•••
Isotope A normalization sample is in detector 1. Spectrum is taken from the last used detector. Counting window is for isotope A.	•••	Isotope A normalization sample is in the last used detector. Spectrum is taken from the last used detector. Counting window is for isotope A.	Isotope B normalization sample is in detector 1. Spectrum is taken from the last used detector. Counting window is for isotope A.	•••	Isotope B normalization sample is in the last used detector. Spectrum is taken from the last used detector. Counting window is for isotope A.
Isotope A normalization sample is in detector 1. Spectrum is taken from detector 1. Counting window is for isotope B.	•••	Isotope A normalization sample is in the last used detector. Spectrum is taken from detector 1. Counting window is for isotope B.	Isotope B normalization sample is in detector 1. Spectrum is taken from detector 1. Counting window is for isotope B.	•••	Isotope B normalization sample is in the last used detector. Spectrum is taken from detector 1. Counting window is for isotope B.
•••	•••	•••	•••	•••	•••
Isotope A normalization sample is in detector 1. Spectrum is taken from the last used detector. Counting window is for isotope B.	•••	Isotope A normalization sample is in the last used detector. Spectrum is taken from the last used detector. Counting window is for isotope B.	Isotope B normalization sample is in detector 1. Spectrum is taken from the last used detector. Counting window is for isotope B.	•••	Isotope B normalization sample is in the last used detector. Spectrum is taken from the last used detector. Counting window is for isotope B.

Only detectors that are in use appear in the matrix. If for one of the isotopes, say B, "Crosstalk correction" is "No", then all elements which have the text "Isotope B normalization sample is in detector X. Spectrum is taken from detector Y." with X different from Y are equal to 0.

Then values in each column of the matrix are divided by the diagonal value in that column. This converts measured activities to relative spillover and crosstalk values. Let us denote this new matrix with the letter **M**. If this matrix is multiplied with the following 1×(2*N) vector **B**

$\mathbf{B} =$

Isotope A activity in detector 1.
•••
Isotope A activity in the last used detector.
Isotope B activity in detector 1.
•••
Isotope B activity in the last used detector.

the result is the following 1×(2*N) vector **A**.

$\mathbf{A} =$

Measured activity in isotope A counting window for detector 1.
•••
Measured activity in isotope A counting window for the last used detector.
Measured activity in isotope B counting window for detector 1.
•••
Measured activity in isotope B counting window for the last used detector.

Thus $\mathbf{A} = \mathbf{MB}$. Then the matrix **M** is inverted. Let us denote the inverted matrix with $\mathbf{M}^{-1}$. When this is multiplied during assay counting with a vector consisting of the measured CPM values in both counting windows and for all detectors, the result vector consists of spillover and crosstalk corrected isotope activities for all detectors, that is $\mathbf{B} = \mathbf{M}^{-1}\mathbf{A}$.

If the matrix **M** cannot be inverted, the message "! The spill matrix could not be inverted. No spill correction is made." is printed. If the absolute value of the determinant of the matrix **M** is smaller than 0.1, the message "! Results for CPM may be inaccurate." is printed.

10.3.2 Setting the assay counting window

The assay counting window is already known in keV's, but it must be converted to MCA channels before the number of counts in it can be determined. To do the converting, the program has to have a value for the effective gain (keV / channel) during this assay. The conversion formula is explained in Section 10.2.4.1.2 on page 137.

10.3.2.1 Fixed window

If "SYSTEM | Isotope | <isotope name> | Counting window" is "Fixed", the keV-values of window boundaries are given by the parameters "SYSTEM | Isotope | <isotope name> | Low boundary (keV)" and "High boundary (keV)". The nominal gain is used as the effective gain value when these are converted to MCA channel numbers. Thus any drifting due e.g. to temperature variations is not taken into account. In dual label counting both isotope counting windows are taken to be fixed if at least one of the isotopes has "Counting window" equal to "Fixed". In

this case the nominal gain is used as the effective gain also for the isotope for which the counting window is not fixed. Nominal gain is explained in Section 10.1.4 on page 132.

10.3.2.2 Dynamic-% and Dynamic-keV window

If "Counting window" is "Dynamic-keV", the keV-values of window boundaries are given by the parameters "Low boundary (keV)" and "High boundary (keV)". If "Counting window" is "Dynamic-%", the window boundary keV-values are determined during isotope normalization on the basis of the parameters "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)" and "SYSTEM | Isotope | <isotope name> | Window coverage (%)".

These window types take into account that the effective gain is not always exactly the same as the nominal gain, but may vary according to e.g. temperature and measured activity. The effective gain is calculated by dividing the main peak energy in keV's by its observed channel number. To do this the program must know the peak energy and be able to find the actual MCA channel number of the peak. The peak energy is given by the parameter "Peak pos. (keV)". The next sections explain how the peak channel number is determined.

Note: If “SYSTEM | Operation mode | No dynamic normalization” is “Yes”, then the same channel number will be used as was found during isotope normalization. This means that counting windows remain the same as they were during isotope normalization, they are not shifted anymore during assay measurement.

10.3.2.2.1 Single label assay

The isotope main peak channel number is determined in the same way as in isotope normalization, see Section 10.2.4.2.1 on page 138. Only the parameter "Max. norm. dev. (%)" in Section 10.2.4.2.1.3 on page 139 is replaced with the parameter "Max. assay dev. (%)".

If the smoothed spectrum height of the isotope main peak does not exceed ThresholdChannelCounts (see Section 10.2.4.2.1.6 on page 140), the activity of the sample is considered to be so low that the so called expected peak position is taken as the position of the peak. The expected peak position is taken from the latest batch where a sample in this detector had a sufficiently high activity for this isotope, so that the peak exceeded ThresholdChannelCounts. If the parameter “SYSTEM | Operating mode | Default is norm window” is “No”, the expected isotope peak positions are stored between assays that use the same isotope. If there has not been any sufficiently active sample in this detector since isotope normalization, the peak position during normalization is used as the expected position. If the parameter “Default is norm window” is “Yes”, the peak determined at isotope normalization time is taken as the expected peak position at the beginning of every assay.

If "SYSTEM | Isotope | <isotope name> | Spectrum type" is "I-125" and the smoothed spectrum height of the isotope main peak exceeds ThresholdChannelCounts, then the existence and height of the coincidence peak is checked. If the coincidence peak is not high enough, the spectrum is considered "bad". (The coincidence peak checking is done in the same way as in I-125 isotope normalization, see Section 10.2.4.2.1.7 on page 140).

Then if the smoothed spectrum height of the isotope main peak exceeds ThresholdChannelCounts, this limit value is increased by an amount this is equal to the smoothed spectrum height of the isotope main peak times multiplied by "SYSTEM | Isotopes | <isotope name> | Threshold level (%)". It is assumed that the isotope activity increases background level outside the main peak area by this amount.

In the Diagnostic info printout those peak channel numbers are printed whose smoothed spectrum height exceeds this new ThresholdChannelCounts. See Section 10.3.8 on page 164.

If "SYSTEM | Isotopes | <isotope> | Spectrum type" is "Single peak" or "I-125" then the smoothed spectrum is scanned for unexpected peaks. If there are channels that are marked as "Maximum" and the channel counts of which exceed ThresholdChannelCounts and which are not the main peak or the coincidence peak, then these are marked as unexpected. If such peaks are found, the spectrum is considered "bad" and measured counts are set to 0 in the assay printout. The possible first unexpected peak is included in the diagnostic info printout.

10.3.2.2.2 Dual label assay

First isotope main peaks are determined separately for both isotopes in the order the isotopes are in the assay protocol. This is done as explained in the previous paragraph.

If both isotopes would have the same peak as the main peak, this means that for them both the intervals where the highest (most active) peak is assumed to be the main peak overlap and the same peak is the highest one in both of them. In this case a dividing line is set at the geometric mean of the expected peak positions. The highest (most active) peak is assumed to belong to that isotope on whose side of the dividing line it lies. The geometric mean of two numbers *a* and *b* is equal to $\sqrt{ab}$. If the highest isotope is I-125, its coincidence peak is searched for as explained in Section 10.2.4.2.1.5 on page 140. Then the main peak of the other isotope is searched for again among the remaining peaks in the interval where the highest (most active) peak is assumed to be the main peak for that isotope. If this other isotope is I-125, its coincidence peak is also searched for.

For both isotopes the threshold level is calculated separately and the height of the isotope peak is checked against it as described in the preceding paragraph for the single label case. Unexpected peaks are searched for if for neither isotope "Spectrum type" is "Many peaks". The level that a peak height must exceed in order for the peak to be considered an unexpected peak is the sum of the individually calculated threshold counts values for both isotopes. If at least one unexpected peak is found, the spectrum is considered "bad". In this case a note about this is included in the assay printout and the corrected CPM values are set to 0.

If both isotopes exceed their threshold counts levels, the program makes one more check to ascertain that one of the peaks is not a spillover peak from the spectrum of the other isotope. If at least 1/3 of counts in either window are spillover counts then that peak channel number is considered unreliable and the expected peak channel number is used instead.

10.3.3 Calculating corrected CPM values

Once the effective gain value has been obtained, the counting window boundaries are converted from keV to MCA channels and counts are summed over the window. Counting window boundaries are determined to a fraction of channel. When counts are summed over a counting window, counts from the low and high boundary channels are added only in the proportion that these channels are included inside the counting window.

The sum is corrected for dead time, converted to CPM, the background activity is subtracted and this value is divided by the relative detector efficiency, then correction is made for isotope decay, if such has been selected.

Assay measurements are corrected for isotope decay if "SYSTEM | Isotopes | <isotope name> | Decay correction" is "Yes" and "SYSTEM | Isotopes | <isotope name> | Assay zero time" is not "None". If "Assay zero time" is "Start", decay correction is done to the start of the assay. The starting time is printed in the title line of the assay printout. If the value of "Assay zero time" is an explicit time, decay correction is done with reference to that time.

After this spillover and crosstalk are eliminated as follows.

- For single label assays that do not correct for crosstalk the CPM values are printed as such.
- For dual label assays that do not correct for crosstalk (that is, for both isotopes "SYSTEM | Isotopes | <isotope name> | Crosstalk correction" is "No"), the CPM values of both counting windows are used to form for each detector a 1×2 vector $\mathbf{A}$, as described in Section 10.3.1.2.1 on page 158. It is multiplied by the spillover correction matrix $\mathbf{M}^{-1}$ to get a vector $\mathbf{B}$ of spillover corrected CPM values. These are printed.
- For single label assays that correct for crosstalk, the CPM values from all used detectors are used to form a 1 ×N vector $\mathbf{A}$ (N is the number of detectors in use), as described in Section 10.2.13 on page 147. It is multiplied by the crosstalk correction matrix $\mathbf{M}^{-1}$ to get a vector $\mathbf{B}$ of crosstalk corrected CPM values. These are printed.

- For dual label assays that correct for crosstalk (that is, for at least one of the isotopes "SYSTEM | Isotopes | <isotope name> | Crosstalk correction" is "Yes"), the CPM values of both counting windows from all used detectors are used to form a 1×(2*N) vector **A** (N is the number of detectors in use), as described in Section 10.3.1.2.2 on page 159. It is multiplied by the spillover and crosstalk correction matrix $\mathbf{M}^{-1}$ to get a vector **B** of spillover and crosstalk corrected CPM values. These are printed.

10.3.4 Bad spectrum

During assay counting a spectrum is considered "bad", if an I-125 coincidence peak is not found or if it is too small or if an unexpected peak is found. (The I-125 coincidence peak is not checked in dual label assays if for the other isotope "SYSTEM | Isotopes | <isotope name> | Spectrum type" is "Many peaks".) In this case the printed corrected CPM values are 0. If "SYSTEM | Isotopes | <isotope name> | Counting window" is "Fixed", a spectrum is never considered to be "bad".

10.3.5 "TOTAL CPS" and "TOTAL DPS"

These values are stored in the file WASTELOG.TXT in the root directory of the instrument hard disk. "TOTAL CPS" is the sum of all printed corrected CPM values of measured tubes in the assay. The DPS value is obtained by dividing the printed corrected CPM value by the actual coverage of the isotope counting window and by "SYSTEM | Isotope | <isotope name> | Efficiency (%)". "TOTAL DPS" is the sum of all DPS values of measured tubes in the assay.

10.3.6 Storing the latest counting window

At the end of the assay the latest observed isotope main peak position in the MCA channels is stored for each detector used (and isotope, if the assay is dual labelled). This is used as the expected peak position in the next assay using the same isotope until a sufficiently strong isotope peak is observed again. This is done even if the parameter "SYSTEM | Operating mode | Default is norm window" is "Yes".

10.3.7 How the relative CPM error is calculated in RIA/IRMA/RATIO CPM printout

In CPM printout this quantity has the title ERROR %. The following formulas are used.

```
Variance1 = SQUARE(DeadTimeFactor * (60 / CountingTimeInSeconds)) * CountsInWindow
BVariance = SQUARE(BgrdDeadTimeFactor * (60 / BgrdCountingTimeInSeconds)) * BackgroundCountsInWindow
Variance2 = Variance1 + BVariance
RelativeError1 = SQUAREROOT(Variance2) / CorrectedCpm1
RelativeCpmError = RelativeError1 + RelativeErrorInRelativeDetectorEfficiency
```

Here RelativeErrorInRelativeDetectorEfficiency = 0 if the counter has only one detector in use. CorrectedCpm1 is corrected for dead time and background but not for relative detector efficiency, spillover, crosstalk or isotope decay. Note that if the final corrected CPM in the RIA/IRMA/RATIO CPM printout includes spillover or crosstalk corrections, the ERROR % printed still refers to the CPM value which has only dead time, background and relative detector efficiency corrections, but not spillover or crosstalk corrections. In this case it is not possible to also output that CPM value to which ERROR % refers.

10.3.8 Values that are printed in diagnostic info

Diagnostic info is used to show some measurement results that are not included in background or isotope normalization printouts or assay printouts. Diagnostic info is printed if "SYSTEM | Diagnostic output | Print diagnostic info." is "Yes". For 1470 you can select the detectors for which diagnostic info is printed in the menu "SYSTEM | Diagnostic output | Select detectors for info.". The parameter "SYSTEM | Diagnostic output | Level of diag. info." is used to select how much data is printed. Below are two examples of assay diagnostic info printout.

```
ASSAY                          26-Aug-1994 10:38:01

Protocol id          29 PROT01
Time limit           20
Count limit          99999999
Isotope              I-129
Protocol date        22-Aug-1994 10:59:52
Run id              6

DIAGNOSTIC INFO
Dead time factor      1.00011 Open window counts       340
Spectrum assessment   Spectrum OK
ISOTOPE I-129:
Expected peak chn     32.2    Low channel limit         24
High channel limit    41      Used peak channel       31.5
Peak begin channel    -1      Peak end channel          42
This peak has noise           Window shifted

Peaks in smoothed spectrum that exceed 7
Channel      Counts
     31          18 <<
END OF DIAGNOSTIC INFO


   POS RACK DET BATCH  TIME   COUNTS       CPM  ERROR %
     1    1   1     1    20      217     643.0     7.35
     2    1   2     1    20     9239   27736.3     1.47
     3    1   3     1    20     5586   16800.4     1.76
     4    1   4     1    20     3898   11711.4     2.03
     5    1   5     1    20     2262    6748.4     2.54

DIAGNOSTIC INFO
Dead time factor      1.00090 Open window counts       2822
Spectrum assessment   Spectrum OK
ISOTOPE I-129:
Expected peak chn     31.5    Low channel limit         24
High channel limit    40      Used peak channel       32.2
Peak begin channel    -1      Peak end channel          55
This peak has noise           Window shifted

Peaks in smoothed spectrum that exceed 46
Channel      Counts
     32         216 <<
END OF DIAGNOSTIC INFO


   POS RACK DET BATCH  TIME   COUNTS       CPM  ERROR %
     6    1   1     2    20     2667    8031.6     2.36
     7    1   2     2    20     2596    7766.2     2.40
     8    1   3     2    20      226     673.2     7.13
     9    1   4     2    20    14997   45267.7     1.24
    10    1   5     2    20     2332    6958.6     2.51

END OF ASSAY
```

Figure 10. Diagnostic info. printout of a CPM assay.

```
NORMALIZATION OF  4 I-129        26-Aug-1994 10:25:45

DIAGNOSTIC INFO FOR DETECTOR 1

ISOTOPE I-129:
Actual coverage       63.42
END OF DIAGNOSTIC INFO

Total counting time           45
Repeat times                  3
Detectors not in use          6, 7, 8, 9, 10

      DECAYED   MEASURED   DETECTOR   RELATIVE SIGNIF.
DET   ACTIVITY   COUNTS   EFFICIENCY  ERROR %  LEVEL %
  1     1.0000     68182      1.0138      0.31  78.252
  2     1.0000     68475      1.0182      0.30   0.279
  3     1.0000     65925      0.9804      0.31  68.787
  4     1.0000     65654      0.9763      0.31  35.961
  5     1.0000     68010      1.0113      0.31  20.969
-------------------------------------------------------
AVG                67249      1.0000      0.31

VALUES SAVED

END OF ISOTOPE NORMALIZATION
```

Figure 11. Diagnostic info. printout in conjunction with an isotope normalization. Detector 6 is not active. "SYSTEM | Isotopes | I-129 | Counting window" is "Fixed".

```
NORMALIZATION OF  4 I-129        26-Aug-1994 10:33:07

DIAGNOSTIC INFO FOR DETECTOR 1
Dead time factor      1.01552 Open window counts       35789
Spectrum assessment   Spectrum OK
ISOTOPE I-129:
Expected peak chn     31.0    Low channel limit        20
High channel limit    46      Used peak channel       32.2
Peak begin channel    13      Peak end channel         56
Resol start chn       26      Resol end chn            38
Actual coverage       98.51   Window shifted

Peaks in smoothed spectrum that exceed 576
Channel      Counts
    32         2867 <<
END OF DIAGNOSTIC INFO

Total counting time           15
Detectors not in use          6, 7, 8, 9, 10

      DECAYED   MEASURED   DETECTOR   RELATIVE
DET   ACTIVITY   COUNTS   EFFICIENCY  ERROR %
  1     1.0000     35254      0.9955      0.43
  2     1.0000     35478      1.0019      0.42
  3     1.0000     35377      0.9991      0.43
  4     1.0000     35356      0.9984      0.43
  5     1.0000     35585      1.0050      0.42
-----------------------------------------------
AVG                35410      1.0000      0.43

VALUES SAVED

END OF ISOTOPE NORMALIZATION
```

Figure 12. Diagnostic info. printout in conjunction with isotope normalization. Detector 6 is not active. "SYSTEM | Isotopes | I-129 | Counting window" is "Dynamic-%".

The following values are printed in diagnostic info. "Level" is the minimum value that the parameter "SYSTEM | Diagnostic output | Level of diag. info." must have in order that the value is printed. "Assay" indicates whether a value is printed also during a RIA/IRMA/RATIO assay or only during isotope normalization. "Isotope" indicates whether a value is printed for both isotopes in a dual labelled assay.

Name	Level	Assay	Isotope
Dead time factor	0	Yes	No
Open window counts	0	Yes	No
Spectrum assessment	0	Yes	No
Unexpected peak	0	Yes	No
Expected peak chn	3	Yes	Yes
Low channel limit	5	Yes	Yes
High channel limit	5	Yes	Yes
Used peak channel	4	Yes	Yes
Peak begin channel	6	Yes	Yes
Peak end channel	6	Yes	Yes
Resol start chn	7	No	Yes
Resol end chn	7	No	Yes
This peak has noise	0	Yes	Yes
This is abnormal peak	0	Yes	Yes
Actual coverage	0	No	Yes

Dead time factor is the factor by which measured counts must be corrected to account for pulses that are lost because they occurred when a previous pulse was being processed. You can include the dead time factor in a RIA/IRMA/RATIO assay printout by setting the parameter "SYSTEM | Diagnostic output | Print dead time factor" to "Yes".

Open window counts is the total number of counts in all channels of the multi channel analyser. It also includes those pulses that are beyond the MCA high energy limit.

Spectrum assessment can have one of the following values: "Spectrum OK", "Bad spectrum", "No coincidence peak", "Too small coincidence peak", "Too low activity" (this can appear only during isotope normalization), "Too high activity".

Unexpected peak gives the peak channel number of a peak that was not expected. In this case "Spectrum assessment" is "Bad spectrum".

Expected peak chn is in isotope normalization the main peak set by "SYSTEM | Isotope | <isotope name> | Peak pos. (keV)" converted to a MCA channel number using the nominal gain. If an isotope uses a fixed window, then expected peak channel is not used.

In RIA/IRMA/RATIO assay counting the expected peak channel is the MCA channel number where the isotope peak was in the latest batch where it was sufficiently high. The peak position is saved at the end of each assay and if the parameter “SYSTEM | Operating mode | Default is norm window” is “No”, the expected position is retrieved at the beginning of next assay using the same isotope. If the parameter “Default is norm window” is “Yes”, the peak determined at isotope normalization time is taken as the expected peak position at the beginning of an assay The expected peak channel is used as a substitute for the observed peak channel, if the current tube's activity is so small that the isotope peak does not exceed the threshold level (see Section 10.2.4.2.1.6 on page 140). The isotope peak in turn is used to calculate the effective gain, if "SYSTEM | Isotope | <isotope name> | Counting window" is "Dynamic-keV" or "Dynamic-%". The gain value is used to convert counting window boundaries from keV to MCA channel numbers. In 1480 multiple isotope assays (MIA) the windows determined at isotope normalization time are always used, so expected peak position has no meaning there.

Low channel limit and **High channel limit** define the interval inside which the isotope main peak should be. They are obtained with the following formulae:

LowChannelLimit = ExpectedPeakChn / (1.0 + MaxPeakDev / 100)
HighChannelLimit = ExpectedPeakChn * (1.0 + MaxPeakDev / 100)

In isotope normalization MaxPeakDev is equal to "SYSTEM | Isotope | <isotope name> | Max. norm. dev. (%)". In RIA/IRMA/RATIO assays MaxPeakDev is equal to "SYSTEM | Isotope | <isotope name> | Max. assay dev. (%)". The highest isotope peak between LowChannelLimit and HighChannelLimit is assumed to be the isotope main peak. If an isotope has been set up to use fixed windows, there is no need to determine the isotope main peak channel number and hence neither LowChannelLimit or HighChannelLimit.

Used peak channel is the highest isotope peak between LowChannelLimit and HighChannelLimit. It is assumed to be the isotope main peak channel number, provided that such a peak exists and it is sufficiently high (see Sections 10.3.2.2.1 on page 162 and 10.3.2.2.2 on page 163). If no such peak exists, “Used peak channel” is set equal to “Expected peak chn”. In both cases the counting windows determined during isotope normalization are shifted by the same relative amount that “Used peak channel” differs from the main peak channel determined during isotope normalization.

Peak begin channel and **Peak end channel** are the nearest local minima around Found peak channel.

Resol start chn and **Resol end chn** give the interval within which the peak is over 67% of its maximum height in the smoothed spectrum. During isotope normalization the program fits a bell-shaped Gaussian curve over this area and gives the calculated resolution of the fitted curve as the peak resolution. See Section 10.2.4.2.5 on page 142.

This peak has noise is printed if the smoothed spectrum between Peak begin channel and Found peak channel and again between Found peak channel and Peak end channel is not monotonous. See Section 10.2.4.2.1.4 on page 139.

This is abnormal peak is printed during isotope normalization if the smoothed spectrum counts at channels Peak begin channel or Peak end channel are over 67% of the height of the peak in the smoothed spectrum. It is also printed during a RIA/IRMA/RATIO assay if the nearest local maximum or minimum to Found peak channel is a local maximum.

Actual coverage is the ratio of measured counts in a window to Open window counts. Neither of these counts values is corrected in any way, e.g. for dead time or for background. The measured counts in a window has in the isotope normalization printout the title "MEASURED COUNTS". If "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-%", then Actual coverage is always slightly greater than "SYSTEM | Isotopes | <isotope name> | Window coverage (%)".

Window shifted is printed if the observed isotope main peak was used to shift the counting window determined at isotope normalization.

Peaks in smoothed spectrum that exceed... This line in the diagnostic info printout is used to show some prominent peaks in the spectrum. The limit above which peaks are shown is obtained in the following way. Using the parameters "SYSTEM | Isotopes | <isotope name> | Signif. cpm per keV" and "SYSTEM | Isotopes | <isotope name> | Threshold level (%)", first convert the former parameter to a counts value and add to it the fraction given by the latter parameter of the height of the isotope main peak in the smoothed spectrum. This sum is the limit value. In dual label RIA/IRMA/RATIO assays the limit is the smaller of the two limits.

The parameter "Signif. cpm per keV" is used to indicate the amount of background activity at the energy of the isotope main peak. Local maxima that are smaller than this are not considered eligible for being the isotope main peak. Once the isotope main peak has been found, it is assumed that stray counts due e.g. to Compton scattering are raising the background activity level outside the main peak by the fraction given by "Threshold level (%)".

If in isotope normalization and in single label RIA/IRMA/RATIO assay measurement the "SYSTEM | Isotopes | <isotope name> | Spectrum type" is "Single peak" or "I-125" for the isotope used, any extraneous peaks in the spectrum that rise above the calculated limit value cause the spectrum to be regarded as "bad" because it has an unexpected peak. If in dual label RIA/IRMA/RATIO assay measurement neither isotope is of the type "Many peaks", the search for extraneous peaks is made using the sum of the limits for both isotopes. In the diagnostic info printout, below the title Peaks in smoothed spectrum that exceed... are listed the channel number and smoothed spectrum counts of local maxima for which the counts value exceeds the limit value, or in the case of dual labelled RIA/IRMA/RATIO assays, the smaller of the two limit values.

10.4 Multiple isotope assays (MIA)

When a rack having an ID clip with a numeric label in the range from 1 to 99 stuck in the area marked "PROTOCOL" is encountered during automatic measurement, WIZARD starts measuring the assay that has this protocol number. An optional rack number can be given by placing a numeric code in the "RACK/SPECIAL" area of the ID clip; otherwise the first rack is assumed to have the number 1 and this increases by one for each subsequent rack.

The possible assay types for an assay protocol are RIA, IRMA, RATIO and MIA. The assay type is determined when a protocol is created. MIA protocols can only be created on a 1480 counter with RiaCalc WIZ.

A multiple isotope assay consists of standard samples followed by unknown samples. Both standard and unknown samples can comprise several replicate tubes. The number of replicates for each isotope standard can be set independently. At the beginning of a multiple isotope assay isotope standards are normalized in order to determine counting windows and spillover. For each standard sample the replicate tube spectra are summed and this sum spectrum is used when that isotope is normalized (see Section 10.2.3 on page 136). The replicate number for a standard can also be set to 0. This means that there are no tubes for this isotope standard in the multiple isotope assay and the latest previously done normalization for that isotope is used instead.

10.4.1 Spillover correction

If spillover correction has been specified in the MIA protocol, the spillover correction factors are calculated in the following way. During isotope normalization the spectrum of the normalization sample is stored in the instrument hard disk. Latest background spectra are also copied and stored with the normalization spectra.

The program determines counting windows in terms of MCA channels. This is done by converting the stored keV values of counting window boundaries by using the effective gain during the isotope normalization during which the counting window was determined. This is discussed further below.

If "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-%", the window boundary keV values are determined during isotope normalization on the basis of the parameters "SYSTEM | Isotope | <isotope name> | Window coverage (%)". For window types "Dynamic-keV" and "Fixed", the boundaries are given by the isotope parameters "SYSTEM | Isotopes | <isotope name> | Low boundary (keV)" and "High boundary (keV). If "Counting window" is "Dynamic-%" or "Dynamic-keV", then the effective gain is calculated by dividing the energy of the main isotope peak in keV's by the MCA channel number of the main peak. If "Counting window" is "Fixed", then the nominal gain is used.

Next the activities of all standards in all counting windows are determined. This is done in the following way. For each isotope standard spectrum, counts are summed over all isotope counting windows. They are corrected for dead time, converted to CPM and the background activity is subtracted.

Dead time factor, background activity and possible isotope decay correction factor are all determined by the isotope spectrum used. The effective gain, which is used to convert counting windows from keV's to MCA channels, is taken from the isotope normalization which produced the counting window and not by the isotope spectrum to which the counting window is applied. In RIA/IRMA/RATIO dual label assays the gain is determined by the spectrum on which a counting window is applied and theoretically it should be this way. But since isotope normalizations usually are done at the beginning of a MIA so that the effective gain has no time to change and because the detector unlinearity correction described in Section 10.2.4.1.2 on page 137 may not be entirely accurate, the implementation chosen for MIA in practice gives better results.

The following N×N matrix of CPM activities is set up, where N is the number of isotope standard samples.

Spectrum of isotope standard 1. Counting window of isotope standard 1.	Spectrum of isotope standard 2. Counting window of isotope standard 1.	•••	Spectrum of isotope standard N. Counting window of isotope standard 1.
Spectrum of isotope standard 1. Counting window of isotope standard 2.	Spectrum of isotope standard 2. Counting window of isotope standard 2.	•••	Spectrum of isotope standard N. Counting window of isotope standard 2.
•••	•••	•••	•••
Spectrum of isotope standard 1. Counting window of isotope standard N.	Spectrum of isotope standard 2. Counting window of isotope standard N.	•••	Spectrum of isotope standard N. Counting window of isotope standard N.

Then values in each column are divided by the diagonal value in that column. This converts measured activities to relative spillover values. Let us denote this new matrix with the letter **M**. If this matrix is multiplied with the

$$\mathbf{B} = \begin{bmatrix} \text{Isotope 1 activity.} \\ \text{Isotope 2 activity.} \\ \bullet\bullet\bullet \\ \text{Isotope N activity.} \end{bmatrix}$$

following 1×N vector **B**

the result is the following 1×N vector **A**

$$\mathbf{A} = \begin{bmatrix} \text{Measured activity in the counting window of isotope 1.} \\ \text{Measured activity in the counting window of isotope 2.} \\ \bullet\bullet\bullet \\ \text{Measured activity in the counting window of isotope N.} \end{bmatrix}$$

Thus $\mathbf{A} = \mathbf{MB}$. Then the matrix **M** is inverted. Let us denote the inverted matrix with $\mathbf{M}^{-1}$. When this is multiplied during assay counting with a vector consisting of the measured CPM values in all isotope counting windows, the result vector consists of spillover corrected isotope activities, that is $\mathbf{B} = \mathbf{M}^{-1}\mathbf{A}$.

If the matrix **M** cannot be inverted, the message "! The spill matrix could not be inverted. No spill correction is made." is printed. If the absolute value of the determinant of the matrix **M** is smaller than 0.1, the message "! Results for CCPM, DPM and RATIO may be inaccurate." is printed.

```
MULTI-ISOTOPE ASSAY  10 PROT01  -- STANDARDS

NORMALIZATION OF  1 I-125        04-Oct-1994 14:53:59

Total counting time           600
Nominal gain                  1.00 keV/channel
Main peak                     29.0 keV
Main peak at nominal gain     29.0 channels

   PEAK  PEAK   RESOL    WINDOW keV   DECAYED   MEASURED HORROCKS   STANDARD
   CHN   DEV %    %       LOW   HIGH   ACTIVITY   COUNTS  EFFICIENCY    CPM
   22.9  -21.1   29.8   15.0   35.0     1.0000    417276       84.1     42115

VALUES SAVED

END OF ISOTOPE NORMALIZATION

NORMALIZATION OF  2 Co-57        04-Oct-1994 14:54:06

Total counting time           600
Nominal gain                  1.00 keV/channel
Main peak                     122.0 keV
Main peak at nominal gain     135.0 channels
Nominal window coverage       92.00 %

   PEAK  PEAK   RESOL    WINDOW keV   DECAYED   MEASURED  STANDARD
   CHN   DEV %    %       LOW   HIGH   ACTIVITY   COUNTS     CPM
  100.9  -25.2   16.2   92.2  177.1     1.0000   1621968    165162

VALUES SAVED

END OF ISOTOPE NORMALIZATION

NORMALIZATION OF  3 Cr-51        04-Oct-1994 15:04:19

Total counting time           600
Nominal gain                  1.00 keV/channel
Main peak                     320.0 keV
Main peak at nominal gain     335.0 channels
Nominal window coverage       80.00 %

   PEAK  PEAK   RESOL    WINDOW keV   DECAYED   MEASURED  STANDARD
   CHN   DEV %    %       LOW   HIGH   ACTIVITY   COUNTS     CPM
  254.7  -24.0   11.6  188.2  428.4     0.9997    460344     46227

VALUES SAVED

END OF ISOTOPE NORMALIZATION

NORMALIZATION OF  4 I-129        04-Oct-1994 15:14:31

Total counting time           600
Nominal gain                  1.00 keV/channel
Main peak                     29.0 keV
Main peak at nominal gain     29.0 channels

   PEAK  PEAK   RESOL    WINDOW keV   DECAYED   MEASURED HORROCKS   STANDARD
   CHN   DEV %    %       LOW   HIGH   ACTIVITY   COUNTS  EFFICIENCY    CPM
   22.9  -21.1   29.8   15.0   35.0     1.0000    417276       84.1     42115

VALUES SAVED

END OF ISOTOPE NORMALIZATION

MULTI-ISOTOPE ASSAY  10 PROT01  -- UNKNOWN SAMPLES  04-Oct-1994 15:39:48

! Determinant of spillover matrix: 0.00245
! Results for CCPM, DPM and RATIO may be inaccurate.

Time limit          600
Max. count limit    9999999
Low count time      6
Low count limit     10
Replicates          1
Subtract background YES
Eliminate spillover YES
```

```
Spill determinant   0.002

Sequence number     1
Sample number       1
Replicate number    1
Elapsed time        0.00 hours
Counting time       600 seconds
Dead time           0.952 %

ISOTOPE     COUNTS  BGRD CPM        CPM   ECPM %       CCPM        DPM     RATIO  CCPM/DPM
I-125       417441       9.4    42132.0     0.15    42117.9   109101.5    1.0001    0.3860
Co-57         1661      38.9      128.8     3.54       0.5        0.6    0.0000    0.8528
Cr-51          879      88.3        0.5   860.58       4.6       80.6    0.0001    0.0565
I-129       418891       9.4    42278.3     0.15      14.1       34.0    0.0006    0.4137

Sequence number     2
Sample number       2
Replicate number    1
Elapsed time        0.17 hours
Counting time       600 seconds
Dead time           1.852 %

ISOTOPE     COUNTS  BGRD CPM        CPM   ECPM %       CCPM        DPM     RATIO  CCPM/DPM
I-125         3689       9.4      366.4     1.71       19.2       49.7    0.0005    0.3860
Co-57      1621556      38.9   165119.8     0.08   165123.9   193615.5    0.9998    0.8528
Cr-51        10583      88.3      989.6     1.10      -12.7     -224.2   -0.0003    0.0565
I-129         3698       9.4      367.3     1.71       -8.8      -21.2   -0.0004    0.4137

Sequence number     3
Sample number       3
Replicate number    1
Elapsed time        0.34 hours
Counting time       600 seconds
Dead time           0.609 %

ISOTOPE     COUNTS  BGRD CPM        CPM   ECPM %       CCPM        DPM     RATIO  CCPM/DPM
I-125        15385       9.4     1538.5     0.81       15.8       41.0    0.0004    0.3860
Co-57        43628      38.9     4350.5     0.49       44.2       51.9    0.0003    0.8528
Cr-51       459499      88.3    46141.5     0.15    46157.7   817572.5    0.9985    0.0565
I-129        15457       9.4     1545.7     0.81      -10.6      -25.7   -0.0004    0.4137

Sequence number     4
Sample number       4
Replicate number    1
Elapsed time        0.51 hours
Counting time       600 seconds
Dead time           0.409 %

ISOTOPE     COUNTS  BGRD CPM        CPM   ECPM %       CCPM        DPM     RATIO  CCPM/DPM
I-125       242786       9.4    24368.5     0.20     -424.5    -1099.6   -0.0101    0.3860
Co-57          425      38.9        3.7    76.65       2.7        3.1    0.0000    0.8528
Cr-51          955      88.3        7.6    56.20       1.1       18.7    0.0000    0.0565
I-129       244239       9.4    24514.3     0.20    24940.1    60279.4    1.0152    0.4137


END OF MULTI-ISOTOPE ASSAY
```

Figure 13. A MIA printout. The parameter "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-keV" for I-125 and I-129 and "Dynamic-%" for Co-57 and Cr-51. Since I-125 spillover to I-129 window is very large, the warning "Results for CCPM, DPM and RATIO may be inaccurate" is printed.

10.4.2 Calculating corrected CPM values (CCPM) for unknown samples

The counting windows used for unknown samples are the same as those that were used to make up the spillover correction matrix. Isotope peaks are not searched for, so possible variations in effective gain are not taken into account; i.e. the windows are not shifted once they have been determined.

Counting window boundaries are determined to a fraction of channel. When counts are summed over a counting window, counts from the low and high boundary channels are added only in the proportion that the channels are included inside the counting window.

The counts sum is corrected for dead time, converted to CPM and background activity is subtracted (if selected in the MIA protocol). If spillover correction has been specified in the MIA protocol, these CPM values from all counting windows are used to form for each measured tube a 1×N vector **A**, as described in the preceding paragraph. It is multiplied by the spillover correction matrix $\mathbf{M}^{-1}$ to get a vector **B** of spillover corrected CPM values. If decay correction has been selected for this isotope, (that is, if "SYSTEM | Isotopes | <isotope name> | Decay correction" is "Yes" and "SYSTEM | Isotopes | <isotope name> | Assay zero time" is not "None") the CPM values are further corrected for isotope decay. Then the CPM values are printed, if selected in the MIA protocol. The title used for these values is CCPM.

10.4.3 The printout fields DPM, RATIO and CCPM / DPM

In a MIA protocol these fields can selected to be included in the MIA printout.

DPM is CCPM (the corrected CPM) divided by the actual isotope window coverage and by the parameter "SYSTEM | Isotopes | <isotope name> | Efficiency". The actual isotope window coverage is the ratio of counts in the isotope window to counts in the open window that contains all channels of the multi-channel analyzer. You can print the actual coverage during isotope normalization by setting "SYSTEM | Diagnostic output | Print diagnostic info" to "YES". If "SYSTEM | Isotopes | <isotope name> | Counting window" is "Dynamic-%", then actual coverage is always slightly larger than "SYSTEM | Isotopes | <isotope name> | Window coverage (%)".

RATIO is the relative amount of isotope present in an unknown sample (given by CCPM) as compared to the standard sample for this isotope. The standard sample CPM is included in isotope normalization printout.

The ratio CCPM / DPM gives the absolute efficiency of the instrument using the current isotope counting window.

10.4.4 Replicate averages

In a MIA protocol the number of unknown replicates can be specified. If it is greater than 1, unknown replicate averages are calculated. The averages are weighted according to the measurement time used to measure each individual replicate tube. The counting times can be different if counting is terminated because the max. counts limit has been reached or if the tube has been rejected because of low activity. The replicate averages are printed if this has been specified in the MIA protocol.

10.4.5 "TOTAL CPS" and "TOTAL DPS"

These two values are stored in the file TOTALCPS.TXT in the root directory of the instrument hard disk. "TOTAL CPS" is the sum of all printed corrected CPM values of measured unknown tubes in the assay. These CPM values are corrected for dead time, for background (if selected in MIA protocol), for spillover (if selected in MIA protocol) and for isotope decay (if selected in isotope parameters). The DPS value is obtained by dividing the printed corrected CPM value by the actual coverage of the isotope counting window and by "SYSTEM | Isotope | <isotope name> | Efficiency (%)". "TOTAL DPS" is the sum of all DPS values of unknown tubes in the assay.

10.4.6 How CPM error is calculated when 1480 MIA unknown samples are measured

In MIA printout this quantity has the title ECPM %. The following formulae are used.

```
Variance1 = SQUARE(DeadTimeFactor * (60 / CountingTimeInSeconds)) * CountsInWindow
RelativeErrorInBackgroundCpm = 1.0 / SQUAREROOT(BackgroundCountsInWindow)
Variance2 = Variance1 + SQUARE(BackgroundCpmInWindow * RelativeErrorInBackgroundCpm)
```

Background error is added only if background correction has been selected.

RelativeCpmError = SQUAREROOT(Variance2) / CorrectedCpm

CorrectedCpm is corrected for dead time and background (if background correction is selected) but not for spillover, crosstalk or isotope decay. If CCPM, the final corrected CPM in the MIA printout includes spillover corrections, the ECPM % printed refers to the quantity CPM, which does not have this correction.

If an unknown MIA sample consists of several replicate tubes, the error in the replicate average is calculated in the following way. For each replicate measurement, define

Variance3 = SQUARE(DeadTimeFactor) * CountsInWindow

Let TotalVariance be the sum of Variance3's for all replicate measurements and let TotalCountingTimeInSeconds be the sum of CountingTimeInSeconds's for all replicate measurements. Then

RelativeReplicateAverageCpmError = SQUAREROOT(TotalVariance * SQUARE(60 / TotalCountingTimeInSeconds)
+ SQUARE(BackgroundCpmInWindow * RelativeErrorInBackgroundCpm)) /
CorrectedReplicateAverageCpm

Background error is added only if background correction has been selected. CorrectedReplicateAverageCpm is corrected for dead time and background (if background correction is selected) but not for spillover or isotope decay.

One could think that the variance of the replicate average could be calculated by summing the variances of individual replicate sample CPM's and dividing the result by the square of the number of replicates (assuming that all replicate sample CPM's were obtained using the same counting time). But implicit in this is the assumption that the background CPM's that were subtracted from individual replicate sample CPM's were all measured separately. This would be equivalent to the total background counting time for the replicate average being obtained from the number of replicate tubes multiplied by the background counting time used for a single replicate tube. This would have greater accuracy, but in fact we have only measured background once. The formulae above takes this into account.

10.5 Appendix: Some basic mathematical formulas

10.5.1 Error calculation

Isotope decay has Poisson statistics so that the variance of a measured counts value is equal to the counts value itself. Standard deviation is by definition equal to the square root of variance. Relative error is defined to be equal to standard deviation divided by the counts value.

If a random variable is multiplied by a constant, the corresponding variance is multiplied by the square of that constant. If two independent random variables are summed, their variances are also summed to get the variance of the sum value.

10.5.2 Matrix calculation formulas

The product of an N×N matrix with an 1×N vector is defined as follows

$$\begin{bmatrix} m_{11} & \bullet\bullet\bullet & m_{1N} \\ \bullet\bullet\bullet & \bullet\bullet\bullet & \bullet\bullet\bullet \\ m_{N1} & \bullet\bullet\bullet & m_{NN} \end{bmatrix} \begin{bmatrix} v_1 \\ \bullet\bullet\bullet \\ v_N \end{bmatrix} = \begin{bmatrix} \sum_i m_{1i} v_i \\ \bullet\bullet\bullet \\ \sum_i m_{Ni} v_i \end{bmatrix}$$

We can thus write a system of N linear equations each having N unknowns

$$\begin{cases} a_{11}x_1 + a_{12}x_2 + \bullet\bullet\bullet + a_{1N}x_N = b_1 \\ \bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet \\ a_{N1}x_1 + a_{N2}x_2 + \bullet\bullet\bullet + a_{NN}x_N = b_N \end{cases}$$

as the matrix equation

$$\begin{bmatrix} a_{11} & \bullet\bullet\bullet & a_{1N} \\ \bullet\bullet\bullet & \bullet\bullet\bullet & \bullet\bullet\bullet \\ a_{N1} & \bullet\bullet\bullet & a_{NN} \end{bmatrix} \begin{bmatrix} x_1 \\ \bullet\bullet\bullet \\ x_N \end{bmatrix} = \begin{bmatrix} b_1 \\ \bullet\bullet\bullet \\ b_N \end{bmatrix}$$

If we denote

$$\mathbf{A} = \begin{bmatrix} a_{11} & \bullet\bullet\bullet & a_{1N} \\ \bullet\bullet\bullet & \bullet\bullet\bullet & \bullet\bullet\bullet \\ a_{N1} & \bullet\bullet\bullet & a_{NN} \end{bmatrix}$$

$$\mathbf{X} = \begin{bmatrix} x_1 \\ \bullet\bullet\bullet \\ x_N \end{bmatrix}$$

$$\mathbf{B} = \begin{bmatrix} b_1 \\ \bullet\bullet\bullet \\ b_N \end{bmatrix}$$

we can write the equation as

$$\mathbf{AX} = \mathbf{B}$$

The inverse of a matrix $\mathbf{A}$ is denoted with $\mathbf{A}^{-1}$ and is defined so that

$$\mathbf{X} = \mathbf{A}^{-1}\mathbf{B}$$

for all $\mathbf{X}$ and $\mathbf{B}$ that fulfill the previous equation. It is possible to find an inverse for every N×N matrix for which the determinant is not zero.

Matrix inversion and multiplication can be used to solve a system of N linear equations each having N unknowns. In this Calculation Methods manual the following situations are modelled with a system of linear equations.

- A sample consists of several (say N) isotopes and as many counting windows are used when it is measured. Each isotope is assigned one counting window. When pure isotope samples are measured, the spillover factors giving the relative activity of an isotope in the counting windows of other isotopes as compared to the isotope's own window can be determined and an N×N matrix can be set up, as descibed in Section 10.3.1.2.1 on page 158 and Section 10.4.1 on page 171. The spillover factors can be assumed to independent of isotope activity, at least as long as the isotope activities are not very large. When an unknown sample containing several isotopes is measured, the inverse of the spillover factor matrix can be used to solve the individual isotope activities in the sample.
- Single labelled samples are measured in a counter having several (say N) detectors. There is crosstalk between the detectors. When the same sample is measured successively in each detector, the remaining detectors being empty, the crosstalk coefficients giving the relative activity measured in the empty detectors as compared to the activity measured in the detector having the sample can be determined and an N×N matrix can be set up, as described in Section 10.2.12 on page 147. The crosstalk coefficients can be assumed to independent of isotope activity, at least as long as the isotope activities are not very large. When samples are measured in all the detectors simultaneously, the inverse of the crosstalk coefficient matrix can be used to solve the activity of each of samples, just as if all the other detectors were empty.
- Samples consist of several (say M) isotopes and as many counting windows are used when they are measured in a counter having several (say N) detectors. Each isotope is assigned one counting window. There is spillover between isotope counting windows and crosstalk between the detectors. When pure isotope samples are measured successively in each detector, the remaining detectors being empty, the coefficients giving the relative activities in all counting windows of all detectors can be determined and an (M*N)×(M*N) matrix can be set up, as described in Section 10.3.1.2.2 on page 159 for the case where M=2. The spillover and crosstalk coefficients can be assumed to independent of isotope activities, at least as long as they are not very large. When samples containing several isotopes are measured in all the detectors simultaneously, the inverse of the spillover and crosstalk coefficient matrix can be used to solve the individual isotope activities in each of the samples, just as if all the other detectors were empty and only one isotope present.

For typographical reasons the matrices and vectors in this manual are represented as tables, i.e.

$$\begin{bmatrix} a_{11} & \bullet\bullet\bullet & a_{1N} \\ \bullet\bullet\bullet & \bullet\bullet\bullet & \bullet\bullet\bullet \\ a_{N1} & \bullet\bullet\bullet & a_{NN} \end{bmatrix} =$$

a_{11}	⋯	a_{1N}
⋯	⋯	⋯
a_{N1}	⋯	a_{NN}

and

$$\begin{bmatrix} b_1 \\ \bullet\bullet\bullet \\ b_N \end{bmatrix} =$$

b_1
⋯
b_N

11 Installation

11 Installation

11.1 Installation procedure

Make the installation according to these instructions and complete the Installation Qualification and Operation Qualification (IQOQ) report form in parallel.

11.2 Environment

Although normal clean laboratory conditions are usually quite satisfactory as an operational environment, it is useful to take the following points into consideration.

If possible, a separate room should be provided for Wallac 1470 WIZARD as this allows the best control over the immediate environment. Ventilation in the room should be reasonably constant at about 22°C, relative humidity should not be excessive and direct sunlight should not be able to reach the instrument. It is also important that the various isotopes are stored well away from the instrument in another room. Only those radioactive samples that are actually measured should be in the laboratory at any time in order to keep the background at a low level.

11.3 Electric power

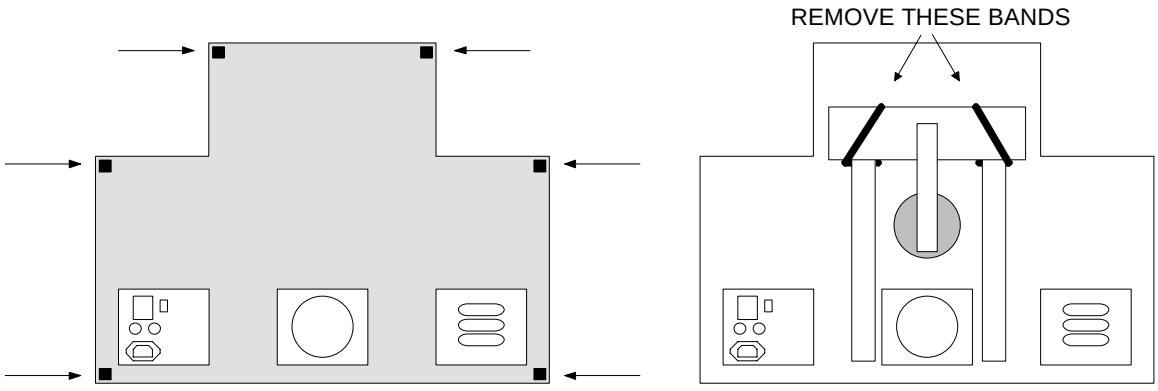
Three electrical outlets, each with a protective earth, should be available, with, if possible, a separate power line for the instrument itself having an isolation switch and a fuse box. If excessive fluctuations in the mains voltage are anticipated, a mains stabilizer may be necessary.

11.4 Unpacking

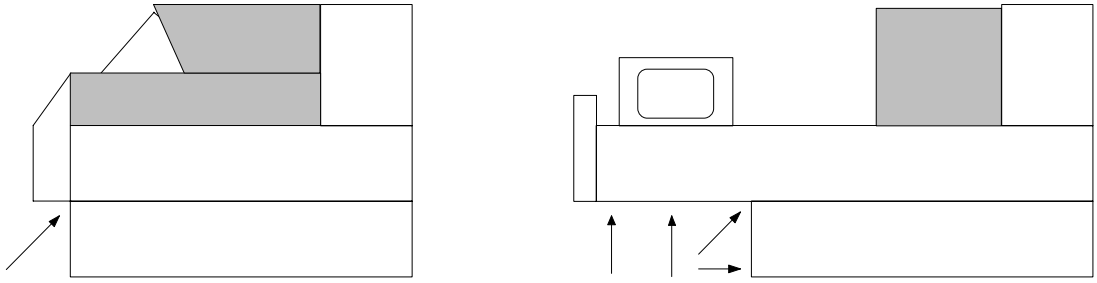
Unpack all units and accessories and check them according to the packing list also noting any possible transport damage.

Move the instrument to its place of operation.

Remove the rear panel of the instrument by loosening the captive screws. Remove the two plastic bands which keep the elevator at the lowest position during transportation (see the figure below).

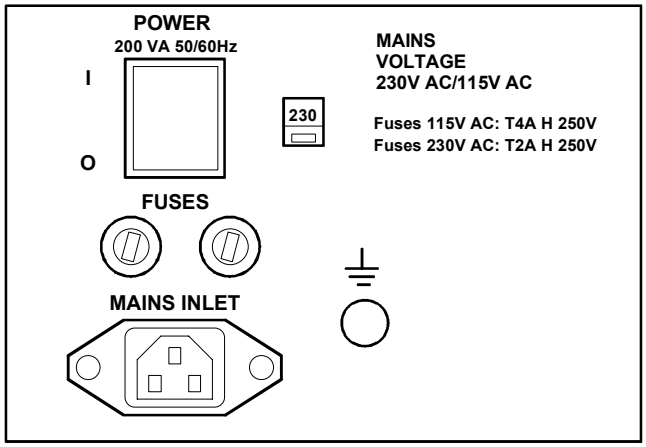


Remove the front lower cover by unscrewing the screws at the front of the instrument (see the figure below). Check that all the boards in the electronic racks are firmly in their positions. Check also all the cable connections. You can now replace the back panel and front cover.

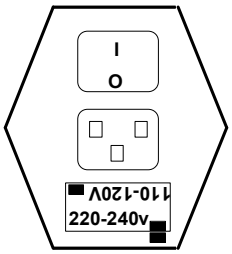


11.5 Checking the mains voltage setting

Measure and note the mains voltage at the outlets to be used. Locate the mains selector switch, see the figure below, this is on the left side when looking from the rear of the instrument. If necessary, adjust the mains selector switch to correspond with the measured supply. Check that the fuses fitted in the fuse carriers are of the correct rating for the local supply, and according to the label.



The mains selector in older instruments has the voltage marked on it and is combined with the fuse carriers. To adjust the mains selector, pull it out, turn it through 180 degrees and replace it.



11.6 Connecting up the counter and peripherals

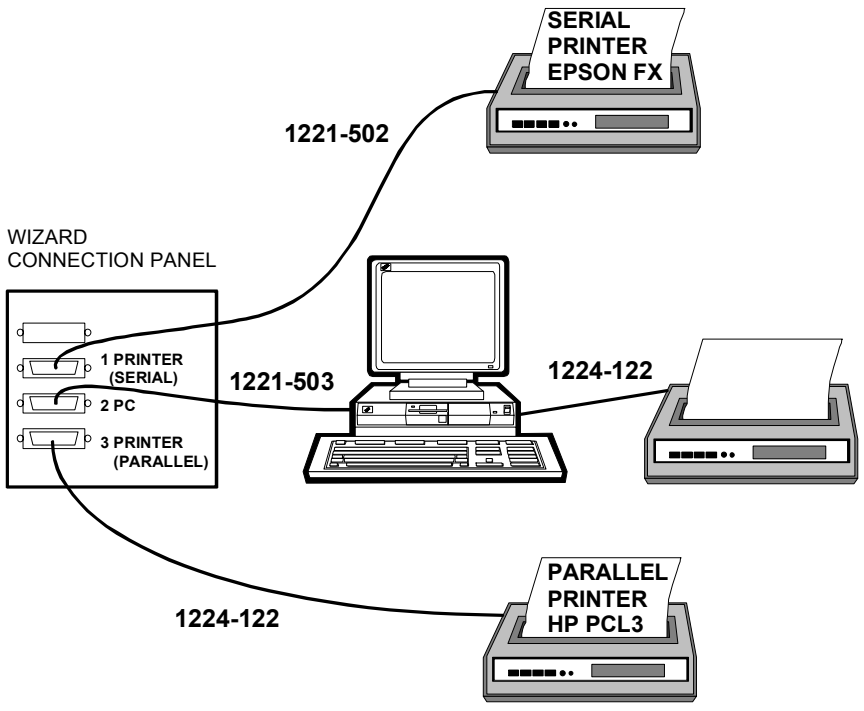
Connect the counter to the PC (if supplied) and the printer using the cables shown in the Peripheral Installation Sheet. Further details of the settings for the PC and printer are given in their respective installation sheets which follow these installation instructions.

WIZARD has three communication ports. They are:

1 PRINTER (SERIAL)	RS-232 port to connect a **serial** printer
2 PC	RS-232 port to connect the PC
3 PRINTER (PARALLEL)	Parallel port to connect a **parallel** printer HP PCL 3

The parallel port is standard in new instruments where the software version V3.5 is factory installed. The third communication port in older instruments is a RS-232 serial port and is marked as “MAINFRAME”. V3.5 does not support the mainframe port. The printout selection “Use parallel pr. port?” is available only if the microprocessor board has a parallel port (DCE CPU board).

WARNING: Do not in any circumstances connect a parallel printer to the RS 232 serial port i.e. to the Printer (serial), PC or Mainframe port. This may damage the parallel printer. The parallel printer can be connected only to the port marked as “PRINTER (PARALLEL)”.



1

CPM or RiaCalc WIZ

Serial printer

2

MultiCalc

3

CPM or RiaCalc WIZ

Parallel printer

Plug in the power cables for each device.

Switch on the printer and PC.

11.7 Switching on WIZARD

If you have WIZARD without a hard disk, you must insert the program disk into the drive located in the microprocessor rack. This disk remains in place permanently. The latest software version for WIZARD without a hard disk is V2.2B FL. This version does not support the parallel port.

Insert the data disk into the disk drive located in the front cover of the instrument.

Switch on WIZARD. Loading takes about three minutes before the main menu appears on the built-in display.

Set the SYSTEM parameters “Operation mode” and “Printout selection” according to the current hardware. The typical configurations are:

1. Operation mode = CPM or RiaCalc WIZ. Serial printer

SYSTEM | Printout selections | Use serial printer port? = Yes

SYSTEM | Printout selections | Use parallel pr. port? = No

SYSTEM | Printout selections | Without buffering to PC = No

SYSTEM | Printout selections | Printer type = Epson FX

2. Operation mode = MultiCalc

SYSTEM | Printout selections | Use serial printer port? = No

SYSTEM | Printout selections | Use parallel pr. port? = No

SYSTEM | Printout selections | Without buffering to PC = No

3. Operation mode = CPM or RiaCalc WIZ. Parallel printer

SYSTEM | Printout selections | Use serial printer port? = No

SYSTEM | Printout selections | Use parallel pr. port? = Yes

SYSTEM | Printout selections | Without buffering to PC = No

SYSTEM | Printout selections | Printer type = HP PCL3

11.8 Installing MultiCalc

WIZARD can be connected to an external computer running the MultiCalc (or RiaCalc) software. The installation instructions are given in the MultiCalc manual 1224 – 930.

During the installation procedure you will be asked amongst other questions to select the "Technology" and the "Counter". Technology" describes the counting process to be used. In this case it is gamma counting so you must select GAMMA. "Counter" is the type of counter MultiCalc is to be working with. You must select "WIZARD". Note that you can select more than one type of technology and counter at the same time.

If you have a RiaCalc or MultiCalc version that does not include a communication protocol for WIZARD you can copy it from the instrument program diskette. The communication protocols for the particular program are stored on the WIZARD Main Program diskette, in file 1470.C00 for RiaCalcV2.65 and file \INST\0COM\ GAMMA\WIZARD.C00 for MultiCalc V2.0.

Write-protect your WIZARD Main Program diskette and put it in drive A: (or B:) in the PC where you have RiaCalc or MultiCalc. Copy the particular communication protocol to C:\WIACALC\0COM\ (C:\RIACALC2\0COM if RiaCalc).

If MultiCalc is already installed in the PC but not for WIZARD, you must go through a similar procedure to that described above but select "Setup only" rather than "Installation" from the main installation menu.

Start MultiCalc.

Press F1 (= COUNTER) to get to counter control.

Move the cursor to WIZARD in the counter list.

Press F5 (= INSTALL) to install WIZARD for MultiCalc. In step a) installation, you were adding WIZARD to the list of possible counters to be operated with MultiCalc. Now with this instruction you are telling which counter you are actually going to work with.

If MultiCalc cannot get WIZARD into the Ready state it will ask you to press F3 to get into the terminal mode.

Press F3 and try to get WIZARD to the Ready state. You can also try pressing the Esc-key several times.

At the end of installation you will see instructions about MultiCalc/WIZARD use.

For more details about MultiCalc installation see the MultiCalc Supervisor's Manual, 1224-930.

11.9 Functional check

Carry out the functional check and the performance test of the instrument. For checking the instrument, prepare three racks with the following labels:

BKG

NORM 4

STOP

Place the I-129 standard supplied with the instrument in the last position of the normalization rack. Remove the holders from positions 1 to 9 in the rack.

The background rack must be an empty rack with the BKG ID label.

The STOP rack is a totally empty rack or a rack with a STOP ID label. In the latter case it does not matter if there are holders or not.

Load the racks onto the conveyor in the order BKG, NORM and STOP. To obtain a full printout follow the procedure below.

Go to the SYSTEM menu, select "Printout selections" and change "Extended norm. printout" to "Yes". Then press EXIT and SAVE changes. Set SYSTEM parameters "Isotopes/I-129/Norm. counting time(s)" to 180 and "Background/Bgrd. Counting time" to 600s. Press EXIT and SAVE changes.

Start counting and check that the instrument reads the ID labels correctly. Compare the results obtained with those on the final test data sheet from PerkinElmer Life Sciences, Wallac Oy. Send the printout to PerkinElmer Life Sciences, Wallac Oy with the IQOQ report.

Finish the test by changing "Extended norm. printout" back to "No".

12 Declaration of Conformity for CE-marking

PerkinElmer life sciences

DECLARATION OF CONFORMITY FOR CE-MARKING
INSTRUMENTS

We

Supplier's name
WALLAC OY
Address
PL 10, 20101 TURKU, FINLAND

declare under our sole responsibility that the product

Name, type or model, lot, batch or serial number, possibly sources and numbers of items
1470 Wizard, Gamma Counter Valid from serial number 4700909

to which this declaration relates is in conformity with the following standard(s) or other normative document(s)

TItle and/or number and date of issue of the standard(s) or other normative document(s)
EN 50082-1 :1992; EN 50081-1 :1992 EN 61000-3-2 :1995 + A1 :1998 + A2 :1998 + A14 :2000; EN 61000-3-3 :1995 EN 61010-1 :1993

(if applicable) following the provisions of the following directives

Electromagnetic compatibility (EMC), 89/336/EEC Low voltage (LV), 73/23/EEC

Date and place of issue
01 June 2001 TURKU, FINLAND
Name and signature or equivalent marking of authorized person
Pekka Mäkinen, Quality Assurance Manager 

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