University of Aberdeen
Institute of Medical Sciences

LOCAL RULES

These rules apply to the following areas:-

Polwarth Building, 3rd floor

Issue date: November 2018

Review date: March 2020

Overview

- Only registered and suitably trained workers are permitted to work with radioisotopes in the Polwarth Building.

- Registration is initiated using the Isoinventory system. (http://isoinventory.abdn.ac.uk/). Please see Gary Cameron to initiate registration.

- Completion of the online radiation user training course (https://www.abdn.ac.uk/safety/resources/radiation/ionising/) is mandatory before users can be registered. It is also mandatory for new users even if they have completed a similar course elsewhere.

- Supervisors and/or line-managers are responsible for ensuring that all technical or research staff and post-graduate students in their groups are registered to use isotopes **before** any such work commences.

- A risk assessment (http://isoinventory.abdn.ac.uk/) must be completed before any new radiochemical work can be carried out.

- Gary Cameron is responsible for the ordering and registration of all radiochemicals as well as recording their usage and disposal on the Isoinventory system. No other staff or students should access the Isoinventory system.

- Completion of a refresher course online is required every 3 years.

Isotope users who do not comply with these rules may be subject to disciplinary action including being barred from working with isotopes.

1. Radiation protection supervisors:

If you have any problems with the safety aspects of the use of isotopes please contact your local RPS.

Gary Cameron	RINH 2.028	8615	g.a.cameron@abdn.ac.uk

In Gary Cameron's absence, the following people may be contacted for advice/information or in the case of an emergency

Isabel Crane	room 5.19	7529	i.j.crane@abdn.ac.uk
Lindsay Hall	room 3.20	7510	lhall@abdn.ac.uk
Aivaras Ratkevicius	room HSB 1.07	8023	a.ratkevicius@abdn.ac.uk

2. Designated areas

Controlled radiation areas	Supervised radiation areas
None	3.020, 3.022 and 3.025

3. Unsealed Radionuclides used

Radionuclide	Half Life	Emissions	Contamination monitor
H^3	12.3 years	β	Wipe tests, liquid scintillation counting
C^{14}	5570 years	β	Wipe tests, liquid scintillation counting

4. Holding and disposal limits for the Polwarth (MBq)

Radionuclide	Holding	Drain disposal
H^3	740	740
C^{14}	200	200
P^{32}	1	1
P^{33}	50	10
S^{35}	20	10
I^{125}	50	125

5. Radiation Equipment used in the Polwarth

None

6. General Lab arrangements

These rules must be posted in each laboratory where radioactive materials are handled. A prior risk assessment must be carried out before commencing new work activities and recorded using the forms on the Isoinventory system.

Access to the lab

- Access to controlled radiation areas should be restricted to those who have been trained and are directly involved in the experiment. Only trained, registered workers may handle radioactive isotopes.

General

- Work with radioactive materials should only be carried out in designated areas identified in section 2. If you wish to carry out work in an area not identified in section 2 then contact your RPS for advice
  .
- Principal Investigators are responsible for ensuring that all University and local rules regarding use of radioisotopes within their laboratories are adhered to. Principal Investigators are also responsible for the day-to-day practical training of those workers in their groups who use radioisotopes.

- Experiments should be carefully planned and should only take place if no other equivalent experiment which does not involve radioactive substances exists. We are obliged by SEPA to ensure that any experiments that require the use of an isotope utilises the minimum quantity of radioactivity that will ensure a viable result. Consequently **all** isotope users must have **written** evidence that they are complying with this requirement. Examples of the information required are given below (Appendix 2). Please note that this information must be provided whenever a new technique requiring isotope usage is started.

- Users should limit the amounts of radioactive stock solutions kept within the Polwarth to that required in the immediate future. Stocks stored for several years, even those containing isotopes with long half-lives, are unlikely to retain any biological activity.

- Consideration should always be given to using the least hazardous radionuclide for example P-33 should be used in preference to P-32.

- Experiments involving radioactive materials must only be carried out by suitably trained staff/students. A member of staff or student wishing to undertake work with unsealed radioactive substances **must** first have completed the basic radiation safety course, available online at https://www.abdn.ac.uk/safety/resources/radiation/ionising/. A refresher course should be completed every 3 years. Previous experience/training elsewhere is **not** sufficient. Additionally the principal investigator must ensure that all staff or students working on the experiment are proficient in basic laboratory techniques, and in the protocols required for the experiments with isotope, before they start manipulation of radioactive substances unsupervised. It is important that all staff involved in this work are suitably trained in carrying out contamination monitoring.

- All radiochemical users must inform Gary Cameron of their intention to use radiochemicals to ensure that the storage, usage and disposal of radioisotopes are recorded promptly on the Isoinventory system. In the case of disposal of radioisotopes to drain, Gary Cameron must be informed prior to disposal. This is to ensure that we do not exceed the monthly discharge limits as determined by SEPA.

- From time to time it may be necessary to decommission labs or areas in labs no longer required for isotope work. When this is done it is vital that comprehensive monitoring is performed to ensure that all working surfaces, floors, equipment, drains and flow hood are free of radioactive contamination. A form must be completed to document these checks and is available from the RPS. You are advised to speak to Stephen McCallum and the lead RPS before commencing decommissioning of any lab in the Polwarth.

Lab Procedures

- Observe all the basic laboratory safety procedures:
    - There must be **no** eating, drinking or applying cosmetics in the laboratory
    - Never use your mouth to pipette
    - If you see a colleague doing something dangerous, point it out to him/her immediately and if necessary report it to your Principal Investigator. Your RPS can also be contacted if you feel that this is necessary in order to avoid dangerous practices.
    - Work must not be carried out by a person with an undressed cut or abrasion below the wrist

- Lab coats or other suitable protective clothing should be worn at all times when entering a supervised area. Disposable gloves and protective eyeglasses should be worn whenever unsealed sources are being handled or manipulated.

- Work should be carried out over trays wherever possible.

- Contamination monitoring should take place **before** starting work and **after** the work is completed. Procedures for carrying out and recording contamination monitoring are explained in section 11, appendix 1. If significant contamination is found then decontaminate following the advised procedure.

- If using isotopes other than Tritium always check your gloves, hands and laboratory coat for radioactive contamination before leaving the laboratory.

- Wash your hands using the hand wash sink before leaving the laboratory.

- All apparatus being used with radioactive materials must be labelled using “radioactive” warning tape. The tape must be removed when the apparatus has been washed and found to be clear of contamination.

- Radioactive substances must only be removed from controlled or supervised areas in closed uncontaminated containers.

- Radionuclides emitting penetrating radiations must be adequately shielded. Lead shielding must be used for gamma emitters and Perspex shielding for beta emitters.

- Containers for radioactive materials other than Carbon - 14 and tritium should not be directly held in the unprotected hand. (Note: the outside of containers of Carbon-14 and H-3 can become contaminated so it is good practice to wear gloves when handling them). Tweezers should be used for handling sealed radioactive sources.

- Contamination must be contained without delay and you must be familiar with the contingency procedures given in section 15

- Keep the time spent manipulating radioactive substances to a minimum.

- Place any waste items in the appropriate bin as described in section 14

- Keep all radioactive materials in labelled containers and stored in a designated fridge. In general, fridges that are used to store radioactive materials should not be used to store non-active items. If it is necessary to use a fridge for active and non-active items there should be clear demarcation and additional containment for the active items.

- In case of emergency remain calm and follow the contingency procedures.

7. Local arrangements and procedures

All radiochemicals must be stored in Room 3.025. See section 13 below.

8. Pregnant and breast feeding females

Any worker who becomes pregnant should inform the Radiation Protection Supervisor as soon as possible and discuss the situation. It is also the University's policy that anyone who works with any form of ionising radiation and becomes pregnant should be given the option of alternative work. This recommendation would also apply to breast feeding mothers. However if the pregnant or breast feeding female continues working, a risk assessment should be carried out to assess the hazard and additional protection measures that may be required. The RPA can advise.

9. Personal Monitoring

If you are issued with a personal dose monitor you must wear it and it is your responsibility to look after it. These badges should be worn at hip or waist level. For work with certain isotopes, additional dosimeters may have to be worn on the fingers or at neck level. If you lose your dosimeter or it is damaged (or goes through a washing machine) tell Stuart Gray (Section 1) without delay and arrangements will be made to issue a replacement. You should stop working with radioactive materials until a replacement monitor has arrived.

If you consider that your work requires a personal dose monitor, contact your relevant floor RPS. If it is agreed that you require a dosimeter, then the RPS will arrange for one to be issued to you and for collection at the required intervals.

10. Dose investigation levels

The following dose investigation levels apply.

	Effective whole body dose (mSv)	Equivalent dose to the skin (averaged over <100cm^2) (mSv)	Equivalent dose to lens of the eye. (mSv)	Equivalent dose Hands, forearms, feet and ankles (mSv)
Investigation level (over the wear period of the dosimeter[1])	0.3	7.5	2	7.5

[1]wear period will either 1 or 2 months as directed by RPA

If one of these levels is exceeded an immediate investigation should take place to establish why the level has been exceeded and any preventative actions that are required.

11. Contamination monitoring

Contamination monitoring must be carried out **before** commencing any work with unsealed radioisotopes and **after** completion of the work. Routine monitoring of the area should also take place every two weeks unless the area is not being used for radioisotope work for a period of time. Users should also monitor themselves when work is completed or during work if contamination is suspected.

See **Appendix 1** for a detailed description of contamination monitoring procedures.

12. Ordering radioactive materials

Sealed sources must not be ordered

Ordering of unsealed sources

The amount of a radioisotope that can be held in the Polwarth is limited by the Radiation Protection Service (Section 4). To place an order for an unsealed radioisotope, please contact Gary Cameron first and provide all the relevant details. Gary Cameron will then register the order on the Isoinventory system. Radiochemicals cannot be ordered without first being registered on the Isoinventory system.

Vial arrival: Parcels must be signed for by one of the stores staff. If no-one is available to sign for radioactive source parcels they are returned with the courier. Radioactive source parcels are never left outside stores. The box containing the radioactive source is opened and inspected by stores staff, wearing gloves, to ensure that the package contents are the same as on the delivery sheet and to ensure that the package is undamaged. If the package appears damaged the box is closed up without removal of the radioactive source and the end-user is notified. If the package is undamaged the box is delivered to the lab by stores staff. Parcels containing radioactive material must be signed for by a member of the lab. If no-one is available to sign for the parcel, the parcel is brought back to stores for safe-keeping until a lab member can be contacted.

Gary Cameron must be informed of the receipt of any radiochemicals in order that the Isoinventory system can be updated. In Gary Cameron's absence, the radiochemical should be stored in the fridge or freezer in room 3.025. A key is available in room 3.022 and should be signed in and out. **However, no isotope work should be carried out until the radiochemical has been logged into the Isoinventory system.**

Radioisotopes from non-commercial sources

Before any gifts of radioisotopes are accepted they must be discussed with an RPS. This is to ensure that we do not receive any radioisotopes that are not covered by our registration and also to ensure that our storage limits (Section 4) are not exceeded.

13. Storing radioactive materials

Isotopes stored in the Polwarth are kept in room 3.025. **All isotopes must be placed in 3.025 upon arrival.**

Isotopes should be removed from 3.025 to other supervised radiation areas for aliquot removal but must be returned to 3.025 immediately after the aliquot is removed.

Entry to 3.025 is made using the key kept in 3.022. All users must sign in and out using the sheet provided and make a note of the S/N of the radiochemical used.

Room 3.025 is a multi-user facility and should be kept in a clean, tidy and contamination-free state.

14. Disposing of radioactive waste

Liquid Waste

Aqueous liquid waste and biodegradable scintillation fluid should be disposed of via the designated sink in 3.022, taking care that the liquid is poured directly and carefully into the waste outlet.

- The total activity of waste discharged per month must not exceed the maximum permitted under the terms of the Authorisation Certificate for the Polwarth (section 4).
- If radioactive liquid waste also contains chemicals which are not allowed to be disposed of down drains please contact the RPA.
- Liquid waste disposals should be logged onto the Isoinventory system by Gary Cameron before disposal is made to ensure limits are not breached.
- The remaining waste should be considered as solid waste.

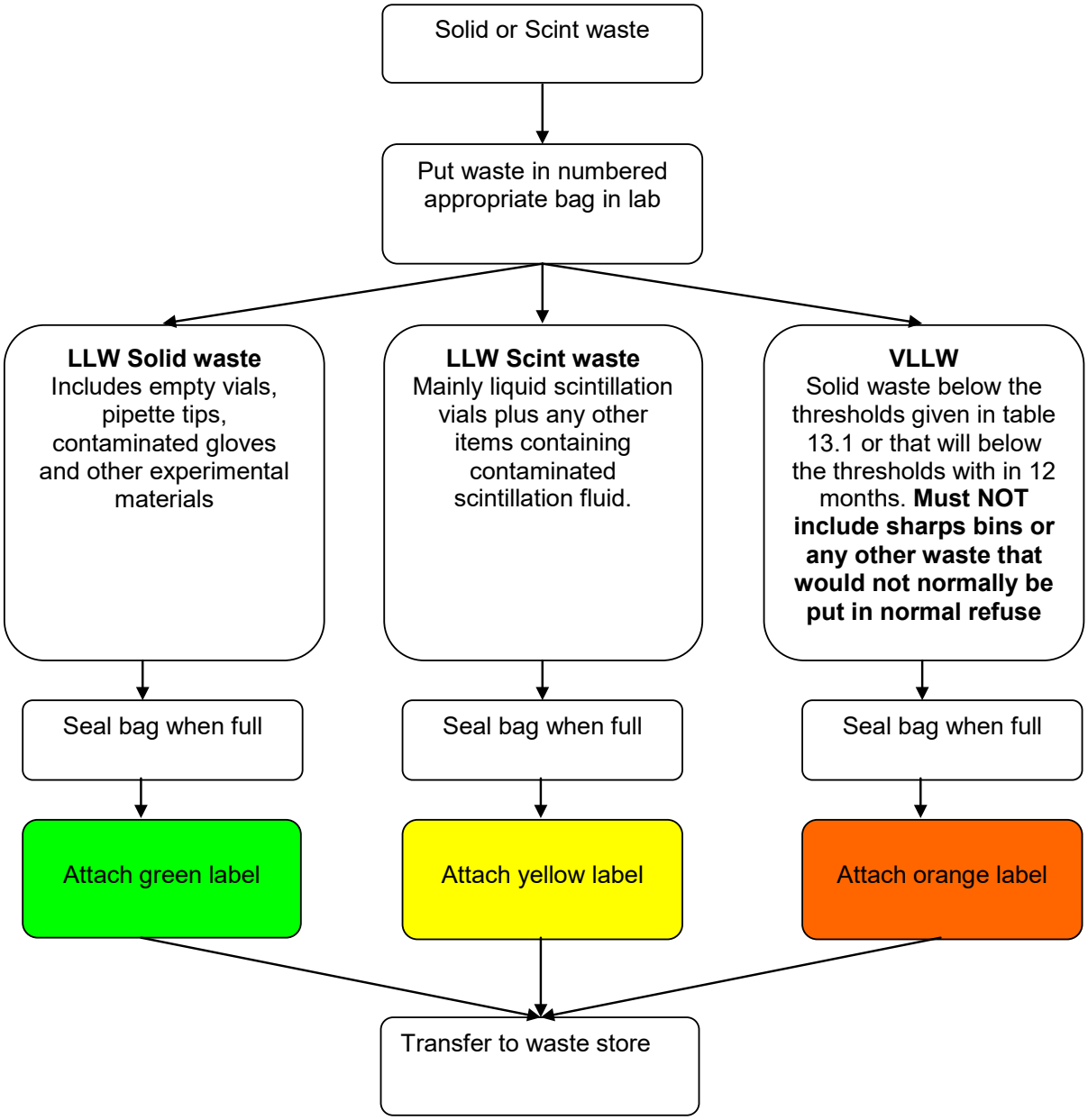
Solid waste

Solid waste should be placed in the designated bin in 3.022

In a series of experiments we have calculated that the solid waste from experiments is always less than 1% of the total radioactive waste. This was done by re-analysing scinitillation vials after disposal of radioactive scintillant, as well as rinsing out diluted stock vial at the end of the experiment and determining the radioactivity in the rinses as a percentage of the total radioactive waste. This value of 1% can be used for estimating residual activity in solid waste.

This documented method must be entered into the Assessment required to be completed within the Isoinventory software for the use of each radioisotope by each group, under the "Description of Work" section within the Assessment. Assessments will not be approved if they do not contain explicit methods for such estimations.

Gary Cameron is responsible for the monitoring, removal and disposal of the designated waste bin in 3.022.



Heavy duty plastic bags suitable for use as radioactive waste bags are available from the IMS stores. Bags should be sealed using blue zip ties, also available from the IMS stores.

Scintillation waste and vials must be disposed of using a solid plastic bin with a sealable lid to prevent leakage in the waste store.

Never dispose of non-radioactive waste with radioactive waste. If you are unsure check the waste with a suitable contamination monitor. Cans and packaging in which radioactive material has been supplied are not normally contaminated. These should be checked with a suitable monitor and, if no contamination is detected, treated as non-radioactive waste. Be sure to remove references to radioactivity; for example, the outer labels of cans should be removed or obliterated or otherwise defaced.
If solid radioactive waste also contains any bio hazardous, clinical or infectious waste please contact the RPA for advice on disposal.

Table 14.1. Thresholds for VLLW.

Radionuclides	Maximum Activity in 0.1 m^3 (kBq)	Maximum activity for a single item (kBq)
All radionuclides taken together except H-3 and C-14	400	40
H-3 and C-14 taken together	4000	400

15. Contingency arrangements

RADIATION SPILLAGE

1. Immediately alert personnel working near the area of the radiation spill and if possible alert your nearest RPS. If in doubt contact the radiation protection service for help and advice (the Radiation Protection Adviser, Stephen McCallum, can be reached on 53109). Any personnel not required to deal with the spillage should remove themselves from the area after checking themselves for contamination.

2. Put on lab coat, plastic apron, gloves and overshoes. Overshoes are important to ensure that your footwear does not become contaminated. Please ensure that there is a supply of these in your lab. In an emergency your nearest RPS will have some spares.

3. Do not allow anyone to walk through the spillage and spread the contamination. If possible isolate and cordon off the area.

4. Use a contamination monitor to locate areas of contamination on the work bench, floor and workers.

5. **If a worker has become contaminated deal with them first** (although it would be prudent to cover the spillage with absorbent material such as paper towels to prevent it from spreading.)

    - If a worker believes they are contaminated they should always attempt to locate the contaminated area and decontaminate just that area. Only if large areas of the body are contaminated should staff resort to a full body shower.

    - ***Contamination of the skin, hands, arms.*** If significant contamination is found on the hands staff should remove and discard gloves and re-monitor their bare hands. If still contaminated then the hands should be washed using a suitable detergent and then re-monitored and if necessary a soft brush should be used. Care should be taken not to break the skin. Other areas of exposed skin should be washed in a similar manner and re-monitored. The RPS should make a suitable report of any incident, including an estimation of dose, and submit to the RPA.

    - ***Contamination in the eyes.*** If a member of staff suspects that radioactivity has splashed into their eyes, they should use an eye bath. Another member of staff should then take a reading using the contamination monitor. If contamination persists then contact the RPA.

The RPS should make an appropriate report any incident, including an estimation of the dose, and submit it to the RPA.

- ***Contamination on clothing.*** If contamination is found on a lab coat or other clothing it should be removed, bagged and either disposed of or be allowed to decay.

6. Cover the spillage with absorbent material such as paper towels to prevent it from spreading.
7. Remove as much contamination as possible by absorbing the spill on paper towels. Contaminated towels should be disposed of as radioactive waste.
8. Ensure that any glass that has broken is placed in a sharps bin and labelled as radioactive.
9. Any residual contamination should be cleaned using a detergent (eg 5% Decon). When mopping up always work from the outside in.
10. Monitor the area to ensure that all the activity has been removed.
11. If the area has been cleared of radioactivity, remove the tapes and signs.
12. Remove apron, overshoes, gloves and bag, monitor and dispose as radioactive waste if necessary
13. Monitor hands, lab coat, clothes and feet to ensure that they are not radioactive.
14. If clothes or shoes become contaminated, remove them and bag them. If mildly contaminated they should be washed as normal before they are worn again.

Appendix 1

Contamination Monitoring Procedures

A1.0 Introduction

Contamination monitoring should be carried out **before** commencing any work with unsealed radioactive material and **after** completion of the work (see A1.1). In labs where isotopes with half-lives greater than 24 hours are used, a check of the area should be made every 2 weeks (see A1.2). Users should also monitor themselves when work is completed or during work if contamination is suspected. If a significant spill occurs then follow the lab contingency plans given in the local rules.

Contamination monitoring must be recorded in each lab or work area on the contamination monitoring record provided at the end of this appendix. In labs where both tritium and other radionuclides are used it may be helpful to use a separate form for tritium. Before and after work checks and area checks should be recorded on this sheet. Each column should be dated and records for that day entered in that column. If multiple experiments take place or if contamination is found then more than one column can be used for each day.

For before and after work monitoring the PI/lab supervisor should decide in consultation with the RPS the areas and equipment that should be checked and they should be entered into the first column for the record sheet under readings before experiment and readings after experiment.

For area checks the PI/lab supervisor should decide in consultation with the RPS which areas should be monitored and a plan should be drawn up on the reverse of the monitoring record sheet indicating the areas to be monitored and allocating them a number. If you require more than 5 areas add them to the first column on the monitoring sheet under weekly check.

A1.1 Contamination Monitoring Before and After Work with Radionuclides

Instructions for the monitoring of 3-H and 14-C

Contamination monitors are not sensitive enough to detect the low energy beta radiation emitted by tritium. Monitoring must therefore be done using wipe tests. It is normally assumed that 10% of any contamination will have been transferred to the wipe.

1. Take 2 steret wipes or swabs (tissue dampened with 70% ethanol) and place each straight into a separate scintillation vial with an appropriate quantity of liquid scintillant to obtain 2 background readings. The background readings should be entered on the monitoring sheet as background 1 & 2.
2. Before starting work use a steret wipe or swab to wipe an area of about 100 cm^2 for small objects or surfaces and 1000 cm^2 for larger surfaces such as benches or floors. Use a separate wipe or swab for each item listed on the monitoring sheet
3. Place the wipe in a scintillation vial with appropriate quantity of liquid scintillant.
4. Count the samples in a liquid scintillation counter. The action level is set at 2 times the average background reading.
5. If the area is contaminated, note this on the monitoring record. Wearing gloves, decontaminate any areas where the reading is more than 2 times the background. Wipe the area using a paper towel and 5% decon solution or other suitable cleaning agent. Dispose of the paper as radioactive waste. Monitor the area again and repeat

this process until the reading is below the action level and record the result on the record sheet.

6. **If you are unable to decontaminate successfully, contact your RPS for advice and ensure no further work is carried out in the area until the issue has been resolved, make a note of this action on the monitoring record**
7. Take further wipe tests after completing the work, including the work surface, floor area, disposal sink and any other item noted on the monitoring sheet.
8. If necessary, decontaminate the area as described in 5.0 and record actions on monitoring sheet.
9. **If the decontamination was unsuccessful then contact your RPS for advice and ensure no further work is carried out in the area until the issue has been resolved.**

A1.2 Area checks

In addition to the monitoring described above, in labs where long lived radioisotopes are used, checks of a larger area should be undertaken every 2 weeks or after every experiment if work is infrequent. This is to ensure that there is no build-up of radioactivity over time. Checks should extend into 'clean' areas and include 2 or 3 random areas of the lab to confirm that there is no contamination outside the normal work areas such as door handles, telephones and fridges. Monitoring should be carried out as shown below:

Radioisotope	Routine monitoring method
Tritium (H-3)	Wipe tests, liquid scintillation counter
Carbon-14	
Phosphorus-32	
Phosphorus-33	
Sulphur-35	
Iodine-125	Wipe tests with gamma counter if available, or scintillation detector
Iron-59	

A plan of the lab should be drawn on the back of the monitoring record sheet with the areas that are monitored marked on it see A1.0. An entry should be made on the record sheet every time monitoring is carried out, whether contamination is found or not. If a lab is not used for a period of time, there is no need to carry out routine contamination checks, but this should be indicated on the record sheet.

Contamination Monitoring

Lab/Lab area				Radionuclides				
Monitoring method								
Enter counts recorded in each column. Where counts are over 2 times above background average please decontaminate, recount and enter new count in next column.								
Name								
Signature								
Initials								
Date								
Readings before experiment								
Background 1								
Background 2								
Work area								
pipettes								
container								
Other equip -specify								
Other equip -specify								
Floor in front of exp								
Contaminated (Y/N)								
Readings after experiment								
Background 1								
Background 2								
Work area								
pipettes								
container								
Other equip -specify								
Other equip -specify								
Floor in front of exp								
Contaminated (Y/N)								
Twice weekly lab check or with every experiment if experiments are less frequent								
Background 1								
Background 2								
Area 1 on lab plan								
Area 2 on lab plan								
Area 3 on lab plan								
Area 4 on lab plan								
Area 5 on lab plan								
Contaminated (Y/N)								

Radionuclides used:_____ Date:_____

Plan of lab showing areas to monitored for radiation contamination once every 2 weeks:

Appendix 2

Minimum isotope usage - Example 1

γ-[^{33}P]-ATP	end-labelling of DNA oligonucleotides, protein phosphorylation
α-[^{32}P]-UTP	labelling of RNA transcripts
^{35}S-methionine	labelling of proteins

γ-[^{33}P]-ATP (>2500 Ci/ mmol)
End-labelling of DNA oligonucleotides for protein-DNA studies.

DNA oligonucleotide:	10 pmoles/ labelling reaction
γ-[^{33}P]-ATP (>2500 Ci/ mmol):	10 μCi/ labelling reaction
Labelling efficiency	25%

After labelling, equal amounts of oligonucleotides are annealed to give 01.-0.2 pmoles of double-stranded DNA. 20, 000 cpm of labelled dsDNA is then used per reaction, with up to 10 reactions per experiment (~ 0.18 μCi/ ~ 0.037 MBq)

In vitro kinase assay: post-translational modification of proteins.

Protein substrate:	2-4 μg/ reaction
γ-[^{33}P]-ATP (>2500 Ci/ mmol):	10 μCi/ reaction
Labelling efficiency	2-4 %

Labelled proteins are analysed by SDS-PAGE and detected 24-36 hours by autoradiography.

α-[^{32}P]-UTP (~3000Ci/ mmol)
Labelling of RNA: measuring transcription initiation and elongation.

DNA template:	100 ng / reaction
α-[^{32}P]-UTP (~3000Ci/ mmol)	0.5 μl (5 μCi)/ reaction

Typical experiment involves up to 10 reactions (50 μCi/ 1.85 MBq). RNA transcripts are resolved by denaturing polyacrylamide gel electrophoresis and visualised overnight by autoradiography.

^{35}S-methionine (>1000Ci/mmol)
Labelling of recombinant proteins for protein-protein interaction studies.
According to manufacturer (Promega) in a 50 μl standard *in vitro* translation reaction 2 μl of ^{32}S-methionine (>1000Ci/mmol) are included. This allows for visualisation of proteins (2-5 μl/ lane) by SDS PAGE.

Minimum isotope usage - Example 2

Principal isotopes

$^{3}H_2O$ - measurement of cell volume for cytoplasmic pH determination
^{14}C benzoic acid - measurement of cytoplasmic pH
^{14}C acetate - measurement of cytoplasmic pH
^{14}C-labelled amino acids - assay of transport activity

$^{3}H_2O$/^{14}C benzoic acid and ^{14}C acetate

Measurement of cytoplasmic pH - uses double labelling ^{3}H/^{14}C mixtures. The amount of radioactivity used per experiment has to take into account the magnitude of the difference

between the external pH and the cytoplasmic pH as the method used measures the difference across the membrane and the ratio of incubation volume to cytoplasmic volume (approx 1000:1). Knowledge of the external pH and the measured pH gradient gives the value for the cytoplasmic pH. The method is applied to very low volumes of cells and must use relatively large amounts of radioactivity per experiment to reduce the error in the measurement.

We use $^{3}H_2O$ to measure the trapped water in cell pellets when we use centrifugation-based assays for determination of cytoplasmic pH. The organic acids are used to measure the transmembrane pH gradient in bacterial cells.

Approx 0.5 mg/ml cells are used in the assay and triplicate 1 ml samples are centrifuged to measure the cytoplasmic pH. The total incubation is usually 5 ml if a single time point is to be measured and the volume of cell suspension to be used is increased *pro rata* if a longer series of time points is required from the same cell suspension. We use approx. 1 μCi/ml $^{3}H_2O$ and 0.2 μCi/ml ^{14}C benzoate or acetate. After the brief incubation the cells are separated from the supernatant be centrifugation in an Eppendorf centrifuge and 100 μl samples of supernatant are counted to give a measure of the radioactivity/μl of supernatant for both ^{3}H and ^{14}C. The pellet of cells is suspended in 100 μl buffer and the counts determined. Typical raw data would be:

^{3}H in supernatant	^{14}C in supernatant	^{3}H in pellet	^{14}C in pellet
116866	16520	7091	1597

The critical data are the pellet values, which actually arise from the radioactivity inside the cells and radioactivity trapped between the cells. A correction is applied based on the ^{3}H counts. Using this information we can calculate the cytoplasmic pH in the cells. The level of radioactivity in the experiment is set by the need for accuracy when counting the small number of counts insider the cells against a background of trapped radioactivity.

^{14}C amino acids
We use these to analyse the rate of transport of the amino acids across the membrane.

Cell density = 0.25 mg cells/ml = ~ 0.4 μl cytoplasm/ml cell suspension.
Intracellular concentration in the range 20-200 mM = 20-200 nmol/μl cytoplasm.

Assume 20 nmol accumulated:
Filter 0.5 ml cells at each time point = 0.2 x 20 nmol per filter = 4 nmol;
Need approx. 4000 cpm/filter for accuracy - therefore, maximum specific activity usually around 2 nmol/nC;
Radioactivity accumulated by cells must be less than 5% of the total present to prevent change in external concentration 4000 cpm/filter = 8000 cpm/ml cells = 8000 x 20 = 160000 cpm/ml experiment. Assay volume usually 3 ml, therefore the maximum radioactivity per assay is 480000 cpm ~ **200 nC/assay.**

When the anticipated pool of solute is higher than 20 mM the amount of radioactivity is reduced *pro rata*, so that one still uses approx 200 nC/assay.