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BioAMS Training

Accelerator Mass Spectrometry is an analytical technique for the quantification of rare isotopes in an elemental sample. In the biosciences the uniqueness of this technique allows for tracer studies to be performed at a sensitivity of parts per quadrillion ($1:10^{15}$.) AMS directly counts the number of rare isotope nuclei in the sample and expresses this as a ratio of rare to more abundant isotopic species. This direct quantification obviates the need for the long counting periods found with decay-based techniques. For example, the amount of ^{14}C in a milligram sample of carbon can be measured in 30 seconds as opposed to the week it would take to measure a similar sample at the same activity level using liquid scintillation counting methods.

I. Introduction to Radioactivity in the Accelerator Mass Spectrometry (AMS) lab

A. Radioactivity

1. The level of radioactivity that is worked with in AMS labs is extremely low, to the point of being undetectable by standard detection methods, e.g. LSC.

2. Radioisotopes used:

a. ^{14}C, 5730 year half-life - most commonly used in biological studies utilizing AMS measurement (BioAMS)

b. ^{3}H (Tritium), 12.3 year half-life

c. Other radioisotopes may be used in biological experiments for measurement by AMS (i.e.-^{41}Ca, ^{36}Cl).

3. These low levels are generally not a health or major safety concern, however, one should be aware of the nature of what is being handled and follow good lab practices. These practices include wearing lab coats, safety glasses, and gloves. This should always be a priority because in BioAMS laboratories the concern is not what the sample may do to us, but what we may do to the sample.

B. Contamination

1. Radioactivity is easily spread, often unknowingly. Workers can carry previously undetectable contamination between laboratories on their gloves or hands [or shoes]. Work bench areas receive such human

carryover but are also recipients from contaminated equipment, trays, jars, etc. that are placed on them. Spills, however small and however meticulously cleaned, can leave picocuries of ^{14}C on a bench.

2. Contamination can arise from three primary sources: the laboratory and its furnishings, the air, and the tools or vessels used in experiments.

3. Contamination is often found on routinely handled surfaces: door knobs, light switches, drawer handles, telephone receivers, water faucets, instrument lids, etc.

C. Contamination Control

1. No container, surface or tool is trusted unless the user personally knows the complete history of the surface that touches the sample.

2. Use of shoe covers is strongly recommended.

3. Gloves should be changed frequently, at least once per hour or whenever going between work with materials that are higher in activity to work with lower activity materials.

4. Fresh bench paper should be used under all work areas.

5. Touch pad control panels (i.e. keyboards) are operated through transparent plastic wrap that is changed frequently.

6. No level of paranoia is too great!!

II. Lab Policies and Procedures

A. Establish a "Hot Lab" separate from the low level sample preparation laboratory

1.The "Hot Lab" is an isolated lab, separate from the laboratory used for sample preparation and is used for storing and reducing stocks of radiological material to lower levels creating working stocks for in vivo or in vitro experiments.

2. Have a LSC in the "Hot Lab" so radiological material from outside sources can have radioactivity levels verified or to establish baseline information on the material.

3. Products generated in the hot lab for use somewhere else (i.e.-dosing solutions, samples) can spread contamination. Before removing items from the hot lab, do everything possible to ensure the container is free

from contamination. Secondly, have a secondary container (could be a bag) outside of the lab (which has not entered the lab) in which the item can be placed for transfer.

4. In order to minimize the potential of spreading contamination, after entering the hot lab, you should not, in the same day, enter the low rad level sample preparation labs.

5. Should a product generated in the hot lab need to enter the low level sample preparation laboratory on the same day, follow the above "bagging" out procedures and arrange for someone else who has not been in the hot lab to place them in the AMS sample preparation laboratory. Once there, the item should be "bagged" a third time and identified as having come from the "hot lab". Do not place the item in any refrigerator, freezer or area of the lab where finished or in-process AMS samples are stored or prepared.

6. It is critical that product generated in the hot lab be appropriately labeled: name of item, volume, amount of radioactivity (DPM's, specific activity, Modern), etc.

7. Before leaving the lab, you should survey yourself and your work area with the Geiger counter. Should you find your area contaminated, do your best to clean it and survey again. Should the problem persist, seek guidance on further decontamination procedures

8. Do not leave a contaminated area unattended. Should you need to leave the area, identify the area clearly so that others entering the lab may know to avoid the area.

B. Policies in the Low Level Sample Preparation Laboratory

1. This lab should be operated as if it were a clean lab facility.

2. Shoe covers should be donned before entering the lab and removed upon exiting. Exercise care when walking as shoe covers may cause floor to be slippery.

3. Absorbent bench paper should be used under all work surfaces. Bench Paper should cover all counter spaces and should be changed on a regular basis or when soiled. A SECOND LAYER of bench paper should be placed on the counter for a workspace surface PRIOR to initiating any work or setting anything down. It is important to use pieces that are large enough to adequately accommodate your workspace needs. Change this workspace paper daily or when working with a different sample set.

4. Items originating from other labs (i.e.- equipment, chemicals, etc.) should not enter and CANNOT be used in the low level labs until they have been determined to be free from contamination. This is done by taking swipes (see Section III part D) of the surfaces. If possible, clean the item with soap and water.

5. Do not share equipment, chemicals, or other such items with lab users that are not conversant with AMS laboratory practices. They might inadvertently contaminate them. Chemicals should be purchased by the user and be identified as "for AMS use only".

6. Access control to this lab should be maintained

C. Personal Protective Equipment (PPE): Disposable lab coats should be used when working with radiological materials. The disposable coats may be reused if not soiled/contaminated. If in doubt, throw it away. Washable lab coats can be used for other non-radiological work being performed. Gloves shall be used at all times while performing tasks.

D. Designate areas of all labs "for AMS work only" and do not perform other types of work in these areas or use them for general storage. Delineate these areas with tape or other identifying marking materials.

III. Conducting Experiments

A. Experimental Design

1. It is important to discuss your AMS experiment with a qualified (experienced in AMS) individual well in advance of beginning your work. The individual will want to verify the project is suitable for AMS measurements and ensure that the dosing regimen will provide sample that are neither too "Hot" nor too "Cold" for AMS measurement. Ideally, AMS samples should result in values of 0.1-100 Modern.

2. All AMS experiments should include blank experimental controls. "AMS controls reflect the isotope concentration of undosed or predosed tissues, cells, etc." (*B.A. Buchholz et al. Nucl. Instr. And Meth. In Phys. Res. B172(200) 404-408)*

B. Equipment

1. When conducting AMS experiments, use brand new plastic or disposable items. This practice is key to preventing contamination of current and future work.

2. It is critical that there be separate set of pipettes used for dosing and conducting experiments from those used to submit AMS samples. Pipettes marked “AMS Only” should be used only for the preparation of AMS samples for submission and not for general lab work.

3. When conducting experiments as well as submitting samples for AMS, aerosol resistant pipetter tips are required.

C. Sample Storage

1. AMS Sample storage areas should be segregated from other storage areas

2. Higher level radioactive samples or solutions should also be segregated from low level samples that are to be measured by AMS.

D. Swipes - It is good lab practice to conduct swipe surveys in areas you have been using in order to ensure that you have left the area free of contamination.

1. To conduct a swipe survey:

a. Clean area as if it were going to be used.

b. Using gloved hands, wet a single 6cm Whatman GF/C glass fiber filter with alcohol (methyl, ethyl, or isopropyl).

c. Place this swipe in a scintillation vial, seal, and label. This will be your blank control.

d. Remove your gloves and don a new pair and avoid touching anything prior to handling your next swipe.

e. Again wet the filter and swipe or lightly rub the filter over a few cm^2 of the surface area or object to be swiped.

f. Place swipe in a scintillation vial, cover, and label –only one swipe per vial.

g. Repeat with all areas to be surveyed, removing and re-donning gloves between each swipe.

h. Take enough swipes to cover the area and items with which you worked.

i. Submit swipe samples for AMS analysis.

2. If you have any questions please contact the Research Resource staff.

IV. AMS Sample Submissions

A. Sample Preparations

1. Samples labeled with ^{14}C are measured in either gaseous or solid forms and sample material must be converted to CO_2 for gaseous sample analysis or graphite for solid sample analysis. This conversion is performed by either the liquid sample interface or the utilization of a complex graphitization technique. All material submitted must be amenable to conversion to either of these two forms. Submitted material must also have an expected activity level commensurate with the extreme sensitivity of AMS detection, which is between 0.1 and 100Modern for ^{14}C.

2. Samples labeled with ^{3}H must have the material converted to TiH_2 for solid sample analysis since there is no gaseous capability for this type of analysis at present. Samples labeled with other isotopes are also converted to solid forms for AMS measurement. The expected activity levels present in these samples should be established in consultation with Research Resource staff.

3. Generally all conversions to solid or gaseous forms are performed in our laboratories. However, personnel are encouraged to visit the resource and be trained in these procedures. After this training, the resource is also ready to assist with the establishment of satellite graphitization or tritiated sample preparation laboratories

4. Since AMS measures an isotopic ratio, a measurement can be made from a very small amount of analyte. The amount of material to submit for AMS measurement should be determined in consultation with Research Resource staff.

5. Before submitting samples for AMS analysis, a portion of samples with the highest expected radiation levels should be counted by LSC. The purpose of the LSC in this case is to give a “rough estimate” of how hot your samples are. If you are striving for precise LSC results and your samples are readily detectable by LSC, you should not be submitting these samples for AMS.

6. Things to note:

a. The utmost care should be taken when preparing samples for AMS. Be aware of every aspect of your work (i.e.-chemicals, lab ware, etc.) and how they are interacting with your samples.

1.Plastics have been known to leach, affecting the AMS results.

2.Chemicals have been found to be contaminated with 14C direct from the manufacturer.

3.Some chemicals may remain in your final sample, unknowingly adding excess carbon and altering the final AMS result.

b. Some buffer systems may not be usable when trying to make AMS measurements. Consult the Research Resource staff on compatible buffer systems.

c. The suggested sample size for measurement by the graphitization process is 0.5-2mg carbon. When this sample size cannot be achieved the carbon mass is amplified by adding Tributyrin to the sample volume.

B. Submission Form

1. All samples submitted for AMS analysis must be accompanied by a submission form. We have a standardized form, which is available from the Research Resource staff. It is important to fill it out completely and correctly.

2. The Research Resource staff reserves the right to prevent any samples from being analyzed until a properly completed form is received. Also, should any information on the sheet be of concern to them, they may hold samples until clarification or resolution has been made.

3. A paper copy of completed forms should be enclosed in the sample package and an electronic copy sent to the Research Resource staff at the time of submission.

C. Pre-submission calculations

1. It is critical that calculations should be done to establish the Fraction Modern of AMS samples prior to beginning an experiment as well as prior to submitting samples. Just knowing the DPM value of a sample is not enough information. For tables of important numbers, please see appendix.

2. Please talk with RR personnel if you have any questions regarding calculations.

3. The following examples demonstrate calculations for a sample labeled with ^{14}C.

Example 1: DPM to Fraction Modern

Step 1. Subtract out control

DPM test sample –DPM control sample = DPM/vol counted

Step 2. Determine DPM for AMS submission

(DPM/vol counted)(vol for AMS submission) = DPM/AMS submission vol

Step 3. Determine DPMs per g Carbon

(DPM/AMS submission vol) / (mg of sample) (1000)= DPM of sample/g Carbon

Step 4. Determine Fraction Modern

1 Modern = 13.56 DPM/g Carbon

(DPM of sample/g Carbon)/13.56=Modern value of AMS sample

Anything over 100 Modern should not be submitted for AMS

Example 2: Percent Labeling

1 atom of ^{14}C per molecule = 62.4 mCi/mmol

Specific Activity of compound (mCi/mmol) / 62.4 = % Labeled

Example 3: Percent Carbon of a Compound

a. Example Calculation: Peptide, mass of 957g/mol

Step 1. Count the number of carbon atoms

CH-C-L-A-E-Y-H-A-K-C-CH_3
(1 1 6 3 5 9 6 3 6 1 1) # of carbons

Total: 43 ^{12}C + 1 ^{14}C atoms

Step 2. Determine Mass of Carbon in the peptide:

12g/mol ^{12}C atom x 43 atoms = 516 g/mol
14g/mol ^{14}C atom x 1 atom = 14 g/mol

Total: 530 g/mol Carbon

Step 3. Calculate Percent Carbon

530g/mol Carbon / 957g/mol Peptide x 100 =

55.4% Carbon

Appendix:

Useful Radiocarbon Information

	Years	Days	Hours	Minutes	Seconds
Half life	5730	2.09E+07	5.02E+07	3.01E+09	1.81E+11

	DPM	DPS
1 Curie =	2.22E+12	3.70E+10
1 µCi =	2.22E+06	37000

	atoms	moles
1Curie of ^{14}C =	9.65E+21	0.016
1 µCi of ^{14}C =	9.65E+15	1.60E-08

Modern =	^{14}C in 1950 AD air	13.56 DPM/g carbon
		5.89E+10 ^{14}C atoms/g carbon
		97.9 fmol ^{14}C/g carbon
		97.9 amol/mg carbon
		6.11 pCi/g carbon
		6.11 fCi/mg carbon
		73.32 fCi/mmol carbon

Contemporary =	1.045 Modern as of 2013

AMS Range =	0.1 Modern - 100 Modern
	611 aCi/mg carbon - 611 fCi/mg carbon
	9.8 - 9800 amol^{14}C/mg carbon

Useful Tissue Information

% Carbon, Human

Organ/tissue	Mass (g)	Carbon mass (g)	% Carbon
total body	70000	16000	22.9
total soft tissue	60000	14000	23.3
Adipose tissue	15000	9600	64
Subcutaneous (hypodermis)	7500	4800	64
other separable	5000	3200	64
Interstitial	1000	640	64
Adrenals (2)	14	4	28.6
Aorta	100	15	15
Blood (arterial + venous)	430	43	10
Blood (whole = 5200 ml x 1.06g/ml	5500	540	9.8
Plasma (3000 ml x 1.03 g/ml)	3100	130	4.2
Erythrocytes (2200 x 1.09 g/ml)	2400	410	17.1
Blood vessels (dissectable)	200		
Connective tissue	3400	650	19.1
Separable connective tissue	1600	320	20
Central Nervous system (+spinal cord)	1430	180	12.6
Brain	1400	170	21.1
Cerebrum	1200	160	13.3
Cerebellum	140	19	13.6
Brain stem	30	4	13.3
Contents (cerebrospinal fluid)	120	0.02	0
Eye lenses (2)	0.4	0.08	20
Gall bladder	10		
GI tract	1200	140	11.7
Esophagus	40		
Stomach	150	18	12
Intestine	1000	94	9.4
small intestine	640	74	11.6
Duodenum	60	6.9	11.5
Jejunum	280	32	11.4
Ileum	300	35	11.7
Large intestine	370	43	11.6
Upper large intestine	210	24	11.4
Ascending colon and cecum	90	11	12.2
Transverse colon	120	14	11.7
Lower large intestine	160	19	11.9
Descending colon	90	11	12.2
Sigmoid colon	50	5.8	11.6
Rectum	20	2.3	11.5
Hair	20	9.8	49
Heart	330	54	16.4
Kidneys (2)	310	40	12.9
Larynx	28		
Liver	1800	260	14.4
Lung	1000	100	10
Parenchyma + capillary blood & bronchus	570	58	10.2
Lymph nodes (dissectible)	250		
Muscle (skeletal)	28000	3000	10.7
Pancreas	100	13	13
Pituitary	0.6		
Prostate	16	1.4	8.8
Sleleton	10000	2500	25
Bone	5000	740	14.8
Cortical	4000	550	13.8
Trabecular	1000	130	13
Red marrow	1500	620	41.3
Yellow marrow	1500	950	63.3
Cartilage	1100	110	10
Periarticular tissue (skeletal)	900	82	9.1
Skin	2600	590	22.7
Epidermis	100		
Dermis	2500		
Spleen	180	20	11.1
Teeth (32)	46	4.3	9.3
Enamel	35	0.06	0.2
Dentin	10	0.83	8.3
Testes (2)	35	3.1	8.9
Thymus	20		
Thyroid	20	2.1	10.5
Tongue	70	17	24.3
Trachea	10		
Urinary bladder	45		
Urine	102	0.34	0.3

% Carbon, Rat

Organ/tissue	% carbon
Albumin	48.4
Blood	8.4
Blood plasma	3.4
Bone Marrow	30
Brain	14.9
Breast	10
DNA	29
Fat	11
Feces	24
Heart	24.9
Hemoglobin	50.4
Kidney	23.3
Liver	29.7
Lung	14.8
Protein (liver)	31.4
Red blood cells	21
Skin/fur	29.4
Spleen	22.8
Thymus	21.3

Tributyrin (TB)
1 µl 100% TB = 0.615 mg Carbon

Human tissue information obtained from: Standard Man, International Commission on Radiological Protection (ICRP) 23, Report of the Task Group on Reference Man Chapter 2. Gross and Elemental Content of Reference Man pp. 273-334. Published for ICRP by Pergamon (1975) Elsevier Science Inc. Tarrytown, NY.
Other information obtained from internal LLNL measurements.