RDM Division of Cardiovascular Medicine
University of Oxford

Radiation Risk Assessment

		DATE:	21st Aug 2017
DEPARTMENT: Cardiovascular Medicine Wellcome Trust Centre for Human Genetics **LOCATION OF WORK:** Rooms 10/088 and 10/109 (Lab 3)		**PERSONS INVOLVED:** See attached training record	**OTHERS AT RISK:** None

DESCRIPTION OF PROCEDURE:

Arginine-citrulline radiochemical HPLC for measurement of NOS activity.

SUBSTANCES USED	QUANTITIES USED	FREQUENCY OF USE	HAZARDS IDENTIFIED	EXPOSURE ROUTE	DOSE PER PROCEDURE (µSv)
L-[U-^{14}C] Arginine	Stock pot is 1.85mBq maximum. 3.7-18.5 kBq (0.1-0.5 µCi) per rxn (average 50 rxns each time - 925 kBq or 25 µCi)	Approximately twice per month	Low energy β-emitters (^{14}C 157 keV, ^{3}H 19keV). Skin dose rate from 1MBq point source at 30cm is 0 mSv/h. Bremsstrahlung radiation may be significant. [Delacroix et al. *Radiat. Prot. Dosim.* **98**, 2002]	Skin (mostly fingers)	*Whole body:* Effectively zero
Citrulline L-[Ureido-^{14}C]	3.7-18.5 kBq or 0.1-0.5 µCi	Rarely (calibration)			
D-[6-^{3}H]Glucosamine hydrochloride	7.4 kBq or 0.2 µCi				*Extremities:* Effectively zero
L-[U-^{14}C]Ornithine hydrochloride	3.7-18.5 kBq or 0.1-0.5 µCi				

COULD A LESS HAZARDOUS SUBSTANCE (OR FORM OF THE SUBSTANCE) BE USED INSTEAD? No

COULD A LOWER ACTIVITY BE USED? No

JUSTIFY QUANTITY OF MATERIAL IN USE: Quantities used are already very low and cannot be further reduced due to detection limits of HPLC radiometric detector.

WHAT MEASURES HAVE YOU TAKEN TO CONTROL RISK?

ENGINEERING CONTROLS:

Work carried out in designated areas on radioactivity spill trays.
Reduce dosage by control of distance, time and shielding.
Liquid waste goes directly to drains, reducing risks associated with handling, spillage and contamination (personal or lab).

PPE:

Lab coat, nitrile gloves and safety eyewear

PROCEDURAL & MANAGEMENT CONTROLS:

Follow the As Low As Reasonably Practicable (ALARP) rule
Adhere to local rules, EPR2010 and IRR99
Monitor work area before, during and after use. Wipe tests taken on HPLC equipment and floor close to waste pot after each set of experiments.
Lone working prohibited without prior arrangement with RPS and SRPS.
Work only in designated Supervised areas
Radioactivity stock pot stored in locked refrigerator in Room 10/088
Adhere to limits of designated sink and bins
Adhere to storage time limits for bins [12 months for both 'solids' green/yellow band bin & 'organic liquids' orange bin]

CHECKS ON CONTROL MEASURES:

Regular monitoring (including wipe tests by users and RPSs) and supervision
Checks on documentation – control of Monthly returns

RADIATION MONITOR: EP15	**TRAINING REQUIREMENTS:** URPO lecture "Working with unsealed radioactive sources" and in-house training.
IS DOSIMETRY REQUIRED? No	
EMERGENCY PROCEDURES: Refer to Contingency Plans in Local Rules & University Policy Statement S8/05: Appendix 16. **DECONTAMINATION PROCEDURES:** Decontamination where necessary with Count-Off and/or Decon90 decontaminants as recommended. Dispose of contaminated materials in designated bins and sinks. For any incident beyond minor contamination of radioactive workspaces alert RPS (and SRPS/URPO if appropriate).	**WASTE DISPOSAL:** See attached waste stream flowchart. Solid 50% to green-yellow band bin Aqueous 2.5% to designated drain* Scintillant 47.5% to designated drain* *Liquid waste disposed of to drains totals 50%

Are overall risk control measures adequate?	Yes	
NAME OF RADIATION PROTECTION SUPERVISOR:	Dr James Brown	**SIGNATURE**: 

Date of routine review	**DATE:**	/ / 2018	/ / 2019	/ / 2020	/ / 2021	/ / 2022
	BY:					

RDM Division of Cardiovascular Medicine
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Radiation Risk Assessment Training Record

Title: Arginine-citrulline radiochemical HPLC - measurement of NOS activity

The undersigned have read the above radiation risk assessment and understand the safety arrangements required and their own obligations in ensuring compliance.

DATE	NAME & SIGNATURE	TRAINED BY (NAME & SIGNATURE)

Carbon - 14

$^{14}C_6$

Half life: 5730 years
Specific activity: 1.65E+11 Bq.g^{-1}

Risk group: 4
Risk colour: Green

Main emissions (keV)	Gamma or X		Beta (Emax)		Electrons		Alpha	
	E	%	E	%	E	%	E	%
E1			157	100				
E2								
E3								
% omitted			0					

Exemption levels	
Quantity (Bq)	1E+07
Concentration (Bq.g^{-1})	1E+04

Transport (TBq)	
IAEA ST1 A_1 value	4E+1
IAEA ST1 A_2 value	3E+0

EXTERNAL EXPOSURE (mSv.h^{-1}) for an activity of 1 MBq or 1 MBq.m^{-2} (as appropriate)

Point source (30 cm)

Betas, electrons (skin dose): 0.00E+0

Gammas, X rays (deep tissue dose): 0.00E+0

Infinite plane source

Betas, electrons (skin)

10 cm	0.0E+00
1 m	0.0E+00

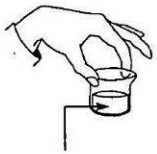
Photons (skin)

10 cm	0.0E+00
1 m	0.0E+00

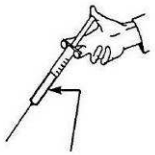
Photons (deep dose)

10 cm	0.0E+00
1 m	0.0E+00

10 ml glass vial: Brem. Rad.



Contact with 50 ml glass beaker: Brem. Rad.



Contact with 5 ml plastic syringe: Brem. Rad.

The values above do not include Bremsstrahlung radiation. Brem. Rad. indicates that it may be significant.

CONTAMINATION

Contamination skin dose (mSv.h^{-1})	
Uniform deposit (1kBq.cm^{-2})	3.24E-1
0.05 ml droplet (1 kBq)	2.70E-3

Detection: Recommended probes*	
Alpha	
Beta	+ +
Gamma	
X rays	

Derived limits (Bq.cm^{-2})	
Removable contamination	4E+2
Fixed contamination	4E+4

* If no probes are indicated the recommended technique is to use a wipe test in association with a probe or liquid scintillation technique

SHIELDING (mm)

Betas and electrons (Total absorption)	
Glass	0.2
Plastic	0.3

Gamma and X rays (half and tenth value thickness)

	½	1/10
Lead	-	-
Steel	-	-

INTERNAL EXPOSURE FOR WORKERS

COMMITTED EFFECTIVE DOSE PER UNIT INTAKE (Sv.Bq^{-1})

Ingestion	f_1	
Labelled organic compounds	1.000	5.8E-10

Inhalation (for soluble and reactive gases and vapours)	
Vapour	5.8E-10
Dioxide	6.2E-12
Monoxide	8.0E-13

Highest dose organ Whole body **20 mSv ALI$_{ingestion}$** 3.4E+07 (Bq) **20 mSv ALI$_{inhalation}$** 3.4E+07 (Bq)

MAXIMUM RECOMMENDED ACTIVITIES IN LOW LEVEL OR INTERMEDIATE LEVEL LABORATORIES (Bq)

PHYSICOCHEMICAL STATE	*Subject to external exposure requirements which may be more restrictive*					
	Volatility factor (k)	Supervised area		Controlled area		
		Bench	Fume hood	Bench	Fume hood	Glove box
All compounds except below	0.01	1E+07	1E+08	3E+07	3E+08	5E+09
Oxide & anhydride	1	Forbidden	1E+06	Forbidden	3E+06	3E+08

Tritium

$^{3}H_1$

Half life: 12.3 years
Specific activity: 3.59E+14 Bq.g^{-1}

Risk group: 5
Risk colour: Blue

Main emissions (keV)	Gamma or X		Beta (Emax)		Electrons		Alpha	
	E	%	E	%	E	%	E	%
E1			19	100				
E2								
E3								
% omitted			0					

Exemption levels	
Quantity (Bq)	1E+09
Concentration (Bq.g^{-1})	1E+06

Transport (TBq)	
IAEA ST1 A_1 value	4E+1
IAEA ST1 A_2 value	4E+1

EXTERNAL EXPOSURE (mSv.h^{-1}) for an activity of 1 MBq or 1 MBq.m^{-2} (as appropriate)

Point source (30 cm)

Betas, electrons (skin dose): 0.00E+0

Gammas, X rays (deep tissue dose): 0.00E+0

Infinite plane source

Betas, electrons (skin)

10 cm	0.0E+00
1 m	0.0E+00

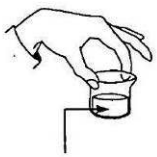
Photons (skin)

10 cm	0.0E+00
1 m	0.0E+00

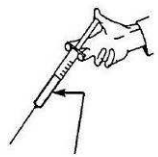
Photons (deep dose)

10 cm	0.0E+00
1 m	0.0E+00

10 ml glass vial: Brem. Rad.



Contact with 50 ml glass beaker: Brem. Rad.

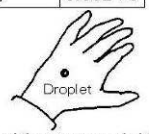


Contact with 5 ml plastic syringe: Brem. Rad.

The values above do not include Bremsstrahlung radiation. Brem. Rad. indicates that it may be significant.

CONTAMINATION

Contamination skin dose (mSv.h^{-1})	
Uniform deposit (1kBq.cm^{-2})	0.00E+0
0.05 ml droplet (1 kBq)	0.00E+0



Detection: Recommended probes*	
Alpha	
Beta	
Gamma	
X rays	

Derived limits (Bq.cm^{-2})	
Removable contamination	2E+4
Fixed contamination	2E+6

* If no probes are indicated the recommended technique is to use a wipe test in association with a probe or liquid scintillation technique

SHIELDING (mm)

Betas and electrons (Total absorption)	
Glass	<0.1
Plastic	<0.1

Gamma and X rays (half and tenth value thickness)

	½	1/10
Lead	-	-
Steel	-	-

INTERNAL EXPOSURE FOR WORKERS

COMMITTED EFFECTIVE DOSE PER UNIT INTAKE (Sv.Bq^{-1})

Ingestion	f_1	
Tritiated water	1.000	1.8E-11
Organically bound tritium	1.000	4.2E-11

Inhalation (for soluble and reactive gases and vapours)	
Elemental hydrogen	1.8E-15
Tritiated water	1.8E-11
Organically bound tritium	4.1E-11

Highest dose organ Whole body **20 mSv ALI$_{ingestion}$** 4.8E+08 (Bq) **20 mSv ALI$_{inhalation}$** 4.9E+08 (Bq)
Gaseous form 1.1 E+13 (Bq)

MAXIMUM RECOMMENDED ACTIVITIES IN LOW LEVEL OR INTERMEDIATE LEVEL LABORATORIES (Bq)

PHYSICOCHEMICAL STATE	*Subject to external exposure requirements which may be more restrictive*					
	Volatility factor (k)	Supervised area		Controlled area		
		Bench	Fume hood	Bench	Fume hood	Glove box
Tritiated water	0.01	3E+06	3E+07	1E+07	1E+08	1E+10
Elemental hydrogen	1E+00	Forbidden	2E+08	Forbidden	5E+08	5E+10

Arg-Cit radiochemical HPLC for measurement of NOS activity – ^{14}C dose estimation

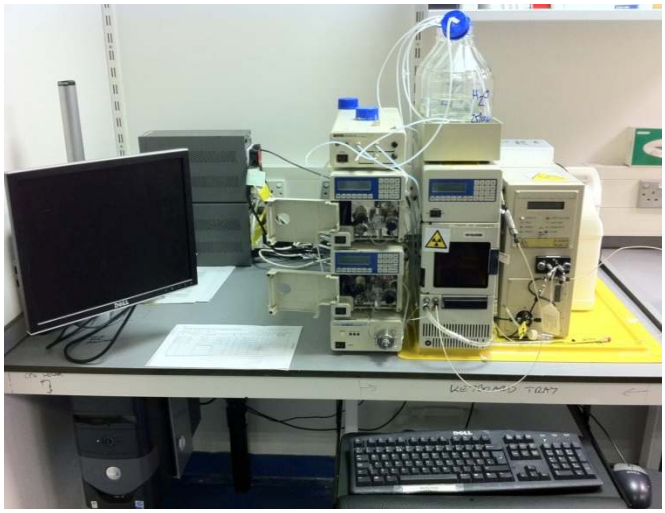
MOST WORK IS CARRIED OUT EITHER BEHIND A PERSPEX SHIELD OR USING PERSPEX TUBE RACKS SO THE DOSE SHOULD BE LIMITED TO FINGERS; WHOLE BODY DOSE WILL BE INSIGNIFICANT.

BREMSSTRAHLUNG RADIATION IS UNLIKELY TO BE SIGNIFICANT DUE TO THE LOW ENERGY OF THE RADIATION AND THE USE OF PERSPEX SHIELDING.

The external dose will be effectively zero since Perspex shielding, assay tubes, gloves and skin will all stop low energy beta-particles arising from ^{14}C decay.

The maximum dose likely to be encountered arises from handling the stock material which is a plastic container at a maximum activity of 1.85MBq (500µl @ 3.7MBq/ml). This is handled for a matter of seconds and estimation of the dose is impractical based on the lack of dose values from Delacroix *et al*.

ARGININE-CITRULLINE RADIOCHEMICAL HPLC MEASUREMENT OF NOS ACTIVITY



HPLC equipment is on a drip tray to contain any leaks. Waste disposal is directly to drains below the system. Water flushes from the autosampler drain through one waste tube. Solvent/scintillant waste from the detector drains through a second tube. Both tubes enter the drains via a swept tee and are sealed (non-permanently) in place. The scintillation cocktail, FlowLogic 1:1 (MSDS attached) is a biodegradable, low viscosity, non gelling fluid designed for flow-counting applications; some components are incompatible with oxidising agents so no bleach, Virkon or other oxidising agents should be poured down either of the sinks in room 10/109.

MAJOR RULES

- Wear gloves for handing HPLC sample vials and the sample tray.
- Remove gloves for touching HPLC equipment after loading sample tray into autosampler.
- Never wear gloves when using the computer keyboard.
- Radioactive stocks must always be stored in locked fridge.
- Label all tubes/bottles/boxes containing radioactivity with isotope, date, activity and name.

RECORD KEEPING

- Monitor and complete monitoring sheets in radiation suite 10/088 before and after each use.
- Record all isotope usage on the stock sheets on the storage fridge. EVERYTIME isotope is taken from a stock pot it must be recorded on the stock sheet IMMEDIATELY.
- Record waste on stock sheet AND on yellow sheet for solid waste AND on green sheet (on the designated sink/drain) for liquid waste.
- For samples stored in freezer before HPLC processing, record details on stock sheet under ‘Subsequent Containers’ columns. Sample box should be uniquely labelled (eg name & date).
- Record all samples that are processed through the equipment, with date.

- Store records of wipe tests with the HPLC.

WIPE TEST TO CHECK FOR CONTAMINATION

After each assay, swabs should be taken of the HPLC equipment using detergent (Decon90)-soaked tissue and counted in liquid scintillation vials with 3 ml of water mixed with 3ml of scintillation fluid.

^{14}C
Very difficult to detect using Geiger counter.
Maximum range in air: 24 cm
Maximum range in water: 0.28 mm

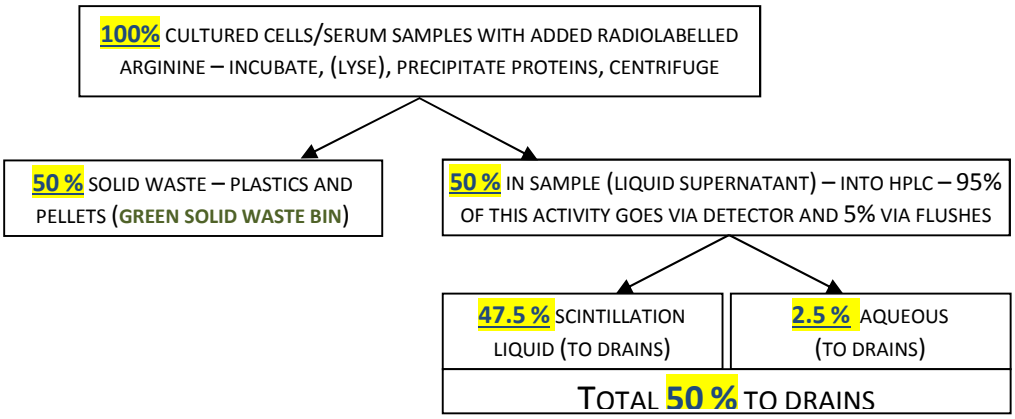
^{3}H
Not detectable using Geiger counter
Maximum range in air: 6 mm
Maximum range in water: 6 x 10^{-3} mm

The Geiger counter probe must virtually touch the spill, which needs to be dry before it can be detected.

DON'T RELY ON GEIGER COUNTER - DO A WIPE TEST!

WASTE DISPOSAL - DISPOSE OF ALL WASTE AT THE END OF EACH ASSAY

- For all waste records, record the original stock container number for each isotope.
- 50% of the radioactive waste is in the solid plastics of the assay. The remaining 50% is liquid sample transferred to HPLC vials.
- Plastics and tips should be disposed of into the solid waste bin.
- Liquid flush waste from the autosampler (2.5% of the total radioactive waste) and liquid scintillant waste from the detector (47.5% of the total radioactive waste) are piped directly to drains.
- Flush drains before AND after each experiment with 5 litres of 1% Decon90.
- Record the activity on the sink record sheet, solid waste record sheet AND stock sheet.
- **Ensure that disposal amounts do not exceed the limits of the bin or the sink.**



Dry disposal items: 24 well plates, pipette tips, gloves, Eppendorf tubes.

Dry disposal activity: 1.85 – 9.25 kBq per reaction, tube, sample.

Liquid disposal: maximum activity per sample of 18.5 kBq (5µl stock @ 3.7 kBq/µl)
As a guide, waste liquid generated per sample consists of approximately 45 ml aqueous mobile phase, 22.5ml scintillation fluid and 1ml aqueous sample.

ISOTOPES USED:

Products:	^{14}C-L-arginine	Hartmann	MC137	3.7 MBq per ml
	^{14}C-citrulline	NEN	NEC214	3.7 MBq per ml
	^{3}H-glucosamine	GE Healthcare	TRK398	37 MBq per ml
	^{14}C-Ornithine HCl	GE Healthcare	CFT180	3.7 MBq per ml

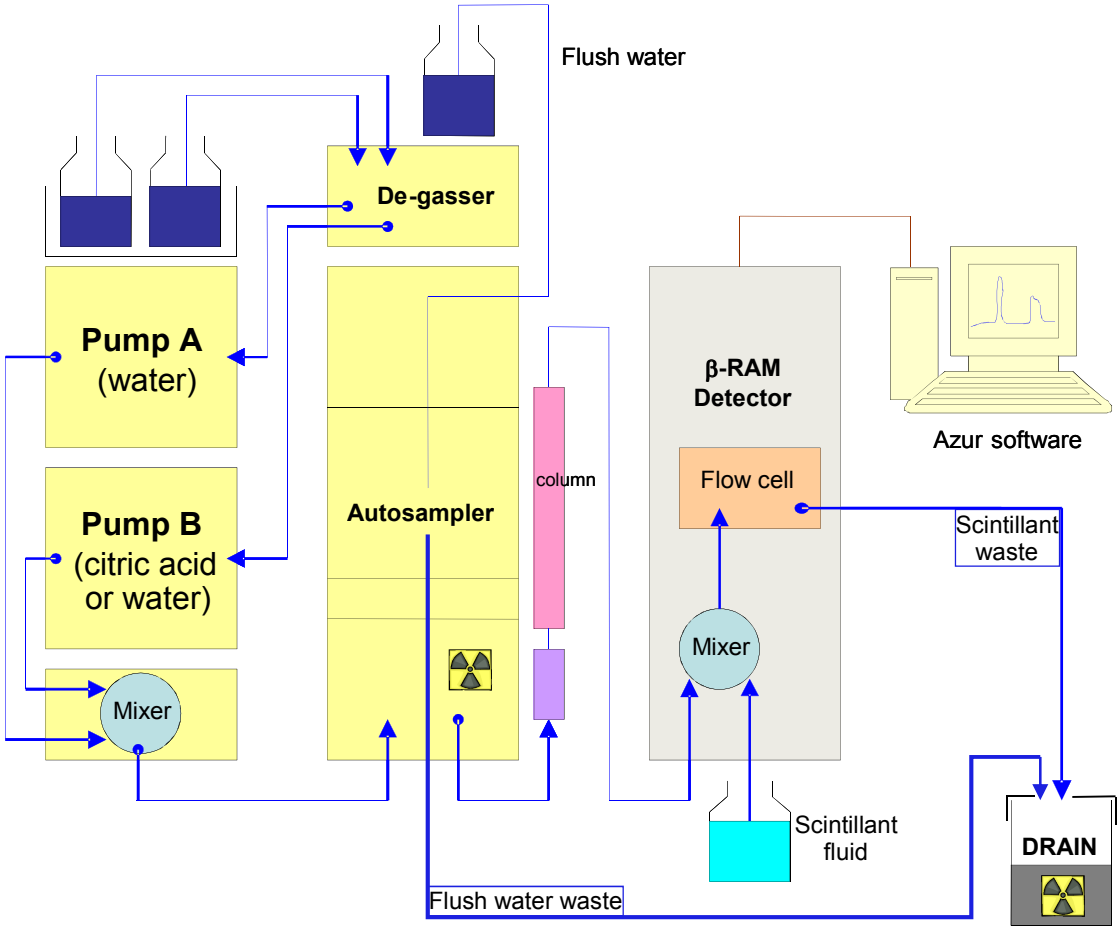
Quantity: (per reaction, tube, sample) ^{14}C 3.7 – 18.5 kBq
^{3}H 7.4 kBq

Storage: Locked radioisotope storage fridge in room 10/088.

EQUIPMENT

Degasser	Jasco DG-980-50
Pumps	Jasco PU-2080 Plus (x2)
Dynamic mixer	Jasco MX-2080-32
Autosampler	Jasco AS-2057 Plus
Guard column	Supelco Supelguard LC-SCX (2 cm)
Column	Supelco Supelcosil LC-SCX 5 mcm (25x0.46 cm)
Detector/Scintillation pump	Lab Logic β-RAM Model 3

SCHEMATIC OF HPLC SETUP



HPLC SETTINGS

Follow laminated guide sheet. Each pump can be controlled independently in manual settings. For setting gradient runs, pump A acts as 'master' and controls pump B as a 'slave'. All activity in 'program – PRGM' mode is performed using the keyboard on pump A.

You can set solvent gradient composition, flow rate, and events (such as turning the detector scintillant pump on or off at specified times).

Press PRGM-RUN to set up the pumps to run in initial conditions. They will start a program when the autosampler injects a sample.

AUTOSAMPLER SETTINGS

- Set up number of samples, number of flushes, run time.
- The autosampler starts the pump programs and Azur software data collection.
- Press Run to initiate assay.

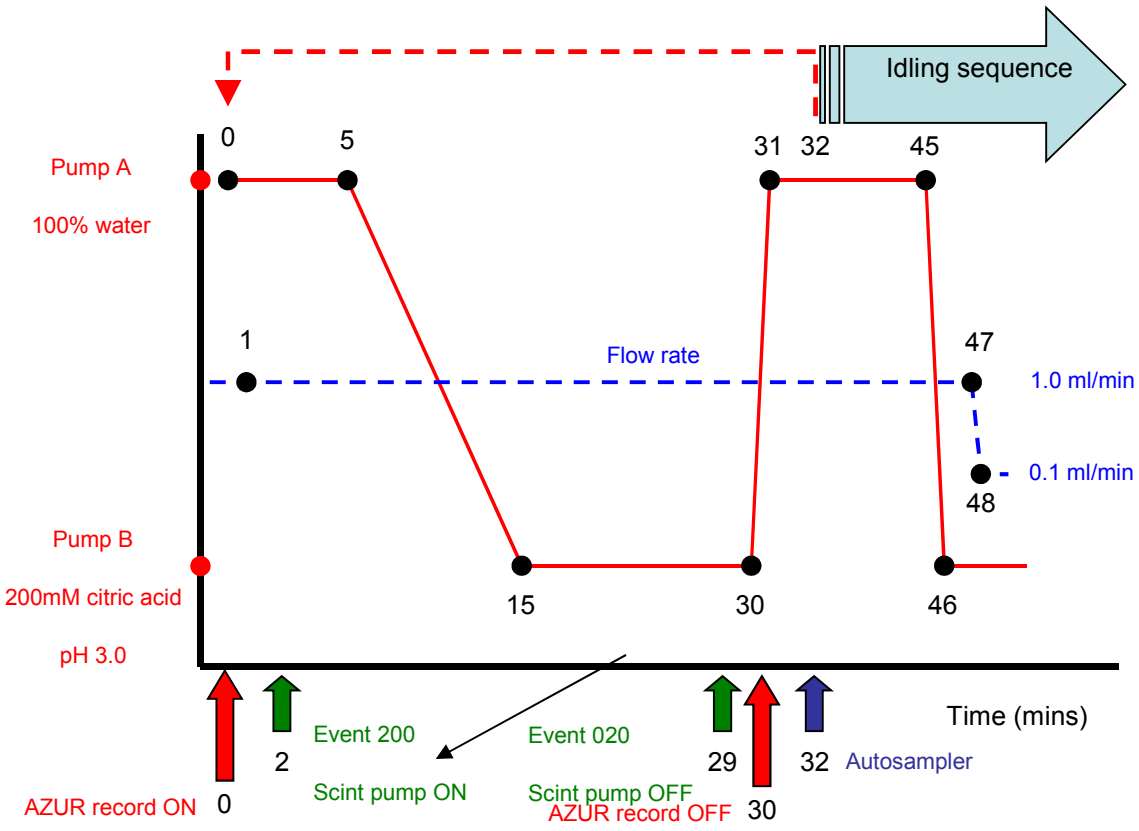
DETECTOR SETTINGS

Can be changed using Scintflow software.

- First open communications tab. Open COM port.
- Adjust Run set up, Channel set up, and Configuration as required. 2 Channels can be set up in the detector.
- Click download to activate new settings.
- File-exit to close.

Current settings include 0.5 ml/min scintillation fluid with a solvent flow of 1.0 ml/min.

HPLC GRADIENT PROTOCOL



Chromatographic analysis

Citrulline elutes at 16.5 minutes
Ornithine elutes at 19 minutes
Arginine elutes at 21 minutes

Software collects data from 2 channels: a 1 volt range and a 10 volt range.

Express results as % area of citrulline peak as a % of the total.

Starting from scratch

- Turn on all equipment. They will go through initialization steps.
- Turn manual pump valve on pump B (Citric acid or water) to the citric acid input (yellow marker on tube).
- Run the pump for 5 mins at 1 ml/min to prime the pump heads with citric acid.
- Stop pump B.
- Start pump A (water/water) to run in manual at 1 ml/min for 10 mins, to run water right through the column.
- Stop pump A, then press program (PRGM), then PRGM/RUN button. Light should flash, and PUMP light should be on, for both pump A and pump B.
- Program autosampler according to number of vials in the assay. Analysis time 32 minutes. Injection volume 700 mcl. 1 flush. 1 injection per sample.
- Set up software in Azure: Program a new sequence for the assay. Click on 'start' arrow. The software will wait for the autosampler to inject a sample before recording data.
- Press RUN on autosampler.

Procedure for turning off equipment at end of assay

- Pump B needs to have the pump heads flushed through with water. Turn the manual valve to the water input (brown marker on tube).
- Run water through for 5 mins at 1 ml/min.
- Turn off pump A and B, and all other equipment.

Making solvents

- **Distilled water**: from MilliQ machine. Add 1ml of 10% sodium azide (anti-bacterial) per litre, to a final concentration of 0.01%. Filter through filter apparatus into new bottle.
- **200mM citric acid**. Make up using 1M solution or fresh powder in MilliQ water. Add 1ml of 10% sodium azide per litre, to a final concentration of 0.01%. Adjust pH using 3M NaOH – will need about 25 ml of NaOH per litre to bring pH up from 2.1 to 3.0. Filter.
- **Pump flush recirculating water**: MilliQ water. Replace with fresh water every month; Add 1ml of 10% sodium azide per litre, to a final concentration of 0.01%.

SAMPLE PREPARATION - CULTURED ENDOTHELIAL CELLS

1. Seed cells into 6-well culture plates ($1x10^5$ cells per cell: containing DMEM, supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin). Maintain cultures at 37 °C in a humidified 5% CO_2/air atmosphere for 24 h prior to measuring NOS enzymatic activity.
2. Remove culture media and rinse with Krebs Hepes buffer (NaCl 99 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.0 mM, $CaCl_2$ 1.9 mM, $NaHCO_3$ 25 mM, glucose 11.1 mM, and Na-Hepes 20 mM).
3. Pre-incubate cells with 600 µl of KHB containing 5 mM of the arginase inhibitor, nor-NOHA (25 µl in 50 ml KHB, Calbiochem, UK) in the absence or presence of 1 mM of L-NMMA or L-NAME for 30 min at 37 °C.
4. Add 4 µl of ubiquitously labeled ^{14}C L-arginine (1.85 MBq/ml, Amersham Biosciences UK Ltd., Chalfont St. Giles, UK) to each well and incubate for 5 min.
5. Add 6 µl of 100 µM calcium ionophore to each well and incubate at 37 °C for 2 h.
6. Transfer the supernatant to 2 ml eppendorf tube.
7. Lyse cells - add 300 µl water and freeze-thaw on dry ice.
8. Deproteinate samples - add 300 µl of 10% trichloroacetic acid.
9. Transfer to sample from well into the original tubes and centrifuge (13,200 rpm, 5 min).
10. Store samples at -80 °C until required for analysis.
11. Measure the conversion of radioactively labeled arginine to citrulline by HPLC.

Sample fluid is then transferred to 750 µl plastic HPLC vials. The total volume in each vial is made up to 750 µl with MiliQ water. Orange snap-caps are clicked onto each vial. HPLC vials are placed in the autosampler rack.