

# <sup>14</sup>CHRONO, Centre for Climate, the Environment and Chronology

## - Quality Assurance/Control –



The <sup>14</sup>CHRONO Centre currently analyses approximately 2600 unknown samples per year and > 350 secondary standards not including the primary standard. The average turnaround time is 10-12 weeks. Of the unknown samples analysed in 2012-2017 to date, approximately 32% were for collaborative research projects, 57% were for commercial customers and the remainder for pilot projects, student research projects and laboratory test samples.

The <sup>14</sup>CHRONO Centre Radiocarbon Laboratory, AMS facility and stable isotope facility are committed to high standards of practice and quality. Quality assurance/control is put in place in order to ensure that:

- correct procedures are followed for each sample
- accurate data is kept for each sample at every stage of processing
- quoted dates and uncertainties are accurate, and
- systems errors are properly identified and corrected

The <sup>14</sup>CHRONO Centre successfully participated in the AMS portion of the Fifth International Radiocarbon Intercomparison exercise (VIRI) which can be seen, along with re-measurements of the VIRI bone samples in 2013. Our excellent results for the Sixth International Radiocarbon Intercomparison exercise (SIRI) are shown in the section 'Quality assurance data demonstrating the accuracy of measurements and errors'.

### **Description of technical facilities and equipment employed**

The <sup>14</sup>CHRONO Centre has a recently refurbished, well-lit and clean Radiocarbon Laboratory for sample preparation, a basement laboratory where stable isotope analysis equipment is housed and the AMS facility which was purpose built in 2006.

<sup>14</sup>C/<sup>12</sup>C and <sup>13</sup>C/<sup>12</sup>C measurements will be made on the <sup>14</sup>CHRONO Centre Ionplus Mini Carbon Dating System (MICADAS) which was commissioned in May 2022. The radiocarbon ages are corrected for isotope fractionation using the AMS-measured  $\delta^{13}\text{C}$  which accounts for both natural and machine fractionation.

For all bone samples with sufficient material C:N, %C, %N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  will also be measured on a Thermo Delta V elemental analyser - isotope ratio mass spectrometer (EA-IRMS).

Two graphitisation methods are available – An Ionplus AGE3 automated graphite system (7 reactors) and a line using the hydrogen reduction method (12 reactors). A ramped pyrooxidation/combustion apparatus with inline hydrogen graphitisation line is also available. Please see Methods Statement for details.

Other equipment utilised includes:

Centrifuges: Jouan B4i & Thermo B4i
Martin Christ freeze drier (2)
Sartorius CP2P microbalance for stable isotope analysis
Denver TL64 & TB60 Microbalances (2)
2 Millipore water purification systems (Direct Q UV)
Soxhlet extractor
Hot water bath: Grant Sub Aqua 5 Plus
2 Clifton Hotplates – Watlow Tube Heaters
Vacuum filter: KNF Neuberger VP series
Vortex stirrer
Drying oven: Genlab ovens
2 Carbolite combustion ovens
Vacuum tube sealing line (QUB manufacture) and Junior Jet 7 torch
Buchi R-210 Rotoevaporator
Microflow Laminar microflow cabinets and Labcaire Aura 750E
Thermofisher ATR Fourier Transform Infrared Spectrometer (FTIR)
Fume cupboards (6) Manufactured by Sarmieke Ltd
Metkon Micracut 152 precision cutter

#### **Principal Staff involved in quality control**

Mrs Michelle Thompson – overall QA/QC for sample pre-treatment, graphitisation, stable isotope analysis

Ms Niamh O’Neill Munro – QA/QC for bone collagen extraction

Dr. Gerard Barrett – QA/QC for AMS measurements and calculations

#### **Methods – Radiocarbon Dating (including pre-treatment methods for all sample types, methods of combustion and graphitisation, and methods of AMS measurement)**

Sample preparation and pre-treatment methods for all sample types, as well as combustion, graphitisation, and methods of AMS measurement are briefly outlined below. In-depth procedures are given in Historic England Research Report 5/2015 (Reimer et al. 2015) with the exception of shells and chitin which are given in more detail below. Please note that pre-treatment for charred organic residues on potsherds has been updated to include removal of lipids unless otherwise stated in the sample submission. All chemicals used are scientific grade. References in this section are given in Appendix A.

- **Bone collagen**

Collagen is extracted from the bone samples based on the method of Brown et al. 1988 using a Vivaspin® filter cleaning method introduced by Bronk Ramsey et al. (2004). An alkali step has been added since 2014 following Brock et al. (2010). Measurement of C:N ratios,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  is described in the following section, ‘Method - Stable Isotopes / FTIR’.

- Charred organic residues on potsherds  
For sufficiently robust samples, charred organic residues on potsherds will normally be treated to remove lipids in order to avoid reservoir offsets from utilization of marine or freshwater resources. The samples are treated with a 2:1 (v/v) mixture of chloroform and methanol and sonicated for 30 minutes, then filtered and the remaining solvent evaporated following the method of Boudin et al. 2010. The samples are then given the standard acid, alkali, acid (AAA) treatment (de Vries and Barendsen 1958; Fischer and Heinemeier 2003). Samples which cannot withstand such treatment are normally treated using the AAA method only.
- Charcoal and other plant material  
AAA (Acid-Alkali-Acid) pre-treatment is the standard treatment for charcoal, wood, peat and most plant macrofossils as in de Vries and Barendsen (1958) and Fischer and Heinemeier (2003). For macrofossils that have been separated from sediment using alkali treatment, an acid-only treatment is sufficient.
- Cremated bone  
Cremated bone procedure follows the method of Lanting and Brindley 1998; Lanting *et al.* 2001. As sulfur in bone interferes with the graphitisation, the CO<sub>2</sub> gas generated by hydrolysing the sample is treated further by heating with silver under vacuum.
- Bulk sediments (various fractions)  
For peat or lake sediments various fractions can be separated and dated. Separation of humic acid (alkaline soluble) and humin (non-soluble) fractions of bulk sediments follow the method of (Lowe et al., 2004). Bulk sediments are given an acid-only or acid fumigation pre-treatment.
- Shells and other carbonates  
Mollusc shells and other biogenic carbonates are cleaned in Milli-Q water in an ultrasonic bath to remove surface dirt and then dried. The shell sample is placed in a septa seal vial (exetainer) and etched with 1% HCl to remove about 25-30% of the outer surface, rinsed and the exetainer is evacuated. The samples are then hydrolysed with phosphoric acid on a heating block at 90°C to evolve carbon dioxide as described as described in Santos et al. 2004. The sample contained in the exetainer is evacuated by puncturing the septum with a hypodermic syringe attached to the vacuum line (Fig. 3). When a good vacuum pertains, the sample is removed and 2 ml of phosphoric acid is introduced through the septum. The vacuum syringe is then carefully inserted into the rubber of the septum (doesn't go right through the septum) to evacuate everything down to the seal. The syringe is then fully inserted and the CO<sub>2</sub> is drawn out under liquid nitrogen

Note: Mollusc samples with aragonitic shells should generally be analysed by x-ray diffraction (XRD) to determine if they have been recrystallized to calcite before dating. This is not possible for molluscs with calcite shells. This analysis can be arranged through the British Geological Survey, if desired.

- Chitin  
Insect chitin pre-treatment follows the Tripp et al. (2004) procedure for fragile samples. The sample is rinsed sequentially with acetone, methylene chloride, and acetone again, freeze-dried for 5 hrs and placed in 0.5 M HCl for 3 days. The sample is filtered, rinsed with Milli-Q water and freeze-dried overnight. The procedure has been found to be successful on insect remains from archaeological sites provided they have not been stored in organic solvents such as alcohol (Panagiotakopulu et al. 2015).
- Consolidated or otherwise contaminated samples  
For samples consolidated or contaminated with various organic substances such as PEG or PVA, a Soxhlet extraction is done with increasing polarity solvents ending in distilled water (Bruhn et al. 2001). The thoroughness of the extraction of the contaminants by solvent extraction is then tested by analysing the treated sample using Fourier transform infrared spectroscopy (FTIR) (D'Elia *et al.* 2007).
- Combustion  
There are 2 combustion methods currently employed at 14CHRONO.  
  
Dried organic samples are weighed into pre-cleaned tin capsules and combusted in oxygen with a helium carrier gas in an Elemental Analyser (Elementar Vario Isotope) before transfer to the AGE3 automated graphite system.  
  
Dried organic samples are weighed into pre-combusted quartz tubes with an excess of copper oxide (CuO) and silver (Ag) foil, sealed under vacuum, and combusted to carbon dioxide (CO<sub>2</sub>) at 8500C for 8 hours.
- Graphitisation  
Small to standard sized organic samples (0.3 – 1.0 mg C) are reduced to graphite on the AGE3 automated graphite system, which uses the hydrogen reduction method (Wacker et al. 2010).  
  
Small to standard sized organic samples are combusted in quartz tubes and reduced to graphite on iron catalyst using the hydrogen reduction method on a graphitisation line (Vogel et al. 1987). Carbonate samples are hydrolysed and reduced with the hydrogen method on this line.
- Target Preparation  
Graphite is pressed into vacuum cleaned aluminium holders (known as targets or cathodes) using an automated hydraulic press with a clean pin mounted in it. After pressing, the targets are stored in a compartmented box in a desiccator until they are ready to be inserted into an AMS magazine. The sample UB number is recorded on the target and in the appropriate position on a run list. The sticker

with the sample UB number is also transferred to the run list to double check the number. Once the targets have been transferred to the magazine, it is immediately loaded into the AMS.

#### AMS measurement

Sample magazines going into the AMS will generally contain targets produced within 1-2 weeks from either of our two graphitization rigs.

The magazine has 39 positions available for targets. The structure of our magazine is the following: five HOX-II (OX2) standards at regular intervals; minimum of two blanks (background) with extra backgrounds dependent on the unknown sample types being run; two pairs of secondary standards. Tuning and measurements are carried out on a single OX2 and background until satisfactory 14/12 and 13/12 ratios are obtained. Then an initial pass is carried out on the entire magazine with each target exposed for 2.5 minutes. If results are satisfactory, the full run is allowed to proceed. All targets are run for a minimum of eight 2.5 minute exposures until F14C uncertainties of typically <0.003 are achieved.

The  $^{14}\text{C}/^{12}\text{C}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios are measured by accelerator mass spectrometry (AMS) on an *Ionplus* MICADAS. The sample  $^{14}\text{C}/^{12}\text{C}$  ratio is background corrected and normalised to the HOXII standard (SRM 4990C; National Institute of Standards and Technology). The radiocarbon ages are corrected for isotope fractionation using the AMS measured  $\delta^{13}\text{C}$  which accounts for both natural and machine fractionation. Backgrounds (blanks) used are anthracite, Icelandic Spar calcite, wood or bone samples known to be >50,000 years old and pre-treated following the same methods described above for the samples.

To account for variability in the chemistry procedures we track the long-term variance in the reported  $\text{F}^{14}\text{C}$  of several secondary standards. This variance is taken in ratio with the average long-term average of these measurements to arrive at an error multiplier that we apply to unknown sample uncertainties of the same or similar sample types. Following this we estimate the uncertainty of our background measurement by determining the long-term variance of our measured backgrounds and obtain a ratio of this variance with the long-term average of our measured backgrounds. This ratio is multiplied with each day's measured background value and the result added in quadrature with the day's measured background uncertainty.

#### **Method - Stable Isotopes / FTIR**

- Measurement of C:N ratios,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$

C:N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  will also be measured on a Thermo Delta V elemental analyser - isotope ratio mass spectrometer (EA-IRMS). Samples and standards are sealed into tin capsules and combusted in the elemental analyser which yields %C and %N values and C:N ratios are calculated from these. The EA is connected to the IRMS for measurement of the stable isotope ratios. Three blanks are measured at the start of the run followed by three standards of Nicotinamide (known values of 59.01%Carbon and 22.94%Nitrogen) for the % element values. Standards bracket blocks of 8-10 samples. The number

measured depends on the size of the run. Standards used for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of collagen are IA-R041 L-Alanine ( $\delta^{15}\text{N}$ ,  $-5.56 \pm 0.14\text{‰}$ ;  $\delta^{13}\text{C}$ ,  $-23.33 \pm 0.10\text{‰}$ ), IAEA-CH-6 Sucrose ( $\delta^{13}\text{C}$ ,  $-10.449 \pm 0.033\text{‰}$ ) and IAEA-N-2 Ammonium Sulphate ( $\delta^{15}\text{N}$ ,  $+20.3 \pm 0.2\text{‰}$ ). An in-house fish bone standard (Fish) is also run for quality control ( $\delta^{13}\text{C}$ ,  $-31.44$ ;  $\delta^{15}\text{N}$ ,  $+17.78$ ; ( $n > 100$ )). For %C and %N determinations nicotinamide is used (%C, 59.01%; %N, 22.94%). For carbon stable isotope analysis of wood, charred seed and charcoal IA-R041 L-Alanine, IAEA-CH-6 Sucrose and IAEA-CH-7 polyethylene ( $\delta^{13}\text{C}$ ,  $-32.15 \pm 0.05\text{‰}$ ). For collagen typically 8-10 replicate measurements are made on R041, 3 replicates of IAEA-N-2, 3 replicates of IAEA-CH-6 and 5 replicates of Fish. For charcoal, seeds, wood (carbon only) standards typically include 8-10 replicates of R041, 6 replicates IAEA-CH-6, 6 replicates IAEA-CH-7 and 5 replicates of Fish.

The machine uncertainty is reported for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . This has been validated by the observed reproducibility of measurements on 10 replicate aliquots of seven different bone samples, which show no additional variability.

- Fourier Transform Infrared Spectrometer (FTIR)

Samples that have been treated with preservatives or consolidating agents will be analysed using FTIR after removal using organic solvents in a Soxhlet extraction apparatus. Approximately 1mg of sample  $< 0.63\mu\text{m}$  is mixed with approximately 0.25g potassium bromide. They are ground and mixed together using a pestle and mortar. A pellet is made using a manually operated hydraulic press at 10 tons for 3 minutes. The samples are analysed using a Thermofisher ATR FT-IR and the spectrum compared to standard material (e.g. collagen or cellulose).

### Measures for ensuring quality

Sample pre-treatments follow our standard operating procedure (see above) except in special cases which are discussed by laboratory staff and documented carefully. Laboratory staff meet weekly to discuss any problems or questions that arise and to be sure that all are aware of any procedural changes. Processes and procedures are reviewed annually.

### Sample handling

Samples are assigned a tracking number (UB number) at submission. This identifies the samples throughout the entire process including stable isotope measurements. The submitter is expected to label the UB number on the sample bags or vials. In the Radiocarbon Laboratory the Sample Curator checks that all sample ID's and UB numbers on the samples received agree with the database. The date received is entered and pre-treatment type is then selected. The UB number and sample ID is automatically printed on the sample pre-treatment sheets which includes pre-treatment specific fields. The sample pre-treatment sheets are used to document the sample processing throughout the procedure. When the pre-treatment and graphitization is completed the data is entered into the laboratory database and the sheets stored in binders. This ensures that data gathered for each sample is both complete and easily recalled.

and avoids potential sample mix-up. The UB number is also printed on sticky labels to transfer onto subsamples. An automatic check is made that all pre-treatment data has been entered into the database before the samples are analysed by AMS <sup>14</sup>C. Excess sample material is held in a safe storage location until analyses are completed and then returned if requested. Excess pre-treated sample material is held in labelled vials for the length of the project and beyond in some cases. Collagen and dentine samples are kept in a desiccator to minimize moisture and potential mould growth.

#### <sup>14</sup>C AMS QA/QC

To assure accuracy of the AMS results the laboratory routinely includes a number and variety of secondary standards running in the same wheel with unknowns. We have accumulated considerable data for these quality assurance/control samples and if there are any problems with these samples included in each machine run then we don't proceed to analyse the samples. Instead the problem (usually instrument related) is found and cured before we proceed. In addition to our routine internal quality assurance programme the <sup>14</sup>CHRONO Centre successfully participated in the AMS portion of the Fifth International Radiocarbon Inter-comparison exercise (VIRI). Results of our equally successful participation in the Sixth International Radiocarbon Inter-comparison exercise (SIRI) are given in Table 2.

Accuracy of dates and systems precision are internally monitored on a regular, ongoing basis and assessed whenever possible in relation to other facilities. The <sup>14</sup>CHRONO Centre adheres to the following aspects of quality management:

- a. The use of internationally agreed standards and backgrounds to maintain reliability and reproducibility. The primary standard is N.I.S.T (National Institute of Standards and Technology) SRM 4990C -oxalic II.
- b. The use of known-age or consensus standards (including dendrochronologically dated wood, IAEA C-6 sucrose, FIRI-C turbidite and a VIRI whale bone sample) to check for bias and sample size effects.
- c. The use of appropriate background materials to check for additions to the background (blanks) during processing.
- d. Replicate analysis of consensus material covering various materials and time-scales.
- e. A commitment to remain informed about new or improved methods and equipment for radiocarbon dating, and to carry out improvement-related research and design.
- f. Strong communication between research and technical staff.
- g. Participation in major and specific radiocarbon inter-comparison exercises.

### Stable isotope QA/QC

Quality assurance/control of the stable isotope analyses is achieved by the following:

- a. Analysis of blanks to obtain a baseline from which to correct subsequent samples.
- b. Analysis of standard of known elemental composition.
- c. Analysis of a pair of international standards of known isotopic composition every 10 samples. Sample isotopic values are calculated from values obtained for the standard. Periodic measurement of the standard also checks for machine drift which though rare can occur. If drift is present, then intervening sample values can be corrected. Copies of the standard certifications are attached.
- d. Mass spectrometer performance integrity (stability and linearity) is checked at the beginning of each working week.
- e. Annual preventative and emergency maintenance for the EA-IRMS is done by a certified engineer.
- f. Regular maintenance on balances is undertaken to ensure accurate weights for %C and %N measurements.

### **Quality assurance data demonstrating the accuracy of measurements and errors**

Data demonstrating the accuracy of quoted precision of from analysis of secondary standards, the SIRI intercomparison exercise, an interlaboratory cremated bone exercise and an intercomparison on PVA treated bone are given in Tables 1-4. Quoted precision are estimates of total error including the long-term background uncertainty and an error multiplier by material type. The background uncertainty and error multipliers are re-evaluated on a six-month basis.

**Table 1. Accuracy, precision and laboratory error multiplier of the main secondary standards and backgrounds analysed since 2012. Background values are not corrected.**

Secondary Standards	Consensus value	QUB Mean	Standard Deviation	Error Multiplier	number
TIRI-B pine	4508	4507	44	1.3	542
VIRI-I bone	8330	8331	56	1.3	79
Mammoth bone	infinite	45324	2473	1.3	32
Spar Calcite	infinite	47929	3598	1.4	94
FIRI-C turbidite	18176	18167	113	1.3	271
IAEA-C6 sucrose	F <sup>14</sup> C = 1.503	1.503	0.0078	1.7	1868



**Table 2. Accuracy and precision from most recent international radiocarbon intercomparison (SIRI) samples. Samples were treated as unknowns, so background corrections were made to all samples. (c) is infinite age, (nc) is finite age.**

CHRONO designation	Sample type	CHRONO value	SIRI value*	SIRI designation
UBA-23942	Miocene wood	51088±2945	50864(c) 51697(nc)	A
UBA-23943	Mammal bone	40876±1506	39165±2301	B
UBA-23944	Mammoth bone	>49275	46550(c) 45347(nc)	C
UBA-23945	Barley mash	1.0262±.0035	1.039±.0063	D
UBA-23946	Kauri wood	10787±60	10827±77	E
UBA-23947	wood	369±26	370±34	F
UBA-23948	wood	349±26	378±40	G
UBA-23949	wood	389±31	385±36	H
UBA-23950	wood	10011±62	9987±49	I
UBA-23951	charcoal	32633±355	31768±1067	J
UBA-23952	Doublespar carbonate	>55483	51603(c) 53532(nc)	K
UBA-23953	wood	>52342	51989(c) 50195(nc)	L
UBA-23954	Peat – humic acid	3264±32	3370±52	N

\*Scott et al. 2017

**Table 3. Accuracy and precision of cremated bone intercomparison studies.**

QUB lab code	Material	QUB age	Intercomparison sample	Comparison age
UB-S2KA	Cremated bone*	1496 ± 32	VIRI Sample 2	1496 ± 54
UBA-8436	Cremated bone**	5854 ± 38	GrA-20197	5780 ± 45

\*sample analysis too late for inclusion in Naysmith et al. 2007

\*\*Schulting et al. 2009

**Table 4. Results of second inter-comparison dating of two PVA treated Miesenheim IV elk bones found directly beneath Laacher Sea tephra (ca. 11,060 <sup>14</sup>C BP) Kuzmin et al. in review.**

Laboratory	Sample	<sup>14</sup> C age BP	Lab Code
Brussels	91/110-1	11,025 ± 48	RICH-22120.1.1
		11,060 ± 40	RICH-22120.2.1
	91/111-3	11,050 ± 49	RICH-22121.1.1
		11,100 ± 45	RICH-22121.2.1
Groningen	91/110-1	11,030 ± 50	GrA-64379
	91/111-3	11,190 ± 50	GrA-64380
Arizona	91/110-1	11,265 ± 67	AA-106555
		11,145 ± 65	AA-106555-UF**
	91/111-3	11,270 ± 69	AA-106554
		11,140 ± 66	AA-106554-UF**
Belfast	91/110-1	11,240 ± 62	UBA-30011**
	91/111-3	11,080 ± 63	UBA-30012**
Novosibirsk	91/110-1	11,080 ± 33	NSK-1352/UGAMS-23137
	91/111-3	10,920 ± 31	NSK-1618/UGAMS-27119

\*\*Ultrafiltered collagen.

## Average precision statements

**Table 5. Expected average precision achievable (for each sample type with regard to the timescales) including laboratory error multipliers and number of samples (n) included in average based on unknowns analysed 2012-2017**

Sample type	Average precision 0-2000 <sup>14</sup> C BP	Average precision 4000-6000 <sup>14</sup> C BP	Average precision 9000- 11000 <sup>14</sup> C BP
Wood, charcoal, charred seeds, macrofossils	31 (n=2399)	40 (n=885)	55 (n=108)
Bone collagen	33 (n=1078)	43 (n=489)	58 (n=16)
Cremated bone	31 (n=20)	35 (n=46)	43 (n=1)
Sediment fractions	30 (n= 367)	38 (n=74)	53 (n=53)
Carbonates	27 (n=208)	35 (n=130)	49 (n=160)

## Appendix A

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