

QUALITY ASSURANCE OF ULTRAFILTERED BONE DATING

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ABSTRACT. Ultrafiltration of bone collagen provides a method of purification that can be very effective in reducing environmental contamination from soil-derived amino acids as well as removing degraded collagen or other short-chain proteins. The Oxford Radiocarbon Accelerator Unit (ORAU) first implemented ultrafiltration in the pretreatment of bone material for accelerator mass spectrometry (AMS) radiocarbon dating in 2000. However, the filters themselves contain carbonaceous material, and thus stringent quality control is required to demonstrate that this does not affect the accuracy of the dating. Here, we present quality assurance data from the bone pretreatment and dating program at ORAU, including dates on known-age and background-age bones over a range of sample sizes, and measurements of residual carbon contamination present in the filters after cleaning.

INTRODUCTION

Bone is one of the most commonly dated archaeological materials and a great deal of research has been undertaken into identifying the most effective prescreening and pretreatment methods prior to radiocarbon dating (e.g. Brown et al. 1988; Hedges and van Klinken 1992; Tisnérat-Laborde et al. 2003; Bronk Ramsey et al. 2004). At the Oxford Radiocarbon Accelerator Unit (ORAU), our routine bone pretreatment involves a simple acid-base-acid (ABA) treatment followed by gelatinization and ultrafiltration, and is described in detail in Bronk Ramsey et al. (2004). Briefly:

- Coarsely ground bone powder (~0.5–1.0 g) is sequentially treated with hydrochloric acid (0.5M), sodium hydroxide (0.1M), and hydrochloric acid (0.5M) with thorough rinsing with ultrapure (MilliQ™) water between each reagent;
- Crude collagen is gelatinized in pH 3 solution at 75 °C for 20 hr;
- The gelatin solution is filtered using a 9-µm polyethylene Eezi-filter™ that has been cleaned by ultrasonication in ultrapure water for 20 min;
- The filtered gelatin is transferred into a precleaned (see below) ultrafilter (Vivaspin™ 15, 30 kD MWCO [molecular weight cut off]) and centrifuged at 2500–3000 rpm until 0.5–1.0 mL of the >30-kD gelatin fraction remains (typically 20–40 min);
- This gelatin is freeze-dried and ready for combustion in a CHN analyzer prior to graphitization.

Brown et al. (1988) originally proposed the addition of an ultrafiltration step to Longin (1971) collagen extraction. By selecting only higher molecular weight (>30 kD) proteins, they hoped to remove contaminants that were considered likely to be of relatively low molecular weight, such as short-chain proteins from degraded and potentially contaminated collagen, salt products, fulvic acids, etc. Initial dating of archaeological bone samples using ultrafiltration at ORAU showed that this method more successfully removed contaminants than simple Longin collagen extraction did not remove (Bronk Ramsey et al. 2004; Higham et al. 2006a,b; Jacobi et al. 2006). In addition, the method is simpler to apply than others such as single amino acid dating or ion exchange chromatography. However, the polyethersulfone filter membranes of the ultrafilters are coated with a humectant (glycerol) by the manufacturers to prevent the membrane drying out. This requires effective removal prior to use to avoid contamination.

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It became apparent during an intercomparison exercise between 3 laboratories in late 2002, that known-age samples from dendrochronologically dated coffins were producing dates at ORAU that were 100–300 yr too old for their expected age or context. A thorough investigation revealed that the source of the contamination was the humectant applied to the ultrafilter membrane. The manufacturer's instructions for cleaning the ultrafilters did not result in effective removal of all the humectant. This finding along with a revised cleaning protocol for the ultrafilters (given below) and details of quality control procedures initially implemented were described by Bronk Ramsey et al. (2004).

Bronk Ramsey et al. (2004) suggested that the average offset due to the original cleaning method might well have been as high as a hundred years until the yield rose above 40 mg collagen. This has been confirmed in a major redating exercise. For yields less than 10 mg collagen, the offset seems to have averaged about 200 yr, for those greater than 30 mg collagen it reduces to about 55 yr, and when yields are greater than 60 mg it drops to about 30 yr. Overall, the average shift seems to be about 120 yr. However, in all instances there is a lot of variability in the offsets, with some shifts being greater than the average and some almost not shifted at all; this precludes precise correction of the results. A number of these redeterminations are published and discussed elsewhere (Higham et al. 2004, forthcoming; Bayliss et al. 2007; Bronk Ramsey et al., forthcoming a,b).

Since 2002, we have continued to use ultrafiltration for all bone samples regardless of age, and for redating bone previously analyzed at ORAU and other facilities. It is clearly crucial to implement an effective cleanup of the ultrafilters used in the dating process, and to monitor closely the carbon content of the ultrafilters and test the effectiveness of the cleaning procedures. Here, we present our ultrafilter cleanup procedures and quality assurance data for ultrafilters used and known-age bones dated.

CLEANING ULTRAFILTERS

The humectant used to coat the filter membranes of the ultrafilters is highly water-soluble. The manufacturers recommend that the glycerol can be removed effectively by centrifuging twice with ultrapure water. When we first began to use the ultrafilters in 2000, we decided to centrifuge 3 times, but when the problems with contamination became apparent we discovered that even this failed to remove all the glycerol. We now clean the ultrafilters as detailed in Bronk Ramsey et al. (2004) as follows:

- Centrifuge twice with ultrapure (MilliQ) water;
- Ultrasonicate in a large volume of ultrapure water for 1 hr;
- Centrifuge once with ultrapure water;
- 1 mL of ultrapure water is then added to 1 filter from each batch cleaned, swirled over the filter membrane, and then removed with a glass pipette for carbon content analysis;
- Centrifuge twice more with ultrapure water.

Ultrafilters are cleaned no more than 24 hr prior to use and are kept wet to prevent from drying out whenever possible.

The water removed for carbon content analysis is combusted in a CHN analyzer on a Chromosorb® pellet. The amount of carbon has remained fairly consistently below 10 µg since 2003, despite changes in ultrafilter batches (Figure 1). “Blank” samples of Chromosorb on which the water is analyzed typically yield ~3 µg of carbon. Where greater than 15 µg of carbon is detected, the dates produced for the entire batch of bones treated are considered individually, taking into account their expected ages, the ages of other samples from the same site, the ages of the known- or background-

age bones dated alongside them, and the age of the humectant present in that batch of ultrafilters. If there is any suspicion of contamination from the humectant, additional subsamples of bone undergo full pretreatment and dating. (It should be noted, however, that this has only rarely produced significantly different dates, suggesting that contamination from the ultrafilters was negligible or absent.)

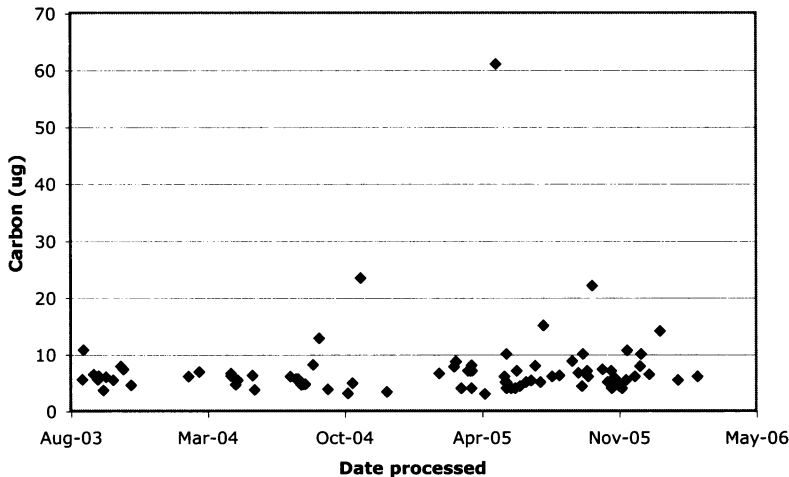


Figure 1 Levels of carbon remaining on ultrafilters after 2 centrifuge rinses, 1-hr ultrasonication in ultrapure water, and a further centrifuge rinse. Where greater than 15 μg carbon is detected, the entire batch of bone samples are considered individually.

We also extract and date the glycerol present in each new batch of ultrafilters received at ORAU. Initially, this produced older dates, often >35 kyr BP, although there were variations within and between batches. However, since 2005 all batches have contained humectant with a modern organic origin (Table 1). The total amount of humectant present within the ultrafilters is not measured, but appears to have increased in recent batches. However, we are confident that our cleanup process remains effective as, excluding 1 background value of 61 μg of carbon (which may not have originated from the ultrafilter itself), there is no significant difference in background carbon levels detected between the pre-March 2005 (background age) and the post-March 2005 (modern) ultrafilters.

Table 1 Age of humectant recovered from each batch of ultrafilters used at ORAU since 2003.

Batch	In use at ORAU	Age
02VS1538	2003–2004	Ranging from 25,540 to >35 kyr BP
03VS1539	2003–2004	Ranging from 12,325 to >35 kyr BP
05VS1510	2005	103.95 pMC
05VS1521	2005–2006	103.20 pMC
06VS1506	2006	101.46 pMC

QUALITY ASSURANCE USING KNOWN-AGE AND BACKGROUND-AGE BONES

Known-age and background bone samples are routinely analyzed alongside archaeological bone samples in batches. This enables us to monitor a) our precleaning of the ultrafilters used in the pretreatment (regardless of the origin of the humectant) and b) the reproducibility of bone determinations prepared under identical conditions to those unknown-age samples we analyze routinely.

Bones with a range of sample starting weights are analyzed to consider the reproducibility of dating bones with low collagen yields (about 1–3 wt% [weight percent] collagen) and to provide a suitable background correction for old (>4 half-lives of ^{14}C) samples. We pretreat samples of 2 starting masses, a “high” mass (~600 mg) similar to that used for most bones dated at ORAU, and a “low” mass (typically of ~200 mg) to simulate low collagen yields. To test for low levels of old contaminant, we treat and date pig bones recovered from the wreck of the *Mary Rose*, Henry VIII’s flagship. The *Mary Rose* sank in AD 1545, and so the bones should yield a ^{14}C age of ~311 BP. Collagen yields and ages of ultrafiltered *Mary Rose* pig bones are presented in Figure 2.

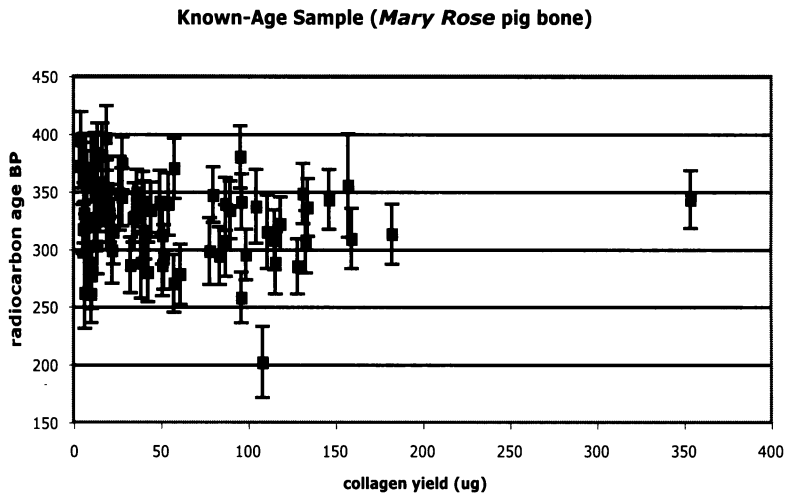


Figure 2 Dates from known-age pig bones found on the wreck of the *Mary Rose* (sank in AD 1545, so should be ~311 BP) analyzed using ultrafiltration since 2003.

We also routinely date high and low mass samples of 2 Alaskan bison bones of background age alongside each batch of bones treated (Figure 3a,b). Where these bones yield erroneous ages (>0.5 pMC), low-yielding or old samples are repeated where possible from the start of the pretreatment process. These results may indicate contamination by modern humectant.

Additional Quality Assurance Checks

In addition to the procedures that we follow to identify any contamination of collagen from ultrafilter humectant, we carry out several other quality assurance checks during and after our bone pretreatment preparation (see van Klinken 1999). These include an assessment of the weight percent of collagen in each sample. Any sample yielding <10 mg of collagen or <1% of the starting weight of bone powder is automatically failed. However, “low” mass quality assurance samples frequently fall below this threshold and therefore test the accuracy of the method over the full range of acceptable sample sizes. The collagen is combusted using a CHN analyzer, which enables an assessment of C:N atomic ratios, the % carbon and nitrogen of the material, as well as the stable isotopes of carbon and nitrogen. These data are collected routinely for all samples. The acceptable range for C:N ratios at ORAU is 2.9–3.5 (van Klinken 1999). High C:N ratios result either in the failing of the bone or lead to its repeat analysis.

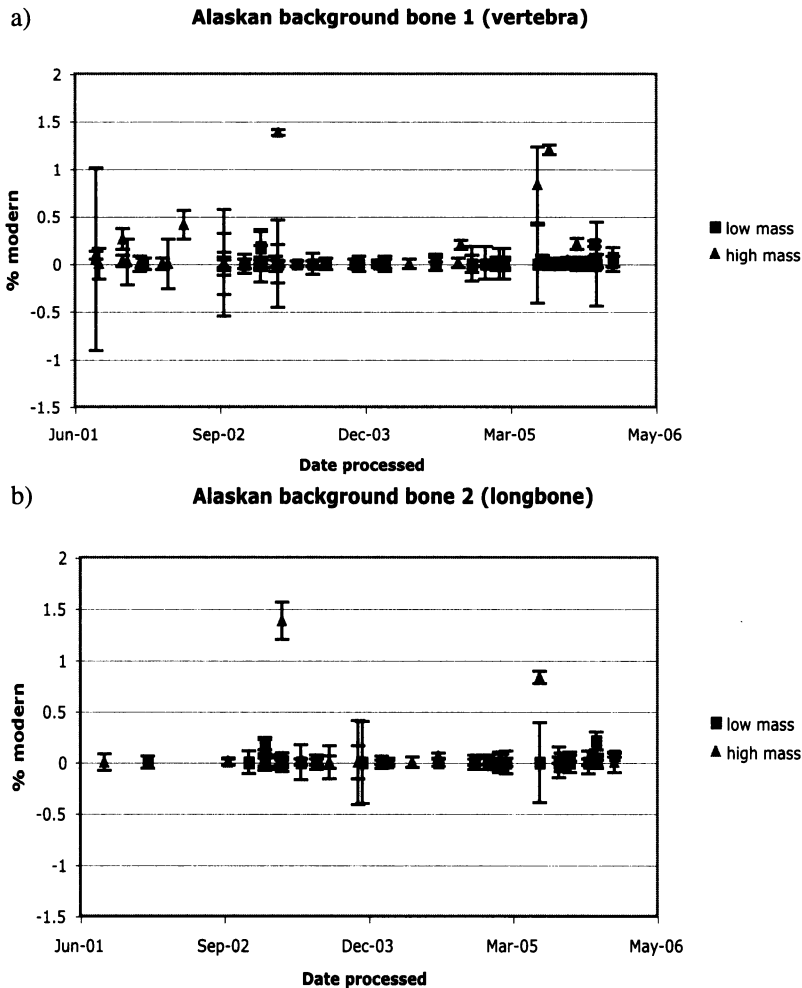


Figure 3 Background-age Alaskan bison bones (a: vertebra; b: longbone) are dated alongside every batch of bones treated at ORAU. Where erroneous dates are produced ($>0.5 \text{ pMC}$), old or low-yielding samples are retreated where possible (NB: a sample-size dependent background has already been subtracted from these data).

CONCLUSIONS

While ultrafiltration will not remove high-molecular weight contaminants, such as cross-linked humic-collagen complexes, it is an effective method of removal of low-molecular weight contaminants from bone collagen. The use of ultrafiltration to redat some bones previously dated at ORAU that were suspected of being problematic due to low collagen yields or aberrant C:N ratios has resulted in some substantial revisions. These are usually restricted to bone of Paleolithic age and have previously been reported (Higham et al. 2006a,b; Jacobi et al. 2006). In addition, when ultrafiltered gelatin determinations are compared with bone determinations obtained in other laboratories using the Longin collagen method, there are often significant differences that we conclude are probably related to improved contaminant removal using ultrafiltration (see Bronk Ramsey et al. 2004).

We suspect that this may be evidence of a more general pattern amongst the corpus of bone determinations in archaeology that could be improved by the wider use of this method in bone pretreat-

ment chemistry. Jöris et al. (2003) have noted that, where charcoal and bone are dated from identical contexts in Paleolithic Europe, bone often produces ages that are younger by comparison. Our results would suggest that because ultrafiltration often increases the measured age compared with Longin collagen techniques, its adoption could contribute to the resolution of this problem.

However, the filters do contain a carbon-containing humectant that must be removed prior to the ultrafiltration of archaeological bone gelatin. The age (and organic origin) and amount of the humectant varies within and between batches of ultrafilters. The manufacturer-recommended cleaning procedures are insufficient to remove all the humectant from the ultrafilters, and so we implement a more rigorous procedure.

Because of the presence of this humectant within the ultrafilters and its variation between batches of ultrafilters, it is important to apply consistent quality assurance checks alongside the pretreatment of bone collagen samples for accelerator mass spectrometry (AMS) dating to allow the detection of potential contamination during the pretreatment process. We implement a range of checks, including monitoring the level of carbon present in cleaned ultrafilters, and dating known-age and background-age bones alongside each batch of bones pretreated, in order to ensure the accuracy of the determinations made by this method.

ACKNOWLEDGMENTS

We acknowledge the careful laboratory work of the ORAU staff, especially A Bowles, C Tompkins, and J Davies, and the Mary Rose Trust for their kind donation of bone specimens.

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