

1 Assessment and development of bone preparation for radiocarbon dating at 2 HEKAL 3

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14

15 Abstract 16

17 Bone is one of the most complex sample materials used for radiocarbon dating. The
18 installation of the EnvironMICADAS AMS at HEKAL (department of ICER) in 2011
19 required the adoption of new sample preparation techniques for small bones samples. Since
20 then, hundreds of procedural background and known-age bones have been processed using
21 our modified Longin method (MLM) and dated along with unknown samples. Their results
22 are used in this study to assess the reproducibility of our current bone preparation method and
23 the real uncertainty of the final age result. In addition, using the background samples, which
24 are included in each bone measurement batch, blank correction of the unknown samples could
25 also be performed. The mean $F^{14}C$ value of our bone blanks is better than 0.005 (~ 42.500
26 BP) alongside 0.0013 SD. Good reproducibility was confirmed by the results of the laboratory
27 known-age bone as well, where the standard deviation of the mean value is better than
28 0.0025. In addition, the results of the three bone samples used in an ultrafiltration (UF) test
29 study have not shown notable differences from the ones obtained by our current protocol in
30 1σ uncertainty range but more experiments will be performed in the near future.
31

32 **Keywords:** blank correction, bone preparation, radiocarbon, quality assurance, ultrafiltration
33

34 Introduction 35

36 Human and animal archaeological remains (bone, tooth, ivory and antler) are very
37 complex materials that are used in the radiocarbon (^{14}C) dating technique. Bones are basically
38 built up by an organic collagen (an amino acid mixture composed mainly of hydroxyproline)
39 and an inorganic mineral (calcium phosphate containing structural carbonate) fraction.
40 Although carbon from the mineral fraction might be suitable for ^{14}C dating (Saliège et al.
41 1995), extraction of the organic collagen fraction is the most preferred method in dating of
42 bones (Longin 1971). Fresh bone contains about 20 wt % collagen but this value drops
43 steadily during burial and its chemical composition can be altered significantly due to
44 diagenetic processes and ambient carbon-containing compounds such as fulvic and humic
45 acids, polysaccharides, lipids and carbonates (van Klinken and Hedges 1995).

46 The earliest ^{14}C dates were obtained on whole bones, thus the dates were often
47 inaccurate, compared to the associated dates on charcoal. Longin (1971) was the first who
48 proposed the separation of the acid-insoluble collagen from the inorganic fraction, using a
49 weak acid decalcification wash. Later, an additional base wash step (using diluted NaOH) was
50 introduced into the protocol to remove humic substances (Arslanov and Svezhentsev 1993).
51 Although this protocol provides collagen that generally yields more accurate ^{14}C dates, NaOH

52 may significantly decreases the collagen yield, and does not completely remove organic
53 contaminants (Brown et al. 1988). Since then, other improved methods for purification of
54 collagen have been developed (e.g. ninhydrin method, step combustion technique or protein
55 remnant cleaning by ion-exchange columns) but ultrafiltration is currently the most popular
56 protocol for collagen purification (Bronk Ramsey et al., 2004). Ultrafiltration separates
57 collagen compounds with high molecular weight (HMW, >30 kD) from low molecular weight
58 (LMW, <30 kD) fraction. The HMW fraction is expected to contain intact bone collagen,
59 while LMW compounds include broken collagen chains and soil-derived organic compounds.
60 However, numerous studies have demonstrated that the glycerol used to keep the cellulose
61 membrane of the ultrafilter moist may contain old or post-bomb organic carbon, of which
62 removing might be problematic (Hüls et al. 2007, 2009). Nonetheless, according to Higham et
63 al. (2006), using ultrafiltration can be strongly associated with the reduction of the ¹⁴C
64 background limit of ancient bones, which has made the investigation of bone background
65 limit and reproducibility a major topic in many laboratories (Wood et al., 2010; Naysmith et
66 al., 2017). For the purpose of background determination, ¹⁴C laboratories have begun to use
67 ¹⁴C free infinite aged bones, which are treated in parallel with the unknown samples to
68 identify any contamination-derived ¹⁴C introduced during the chemical pretreatment, CO₂
69 conversion or graphitization sub-steps (Dunbar et al., 2017). The vast majority of bone
70 samples submitted for dating are less than one ¹⁴C half-life in age, thus younger known-age
71 samples are also worth studying to monitor the variability and uncertainty of the ¹⁴C date
72 determinations. Subsequently, when a large number of results of repeated samples have
73 already been collected, statistical methods may also help to reveal the long-term
74 reproducibility of the measurements, in addition, to determine the most adequate blank
75 correction procedure.

76 The two main purpose of this study is to (1) gain a better understanding of the
77 background and reproducibility of our modified Longin (MLM) bone preparation protocol
78 and suggest an adequate blank correction procedure, and (2) additionally, evaluate and
79 establish an ultrafiltration protocol (UF) at our laboratory, using our internal standard bone
80 samples of different ages and preservation.

81 **Materials and methods**

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83
84 Since the start of the AMS laboratory at the Hertelendi Laboratory of Environmental
85 Studies (HEKAL, Department of the Isotope Climatology and Environmental Research
86 Centre, ICER), numerous internal procedural blank and known-age bones have been prepared.
87 Our study uses 82 background and 70 known-age measurements carried out between February
88 2016 and September 2018 to evaluate mathematically the background and uncertainty of our
89 current protocol. The blank correction process can also be applied to unknown samples.
90 Usually, only the uncertainty of the ¹⁴C measurement is given in analysis reports, which,
91 despite the instrument blank correction and normalization, may not include the standard
92 deviation (SD) related to the sample pretreatment and processing. At HEKAL, background
93 bones with infinite ¹⁴C age are routinely prepared and measured in each AMS measurement
94 batch, which have resulted in a large number of dates over the last two and a half year.
95 Although, our bone preparation protocol appears to be adequate based on the dates of blank
96 and known-age sample, we have recognized the need for the continuous development. Hence,
97 we inserted an ultrafiltration step into our current bone process protocol and tested it on three
98 bone samples covering a wide range of ages from modern to blank. The quality of collagen
99 was monitored by carbon and nitrogen stable isotope ratio, in addition C/N ratio
100 measurements.

101

102 Description of the test samples

103

104 As internal procedural standards, three different blank and one known-age sample
105 applied at our laboratory were studied. All the three blank samples were classified and kindly
106 provided by Mihály Gasparik (Hungarian Natural History Museum, Budapest). The first two
107 samples (ProBl_1 and ProBl_2) belonging to a steppe bison (*Bison priscus*) were found in the
108 same gravel pit in 2012. The third bone blank (ProBl_3) originates from a complete mandible
109 of a woolly mammoth (*Mammuthus primigenius*). This individual was found during a
110 cleaning campaign of the bed of the river Tisza, most probably during the second half of the
111 19th Century. Unfortunately, in both cases, the exact localities of the remains are not known.
112 The age of these bones were considered to be infinite with regard to ¹⁴C dating. As a known-
113 age sample (K-Age), a larger tibia fragment of a Eurasian aurochs (*Bos primigenius*
114 *primigenius*) from the Early Neolithic period was used, which had been offered by János Dani
115 (Déri Museum, Debrecen). Based upon the circumstances of the archaeological excavation, its
116 expected age falls into the period of Late Körös culture (Hencida-Gyűrűszeg archaeological
117 site, Eastern Hungary), which was also confirmed by ¹⁴C age measurements of two other
118 contemporaneous sites (Szentpéterszeg-Körtvélyes and Berettyóújfalú-Morotva-liget) (Gamba
119 et al 2014). For the ultrafiltration tests, a modern pig (*Sus scrofa domestica*) bone (referred as
120 Modern) was purchased at a butcher shop at the beginning of summer 2018. This bone was
121 not identified by a zoologist but it is expected to be a leg bone (humerus or femur) of a 8-9
122 month old individual, which was slain 1 or 2 month before. After a thorough rinsing in
123 ultrapure water, the bone was freeze-dried and the meat was removed by a scalpel.

124

125 Bone preparation using modified Longin method (MLM)

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127 At HEKAL, all bone samples containing collagen are processed as follows. At the
128 beginning of the pretreatment, all samples are visually inspected and all visible marks on its
129 surface are noted along with the initial mass. After repeated ultrasonication in ultrapure water,
130 the bones are dried at 60 °C overnight. The outer surface of the bones is removed by thorough
131 abrasion. Then, larger bone fragments are ground and sieved to get the adequate size fraction
132 of 0.5–1.0 mm. For chemical pretreatment, 500 – 1000 mg (min. 100 mg) of the ground
133 powder is placed in a special designed Omnifit™ glass column. These columns are used as
134 flow cells in our semi-automatic system that was constructed for performing the ABA (acid-
135 base-acid) cleaning method on bone samples (Molnár et al., 2013). At the end of the chemical
136 pretreatment, the pH is adjusted to 3 to eliminate any ambient CO₂ absorbed in the base-
137 treatment step. Subsequently, the acid-insoluble collagen is transferred into a test tube
138 containing 5 ml of pH 3 aqueous solution, and it is put into a block heater at 75°C for 24
139 hours. Dissolved gelatin is then filtered via a 2 µm glass fibre filter (Milles AP20) into a 20
140 mL vial pre-cleaned by nitrogen gas, and after freezing, it is freeze-dried for at least 2 days.

141 Gelatin samples are then combusted using a modified sealed-tube combustion method
142 where the sample and MnO₂ reagent are together placed in a borosilicate test tube. After
143 flame sealing, the closed tubes are placed in a muffle furnace at 550°C for at least 12 hr to
144 combust the gelatin. The CO₂ gas produced is then transferred and purified from any other by-
145 product gases and carbon content quantified using a dedicated vacuum line (Janovics et al.,
146 2018).

147

148 Bone preparation using ultrafiltration (UF)

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150 Regarding the use of ultrafiltration, numerous aspects of its benefits and drawbacks
151 have been presented and discussed in the literature. Bronk Ramsey et al. (2004) found

152 evidence that the ultrafiltration method is a considerable advance in terms of removing
153 environmental contamination and produces more accurate ages (mainly for bones of
154 Paleolithic age) than less rigorous bone pretreatment methods. Nonetheless, the filters can
155 contaminate the collagen with glycerol deriving from the surface of filters, but Brock et al.
156 (2007) have shown that this humectant can effectively be removed applying a profound
157 cleanup procedure before use. Furthermore, Hüls et al. (2009) have shown that the
158 fragmentation of ultrafilter membrane itself, which is ancient in age, could also contaminate
159 the filtrate fraction (<30 kDa) but no significant contamination was seen in the supernatant
160 fraction (>30 kDa). However, this effect has not yet been independently confirmed by other
161 laboratories (Wood 2010). As ¹⁴C investigations regarding VIVASPIN 15R filters appear to
162 give clear evidence for no contamination with either young or old carbon for both the filtrate
163 and supernatant fractions (Hüls et al., 2009), so we used the ultrafilters of this type for our
164 tests.

165 In all cases, the cleaning step occurred on the day before the ultrafiltration of the actual
166 collagen samples. Briefly, the ultrafilters (VIVASPIN 15R, 30,000 MWCO HY, regenerated
167 cellulose membrane) are filled with 15 mL of ultrapure water and centrifuged at 3500 rpm for
168 15 min. This step is repeated two more times. Then, the filters are completely filled with
169 ultrapure water and ultrasonicated for at least 60 min followed by centrifuging 15 mL
170 ultrapure water further two times. Our ultrafiltration protocol was set up based on other
171 already tested protocols published in the peer-reviewed literature (Ramsey et al. 2004). The
172 bone samples were treated in parallel using the MLM and ultrafiltration bone cleaning
173 protocols. The two collagen extraction procedure are the same till to the point of filtration
174 using 2 µm syringe filters. At this point, the collagen solution is transferred to a pre-cleaned
175 ultrafilter and centrifuged at 3000 rpm for 15 min. The filtrate (referred as UFB in the text)
176 and supernatant (UFA) fractions were collected separately into individual 20 mL vials and
177 were freeze-dried for 2 days. Subsequent combustion and CO₂ cleaning steps are the same as
178 described earlier for the non-ultrafiltered collage preparation.

179 180 Applied analytical methods

181
182 Carbon and nitrogen stable isotope measurements were calibrated by control
183 measurements of IAEA 600 (caffeine) and sulfanilamide standards after every fifth unknown
184 sample. Measurements were performed by a Thermo Finnigan Delta Plus XP isotope ratio
185 mass spectrometer. Briefly, gelatin sub-samples (~0.3 mg) are packed into ultraclean
186 aluminum cups and combusted by an elemental analyzer (EA, Fisons NA 1500). The C/N
187 ratio, carbon and nitrogen stable isotope ratios are measured in the same run. The stable
188 isotope results are expressed as delta value which is defined as follows:
189 $\delta(\%) = (R_{\text{sample}}/R_{\text{reference}} - 1) * 1000$, where R_{sample} and $R_{\text{reference}}$ is the ¹³C/¹²C (or ¹⁵N/¹⁴N) ratio in
190 the sample and in the reference material, respectively. The overall uncertainty of the
191 $\delta^{13}\text{C}_{\text{vs. VPDB}}$ and $\delta^{15}\text{N}_{\text{vs. AIR}}$ measurements is ±0.2 and ±0.3 ‰, respectively.

192 For ¹⁴C dating by AMS, graphite targets from the purified CO₂ samples were prepared
193 using a customized sealed tube graphitization method (Rinyu et al. 2013). The ¹⁴C
194 measurements reported below were performed using the EnvironMICADAS AMS instrument
195 at HEKAL (Molnár et al. 2013b). The overall measurement uncertainty for modern samples is
196 < 3.0 ‰, including normalization, background subtraction, and counting statistics. The
197 conventional ¹⁴C ages were evaluated by the “Bats” software package (version 3.66; Wacker
198 et al., 2010, Stuvier and Polach 1977).

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202 **Results**

203

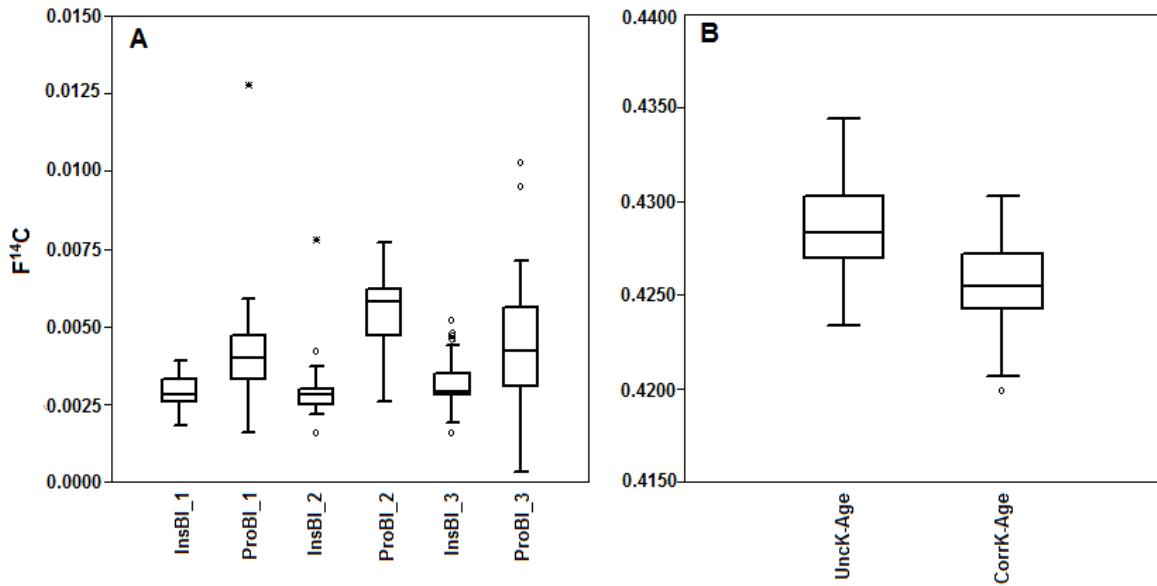
204 Evaluation of the bone procedural blank

205

206 The two bones of a steppe bison (hereafter ProBl_1 and ProBl_2) and the remains of a
207 woolly mammoth (hereafter ProBl_3) were applied sequentially as internal blank samples
208 during the studied two and a half year to assess the procedural background of our current bone
209 preparation method (in total 82 graphite targets). In all cases, the blank samples were assumed
210 to have an infinite age although this was not independently confirmed by archaeological
211 findings or other dating methods. The amount of the extracted gelatins ranged from 10–100
212 mg while the mass of combusted gelatin scattered around 4 mg. For example, Wood et al.
213 (2010) has suggested a new correction method to avoid the under-correction of large and
214 overcorrection of very small samples. In our case, the radiocarbon ages of the three blank
215 samples plotted as a function of the gelatin yield (mg) does not show any significant
216 correlation (max. R^2 is 0.15 for ProBl_3) therefore we did not use any special gelatin yield
217 dependent correction.

218 First, we studied the instrument background (InsBl) itself and compared it to the bone
219 procedural blanks (ProBl, Figure 1/a). The instrument blanks are made from high purity fossil
220 CO_2 borehole gas (Linde Hungary, Répcelak) requiring no chemical pretreatment; the gas
221 samples are just graphitized and AMS measured (Molnár et al., 2013). The long-term mean
222 $F^{14}\text{C}$ for the instrument blank is around 0.0029 ± 0.0007 ($n=78$, 4 outlier) ($F^{14}\text{C}$ values quoted
223 in this paper are all $\pm 1\sigma$). In each measurement batch (22 targets in total), three instrument
224 blanks can be found of which mean value is subtracted from all the unknown samples
225 measured in that certain run. Therefore, all the bone results presented in this paper have
226 already been corrected for the instrument blanks but in case of the blank bones, the
227 uncorrected values are also given. After discarding the apparent outlier results (indicated by
228 circles and dots in Figure 1), bone blank samples coded as ProBl_1, ProBl_2 and ProBl_3
229 gave the following mean $F^{14}\text{C}$ and SD values: 0.0039 ± 0.0012 ($n=25$, uncorrected: 0.0068),
230 0.0054 ± 0.0012 ($n=19$, uncorr.: 0.0082) and 0.0043 ± 0.0013 ($n=35$, uncorr.: 0.0074),
231 respectively. As the mean data show, the corrected $F^{14}\text{C}$ value for the sample ProBl_2 is more
232 than 0.001 higher than that of the other two blank samples. Assuming equal variance, this
233 mean value is significantly different from the other two blanks (two sample t-test, p-value
234 0.1). Based upon archaeological aspects, the two samples should have the same age due to
235 their finding in the same gravel pit. The difference may be caused by a procedural reason, i.e.
236 the cleaning process of glassware of the bone preparation system was changed when we
237 switched from ProBl_1 to ProBl_2. That time, a new decontamination detergent (Contrad 90)
238 was introduced in the protocol instead of chromic acid. Finally, when we changed to the blank
239 bone ProBl_3, the soap and chromic acid reagents were already applied sequentially, thus the
240 blank again achieved a lower value, close to the initial one. Despite the wider interquartile
241 range of the ProBl_3 sample (relative to ProBl_1), there is no statistically significant
242 difference in the SD of these samples (two sample F-test, p-value 0.1).

243



244
 245 Figure 1/a Comparison of the mean $F^{14}C$ values for the associated instrumental (InsBl_1-3,
 246 fossil CO₂) and procedural blanks (ProBl_1-3, fossil bones). The procedural bone blanks are
 247 already ins. blank corrected. 1/b Comparison of the mean $F^{14}C$ values of the known-age bone
 248 sample, before and after procedural blank correction (UncK-Age nad CorrK-Age,
 249 respectively). Circled and dotted results are those that are at least 1.5 or 3 times the
 250 interquartile range(Q3–Q1) from the lower or upper quartile, respectively.
 251

252 Comparing the long-term mean values of 0.0029 ± 0.0007 (n=78) and 0.0041 ± 0.0013
 253 (n=60, ProBl_1 and ProBl_3 discarding the mean value of ProBl_2) for the instrument and
 254 corrected procedural blanks, respectively the former one significantly differs from the latter
 255 one (based on a two sample t-test and F-test, p-value 0.1). Dunbar et al. (2017) reported that
 256 the running mean $F^{14}C$ value of their modified Longin extraction method at SUERC is around
 257 0.0030 ± 0.0009 , which is higher than the value of a wood blank (Heidelberg tree) applied in
 258 parallel. They concluded that the complexity of collagen extraction procedure is unlikely to be
 259 solely responsible for the higher background contribution of bone measurements. Other
 260 studies have also indicated that the slight differences in the procedures undertaken by
 261 different staff members can also result in the variation of the final results (Fülöp et al., 2013;
 262 Dunbar et al. 2017). At our laboratory, bone preparation is performed by only one technician
 263 thus the higher bone blank is likely due to procedural (maybe mixed) factors. In the future, we
 264 would like to perform some physical tests regarding the filamentous structure of the gelatin (it
 265 has a large and adhesive surface relative to charcoal) whether or not it can be responsible for
 266 the increased background.
 267

268 Blank correction for bone samples

269
 270 The results regarding the procedural bone blanks point to the importance of an adequate
 271 background correction method, thus the $F^{14}C$ value of the blank sample measured in each
 272 bone measurement batch was used for blank correction of all the other bone samples to obtain
 273 more accurate ages. The instrumental-only and procedural blank-corrected mean $F^{14}C$ results
 274 for the known-age bone sample are shown in Figure 1/b. The replicated measurements of this
 275 bone offered an average raw $F^{14}C$ value of 0.4286 (n=70), which corresponds to a radiocarbon
 276 age of 6806 BP. The mean measurement uncertainty obtained by the Bats software is around
 277 $0.0017 F^{14}C$ (30 yrs). Although the raw uncertainty of the AMS measurements is of the
 278 correct magnitude, it does not evidently account for the entire variation of the ^{14}C results. If

279 we look at the standard deviations of 0.0020 and 0.0022 (40 yrs) related to the procedural
280 blank corrected and uncorrected $F^{14}C$ values, respectively, these better characterize the
281 variation of the results obtained (in 1σ range). The uncertainty of the procedural blank-
282 corrected result is identical in magnitude with the uncorrected one, but the mean value is a
283 slightly lower. Moreover, the SD of blank samples is also very similar that confirms the
284 magnitude of the estimated uncertainty. We applied the following classical mass-balance
285 equation for contamination correction:

$$F^{14}C_{\text{measured}} * m_{\text{sample+cont}} = F^{14}C_{\text{sample}} * m_{\text{sample}} + F^{14}C_{\text{cont}} * m_{\text{cont}} \quad (1)$$

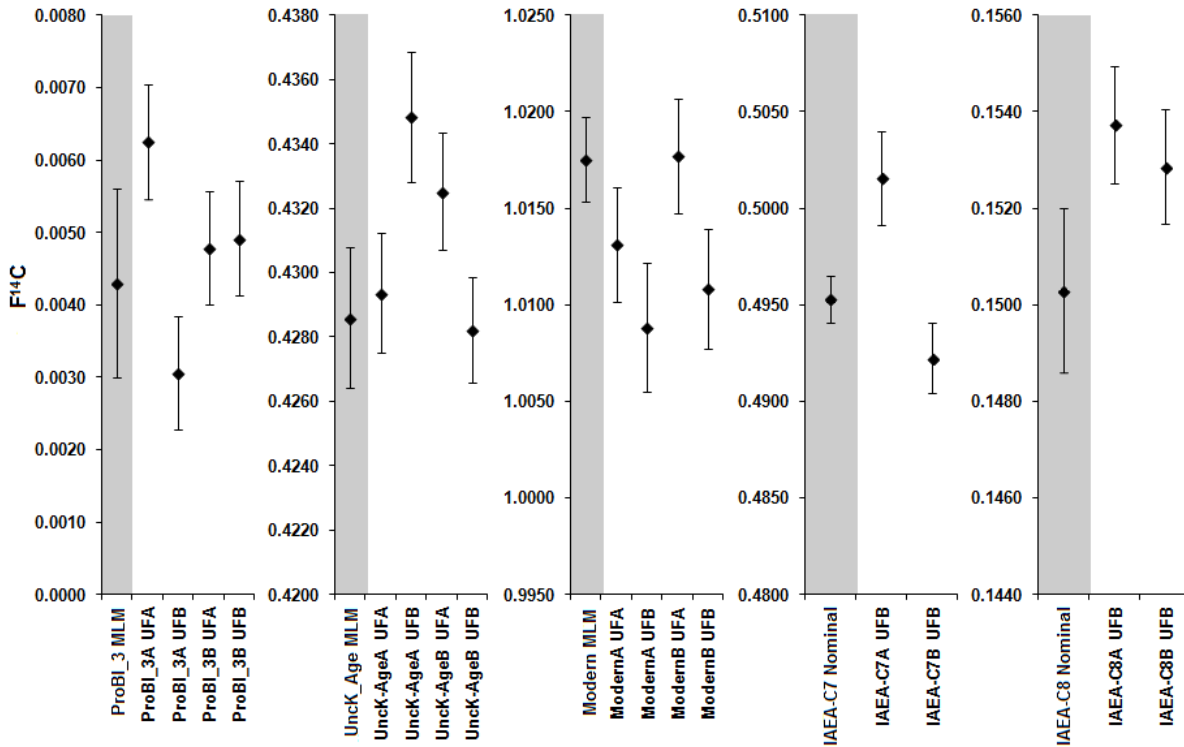
288 where, $F^{14}C$ and m represents the specific ^{14}C activity and mass of the sample and
289 contamination, respectively. Based on the expected and measured mass and ^{14}C data of the
290 blank sample, the mass of the contaminant can be calculated and applied for the unknown
291 samples. As other labs have noted, the contamination added in the laboratory is usually
292 assumed to be modern in age (Vogel et al. 1987). This modern carbon appears to contaminate
293 the bone blank samples with similar amounts as the unknown samples since all samples are
294 handled in the same way. The blank-corrected $F^{14}C$ value of this sample is 0.4256 ± 0.0020
295 corresponding to a date 6860 BP, indicating that this sample is only slightly affected by
296 modern contaminating carbon. The majority of bone samples processed in the laboratory are
297 of Neolithic or younger age, thus the blank correction does not shift significantly the results
298 but these slight changes can gain greater importance in the Paleolithic range.

300 Ultrafiltration tests on bone samples

301 The ultrafiltration protocol was tested on our three internal standard bones of different
302 ages, to see if any contamination remains in the samples (soil-derived) after chemical
303 pretreatment or is introduced during the ultrafiltration step. The results are shown in Table 1.
304 Ultrafiltration is a widely-used procedure for better purification of collagen but due to the risk
305 of contamination derived from the surface of membranes, the filter requires thorough cleaning
306 before use. To check whether or not any supposed humectants come from the surface of the
307 filters and influence the collagen, IAEA-C7 (nominal $\delta^{13}C_{\text{PDB}}$ and $F^{14}C$ values are
308 -14.48 ± 0.21 and 0.4953 ± 0.0012 , respectively) and IAEA-C8 (nominal $\delta^{13}C_{\text{PDB}}$ and $F^{14}C$
309 values are -18.31 ± 0.11 and 0.1503 ± 0.0017 , respectively) oxalic acid reference samples were
310 also prepared in the test process, in parallel with bone samples. As the oxalic acid (dissolved
311 in ultrapure water) has a molecule weight less than 30 kDa, only the filtrate fraction passing
312 through the filters could be ^{14}C dated. According to our assumption, if the humectant has an
313 effect on the filtrate fraction of bones, the supernatant fraction would show the contamination
314 as well. However, if the filtrate fraction is confirmed to have no significant contamination
315 present, the supernatant fraction above the filter can also be regarded as contamination free,
316 as Hüls et al., (2009) has suggested.

317 Looking at the carbon and nitrogen stable isotope data ($\delta^{13}C$, $\delta^{15}N$) there are no
318 significant differences between the values of the Longin and ultrafiltration methods (Table 1).
319 The values obtained for the IAEA C7 and C8 standards are also in good agreement with the
320 nominal values in 1σ range. The %C content, %N content and C/N data for the tested gelatin
321 samples could be indicators for the poorly preserved or contaminated gelatins. We cannot
322 observe significant differences in the values between the two preparation methods but the
323 high C/N ratios might be indicative for the not too well-preserved collagen. In case of the
324 ProBl_3 sample prepared by the Longin method, the C/N ratio is around 4.0 that can be
325 indicative of some extra carbon-containing contamination in the gelatin. However, both of the
326 filtrate and supernatant fractions yielded similar values (3.4-4.1) for the ultrafiltered materials,
327
328

329 thus we can conclude that the high ratios out of the accepted range are not caused by
 330 contaminants having been collected in either fraction. The C/N ratios of the known-age
 331 sample is also slightly higher than the conventionally accepted value (2.9-3.5), but visual
 332 inspection did not show any trace of contamination or poor preservation of the bone.
 333
 334



335
 336 Figure 2 Comparison of the Longin and ultrafiltration methods using bone samples of
 337 different $F^{14}C$ values. The grey bars represents the results obtained by our current method.
 338 UFA and UFB denote the ultrafiltered supernatant and filtrate fractions, respectively.
 339

340 Regarding the ^{14}C data, the procedural blank sample (ProBI_3) has regularly been
 341 prepared using our current method for two and half years and its long-term mean $F^{14}C$ value
 342 of 0.0043 ± 0.0013 is used in the comparisons. The mean values for the ultrafiltered
 343 supernatant (UFA) and filtrate (UFB) fractions are 0.0055 ± 0.0010 and 0.0040 ± 0.0013 ,
 344 respectively, which are very similar to each other and agree in 1σ uncertainty range. Gelatin
 345 extracted using the ultrafiltration protocol produces similar results, suggesting that the bone is
 346 not contaminated appreciably with young carbon. K-Age samples (with an age around one ^{14}C
 347 half life) show a mean $F^{14}C$ value of 0.4286 ± 0.0022 (without ultrafiltration). The mean values
 348 for the ultrafiltered supernatant and filtrate fractions are 0.4310 ± 0.0022 and 0.4315 ± 0.0047 ,
 349 respectively, which are also very similar to each other and to the conventional value. These
 350 ultrafiltered samples were measured in different measurement batches so the external
 351 uncertainty (SD) of different measurements can also be studied. This suggests that the
 352 ultrafilters do not appreciably contaminate the samples with young carbon either. The mean
 353 $F^{14}C$ value for the duplicated modern pig samples prepared conventionally and corrected for
 354 the measurement blank is 1.0175 ± 0.0012 . The mean $F^{14}C$ values obtained for the supernatant
 355 and filtrate fractions of the duplicated samples ultrafiltered are 1.0154 ± 0.0032 and
 356 1.0098 ± 0.0014 , respectively. Seemingly, the filtrate fraction values are lower than that of the
 357 supernatant fraction. This could be an indication that the filter humectant contains a small
 358 amount of “old carbon” (having no large effect on the supernatant fraction) but this was not

359 confirmed by the results of the known-age sample. The mean value of the samples prepared
 360 using our current method is effectively identical with the mean of supernatant fraction within
 361 the uncertainty range; moreover, it also does not differ from the mean of the filtrate fraction.
 362 Unfortunately, we do not yet have sufficient measurements for statistical tests on the modern
 363 bone material.

364
 365 Table 1 Result of the analytical measurements performed on the bone samples included in the
 366 ultrafiltration tests. The uncertainty for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements are ± 0.2 and ± 0.3 ‰,
 367 respectively.

Sample code	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	%C	%N	C/N	F ¹⁴ C	unc.
ProBl_3 MLM	-21.7	10.2	50.3	14.4	4.0	0.0043	0.0013
ProBl_3A UFA	-21.7	10.3	43.0	13.0	3.9	0.0063	0.0008
ProBl_3A UFB	-21.8	10.4	46.3	13.6	4.0	0.0031	0.0008
ProBl_3B UFA	-21.7	10.2	44.2	12.6	4.1	0.0048	0.0008
ProBl_3B UFB	-21.7	10.4	45.6	15.7	3.4	0.0049	0.0008
K-Age MLM	-20.7	6.2	44.8	13.5	3.9	0.4286	0.0022
K-AgeA UFA	-20.7	6.5	45.1	15.4	3.4	0.4294	0.0019
K-AgeA UFB	na	Na	na	na	na	0.4348	0.0020
K-AgeB UFA	na	Na	na	na	na	0.4325	0.0018
K-AgeB UFB	-20.8	6.5	42.7	13.9	3.6	0.4282	0.0016
Modern MLM	-18.1	3.2	44.9	15.2	3.0	1.0175	0.0012
ModernA UFA	-17.8	3.2	46.2	15.8	3.4	1.0131	0.0030
ModernA UFB	na	Na	na	na	na	1.0088	0.0034
ModernB UFA	-17.9	3.4	41.9	15.5	3.2	1.0177	0.0029
ModernB UFB	-18.0	3.3	38.3	14.7	3.0	1.0109	0.0031
IAEA-C7 Nominal	-14.5	Na	19.7	na	na	0.4953	0.0012
IAEA-C7A UFB	-14.7	Na	18.0	na	na	0.5016	0.0024
IAEA-C7B UFB	-14.7	Na	18.9	na	na	0.4923	0.0018
IAEA-C8 Nominal	-18.3	Na	19.7	na	na	0.1503	0.0017
IAEA-C8A UFB	-18.5	Na	18.3	na	na	0.1537	0.0012
IAEA-C8B UFB	-18.5	Na	19.8	na	na	0.1529	0.0012

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 369 If we look at the mean F¹⁴C value of the filtrate fraction of IAEA-C7 standard samples,
 370 we can see a value of **0.4969±0.0066** that is slightly higher than the nominal value
 371 (0.4953±0.0012) but is still within 2σ. These samples were measured in different measuring
 372 batches, thus the higher SD in an indication of the external error. The IAEA-C8 standard
 373 samples gave a mean value of **0.1533± 0.0006**, which is 0.003 higher than the nominal value
 374 (0.1503±0.0017), indicating a small modern effect during the filtration process. The results of
 375 some other studies showed that ultrafiltration did not yield statistically different ages in
 376 comparison to the values obtained for subsamples treated by the Lognin method. It appears
 377 that the ultrafiltration method has reached a point where sample size and AMS precision are
 378 no longer the limiting factors; rather, human error during pretreatment and sample handling
 379 plays a key **role in delivering the expected accuracy of bone ¹⁴C dating (Fülöp et al., 2013).**
 380 **Our results measured for our test bones of different radiocarbon ages also show that the mean**
 381 **values for both the modified Longin and the ultrafiltration gelatin extraction methods are**
 382 **indistinguishable,** but we will continue our tests, involving bones having bad preservation or
 383 low collagen yield. The differences in ages obtained (at 1σ level) could be due to procedural
 384 factors, which has been overlooked, or AMS measurement variability, as we have seen in the
 385 case of background and known-age sample measurements.

386 387 Conclusions

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In the last two and a half years, numerous procedural blank and known-age bones were prepared and measured at HEKAL. In this paper, we tried to evaluate mathematically the results and in addition, an ultrafiltration protocol was also tested. In the first section, we showed that our internal procedural blank samples give a background of around 0.005 F¹⁴C (~42.500 BP) or even better due to the efficient bone pretreatment protocol. A good reproducibility was confirmed by the results of internal laboratory known-age bones as well, where the standard deviation of the sample is better than 0.0025 in F¹⁴C. We presume that any variation is probably caused by natural variability during the sample pretreatment. We have also demonstrated that the actual date of a sample with a Holocene age becomes slightly older after blank correction.

In the second section, we evaluated a new ultrafiltration protocol, which was inserted into our bone processing protocol. The selected bones of different ages were processed in parallel using our modified Longin and ultrafiltration preparation methods, but no notable differences were obtained in 2σ uncertainty range. The results suggest that our modified Longin method remove properly the contamination out of the selected bones but we will continue our tests, involving bones having bad preservation or low collagen yield.

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