^{14}C INTERCOMPARISON EXERCISE ON BONES AND IVORY SAMPLES: IMPLICATIONS FOR FORENSICS

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ABSTRACT. The application of accelerator mass spectrometry radiocarbon (AMS ^{14}C) dating in forensics is made possible by the use of the large excursion of the ^{14}C concentration in the post-WWII terrestrial atmosphere due to nuclear testing as a reference curve for data calibration. By this approach high-precision analyses are possible on samples younger than ~70 years. Nevertheless, the routine, widespread application of the method in the practice of forensics still appears to be limited by different issues due to possible complex interpretation of the results. We present the results of an intercomparison exercise carried out in the framework of an International Atomic Energy Agency (IAEA) CRP-Coordinated Research Project between three AMS laboratories in Italy, Hungary, and Switzerland. Bone and ivory samples were selected with ages spanning from background (>50 ka) to 2018. The results obtained allow us to assess the high degree of reproducibility of the results and the remarkable consistency of the experimental determinations.

KEYWORDS: bomb-peak dating, bones, forensics, intercomparison.

INTRODUCTION

Besides the traditional applications of radiocarbon (^{14}C) dating in archaeology, geochronology, and cultural heritage, different studies have shown the potential of the method in the forensic sciences. In this field, typical applications require the analysis of "recent" samples (at least in the ^{14}C timescale), typically younger than 60–70 years, and high chronological resolutions of the order of a few years or better. Generally speaking, this is beyond the possibilities of ^{14}C-dating which has, at best, resolution of the orders of decades. Indeed, the chronological resolution achievable by ^{14}C-dating is determined by the combination of the uncertainty associated with the measurement of the ^{14}C concentration in the samples (*instrumental precision*) and the effect of its calibration into calendar ages through the calibration curve. Furthermore, while instrumental precision levels of the order of 0.3% in ^{14}C/^{12}C ratio measurement are nowadays routinely achieved, the limitations related to the calibration procedures cannot be easily overcome as they are related to global-scale or even extraterrestrial complex phenomena. And if it is true that higher resolutions, even at the annual level, have been recently shown by using large and rapid excursions of the ^{14}C atmospheric concentrations, they are limited to very particular situations in terms of sample material and time ranges (Wacker et al. 2014; Kuitems et al. 2020).

An example of the negative impact of the flat shape of the calibration curve on the achievable resolution is seen when samples 70–300 years old are dated. This is due to the fluctuations of the atmospheric radiocarbon concentration induced by anthropogenic causes, which make the ^{14}C calibration curve flat in the period between the end of the 16th century and the middle of the 20th

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century. In other words, the uncertainty associated with ^{14}C ages of samples falling in this range can be as large as three centuries.

The possibility to use ^{14}C-dating in forensics is based on the detection of the excess of ^{14}C released into the atmosphere by aboveground nuclear detonations tests carried out from the mid-1950s (Hua et al. 2013). The curve representing the concentration of ^{14}C in the atmosphere, often referred as the "bomb spike" or "bomb curve," is very well known and reconstructed with high resolution through the analysis of terrestrial proxy records such as tree rings (Nydal 1968; Levin and Hesshaimer 2000; Quarta et al. 2005; Levin et al. 2010; Hua et al. 2013) or even by direct atmospheric CO_2 sampling in different locations around the globe. The curve has a steep rising part starting from 1955, reaching a maximum in 1964 CE (at least in the Northern Hemisphere) and then declining to the current level where it is approaching the pre-bomb values. Though the detailed analysis of the shape of the curve is beyond the aims of this paper, we recall here that in 1964 the ^{14}C concentration had almost doubled the pre-bomb value (Hua et al. 2013). After the 1963 ban on atmospheric nuclear tests (the Partial Nuclear Test Ban Treaty), the excess of ^{14}C produced started to decline as it was distributed into different reservoirs such as the hydrosphere and the biosphere. It is also worth mentioning the effect of dilution of the ^{14}C concentration due to the increasing release into the atmosphere of ^{14}C-free carbon dioxide from fossil industrial sources over the same time (Graven 2015).

In forensics, this curve can be used as reference to "calibrate" to calendar years the measured radiocarbon concentrations in a sample. The potential of bomb-spike ^{14}C-dating has been shown by several studies in different fields ranging from medicine (Spalding et al. 2008; Rinyu et al. 2020), the fight against illicit drugs (Zoppi et al. 2004), and contemporary art authentication (Caforio et al. 2014; Brock et al. 2019; Hendriks et al. 2019), which resulted also in a remarkable commitment of the ^{14}C community (Hajdas et al. 2019). In particular, ^{14}C-dating in forensic anthropology has been used at different levels of complexity, from the simple identification of the forensic interest of human remains to the estimation of the year of death or birth supplying information relevant, for example, for the identification of missing persons (Handlos et al. 2018). Indeed, studies have shown how the proper selection and analysis of tissues with different times of (radio)carbon fixation and different turnover rates increases the obtainable information in terms, for instance, of the achievable chronological resolution (Spalding et al. 2005; Cook et al. 2006; Calcagnile et al. 2013; Brock and Cook 2017). Also, in wildlife forensics, ^{14}C bomb-spike dating is extensively used to identify samples or artifacts of endangered species illegally imported by violating international trade bans or national laws. This is the case of the fight against illegal elephant ivory trade and of the legal enforcement of CITES, the Convention on International Trade in Endangered Species of Wild Fauna and Flora. In this case ^{14}C-dating is the only method available to date, for instance, ivory, so it can be established whether it was obtained before or after the trade ban (Cerling et al. 2016; Quarta et al. 2019; Wild et al. 2019).

Nevertheless, despite the significant advantages of the method, such as the low sensitivity to external, environmental, and context factors, it does not appear to be fully exploited in routine forensics practice. This is probably due to the need of dedicated laboratories, the expertise required in data analysis and interpretation and, partially, to the lack of a full awareness of the potential of the method among forensics professionals.

We present the results of an intercomparison exercise run under the auspices of the International Atomic Energy Agency (IAEA) and in the frame of the Coordinated Research Projects (CRP): *Enhancing Nuclear Analytical Techniques to Meet the Needs of*

Table 1   List of the samples.

Sample ID	Sample description	Expected age
Sample #1	Mandible of a woolly mammoth	Background (>50 ka)
Sample #2	Tibia fragment of a Eurasian aurochs	~6800 yr
Sample #3	Ivory sample A	Pre-bomb
Sample #4	Ivory sample C	Post-bomb
Sample #5	Ivory sample B	Post-bomb
Sample #6	Modern pig leg	Sampled in 2018

Forensic Sciences, which has a work-package (WP4) dedicated to accelerator mass spectrometry (AMS) ^{14}C dating. The intercomparison exercise was performed on bone and ivory samples, which are among the sample materials more commonly involved in forensics for issues related to the analysis of skeletal remains in forensic anthropology and the fight against the trade of ivory obtained from endangered species.

The aim of the study was to show the level of achievable precision and the degree of accuracy of the results for the analysis of samples of forensics interest. It is worth mentioning that bone samples were included in different international intercomparison exercises (Scott et al. 2017) and the subject of dedicated studies (Huels et al. 2017) because the effective treatment of bones is of an interest to archaeology (Rubinetti et al. 2020). Here, however, we concentrate mainly on modern samples and an issue relevant in forensic studies.

MATERIALS AND METHODS

The presented study was designed to check the reproducibility of the measurements obtained at the three participating laboratories: CEDAD-Centre for Applied Physics, Dating and Diagnostics at the University of Salento, Lecce, Italy; the Laboratory of Ion Beam Physics at the ETH in Zurich, Switzerland; and the HEKAL-Hertelendi Laboratory of Environmental Studies, Institute for Nuclear Research, Debrecen, Hungary. The laboratories used different procedures to chemically process the samples, as better detailed in the following, and are based on different AMS instruments. In particular, HEKAL and ETHZ are based on a MICADAS (Mini Carbon Dating System) AMS system operated at 200 kV, while CEDAD is based on a 3 MV Tandetron^{TM} type accelerator.

Sample Material

Six bone and ivory samples were selected for this study as summarized in Table 1. The samples were selected to cover a chronological range spanning from background (older than 50 ka) to 2018. This was done to assess the level of background for the three laboratories and the measurement reproducibility for both "archaeological" samples and materials of potential forensics interest. Sample #1 was obtained at HEKAL from a mandible of a woolly mammoth (*Mammuthus primigenius*) found during a cleaning campaign, most probably during the second half of the 19th century, of the bed of the river Tisza (Major et al. 2019). This sample was included to assess the blank level of the three laboratories and its reproducibility. The sample is expected to have an age beyond the limit of the radiocarbon dating method (>50 ka) and should be completely depleted of ^{14}C. Any measured level will then give a direct estimation of chemical processing and machine induced background. Sample #2 was a fragment of a Eurasian aurochs (*Bos primigenius primigenius*) already

dated at HEKAL as part of a previous study to 6862 ± 40 BP, corresponding to a blank corrected ^{14}C concentration of $F^{14}C = 0.4286 \pm 0.0022$ (Major et al. 2019).

Ivory samples #3, #4, and #5 were submitted in the last three years to CEDAD at the University of Salento to be ^{14}C dated and assess whether they were imported in Europe in violation of the CITES treaty. Sample #3 was taken from a rhino horn while #4 and #5 were elephant ivory. According to the information supplied by customers, sample #3 was expected to be "pre-bomb" whereas for the two samples #4 and #5 an age after 1955 CE was expected. The three samples were already dated at CEDAD in 2016, and the measured ^{14}C contents resulted to be $F^{14}C = 0.9859 \pm 0.0055$, 1.5584 ± 0.0055, and 1.11322 ± 0.0052, for #3, #4, and #5, respectively. Sample #6 was a modern pig leg bone (*Sus scrofa domestica*) purchased in a local shop in Debrecen, Hungary, in 2018.

From each of the above-mentioned samples three pieces were obtained with masses ranging from 400 to 600 mg. The 18 samples were then distributed among the three participating laboratories once made anonymous. No information was supplied about the expected ages.

Sample Processing

The 18 samples were processed according to the different procedures in use in the three different laboratories and ^{14}C content measured by using the different instruments as detailed in the following.

CEDAD Sample Processing

Collagen was extracted from bone and ivory samples according to Longin (1971) and then dried and vacuum-sealed in pre-evacuated quartz tubes together with CuO and silver wool. Sample material was then combusted to carbon dioxide at 900°C for 4 hr. Released CO_2 was cryogenically purified and then reduced to graphite at 600°C by using H_2 as reducing agent and 2 mg Fe powder as catalyst (D'Elia et al. 2004). All the samples yielded an optimal quantity of ~1 mg of graphite which was then pressed in the aluminum cathodes of the AMS system (3 MV Tandetron^{TM} Mod. HVEE 4130HC) for the measurement of the isotopic ratios (Calcagnile et al. 2019). Measured $^{14}C/^{12}C$ ratios were then corrected for mass fractionation by using the $\delta^{13}C$ term measured online with the AMS system and for machine and chemical processing background. Uncertainty in measured isotopic ratios was calculated by considering both the scattering of the 10 repeated determinations performed on each sample and the radioisotope counting statistics (Calcagnile et al. 2005).

HEKAL Sample Processing

At HEKAL, all samples were processed in the same way using a procedure standardized for collagen extraction. Briefly, bone fragments are first ground and sieved to get the adequate size fraction of 0.5–1.0 mm. For chemical pretreatment, 600 mg of the ground grains is placed into special designed Omnifit™ glass columns, which can then be attached to a semi-automatic ABA cleaning system (Molnár et al. 2013). Subsequently, the residual collagen is transferred into a test tube containing 5 mL of pH 3 aqueous solution, and it is put into a block heater at 75°C for 24 hr. Dissolved gelatin is then filtered via a 2-µm glass fiber filter (Milles AP20) into a vial precleaned by nitrogen gas, and after freezing, it is freeze-dried for at least 2 days. Approximately 4–5 mg of gelatin together with MnO_2 reagent is then weighted in a mutual borosilicate tube for a modified sealed-tube combustion method. After sealing, the tubes are placed in a muffle furnace at 550°C for at least 12 hr. The CO_2

produced is then purified from any other by-product gases and quantified using a dedicated vacuum line (Janovics et al. 2018). For ^{14}C dating by AMS, graphite targets from the purified CO_2 samples are prepared using a customized sealed tube graphitization method (Rinyu et al. 2013). The ^{14}C measurements reported below were performed using the EnvironMICADAS AMS instrument at HEKAL (Molnár et al. 2013b). The overall measurement uncertainty for modern samples is < 3.0‰, including normalization, background subtraction, and counting statistics. The conventional ^{14}C ages were evaluated by the "Bats" software package (version 3.66; Stuiver and Polach 1977; Wacker et al. 2010a).

ETHZ Sample Processing

The ETH preparation of bones involves an ultrafiltration step, which follows the standard extraction and purification of collagen (Hajdas et al. 2007, 2009). The bones are first placed in MilliQ water and washed using an ultrasonic bath. This step of 15 min is repeated until the water is clear. The bones are then dried and crushed in a steel mortar. The powder is sieved and only fraction <710 μm is taken for preparation. The small portion (4–5 mg) of powder is weighed for combustion using Elemental Analyzer (Elementa varioMICRO) for the estimation of C% and N%. Samples with N%> 1% are considered for preparation, which then follows the protocol in Hajdas et al. (2009). Briefly, 500–1000 mg of powder is placed in 50 mL Falcon© tubes and is treated with acid (40 mL, 1 M HCl, RT) for 20 min. After rinsing, a base wash is applied (40 mL, 0.1MNaOH, RT) for 30 min. Following the rinse, a gelatinization takes place at 100°C in 10–15 mL of 0.001M HCl, for at least 17 hr.

The resulting solution is transferred into the Millipore precleaned UF tubes (30 kDa) using a syringe and Millex Glasfaser filter. The cleaning of UF filters follows the protocol of Brock et al. (2007). The gelatin is centrifuged at 4400 rpm until through and the fraction >30kDa is transferred to the 15-mL Falcon© tubes and frozen prior to freeze drying. Around 3–3.5 mg of dry gelatin was then weighed into Al cups for EA combustion in the AGE system (Wacker et al. 2010b). The graphitized samples were then analyzed using the MICADAS system at ETH (Synal et al. 2007).

RESULTS AND DISCUSSION

The results obtained for the 18 samples in the three laboratories are shown in Table 2. Figure 1a shows the results obtained for sample #1, which was expected to give an infinite age. We can see that the three laboratories gave consistent results overlapping within one standard deviation and corresponding to an average value of 0.00425 ± 0.0002 corresponding to 43.9 ka in the radiocarbon timescale. The measured ^{14}C content is in agreement with previous determinations carried out at HEKAL on a large number of subsamples obtained from the same material (n = 60) and giving a $F^{14}C=0.0041 \pm 0.0013$. The results obtained are then a strong indication of good reproducibility and the capability of the three laboratories to keep the procedural blank under control also when the complex collagen extraction procedure is involved.

Remarkable agreement at the one standard deviation level is obtained among the results also for sample #2 (Figure 1b). In this case, the average ^{14}C age is 6830 ± 32 BP where the uncertainty is calculated as standard deviation of the three measured values. This value is consistent at the 1σ level with the expected age of 6862 BP, measured at HEKAL in a previous study involving a much larger set of samples.

Table 2   Measured ^{14}C concentrations. Conventional ^{14}C ages are given between bracket when positive. Quoted uncertainties refer to one standard deviation confidence level.

Sample	F^{14}C and ^{14}C age (BP) (CEDAD)	F^{14}C and ^{14}C age (BP) (HEKAL)	F^{14}C and ^{14}C age (BP) (ETHZ)
Sample #1	0.0044 ± 0.0006	0.0043 ± 0.0005	0.0040 ± 0.0007
	(43,499 ± 1050 BP)	*(43,773 ± 912 BP)*	*(44,354 ± 1415 BP)*
Sample #2	0.4272 ± 0.0013	0.4256 ± 0.0017	0.4290 ± 0.0015
	(6832 ± 25 BP)	*(6863 ± 30 BP)*	*(6798 ± 29 BP)*
Sample #3	0.9859 ± 0.0055	0.9843 ± 0.0023	0.9850 ± 0.0028
	(114 ± 45 BP)	*(127 ± 18 BP)*	*(121 ± 23 BP)*
Sample #4	1.5584 ± 0.0055	1.5466 ± 0.0038	1.5530 ± 0.0041
Sample #5	1.1322 ± 0.0052	1.1621 ± 0.0025	1.1570 ± 0.0032
Sample #6	1.0110 ± 0.0045	1.0176 ± 0.0031	1.0110 ± 0.0028

Table 3 Comparison between the radiocarbon concentrations measured at CEDAD in 2016 and 2019 for samples #3–5.

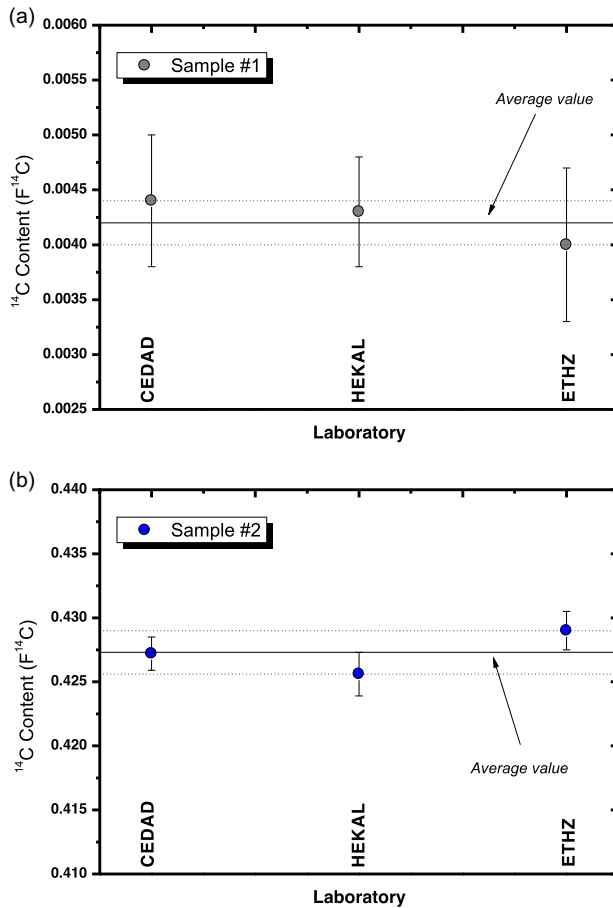| Sample	F^{14}C measured in 2016	F^{14}C measured in 2019
Sample #3	0.9859 ± 0.0055	0.9755 ± 0.0042
Sample #4	1.5584 ± 0.0055	1.5516 ± 0.0055
Sample #5	1.1322 ± 0.0052	1.1288 ± 0.0055

Figure 1 ^{14}C concentrations measured for sample #1 (a) and sample #2 (b).

Samples #3–5 were already measured at CEDAD in 2016 and it was possible to make a comparison with previous results (Table 3). It can be seen that the values measured in 2019 agree within one standard deviation with previous measurements pointing towards a good repeatability of the measurement over time. The results of the intercomparison measurement on the last four samples are shown in Figure 2. For sample #3, all the laboratories measured consistent results corresponding to a combined pre-bomb age of 124 ± 14 yr BP and a relative uncertainty (calculated as standard deviation of the three
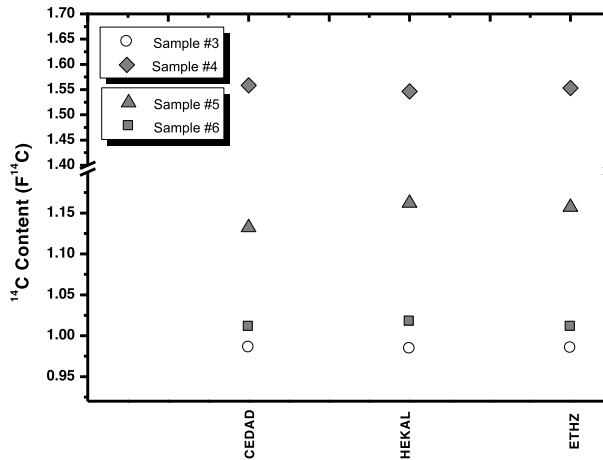
Figure 2 ^{14}C concentrations measured for samples #3–5 (error bars smaller than symbol sizes).

measured values) of 0.8‰. In order to highlight the impact of calibration on the final uncertainty on the calendar age in periods where the curve shows plateaus (such as from the end of the 16th century to 1950 CE), we show that the even the measured uncertainty of only 14 years on ^{14}C age would correspond to two possible intervals: 1686–1732 and 1806–1927. This demonstrates the limitations in resolution achievable by ^{14}C-dating in this time range. Also, for sample #4 the results are fully consistent with an average post-bomb value of 1.5527 and a relative scattering of 0.37%.

For ivory sample #5 the difference between the values measured by the laboratories seems larger than for the other samples and ranges from 1.1322 ± 0.0052 to 1.1621 ± 0.0025. A possible explanation for this may be variations of the radiocarbon concentration due to different time of formation and carbon fixation within the ivory. Indeed, the two values of 1.1322 ± 0.0052 to 1.1621 ± 0.0025 would correspond (Figure 3) to a dating to 1957 or 1957/1958 (then giving consistent ages) or to 1989–1990 and 1990–1994 with a difference of only two years which can be easily explained considering the dimension of the analyzed sample (6–7 cm) and the typical growing rate of elephant tusk of ~4 cm/yr (Uno et al. 2013).

Consistent results are obtained also for sample #6 (a modern pig leg) for which an average value of 1.0132 ± 0.0038 is obtained. This value shows a ^{14}C atmospheric level approaching pre-bomb values and it is in good agreement with the value extrapolated for 2018 from monthly mean atmospheric values measured for the Northern Hemisphere (Hammer and Levin 2017).

A more detailed statistical analysis of the results was performed by using the z-score which give information about precision and accuracy of the results. For this reason, a z-score was calculated for each measurement as:

$$z = \frac{x_m - \overline{x_m}}{\sigma_p}$$

where x_m is the measured value for a single laboratory, $\overline{x_m}$ is the corresponding average value and σ_p the associated uncertainty as reported by each laboratory. It is assumed that z should be
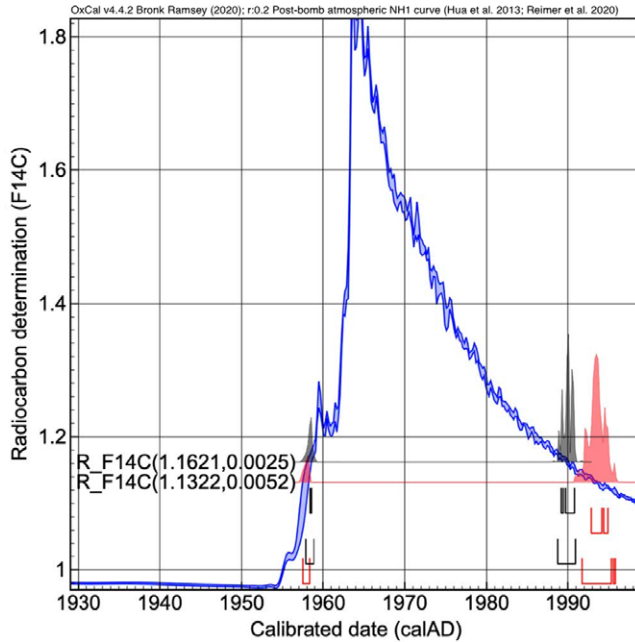
Figure 3   Calibration obtained with the software OxCal v4.4 and the Northern Hemisphere zone 1 bomb curve of the measured ^{14}C concentrations for sample #5.
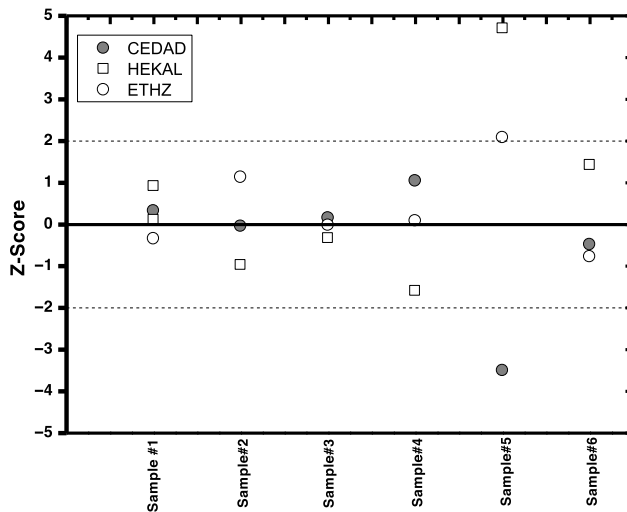

Figure 4   z-score scatter plot for all the analyzed samples and for the three participating laboratories.

normally distributed with zero mean and variance 1, where a z-score between –2 and +2 is considered as complying with fitness purposes (Scott et al. 2010). The scatter plot of z-scores is shown in Figure 4 for all the analyzed samples and for the three participating laboratories. It can be noted that all the values lie within the ±2σ band, indicating a good statistical

agreement between the results. The only exception is represented by the results obtained for the sample #5 of ivory for which a larger scattering of the data is observed. As it has been already discussed this is probably associated with a difference in the ^{14}C content from different parts of the ivory sample. The average Z value is very close to 0 (0.16) and the variance is 1.67 (which becomes 0.8 when the data obtained for sample #5 are removed from calculations), pointing towards a very good consistency between the data obtained by the three laboratories.

CONCLUSIONS

In the framework and under the auspices of IAEA, an intercomparison exercise was designed and run to check the level of reproducibility achievable in the AMS ^{14}C dating of ivory and bone samples of forensics interest. Six samples were selected with ages ranging from background to 2018 and sent to the three participating laboratories. Analysis of the results supports a high level of reproducibility of the measurements, which resulted to be consistent with expected results. The scatter of the data shows that uncertainty levels of 0.1–0.3% are routinely achieved in ^{14}C dating. It is important to note that our intercomparison study has been performed on well-preserved bones, and conclusions do not necessarily apply to all archaeological samples. Further tests are in progress on other materials of forensics interest, such as paper, paintings, food, and bio-based products of industrial interest.

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