# 4 Establishing $^{14}C$ dating at MPI-EVA

In this thesis I focus on the pretreatment of bone to obtain pure collagen and convert it into graphite for AMS measurements to obtain reliable radiocarbon dates. The individual steps of pretreatment involve extraction of collagen from bone, cleaning all the equipment used in the procedures and the conversion of collagen to graphite (graphitization). I chose to use the extraction method (method C in chapter 6; paper (Talamo and Richards, 2011)) that best avoids lab contamination. In the field I collected good quality bone samples, which were selected due to their potential for high carbon yields.

All the bone samples presented in this thesis were subject to the following pretreatment procedures, usually in batches of up to 12 samples:

- Entry in database
- Pulverisation of bone
- Decalcification
- Removal of humics
- Gelatinization
- Cleaning of the filters and checking for the removal of contamination
- Ultrafiltration
- Freeze drying

These procedures are outlined in detail below.

## *4. 1 Database entry*

A S-EVA number is assigned to the sample and it is inserted in our database. Important fields of the sample record are S-EVA number, submitter name, sample code assigned by the submitter, name of the project or site, weight of the sample as received and a photo. All the subsequent pretreatment steps are entered into the database.

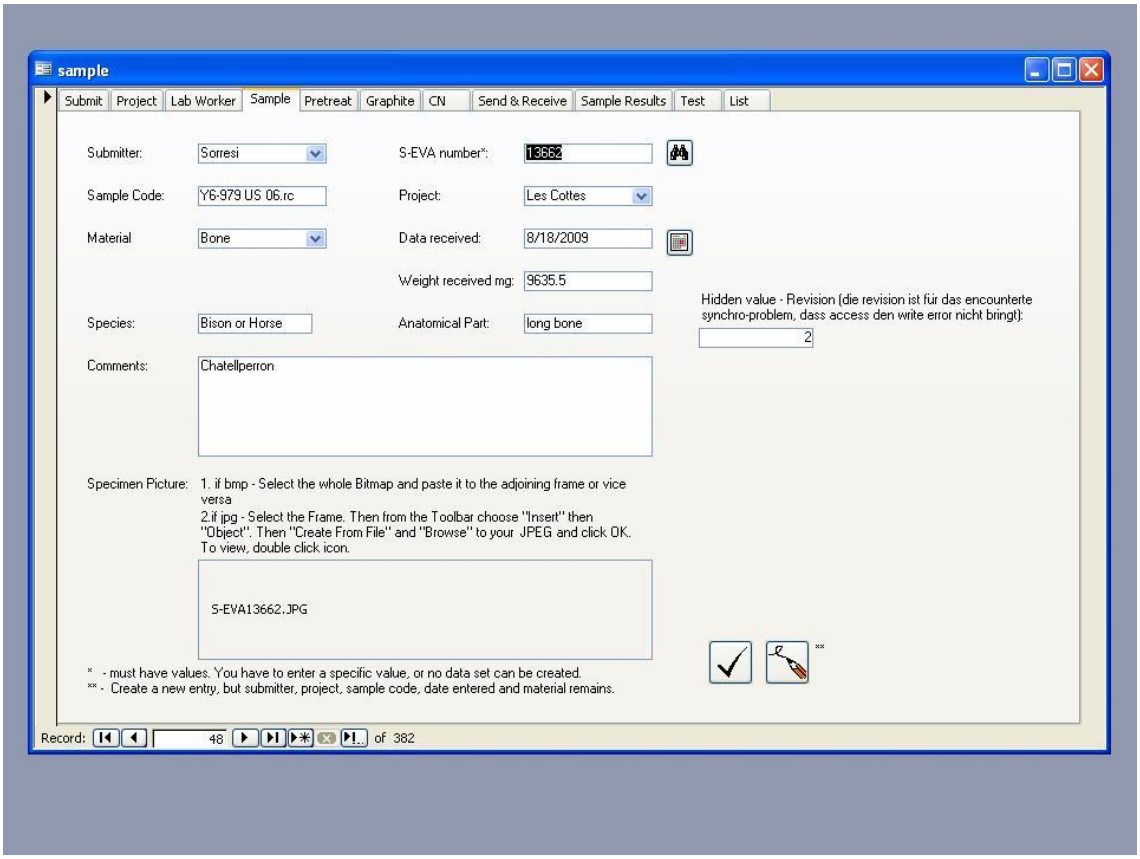


*Figure 4.1 Entry page of the database at MPI*

## *4.2 Pulverisation of bone*

The bone is first cleaned by sand-blasting and then, using a dental drill, 500 mg of bone powder is taken. In the case of bone fragments a mortar is used to grind the bone. Bone powder is essential for a fast and efficient decalcification.

## *4.3 Decalcification*

The sample is kept in 0.5M HCl at room temperature until no $CO_2$ effervescence is observed, which usually takes 4 hours. This interval is divided into two 2-hour segments after which the sample is rinsed in ultrapure water and centrifuged. Then it is kept overnight in 0.5 M HCl in a refrigerator.

## *4.4 Removal of humics*

The following day 0.1M NaOH is added for 30 minutes to remove humics, which could have been introduced to the bone sample by ground water during the interval between burial and excavation. The NaOH step must be complemented by a final HCl step (15 minutes), to remove potential contamination from modern $CO_2$ taken up by the NaOH.

### *4.5 Gelatinization*

The gelatinization step follows the method outlined in Longin (1971), at pH3 in a heater block at 75°C for 20h.

### *4.6 Cleaning of the filters and checking for the removal of contamination*

The cleaning procedures for the ultrafilters are essential for a valid radiocarbon date (Higham, et al., 2006b). The ultrafilters are Sartorius "Vivaspin 15" of 30 KDalton size with 50ml plastic centrifuge tubes. The cleaning is designed to remove carbon-containing humectants. It is very important not to clean the filters more than 24 hours in advance as they may soften or dry out. The ultrafilters are rinsed 5 times in the centrifuge with ultrapure water for 15 to 20 minutes. Then they are bathed in ca 1 liter of ultrapure water in the ultrasonic bath for one hour, and after that rinsed 3 times.

Before the $4^{th}$ centrifuge step, 1 ml of ultrapure water is added to one of the filters, and removed for analysis of remaining carbon. For this measurement the water sample is freeze-dried, another *ca*. 20 μl ultrapure water is added and inserted with *ca*. 8 mg chromosorb into a large tin capsule. The amount of carbon is determined by combustion in the EA (see step below). The burn yield must be below 5 to 10 μg C for this sample to indicate that the filter is not contaminated by carbon-containing humectants.

The Eeze-Filter™' (Elkay Laboratory Products (UK) Ltd.) is bathed for 20 minutes in *ca*.1 liter of ultrapure water.

### *4.7 Ultrafiltration*

The gelatine obtained in step 5 is filtered in the Eeze-filter to remove mineral particles. Then the liquid is transferred to the ultrafilter and centrifuged until the liquid in the filter is below 0.5 ml.

### *4.8 Freeze-drying*

The filtered sample is frozen to a solid. The tube is sealed with parafilm and kept in a -28°C freezer. The tubes are kept in an inclined position so that the solution is thinly distributed along the tube, with no more than 10 mm at the thickest part. The samples stay in the freezer for at least 12 hours so that they are solidly frozen. Then the samples are transferred to the freeze-drier and lyophilized for 48 hours.

The specific lab protocol procedures for each sample (Figure 4.2) is entered into the database (Figure 4.3).

## Collagen extraction protocol

S-EVA ☐ Notes_____

Date _____ Total Bone _____ mg

Sample taken _____ mg Rest _____ mg

Date_____ HCl for 2h ☐ HCl for 2h ☐ Fridge all night_____

Date_____ HCl for 2h ☐ Wash 3 times H2O ☐
NaOH 30min ☐ Wash 3 times H2O ☐
HCl 15min ☐ Wash 3 times H2O ☐

Date Heater in with 10ml of Ph3 _____ Time in _____

Date Heater out _____ Time out _____ 75°C for 20h

**Cleaning Eeze – Filter**

Date _____ 20 minutes in Ultrasonic water

**Cleaning Ultrafilter procedures**

Date_____

15 min centrifuge with H2O pure ☐
15 min centrifuge with H2O pure ☐
**1 Hour in Ultrasonic water ☐**
15 min centrifuge with H2O pure ☐
15 min centrifuge with H2O pure ☐
15 min centrifuge with H2O ☐

Date Centrifuge and Samples in the fridge _____

Date Freeze – dryer in _____ Time in _____

Date Freeze – dryer out _____ Time out _____

**Collagen mg** _____

Circa 0.5mg of Collagen for C/N _____

**Sent Collagen_____ mg Remaining Collagen at MPI _____mg**
**Sent Collagen to_____ Date_____**
**Graphite ☐ Sent graphite to_____ Date_____**

**Radiocarbon Age_____ Collagen back from the AMS Lab _____mg**

*Figure 4.2 Lab protocol with all the procedures made during the pretreatment*

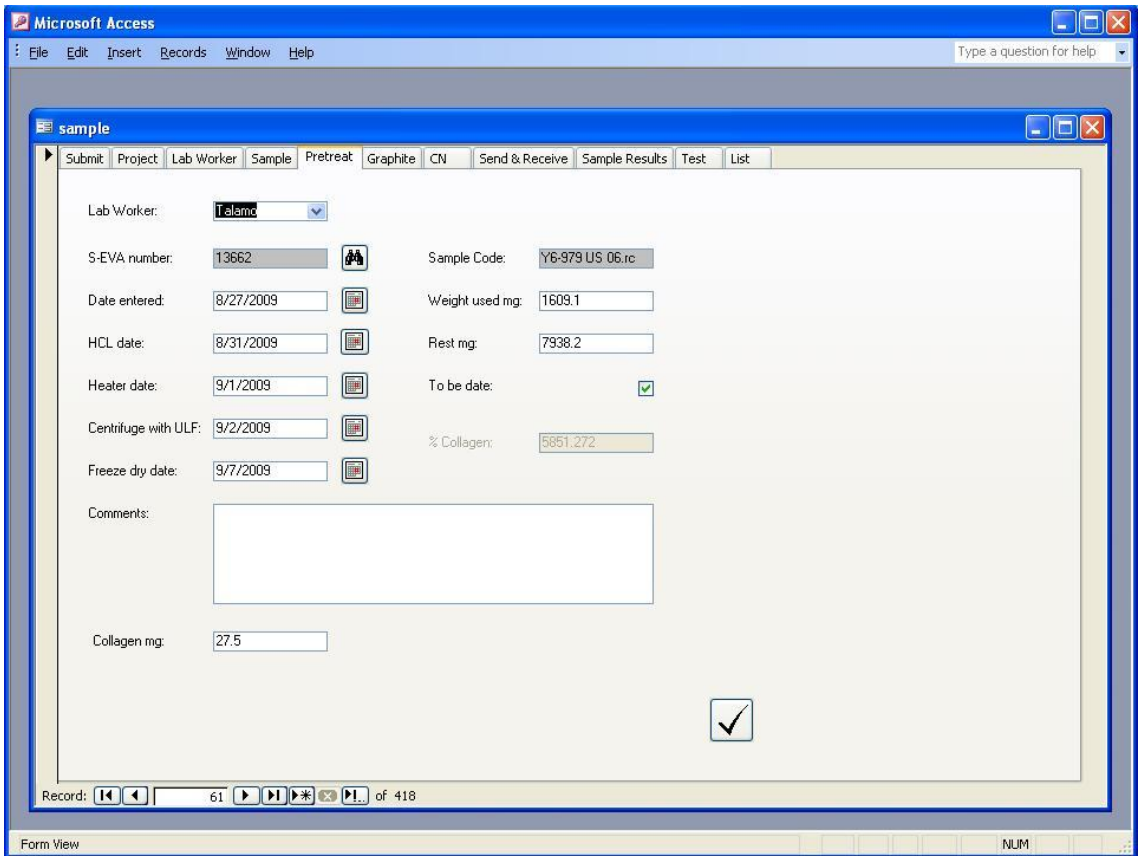


*Figure 4.3 Input of the lab protocol of the pretreatment and calculation of the % collagen*

Important parameters are the date of the various steps, the weight used and the final weight of the collagen. At this point the collagen yield is available, which should be a minimum of 5 mg for 500 mg initial bone powder (1% yield limit). The minimum amount of collagen for graphitization is 3 mg.

# 4.1 Graphitization steps

All the collagen obtained after the pretreatment outlined above is graphitized according to the following procedures:

- Loading of collagen into tin caps
- Combustion in an Elemental Analyser (EA)
- Determination of carbon yield and C:N ratio
- Determination of $\delta^{13}C$ and $\delta^{15}N$ in a mass spectrometer
- Cleaning the $CO_2$ gas containers and conditioning of the iron catalyst
- Collection of $CO_2$ in the rigs
- Addition of hydrogen

- Conversion of $CO_2$ into graphite in the graphitizer
- Check of graphitization parameters
- Preparation of blank samples
- Preparation of shipment to an AMS facility and submission to the AMS laboratory for radiocarbon measurement.

### *4.1.1 Loading collagen into tin caps*

The collagen is loaded into tin capsules, which are pre-cleaned in cyclohexane and acetone. An empty tin capsule is combusted to check that the blank contribution is < 2 µg C.

### *4.1.2 Combustion in Elemental Analyser (EA)*

The collagen is combusted in the EA (CHN analyzer) system, in a sequence of up to 10 samples limited by the amount of available gas containers. Each sample combustion is preceded by the combustion of an empty tin capsule to purge the system. The sample is injected into the furnace together with a stream of helium and oxygen. The combustion furnace is at a temperature of 1000°C and with the addition of tin the combustion temperature reaches 1500°C; the subsequent reduction furnace is used to complete the combustion at 600 °C (Figure 4.4).

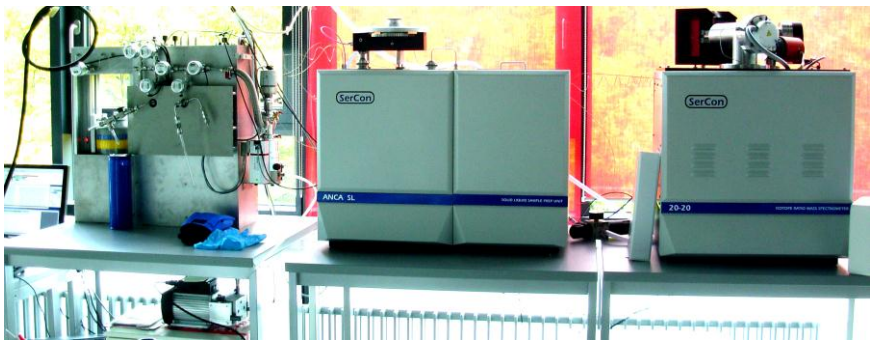


*Figure 4.4 Elements of the graphitization: combustion in the EA (middle), $\delta^{13}C$ and $\delta^{15}N$ determination in the mass spectrometer (right) and the graphitizer (left).*

The helium acts as carrier gas. The combustion products are sent through several gas chromatographic columns (GC) to purify and to separate the components of interest, nitrogen (N), $CO_2$ carbon (C), and hydrogen (H). This information is recorded by the EA software (Figure 4.5).

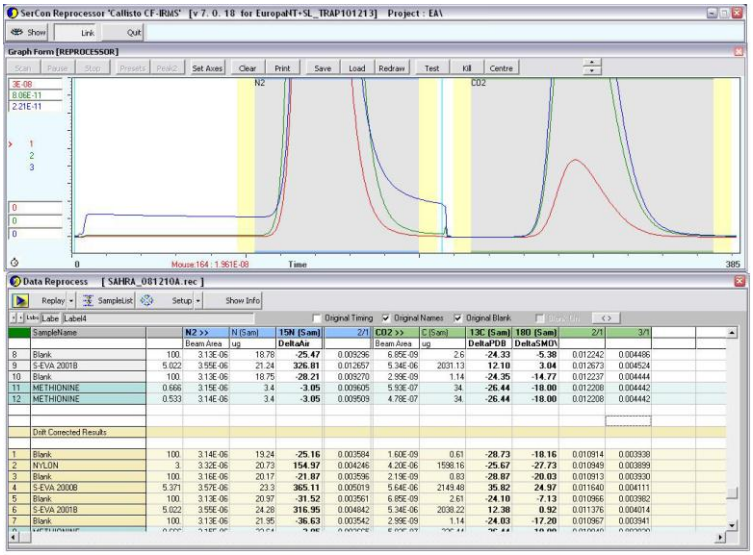


*Figure 4.5 Protocol of the elemental analyser. The peaks represent the separation of C ($CO_2$) and N*

### *4.1.3 Determination of carbon yield and C:N ratio*

After the successful combustion of a sample the key parameters for quality control of bone collagen are available. These are the amount of carbon and nitrogen in the sample, which is used to determine the C:N ratio, These data and isotopic data from the next step are then entered into the database (Figure 4.6)

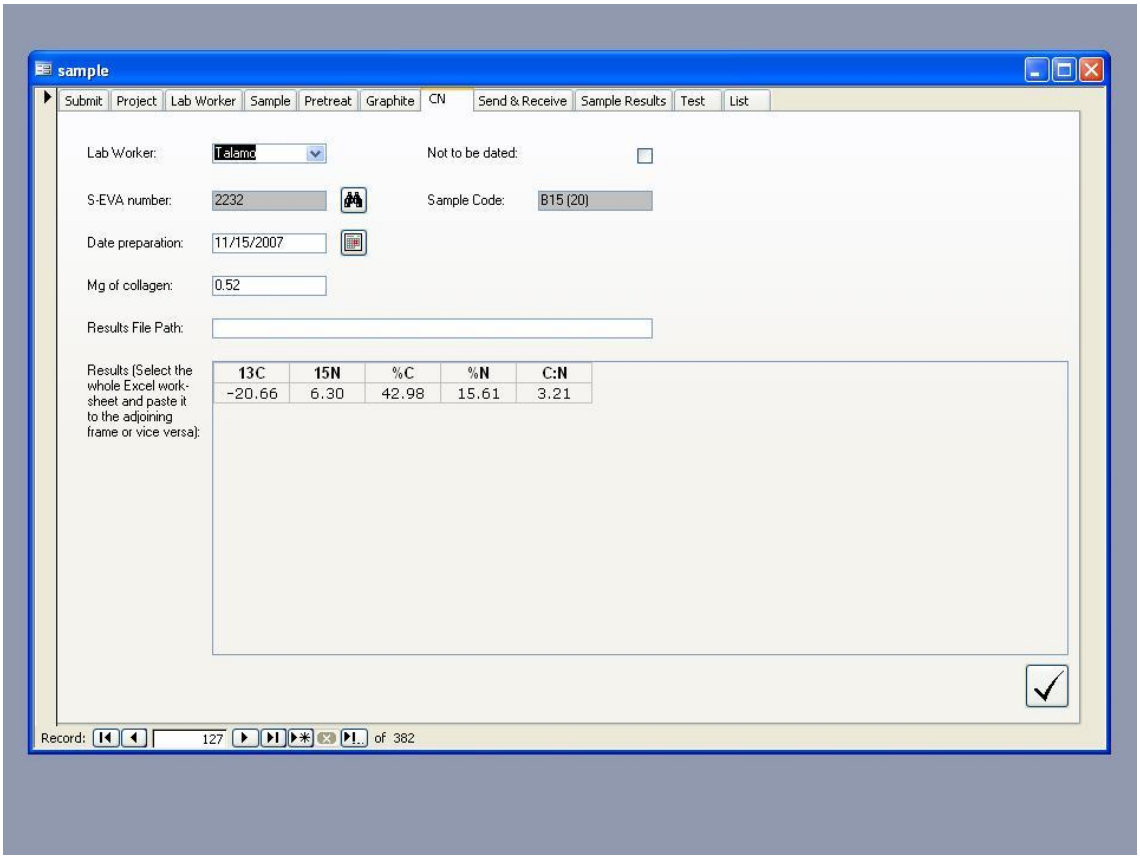


*Figure 4.6 Input of isotope data into the database*

### *4.1.4 Determination of $\delta^{13}C$ and $\delta^{15}N$ in a mass spectrometer*

A small fraction, approximately 1%, of the purified gases are sent to the mass spectrometer (Figure 4.7), connected to the EA, to measure the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) (Table 4.I).

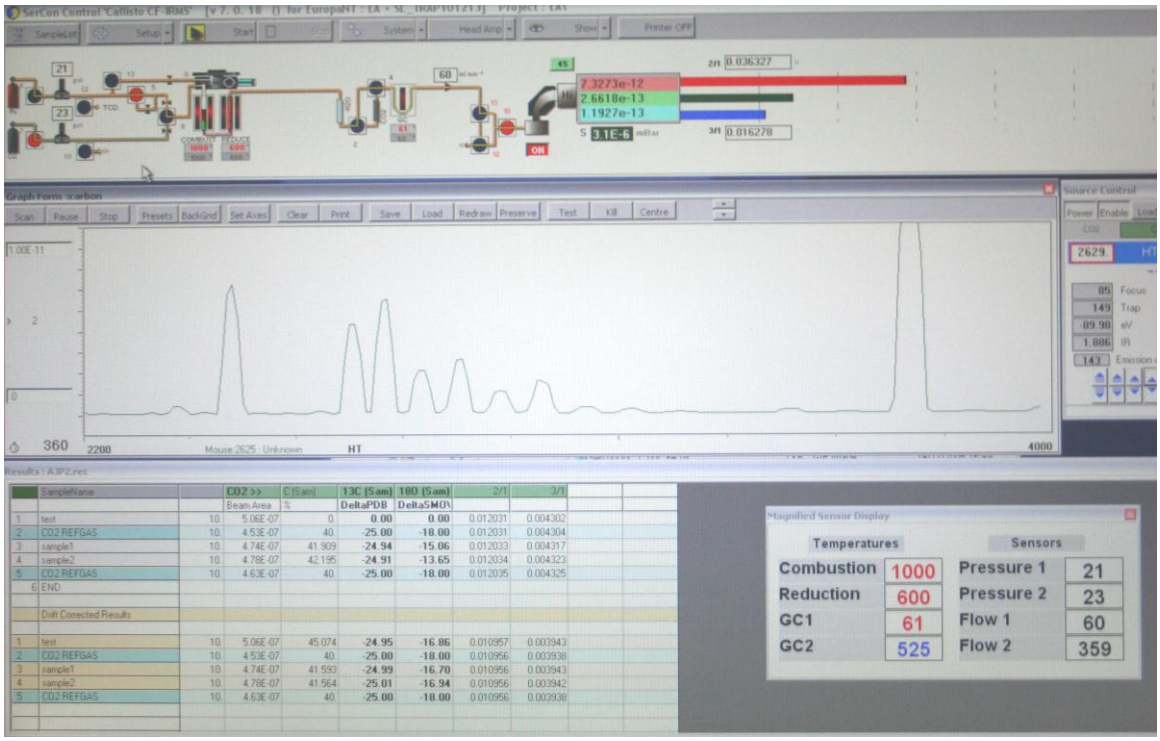


*Figure 4.7Output page of the mass spectrometer for $\delta^{13}C$.*

*Table 4.I Example of a determination of stable isotope $^{13}C$ and $^{15}N$ for the reference material of Nylon 66*

| **Name** | **Weight/Vol** | **$^{13}C$ (Sam)** | **$^{15}N$ (Sam)** | **C** | **N** | **C:N** |
|---|---|---|---|---|---|---|
| | **mg** | **DeltaPDB** | **DeltaAir** | **%** | **%** | |
| EVA 0008 Nylon | 4.092 | -29.5 | 1.6 | 60.90 | 11.80 | 6.02 |
| EVA 0008 Nylon | 4.802 | -29.5 | 1.6 | 60.90 | 11.80 | 6.02 |
| Nylon 66 B2 Sample | 4.708 | -29.5 | 1.2 | 60.81 | 11.85 | 5.99 |
| Nylon 66 B3 Sample | 4.745 | -29.5 | 1.2 | 60.83 | 11.80 | 6.01 |
| | **average** | -29.54 | 1.52 | 60.86 | 11.83 | |
| | **stdev** | 0.04 | 0.24 | 0.05 | 0.03 | |

### *4.1.5 Cleaning the $CO_2$ gas containers and conditioning of the iron catalyst*

The $CO_2$ gas containers are glass tubes closed by metal valves, called rigs (Figure 4.8).

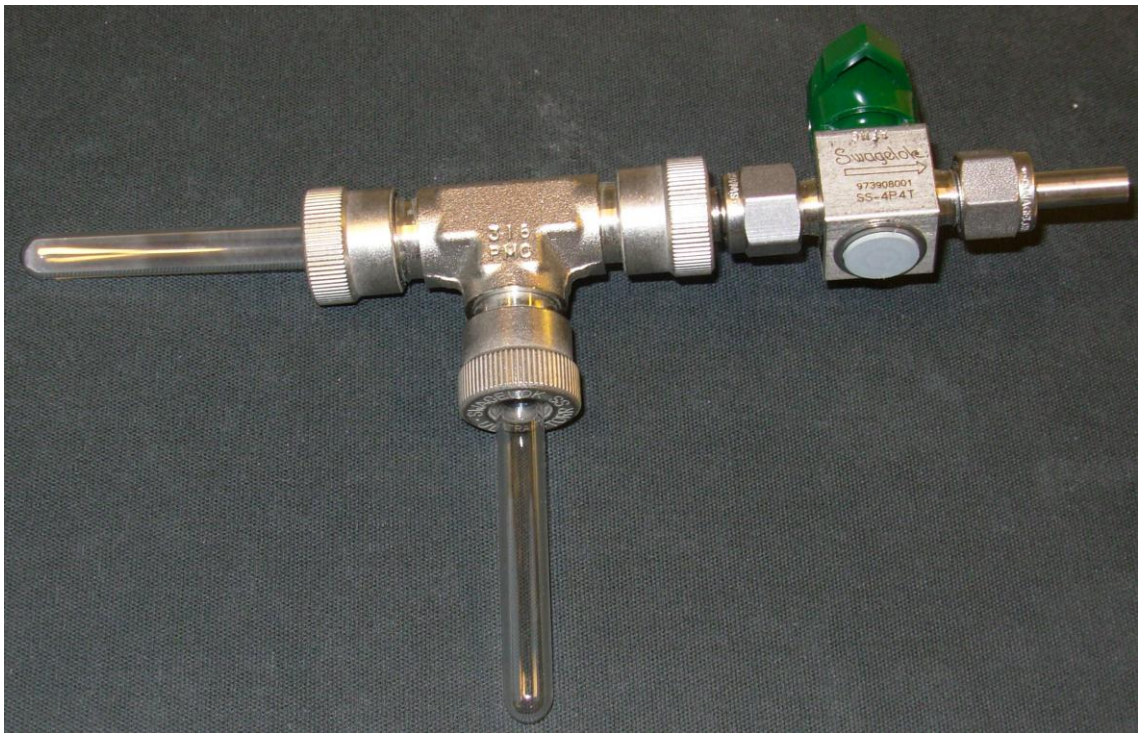


*Figure 4.8 $CO_2$ gas container (rig) filled with iron catalyst.*

The rigs are filled with 1.5 to 3 mg iron catalyst (Aldrich Chem. Co. <10micron 99.9%) and the optimal ratio of iron to carbon was determined to be 3 to 1 (Vogel, et al., 1984). To avoid contamination from absorbed $CO_2$ or particulates the iron and the glass surfaces are cleaned by adding $H_2$ (99.999%) at 500 mbar into the rigs and placing them in the oven at 450 °C for 1 hour.

### *4.1.6 Collection of $CO_2$ in the rigs*

Most of the $CO_2$ is collected in a rig attached to the gas collection system (Figure 4.9) and is trapping using liquid $N_2$.

Hydrogen is added to the frozen $CO_2$ in a quantity sufficient to guarantee a complete reduction of $CO_2$. In our system an excess of $H_2$ is used with the ratio $H_2$:$CO_2$= 2.2 : 1


*Figure 4.9 Graphitization system manufacture by the Oxford laboratory*

### *4.1.7 Conversion of $CO_2$ into graphite in the graphitizer*

The rig is placed in the oven at 560 °C for 6 hours, where $CO_2$ is reduced to carbon and water vapour. The latter is removed by cooling one finger of the rig (Figure 4.10).

*Figure 4.10 Reduction of $CO_2$ to graphite using iron as catalyst in an oven (top section); water vapour is removed by immersing the vertical finger of the rig into a cooling bath (left and right section).*

### *4.1.8 Check of the graphitization parameters*

During the reduction of $CO_2$ to carbon hydrogen is consumed at the ratio 2:1 with respect to carbon. Therefore the pressure in the rigs after reduction will be low reflecting the excess amount of hydrogen. Typically we use 400 mbar of hydrogen which results in a residual pressure of *ca*. 80 mbar. This pressure is checked by reconnecting the rigs to the gas collection system. All these parameters are entered in the database (Figure 4.11).

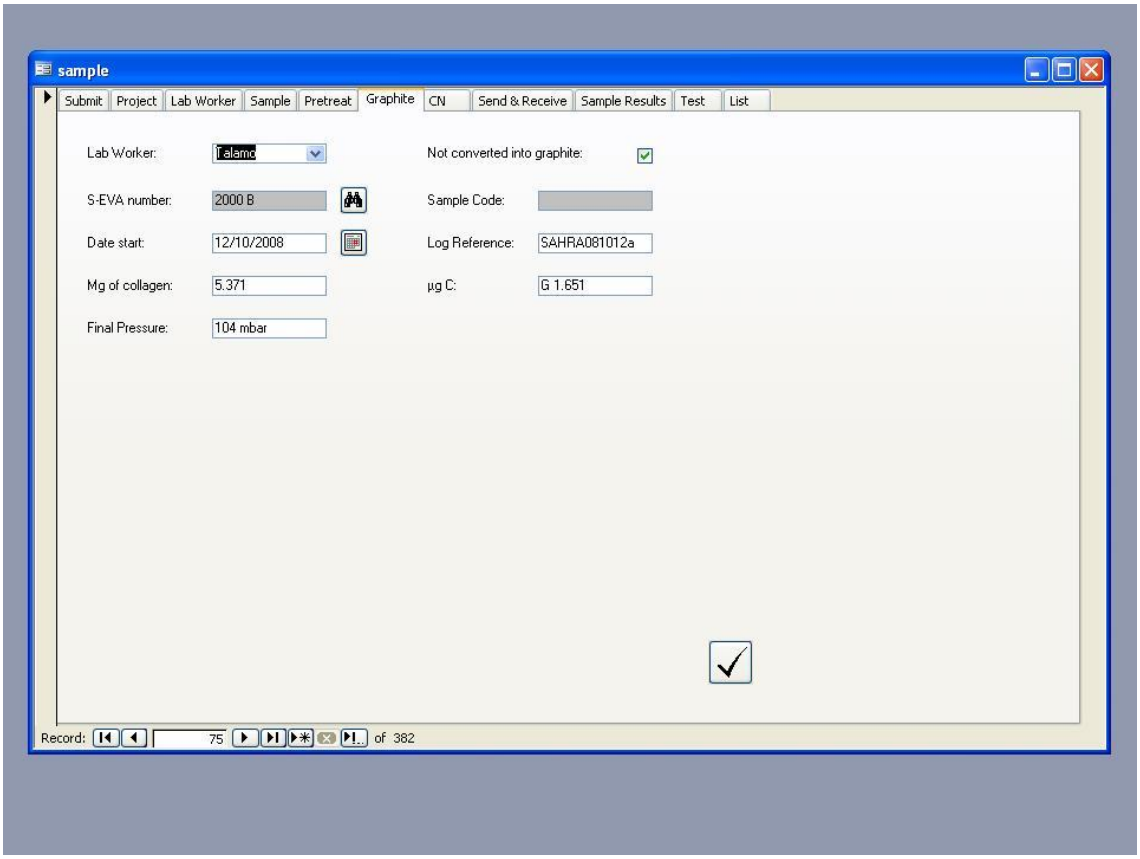


*Figure 4.11 Input of graphitization parameters into the database*

### *4.1.9 Preparation of blank samples*

All steps of pretreatment and graphitization may contribute exogenous carbon (contamination). Therefore $^{14}C$ free material (old bone, Nylon 66, Pliocene wood) is pretreated and graphitized in the lab. These samples are called blank samples and they are prepared at the same time as the archaeological samples, and are also sent to the AMS facilities to establish the level of $^{14}C$ activity in the blanks.

### *4.1.10 Preparation of shipment to an AMS facility and submission*

At this point the samples are ready to be sent to an AMS facility, where the graphite will be pressed into a target and measured in batches in the accelerator. A batch of target usually consists of a number of samples, standards and blanks. For this thesis the samples were submitted to the laboratories of Oxford. Kiel and Mannheim/Zurich. All dates of shipments and the dating results are recorded in the database providing the final list of samples and their ages (Figure 4.12 – 4.13 – 4.14)

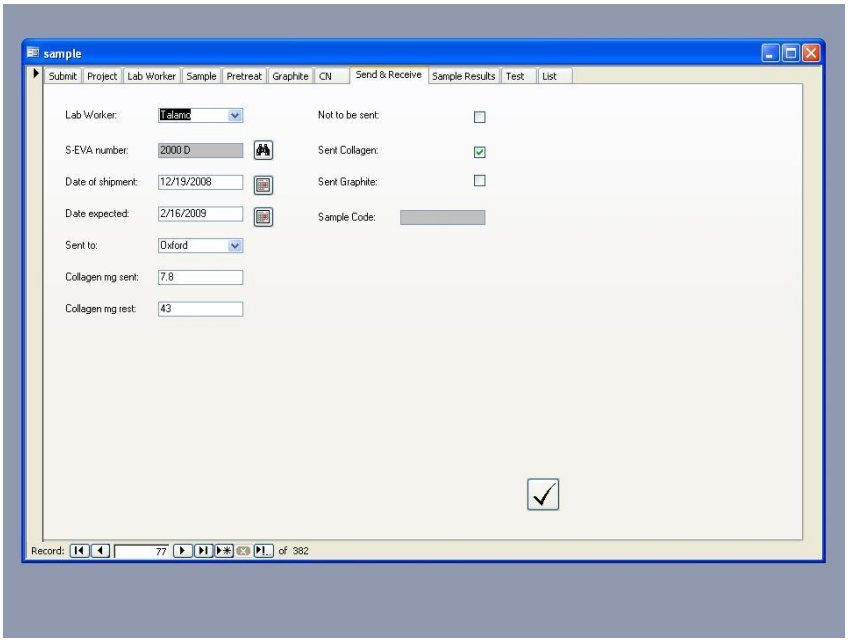


*Figure 4.12 Table of shipment and dating results of a sample*

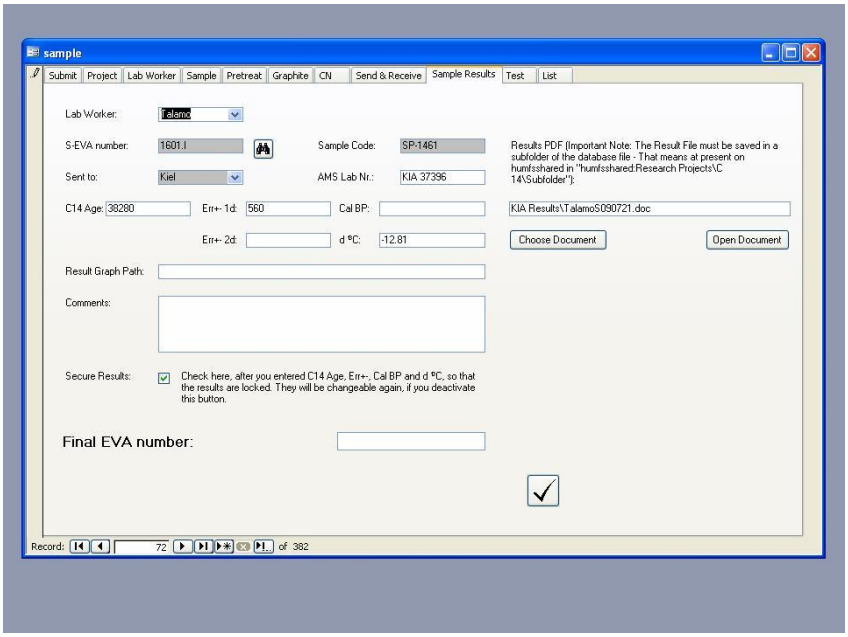


*Figure 4.13 Input of data as reported by the AMS facility*

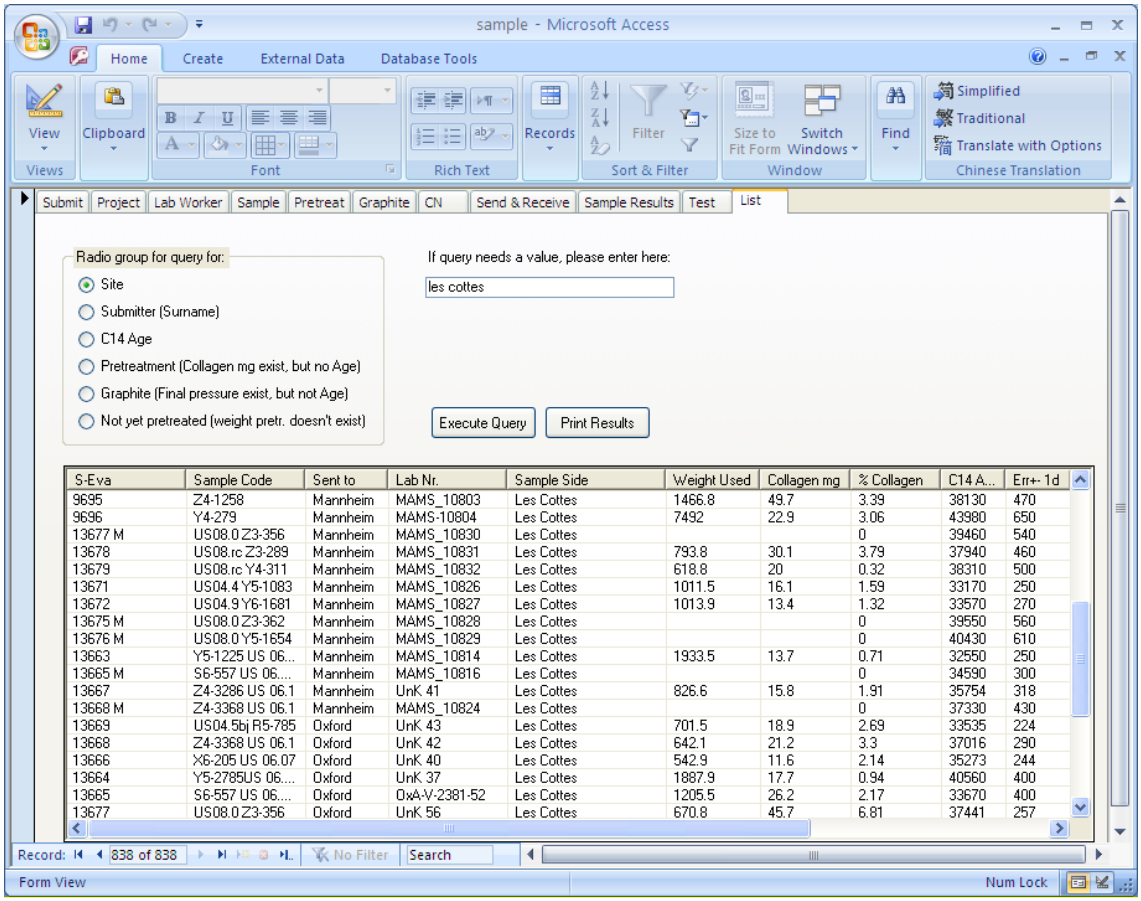


*Figure 4.14 Example of a summary sheet of an archaeological site (Les Cottés)*