

## Explanation of the Results Summary Table

### (Illustration)

**This report summarizes the results of amino acid tests performed for the purpose of identifying possible candidates for DNA preservation in ancient bones.**

**Submitter:** International Archaeology &

**Report Date:** August 7, 2002

Recommendation on proceeding with a DNA analysis on the sample.

Sample Designation			Racemization Results		Concentration Ratios					
PaleoDNA Number	Submitter Number	Material	1st Test: Is D/L Asp < 0.1?		Published Reference Values	Asp/Glu	Ser/Glu	Ala/Glu		
			2nd Test: Is D/L Ala < D/L Asp?		Modern Reference Standard	Your Samples				
			D/L Asp	D/L Ala				Asp/Glu	Ser/Glu	Ala/Glu
12345	FS 241	Bone	0.27	0.327		1.36	0.41	0.84		
12345	FS 241	Bone	0.24	0.268		1.35	0.47	0.88		
<b>Candidate for DNA Preservation?</b>			<b>No.</b>							
0	0	0	0.00	0.000		0	0	0		
0	0	0								

Racemization values for Aspartic Acid and Alanine. Since each sample is analyzed twice there will be two values for each sample.

If the values in the D/L Asp column are less than 0.1, there is a possibility for DNA preservation. DNA preservation is progressively less likely as the values increase above 0.1.

The values in the D/L Ala column should be less than the values in the D/L Asp column. If this trend is not present, any DNA extracted would be suspect as being from contaminating sources.

These are the reference values to compare with the sample values. They represent contemporary bone collagen without any decay or alteration. Since DNA is abundant in contemporary bones, ancient bones with identical values are likely candidates for a subsequent DNA test.

The modern reference is a bone analyzed along with the samples. This provides validation that the analysis was done correctly. The modern standard values should be almost the same as the published values.

These are the sample values to compare with the reference values. Since each sample is analyzed twice there will be two values for each ratio.

If the values for the sample are very close to the reference materials, the bone is well preserved and a likely candidate for a DNA study.

These values are averaged and then plotted on a histogram for each sample. It's easiest to compare by looking at the height of the bars on the graphs.

### Test for Amino Acid Racemization of Aspartic Acid and Alanine

Racemization results for Aspartic acid (D/L Asp) which are less than 0.1 suggest possible DNA preservation. Increasing values above 0.1 indicate progressively less likelihood of DNA preservation. Racemization of Alanine (D/L Ala) should be less than D/L Asp in ancient bone collagen. In cases where D/L Ala is greater than D/L Asp, contaminating amino acids may be present, indicating the potential for contaminating DNA to be present in the final extract, lending suspicion to any DNA result.(1)

### Test for Amino Acid Concentration Ratios

Contemporary bone collagen contains amino acids in consistent relative proportions. For example, Aspartic Acid is 5.1% of the total amino acid concentration, Alanine is 11.3%, Serine is 3.5% and Glutamic Acid is 7.5%. By comparing the relative proportions of these amino acids in the ancient sample to these expectations, a "degree of preservation" is established.(2) A higher degree of preservation logically leads to a higher inference for DNA preservation. Concentration ratio analysis compares Aspartic Acid, Serine, and Alanine concentrations to Glutamic Acid in the sample (Asp/Glu, Ser/Glu, Ala/Glu) with that of a contemporary bone analyzed along with the sample and with published expectations for bone collagen. Where all three ratios closely match that of the two reference sources, the sample is a better candidate for DNA preservation, when compared with racemization ratios, than those that do not match closely.

#### References:

- (1) Poinar, Hendrik N., Hoss, Matthias, Bada, Jeffrey L., Paabo, Svante, *Amino Acid Racemization and the Preservation of Ancient DNA*, Science 1996 272: 864-866
- (2) Klinken, Gert J., Mook, Willem G., *Preparative High-Performance Liquid Chromatographic Separation of Individual Amino Acids Derived from Fossil Bone Collagen*, Radiocarbon 1990, Vol 32, No. 2, 155-164

**MicroAnalytica, LLC**

4989 SW 74 Court, Miami, Florida, 33155 USA Tel: 305-479-1259, Fax: 305-675-2205  
dhood@paleoDNA.com

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PaleoDNA Number	Submitter Number	Material	1st Test: Is D/L Asp < 0.1?		Published Reference Values	Asp/Glu	Ser/Glu	Ala/Glu
			2nd Test: Is D/L Ala < D/L Asp?			0.68	0.47	1.51
					Modern Reference Standard	0.69	0.48	1.58
					Your Samples			
					Asp/Glu	Ser/Glu	Ala/Glu	
12345	FS 241	Bone	0.27	0.327		1.36	0.41	0.84
12345	FS 241	Bone	0.24	0.268		1.35	0.47	0.88
<b>Candidate for DNA Preservation?</b>			<b>No.</b>					
0	0	0	0.00	0.00		0	0	0
0	0	0	0.00	0.00		0	0	0
<b>Candidate for DNA Preservation?</b>								
0	0	0	0.00	0.00		0	0	0
0	0	0	0.00	0.00		0	0	0
<b>Candidate for DNA Preservation?</b>								
0	0	0	0.00	0.00		0	0	0
0	0	0	0.00	0.00		0	0	0
<b>Candidate for DNA Preservation?</b>								
0	0	0	0.00	0.00		0	0	0
0	0	0	0.00	0.00		0	0	0
<b>Candidate for DNA Preservation?</b>								

## Test for Amino Acid Racemization of Aspartic Acid and Alanine

Racemization results for Aspartic acid (D/L Asp) which are less than 0.1 suggest possible DNA preservation. Increasing values above 0.1 indicate progressively less likelihood of DNA preservation. Racemization of Alanine (D/L Ala) should be less than D/L Asp in ancient bone collagen. In cases where D/L Ala is greater than D/L Asp, contaminating amino acids may be present, indicating the potential for contaminating DNA to be present in the final extract, lending suspicion to any DNA result.<sup>(1)</sup>

## Test for Amino Acid Concentration Ratios

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### References

- <sup>(1)</sup> Poinar, Hendrik N., Hoss, Matthias, Bada, Jeffrey L., Paabo, Svante, *Amino Acid Racemization and the Preservation of Ancient DNA*, Science 1996 272: 864-866
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Submitter	International Archaeology & Ecology
Material:	Bone
Submitter No.	FS 241
Date Reported	August 7, 2002

### **Amino Acid Racemization Results**

***Racemization of Aspartic Acid (D/L Asp): 0.26***

Based on the average of two measurements of 0.27 and 0.24

***Racemization of Alanine (D/L Ala): 0.298***

Based on the average of two measurements of 0.327 and 0.268

Analysis variables: The sample was powdered, washed, vacuum dried, weighed, and hydrolyzed in 6M HCl for 24 hours at 100 C. Racemization was performed on an HP 1100 using reverse phase HPLC with a C18 column.

### **Theory<sup>(1)</sup>**

#### **Two parameters are considered for DNA preservation based on racemization results**

- 1. If the racemization of Aspartic Acid (D/L Asp) is less than ~ 0.1, then there is a good probability of paleoDNA preservation.**

Research indicates the highest probability of paleoDNA preservation occurs when the D/L Asp is less than 0.1. As the ratio increases, there is progressively less probability of DNA preservation. Note that the D/L Asp reference value of 0.1 is qualitative. DNA has been obtained from bones with D/L Asp ratios of 0.12 and DNA has been void in bones having D/L Asp ratios less than 0.1, due to indeterminate factors controlling DNA preservation and racemization processes. However, a value of less than 0.1 means the sample is a candidate for a paleoDNA study and an extreme value such as 0.2 or greater would indicate the sample should be excluded from DNA analysis.

- 2. If the racemization of Alanine (D/L Ala) is greater than that of Aspartic Acid, the bone is probably contaminated.**

Research indicates that degradation of bone collagen exhibits less racemization in Alanine than racemization of Aspartic Acid (D/L Ala < D/L Asp). If this trend is not present, and the D/L Ala is greater than D/L Asp, it suggests the bone contains contaminating organics and that the sample should be excluded from paleoDNA analysis.

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4989 SW 74 Court, Miami, Florida, 33155 USA Tel: 305-479-1259, Fax: 305-675-2205  
dhood@paleoDNA.com

(page 2 of 2, continuation of testing)

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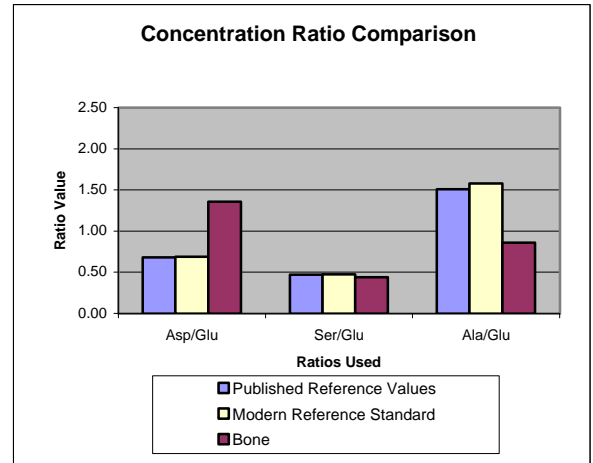
Submitter No. FS 241

Material: Bone

Date Reported August 7, 2002

### Concentration Ratio Analysis Results

Concentration Ratios			
	Asp/Glu	Ser/Glu	Ala/Glu
Published Values	0.68	0.47	1.51
Internal Bone Reference Values	0.69	0.48	1.58
First Sample Analysis	1.36	0.41	0.84
Second Sample Analysis	1.35	0.47	0.88
Average of the two Sample Analyses	1.36	0.44	0.86
% of Standard	199%	94%	57%



### Theory<sup>(2)</sup>

Contemporary bone collagen contains amino acids in consistent relative proportions. For example, Aspartic Acid is 5.1% of the total amino acid concentration, Alanine is 11.3%, Serine is 3.5% and Glutamic Acid is 7.5%. By comparing the relative proportions of these amino acids in the ancient sample to these expectations, a "degree of preservation" is established. A higher degree of preservation logically leads to a higher inference for DNA preservation. Concentration ratio analysis compares Aspartic Acid, Serine, and Alanine concentrations to Glutamic Acid in the sample (Asp/Glu, Ser/Glu, Ala/Glu) with that of a contemporary bone analyzed along with the sample and with published expectations for bone collagen. Where all three ratios closely match that of the two reference sources, the sample is a better candidate for DNA preservation, when compared with racemization ratios, than those that do not match closely.

The table shows the values obtained from the analyses and how well the sample ratio matches the reference ratios (100% being a perfect match). On the graph, the blue bar is the published expectations, the yellow bar is the contemporary bone analyzed along with the sample, and the maroon bar is the average of two analyses on the unknown sample. A well preserved bone will show maroon peaks very close in height to the blue and yellow reference bars.

### CONCLUSION & COMMENTS : IS THIS SAMPLE A CANDIDATE FOR DNA PRESERVATION?

**The results indicate that this sample is not a suitable candidate for paleoDNA preservation. The Aspartic Acid D/L ratio is above 0.1 (ie: 0.26), and the concentration ratios for the sample diverge significantly from the reference standards. Furthermore, the D/L ratio for Alanine (ie: 0.298) is greater than the Aspartic Acid D/L ratio suggesting exogenous contamination.**

#### References:

1. Poinar, Hendrik N., Hoss, Matthias, Bada, Jeffrey L., Paabo, Svante, *Amino Acid Racemization and the Preservation of Ancient DNA*, Science 1996 272: 864-866
2. Klinken, Gert J., Mook, Willem G., *Preparative High -Performance Liquid Chromatographic Separation of Individual Amino Acids Derived from Fossil Bone Collagen*, Radiocarbon 1990, Vol 32, No. 2, 155-164