Queensland Health
HealthSupport Queensland

Project Report #192

Validation of QIAsymphony® SP for Bone Extraction

Supplementary Repeatability and Reproducibility

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Document Details

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Contents

Abstract

Forensic DNA Analysis currently uses an organic extraction for the extraction of DNA from bone and teeth. Phenol chloroform isoamyl alcohol is used in the organic extraction process and is a chemical hazard to the operator. The organic extraction process is time consuming and labour intensive. One organic extraction batch contains a maximum of 12 bone/teeth samples and takes an operator a full day to complete which is relatively inefficient and is the rate limiting step in the processing of bone/teeth samples in the Analytical Team.

Forensic DNA Analysis currently uses the QIAsymphony® SP instrument for automated DNA extraction of a range of substrate and sample types (QIS# 33758), but not for bones/teeth. QIAsymphony® SP DNA extractions can process up to 96 samples per batch, and it is possible for one operator to run up to two full runs of 96 samples in a day. QIAGEN have developed protocols for pre-lysis and on-deck protocols for bones/teeth and other casework samples which have been used as the basis for the protocols to be tested in this validation.

The purpose of this project was to conduct further repeatability and reproducibility experiments for the QIAsymphony® SP bone extraction using both the 5 hour and overnight pre-lysis protocols, and to compare these results to the current organic extraction protocol.

The results obtained from this experiment show the 5 hour and overnight pre-lysis QIAsymphony® extractions are comparable to the current organic extraction with the overnight pre-lysis QIAsymphony® protocol the preferred method for routine processing.

Introduction

Forensic DNA Analysis currently performs automated DNA extractions on a range of sample types and substrates using a QIAGEN® QIAsymphony® SP/AS instrument. The QIAsymphony® SP/AS instrument is a modular automated system which enables the processing of up to 96 samples on a single run. The QIAsymphony® SP module is used for the extraction and purification of DNA from forensic casework and reference samples. It uses pre-programmed optimized protocols and the QIAGEN® cartridge-based magnetic-particle chemistry kit, the QIAsymphony® DNA Investigator Kit.

The original validation of the QIAsymphony® SP/AS did not include bone or teeth extraction. Forensic DNA Analysis currently have two QIAsymphony® SP/AS instruments and the use of these instruments for bone/teeth extraction would be particularly beneficial in the event of a large scale disaster victim identification (DVI), as it will dramatically increase the efficiency and processing capacity of bone/teeth DNA extractions. Furthermore, organic extraction involves the use of phenol chloroform isoamyl alcohol which is a chemical hazard, therefore implementing an alternative protocol would remove this hazard.

Processing bone extractions on the QIAsymphony® SP would also provide benefits and efficiencies to training and maintenance of competency. The low numbers of routinely submitted bones/teeth make initial training, and subsequent maintenance of competency, lengthy and difficult to coordinate. Extraction of bones/teeth on the QIAsymphony® would be included in the standard QIAsymphony® casework training module, and not a separate organic extraction competency as it currently is.

Following the completion of the first validation experiments it was decided additional repeatability and reproducibility experiments were required. The following experiments were performed to test and compare repeatability and reproducibility of three extraction protocols:

- Repeatability Experiment:
  - Current organic extraction
  - QIAGEN pre-lysis with overnight incubation and QIAsymphony® SP extraction
  - QIAGEN pre-lysis with 5 hour incubation and QIAsymphony® SP extraction
- Reproducibility Experiment over 5 days:
  - Current organic Extraction
  - QIAGEN pre-lysis with overnight incubation and QIAsymphony® SP extraction
  - QIAGEN pre-lysis with 5 hour incubation and QIAsymphony® SP extraction

Resources and Methods

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project #192 Validation of QIAsymphony® Bone Extraction - Supplementary R&R. This document will be referred to as the experimental design.

All samples used in this verification were selected, analysed and interpreted as outlined in the experimental design.

Sample Selection

Five powdered bone samples were retained from the Freezer Mill Project #209.

Bone Sample	Laboratory Number
Bone 1	[redacted]
Bone 2	
Bone 3	
Bone 4	
Bone 5	

(*Exhibit registered in Auslab)

Table 1: Bone samples used in this Validation

Experiments and Results

Experiment 1 – Repeatability

Purpose

The purpose of the repeatability experiment was to extract human genomic DNA from powdered bone using three different extraction methods and compare the results. The compared methods were:

- The current validated method of extracting DNA from bone and teeth using organic extraction.
- The QIAGEN pre-lysis method with the samples being incubated for 5 hours only and then extracted on the QIAsymphony® SP instrument.
- The QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Tabulated results are provided in Appendices 1-5. The repeatability quantification results for bones 1-5 are shown in Figures 1-5. The number of alleles obtained for bones 1 to 5 are shown in Figure 6. It should be noted the allele count for some samples were obtained after a microcon concentration procedure (refer to tabulated results in Appendices 1-5).

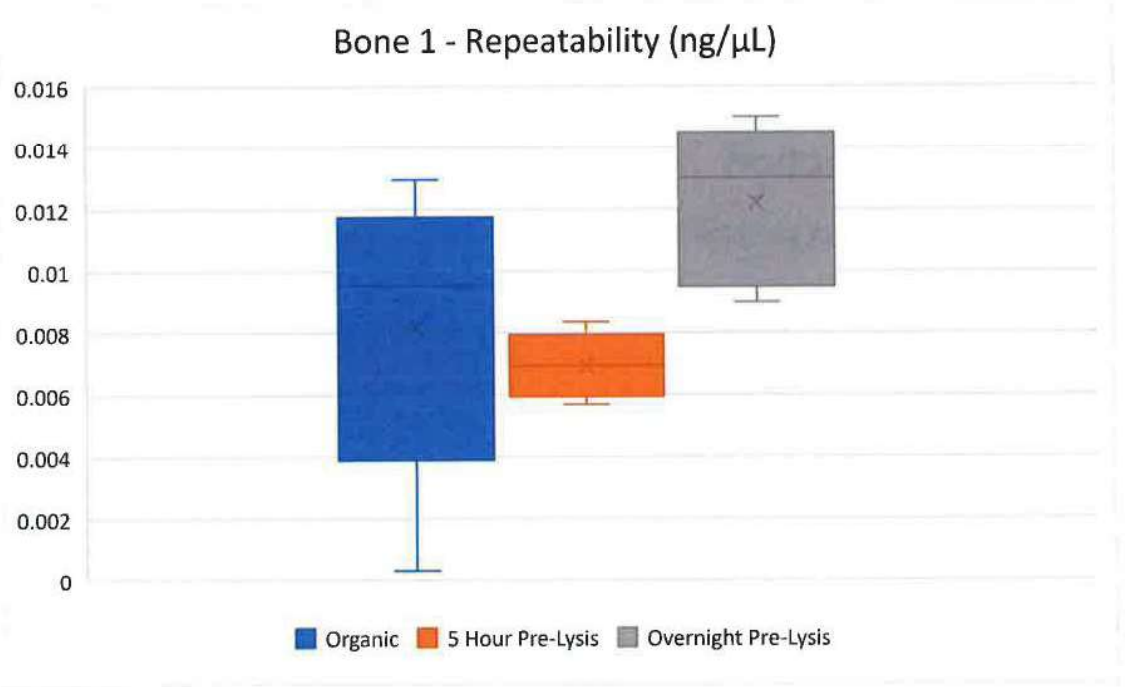

Figure 1: Representation of repeatability data for Bone 1 using Quant Values

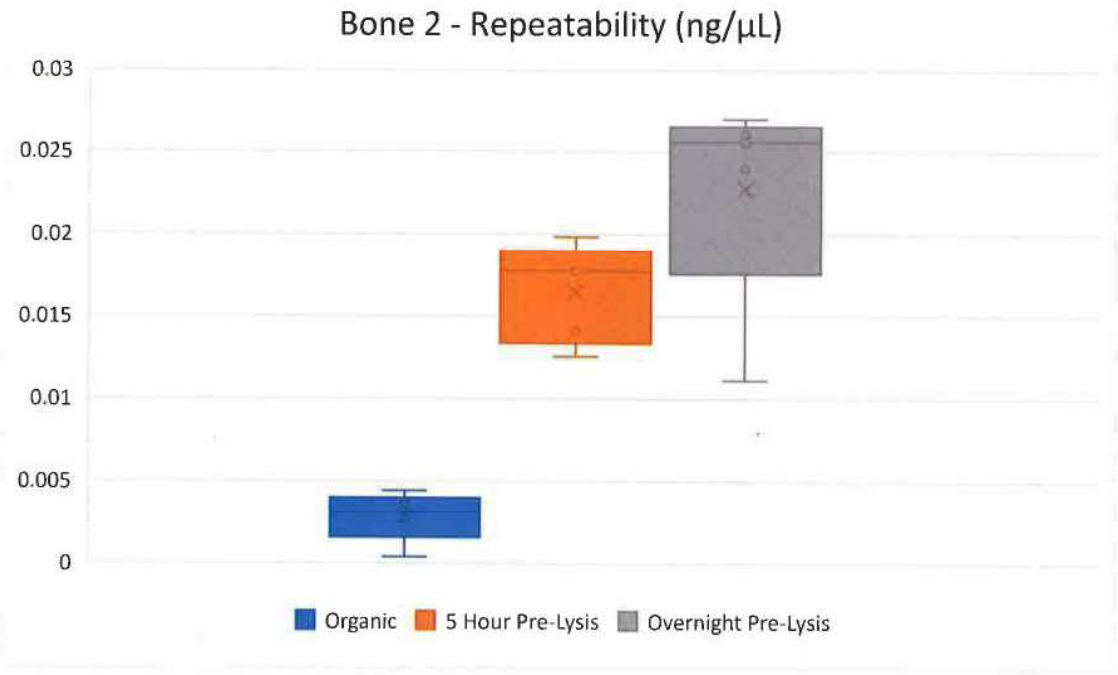

Figure 2: Representation of repeatability data for Bone 2 using Quant Values

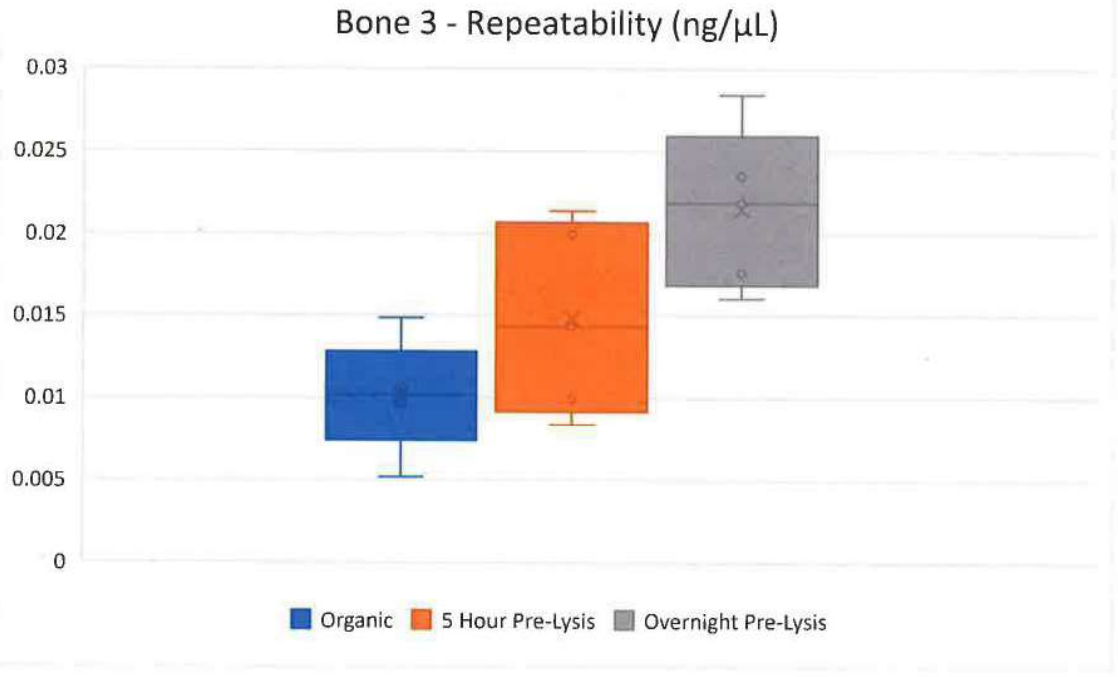

Figure 3: Representation of repeatability data for Bone 3 using Quant Values

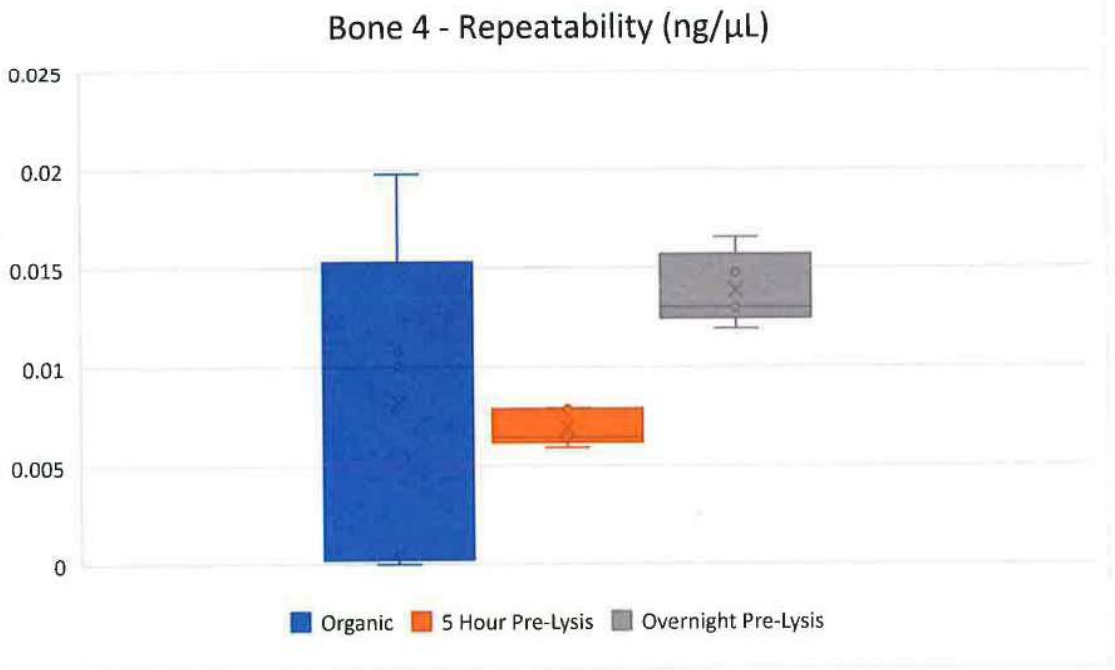

Figure 4: Representation of repeatability data for Bone 4 using Quant Values

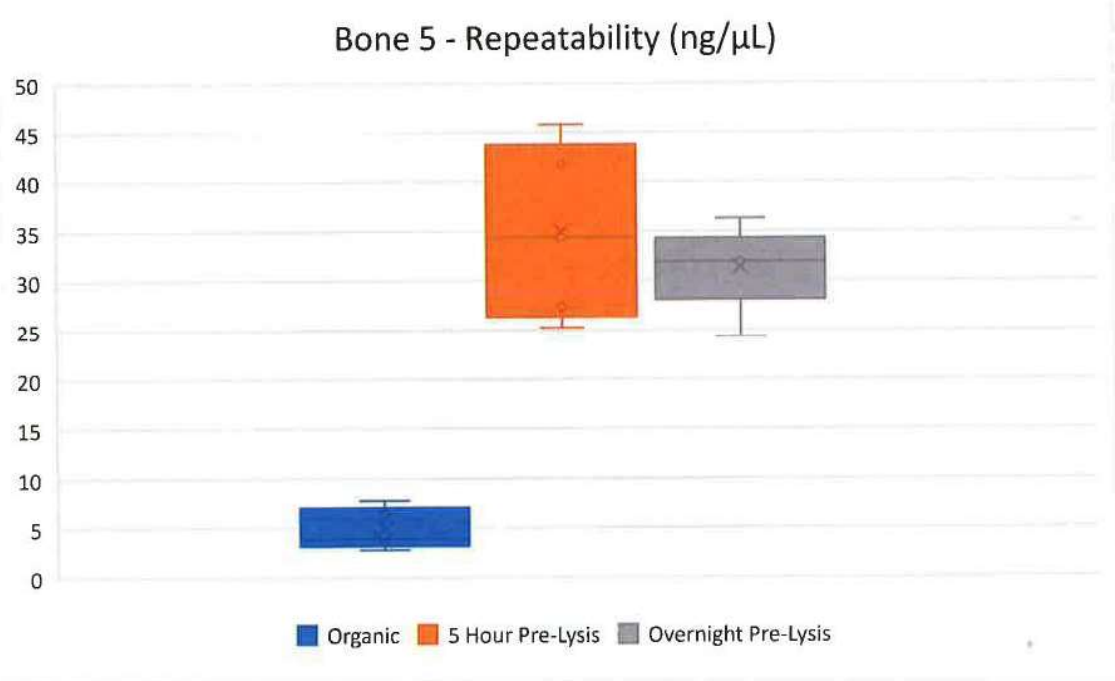

Figure 5: Representation of repeatability data for Bone 5 using Quant Values

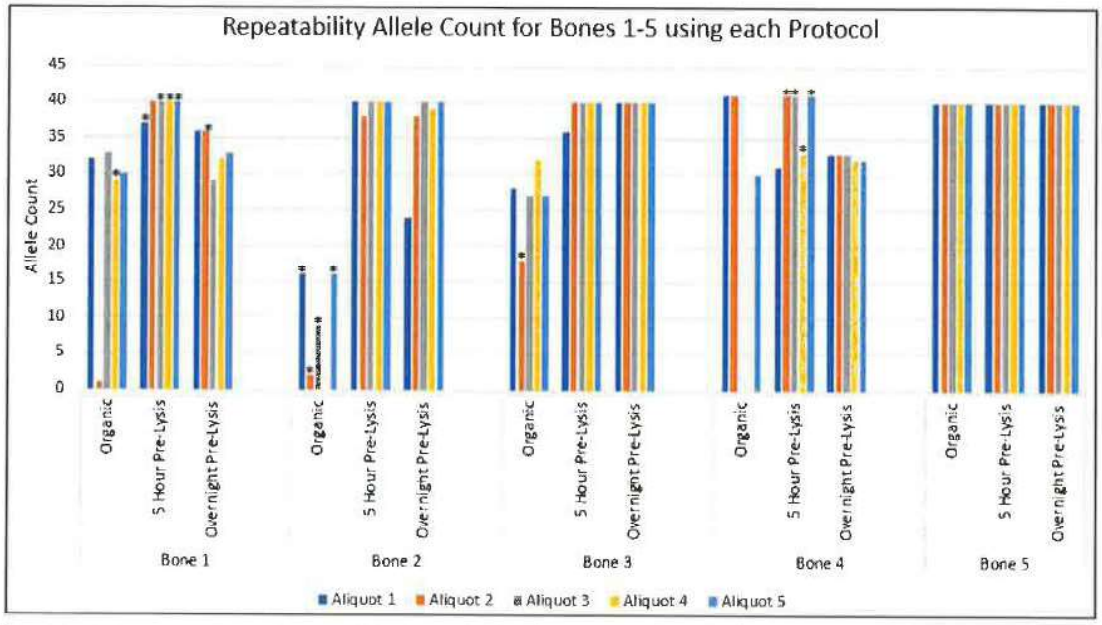

Figure 6: Representation of repeatability data for Bones 1-5 using Allele counts. * indicates samples which have undergone microcon concentration.

Discussion

The results from the current validated method (organic extraction) were used as a benchmark to compare the results from the QIAsymphony® protocols.

Repeatability for each extraction protocol varied between samples with no apparent consistency or trend. The organic and both QIAsymphony® protocols appeared to have a comparable level of repeatability with no one protocol being more or less repeatable consistently across the 5 samples (refer to Figures 1 - 5).

The quantification results for bones 1-4 were lower than bone 5 for all extraction protocols. This is likely due to the quality of the bone samples given the consistency across each of the three extraction protocols.

The overnight pre-lysis QIAsymphony® extraction gave higher quantification results for each of the 5 replicates of bones 2, 3 and 5 (as per Figures 2, 3 and 5) than the organic extraction. For bones 1 and 4, the mean quantification results (across the 5 replicates) were higher for the overnight pre-lysis QIAsymphony® than the organic extraction (as per Figures 1 and 4).

The QIAsymphony® extraction with 5 hour pre-lysis gave higher mean quantification results (across the 5 replicates) than the organic extraction for bones 2, 3 and 5. For bones 1 and 4, although the mean quantification result was lower for the QIAsymphony® extraction, quantification results overall overlapped and were comparable. It should be noted for bones 1 and 4, the range of results for the organic extraction were much wider than the QIAsymphony® extraction with 5 hour pre-lysis, which meant that although some organic replicates gave higher quantification results, some also gave lower quantification results.

Sample extracts quantified in the range 0.001-0.0088 ng/µL underwent microcon concentration prior to amplification to mimic real processing conditions. As stated

previously, bones 1-4 gave low quantification results which resulted in a number of samples undergoing microcon concentration. Across all samples tested, 6 organic extraction samples and 8 QIAsymphony® 5 hour pre-lysis extraction samples underwent microcon concentration. No QIAsymphony® overnight pre-lysis samples underwent microcon concentration (see Appendices 1 – 5). Given the final DNA profile results include samples which have and have not undergone microcon concentration, the final profile and allele count results (refer to Figure 6) have only been used to assess any negative impact the extraction protocols may have had on profile quality. No negative impact on profile quality was noted for any of the extraction protocols.

Overall this repeatability experiment has shown that the organic and both QIAsymphony® protocols are comparable, with the overnight lysis generally giving higher quantification results than the 5 hour lysis. This fits with intuitive expectations as increased reaction time could be expected to give higher yields.

Experiment 2 - Reproducibility

Purpose

The purpose of the reproducibility experiment is to test the reproducibility of results from each extraction protocol when performed by five independent scientists. One aliquot from each sample was tested per protocol for the reproducibility experiments (75 aliquots in total not including controls).

The compared methods were done over a 5 day period by 5 different operators:

- Current organic extraction
- The QIAGEN pre-lysis method with the samples being incubated for 5 hours only and then extracted on the QIAsymphony® SP instrument.
- The QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

The five independent analytical scientists who conducted each of the reproducibility experiments are:

1	Scientist 1
2	Scientist 2
3	Scientist 3
4	Scientist 4
5	Scientist 5

Table 2: The five independent scientists used for the reproducibility validation

Results

Tabulated results are provided in Appendices 6-8. The reproducibility quantification results for bones 1-5 are shown in figures 7-11. The number of alleles obtained for bones 1-5 are shown in Figure 12. It should be noted the allele count for some samples were obtained after a microcon concentration procedure (refer to tabulated results in Appendices 6-8).

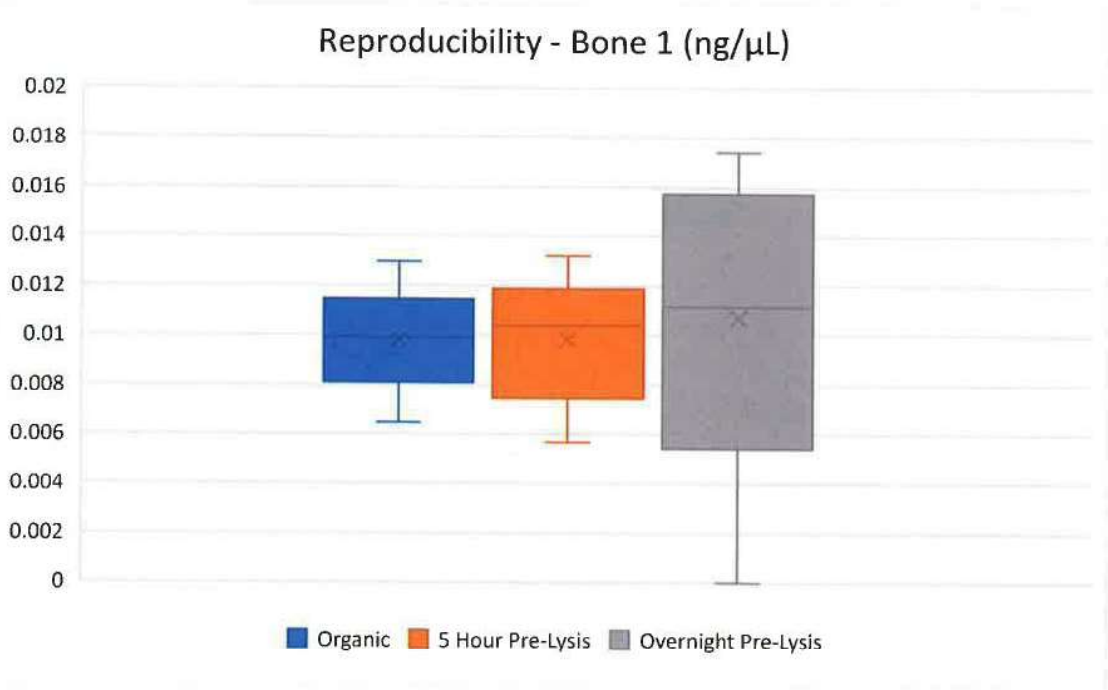

Figure 7: Reproducibility results for Bone 1 using each protocol and quant values

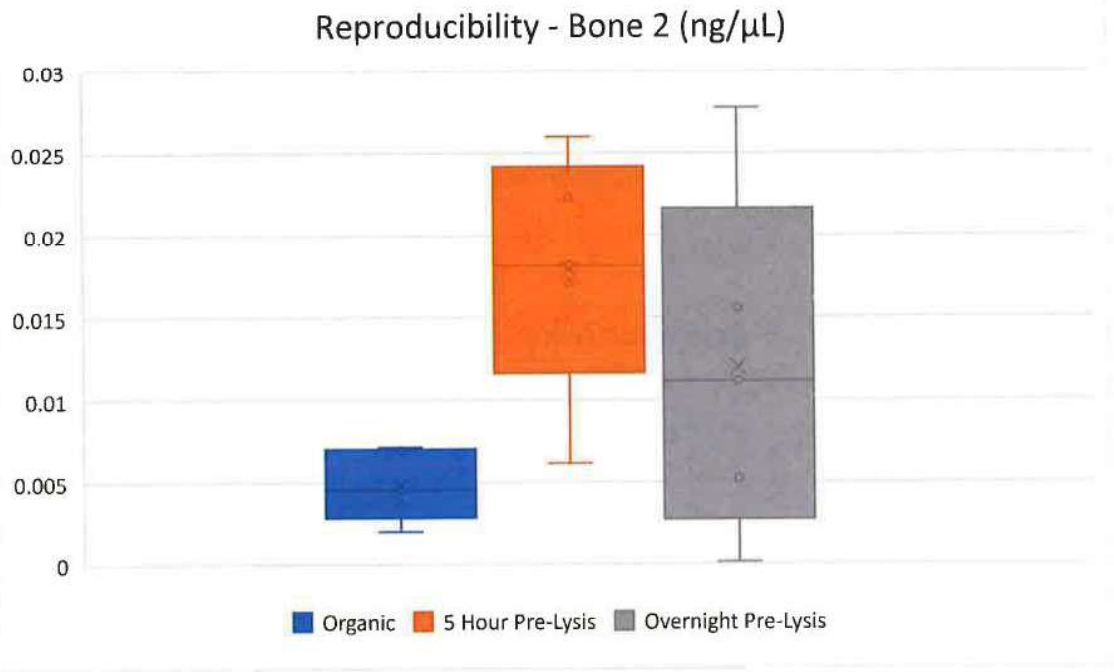

Figure 8: Reproducibility results for Bone 2 using each protocol and quant values

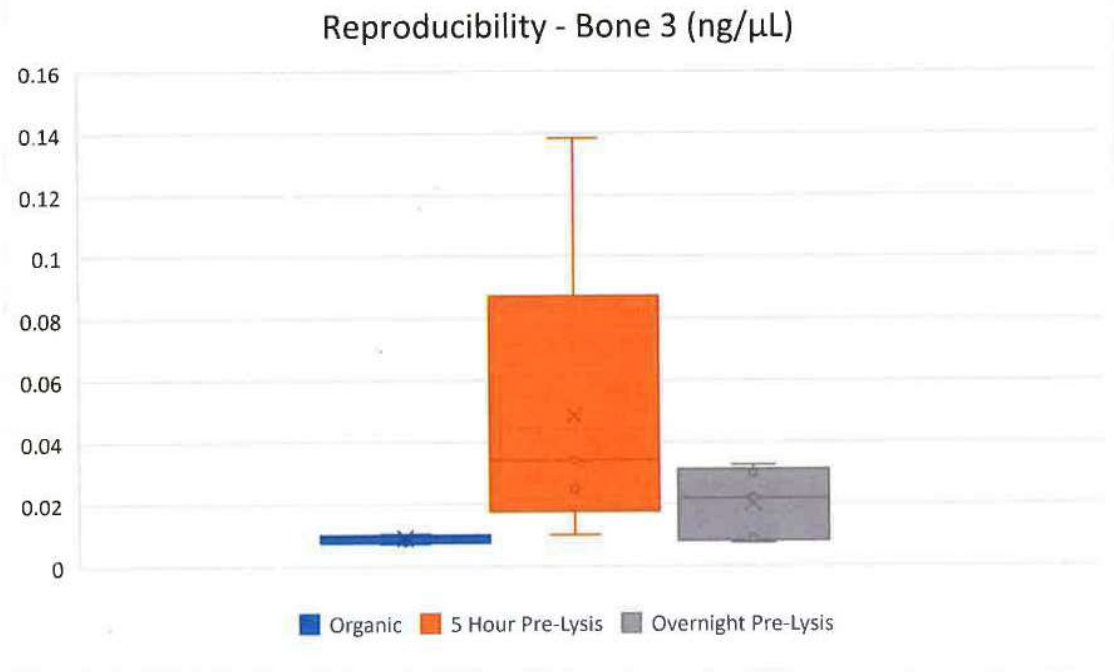

Figure 9: Reproducibility results for Bone 3 using each protocol and quant values

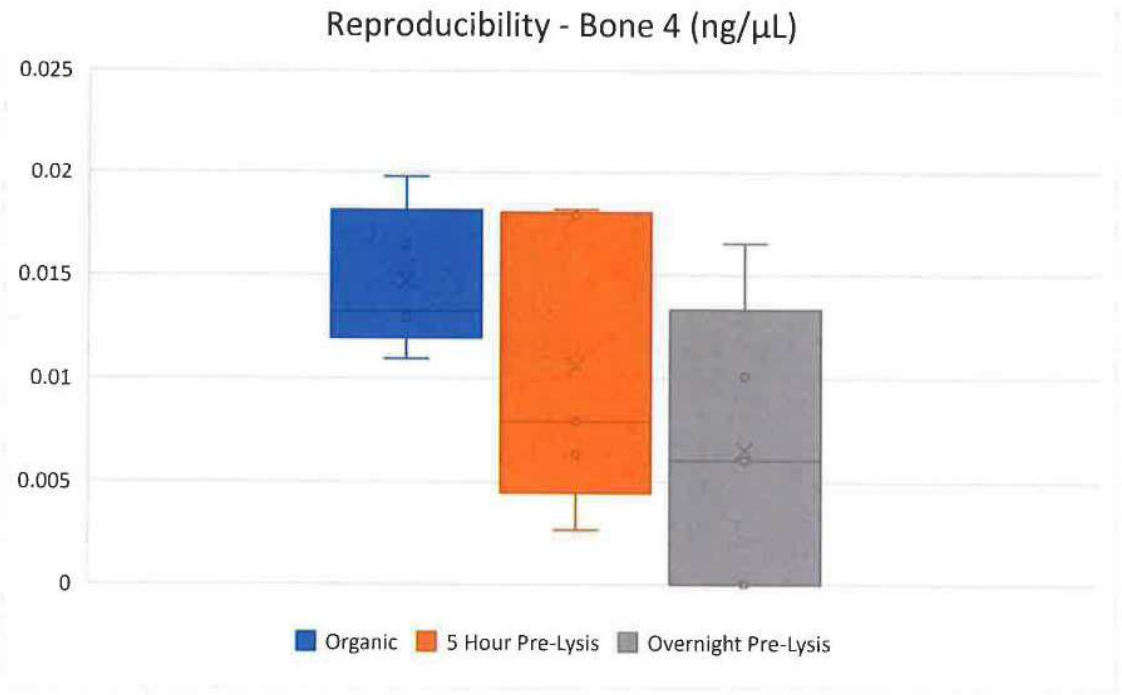

Figure 10: Reproducibility results for Bone 4 using each protocol and quant values

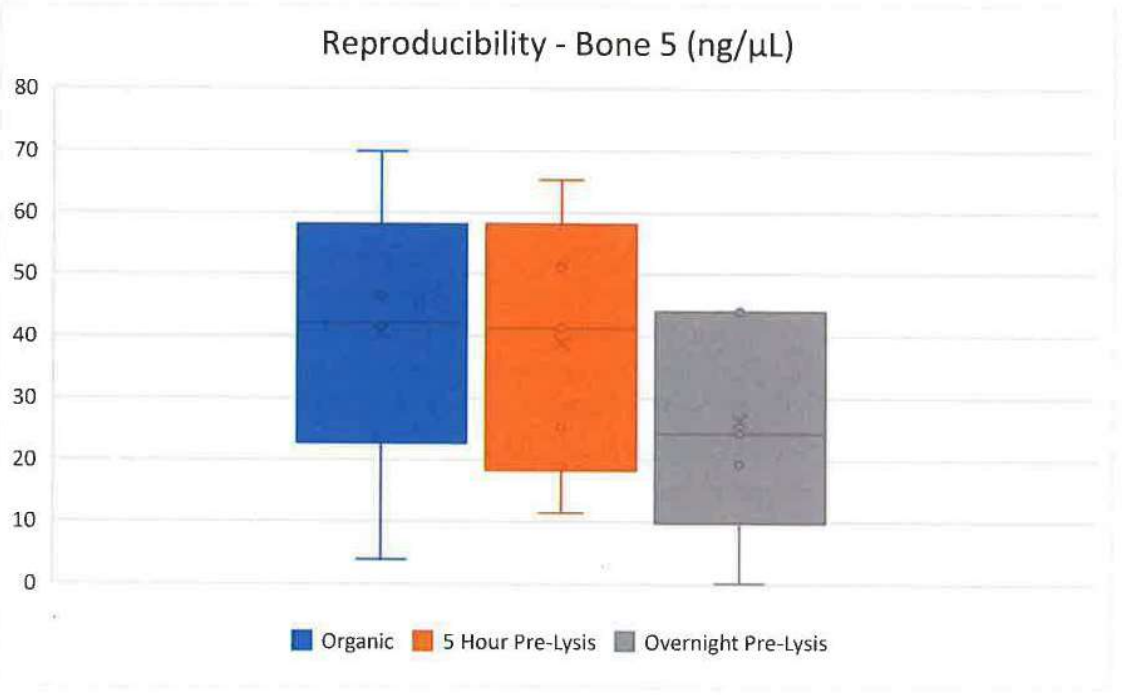

Figure 11: Reproducibility results for Bone 5 using each protocol and quant values

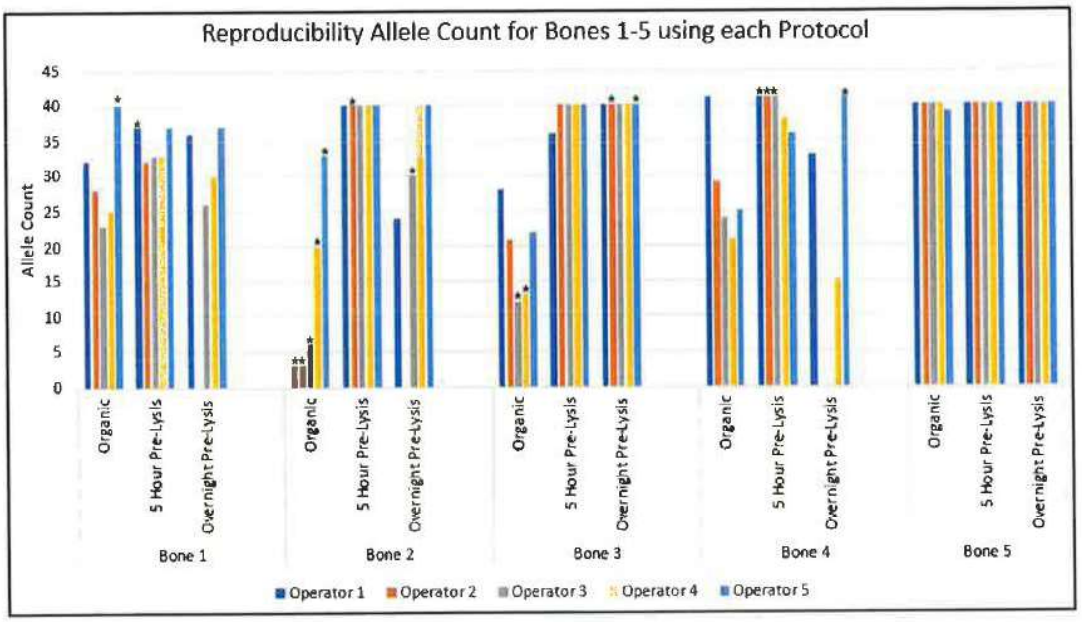

Figure 12: Reproducibility results for Bones 1-5 using allele counts for each protocol. * indicates samples which have undergone microcon concentration.

Discussion

Similar to repeatability, the reproducibility for each extraction protocol varied between samples with no apparent consistency or trend. No one protocol appeared to be more or less reproducible with consistency across the 5 samples. The organic and both QIAsymphony® protocols appeared to have a comparable level of reproducibility.

As with repeatability, the quantification results for bones 1-4 were lower than bone 5 for all extraction protocols. This is likely due to the quality of the bone samples given the consistency across each of the three extraction protocols.

For bones 1, 2 and 3, both QIAsymphony® protocols gave higher mean quantification results (across the 5 operators) than the organic extraction. For Bone 4, the organic protocol gave high average quantification results than both QIAsymphony protocols. The maximum quantification results were comparable across the three protocols protocols (0.01979, 0.01821 and 0.01660 ng/µL for organic, QIAsymphony 5 hour pre-lysis and respectively) however both QIAsymphony protocols gave more samples with lower quantification results (when compared to the Organic protocol). This was particularly evident for the overnight protocol, where two replicates gave a zero quantification result. This may be a sample specific issue as this trend was not replicated in the other bones.

For bone 5, the organic and QIAsymphony® with 5 hour pre-lysis gave comparable results, while the QIAsymphony® overnight pre-lysis extraction gave a lower mean quantification result.

Sample extracts quantified in the range 0.001-0.0088 ng/µL underwent microcon concentration prior to amplification to mimic real processing conditions. Bones 1-4 gave low quantification results which resulted in a number of samples undergoing microcon concentration. Across all samples tested, 7 organic, 5 QIAsymphony® 5 hour pe-lysis and 4 QIAsymphony® overnight lysis samples underwent microcon

concentration (see Appendices 6 - 8 for details of specific samples). Given the final DNA profile results include samples which have and have not undergone microcon concentration, the final profile and allele count results have been used only to assess any negative impact the extraction protocols may have had on profile quality. No negative impact on profile quality was noted for any of the extraction protocols.

Overall this experiment showed the QIAGEN protocols using either the 5 hour or overnight incubations gave DNA quantification results which were comparable to the organic extraction.

Additional Analysis – IPCCT

Purpose

To provide comparative analysis of IPCCT results for the tested bone extraction protocols. The compared methods were:

- The current validated method of extracting DNA from bone and teeth using organic extraction.
- The QIAGEN pre-lysis method with the samples being incubated for 5 hours only and then extracted on the QIAsymphony® SP instrument.
- The QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Raw data IPCCT results can be located in the Change Management folder (I:\Change Management\Proposal#192 - QIAsymphony Bone Extraction\Supplementary R&R\Results – Supp R&R.xls). Figures 13-17 below contain the IPCCT results for bones 1-5.

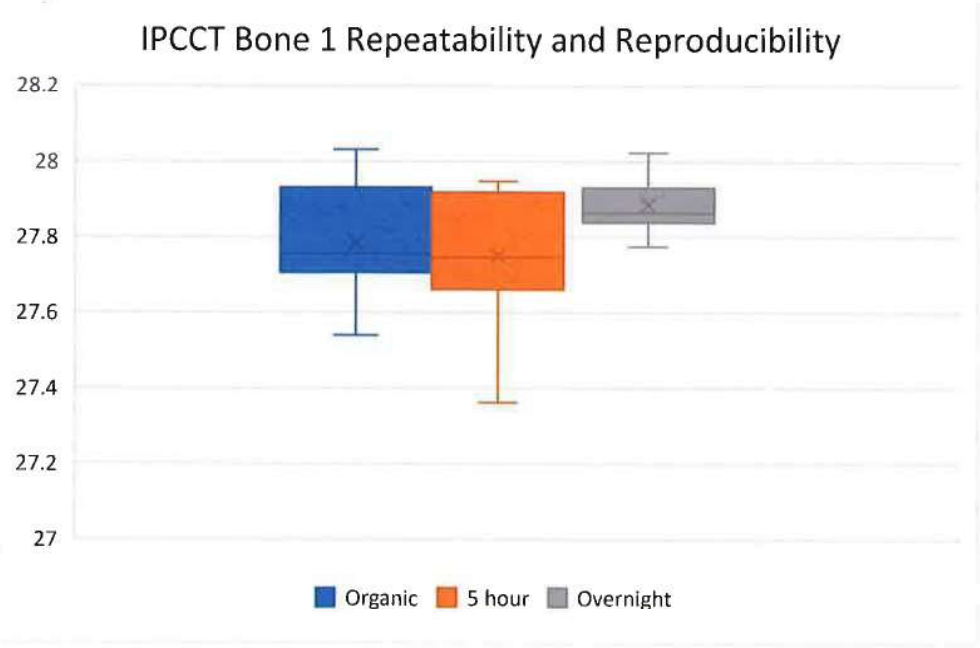

Figure 13: IPCCT results for Bone 1 – Combined Repeatability and Reproducibility data.

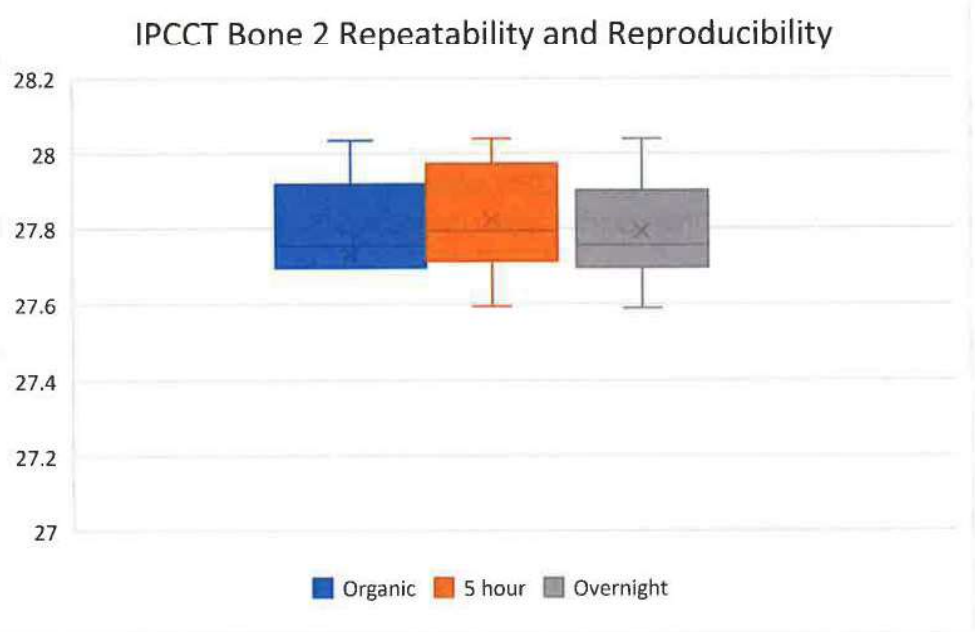

Figure 14: IPCCT results for Bone 2 – Combined Repeatability and Reproducibility data.

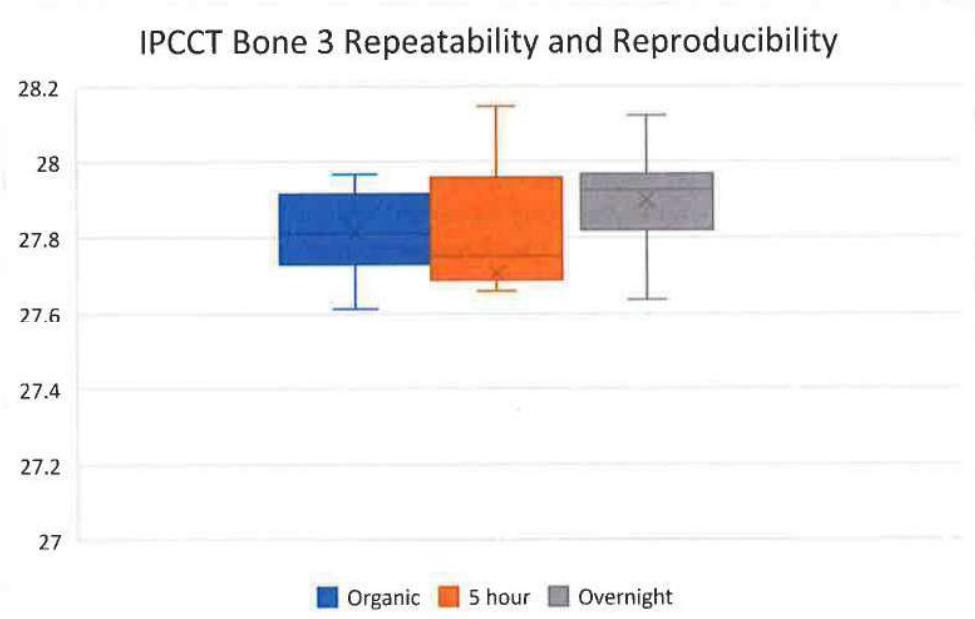

Figure 15: IPCCT results for Bone 3 – Combined Repeatability and Reproducibility data.

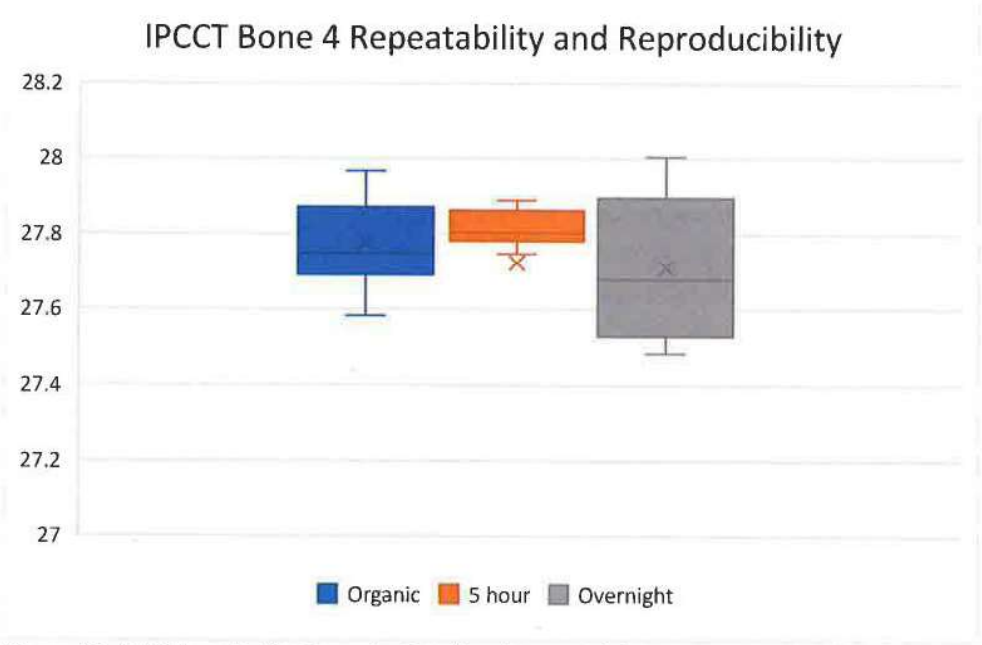

Figure 16: IPCCT results for Bone 4 – Combined Repeatability and Reproducibility data.

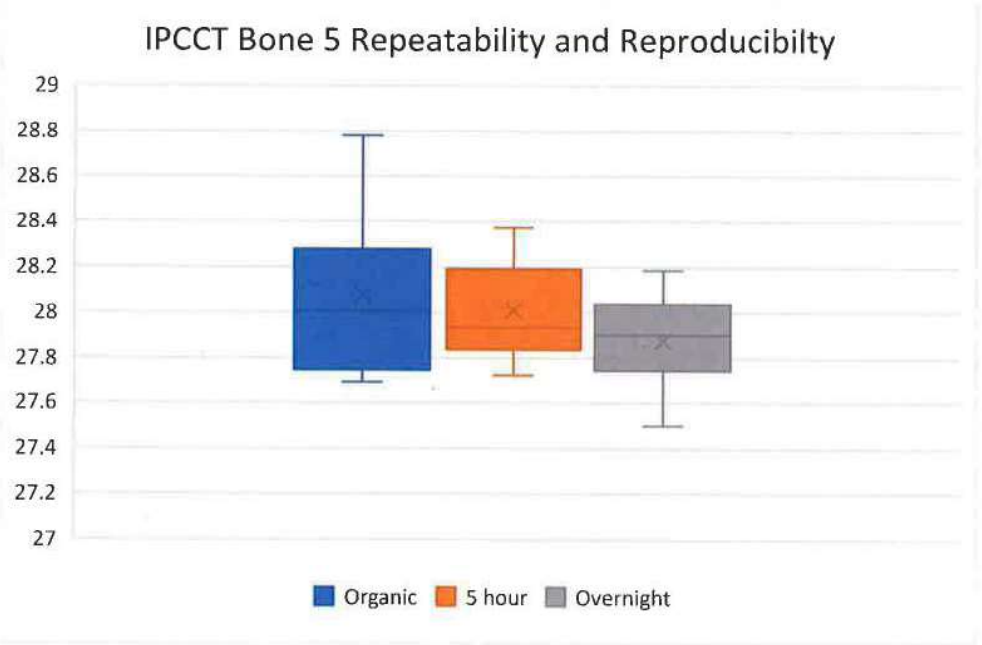

Figure 17: IPCCT results for Bone 5 – Combined Repeatability and Reproducibility data.

Discussion

The combined repeatability and reproducibility IPCCT results for bones 1-5 were compared across the three extraction protocols. Bones 1-5 showed comparable IPCCT results across each of the three tested protocols, with no indication of inhibition.

Conclusion and Recommendations

Overall the results of these additional experiments have shown that the QIAsymphony SP extraction with both 5 hour and overnight pre-lysis produce comparable DNA yields and repeatability and reproducibility to the current organic extraction. There was some evidence that the overnight pre-lysis produced higher DNA yields than the 5 hour pre-lysis and this fits with intuitive expectations given the longer reaction time. It should be noted that it is routine practice for multiple samples from a single bone to be submitted for DNA analysis, which may mitigate and/or compensate for some of the sample to sample variability observed in this validation.

As noted in the discussion, sample extracts quantified in the range 0.001-0.0088 ng/µL underwent microcon concentration. Samples underwent microcon concentration 13 times for the organic extraction, 8 times for the QIAsymphony 5 hour pre-lysis protocol and 4 times for the QIAsymphony overnight pre-lysis protocol. This indicates the QIAsymphony overnight pre-lysis protocol gave extracts with less samples in the 0.001-0.0088ng/µL microcon concentration range.

In addition to workflow efficiency improvements, implementation of the QIAsymphony for bone extraction also improves occupational health and safety for staff by removing the use of phenol chloroform in the organic extraction.

It is therefore recommended that:

- The DNA extraction of bones on the QIAsymphony SP is implemented as a replacement for organic extraction.
- The organic extraction SOP is archived.
- The overnight pre-lysis is used for routine, non-time critical bone processing given the evidence of higher DNA yields.
- The 5 hour pre-lysis protocol is considered for use where
    - there is a large number of samples and/or where time critical processing is required (i.e. for DVIs), or
    - samples are expected to provide good DNA yields and there is sufficient material for retesting if required.

References

Aguilera, M., Micic, B., Acedo, P., Ryan, L. and Allen, C. (2016) Validation of the QIAsymphony6® SP/AS Modules [Final Report].

- QIS 34039 Extracting DNA from Bone and Teeth
- QIS 34045 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit
- QIS 34052 Amplification of Extracted DNA using the PowerPlex®21 System
- QIS 34112 STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software
- QIS 34131 Capillary Electrophoresis Quality (CEQ) Check
- QIS 34132 DNA Extraction and Quantification of samples using the QIAsymphony® SP and AS – FR

Appendix 1 - Table of Results: Bone 1

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
1	Organic	[redacted]	0.01296		32
1	Organic		0.00032		1
1	Organic		0.00747	Microcon	33
1	Organic		0.01054		29
1	Organic		0.00956		30
1	Pre-Lysis 5 hour		0.00568	Microcon	37
1	Pre-Lysis 5 hour		0.00835		40
1	Pre-Lysis 5 hour		0.00694	Microcon	40
1	Pre-Lysis 5 hour		0.00619	Microcon	40
1	Pre-Lysis 5 hour		0.00754	Microcon	40
1	Pre-Lysis Overnight		0.014		36
1	Pre-Lysis Overnight		0.015		36
1	Pre-Lysis Overnight		0.009		29
1	Pre-Lysis Overnight		0.010		32
1	Pre-Lysis Overnight		0.013		33

Table 3: Repeatability for Bone 1 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction.

Appendix 2 - Table of Results: Bone 2

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
2	Organic	[redacted]	0.00361	Microcon	16
2	Organic		0.00274	Microcon	2
2	Organic		0.00311	Microcon	9
2	Organic		0.00038		0
2	Organic		0.00444	Microcon	16
2	Pre-Lysis 5 hour		0.01811		40
2	Pre-Lysis 5 hour		0.0177		38
2	Pre-Lysis 5 hour		0.01412		40
2	Pre-Lysis 5 hour		0.01253		40
2	Pre-Lysis 5 hour		0.01984		40
2	Pre-Lysis Overnight		0.01112		24
2	Pre-Lysis Overnight		0.02394		38
2	Pre-Lysis Overnight		0.02606		40
2	Pre-Lysis Overnight		0.02558		39
2	Pre-Lysis Overnight		0.02697		40

Table 4: Repeatability for Bone 2 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 3 - Table of Results: Bone 3

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
3	Organic	[redacted]	0.00965		28
3	Organic		0.00520	Microcon	18
3	Organic		0.01075		27
3	Organic		0.01485		32
3	Organic		0.01017		27
3	Pre-Lysis 5 hour		0.00996		36
3	Pre-Lysis 5 hour		0.00840		40
3	Pre-Lysis 5 hour		0.02136		40
3	Pre-Lysis 5 hour		0.01434		40
3	Pre-Lysis 5 hour		0.01998		40
3	Pre-Lysis Overnight		0.02185		40
3	Pre-Lysis Overnight		0.01757		40
3	Pre-Lysis Overnight		0.02838		40
3	Pre-Lysis Overnight		0.02348		40
3	Pre-Lysis Overnight		0.01603		40

Table 5: Repeatability for Bone 3 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 4 - Table of Results: Bone 4

Bone	Method	Barcode	Quant Value ng/µL	Rework	Allele Count
4	Organic	[redacted]	0.01979		41
4	Organic		0.01077		41
4	Organic		0.00044		0
4	Organic		0.0000		0
4	Organic		0.00999		30
4	Pre-Lysis 5 hour		0.00792		31
4	Pre-Lysis 5 hour		0.00643	Microcon	41
4	Pre-Lysis 5 hour		0.00787	Microcon	41
4	Pre-Lysis 5 hour		0.00591	Microcon	33
4	Pre-Lysis 5 hour		0.00643	Microcon	41
4	Pre-Lysis Overnight		0.01660		33
4	Pre-Lysis Overnight		0.01300		33
4	Pre-Lysis Overnight		0.01471		33
4	Pre-Lysis Overnight		0.01193		32
4	Pre-Lysis Overnight		0.01296		32

Table 6: Repeatability for Bone 4 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 5 - Table of Results: Bone 5

Bone	Method	Barcode	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
5	Organic	[redacted]	3.90037			40
5	Organic		7.82573	Dilution	714153595	40
5	Organic		2.79068			40
5	Organic		3.52519			40
5	Organic		6.41383	Dilution	714153603	40
5	Pre-Lysis 5 hour		25.25375	Dilution	714153523	40
5	Pre-Lysis 5 hour		27.41910	Dilution	714153534	40
5	Pre-Lysis 5 hour		45.80926	Dilution	714153540	40
5	Pre-Lysis 5 hour		41.76097	Dilution	714153556	40
5	Pre-Lysis 5 hour		34.41719	Dilution	714153567	40
5	Pre-Lysis Overnight		24.29558	Dilution	724204372	40
5	Pre-Lysis Overnight		31.82610	Dilution	724204381	40
5	Pre-Lysis Overnight		31.86150	Dilution	724204390	40
5	Pre-Lysis Overnight		32.42724	Dilution	724204407	40
5	Pre-Lysis Overnight		36.27103	Dilution	724204416	40

Table 7: Repeatability for Bone 5 using the three different methods tested including the Organic Extraction, the QIAsymphony® Pre-Lysis (5 hour) and Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction

Appendix 6 - Reproducibility Table of Results for Organic Extraction

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	1	[redacted]	0.01296		32
Day 2	Scientist 2	1		0.00999		28
Day 3	Scientist 3	1		0.00990		23
Day 4	Scientist 4	1		0.00974		25
Day 5	Scientist 5	1		0.00647	Microcon	40

Table 8: Reproducibility results for the Current Organic Extraction for Bone 1

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	2	[redacted]	0.00361	Microcon	3
Day 2	Scientist 2	2		0.00196	Microcon	3
Day 3	Scientist 3	2		0.00458	Microcon	6
Day 4	Scientist 4	2		0.00713	Microcon	20
Day 5	Scientist 5	2		0.00692	Microcon	33

Table 9: Reproducibility results for the Current Organic Extraction for Bone 2

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	3	[redacted]	0.00965		28
Day 2	Scientist 2	3		0.00953		21
Day 3	Scientist 3	3		0.00785	Microcon	12
Day 4	Scientist 4	3		0.00723	Microcon	13
Day 5	Scientist 5	3		0.01027		22

Table 10: Reproducibility results for the Current Organic Extraction for Bone 3

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele Count
Day 1	Scientist 1	4	[redacted]	0.01979		41
Day 2	Scientist 2	4		0.01093		29
Day 3	Scientist 3	4		0.01657		24
Day 4	Scientist 4	4		0.01294		21
Day 5	Scientist 5	4		0.01324		25

Table 11: Reproducibility results for the Current Organic Extraction for Bone 4

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	5	[redacted]	3.90037		[redacted]	40
Day 2	Scientist 2	5		69.92214	Dilution		40
Day 3	Scientist 3	5		42.16170	Dilution		40
Day 4	Scientist 4	5		46.41128	Dilution		40
Day 5	Scientist 5	5		41.47680	Dilution		39

Table 12: Reproducibility results for the Current Organic Extraction for Bone 5

Appendix 7 - Reproducibility Table of Results for the QIAsymphony Pre-Lysis (5 hour incubation)

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	1	[redacted]	0.00568	Microcon		37
Day 2	Scientist 2	1		0.00922			32
Day 3	Scientist 3	1		0.01038			33
Day 4	Scientist 4	1		0.01321			33
Day 5	Scientist 5	1		0.01056			37

Table 13: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 1

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	2	[redacted]	0.01811			40
Day 2	Scientist 2	2		0.00614	Microcon		40
Day 3	Scientist 3	2		0.02598			40
Day 4	Scientist 4	2		0.02227			40
Day 5	Scientist 5	2		0.01705			40

Table 14: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 2

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	3	[redacted]	0.00996			36
Day 2	Scientist 2	3		0.1384			40
Day 3	Scientist 3	3		0.02495			40
Day 4	Scientist 4	3		0.03412			40
Day 5	Scientist 5	3		0.03583			40

Table 15: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 3

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	4	[redacted]	0.00792	Microcon		41
Day 2	Scientist 2	4		0.00594	Microcon		41
Day 3	Scientist 3	4		0.00632	Microcon		41
Day 4	Scientist 4	4		0.01793			38
Day 5	Scientist 5	4		0.01821			36

Table 16: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 4

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele count
Day 1	Scientist 1	5	[redacted]	25.25375	Dilution	[redacted]	40
Day 2	Scientist 2	5		11.38112	Dilution		40
Day 3	Scientist 3	5		41.33673	Dilution		40
Day 4	Scientist 4	5		51.12676	Dilution		39
Day 5	Scientist 5	5		65.29295	Dilution		40

Table 17: Reproducibility results for the QIAsymphony® Pre-Lysis (5 hour incubation) and QIAsymphony® SP Extraction for Bone 5

Appendix 8 - Reproducibility Table of Results for the QIAsymphony Pre-Lysis (Overnight incubation)

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele count
Day 1	Scientist 1	1	[redacted]	0.01409		36
Day 2	Scientist 2	1		0.00000		0
Day 3	Scientist 3	1		0.01114		26
Day 4	Scientist 4	1		0.01741		30
Day 5	Scientist 5	1		0.01084		37

Table 18: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 1

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	
Day 1	Scientist 1	2	[redacted]	0.01112		24
Day 2	Scientist 2	2		0.00013		0
Day 3	Scientist 3	2		0.00521	Microcon	30
Day 4	Scientist 4	2		0.02773		40
Day 5	Scientist 5	2		0.01552		40

Table 19: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 2

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele count
Day 1	Scientist 1	3	[redacted]	0.02185		40
Day 2	Scientist 2	3		0.00865	Microcon	40
Day 3	Scientist 3	3		0.02976		40
Day 4	Scientist 4	3		0.03259		40
Day 5	Scientist 5	3		0.00743	Microcon	40

Table 20: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 3

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Allele count
Day 1	Scientist 1	4	[redacted]	0.01660		33
Day 2	Scientist 2	4		0.0000		0
Day 3	Scientist 3	4		0.0000		0
Day 4	Scientist 4	4		0.01010		15
Day 5	Scientist 5	4		0.00604	Microcon	41

Table 21: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 4

Day	Operator	Bone Sample	Barcode Number	Quant Value ng/µL	Rework	Rework Barcode	Allele Count
Day 1	Scientist 1	5	[redacted]	24.29558	Dilution	724204372	40
Day 2	Scientist 2	5		0.11813			40
Day 3	Scientist 3	5		44.00444	Dilution	723695325	40
Day 4	Scientist 4	5		44.07124	Dilution	718880557	40
Day 5	Scientist 5	5		19.29339	Dilution	718880524	40

Table 22: Reproducibility results for the QIAsymphony® Pre-Lysis (Overnight incubation) and QIAsymphony® SP Extraction for Bone 5

1.1 APPENDIX 3: Implementation Plan for project leaders

Successful project implementation may require numerous tasks to be completed either prior to implementation, or shortly after the implementation date. Some of the considerations/tasks that may be required are listed below; however this is not intended to be a comprehensive list of tasks as each project will have different implementation requirements. Project leaders should devise and submit a comprehensive implementation plan for management review. Once complete, the checklist should be submitted to the quality team for filing with the signed project documents.

Task	Details	Date Completed
Staff Training	All current QIAsymphony trainers to be assessed at CTT using RCC given similarity of bone and other substrate protocols.	24/03/2020
Staff Training	All current QIAsymphony operators (assessed as competent) will be assessed as competent using RCC given similarity of bone and other substrate protocols.	24/03/2020
Add to minor change register	Ensure that implementation has been added to the minor changes register	24/03/2020
Communication	Communicate to staff and other stakeholders – by meetings and emails.	24/03/2020
SOP	Archive Organic extraction SOP (QIS# 34039)	24/03/2020
SOP	Add bone extraction protocols to QIS # 34132 DNA Extraction and Quantification of Samples Using the QIAsymphony® SP and AS Modules	23/03/2020