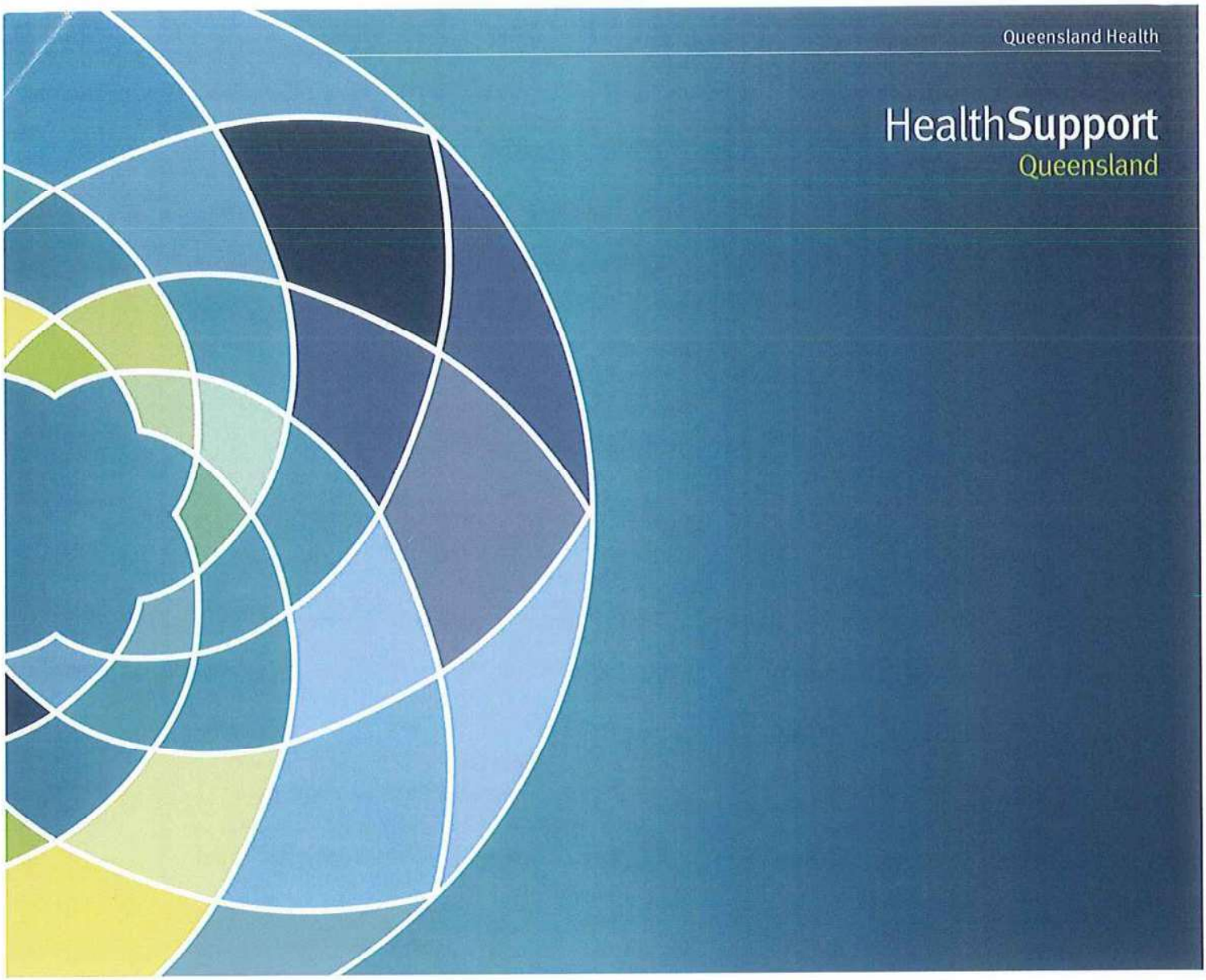

Project Report #192

Validation of QIAsymphony SP for Bone Extraction

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Queensland Government

Document Details

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Version history

Version	Date	Changed by	Description
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Document sign off

A quorum was reached for the approval of this Project Report in accordance with Section 4.1 of QIS 22871 'Procedure for Change Management in Forensic DNA Analysis'. The quorum was reached based on those officers who have provided feedback on this document and includes those members of the Management Team who have approved and/or endorsed this document below.

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Abstract

The purpose of this project was to validate bone DNA extractions on the QIAsymphony SP instrument, as this would dramatically increase the efficiency and processing capacity of extracting DNA from bone samples when compared to the current organic extraction protocol. Further, organic extraction involves the use of phenol chloroform isoamyl alcohol which is a chemical hazard, therefore implementing an alternative protocol would remove this hazard.

This project had quantitative acceptance criteria, and these criteria were assessed on completion of the data analysis for each experiment performed. The following experiments were performed to test and compare a number of extraction protocols:

- Experiment 1: Current Organic Extraction
- Experiment 2: QIAGEN Pre-Lysis with Overnight Incubation and QIAsymphony SP Extraction
- Experiment 3: QIAGEN Pre-Lysis with 5 hour Incubation and QIAsymphony SP Extraction
- Experiment 4: Organic Pre-Lysis with Overnight Incubation and QIAsymphony SP Extraction
- Experiment 5: Organic Pre-Lysis with 5 hour Incubation and QIAsymphony SP Extraction

The results of this project have shown the protocol tested in Experiments 2 and 3 (QIAGEN Pre-Lysis with 5 hour and overnight Incubation and QIAsymphony® SP Extraction) should be implemented as they gave DNA quantification and final profile results which were comparable to or better than the other tested protocols. Further, use of the Experiment 3 protocol (5 hour pre-lysis incubation) would dramatically increase the efficiency and processing capacity of bone DNA extractions from 24 hours (using current organic extraction) to under 8 hours which may be critical for disaster victim identification (DVI) cases.

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 original validation of the QIAsymphony® SP/AS did not include bone or teeth extraction. Forensic DNA Analysis currently have two QIAsymphony® SP/AS instruments the use of these instruments for bone/teeth extraction would be particularly beneficial in the event of a large scale DVI as it will dramatically increase the efficiency and processing capacity of bone/teeth DNA extractions.

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 SP module was the only module tested in this validation.

The original scope of this validation included both teeth and bone extractions. However due to time and resource limitations, only bone extractions were tested. Teeth extractions will be tested in the future and reported in a separate report.

Resources and Methods

All reagents, materials and equipment used in this project were as specified in the approved in-house document Project Proposal #192: Validation of QIAsymphony SP for bone extraction (November 2017). This document will be referred to as the Experimental Design. The following QIS documents are referenced throughout this 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

Sample Selection

Ten powdered bone samples were retrieved from storage (from previously examined case files) and used as per the experimental design. The quantification results from original testing of these bone sample aliquots was used to ensure that sample selection included a range of sample qualities. Table 1 contains the bones selected for this project (for simplicity in the remainder of this document, these samples will be referred to using their Sample Number rather than Lab Number).

Please note that for each of these samples a number of bone aliquots were originally tested, and differing quantification results were obtained from each aliquot. For this reason, quantification results are given as a range.

Table 1: Bone samples used in this validation

Lab Number	Sample Number	Original Quant Range (ng/µL)
[redacted]	Sample 1	>50
	Sample 2	10 – 20
	Sample 3	20 – 30
	Sample 4	0.10 – 0.15
	Sample 5	7 – 12
	Sample 6	0.08 – 0.40
	Sample 7	4 – 5
	Sample 8	0.03 – 0.30
	Sample 9	0.00
	Sample 10	0.001 – 0.003

Experiments and Results

Experiment 1: Current Organic Extraction

Purpose

The purpose of this experiment was to extract human genomic DNA from powdered bone using the current validated method of extracting DNA from bone and teeth using phenol chloroform isoamyl alcohol (PCIA), this is also referred to as an organic extraction.

Results

The results from Experiment 1 provide a benchmark to compare the results from Experiments 2, 3, 4 and 5.

Samples 1 and 3 gave average quantification values >10 ng/µL. These samples were diluted and quantified. The quantification values were multiplied by the dilution factor to give a more accurate final quantification result. The average quantification results and allele counts obtained for all the samples that underwent organic extraction are outlined in Table 2.

Table 2: Average quantification results and allele count for Experiment 1

Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	1.648			1.648	40
Negative Control	0.001			0.001	9
Sample 1	53.879	1:150	0.319	47.793	40
Sample 2	1.883			1.883	32
Sample 3	58.155	1:150	0.321	48.174	40
Sample 4	0.072			0.072	35
Sample 5	4.448			4.448	40
Sample 6	0.004			0.004	12
Sample 7	1.618			1.618	39
Sample 8	0.189			0.189	40
Sample 9	0.000			0.000	0
Sample 10	0.002			0.002	0

The positive extraction control gave a full expected profile, however given this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.001 ng/µL quantification result and a DNA profile with 9 peaks present (analysed at 16 RFU as per QIS #34131). The negative extraction control was reamplified and the contamination was reproduced, confirming

the contamination is present in the extract. A quality search was conducted and the peaks were found to be consistent with an Analytical Team staff member. Sample 4 on this batch gave a low level mixed DNA profile, which was consistent with a mixture of the expected profile and the staff sample which had contaminated the negative extraction control.

No other samples on this batch show the presence of contamination from this staff member or from any other source. The staff member whose DNA has contaminated these samples did not process any of these batches, and was not rostered in the main laboratory during the processing of these samples. Given two samples on this batch have been contaminated from the same staff member, it is likely the mechanism of contamination is the same for both samples. However, as only two samples were contaminated, and the remaining samples showed no evidence of contamination (even those samples which gave No DNA Profile final results), it is unlikely one of the reagents used for the entire batch was contaminated. The contamination may be due to a pipette, tip, tube or other consumable used in the process. The exact mechanism of contamination was not able to be determined.

Given the Experimental Design called for each protocol to be tested with at least four different bone samples, and that ten samples were actually tested in this validation, Sample 4 has been excluded from the remainder of this validation. Given that the remainder of samples on this batch gave expected profiles and did not show signs of contamination, they were accepted as valid.

Experiment 2: QIAGEN Pre-Lysis with Overnight Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the QIAGEN pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Samples 1 and 3 gave quantification values >10 ng/µL. These samples were diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 2 are outlined in Table 3.

Table 3: Average quantification results and allele counts for Experiment 2

Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	0.844			0.844	40
Negative Control	0.000			0.000	0
Sample 1	49.623	1:150	0.329	49.341	40
Sample 2	1.831			1.831	40
Sample 3	13.800	1:20	0.773	15.465	40
Sample 5	8.088			8.088	40
Sample 6	0.236			0.236	40
Sample 7	1.447			1.447	40
Sample 8	0.120			0.120	40
Sample 9	0.000			0.000	0
Sample 10	0.007			0.007	18

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and No DNA Profile final result as expected.

Experiment 3: QIAGEN Pre-Lysis with 5 hour Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the QIAGEN Pre-lysis method with the samples being incubated for 5 hours and then extracted on the QIAsymphony® SP instrument.

Results

Samples 1 and 3 gave quantification values >10 ng/µL. These samples were diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 3 are outlined in Table 4.

Table 4: Average quantification results and allele counts for Experiment 3

Sample	Initial Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	0.920			0.920	40
Negative Control	0.000			0.000	0
Sample 1	57.257	1:150	0.382	57.257	40
Sample 2	2.344			2.344	40
Sample 3	25.345	1:100	0.253	25.345	40
Sample 5	8.101			8.101	40
Sample 6	0.261			0.261	39
Sample 7	2.299			2.299	40
Sample 8	0.073			0.106	40
Sample 9	0.000			0.000	0
Sample 10	0.261			0.009	12

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and No DNA Profile final result as expected.

Experiment 4: Organic Pre-Lysis with Overnight Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the current organic extraction pre-lysis method with the samples being incubated overnight and then extracted on the QIAsymphony® SP instrument.

Results

Sample 1 gave a quantification value >10 ng/µL. This sample was diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 4 are outlined in Table 5.

Table 5: Average quantification results and allele counts for Experiment 4

Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	2.218			2.218	40
Negative Control	0.000			0.000	1
Sample 1	14.430	1:20	0.721	14.430	39
Sample 2	1.184			1.184	39
Sample 3	8.468			8.468	40
Sample 5	3.259			3.259	40
Sample 6	0.047			0.047	34
Sample 7	0.512			0.512	35
Sample 8	0.015			0.015	35
Sample 9	0.000			0.000	0
Sample 10	0.000			0.000	0

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and a DNA profile with one peak present. The negative extraction control was accepted as per routine negative extraction control acceptance criteria (see QIS #34131).

Experiment 5: Organic Pre-Lysis with 5 hour Incubation and QIAsymphony® SP Extraction

Purpose

The purpose of this experiment was to test the extraction of human genomic DNA from powdered bone using the current organic extraction pre-lysis method with the samples being incubated for 5 hours and then extracted on the QIAsymphony® SP instrument.

Results

Samples 1 and 3 gave quantification values >10 ng/µL. These samples were diluted and quantified. The quantification value was then multiplied by the dilution factor to give a more accurate overall quantification value.

The average quantification results and allele counts for all the samples in Experiment 5 are outlined in Table 6.

Table 6: Average quantification results and allele counts for Experiment 5

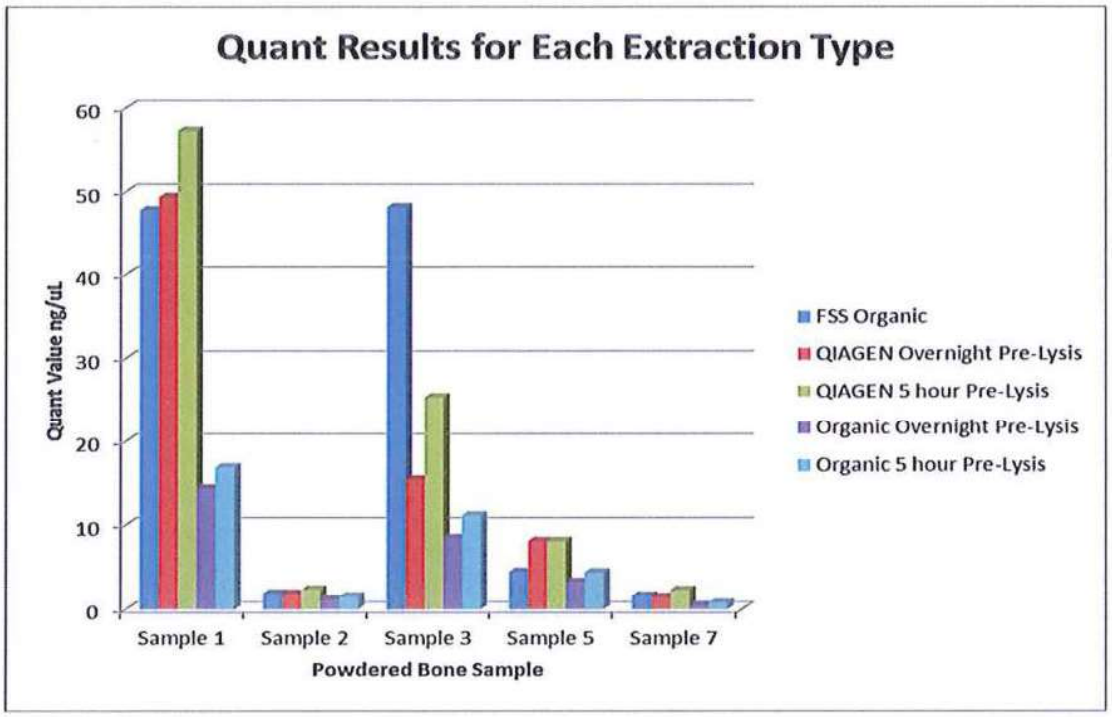
Sample	Initial Average Quant Result (ng/µL)	Dilution factor	Dilution Quant Result (ng/µL)	Final Quant Result (ng/µL)	Allele Count
Positive Control	0.481			0.481	40
Negative Control	0.000			0.000	0
Sample 1	16.935	1:20	0.847	16.935	40
Sample 2	1.508			1.508	40
Sample 3	11.148	1:20	0.557	11.148	40
Sample 5	4.405			4.405	40
Sample 6	0.047			0.047	35
Sample 7	0.779			0.779	39
Sample 8	0.014			0.014	40
Sample 9	0.000			0.000	0
Sample 10	0.000			0.000	0

The positive extraction control gave a full expected profile, however given that this is a blood sample, this was not used to compare/assess the bone extraction protocols. The negative extraction control gave a 0.000 ng/µL quantification result and No DNA Profile final result as expected.

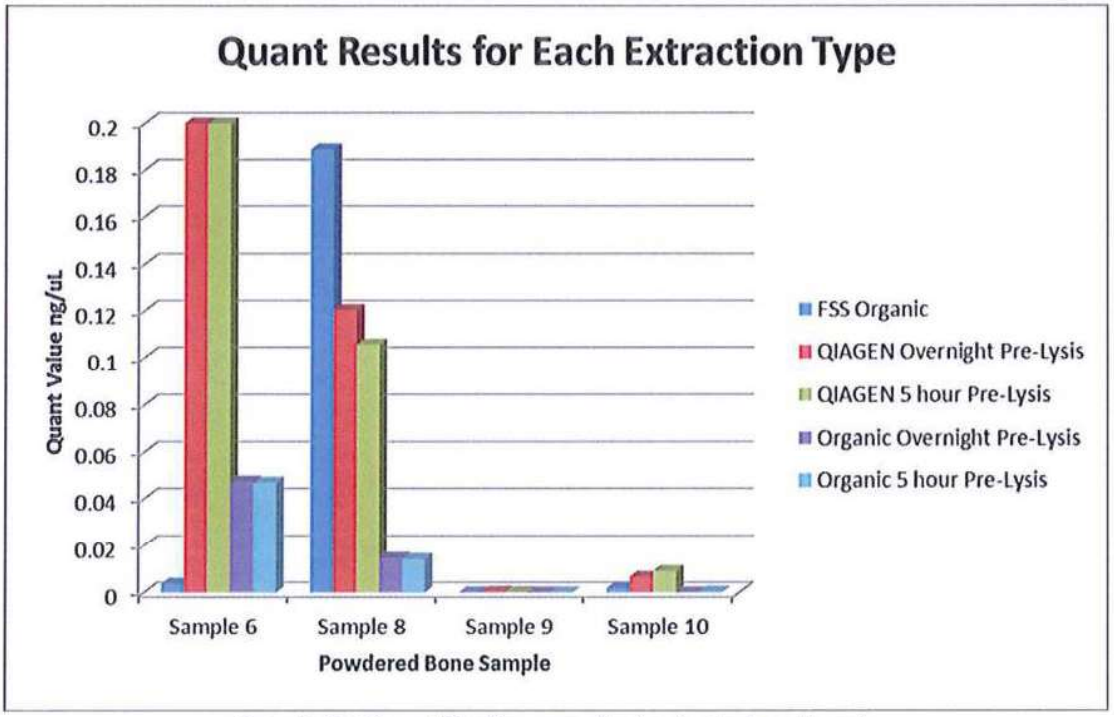
Discussion

The protocols tested in Experiments 1, 2, 3, 4 and 5 were compared using DNA quantification results and allele counts obtained in the final DNA profile. Generally, an extraction protocol was assessed as performing better if it gave higher DNA quantification results and more alleles in the final DNA profile. Graphs have been prepared to display and compare the quantification and allele count results for each of the protocols.

Graphs 1 and 2 below provide a summary of the average quantification results for the samples tested in this validation.

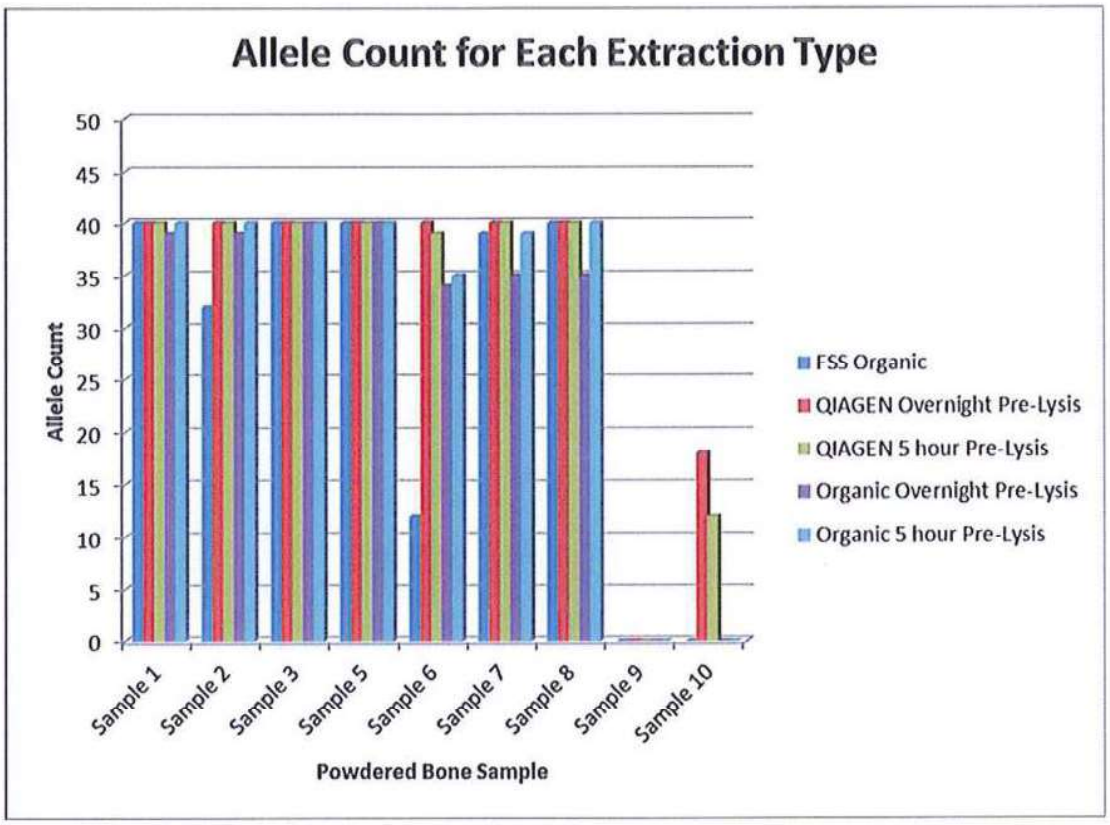


Graph 1: Quantification results for tested protocols



Graph 2: Quantification results for tested protocols

Graph 3 below provides a summary of the alleles obtained in final DNA profiles for each of the samples included in this validation.



Graph 3: Allele Count for each extraction type

Results showed that Organic Pre-Lysis with 5 hour and overnight incubations with QIAsymphony® SP extraction (Experiments 4 and 5) gave the lowest DNA quantification results when compared to other protocols. This may be due to incompatibility between the Organic Pre-Lysis reagents and QIAsymphony® reagents. These two protocols have therefore been excluded as possible options for implementation.

The QIAGEN 5 hour and/or overnight Pre-Lysis with QIAsymphony® SP extraction (Experiments 2 and 3) gave DNA quantification results which were comparable to or higher than the Organic extraction for Samples 1, 2, 5, 6, 7, 9 and 10. The QIAGEN protocols also gave the same or higher number of alleles in the final DNA profile:

- Sample 1 – all protocols gave full profiles (40 alleles)
- Sample 2 – both QIAGEN protocols (Experiments 2 and 3) gave full profiles (40 alleles), whereas the Organic extraction gave 32 alleles.
- Sample 5 - all protocols gave full profiles (40 alleles)
- Sample 6 – the QIAGEN overnight incubation (Experiment 2) gave a full profile (40 alleles) and the QIAGEN 5 hour (Experiment 3) gave 39 alleles, whereas the Organic extraction gave 12 alleles.
- Sample 7 – both QIAGEN protocols (Experiments 2 and 3) gave full profiles (40 alleles) and the Organic extraction gave 39 alleles.

- Sample 9 – both QIAGEN protocols (Experiments 2 and 3) and the Organic extraction gave undetermined quantification result and No DNA Profile final result.
- Sample 10 – the QIAGEN overnight incubation (Experiment 2) gave 18 alleles and the QIAGEN 5 hour (Experiment 3) gave 12 alleles, whereas the Organic extraction gave No DNA Profile.

The Organic extraction gave higher DNA quantification results than both the QIAGEN 5 hour and overnight Pre-Lysis with QIAsymphony® SP extraction (Experiments 2 and 3) for Samples 3 and 8. Of these samples, there was no difference in the number of alleles obtained in the final DNA profiles:

- Sample 3 - all protocols gave full profiles (40 alleles)
- Sample 8 - all protocols gave full profiles (40 alleles)

In addition to allele count data, profile quality (i.e. preferential amplification, degradation and peak morphology) were not noticeably different for the samples in Experiments 1, 2 and 3.

When comparing the quantification results for the two QIAGEN protocols (Experiments 2 and 3):

- The 5 hour pre-lysis incubation protocol (Experiment 3) gave higher DNA quantification results for Samples 1, 2, 3 and 7
- The 5 hour and overnight pre-lysis incubations gave similar DNA quantification results for Samples 5, 6, 8, 9 and 10.

When comparing the number of alleles in final DNA profiles for QIAGEN pre-lysis 5 hour and overnight incubations:

- Both the 5 hour pre-lysis and overnight incubations gave full DNA profiles (40 alleles) for Samples 1, 2, 3, 5, 7 and 8.
- The overnight pre-lysis incubation (Experiment 2) gave more alleles than the 5 hour incubation for Sample 6 (40 and 39 alleles respectively) and Sample 10 (18 and 12 alleles respectively).
- Both the 5 hour pre-lysis and overnight incubations gave No DNA Profile for Sample 9.

T-tests were conducted to compare the quantification results for the two QIAGEN and Organic extraction protocols (see Table 7 below). The T-test results show although there were differences between the Organic and QIAGEN protocols (overnight and 5 hour incubations), these differences were not statistically significant. Further, the T-test results also showed any differences between the two QIAGEN protocols (overnight and 5 hour incubations) were not significantly different.

Table 7: T-tests comparing QIAGEN and Organic extraction protocols

Comparison of Quantification Results	T-test
Organic Extraction – QIAGEN 5 hour Incubation	0.435044
Organic Extraction – QIAGEN Overnight Incubation	0.922513
QIAGEN Overnight – 5 hour Incubation	0.802843

The quantification results, final DNA profile allele counts and T-test results support the conclusion that the QIAGEN protocols (Experiments 2 and 3) performed as well as or better than the Organic extraction protocol (Experiment 1).

Conclusion

Overall, the QIAGEN protocols tested in Experiments 2 and 3 were shown to be comparable to or better than the current Organic extraction protocol (Experiment 1) in terms of DNA quantification and final profile allele count.

The protocols tested in Experiments 4 and 5 (Organic pre-lysis with QIAsymphony® SP extraction gave poorer quantifications results overall when compared to the results of Experiments 1, 2 and 3.

Implementation of either QIAGEN protocol (Experiments 2 and 3) would increase efficiency and throughput of bone extractions and also eliminate the use of the hazardous phenol chloroform isoamyl alcohol (used in Organic extractions).

Recommendations

It is recommended that:

- The protocols tested in Experiments 2 and 3 are both implemented and can be selected based on staffing and operational requirements.
- Organic extractions are ceased, QIS# 34039 archived and phenol chloroform isoamyl alcohol is disposed.
- Further validation is conducted to assess the ability to store lysates for up to 8 days.

References

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