Development and Internal Validation of a Chelex® DNA Extraction Protocol for Reference Oral Swabs

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Abstract

Forensic DNA laboratories have limited budgets, analysts and time with an abundance of samples to be examined. Therefore, it is necessary to implement procedures in a lab that maximize efficiency while minimizing cost. Additionally, any procedure used in a lab must be validated following SWGDAM's Validation Guidelines to show it is a reliable and robust method and to determine any limitations. The purpose of this research was to devise and validate such a procedure for the Boston Police Department Crime Laboratory DNA Section. Specifically, a BioRad Chelex® resin based extraction procedure for reference oral swabs.

Introduction

This project had three stages: method development, validation and addressing any additional questions that arose during the course of the project.

Development Studies

–Swab sampling type
–Room temperature incubation length
–56°C incubation length
–Boiling method

Validation Studies

–Concordance
–Contamination risk assessment
–Ability of Chelex® to extract DNA from potentially compromised swabs – artificially aged swabs
  • Original and revised protocol
–Stability of Chelex® extract

Studies to Address Additional Questions

–Stability of 5% Chelex® Solution
–Amplification target assessment
–Post-Extraction interference studies
  • Biological sample type - Blood vs. Saliva
  • Extraction method - EZ1® vs. Chelex®
  • Sample substrate type – Liquid vs. Stained Swab

Materials and Methods

Kits, Instruments and Software:

–BioRad BT Chelex® 100 Resin
–Qiagen EZ1® Advanced
–EZ1® DNA Investigator Kit
–Applied Biosystems® Quantifiler® Duo Quantification Kit
–Applied Biosystems® 7500 Real-Time PCR system
–AmpFLSTR® Identifiler Plus® Amplification Kit
–Applied Biosystems® 9700 Thermal Cycler
–Applied Biosystems® 3130xl Genetic Analyzer
–GeneMapper® ID v3.2.1

Sample Materials:

–Previously collected oral swabs refrigerated for 0-5 years
–Freshly collected oral swabs from known donors
–Saliva samples from known donors
–Control rbs fabrics of known donor
–Liquid blood control samples from serology section

Method Development Results

Parameter Evaluated	Original Protocol	Developed Protocol
Swab Sample Type	Undefined for Swab	Tip of Swab Cutting
Room Temperature Incubation Length	15-30 min	15 min
56°C Incubation Length	15-30 min	15 min
Boiling Method	Boiling Water Bath	100°C Dry Bath

Method Validation Results

Concordance

–All samples of known profile were found to be concordant.

Contamination Risk Assessment

–All reagent blanks met BPD's requirements for uncontaminated reagent blanks.

Stability Study: Substrate Variability – Revised Procedure

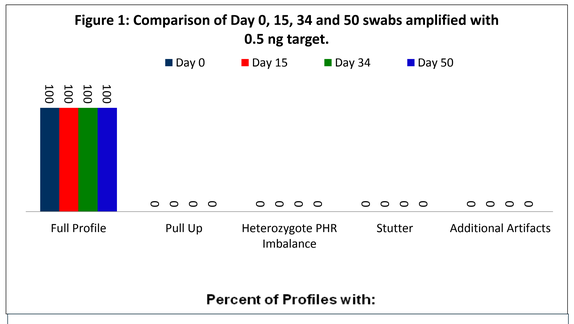

Figure 1 compares characteristics of DNA profiles obtained from swabs that were artificially aged for 0, 15, 34 and 50 days in a 37°C incubator and then extracted with the revised Chelex® procedure.

Stability Study: Substrate Variability – Original Procedure

- Similar results to Revised Procedure Study.

Extract Stability Study

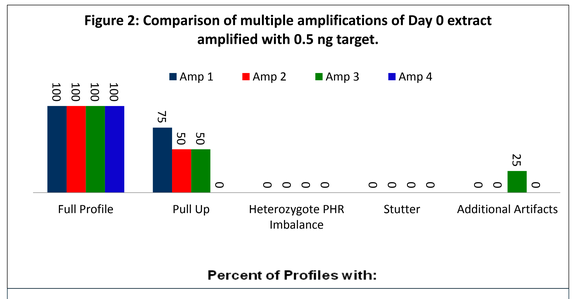

Figure 2 compares characteristics of DNA profiles after refrigeration of Day 0 (original procedure) extracts for approximately 0, 15, 34 and 50 days.

Additional Studies Results

5% Chelex® Solution Stability Study

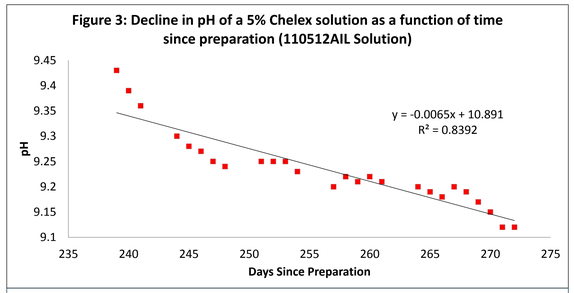

Figure 3 depicts the behavior of the pH of a 5% Chelex® solution up to 272 day after preparation.

Amplification Target Assessment Study

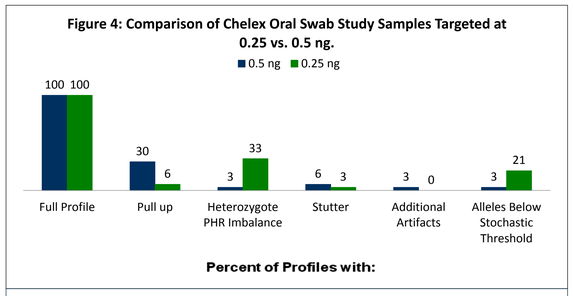

Figure 4 compares the quality of the DNA profiles obtained from oral swabs when extracted with Chelex® and amplified with either 0.25 ng or 0.5 ng of DNA.

Post-Extraction Interference Studies

Hypothesis: Source of disconnect between quantification values and amplification results is due to underestimation of quantification results due one or a combination of:

- sample type
- substrate type
- extraction method.

Sample Type – blood vs. saliva
- Relative underestimation of blood

Extraction Method – Chelex® vs. EZ1®
- Slight indication of relative underestimation of Chelex®

Substrate Type – liquid vs. stained swabs
- Relative underestimation of stained swabs

Cost Analysis Assessment

	Qiagen DNA Investigator Kit	Chelex® 100 Resin (100 g Bottle)
Cost of Kit/Bottle	$414.72	$397.10
Number of Samples per Kit/Bottle	48	11765
Cost per Sample	**$8.64**	**$0.03**

Conclusions

A protocol was developed based on BPD's Chelex® DNA Extraction from Whole Blood/Bloodstains/Saliva Protocol and previous studies performed by the BPD crime lab DNA section. The changes made in the development of the extraction protocol included use of a tip of swab cutting, a 15 minute room temperature incubation, a 15 minute 56°C incubation and use of a dry bath set at 100°C.

This protocol was evaluated to determine whether it was a reliable method and to determine its limitations.

–Sensitivity of this protocol is sufficient to extract amplifiable quantities of DNA from oral swabs in sufficient quality to be used for DNA analysis and provide interpretable, concordant DNA profiles.

–Low risk of contamination

–Capable of extracting DNA from artificially-aged swabs.

–Extract is stable when stored at 2-8°C for at least 50 days.

Additional studies were performed to answer questions that arose during the project.

–An intermediate amplification target amount, perhaps 0.35 ng, should be evaluated.

– 5% Chelex® solution should be made more than once a year with a pH monitoring system set in place.

–Possible relative underestimation of quantification values is associated with stained swabs (compared to liquid samples) and blood based samples (over saliva samples).

–Slight indication that Chelex® extracted samples have underestimation in quantification values compared to EZ1 extracted samples.

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