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## Effects of sampling, DNA amount and Reduced volume of AmpFLSTR Identifiler Plus kit on STR profile

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### ABSTRACT

This research showed two examined blood sampling option, 20 finger-prick and 20 four-month freezers venous blood and its effect on the recovery of isolated DNA preserved on FTA Cards. also, to reduce the cost of DNA profile, we estimated kit efficiency using a reduced volume of PCR reaction (12.5 $\mu$ L) with different DNA concentration. A prepare series of DNA dilution (0.01, 0.1, 0.5, 1 and 10 ng/  $\mu$ L) to amplified DNA with two volume 12.5 and 25  $\mu$ L of AmpFSTR® Profiler Plus™ Kit. Our research observed that sampling methods and storage time led to decreased DNA. The full and half PCR volume reaction was produced a complete DNA profile with both 0.5 and 1  $\mu$ L DNA concentration. The loss of DNA profile was detected in samples with 0.01  $\mu$ L of DNA concentrations, while 0.1  $\mu$ L of DNA concentration was produced partial DNA profile. The optimal DNA quantity which yielded full DNA profile were 0.5 and 1 ng/  $\mu$ L with both 12.5 and 25  $\mu$ L volume of PCR. Generally, our data indicated that the peak height of STR loci in histogram results, lowering progressively with decreasing of DNA amounts. Accordingly, this modified method decreases the cost of DNA profile to half, while maintaining its performance.

### 1.Introduction

Short tandem repeat (STR) detection is a routine procedure in forensic science and plays an irreplaceable role in individual identification. The workflow of STR profiling for DNA fingerprint samples essentially consists of sample collection, storage condition, DNA extraction/quantification, amplification of STR loci, capillary electrophoresis, and Data analysis (Butler 2011; Kitayama *et al.*, 2013; Liu, 2014). Storage condition for the medium or long period of biological samples is actual challenging labour in the STR analysis (Corradini *et al.*, 2019). Since the 1960s a blood sampling for new newborn screenings using the technology of dried blood spot to preserve samples (Guthrie, 1961). FTA® technology is an unprecedented technique deliberated to simplify the collection, transport, saving and purification of DNA from a different type of biological sources (Yoshida *et al.*, 1995). FTA cards are vastly used in genetics studies to maintain human DNA. They introduce an easier and flexible collection, small amounts of blood, conveyance, and storage manner than other famous protocols (Rahikainen *et al.*, 2016; Stowell *et al.*, 2018). Blood samples on FTA® cards can be stored under dark and dry conditions at room temperature, also if an error occurred, a small piece of FTA® cards require to re-extraction. Currently, several manual methods are

available and commercially kits for DNA extraction from blood FTA cards. DNA isolation using Chelex 100 resin is a common manual procedure for DNA extraction.

The STR loci were well-characterized, broadly approved, and are commonly used in the forensic studies (Connon *et al.*, 2016; Ricke *et al.*, 2016; Ye *et al.*, 2017). Now Several commercially PCR kit is available for analysis of STR loci. However, in our study DNA samples was performed with the Amp FLSTR Identifiler Plus kit (Applied Biosystems, Foster City, USA). Generally, STR profiles generated from blood spotted on FTA card usually produce sharp and higher peaks, and confirm better than isolated DNA in an EDTA (stored at EDTA tube) titration investigation exhibit a higher allele performance rate even in the existence of inhibitors (Oostdik *et al.*, 2014).

This research aimed to investigate the effect of sampling methods and stored condition in the quality and quantity of DNA. then, estimated the effect of reducing the volume of the PCR reaction without changing the quality of the results.

### 2. Material and Methods

#### 2.1 sample collection

Bloodstains spotted on FTA Card (GE Healthcare, UK) were collected by two methods; (1) directly,

using the finger-prick method for 20 individual and storage at room temperature. (2) Venous Blood Collection from 20 individual (3 ml) in EDTA Tube and keep in a freeze for 4 months, then transfer 100 microliters to FTA Card. Label necessary information and date collect on the barcode of FTA card. FTA card Blood spotted storage in dry and dark conditions at room temperature.

### 2.2 DNA extraction.

According to the manufacturer's protocol, a 1.2 mm FTA punch contains about 5-20 ng of DNA (Alaeddini, 2012). DNA isolation using Chelex 100 resin (GE Healthcare, 2010), with some modified in a wash of blood disc include; (1) sample from a finger prick, Wash the disc by adding 500 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and incubating at room temperature for 10 min with occasional vortex for only one time. (2) sample from Venous Blood washing for three times.

### 2.3 Amplification volume.

The extracted DNA samples were diluted for four concentration 0.1 ng, 0.5 ng, 1 ng and 10 ng per / µL. Also, DNA mixtures, which included 1 ng and 0.5 ng also tested. Two sets of PCR reaction mix were used to amplified the samples; 25 µL PCR reaction volume and reduced PCR reaction volume of 12.5 µL. Amplified sample using GeneAmp1 PCR System 9700 thermal cycler (Applied Biosystems).

### 2.4 Electrophoresis and analysis

STR products were prepared by adding 1µL of PCR product to 9µL Hi-Di™ Formamide (Applied Biosystems) and 0.75µL internal size standard ILS 500 (Applied Biosystems) and Electrophoresis through the 16-capillary ABI 3500xl Genetic Analyzer. for higher resolution separations used POP™-4 (Applied Biosystems) on a 36 cm array. Alleles analysis: were using GeneMapper1 ID v3.00 software (Applied Biosystems).

### 2.5 Statistical analysis

Data from DNA concentration were performed with IBM SPSS Statistics ver. 22 (IMB corp. 2013, Armonk, NY, USA) software to analyze the effect of storage time and sampling methods on DNA yield.

## 3.Results and discussion

The first phase of our study was to estimate the amount of DNA isolation by Chelex 100 resin from the FTA card. Generally, our results prove the previously confirmed observations of potent and dependable DNA recovery from blood samples spotted on FTA card (Tack *et al.*, 2005; Sahajpal *et al.*, 2019; Turingan *et al.*, 2019). FTA cards are treated with a mix of chemical material, such as detergents, and chelating agents, the detergent lyses cells membrane on contact denatures proteins, and EDTA works as a chelates factor to inhibit nucleases, also preventing the growth of microorganism (Burgoyne, 1996; Smith *et al.*, 2004). However, our data indicates that studies on microsatellite loci require only a few amounts of DNA well-preserved with a medium period on FTA card.

Results of the DNA amount ranged from 3.23 to 10.61 ng/ µL with a mean of 8.363 ng/ µL (Table 1). The part of the input FTA card material was commensurate (two 1.2 mm punches/extraction), and isolated with Two repetitions for each sample. From data research, we can see the variation in both within and among the amount's DNA samples. Some variation in DNA concentration is expected with limited range. However, medium internal variation among two repetitions (SD values between 0.67 to 3.81). DNA recovery can affect by many compounds were implicated as an inhibitor factor, such as haemoglobin (Al-Soud and Rådström, 2001), immunoglobulin G (IgG) (Al-Soud *et al.*, 2000) heme compound (Akane *et al.*, 1994) and viscosity of blood (Wilhelm *et al.*, 2014). Also, increase over time and store condition effect on the concentration and purity of the DNA recovered from FTA card. Therefore, the FTA cards Chemical material and dry storage conditions may not be enough for preserving the safety of DNA fragments.

Quantification data for DNA concentration indicate that (1) the DNA amount is decreased over the storage period, and (2) over storage time led to DNA degradation.

**Table (1) the variation of DNA concentrations.**

Samples collection	Months stored on FTA card	samples	Mean of Two repetitions ng/ µL	SD
*finger prick	2 months	1	10.65	2.2
		2	9.77	3.81
	10 months	1	7.3	1.2
		2	7.6	1.4
**Venous Blood	2 months	1	9.82	0.91
		2	9.11	1.1
	10 months	1	3.23	1.26
		2	3.43	0.67

\*Samples stored at room temperatures.

\*\*Samples stored in freeze for 4 months with EDTA tube before transfer to FTA card.

Reduction of AmpFSTR® Profiler Plus™ Kit reaction volume, which is used as a cost-saving style, can reduce the concentration of PCR components. Two approaches of PCR reaction were used, the

manufacturer recommended for this kit (25 µL) or Reducing reaction volumes to half 12.5 µL, all experiments of STR profile were conducted in two repetitions (n=2). STR profile with DNA

concentration values 0.5 and 1 ng/  $\mu\text{L}$ , in both samples (finger prick and Venous Blood) approximately there were no differences in the electropherograms obtained for both samples amplified in a final reaction volume of 25  $\mu\text{L}$  and Reducing reaction volumes to half 12.5  $\mu\text{L}$ . Thus, the full STR profile obtained from our results supports

evidence from previous studies (Iyavoo *et al.*, 2015; Caputo *et al.*, 2017; Brito *et al.*, 2017).

Generally, our data indicated that the peak height of STR loci in histogram results, lowering progressively with decreasing of DNA amounts. Two repetitions amplifications performed on DNA, with input ranging 0,1-10 ng, all the data were on scale Table 2.

**Table 2: Sensitivity of the AmpFSTR® Profiler Plus™ Kit for DNA amount**

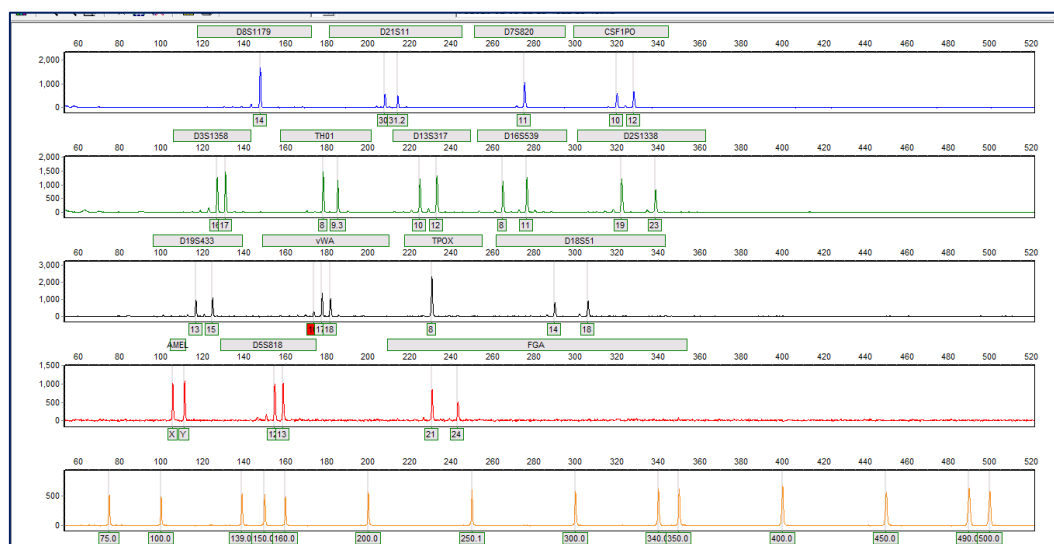
Amount of DNA (ng)	Samples	Stored date	Relative fluorescence units (RFU)
1	finger prick Venous Blood	2 and 10 months	490 -2400
0.5	finger prick Venous Blood	2 and 10 months	390-1700
0.1	finger prick	2 months	85-1950
0.1	Venous Blood	10 months	85-700
10	finger prick	2 months	4900-35000
10	Venous Blood	10 months	5000- 34000

\*Reaction volume fixed at 12.5  $\mu\text{L}$ .

Generally, 1 ng/ $\mu\text{L}$  from finger prick (2 months) gave better preferable peaks due to ideal DNA amount and low level of inhibitors competing with PCR products. Under these conditions, a product of the STR markers was well balanced and absent of non-specific or dropout alleles. for example, DNA profile in figure (1) At a threshold of 85 RFU, “drop-out” begins to occur. minimum peak height was listed as 490 rfu at FGA, maximum peak height listed as 2,400 rfu at TPOX at high RFU thresholds. Also, six of the sixteen peaks below 1000 RFU, whereas only one peak over 2000 RFU. Figure (2) showed a histogram for DNA profile when used 0.5 ng of DNA. minimum peak height was listed as 390 rfu at D19S433, maximum peak height listed as 1700 rfu at D3S1358

at high RFU thresholds. Besides, nine of the sixteen peaks below 1000 RFU, whereas only six peaks over 1000 RFU.

As histogram data, Homozygotes loci are exactly twice heterozygotes due to allele sharing. Therefore, the visible comparison of peak height distributions showed that heterozygous peak altitudes were smaller than those obtained from homozygous loci. Also, the peak high of heterozygous were more identical in DNA profile used 1 ng compare to 0.5 ng of DNA, due to the balance of heterozygote peaks typically declines progressively with decreasing DNA amount. These data support the approach of previous studies (Bregu *et al.*, 2013; Kirkham *et al.*, 2013; Ye *et al.*, 2015, Liu *et al.*, 2019).



**Figure (1): sample of DNA profile, half PCR volume (12.5  $\mu\text{L}$ ) with 1 ng/  $\mu\text{L}$  of DNA concentration.**

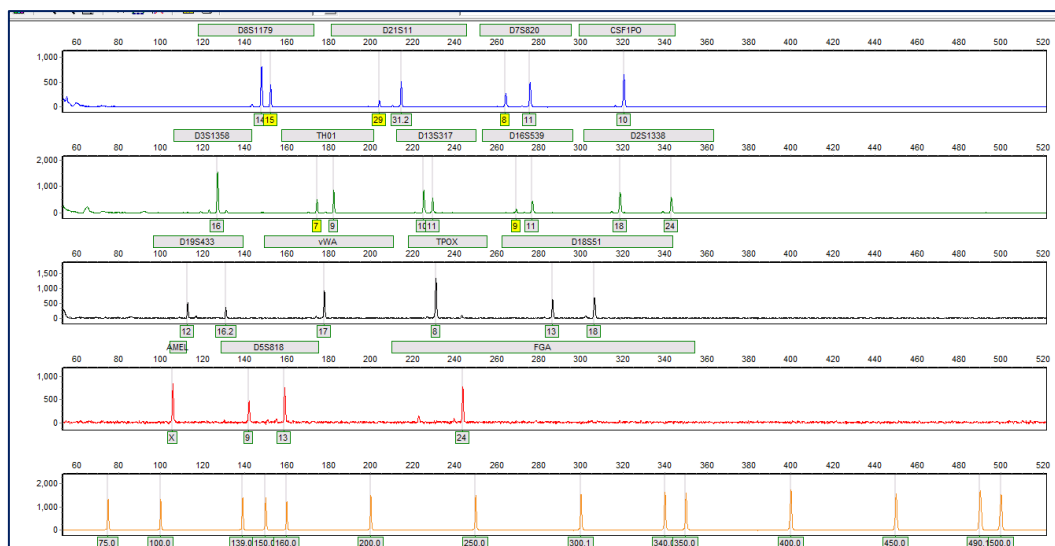


Figure (2): sample of DNA profile, half per volume (12.5  $\mu$ L) with 0.5 ng/  $\mu$ L of DNA concentration.

The threshold used in this study is called the stochastic threshold (SWGDM 2010) or low template DNA threshold (Gill *et al.*, 2009). In general, the threshold is put at around 100 RFU to 150 RFU and determines the minimum peak altitude for alleles such that it can be confidently concluded all alleles at a locus are present and no genetic loci have unsuccessful amplify during PCR due to a few amounts of templet DNA, degradation of DNA or PCR inhibition (Budowle *et al.*, 2009). In general, no

differences in example electropherograms from a finger prick and Venous Blood samples at 0.1 ng/  $\mu$ L of DNA concentration. Absent or very low peak on 0.1 ng/  $\mu$ L of DNA concentration Peak heights of amplification products and the level of background noise depending on the instruments and settings of individual laboratories. As a figure (3), We, therefore, recommend evaluating a suitable threshold for detection of alleles based on results obtained during an internal validation in the laboratory.

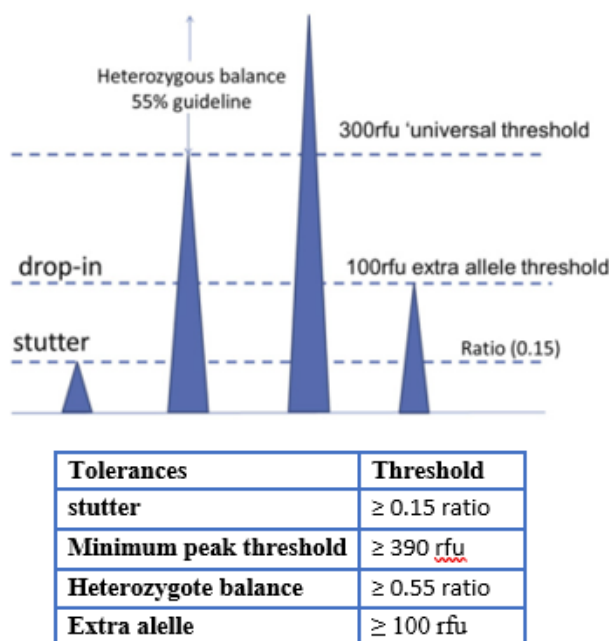


Figure. 3. Diagrammatic representation of thresholds. The 390 rfu threshold. Below this level no alleles are reported. The 100 rfu is threshold of the drop-in (extra allele). Modified from (Kirkham *et al.*, 2013).

Below figure (4) show a clear effect of amount and purity of DNA when used 0.1 ng/  $\mu$ L of DNA concentration. Thus, Amplification of 0.1 ng/ $\mu$ L DNA may lead to incomplete profiles, lacking one or more alleles. Furthermore, allele dropout, significantly increased as the amount of starting

template was reduced. The imbalance peak high in heterozygous can be returned to preferential amplification of one locus over the second locus. Thus, some alleles may be sufficient for detection as a normal peak due to sufficiently replicated via PCR amplification. Sometimes in heterozygous STR locus

may appear to be homozygous falsely due to only one of the two alleles amplified will be (Levine, 2012). Quantity and quality of DNA lead to Allele drop out. no locus drop out, locus drop out was defined as both

alleles from a heterozygous or the single allele from a homozygous locus missing from the profile (Grisedale, 2014).

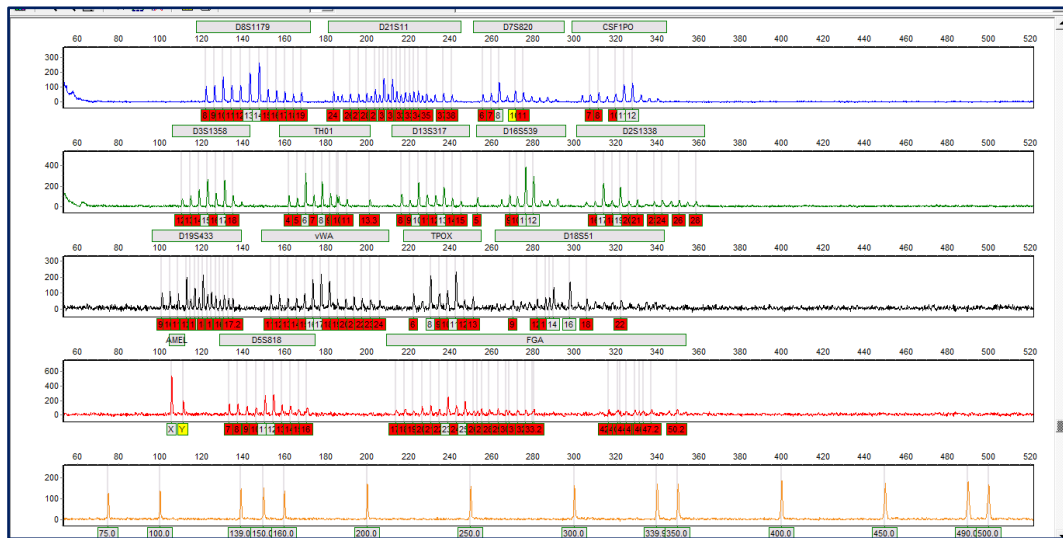


Figure (4): sample of DNA profile, half PCR volume (12.5  $\mu$ L) with 0.1 ng/  $\mu$ L of DNA concentration.

Whereas high concentration of DNA (10 ng /  $\mu$ L) show Amplifying higher quantities of DNA than the optimal amount suggested by the manufacturer's protocols can result in incomplete 3' A nucleotide addition and therefore split peaks. The addition of 10 ng of template DNA to a PCR reaction results in split peaks compared to using only 2 ng of the same template DNA (butler 2005). Also, dilution DNA amounts lead to dilution amount of PCR inhibition. Thus, add 10 ng /  $\mu$ L DNA means also increase the level of PCR inhibitor. figure (5) and (6) represent the STR profile from two sources of DNA (finger prick and Venous Blood) with 10 ng /  $\mu$ L of DNA and

different stored condition. Although difference source of DNA in two histograms (figure 5 and 6) but our results show split peaks in all loci, while the low peak in CSF1PO, D18S51 and FGA loci. In blood, many factors described as inhibitors of PCR. Anticoagulants, for example, heparin, may also inhibit the PCR (Costafreda *et al.*, 2006), humic acid and hematin impair DNA polymerase activity, and immunoglobulin G binds single-stranded genomic DNA thus lowering the amount of available template (Al-Soud *et al.*, 2001; Kermekchiev *et al.*, 2009; Sidstedt *et al.*, 2018).



Figure (5): sample of DNA profile, half PCR volume (12.5  $\mu$ L) with 10 ng/  $\mu$ L of DNA concentration. DNA extraction from finger prick Blood stored in FTA card for 2 months at room temperature



Figure (6): sample of DNA profile, half PCR volume (12.5  $\mu$ L) with 10 ng/  $\mu$ L of DNA concentration. DNA extraction from Venous Blood stored in EDTA tube for 4 months in freeze and then spotted in FTA card to store at room temperature.

Mixture STR profile, Mixture analysis has an important role in many casework studies. We prepared and tested a mixture from two sources of DNA. This included 1 ng and 0.5 ng, with the total DNA amount kept constant at 1 ng per reaction. In figure (7), uses guidelines development by German Institutes of Forensic Science and Legal Medicine to Classification of mixed alleles (Schneider *et al.*, 2009), type A, in D8S1179 loci three alleles. therefore, no evident main contributor allele and

absent of stochastic effects. this results also found in D21S11 and D3S1358 loci. Type B includes a high ratio of consistent in loci (TH01, D13S317 D16S539, D19S433, VWA, TOPX, AMEL and D5S818). therefore, we can notice distinguish between major and minor contributors of alleles; for all peaks in heterozygous loci, absent stochastic effects. Type C: mixtures alleles without main contributor (s) in CSF1PO, D2S1338, D18S51 and FGA loci, an indicator for stochastic effects.

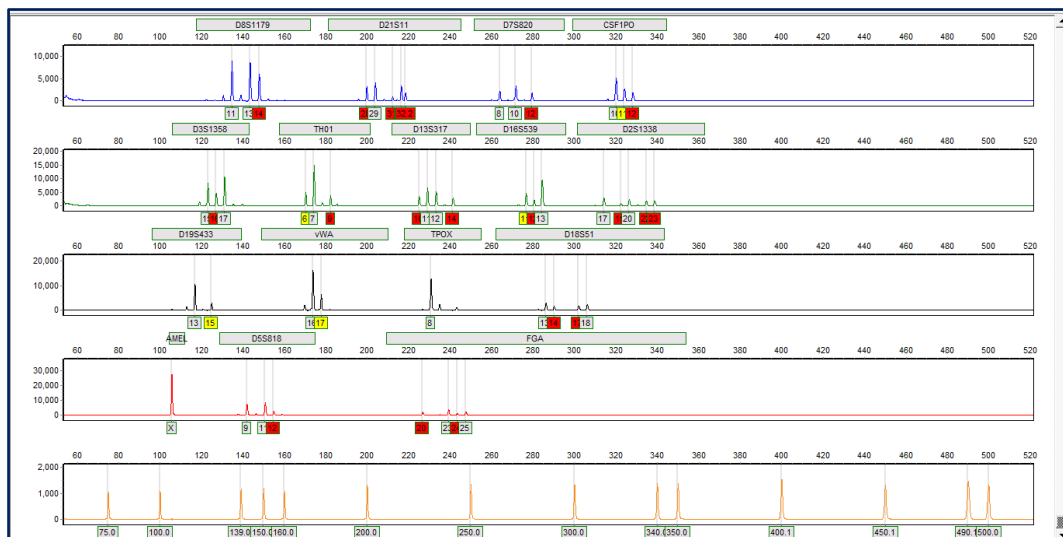


Figure (7): Mixture STR profile

### Conclusion

The present investigation was designed to determine the effect of sampling methods and store period of blood FTA card on DNA concentration. Also, estimate the effects of reduced volume PCR on a DNA profile. As our results suggest, this study has found that store sample for the medium period has a minor effect in decrease amount of DNA, but its

relative role increases when start amplified of shorter amplicons. Thus, we can conclude that the FTA card is a reliable method for collecting samples and then DNA extraction. In addition, this study supports the reduction of reaction volume, which is benefit protocol used to save 50% cost of the reagent when used a high number of a population study.

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## تأثيرات أخذ العينات وكمية الحمض النووي وتقليل حجم عدة Amp FLSTR Identifiler Plus على ملف تعريف STR

مع حسن صالح ، عقيل حسين على العاصي ، مجيد أرشيد سبح

### الملخص

تهدف هذه الدراسة الى اختبار خيارين لجمع نماذج الدم، 20 نموذج من وخز الإصبع و 20 نموذج من الدم الوريدي مجمداً لمدة أربعة أشهر وتأثيرهما على عزل الحمض النووي الذي تم حفظه على بطاقات FTA. أيضاً ، لتقليل تكلفة الحصول على الملف التعريفي للحمض النووي، قيمنا كفاءة المجموعة بتقليل حجم مزيج تفاعل PCR الى النصف (12.5 ميكرو لتر) مع تراكيز مختلفة من الـ DNA (0.01، 0.1، 0.5، 1 و 10 نانوغرام / ميكرو لتر) لتضخيمها بتركيزين 12.5 و 25 ميكرو لتر من مجموع (AmpFSTR® Profiler Plus™ Kit). تلاحظ دراستنا أن طرق أخذ العينات ووقت التخزين أدى إلى انخفاض في تركيز الـ DNA. تم الحصول على ملف تعريف كامل للـ DNA عند استخدام التركيزين 0.5 و 1. بينما لم يتم الحصول على ملف الحمض النووي في عينات الـ DNA ذات التركيز 0.01 ميكرو لتر، اما بالنسبة لتركيز 0.1 ميكرو لتر فقد انتج ملف تعريف بشكل جزئي للحمض النووي. لذلك كانت كمية الحمض النووي المثلّي التي أسفرت عن ملف تعريف كامل للـ DNA هي 0.5 و 1 نانوغرام / ميكرو لتر مع حجم 12.5 و 25 ميكرو لتر من مزيج تفاعل الـ PCR. بشكل عام، أشارت بياناتنا إلى أن ذروة ارتفاع مواقع الـ STR في نتائج الرسم البياني، تنخفض تدريجياً مع تناقص كميات الحمض النووي. وفقاً لذلك، تقل هذه الطريقة المعدلة من تكلفة ملف تعريف الحمض النووي إلى النصف، مع الحفاظ على أدائها.