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Isolation of DNA using IsoCode cards

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Abstract. The use of cards for the collection of different human biological samples has become a frequent and very useful practice in forensic laboratories. In this work, different biological fluids collected in IsoCode cards and in FTA cards were assayed, and results obtained after amplifying the DNA with the use of two commercial genotyping kits were compared. © 2006 Published by Elsevier B.V.

Keywords: IsoCode cards; FTA cards; Validation; Forensic applications

1. Introduction

The collection of fluids for forensic genetic analysis is constituted in an important procedure that should guarantee the preservation of the sample, to facilitate its transport, its analysis and its file.

The samples collected on the support of the IsoCode cards stored in different banks of biological samples have been used for several years in different international programs [1,2]. In our laboratory, the use of cards for the collection of biological samples has allowed us to speed up processes of massive work, specifically cases of filiation, easy storage, and practical filing of evidences to ambient temperature and for long periods of time at a low cost. In our work, different biological fluids collected in IsoCode cards and in FTA cards were analyzed with two commercial genotyping kits.

2. Materials and methods

20 samples of different biological fluids were processed (5 stains of stain of periferical blood, 5 stains of blood postmortem, 5 stains of powdered bone tissue decalcified and 5 stains of rasped of epithelial cells from internal buccal cavity) of cases of filiation and of individuals that consented voluntarily to the study. The samples of periferical blood,

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epithelial cells and the powdered bone tissue decalcified were put simultaneously on support of FTA cards and of IsoCode cards.

FTA cards were treated according to the PET (standardized protocol of work) of the laboratory of the Group of Forensic Genetics of INML and CF (National Institute of Legal Medicine and Forensic Sciences of Colombia).

IsoCode cards was carried out on two assays: one according to the protocol recommended by the commercial house (S&S IsoCode) (assay 1) and the other using half of elution volume (50 μL) (assay 2) [1,3].

Amplification was done using the genotyping kits: AmpF*l*STR Identifiler and Power Plex 16, according to the PET of INML and CF and using 1 and 2 μL of the obtained dilution of DNA from isolation of the IsoCode cards. The amplified products were detected with a genetic analyzer, ABI-3100. The genetic profiles of the samples for copy in support of FTA and IsoCode were compared. A profile with all the systems amplified (picks above 50 UF) was considered indicative of sufficiency of quality and quantity of the DNA.

2.1. Statistical analysis

Analysis of paired *t*-test was used for evaluating significant differences among the results obtained for the samples by copy in support of FTA and IsoCode.

3. Results

There were significant differences in the results of the amplifications for the 5 stains of blood and the 5 stains of epithelial cells of buccal cavity, in support of the FTA and IsoCode cards and among the two assays of isolation of the carried out IsoCode cards.

Table 1
Results of PCR obtained with Power Plex 16 and Identifiler kits in human biological samples

Samples	Power Plex 16 systems			Identifiler		
	IsoCode		FTA	IsoCode		FTA
	Assay 1	Assay 2		Assay 1	Assay 2	
B1	+	+	–	+	+	+
B2	+	+	+	+	+	+
B3	+	+	+	+	+	+
B4	+	+	–	+	+	+
B5	+	+	+	+	+	+
EP1	–	+	+	–	+	+
EP2	+	+	+	+	+	+
EP3	+	+	+	+	+	+
EP4	–	+	+	–	+	+
EP5	–	+	+	–	+	+
PMB1	+	+	NP	+	+	NP
PMB2	+	+	NP	+	+	NP
PMB3	+	–	NP	–	+	NP
PMB4	–	–	NP	–	–	NP
PMB5	–	–	NP	–	–	NP

B, blood sample; EP, epithelial cells; PMB, postmortem blood; NP, no processed.

Amplifications were obtained for 100% of the stains of epithelial cells of buccal cavity in support of IsoCode subjected to assay 2, while the samples subjected to assay 1 offered 40% of amplification results.

The same stains on FTA cards showed successful results for all the amplifications with the used kits (Table 1). The stains of blood showed complete genetic profiles in 100% of the samples processed in support of IsoCode with the assay of isolation 1 and 2 for the two used kits. The amplification of blood samples in support of FTA was of 60% with the kit Power Plex 16 and of 100% with the kit Identifiler. Likewise, there were differences in the signs obtained for the results of the assays of isolation of DNA from the IsoCode cards, being superior to the signs of the assay 1 (Table 1).

The success of the amplification of the stains of blood postmortem was 50% in IsoCode cards. These samples did not have replicas in FTA cards to compare. In the samples of powdered decalcified bone in the two used supports, amplification was not observed.

4. Discussion

The stains of blood in support of cards can present variability with its relationship to the volume of sample adsorbed. Different results were reached during the amplification of obtained DNA of blood samples, hence supporting a similarity between FTA and IsoCode cards. However, the biggest difference was evidenced in cases of saturated cards of blood with its relationship to the used support.

In an isolation of FTA, residual proteins can be liberated during PCR and they could limit the quality of the amplification, mainly in saturated stains. Stains of blood saturated on card in support of FTA offer an excess of DNA that it cannot control (since the complete cut is added to the reaction of PCR); they generate products of PCR with preferential amplifications and, in general, of low quality. On the other hand, the elutions of the isolations of IsoCode supports allowed handling of different volumes with template in the PCR, regulating the concentration of DNA to amplify. The above-mentioned case could have generated the differences observed in our results.

Differences were also observed in the amplifications obtained with the two used kits. The kit Identifiler offered similar results with anyone of the used supports. The kit Power Plex showed better results using the IsoCode cards, which suggests bigger demand as soon as quantity and purity of the DNA.

Our results allow to conclude that the routine use of a support for stains in filiation tests should be standardized with the kit to use and depending on the type of fluid, since we find differences, important recommendation mainly for those laboratories that lack a system of quantification of DNA.

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