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Automation of postmortem or non standard reference samples genotyping using FTA®

B. Pesquier *, A. Taillé, G. Garcin, S. Frackowiak, P.-E. Coiffait

Laboratoire de Police Scientifique de Marseille – Section Biologie – 13245 Marseille Cedex 04 – France

Abstract. We describe here our work toward automation of the analysis of postmortem samples or buccal swabs and brushes, using FTA® cards. First we manually spot the samples on the FTA®. Then we perform their analysis, according to our high-throughput process. The genotypes of spotted samples are compared to those obtained from the original samples manually extracted, to estimate the reproducibility. Then, the impact on the genotypes' quality of the amplification of 1, 2 or 3 FTA® punches in the same well is assessed. Our results show that: 1) The quality of the profile from a FTA is positively correlated to the quantity of DNA obtained by manually treatment of the sample. 2) No sample leads systematically to acceptable results. 3) In few cases, the addition of more than one punch in the same reaction can ameliorate the profiles. However, the improvement is not correlated to the quantity of DNA in the original samples. In conclusion, this technique is convenient for DNA typing as it offers all the advantages of the subsequent automated treatment. Yet, some cases may need multiple analysis to obtain a reliable profile.

Keywords: FTA; Autopsic blood; Non standard reference samples; Genotyping

1. Introduction

In France, the majority of reference samples are saliva applied on FTA cards, and their treatment have been automated in order to get high throughput. Nevertheless, some of them – like postmortem samples (autopsic blood or tissues) or buccal swabs and brushes – are not standardized, and still manually processed. It is a time consuming process and has a higher risk of error compared to automation. So, we have developed a technique to automatically analyze these samples. We have first transferred them on FTA®. Then we have assessed the ability of our automated process to give a reliable profile of these samples. So, we describe in the first part the methodology used to apply these different samples on FTA®. Then we compare the results of the technique we develop with those obtained on the original samples

* Corresponding author. Tel.: +33 491628538; fax: +33 491629770.
E-mail address: Benjamin.Pesquier@interieur.gouv.fr (B. Pesquier).

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category name	Non organic extraction result (reference)	Autopsic blood	Blood on gauze	Brushes	buccal swabs	soft tissue (muscle and marrow)	Nb of tested samples
Cat 0.1	Full amplification with 0.1µL of extracted DNA	6	0	5	4	1	16
Cat 1	Full amplification with 1µL of extracted DNA	10	4	1	2	2	19
Cat 10	Full amplification with 10µL of extracted DNA	3	0	0	1	0	4
Cat -	unsuccesfull amplification	1	0	0	0	3	4
	Total	20	4	6	7	6	43

Fig. 1. Number and types of samples tested.

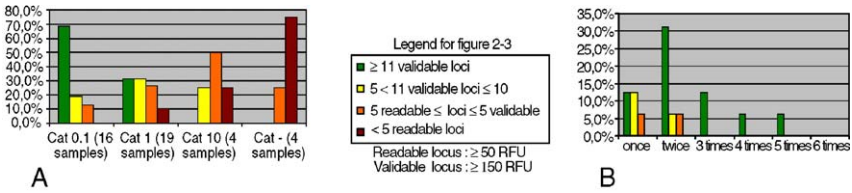

Fig. 2. (A) Quality of profiles obtained. (B) Reproducibility category 01.

manually treated. In a last part, we try to improve results by assessing amplification of 1, 2 or 3 punches in the same well.

2. Material and methods

50 µL of liquid blood were spotted on the paper and the dried blood on gauze was re-hydrated using 50 µL of sterile deionized H_2O and then strongly pressed on FTA®. For the bone marrow, 30 µL were directly put on the FTA®. The other postmortem tissues were cut (1 cm^3) then strongly rubbed on the paper. Buccal swabs or brushes were moistened before application. All the samples were allowed to dry at least overnight. Then the FTA paper was processed according to the high throughput process we developed for standardized reference samples [1]. All the original samples have been analyzed, in parallel, using the magnetic

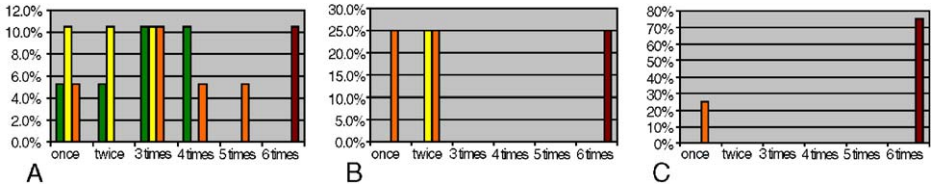

Fig. 3. (A) Reproducibility category 1. (B) Reproducibility category 10. (C) Reproducibility category – Abscissa for Figs. 2B and 3A–C: percentage of sample giving this result according to the number of times.

	Autopsic blood		Buccal swabs		Brushes		Blood on gauze		Soft tissues	
Category Name	improvement	no improvement	improvement	no improvement	improvement	no improvement	improvement	no improvement	improvement	no improvement
Cat 0.1	0	3	1	3	0	5	0	0	0	1
Cat 1	4	2	0	2	0	1	2	2	0	2
Cat 10	1	2	0	1	0	0	0	0	0	0
Cat -	5	0	0	5	0	0	0	0	0	3
Total	5	7	1	5	0	6	2	2	0	5
Total (%)	42%	58%	14%	86%	0%	100%	50%	50%	0%	100%

Fig. 4. Multi punches assay.

	Blood		Swabs		Brushes		Blood on gauze		Soft tissue	
	reference extraction	FTA	reference extraction	FTA	reference extraction	FTA	reference extraction	FTA	reference extraction	FTA
≥ 5 validable loci	19	13	7	7	6	4	4	2	3	3
< 5 validable loci	1	7	0	0	0	2	0	2	3	3

Fig. 5. Results of reference extraction against results of automated process.

beads extraction protocol on KingFisher® [2], which is our routine protocol for non standard reference samples. In Fig. 1, the number and types of samples manually applied on FTA® paper are presented.

3. Results

The quality of the profiles obtained by our automated process has been compared to the results obtained by our reference protocol (Fig. 2A). The quality of the profile from a FTA-spotted sample is positively correlated to the quantity of DNA in the reference extract of the corresponding sample.

Then, we have assessed the reproducibility for the categories created (Figs. 2B and 3A–C). These results indicate that FTA technology does not give good reproducibility.

In Fig. 4, the results of the multi punches assay show that, in a few cases, addition of more than one punch in the same reaction leads to better profiles. However, this improvement does not seem to be correlated to the quantity of DNA in original samples.

Finally, Fig. 5 shows that, with this automated process, we have been able to obtain reliable profile for all the different types of samples.

4. Comments and conclusion

We found good positive correlation between the quality of the sample and its ability to amplify by this automated process. However, majority of the samples should be tested multiple times to be sure to obtain reliable profile because of lack of reproducibility of FTA technology (as already noted for saliva). The results with buccal brushes could be improved by a better application on FTA. For example, we could pellet the cells first to ensure that enough cells have been transferred on FTA. Multi punches assay does not give good improvement of critical samples. Indeed, we can notice some inhibition by DNA excess, but no real improvement in case of lack of DNA in the original sample. These data could be explained either by a polymerase inhibition by excess of FTA, or a lack of purification of the sample. For blood samples, this may be ameliorated by the use of the "FTA Purification Reagent®". With this automated process, the analysis of non standard reference samples is significantly less time consuming. As manual handling is reduced, accuracy in sample treatment is increased, putting this step in accordance with quality assurance guidelines. In conclusion, we show that this technique can be routinely used to process non standard reference samples. Yet, optimization of some conditions could improve performance of this protocol for most critical samples.

References

[1] Delpech and coll, INTERPOL Meeting, Lyon, October 2004. "Use of an automated process for DNA typing of buccal samples to supply the French national database".

[2] Pesquier and coll, IASF Meeting, Montpellier, September 2002. "A semi automated DNA extraction method from swabs using magnetic beads".