

A simple DNA isolation method for dried blood spots (DBS) and buccal swab samples (BSS)

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Introduction

Recently, we developed a simple method for DNA isolation from dried blood spots (DBS) using capillary blood.¹ Although needing less blood and being less invasive, the patient is still subjected to a needle. Therefore, we tested this DNA isolation protocol on samples obtained by buccal swab (BS).

Methods

Twenty-five DBS samples were obtained by finger prick, applying blood to sampling paper, and 25 BS samples (BSS) by rubbing a sterile cotton stick (figure 1) against the inside of the subjects' cheek.

A 3 mm disk was cut out of the sampling paper or the tip was cut off the cotton stick. Additionally, the 3 mm disk or the cotton tip (figure 1) was placed into a cup, adding 500 µl sterile water and vortexed 3 times during 5 seconds. The water was pipetted off. After adding 200 µl 10% Chelex-100 solution, the cup was placed in a water bath at 95 °C for 30 minutes.

Finally, this ready for use DNA solution was pipetted into a new cup.



Figure 1. Cotton stick before and during isolation.

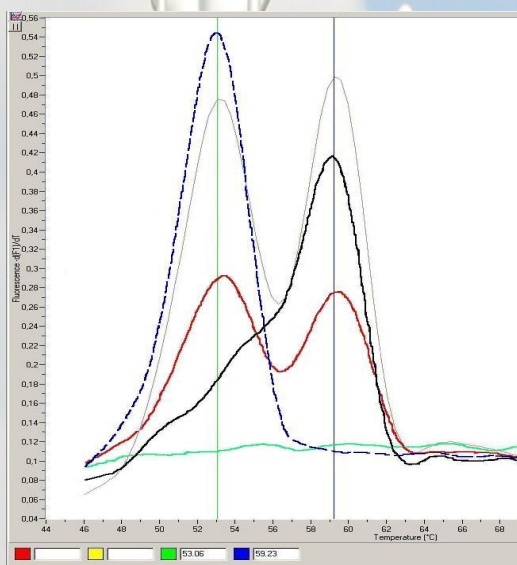


Figure 2. 1 EDTA sample 2 buccal swab 3 dried blood spot 4 positive control 5 blanc

Results

Real-time PCR results obtained for both sample types, DBS and BSS, corresponded completely with results obtained using a commercial DNA isolation kit and EDTA whole blood. Moreover, melting peaks analysis gave good results for all sample types, as shown in figure 2.

Additionally DNA yields were measured using the NanoDrop®. Mean DNA concentrations were 16.1 ng/µl (12.8-19.4) for DBS and 70.2 ng/µl (57.3-83.1) for BSS, respectively ($p < 0.001$), using the isolation method by Wijnen et al.¹

Using the method previously described by Fisher et al.² resulted in a mean concentration of 17.5 ng/µl (12.8-22.2) for DBS. (figure 3)

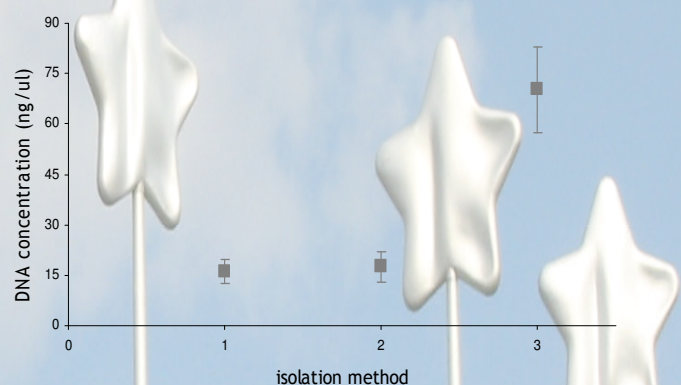


Figure 3. 1 DBS, method Wijnen et al.¹; DNA conc. = 16.1±3.3 ng/µl
2 DBS, method Fisher et al.²; DNA conc. = 17.5±4.7 ng/µl
3 BSS, method Wijnen et al.¹; DNA conc. = 70.2±12.9 ng/µl

Conclusions

1. Both DBS and BSS are useful for this cost-effective DNA isolation method.
2. The DNA yields of BSS were considerably higher compared with the yields of DBS.
3. Especially, the non-invasive BS appeared to be a good alternative for invasive sampling methods.

References

1. Wijnen PA, et al. Genotyping with a dried blood spot method: a useful technique for application in pharmacogenetics. *Clin Chim Acta* 2008;388:189-91.
2. Fisher A. et al. Simple DNA extraction method for dried blood spots and comparison of two PCR assays for diagnosis of vertical human immunodeficiency virus type 1 transmission in Rwanda. *J. Clin Microbiol* 2004;42:16-20.

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