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Assessment of method for buccal swab samples preservation in extreme environmental conditions for population genetics and forensic purposes

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ABSTRACT

The preservation of buccal swab samples in remote environments with high temperature and humidity is a huge problem in samples for forensic and population genetic samples. Since there are no low-cost methods that can generate good results, the action of TNE buffer has been evaluated. TNE buffer has the properties of inactivating nucleases, preventing the growth of microorganisms in the sample and maintaining the integrity of the cells under extreme environmental conditions. The advantage obtained with this poorly diffused method was that the swab does not required a drying process neither low temperatures prior to DNA extraction and analysis showing higher cell integrity and quality of DNA compared with controls.

1. Introduction

A huge problem of swab samples for forensic purposes in tropical extreme weather regions (humidity 86–96%, temperature 36–46 °C) is the lack of a preservation medium, during transportation, that guarantees the physical-chemical integrity of DNA samples. Swabs need to dry as soon as possible but the conditions in these zones prevent the proper drying, which leads to contamination with microorganisms. Hence, a cheap and accessible solutions (buffer) for preserving samples and keep DNA quality, is vitality for forensic and population genetics analysis. In this study, a method for preserving buccal swab samples, under extreme environmental conditions, was evaluated using modified TNE-Ethanol-Buffer; it inactivates nucleases, preventing the development of microorganisms and maintains cells integrity. The advantages of this method is that the swab does not require a drying process and low temperatures prior to DNA analysis. Here, we report the results of swabs transported and maintained in the TNE-Ethanol Buffer from 20 volunteers that were evaluated at 37 °C, 96% humidity and time frame (0–1–3–7 days). After 7 days, it shows complete DNA profiles.

2. Methods

2.1. Samples

Samples were taken from healthy individuals, scrubbing with a cytobrush inside of the cheek; the cytobrush was then washed with 50 mL of saline solution. The remaining liquid was collected and the cell dilution was set to 7×10^5 cell/mL. Sterilized swabs were inserted in the solution so cells adhere to the swab. Besides, field samples were taken with the swab directly from the cheek without subjecting them to a cellular concentration adjustment, at an area with high heat and temperature, maintained at room temperature over 5 days and then placed at 4 °C and the efficiency of the buffer was evaluated over a period of 6 months.

2.2. Experimental conditions

Buffer TNE was prepared as Aidar et al. [1] modified at 2:1 ethanol 99,5%:(TrisHCl6.5 mM/EDTA2.3 mM/NaCl16.6 mM). 500 µL of TNE buffer were placed in various 1.5 mL Eppendor tubes. Three swab samples were obtained from each individual; two swabs were placed in TNA buffer; the remaining swab was left to dry at experimental conditions: temperature of 37 °C and 96% humidity, simulating extreme

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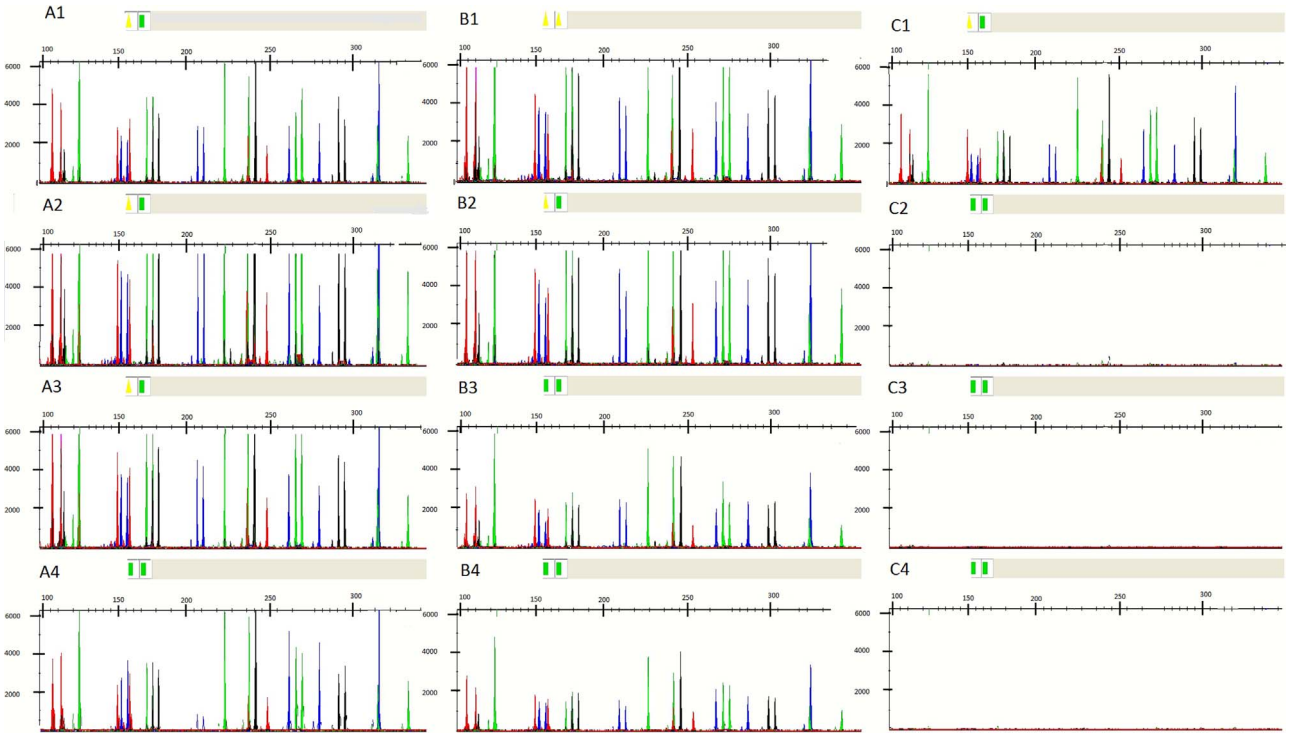

Fig. 1. Identifiler male genetic profile of buccal swab sample. A: Sample preserved with TNE buffer and DNA extracted by Phenol- Chloform method; A1:day0; A2:day1; A3:day3; A4:day7. B: sample preserved with TNE buffer and DNA extracted by Chelex method B1:day0; B2:day1; B3:day3; B4:day7. C: Sample preserved without TNE buffer, under extreme simulated environmental conditions, DNA extracted by the Chelex method. C1:day0; C2:day1; C3:day3; C4:day7.

environmental conditions. For taking out the cells from TNE buffer, tubes were placed in 500 µL of 1 × PBS, then vortex, the swab removed and centrifuged for 2 min, three times to obtain a pellet of cells. The cell integrity was verified every day by Giemsa staining.

2.3. DNA extraction

By Chelex method for inactivating nucleases and making cellular disruption, pellets were subjected three times to a thermal shock of 94 °C for 5 min/–80 °C 5 min; after, 200 uL Chelex was added, incubated for one hour at 54 °C and 94 °C for 10 min then centrifuged 10000RPM for 5 min. For Phenol-Chloroform extraction: 5 ul of Qiagen® proteinase K and 180 ul of PureLink Genomic digestion buffer (Invitrogen®) lysis buffer was added to cell pellet and incubated at 54 °C for 4 h. Sambrock et al. DNA extraction protocol was used [2]. Quantification of DNA was done by spectrophotometry using NanoDrop® as recommended. Integrity DNA was analyzed on a 0.8% agarose gel, run at 10 V/cm for 30 min sybr-safe stained.

2.4. Molecular assessment

DNA (5 ng/µL) was amplified by 30 cycles as follows: Qiagen ®Multiplex PCR 1 ×, 0,5 µL Identifier primer mix (Applied Biosystems®). Amplicon was run on ABI3130 Applied Biosystems® using GeneScan™ LIZ 600® size as internal standard, sized and heighted by Genemapper V3.2 software.

3. Result and discussion

3.1. Cellular integrity

Cellular integrity was lost at day 1 in samples that did not contain TNE buffer (Fig. S1B). On the other hand, samples that were preserved in a TNE buffer maintained their integrity in both the cell membrane and the nucleus (Fig. S1A) this is due to the various compounds that make up the TNE buffer. The ethanol present in the buffer prevents both bacterial proliferation in the sample freezing when stored at −20 °C, a condition that destroy the cellular membrane. Furthermore, in addition to cells with genetic material, saliva also contains cells many enzymes such as: nucleases which have divalent ion cofactors such as Mg^{2+} or Zn^{2+} that degrade nucleic acids, that is why the addition of EDTA in the buffer allows to maintain DNA integrity due to the chelating effect it has.

3.2. Verification of genetic profiles using identifier

The analyzed genetic profiles showed strong differences in peak intensity (Fig. 1), the samples that were preserved in TNE buffer kept the over time, guaranteeing consistent genotypes up to day 7. On the other hand, dried samples under experimental conditions showed low intensity peaks after day 1(Fig. 1C; Table 1S); and allelic dropout due to poor DNA quality (Fig. 1C2, C3, C4). DNA extracted by Chelex showed peaks with less intensity compared to those extracted by Phenol-Chloroform (fig. 1, AvsB). Peak intensity with the Phenol-Chloroform method remains consistent (Table 1S, 2S, 3S).

4. Conclusion

The cell integrity and the quality of genetic material could be maintained over time using TNE buffer. High temperature (37 °C) and humidity (96%), caused the rapid degradation of genetic material in dried swabs, determining the loss of genetic profile after day 1. The method of DNA extraction does not interfere with the quality of the results, but rather on the concentration of the DNA obtained the extraction of DNA with Chelex showed higher concentration of DNA.

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Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fsigss.2017.09.178

References

[1] M. Aidar, S.R.P. Line, A simple and cost-effective protocol for DNA isolation from buccal epithelial cells, Braz. Dent. J. 18 (2) (2007) 148–152.
[2] J. Sambrook, D.W. Russell, Purification of nucleic acids by extraction with Phenol:Chloroform, Cold Spring Harb. Protoc. 2006 (1) (2006) (pdb.prot4455-prot4455. http://www.cshprotocols.org/cgi/doi/10.1101/pdb.prot4455).