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Comparison of different DNA preservation solutions for oral cytological samples

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ABSTRACT

Objective: The objective of this study was to compare the DNA preservation capacity of buccal mucosa exfoliated cells when stored in different solutions under varying time and temperature conditions.
Design: DNA preservation solutions, including Dimethyl sulphoxide disodium-EDTA-saturated NaCl (DESS), Tris-EDTA-NaCl-Tween20 buffer (TENT), Nucleic Acid Preservation Buffer (NAP), and phosphate-buffered saline (PBS), were prepared. Buccal mucosa cells from a single patient were collected, dispensed into these solutions, and stored at room temperature (RT) and 4 °C for 24 h, 72 h, 30 days, 90 days, and 180 days. DNA was extracted using the salting-out method and the QIAamp DNA Mini Kit. DNA concentration and purity were determined using the QuBit device and NanoDrop, while DNA integrity was assessed using the Agilent 4200 TapeStation system. The ability to amplify the IFNA primer was also evaluated by PCR.
Results: The salting-out method yielded better concentration and purity results, with PBS, TENT, and DESS buffers demonstrating superior concentration values when stored at 4 °C, resulting in mean values exceeding 10 ng/μL for up to 30 days. DESS consistently exhibited the best integrity values over time for both temperature conditions. Amplification capacity was enhanced when samples were stored at 4 °C. When stored at RT, PBS achieved 100% amplification within 24 h. NAP yielded the poorest results.
Conclusion: In the context of long-term preservation, the DESS buffer emerges as the most effective solution, maintaining requisite DNA quality and quantity standards for up to 30 days at RT and up to 3 months at 4 °C.

1. Introduction

Exfoliated cells from the buccal mucosa are a promising source of genomic DNA (Borthakur et al., 2008; Shimizu et al., 2022). DNA from oral cells can be used for various purposes such as monitoring cancer (Cortés-Gutiérrez et al., 2021) and DNA damages (Alabi et al., 2020; Bhagwath & Chandra, 2014). These cells can be obtained non-invasively, painlessly, and quickly, which ensures easy acceptance by individuals who can also perform self-collection. Additionally, buccal mucosa exfoliated cells can be collected multiple times over time. The collection procedure can be performed in two ways: dry brushing or using liquid rinse (García-Closas et al., 2001; Huang et al., 1999; Kim et al., 2022).

The dry method is simple, cost-effective, yields sufficient quantity and quality of DNA for genotyping. Furthermore, samples collected by this method showed lower sensitivity to long-term storage effects. Dry collection of oral cells can be carried out using cytological brushes or cotton swabs, yielding a similar amount of DNA in both cases (King et al., 2002; Ambroa-Conde et al., 2022).

After collection, DNA extraction can be performed immediately or the sample can be stored for different periods of time. However, DNA degrades with increasing time and temperature. Ideally, it should be isolated shortly after collection, cryopreserved, or stored in preservation media (Alaeddini et al., 2010; Woo & Lu, 2019). Notwithstanding, in many situations, immediate access to the necessary equipment may not be available, and commercially available preservation cards and

Abbreviations: DESS, Dimethyl sulphoxide disodium-EDTA-saturated NaCl; DIN, DNA integrity number; NAP, Nucleic Acid Preservation Buffer; PBS, Phosphate-buffered saline (PBS); RT, Room temperature; TENT, Tris-EDTA-NaCl-Tween20 buffer.

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solutions are costly, which can hinder their use in epidemiological studies or large-scale population studies (Harvey, 2006; Reeve et al., 2018; Woo & Lu, 2019). In this regard, the use of cost-effective laboratory-formulated DNA preservation solutions that ensure the viability of buccal mucosa exfoliated cells is a promising alternative in the field. Therefore, the objective of this study is to compare the DNA preservation capacity of buccal mucosa exfoliated cells that were kept in different solutions under varying time and temperature conditions.

2. Material and methods

This study was submitted and approved by the Research Committee of the School of Dentistry at the Federal University of Rio Grande do Sul (UFRGS), along with the ethics committee for human research (Protocol No. 3.389.711).

2.1. Preservation solutions

Four different preservation solutions were manipulated, namely: phosphate-buffered saline (PBS - LB LABORCLIN - 50603010), Tris-EDTA-NaCl-Tween20 buffer (TENT buffer), Dimethyl sulphoxide disodium-EDTA-saturated NaCl (DESS), and Nucleic Acid Preservation Buffer (NAP Buffer). The TENT buffer solution consisted of 10 mM Tris base (Sigma-Aldrich - SLBLO891V), 10 mM EDTA (Dinâmica Química Contemporânea - 91001), 100 mM sodium chloride (Sigma Aldrich - SLBR9752V), and 2% Tween 20 (Dako, Santa Clara, United States - 10078013) (Allen-Hall & McNevin, 2012; Sorensen et al., 2016). The DESS solution was composed of 0.25 M disodium EDTA (Dinâmica Química Contemporânea - 91001), 20% DMSO (Sigma Aldrich, Darmstadtt, Germany - RNBD3327), and saturated sodium chloride (Sigma Aldrich - SLBR9752V) (Allen-Hall & McNevin, 2012; Sorensen et al., 2016). Finally, the NAP Buffer solution consisted of 0.019 M disodium EDTA dihydrate (Dinâmica Química Contemporânea, Indaiatuba, Brazil - 91001), 0.018 M dihydrate sodium citrate (Dinâmica Química Contemporânea - 95602), 3.8 M ammonium sulfate (Dinâmica Química Contemporânea - 83521), and sulfuric acid (NEON, Suzano, Brazil - 40670) for pH adjustment to 5.2 (Camacho-Sanchez et al., 2013).

2.2. Collection of cytological samples

Using cytological brushes, collections of exfoliated cells from the buccal mucosa were performed on a single volunteer by the same examiner. The collections alternated between the right and left buccal mucosa, with 10 brush rotations, and the collected material was dispensed against the wall of 1.5 mL Eppendorf tubes containing 1 mL of the respective solutions. Triplicates of the variables to be analyzed were conducted, resulting in a total of 12 collections every 7 days. Prior to the collections, the volunteer rinsed his mouth with water for 1 min, and the same examiner performed all the collections. The volunteer provided informed consent by signing the informed consent form.

The samples were divided into four groups based on the different solutions: PBS, TENT, DESS and NAP. They were then subjected to two different temperature conditions - room temperature (RT) and 4 °C - for five different time periods before DNA extraction: 24 h (h), 72 h, 30 days (d), 90 days, and 180 days. All assays were performed in triplicate.

2.3. DNA extraction, quantification and purity

After the designated time intervals, DNA extraction was performed using the salting out method and a spin column-based nucleic acid purification (QIAamp DNA Mini Kit - Qiagen GmbH, Hilden, Germany). The salting out method involves the dehydration and precipitation of cellular proteins through the addition of a saturated NaCl solution (first described by Miller et al., 1988). The samples were centrifuged, and the resulting cell pellet was resuspended in 3 μL of Proteinase K and 250 μL of Tail Buffer which containing 100 mM TRIS (Sigma-Aldrich, SLBLO891V), 100 mM EDTA pH 7.5 (Dinâmica Química Contemporânea, 9100), 200 mM NaCl (Sigma Aldrich, SLBR9752V), and 1% SDS (Sigma Aldrich, L3771). The solutions were then incubated on a 65 °C heat block for 60 min. Next, 100 μL of 5 M potassium acetate was added followed by centrifugation at 12,000 rpm, 4 °C, during 20 min. The supernatant was transferred to a new tube and 256 μL of cold isopropanol was added separately, both followed by centrifugation at 12,000 rpm, 4 °C, during 15 min. The supernatant was discarded, and 1 mL of 70% ethanol was added, followed by centrifugation at 12,000 rpm, 4 °C, for 2 min. Finally, the ethanol was removed, and the DNA was resuspended in 20 μL of TE Buffer. The spin column-based nucleic acid purification method was performed following the manufacturer's instructions for the extraction of cytological samples from buccal scrapings, resulting in 150 μL of solution containing the DNA.

The DNA concentration and purity were determined immediately by the QuBit 2.0 fluorometric quantification device (Thermo Scientific) and NanoDrop microvolume spectrophotometer (Thermo Scientific), respectively. For the quantification purpose, the Qubit DNA Assay Kit (Thermo Scientific) was used according to the manufacturer's instructions: Qubit™ working solution was prepared by diluting the Qubit™ dsDNA HS Reagent 1:200 in Qubit™ dsDNA HS Buffer and 10 μL of each Qubit™ standard were mixed to 190 μL ok working solution and 2 μL of each sample were mixed to 198 μL of working solution (Qubit dsDNA HS Assay Kit User Guide (Pub.No. MAN0002326C.0).

The purity of the DNA sample was measured using the NanoDrop microvolume spectrophotometer (Thermo Scientific). The ratio of the 260 nm/280 nm peaks indicated the purity of the DNA sample, with a value between 1.8 and 2.0 considered pure (Desjardins & Conklin, 2010).

2.4. Integrity analysis

The analysis of DNA integrity was conducted using the Agilent 4200 TapeStation systems (G2991AA and G2991BA) pipetting 10 μL Genomic DNA Sample Buffer and 1 μL DNA genomic sample in a tube strip for automatized capillary electrophoresis. The assay was performed following the manufacturer's instructions.

2.5. PCR amplification

The PCR reaction was performed in a total volume of 20 μL, containing 10 ng of DNA, IFNA primer (R: GTAAGGTGGAAACCCCCACT; F: FAMTCGGCGTTAAGTTAATTFGTT), buffer solution, oligonucleotides, Taq DNA Polymerase enzyme, and 14.3 μL of ultrapure water. The primer amplification consisted of 30–35 cycles with denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s. Subsequently, agarose gel electrophoresis was performed using a 2% gel, following the same standards described above. However, a ladder was added to each gel to verify the amplification of a band at 150 bp.

2.6. Data analysis

The data from the present study were analyzed using the Statistical Package for the Social Sciences (SPSS). The normality of the distribution of the data obtained for different outcomes was tested through histogram analysis and the Kolmogorov-Smirnov test. The DNA concentration, purity and integrity values were assessed using mean and standard deviation, and the groups were compared using Two-way ANOVA followed by Tukey's post hoc test. Qualitative data regarding IFNA amplification were analyzed descriptively.

3. Results

3.1. DNA concentration and purity

The DNA concentration results indicate the average value of DNA

obtained through the two different extraction methods (Fig. 1). Comparing the methods, it becomes apparent that there is a higher concentration of DNA in the samples subjected to the salting-out method. As a control for the DNA extraction method (Supplemental Table 1), an immediate extraction was conducted using samples in PBS. Although no statistical difference was observed in terms of DNA concentration, a significantly improved purity was achieved with the salting out method ($p = 0.002$).

It is also highlighted that under both temperature conditions NAP exhibits the poorest concentration values (Fig. 1 A, B). On DNA extraction by salting out methodology PBS, TENT, and DESS buffers demonstrated superior concentration values when stored in temperature of 4 °C in comparison to samples stored in room temperature, resulting in mean values over 10 ng/ul up to 30 days (Fig. 1A). This difference is statistically significant for up to 72 h in all solutions (except for TENT at the 24-hour time point) (Fig. 1A).

The ratio between absorbance measurements at 260 nm and 280 nm is the most well-known form of quality and purity control for DNA or RNA extraction. This result division expresses the proportion between the amount of DNA and the amount of extracted proteins. Consequently, values between 1.8 and 2.0 represent an acceptable range and indicate DNA purity.

The salting-out method yielded more adequate results regarding the DNA purity assessment (Tables 1 and 2). Using the salting-out method, PBS exhibited purity values within the standard range under both temperature conditions for up to 30 days. The values remained statistically significant for up to 72 h at RT. The TENT solution showed adequate purity values for up to 90 days under both temperature conditions, except for the 72-hour at 4 °C. TENT values were statistically significant for up to 72 h at RT. The DESS solution displayed better purity values when stored at 4 °C, although without significant differences. However, at RT, the difference was significant, with adequate purity values lasting up to 90 days). Lastly, the NAP solution exhibited the poorest purity values, with none of them falling within the standard range. Based on these results, along with the concentration data, the NAP solution was excluded from further analysis (Table 1).

3.2. DNA Integrity and Amplification

The integrity analysis was performed using the Genomic DNA ScreenTape assay, which is specifically designed to assess the integrity of genomic DNA samples and analyze double-stranded DNA molecules ranging from 200 to > 60,000 base pairs. DNA Integrity number (DIN) starting from 5 suggest more intact bands and, consequently, less fragmented DNA with minimal smearing. As DNA concentration and purity of samples submitted to spin column-based nucleic acid purification were not adequate, the next assays were developed with the samples submitted to salting out methodology.

This assay showed that the DESS solution consistently exhibited the best integrity values over time for both temperature conditions. It was

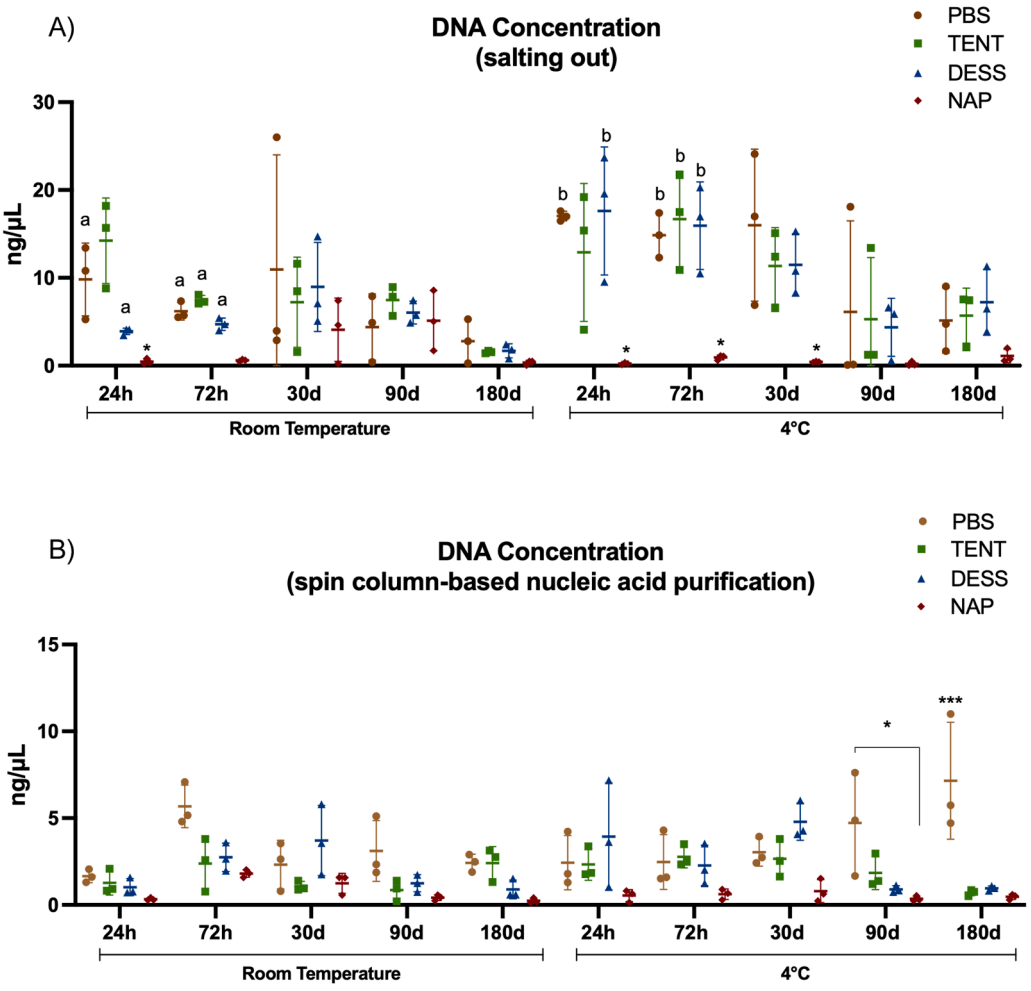

Fig. 1. DNA concentration comparison of the different preservation solutions and temperature storage conditions according to the assessed time points. A) DNA concentration of samples isolated by salting out method (n = 3 for each solution group and each timepoint); B) DNA concentration of samples isolated by spin column-based nucleic acid purification (n = 3 for each solution group and each timepoint). Statistical test Two-way ANOVA. * $p < 0.05$ comparing different preservation solutions within the same temperature. a,b $p < 0.05$ comparing the same preservation solution according to the temperature within the same time point.

Table 1
DNA purity ratio of samples isolated by salting out method. Comparison of the different preservation solutions and temperature storage conditions according to the assessed time points, (n = 3 for each solution group and each timepoint). Statistical test Two-way ANOVA. a,b, $p < 0.05$ comparing different timepoints. A,B, $p < 0.05$ comparing different preservation solutions within the same timepoint. NA, not applicable.

	Solution	24 h	72 h	30d	90d	180d	*p*
	PBS	1.99 (± 0.12)a; A.B	1.96 (± 0.08)a; A	1.88 (± 0.05)a,b	1.63 (± 0.16)a,b	1.34 (± 0.39)b	0.0135
	TENT	1.86 (± 0.04)a; A.B	1.84 (± 0.05)a; A	1.83 (± 0.07)a,b	1.80 (± 0.02)a,b	1.54 (± 0.12)b	0.0015
Room Temp.	**DESS**	2.50 (± 0.48)a; A	1.95 (± 0.09)a,b; A	1.87 (± 0.11)a,b	1.85 (± 0.06)a,b	1.56 (± 0.16)b	0.0090
	NAP	1.42 (± 0.24)B	1.28 (± 0.06)B	NA	NA	1.00 (± 0.13)	0.0548
	p	0.0097	< 0.0001	0.7994	0.0846	0.0629	
	PBS	2.00 (± 0.02)	1.80 (± 0.00)A	1.94 (± 0.04)A	1.78 (± 0.26)	1.39 (± 0.60)A.B	0.1758
	TENT	1.99 (± 0.05)	1.73 (± 0.09)A	1.84 (± 0.11)A	1.8 (± 0.01)	1.52 (± 0.60)A.B	0.3864
4°Celsius	**DESS**	2.01 (± 0.02)	1.81 (± 0.05)A	1.96 (± 0.04)A	1.86 (± 0.16)	1.80 (± 0.35)A	0.5426
	NAP	2.08 (± 0.67)a	1.03 (± 0.06)a,b; B	0.99 (± 0.08)a,b; B	1.54 (± 0.63)a,b	0.46 (± 0.20)b; B	0.0088
	p	0.9885	< 0.0001	< 0.0001	0.7099	0.04	

Table 2
DNA purity ratio of samples isolated by spin column-based nucleic acid purification. Comparison of the different preservation solutions and temperature storage conditions according to the assessed time points, (n = 3 for each solution group and each timepoint).

	Solution	24 h	72 h	30d	90d	180d	*p*
	PBS	2.17 (± 0.74)	2.55 (± 0.41)	1.85 (± 0.16)	1.93 (± 0.72)	1.67 (± 0.16) A,B	> 0.05
	TENT	3.58 (± 0.60)	2.25 (± 0.50)	2.06 (± 0.24)	2.30 (± 0.46)	2.81 (± 0.82) A	> 0.05
Room Temp.	**DESS**	2.06 (± 0.34)	2.26 (± 0.43)	2.32 (± 0.24)	2.00 (± 0.32)	1.58 (± 0.33) A,B	> 0.05
	NAP	2.71 (± 0.38)	2.03 (± 0.31)	2.13 (± 0.38)	2.62 (± 0.74)	0.92 (± 0.23) B	> 0.05
	p	> 0.05	> 0.05	> 0.05	> 0.05	0.048	
	PBS	2.04 (± 0.18)	1.67 (± 0.20)	2.15 (± 0.60)	2.31 (± 0.24)	2.04 (± 0.07)	> 0.05
	TENT	1.88 (± 0.33)	2.31 (± 1.18)	1.58 (± 0.29)	2.25 (± 0.33)	2.77 (± 0.76)	> 0.05
	DESS	2.12 (± 0.15)	1.61 (± 0.37)	2.02 (± 0.17)	2.31 (± 0.20)	2.72 (± 0.57)	> 0.05
4°Celsius	**NAP**	1.95 (± 1.02)	1.57 (± 1.67)	1.57 (± 0.04)	2.20 (± 0.18)	2.50 (± 1.10)	> 0.05
	p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Statistical text Two-way ANOVA. A,B, $p < 0.05$ comparing different preservation solutions within the same timepoint.

statistically significantly superior compared to the TENT solution at 30 and 90 days. TENT values were significantly lower than PBS and DESS at RT in 180 days (Fig. 2A). The bands with less smearing were observed in the PBS and DESS groups (Fig. 2B).

To assess the amplification capacity, the IFNA primer was analyzed. Table 3 shows that the amplification capacity was increased when the samples were stored at 4 °C. In contrast, when stored at RT, the amplification was lower, and PBS was only able to achieve 100%

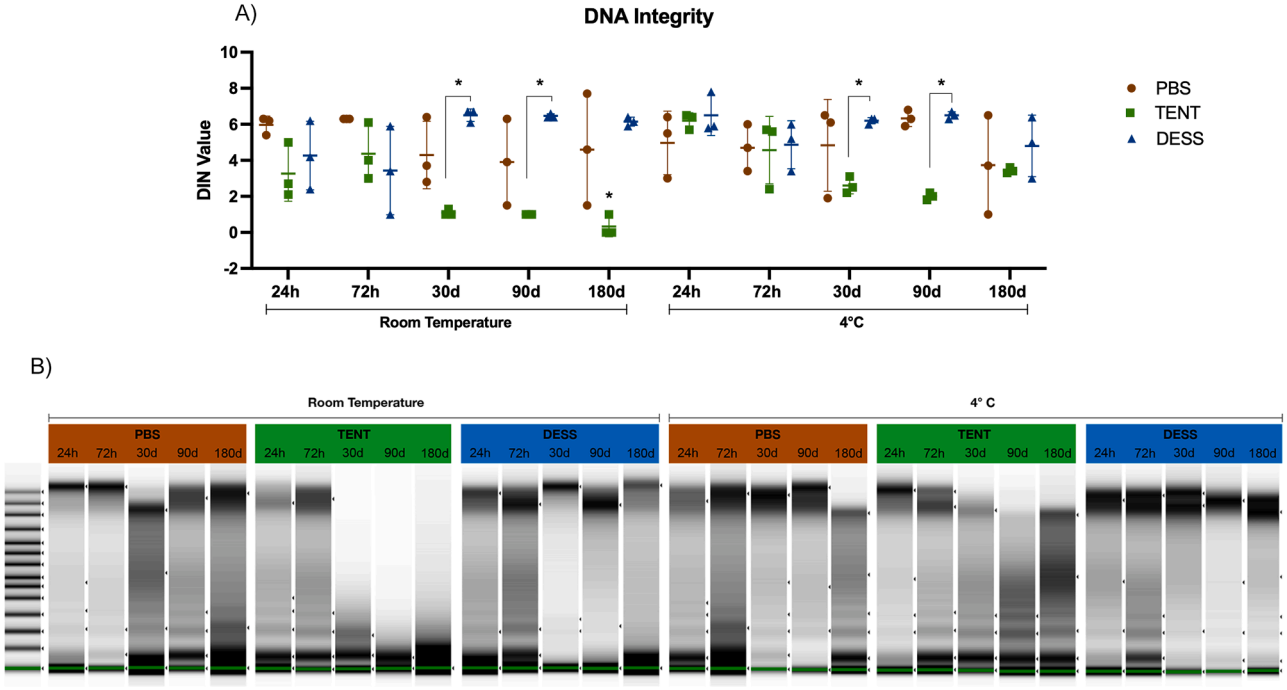

Fig. 2. DNA integrity analysis (n = 3 for each solution group and each timepoint). A) DIN values according to group, temperature, and timepoints. B) Representative DIN bands according to group, temperature, and timepoints. Statistical test Two-way ANOVA. * $p < 0.05$ comparing the different preservation solutions within the same temperature and time point.

Table 3
DNA amplification of IFNA primer in the different preservation solutions and temperature storage conditions according to the assessed time points, (n = 3 for each solution group and each timepoint).

	Solution	24 h	72 h	30d	90d	180d
Room Temp.	**PBS**	100%	66.66%	66.66%	33.33%	0%
	TENT	100%	66.66%	100%	100%	100%
	DESS	100%	100%	100%	66.66%	33.33%
4°Celsius	**PBS**	100%	100%	100%	100%	100%
	TENT	100%	100%	100%	66.66%	100%
	DESS	100%	100%	100%	100%	100%

amplification within 24 h. However, DESS and TENT demonstrated better results, as shown in Table 3.

4. Discussion

DNA analysis is a common and crucial procedure in basic sciences and clinical medicine, with significant relevance to diagnosis, prognosis, and treatments (Franceschini et al., 2018). However, for its analysis, DNA must be isolated immediately, cryopreserved, or maintained in preservation media, as it degrades with an increase in time and temperature (Alaeddini et al., 2010; Oosting et al., 2020). Nevertheless, there are situations in which access to necessary equipment is limited, such as freezers or freezing agents, and commercially available DNA preservation products come at a high market cost (Sharpe et al., 2020). Furthermore, DNA can be obtained from different biological sources and peripheral blood is often the preferred sample due to its abundance. However, its collection can be invasive, less acceptable to patients, and requires trained personnel and specific materials. In that regard, simple and more cost-effective sample procedures as buccal cells scraping offer an excellent alternative as they allow for simple and non-invasive collection, patients can perform self-collection, it is relatively low-cost (Aidar & Line, 2007; King et al., 2002; Trevilatto & Line, 2000). This study assessed the preservation capacity of DNA obtained from exfoliated cells of the buccal mucosa, stored in laboratory-formulated solutions under different storage times and temperatures.

There are different methods for DNA isolation such as the use of commercial kits and those methods developed “in-house”. Although the use of kits is more straightforward, they may be quite expensive. The use of DNA isolation methodology developed in laboratories like the salting out method can be a pivotal factor in planning disease treatment and community disease-prevention programs. By exploring alternative DNA sources like buccal cells, we can potentially simplify and reduce the cost of DNA analysis, making it more accessible and applicable in various research and medical contexts (Aidar & Line, 2007; Saab et al., 2007; Shimizu et al., 2022). Our results demonstrated a superior performance of the salting-out extraction method when compared to the commercial kit, yielding higher DNA concentration and purity standards. This was also observed by Dieki and colleagues (2022), who noted superior performance of the salting-out method for DNA extraction from peripheral blood when compared to the commercial Qiagen kit, as well as other methods (Dieki et al., 2022).

Although buccal cells gave a smaller amount of DNA comparing with blood, developed methods of genotyping use very small amounts of DNA (2–10 ng per assay) and thus allow the use of buccal cells as a source of DNA (Mulot et al., 2005; Shimizu et al., 2022). In our study, all DNA samples obtained from storage at PBS, DESS and TENT buffers presented at least 20 ng of DNA utilizing the salting-out isolation methodology.

DNA quantification, purity, integrity and amplification capacity will be directly linked to the sample storage condition. The degradation and damage of DNA encompass enzymatic processes, oxidative mechanisms, exposure to UV radiation, and hydrolysis phenomena (Schroeder et al., 2006). This process starts shortly after sampling (Graham et al., 2015) and persists irrespective of the chosen preservation methodology (Guo et al., 2018). Nedel et al. evaluated the quality of DNA derived from buccal cells collected through buccal scraping, comparing the integrity of samples preserved for 72 h at room temperature and 4 °C without a preservation medium, with samples extracted immediately. It was observed that DNA degradation occurred even when stored in the refrigerator. Hence, the importance of having a preservation medium for the collected samples (Nedel et al., 2009).

In the same way, the PBS solution would not serve as a preservation medium since it lacks additional components besides water and sodium phosphate. PBS is a very low-cost solution and can be used for collecting buccal scraping cells since it is non-toxic to the cells; however, the collected material should be processed promptly (Martin et al., 2006). Our results presented that PBS at RT was able to perform all the analysis only for 24 h, whereas better performance was achieved if maintained at 4 °C. Probably, during a short period (24 h), there is no advanced DNA degradation, allowing adequate performance in all tests.

Therefore, in order to achieve longer preservation under more viable storage conditions, laboratory-formulated DNA preservation solutions would be essential. Previously, the NAP buffer was analyzed for its DNA preservation capacity in samples obtained from different organs of rats, which were kept at RT for 7 weeks and 10 months, resulting in mean observed DNA concentrations of 70 ng/μL and 50 ng/μL, respectively. Furthermore, the researchers assessed the integrity of this DNA, which exhibited distinct bands with weak "smears" in samples kept at room temperature for up to 7 weeks (Camacho-Sanchez et al., 2013). However, our results presented poorer outcomes with the NAP buffer, as it was unable to support further molecular analyses due to the extremely low DNA concentration and purity. It is important to note that our results may differ from those cited above due to the difference in sample type: their study used tissue samples, while ours used isolated cells.

Another solution formulated in the present study was the DESS buffer which was introduced by Seutin White in 1991. The components within this buffer include EDTA and NaCl, each assumed pivotal roles in the preservation of DNA. EDTA contributes to preservation by chelating divalent cations that are imperative for the enzymatic activity of nucleases. Concurrently, NaCl is anticipated to denature nuclease enzymes, thereby further aiding in DNA preservation. Furthermore, DMSO, another constituent of the DESS buffer, is posited to function as a penetrant, potentially facilitating the transport of these critical preservation ingredients into cellular structures (Carvalhais et al., 2022; Lee et al., 2019; Oosting et al., 2020; Seutin et al., 1991; Stracke et al., 2021).

The results obtained with DESS presented good overall performance, which was even better in 4 °C storage. Under this condition, it exhibited suitable DNA concentration, purity, and integrity for up to 90 days and successfully amplified the IFNA primer at all time points. At RT storage, these qualities were maintained for up to 30 days. This difference can be explained by the sensitivity of enzymes to temperature. Consequently, the DNA degradation process is reduced at lower temperatures (Oosting et al., 2020). Favorable outcomes with the DESS buffer have been observed across various organisms. Furthermore, DESS is a simple and cost-effective solution that can be prepared, stored, and used at room temperature. A review of publications assessing the effectiveness of DESS revealed a median preservation period of 6 months, suggesting that DESS can effectively preserve DNA over time intervals suitable for

many research applications. To date, no analyses have been conducted with buccal cytological scraping cells, which is a significant unique aspect of this study, offering an additional source of DNA and effective preservation using the DESS buffer (Sharpe et al., 2020).

The last buffer to be considered is the TENT buffer which presented suitable concentration and purity values at both temperatures, but the integrity of the DNA was notably compromised. However, this issue did not directly impact the amplification of the IFNA primer. Nevertheless, it's possible that for the analysis of different primers, this factor could have a negative influence. Analyzing the preservation capacity of DNA in muscle tissue samples stored in DESS and TENT solutions for up to 28 days at 35 °C, one study concluded that the DESS solution was capable of producing complete genotyping profiles within 28 days while the TENT solution yielded partial profiles (Allen-Hall & McNevin, 2012). Although this specific analysis was not conducted in our methodology, these findings align with our results, showing the effectiveness of the DESS solution for approximately 30 days and the limited effectiveness of the TENT solution in DNA preservation, due to loss of DNA integrity presented. Nevertheless, it does not directly impact the IFNA amplification capacity and the concentration and purity values were favorable in both RT and 4 °C TENT storage.

The process of sampling for DNA extraction is pivotal, given that DNA deteriorates over time and due to temperature fluctuations. Consequently, there is a strong impetus to streamline this process, reduce associated costs, and ensure the procurement of ample DNA quantities for subsequent PCR analysis. Based on our analyses, it can be unequivocally deduced that buccal mucosal cells serve as a robust source of DNA, and the salting-out DNA extraction methodology proves to be eminently suitable for this purpose. Furthermore, we have observed that samples can be securely stored for up to 24 h in PBS, particularly if the extraction process is conducted within relatively short timeframes. However, with a focus on long-term preservation, the DESS buffer emerges as the most effective solution. It maintains the requisite DNA quality and quantity standards for extended durations, demonstrating remarkable stability for up to 30 days at RT and up to 3 months at 4 °C. These findings represent pioneering contributions to scientific literature. Nonetheless, to validate these results conclusively, further sampling involving diverse subjects and a variety of primers is imperative.

CRediT authorship contribution statement

Nunes Júlia Silveira: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Vera Luisa Pimentel:** Investigation, Methodology, Visualization, Writing – review & editing. **Silva Sabrina Barcelos:** Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **de Bem Prunes Bianca:** Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Rados Pantelis Varvaki:** Conceptualization, Supervision, Validation, Writing – review & editing. **Visioli Fernanda:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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