DNA quality from buccal swabs in neonates: comparison of different storage time

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ABSTRACT

BACKGROUND Genomic medicine has great potential for diagnoses, disease prediction, and targeted treatment. Buccal swabs are a suitable non-invasive method for neonates to obtain DNA samples. Due to Indonesia's geographical conditions, samples require a prolonged time to reach the genetic laboratory. This study aimed to compare the DNA quality of buccal swabs in neonates between immediate and after-storage extraction.

METHODS This study was part of a study about the profile of human milk oligosaccharide and FUT2 genotype in Indonesian mother-infant dyads consisting of 20 neonates. 1 swab stick for each participant was taken using a standardized buccal swabbing protocol and divided into 2 isovolume aliquots, which were grouped into the immediate (extraction was performed within 3 days after sampling) and storage groups (extraction was performed on the 14th day after storage in 4°C). DNA yield and purity A260/280 ratio were measured by spectrophotometry. The PCR amplification and Sanger sequencing were performed to validate the DNA isolate quality for downstream application.

RESULTS The DNA yield for the immediate group was similar compared with the storage group (9.50 [4.89] versus 9.10 [5.05] µg), p = 0.659, as well as DNA purity A260/280 (1.58 [0.24] versus 1.56 [0.28]), p = 0.785. PCR and sequencing of FUT2 results also showed similar quality between both groups.

CONCLUSIONS The similar DNA quality and sequencing results between immediate and storage extraction confirmed that buccal swabs could be stored for 2 weeks, allowing ample time for sample shipping from remote areas to the laboratory.

KEYWORDS buccal, DNA, isolation, quality, swabs

Understanding the genetic basis of disease has substantial healthcare benefits. Exploring single-nucleotide variations to explain human variations in metabolism, physiology, and disease risk factors is another cutting-edge development in the genomic medicine era. Obtaining genetic material for analysis is thus essential and has broad implications for understanding disease pathogenesis, establishing a diagnosis for complicated cases, and designing individualized therapies.1

Buccal swabs are a suitable non-invasive method for obtaining DNA samples from neonates. However, the literature regarding DNA quality from buccal swabs has yielded various results. Siswanto et al2 reported...
much lower DNA concentration in buccal swab than in the whole blood of premature infants. In contrast, Said et al. obtained a significantly higher DNA yield from buccal epithelial cells than that from the whole blood of premature infants. Moreover, buccal swabs pose additional concerns regarding storage time and temperature. As Indonesia has numerous islands, DNA samples often require a prolonged time to reach the genetic laboratory. Various studies have recommended different storage duration and temperature for buccal swabs. Navarro et al. mentioned that buccal swabs could be stored for up to 2 weeks at 4°C before processing without a noticeable loss in DNA yield or quality. Rogers et al. kept buccal swabs at −80°C before extraction but did not compare the storage time. Grujičić et al. compared three groups of different storage time from buccal swabs kept at room temperature. Therefore, this study aimed to compare the DNA quality between immediate and after-storage extractions and to validate the DNA isolate with Sanger sequencing between the immediate extraction (within 3 days) and 2-week-storage extraction groups. This result will help justify the storage of buccal swabs from remote areas before shipment to a genetic laboratory.

METHODS

This research was a part of the main study aiming to learn the profile of human milk oligosaccharides and FUT2 genotype in Indonesian mother-infant dyads. The primary study recruited 120 mother-newborn dyads from Bunda Mother and Child Hospital, Jakarta, Indonesia. However, the participants of this study were a subset of the last 20 participants. All participants provided informed consent. The DNA extraction, polymerase chain reaction (PCR), and sequencing were conducted at the Human Genetic Research Center, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, from December 2021 to August 2022. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: KET-838/UN2.F1/ETIK/PPM.00.02/2021).

Sample collection and storage

Samples were collected by rubbing the inside of the right and left cheeks 10 times each without touching the tongue or lips, as described in the kit manual. The ORAgene® OraCollect OCR-100 (DNA Genotek, USA) used in this study is designed for DNA collection in pediatric patients and is painless because of its soft sponge-tip properties. The swab was directly placed into the 500 μl preservation reagent in the kit and shaken vigorously 10 times. One swab stick was taken for each participant. The swabbing was performed by a trained physician who collected numerous samples for newborns in the main study. The 500 μl raw samples were divided into two aliquots (250 μl each) for each participant. One aliquot was assigned to the immediate group (extracted within 3 days after sampling) and the other to the storage group (extracted on the 14th day after sampling) which was kept in 4°C refrigerator.

DNA extraction from buccal swabs

Raw samples (250 μl) were put into a 1.5 ml microtube. As much as 20 μl of PrepIT.L2P reagent (DNA Genotek) was added to the sample. The microtubes were vortexed for 10 sec to ensure that the reagent and raw samples were homogenized. The samples were incubated for 10 min in grated ice cubes and then centrifuged (Eppendorf 5415R, Germany) for 5 min with 10,000 × g (room temperature); the supernatant was transferred to a fresh, sterilized microcentrifuge tube. As much as 400 μl absolute ethanol (Merck, Germany) was added and mixed thoroughly by inverting the tubes 10 times to precipitate the DNA. The samples were incubated at room temperature (24–26°C) for 10 min and then centrifuged (Eppendorf 5415R) at 10,000 × g (room temperature) again for 2 min. The upper aqueous layer was discarded, and the pellet was stored in microtubes. After decanting the supernatant, 250 μl of 70% ethanol (Merck) was added carefully, and the sample was incubated for 1 min. After 1 min of incubation, the solution was removed. Note that no solution should remain in the microtubes. The pellet was then resuspended in 80 μl 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA) (TE) buffer, and frozen at −20°C or −80°C for further use.

Concentration and purity determination

DNA quality was determined by DNA yield (concentration) and purity. A quantitative spectrophotometric assay of DNA was performed using a Varioskan microplate reader (Thermo Fisher Scientific, USA) to measure the DNA yield and purity. Absorbance was measured at wavelengths of 260 and 280 (A_{260} and A_{280}, respectively) nm. The absorbance quotient ratio (OD_{260}/OD_{280}) was used to express DNA quality.
purity. An absorbance quotient ratio between 1.8 and 2.0 was considered good for purified DNA. A ratio of <1.8 indicated protein contamination, whereas a ratio of >2.0 indicated RNA contamination.\(^9\)

**PCR and Sanger sequencing**

PCR for the FUT2 gene was performed using a primer pair from Lefebvre et al\(^\text{10}\) and its sequences as follows: forward 5’ACACACCCACACTATGCCTG’3 and reverse 5’AAGAGAGATGGGTCCTGCTC’3. MyTaq HS Redmix (Meridian Bioscience, USA)\(^\text{11}\) was used as a PCR mix. The PCR program consisted of pre-denaturation at 95\(^\circ\)C for 1 min, denaturation at 95\(^\circ\)C for 15 sec, annealing at 65\(^\circ\)C for 15 sec, extension at 72\(^\circ\)C for 10 sec, repeated for 35× cycles, and completed by a final extension at 72\(^\circ\)C for 10 min.\(^\text{11}\) Agarose gel electrophoresis was performed to verify the quality of the PCR products. The PCR products were separated on a 0.8% agarose gel at 100 volts for 45 min. The gels were visualized using a gel documentation system (Accuris Instruments, USA).\(^\text{12}\)

**RESULTS**

DNA was successfully extracted from all samples. The DNA yield for the immediate group was similar from that of the storage group (9.50 [4.89] versus 9.10 [5.05] µg, respectively; \(p = 0.659\)). The DNA purity \(A_{260/280}\) ratio was also similar between the immediate and storage groups (1.58 [0.24] versus 1.56 [0.28], respectively; \(p = 0.785\)). A comparison between several studies comparing DNA yield at different storage time and temperature is detailed in Table 1. DNA purity showed no difference between the groups (Table 2).

To evaluate the impact of slight differences in the DNA yield and purity between the immediate and storage groups on the downstream process, PCR and Sanger sequencing results were compared to capture the coding region of the FUT2 gene. Visualization of the PCR products indicated the successful amplification of specific fragments with a size of 1,238 bp, as shown in Figure 1. In addition to successful target amplification, we did not observe the significant appearance of multiple bands, dimers, smears, and off-target fragments. This suggests that even at a purity lower than the recommended value, the presence of chemical contaminants did not significantly interrupt fragment amplification.

Sanger sequencing was performed on each participant to validate whether the DNA quality was sufficient for downstream applications. Sample

### Table 1. Comparison of DNA yield from buccal swabs based on storage time and temperature

| References | Population (n) | Storage temperature | DNA yield (ng/µl) |  
| --- | --- | --- | --- | --- |
| | | | Immediate extraction | Storage group |
| | | | Day 0–3 | Day 7 | Day 14 |
| This study, mean (SD) | Neonates (20) | 4\(^\circ\)C | 9.50 (4.89) | NA | 9.10 (5.05) |
| Mulot et al, mean (range) | Adult (20) | −20\(^\circ\)C | 3.4 (0.4–8.5) | 3.5 (0.9–9.0) | NA |
| Grujičić et al, mean (range) | Adult (7) | 4\(^\circ\)C or −20\(^\circ\)C | 3.91 (1.54–8.3) | 3.98 (1.25–8.1) | 4.29 (0.87–7.9) |
| Ghatak et al, mean (SD) | Adult (5) | 4\(^\circ\)C or −20\(^\circ\)C | 3.55 (0.60) | 2.53 (0.31) | NA |

NA=not available; SD=standard deviation

### Table 2. Comparison of DNA purity (\(A_{260/280}\)) from buccal swabs based on storage time

| References | Population (n) | DNA purity (\(A_{260/280}\)), mean (SD) |  
| --- | --- | --- | --- |
| | | Immediate extraction | Storage group |
| | | | Day 0–3 | Day 7 | Day 14 |
| This study | Neonates (20) | 1.58 (0.24) | NA | 1.56 (0.28) |
| Mulot et al\(^\text{13}\) | Adult (20) | NA | 1.6 (0.2) | NA |
| Grujičić et al\(^\text{4}\), mean (SD), range | Adult (7) | 1.68 (0.15), 1.45–1.88 | 1.64 (0.16), 1.37–1.88 | 1.67 (0.19), 1.32–1.88 |
| Ghatak et al\(^\text{9}\) | Adult (5) | 1.60 (0.05) | 1.70 (0.09) | NA |

NA=not available; SD=standard deviation
 aliquot were randomly assigned to each participant for sequencing. Sequencing was performed using both forward and reverse primers to increase confidence in base calling. High-quality sequencing results were obtained for both groups (Figure 2). No significant noise was observed in the baseline sequence. The average quality value was >20 for a single base and >10 for a mixed base.

**DISCUSSION**

In this study, the DNA yield and purity \( A_{\text{260/280}} \) were similar between the immediate and storage groups. This result aligns with three previous studies that compared

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**Figure 1.** Comparison of polymerase chain reaction (PCR) products extracted (a) within 3 days and (b) on day 14 after sampling

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**Figure 2.** Sanger sequencing results for (a) participant 2: DNA extraction within 3 days and (b) participant 5: DNA extraction after 2 weeks in a 4°C refrigerator
in the DNA yield. Livy et al\textsuperscript{14} obtained a higher DNA yield of 21.03 (17.1), compared with the yield in this study, primarily due to a larger raw sample volume of 400 μl, whereas this study used 250 μl. Ghatak et al\textsuperscript{9} used one cotton swab and put the sample in 500 μl of self-prepared fluid consisting of 10 mM Tris (pH 8.0) + 10 mM EDTA + 2% sodium dodecyl sulfate. Despite using a larger raw sample volume, Ghatak et al\textsuperscript{9} achieved a lower DNA yield than the present study. This difference could be attributed to the swabbing technique used. In contrast to the swabbing technique used by Ghatak et al\textsuperscript{9} in which the participants swabbed themselves, swabbing in this study was conducted by a trained physician following a standardized protocol, as described in the kit manual. In the storage time aspect, immediate extraction in Ghatak et al\textsuperscript{9}\textsuperscript{5} study resulted in a higher average yield of 3.55 (0.60) μg than samples that went to storage before processing (2.53 [0.31] μg). This was probably due to DNA degradation by the bacteria and nucleases in the buccal epithelial cell samples.\textsuperscript{4} Another study by Said et al\textsuperscript{1} in 85 premature infants implied that the average DNA yield from buccal swabs was 25.5 μg (range 8.95 to 42.1 μg), much higher than the present study that obtained a DNA yield of 9.50 μg for immediate extraction and 9.10 μg for after-storage extraction. This was probably because Said et al\textsuperscript{1} used two cotton swab sticks (cytology brushes) with 900 μl cell lysis fluid, whereas this study used one cotton swab with 500 μl preservation reagent, as included in the ORAgen\textregistered{} OraCollect OCR-100 (DNA Genotek) swab kit. Interestingly, significantly higher DNA yields were obtained from buccal swabs than from blood.

All studies revealed DNA purity below the recommended value, between 1.8 and 2.0. Livy et al\textsuperscript{14} who did not compare storage time, also obtained low A\textsubscript{260/280} purity (1.33 [0.32]) from adult buccal swabs. Although the present study showed lower DNA purity, the sequencing results were of high quality (Figure 2). Good sequencing results despite lower than recommended DNA purity was also demonstrated by Grujičić et al.\textsuperscript{6}

According to previous studies, the recommended A\textsubscript{260/280} nucleic acid purity is in the 1.8–2.0 ratio. A lower A\textsubscript{260/280} purity ratio may indicate the presence of protein contaminants that may inhibit downstream applications.\textsuperscript{15} The difference in DNA A\textsubscript{260/280} purity result might be due to the DNA extraction method. We used conventional methods to extract DNA, whereas Livy et al\textsuperscript{14} used QIAamp DNA Blood Kits (Qiagen, Netherlands). The reagents used in this study consisted of PrepIT.L2P DNA reagent (DNA Genotek) for sample preparation, ethanol 100% for precipitation, ethanol 70% as a wash buffer, and TE solution as a DNA elution buffer. The QIAamp DNA Blood Kit (Qiagen) included a lysis buffer, wash buffers 1 and 2, and an elution buffer. Using a column during DNA extraction might increase the DNA purity since the column captures DNA better and leaves the impurities in the flow-through.\textsuperscript{16} In addition to that, adding the wash buffer twice also improves the DNA A\textsubscript{260/280} purity.\textsuperscript{17} To prevent food contamination to purity, the participants were asked to rinse their mouths thoroughly and refrain from eating for around 45 min. In this study, infants were required not to be breastfed for at least 45 min before swabbing; therefore, the possibility of breast milk contamination was considered very low.

The PCR product from a buccal swab was sequenced to validate whether the DNA isolate could be used for genetic analysis. The sequencing quality was at a high confidence level for variant analysis, represented by two common single-nucleotide variants of rs281377 and rs1047781 in the FUT2 gene, which were superimposed on both DNA sample groups.\textsuperscript{16,19}

In conclusion, buccal swabs could be stored for a certain period without affecting DNA quality, which benefits health services and neonatology research. Buccal swabs have great potential as a non-invasive sampling method for genetic analyses in neonates.

Conflict of Interest
The authors affirm no conflict of interest in this study.

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