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# Influence of storage methods of whole blood samples on DNA integrity in epidemiological studies

C. Rahila [a,*], M.B. Aswath Narayanan [b], J. John Kirubaharan [c], S.G. Ramesh Kumar [b], A. Leena Selvamary [b], A. Sujatha [b]

[a] Department of Public Health Dentistry, Vivekanandha Dental College and Hospital for Women, Tiruchengode 637205, India

[b] Department of Public Health Dentistry, Tamilnadu Government Dental College and Hospital, Chennai 600003, India

[c] Department of Veterinary Microbiology, Madras Veterinary College, Chennai 600007, India

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ABSTRACT

*Objectives:* To investigate the influence of storage methods of whole blood samples on Deoxyribonucleic acid (DNA) quality.

*Method and materials:* Whole blood samples were collected from 90 patients reported to the Government Tertiary-care Dental College and Hospital, Tamil Nadu, India. The blood samples were stored at three different temperatures −80 °C, −20 °C, 4 °C which were analyzed for DNA quality immediately within 4 h and after a storage period of 60 days. Quantification of DNA in the sample was recorded using a spectrophotometer.

*Results:* The average concentration of isolated DNA ranged from 0.5 ng/μl to 154.2 ng/μl with purity $A_{260}/A_{280}$ ratio ranging from 0.7 to 2.0. The mean DNA concentration $H = 97.2$ ($p = 0.001$) and purity $H = 80.1$ ($p = 0.001$) obtained from whole blood stored at 4 °C were statistically significant. Thus samples stored at 4 °C did not affect on DNA yield and quality.

*Conclusion:* We conclude that storage at 4 °C temperature for whole blood does not substantially affect the molecular assay measurements. This study enlightens the influence of storage temperatures and duration in genetic epidemiology.



## 1. Introduction

Remarkable advancements in genetics play an indispensable vital role both in Oral health research and Dental practice. To appreciate the genetic aspects of a disease and to create novel methodologies for oral health promotion, the availability of Deoxyribonucleic acid (DNA) is fundamental for epidemiological studies.[1,2] The diagnosis of majority of diseases are nowadays conceivable by direct analysis of DNA and indirectly by investigations of the association between the disease and DNA polymorphisms. Recent advancements are made in our scientific perception of the role of genetics for diagnosis, treatment as well as prevention of oral diseases. Various

* *Corresponding author*. Tel.: +91 9940516751.
E-mail addresses: drrahilaa@gmail.com (C. Rahila), mban1965@yahoo.com (M.B. Aswath Narayanan), jjohnk@gmail.com (J.J. Kirubaharan), ramesh17200@gmail.com (S.G. Ramesh Kumar), leenaselvamary@gmail.com (A.L. Selvamary).

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sources of DNA include saliva, buffy coat, buccal cells, sputum, urine, guthrie cards, hair samples, bone material, cultured cells, amniocytes or amniotic fluid, cerebrospinal fluid (CSF). Saliva may contain extensive proportions of DNA and Ribonucleic acid (RNA) from oral bacteria.[3] In spite of this fact and possibility to get DNA from an assortment of sources such as buccal swabs and saliva, they are often constrained in their usefulness as a result of the reduced DNA yields compared with whole blood.

Numerous research centres receives stored blood samples prior to DNA isolation. Hence, the impact of storage time from blood collection to DNA extraction, as well as temperature on DNA yield and quality are imperative. Usually, collected blood samples are processed immediately to avoid any predictable drop in yield and to minimize impending DNA degradation. When processing cannot be undertaken instantly, as in case of multicentre studies, blood samples are usually frozen in deep freezers. The present conviction is that long-term stockpiling of blood at freezing temperatures has a serious and negative effect on the yield and quality of DNA.[4] Freezing blood for even a brief period of time has been reported to show prompt decrease in DNA yield and potential risk of protein contamination.[4,5] Therefore, the study aimed to assess the influence of storage methods of whole blood samples on DNA integrity.

## 2. Materials and methods

Duplicate 3 ml aliquots of whole blood samples were collected from 90 outpatients reported at Government Tertiary-care Dental College and Hospital, Tamil Nadu, India for the study of Dental fluorosis. Ethical approval for the study was obtained from the Institutional Ethics Committee. Written informed consent was obtained from all the participants after providing them the information sheet of the research. Whole blood was collected from the participants, through venipuncture of the cubital vein in a 5 ml disposable syringe and transferred into 6 ml $K_2$ EDTA vacutainer (BD Vacutainer®plus# 36789) tube by a trained staff nurse. In the research laboratory the collected blood samples were stored for the period of 60 days in three different storage temperatures −80 °C, −20 °C, 4 °C. DNA was retrieved from the blood sample using the buffer solutions available in the DNA extraction kit (Favorprep® Genomic DNA minikit), immediately within 4 h and after the storage period of 60 days.

### 2.1. Initial preparation for frozen blood samples

In case of frozen blood samples stored at −80 °C, −20 °C, 30 μl Proteinase K 10 mg/ml was added to the sample. Later, it was mixed with 200 μl GB buffer and incubated in a 70 °C water bath for 15 min to lyse the sample.

### 2.2. DNA extraction

Three hundred microlitres of fresh blood/frozen blood (after initial preparation) was transferred from the $K_2$ EDTA tube into a 1.5 ml micro-centrifuge tube and was mixed with 900 μl of red blood cell lysis buffer and spun at 3000 rpm for 5 min. The resulting pellet was rewashed and vortexed in 200 μL of Gel DNA Binding GB buffer, and the mixture was then incubated at room temperature for 10 min until the sample lysate cleared. Two hundred μL of ethanol 96–100% was added and mixed immediately. Resuspended with wash buffer solution and spun at 13,000 rpm for 30 s and flow through was discarded. Then, 100 μL of preheated elution buffer 10 mM Tris–HCl, pH 8.5 at 25 °C was added and spun at 13,000 rpm for 30 s to elute the purified DNA from subsequent storage at 4 °C. The yield quantity and purity of the DNA was calculated by Nanodrop spectrophotometer which depicted the concentration of DNA in Nanogram/microliter (ng/μl) and the purity of the sample in $A_{260}/A_{280}$ ratio. A UV absorbance ratio $A_{260}/A_{280}$ ratio of 1.6–1.9 was considered to be good quality DNA.

### 2.3. DNA amplification

DNA was amplified by the conventional polymerase chain reaction (PCR) method which constituted an initial denaturation cycle of 94 °C for 3 min, followed by 30 cycles for denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 45 s, final extension at 72 °C for 5 min and 72 °C for 30 s to assess the quality of DNA for use in molecular techniques.

The PCR was performed in the total volume of 20 μl reaction mixture for samples which constituted varying amounts of genomic DNA (ranging from 2 ng/μl to 25 ng/μl), 10 μl Master mix Thermo scientific Pfu DNA polymerase# EP0501, 2 μl each of a primer pair and distilled water to a final volume of 20 μl. The primers COL1A2 F 5′-GGG ATA TAA GGA TAC ACT AGA GG-3′[6] COL1A2 R 5′-GAA ATA TCG CCG CTG GAA-3′[6] (Shrimpex®) were used to amplify the 771-bp fragment region of COL1A2 PVUII site suspected to exhibit polymorphism. PCR products were run on 2% agarose gel and visualized in the presence of ethidium bromide DNA intercalator. As a quality control measure among six DNA samples, two at each storage temperatures underwent a standard PCR reaction. Gel electrophoresis of the PCR product revealed that a 771-bp DNA product of the COL1A2 gene was successfully amplified from all six samples using between 50 and 120 ng/μl of DNA.

### 2.4. Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences SPSS, ver. 18.0; SPSS Inc., Chicago, IL, USA. According to Shapiro–Wilks test of normality, the sample was found to be in non normal distribution. Thus, Kruskal–Wallis $H$ test was applied to estimate $p$ values for comparing the mean DNA concentration and purity between three storage temperatures and time. Pearson's product-moment correlation coefficient was employed to find correlation between DNA concentration and age.

## 3. Results

A total of 90 persons with mean age of $34 \pm 7.6$ years were enrolled into this study. The mean DNA concentration and purity ($A_{260}/A_{280}$ ratio) was obtained from the aforesaid samples ranged from 0.5 ng/μl to 154.2 ng/μl and 0.7 to 3.0 respectively. Among the three methods of storage group