Editorial

Comparison of three different collection methods for DNA extraction from buccal cells

The number of genetic epidemiological studies in dental research have been increased in the past decade, thus noninvasive and easy methods for DNA collection are required. Although DNA obtainment from blood is possible, this method is invasive and thus less accepted by the volunteers than saliva collection.

The literature on noninvasive DNA collection methods to date has mainly focused on the DNA quantity from buccal (cheek) cells and oral-rinse (mouthwash) samples but not the self-collection of saliva from spit. Therefore comparison of the DNA quantity and quality obtained using a whole-saliva collection from three noninvasive methods was the aim of this study.

Three methods of saliva collection were investigated: 1) cytobrush; 2) oral rinse with 5 ml of 0.9% saline solution and 3) saliva self-collection (spit) of 3 ml of saliva. Each method was tested on 4 adult volunteers (twelve samples) from our center. The DNA from each sample was extracted following the same protocol. The cytobrushes were placed direct in a microtube with extraction solution for the DNA isolation tubes with cytobrush. Tubes containing oral rinse and saliva from the spit self-collection were centrifuged at 3500 rpm for 10 minutes and supernatant was discarded. Then 1 ml of extraction solution (Tris-HCl 10 mmol/L, pH 7.8; EDTA 5 mmol/L; SDS 0.5%, 1 ml) was added to the tubes. Proteinase K (100 ng/ml) was added to all tubes before they were stored at 56°C in an incubator overnight for more than 8 hours. Subsequently 400 μL ammonium acetate was added in order to remove non-digested proteins and the samples were homogenized by inversion for 5 minutes before they were centrifuged at 12000 rpm for 15 minutes. The resulting supernatant was recovered and divided into two microtubes before an equal volume of isopropanol chilled at -20°C was added for DNA precipitation. After vigorous manual agitation or vortexing the tubes were incubated at -20°C for 30 minutes before centrifugation at 4°C and 12000 rpm for 20 minutes. The supernatant was discarded before the DNA was washed with cold 70% ethanol and centrifuged at 12000 rpm and 4°C for 5 minutes. After disposal of the supernatant the tubes were kept upside down for 1 hour or more on absorbent paper in order to let remaining ethanol evaporate completely. The dried DNA was resuspended in 100 μL ultrapure water such as Ampuwa before its concentration in the samples is measured.

DNA quantity and quality were assessed by spectrophotometric analysis using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). Absorbance of ultraviolet light at wavelengths of 230, 260 and 280 nanometers was used to calculate the OD260/OD280 and OD260/OD230 ratios in order to compare the ratio of nucleic acid concentration in the sample (OD260) to that of protein and organics (OD280) and to salt and alcohol (OD230) contaminants. A ratio of 1.7 - 2.0 is generally ideal for the OD260/OD280 ratio (which indicates limited protein and organic contamination). Comparisons were performed using Kruskal-Wallis test with an alpha level of 0.05.

The DNA concentration for the cytobrush ranged from 6.3 ng/ μL to 110.5 ng/μL, for the oral rinse the DNA concentration ranged from 335 ng/μL to 1052.5 ng/μL and for the saliva self-collection ranged from 819.3 ng/μL to 2019.1 ng/μL. A statistical significance was observed between cytobrush and the others methods ($p<0.05$). The DNA concentration according to the method is shown in figure 1. The OD260/OD280 ratio was not statistically different according to the methods ($p>0.05$).

In conclusion, sample collection using the cytobrush method present statistically lower DNA concentration in comparison to oral rinse and the saliva self-collection, suggesting that cytobrush should be used only for small children and patients with particular special needs. Our study also identifies the saliva self-collection to be a good and easy collection method that can be adopted in future genetic studies.

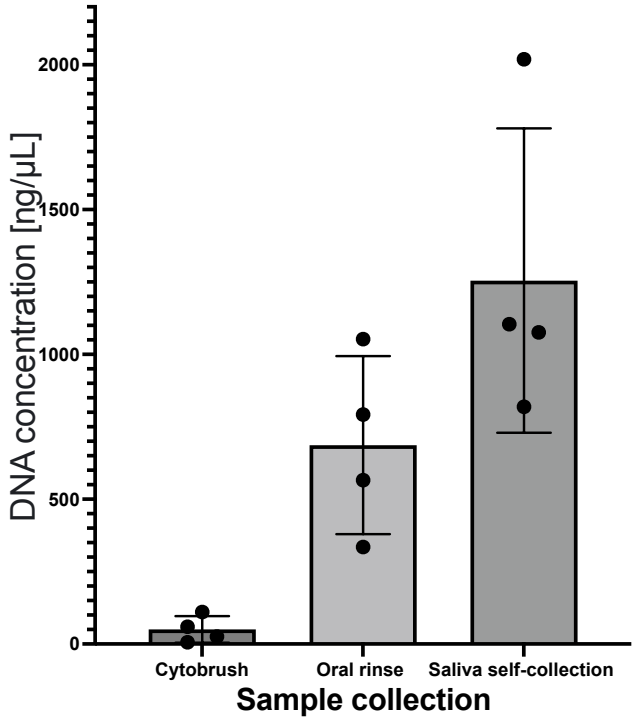

Figure 1 – Comparison of DNA concentrations obtained by 3 different methods of saliva collection

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