



Validation of Collection and Extraction Methods of Saliva for Use in Biomarker Research



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INTRODUCTION

A biomarker is defined as a characteristic that is measured and evaluated as an indicator of a normal biological process, a pathogenic process, or a pharmacologic response to a therapeutic intervention. As such, biomarkers can be a genetic trait, a biochemical change, and/or a change in a structural or functional feature. These parameters are measured either by physical methods, or by using methods of biochemistry and molecular biology providing valuable information on certain disease or metabolic processes. Biomarkers can further be used to identify people who are at risk of a disease years or even decades before symptoms appear. Therefore, one of the most important goals of research in this field is to develop and validate biomarkers that can detect and identify disorders early.

MATERIALS AND METHODS

Saliva Collection

The 1st method is the “drool” method, where the volunteer simply drools into the tube. The 2nd method is the “stimulated” version. Here, the volunteer chews on a piece of parafilm for 1 minute, and then the saliva produced is collected.

Collection Vessel

A commercially available sampling vial made specifically for saliva collection, Oragene•DISCOVER (DNA Genotek) was tested along with disposable centrifuge tubes (Fisherbrand and Corning CentriStar).



Figure 1: (a) Oragene•DISCOVER, (b) Fisherbrand, and (c) Corning CentriStar Tubes

MATERIALS AND METHODS CONTINUED...

RNA Extraction Kits



Figure 2: Rneasy Mini kit (Qiagen), PureLink RNA Mini kit (Life Technologies), SV Total RNA Isolation system (Promega)

DNA Extraction Kits



Figure 3: Ultraclean Tissue & Cells DNA Isolation Kit (Mo Bio), BiOstic FFPE Tissue DNA Isolation kit (Mo Bio), prepIT L2P (DNA Genotek), ReliaPrep Blood gDNA Mini prep system (Promega)

Protein Extraction Buffers

We tested whether or not a lysis buffer was needed for saliva samples. Therefore, the samples tested either had 1X RIPA buffer, or no buffer at all.

RESULTS

Saliva Collection

There is a significant difference in unstimulated vs. stimulated protein expression. This is reasonable as saliva secretion is mainly under autonomic nervous system regulation. Parasympathetic stimulation results in the production of high volume of saliva with low protein concentration while sympathetic stimulation results in low volume, but a greater concentration of proteins.



Figure 4: GAPDH Expression in Unstimulated (Lanes 2 and 3) and Stimulated Samples (Lanes 5 and 6)

RESULTS CONTINUED...

RNA Extraction Kits

Once all the samples were collected, we were able to conclude that there were significant differences in the quality of RNA based on collection tube. Blue-capped tubes yielded lowest quality, while the orange-capped tubes and the Oragene tubes both yielded good quality RNA. For the kits tested, we found that the PureLink Kit (Life Technologies) yielded the highest quality of RNA and out-performed the others significantly.

DNA Extraction Kits

As for the DNA, when we tested differences in the methods, the tubes, and the kits there were no significant differences.

Protein Extraction Buffers

The concentration of protein ranged from 0.5 mg/mL to over 4.0 mg/mL. Protein samples collected in the blue and orange-capped tubes yielded the highest protein concentrations, while the commercial Oragene tubes yielded very little protein. The use of a lysis buffer like RIPA is also not necessary.

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