



## Saliva-Based Diagnostics Can Decrease Generation of Potentially Infectious, Pathological Waste

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Accepted on: 11-07-2013; Finalized on: 30-09-2013.

### ABSTRACT

Saliva, like blood, contains an abundance of biomarkers and has tremendous potential to become an alternative diagnostic fluid to blood. The potential advantages of using saliva as an alternative to blood, in terms of laboratory waste generation were explored in this study. Equal volumes of saliva and blood (30 samples each), were centrifuged, the yields of supernatants from them were measured and compared. The yield of supernatant obtained for testing from saliva was significantly higher ( $p < 0.0001$ ) than the yield of plasma from blood. Based on the volumes of supernatants obtained, it is shown that the volume of saliva sample required to be collected from a patient for testing purpose, could be about 50% less than blood. Saliva-based diagnostics can therefore substantially decrease the volume of potentially infectious, pathological waste generated by laboratories.

**Keywords:** Saliva-diagnostics, waste generation, pathological waste, potentially infectious waste, sharps waste, safety.

### INTRODUCTION

Biomedical waste generated from health care establishments can be hazardous because of its potential for disease transmission and contribution to environmental pollution, necessitating effective management.<sup>1</sup> It is, therefore prudent to explore alternative strategies which have the potential to decrease waste generation without compromising on the issue of reliability which is vital to any health care facility. The prospective use of saliva as an alternative fluid to blood for diagnostic purpose is one such strategy holding promise.

Saliva contains an abundance of biomarkers, like blood. The range of saliva-based diagnostics encompasses protein biomarkers for cancer, autoimmune diseases, cardiovascular diseases and renal disease. In addition several pharmaceutical drugs, hormones, enzymes, minerals and electrolytes have been successfully estimated in saliva.<sup>2-4</sup>

As most analytes are present at lower concentrations in saliva in comparison with blood, saliva assay systems require very high sensitivities for detection of the analytes, a limitation for exploring the diagnostic utility of saliva in earlier times. However, innovative nanotechnology and existing automated analyzers equipped with appropriate limits of detection have recently been used for accurately estimating several salivary biomarkers.<sup>5-7</sup>

In view of the availability of accurate assay methods and mounting evidences of credible saliva-based testing, the present study was designed to explore the potential advantages of using saliva for diagnostic purpose as an alternative fluid to blood, in terms of the volume of waste generated.

### MATERIALS AND METHODS

#### Collection of samples

5 ml of saliva and 5 ml of blood were collected from 30 subjects who attended a clinical biochemistry laboratory. Blood samples were drawn by standard procedure. Unstimulated whole saliva was collected by passive drooling as described previously at least 2 hours after any food intake.<sup>8</sup>

Mouth was rinsed with water 3-4 times, saliva was allowed to accumulate in the floor of the mouth for approximately 2 minutes and repeatedly expectorated into a graduated polypropylene vial to collect 5 ml.

#### Research Ethics

All experimental procedures were conducted in accordance with ethical procedures and policies approved by the Institutional Ethics Committee.

#### Centrifugation and measurement of volumes of supernatants

The saliva and blood samples were centrifuged at 3000 rpm for 15 min on a swing-out rotor centrifuge at room temperature. The respective supernatants were transferred using an Eppendorf autopipette into standard 5 ml graduated cylinder with 0.1 ml divisions and their volumes, measured.

The volumes of supernatants obtained from saliva and blood, were then compared.

#### Statistical analysis

Statistical analysis of data was done using unpaired t test.  $p < 0.05$  was considered as statistically significant.



## RESULTS AND DISCUSSION

The yields of testing fluids obtained that is, supernatant from saliva specimens and plasma from blood, were compared and it was found that the yield was significantly higher from saliva as shown in Table.

**Table 1:** Comparison of yield volumes of supernatant from saliva and plasma from blood

Diagnostic fluid	Supernatant/Plasma yield from 5 ml fluid (in ml)	% increase in yield volume of supernatant from saliva
Saliva <i>n</i> = 30	4.120 ± 0.133* (Mean ± SD)	107.04%
Blood <i>n</i> = 30	1.990 ± 0.116 (Mean ± SD)	

\**p* < 0.0001; highly statistically significant compared to plasma yield from blood

Saliva is known to contain 99% of water whereas plasma volume is only 55% of the whole blood.<sup>3, 9</sup> The sediment obtained after centrifugation of saliva is less than blood. Therefore, significantly higher yield of supernatant is obtained from saliva than the yield of plasma from blood, for testing purpose.

In this study, it has been shown that the yield of supernatant from 5 ml saliva (mean value of 4.12 ml) is significantly more than plasma yield from 5 ml blood (mean value of 1.99 ml). Therefore, it follows that the volume of saliva sample required to be collected to yield 1.99 ml supernatant (as much as the plasma yield from 5 ml blood), would be 51.7 % less than blood.

Percent increase or decrease was calculated using the formula:

$$\frac{\text{Relative Difference}}{\text{Relative Number}} \times 100$$

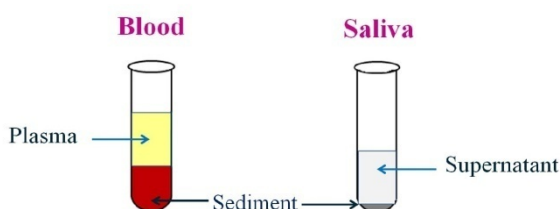
That is,

$$\frac{\text{Difference between yield volumes of supernatant and plasma}}{\text{Volume of supernatant}} \times 100$$

$$\frac{(4.12 - 1.99)}{4.12} \times 100 = 51.7\% \text{ decrease in volume of saliva required to be collected}$$

In other words, if 5 ml blood is required to be collected from a subject for testing purpose, 2.4 ml of saliva (about 50% less than 5 ml) will be adequate for the same purpose as shown in the figure.

**Figure 1:** Illustration of centrifuged sample tubes of 5 ml Blood versus 2.4 ml Saliva



Based on these findings it can be understood that the volume of potentially infectious, pathological waste that would be generated by a clinical laboratory when saliva is used as diagnostic fluid would decrease substantially, by about 50% compared to that generated from blood.

It is already known that that saliva collection method is noninvasive, painless, and convenient to subjects, may be performed several times a day and also allows longitudinal evaluation using minimally stressful sampling methodology.

Significantly lower viral loads of Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) have been reported in saliva than blood or serum.<sup>10-13</sup> The potential risk of infection associated with saliva-handling is therefore, significantly less than blood. In addition, the saliva collection method is needle-free which eliminates the risk of needle stick injuries in health care workers reported to have high occurrence in India and worldwide and results in decreased generation of waste sharps.<sup>14-16</sup> For both these reasons, the use of saliva for testing is less hazardous or offers enhanced safety to handlers.

## CONCLUSION

Saliva-based testing offers combined advantages of decreased generation of potentially infectious, pathological, sharps waste and increased safety to handlers which can revolutionize waste management strategies in health care institutions.

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**Source of Support:** Nil, **Conflict of Interest:** None.

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Dr. Nivedita obtained her Masters and PhD degrees in Medical Biochemistry from Manipal University, in Manipal. She is currently working as Professor of Biochemistry at Yenepoya Medical College, Yenepoya University in Mangalore, India. Her research areas include saliva-based diagnostics, measurement of free calcium at cellular stores and has several publications to her credit. Her report on saliva CRP in Hashimoto, Subacute Thyroiditis has received credit as an emerging diagnostic test in the Best Practice website of BMJ.