*Review Article*

# Salivary Diagnostics Using a Portable Point-of-Service Platform: A Review

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## ABSTRACT

Clinical diagnostics can be improved by faster and more accessible disease detection. Our laboratory has developed a point-of-service (POS) device capable of rapid, sensitive, automated, and multiplexed biomarker detection that uses human saliva instead of other biofluids. Here, we review the technology that led to the development of this POS device. This POS technology can advance clinical diagnostics by saving time because of faster diagnosis, saving money because of a shorter hospital stay, and ultimately improving clinical care. (*Clin Ther*. 2015;37:498–504) © 2015 Elsevier HS Journals, Inc. All rights reserved.

**Key words:** biomarker, detection, device, diagnostic, disease, saliva.

## INTRODUCTION

Human saliva is comprised of 99.5% water that contains electrolytes, proteins, nucleic acids, peptides, polynucleotides, hormones, enzymes, cytokines, antibodies, and other components.[1,2] Saliva is primarily secreted from the parotid, the submandibular, and the sublingual salivary glands.[3] Saliva also contains serumnal components that are transported from blood capillaries into saliva by diffusion, active transport, and/or ultrafiltration via gingival crevices[4]; hence, saliva can be considered a partial filtrate of blood and can provide a window into the health status of a person. Saliva is an attractive diagnostic biofluid because its collection is relatively noninvasive, stress free, and inexpensive and requires minimally trained personnel.[5] Diagnostic methods that use biomarkers from biofluids such as blood and saliva are essential for clinical analyses. Protein and nucleic acid biomarkers can be used to detect medical conditions rapidly, ideally even before the disease presents symptoms in the patient.[6] Many researchers have reported using saliva as a diagnostic fluid. For example, mRNA detection by using saliva samples for oral cancer diagnosis was reported by Matse et al.[7] It was also established in multiple studies that various proteins in saliva correlate with the pathophysiologic state of certain medical conditions.[8–11]

Laboratory testing of clinical samples can be time intensive and expensive.[12] Diagnostic testing is moving toward point-of-service (POS) devices due to the rapid results possible from POS devices when early detection is paramount.[13] Multiple POS devices were developed over the past decade for various diagnostic applications.[14–19] Our laboratory developed a portable POS device that is capable of automated, multiplexed, and sensitive detection of biomarkers present in saliva[19] (**Figure 1**). This review describes the benefits and disadvantages of using saliva instead of blood in a clinical environment, and how a POS device can be used effectively in certain settings. We briefly describe saliva sample preparation, including saliva collection and extraction, a critical aspect of the overall diagnostic process. Next, we discuss the use of protein and nucleic acid biomarkers. We then describe the principles of sandwich immunoassays, ELISAs, digital ELISAs, and microsensor arrays. Finally, we

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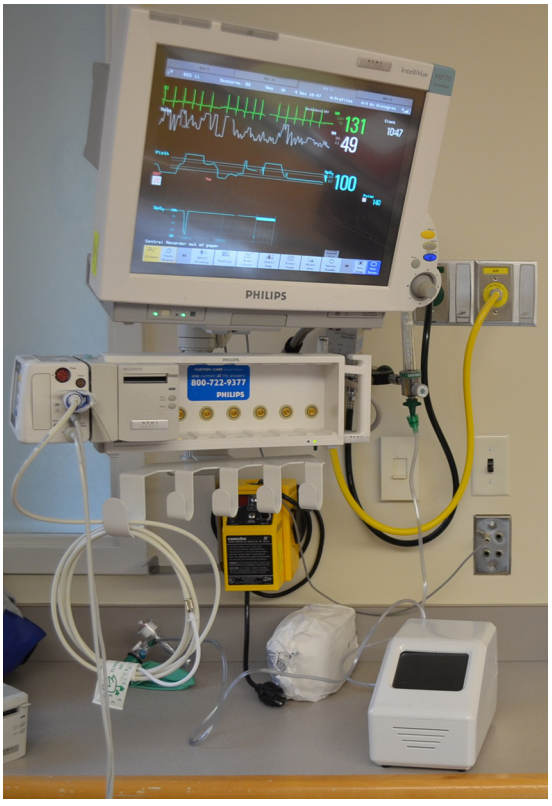


Figure 1. POS device, developed in our laboratory, for salivary diagnostics is shown at a patient's hospital bedside. The device is powered by a conventional AC adapter that provides 12 V DC at 8.5 A (not shown), and the case is 15 cm (wide) × 15 cm (tall) × 25 cm (deep). The weight of the device is 2.7 kg. The concentrations of multiple protein biomarkers in the saliva sample are quantified via sandwich immunoassays conducted on a microfluidic chip present in the device. POS, point-of-service.

discuss the multiplexing capabilities of these assays and their applications to salivary diagnostics.

## SAMPLE PREPARATION

Two essential components of the overall salivary diagnostic process are saliva collection and processing steps. Saliva collection can be done by three established ways, include suctioning, ejecting whole saliva, and swabbing.[20] Saliva can be collected under resting or unstimulated conditions, or it can be stimulated by varied methods, with gustatory and masticatory stimuli being the most common.[21] The method that uses ejection of whole saliva is recommended for collection of both unstimulated and stimulated whole saliva because of its reproducibility and reliability.[21]

Once saliva is collected, it can be analyzed either as whole saliva or separated by centrifugation into the supernatant fluid and pellet and analyzed independently. The supernatant fluid contains dissolved proteins, nucleic acids, organic metabolites, and ions, whereas the pellet contains bacteria and viruses, human cells, debris (such as food particles), and other insoluble components. The composition of whole saliva is complex and, because of its highly proteolytic nature, presents challenges to sample preservation, particularly for disease biomarkers.[22] Unless whole saliva is used immediately, centrifugation is encouraged to separate the cells from the protein-containing supernatant fluid to delay protein degradation.[23] Often, protease and nuclease inhibitors are added to prevent degradation of proteins and nucleic acids, respectively. In addition, cooling the sample on ice reduces the rate of proteolysis and other degradative processes.

Transcriptional and posttranscriptional mechanisms of gene regulation can cause protein and RNA expression levels to be dissimilar.[24] Because of this uncorrelated expression, using proteins as biomarkers for disease detection can sometimes present an incomplete picture. Detecting nucleic acids is not only a potential alternative for clinical biomarker detection in saliva but also a way of providing a more comprehensive picture of the biomarker expression activity by studying the full transcriptome. Numerous studies have successfully detected human mRNA transcripts in cell-free saliva by various methods, including oligonucleotide microarray profiling and reverse transcription–quantitative polymerase chain reaction.[25–29] Nucleic acid detection can be used to complement protein detection methods and to provide additional information about the biomarkers and their posttranscriptional activity.

Proteins are commonly used as clinical biomarkers. The salivary proteome was characterized and reviewed by Helmerhorst and Oppenheim.[22] Analyzing proteins in saliva involves standardizing saliva collection, sample preparation, and protein extraction. Extracting and stabilizing proteins for detection typically involves separating saliva supernatant fluid by centrifugation to prevent protein degradation and analyzing the sample immediately or storing it at 0°C to 4°C for short-term use or –80°C if the sample cannot be analyzed within a few hours. Many aspects about saliva collection, storage, and