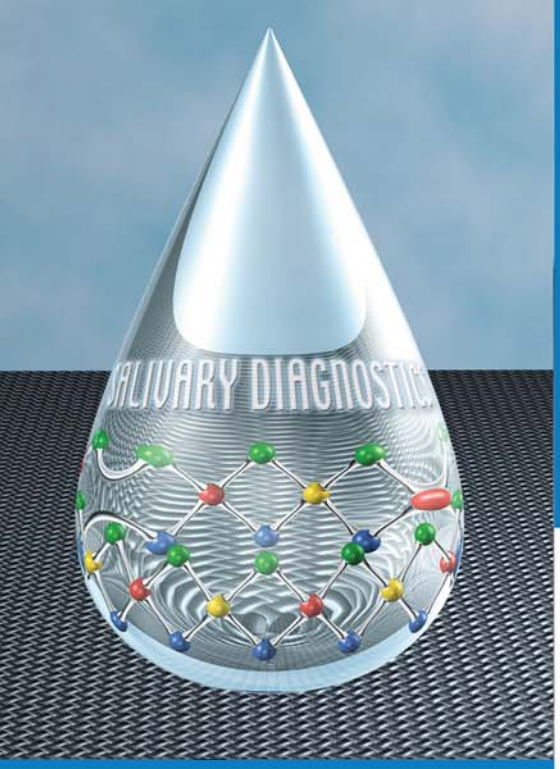

A Primer on Salivary Diagnostics

1. Introduction

Saliva has long been viewed as a unique yet complex bodily fluid, like plasma or serum, although it usually goes unnoticed in daily life. Yet dentists are keenly aware that adequate saliva is essential for maintaining oral health. Scientists have also recognized saliva's utility as a diagnostic fluid for oral or systemic diseases. With expanded research and improved technologies for measuring salivary biomarkers at the molecular level, the field of salivary diagnostics holds tremendous promise for the long-term goal of developing clinically validated, saliva-based tests for health surveillance and early detection of oral disease and systemic conditions.

Saliva is the mixed product of three major salivary glands (parotid, submandibular, and sublingual) and minor salivary glands located throughout the oral cavity.[1] Our average salivary output is approximately one liter per day,[2] and its protective functions include lubricating oral tissues, maintaining a neutral oral pH, remineralizing the dentition, and protecting oral tissue against invasion by microorganisms. Saliva contains enymes that initiate the digestion process, while also moistening food to facilitate swallowing and acting as a solvent for the taste stimuli.

As a common, easily accessible oral fluid, saliva plays a central role in several well-known stories and anecdotes. While "spitting" on something suggests an item is viewed with scorn or disdain, saliva can also appear quite charming when falling from a baby's mouth. Professional baseball players routinely spit (expectorate) on the playing field or on their batting gloves when preparing to hit, either by habit or to improve their grip, or perhaps for good luck. Former professional basketball player, Larry Bird, would spit on his hands at the beginning of every game. In the animal kingdom, dogs and other animals commonly lick their wounds when injured, which provides a first line of defense in preventing the spread of infection. As for Larry Bird's reasons for his pregame spitting ritual, only he could explain.

In the world of science, Ivan Pavlov's classic experiments with

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dogs showed how salivation could be a conditioned reflex to sights or sounds associated with food. More recently, the discovery of growth factors in saliva (nerve growth factor and epidermal growth factor) was partly responsible for the awarding of the 1986 Nobel Prize in Physiology or Medicine to Dr. Rita Levi-Montaicini and Dr. Stanley Cohen.

Despite all the anecdotes and common beliefs about saliva, this oral biofluid is not commonly used for diagnostic or other clinical applications-not yet at least. While there is an FDA-approved product that uses saliva for HIV antibody detection,* and various saliva-based tests for hormonal detection, clinical test applications for saliva remain relatively few at present.

In fact, saliva is often portrayed in a negative light. The verb "spit" is used in many derogatory phrases and insults. There are many social, cultural and psychological stigmas associated with saliva, depending on one's culture or heritage. Spitting in public is also considered a misdemeanor in various municipalities.

On the other hand, some cultures view saliva in a more positive light. For example, in Greek culture, expectorating onto a bride's gown during the wedding ceremony is believed to cast off evil spirits. In a Greek Orthodox baptism ceremony, the priest lifts the infant out of the holy water and expectorates three times on the ground to cast off evil spirits.

As these examples illustrate, there are often polarized images and perceptions associated with saliva among the lay population. By contrast, the dental profession is well aware of the positive value of saliva to one's oral health, from an essential lubricant to its antibacterial and antifungal properties.

Today, a range of interdisciplinary research is well underway that may rapidly change the negative perceptions that some have about saliva. The National Institute of Dental and Craniofacial Research (NIDCR), one of 27 institutes at the National Institutes of Health and the primary funding agency for oral health research in the U.S., has made a significant investment to support scientific research on clinical applications for saliva. Over the past decade, investigators supported by NIDCR have more considerable progress in substantiating the potential of, and expanding the scientific foundation for, saliva's use as a diagnostic fluid.

This primer presents an overview of the significant progress toward the scientific substantiation for the use of saliva in the early detection of oral and systemic diseases. This ongoing research, and the studies to come, will change the relationship between dentistry and medicine, and provide significant benefits to patients.

2. Salivary Diagnostic Science and Technology

With new techniques for detecting small quantities of salivary components, including proteins and messenger RNA (mRNA), the field of salivary diagnostics has emerged as one of dentistry's most promising areas of research. The "modern age" of salivary diagnostics dates back to the early 1900's, when Michaels and Kirk evaluated saliva samples to identify diagnostic biomarkers for rheumatism and gout.[3,4] Today, scientists and engineers worldwide are pursuing initiatives to define and substantiate two critical scientific foundations to support clinical applications that use saliva as a diagnostic biofluid:

- developing the diagnostic "alphabets" for saliva; and
- the design and development of new biosensor technologies capable of fast and effective saliva-based testing for use in clinical settings.

The first step in establishing the scientific foundation was assembling the "diagnostic alphabets" contained in saliva. Saliva is an amorphous fluid and, while we know much about its properties, relatively little is known about the detail of its molecular content. This knowledge gap made it difficult or even impossible to conduct scientific discovery studies to find salivary biomarkers that could be used for disease detection.

With funding support from NIDCR, several research centers in the United States tackled this challenge by first defining and cataloging all the proteins in saliva. This highly successful effort led to the identification of 1166 proteins in human saliva, a landmark accomplishment that has started to provide definition and boundaries for clinical diagnostic applications.[5] This toolbox is known as the salivary "proteome,"

* OraQuick Advance® Rapid HIV-1/2 Antibody Test by Orasure Diagnostics (Bethlehem, PA)

a complete set of proteins expressed and modified following their expression by the genome. The salivary proteome has already been used successfully to identify highly discriminatory biomarkers for the detection of oral cancer and Sjögren's syndrome.[6,7]

Completion of the human salivary proteome project was soon followed by the discovery and development of a number of additional "diagnostic toolboxes":

- the "transcriptome" (a collection of all the gene transcripts present in a given cell);
- the "metabolome" (used to describe the metabolite complement of living tissues); and
- microbes and micro-RNAs (ribonucleic acids).

Details are emerging that will provide additional diagnostic dimensions for saliva and increase its diagnostic utility. Based on these findings and with the support of novel statistical and bioinformatics tools, biomarkers for major human disease --including a distal systemic disease such as breast cancer[8,9]--has been detected in saliva with robust clinical sensitivity and specificity. Figure 1 depicts information from a Web site at UCLA that illustrates the scientific development of the salivary diagnostic toolboxes (www.skb.ucla.edu).

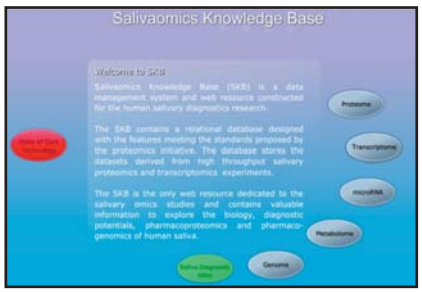

***Figure 1**. Salivaomics Knowledge Base website (http://www.skb.ucla.edu)*

With the establishment of the saliva research base, dental and biomedical researchers now have the scientific foundation upon which it can develop, clinical tools that accurately screen salivary samples for the presence or absence of disease-specific biomarkers. Fortunately, NIDCR is addressing this essential need by calling for the development of point-of-care biosensor technologies that will permit the use of a drop of saliva for the concurrent detection of multiple salivary biomarkers in real time and in a cost-effective manner. These saliva-based biosensor technologies are now under development, and products that use microchip technologies to analyze oral fluid samples may be ready for commercialization in the near future.

A prototype for one of these technologies is shown in Figure 2. Developed by the UCLA research group, the system is called the "Oral Fluid NanoSensor Test" (OFNASET). This device is currently being optimized for detection of oral cancer and Sjögren's syndrome.

***Figure 2**. OFNASET prototype*

3. Clinical Applications: Today and Tomorrow

Salivary constituents are increasingly investigated to aid in the diagnosis of oral and systemic diseases. To date, saliva has been studied to detect caries risk, periodontitis, oral cancer, salivary gland diseases, and viral infections such as HIV and HCV. Another important development in oral disease diagnosis has been the identification of salivary markers for the detection of oral cancer. [10,11]

Based on levels of specific molecular markers, molecular staging profiles have been identified in saliva as objective prognostic indicators. Researchers have found that panels of salivary mRNA (messenger ribonucleic acid) and protein markers can serve as biomarkers for oral cancer detection.[12] Advancements in nanoscale technologies have allowed high-throughput biomarker screening studies to be performed, including RNA detection using microarrays.* Protein detection using highly sensitive mass spectrometry (MS) technologies has allowed researchers to accurately monitor changes in biomarker proteins that are representative of disease.

Another oral disease where salivary diagnostic technology is showing promise is the detection and monitoring of periodontal disease. This application includes the assessment of molecules related to the host inflammatory response and the destruction of associated connective tissue, as well as detection of specific pathogenic bacteria and bacterial products.

Saliva is also providing clues to systemic diseases and disorders. For example, rapid point-of-care HIV tests

* A microarray is a tool for analyzing gene expression that consists of a small membrane or glass slide containing samples of many genes arranged in a regular pattern.

utilize saliva, gingival crevicular fluid, or oral mucosal fluid. The ease of collecting saliva is proving useful for determining hormone levels, including estrogen (estradiol), progesterone, testosterone, DHEA, and cortisol. This is particularly important in the case of estradiol, as it can be an indicator of premature birth and low birthweight babies. A number of drugs are detectible in oral fluid. Salivary drug levels can even be quantified as a viable substitute to blood tests in blood in many cases. Saliva assessment technology is currently used to test for drugs of abuse such as cocaine, ethanol and opiates. The technology can also be used for therapeutic monitoring of drugs such as digoxin, methadone and some anticonvulsants.

As the scientific knowledge base of salivary biomarkers expands and matures, so too does the unprecedented potential for rapid diagnosis and disease progression monitoring of an increasing number of oral and systemic conditions.

Furthermore, saliva may be useful for discovering the genetic causes of disease, including the determination of genetic risk factors. These expanded uses of saliva will no doubt enhance patient satisfaction and cooperation since saliva can be collected noninvasively. One case in point is the current rapid HIV oral fluid test (OraQuick Advance® Rapid HIV-1/2 Antibody Test), which facilitates sample collection and allows the screening of more individuals for HIV in non-traditional settings. This may also improve access to care for at-risk and medically underserved populations, as well as to aid with rapid assessments in public health emergencies. With continued progress in proteomic research and nanotechnology, there will be greater potential to develop saliva-based tests that could be used for normal health surveillance and routine screenings for oral cancer and other life-threatening conditions.

Saliva diagnostics also holds great promise in the fight against bioterrorism. Determining the scope of exposure to toxins requires a technology that is fast, highly sensitive and specific, portable, user-friendly, and capable of screening for multiple agents simultaneously. Saliva and other oral fluids are well suited for this purpose because they can be collected under observation (with or without stimulation), and are less susceptible to adulteration.

4. Implications of Salivary Diagnostic Research for the Dental Profession

The field of salivary (oral fluid-based) diagnostics is a broad, complex and cross-cutting area of scientific research with enormous potential to impact the practicing dentist and health care in general. The role of rapid, in-office screening or chairside diagnostic testing comes down to an important bottom line: improved access and health care outcomes for patients. Saliva-based tests also offer the following advantages for patients and providers:

- Ease of collection;
- Elimination of the common fear of needlesticks
- Lower costs for sample collection; and
- Reduced risks of percutaneous injury.

Salivary diagnostics could dramatically change clinical practice by introducing point-of-care testing and real-time disease surveillance. Dentistry can help this vision become reality, in part because many patients see their dentist more often than their physician because of the prevention focus and the need for regular visits to maintain oral health.

While relatively inexpensive, non-invasive, simple and accurate screening/diagnostic test methods provide obvious patient benefits, there are also challenges and other considerations that are also associated with their routine use in dental practice. Importantly, the dental profession must address knowledge gaps that may exist regarding not only the conditions for which tests are designed, but also a range of economic (reimbursement) issues and various interprofessional practice implications. Although the dental office can be a primary setting for the implementation of oral fluid-based tests, some may feel that embracing a broader base of medical diagnostics, such as breast cancer screening or drug monitoring, rather than a focus on tests for oral conditions (e.g., dental caries and periodontal disease), may not be appropriate for their practice.

For clinicians that begin to incorporate oral fluid-based tests, salivary diagnostics are a “natural” for such applications as: disease surveillance, screening and detection; treatment/outcome monitoring; environmental exposure applications; and home monitoring of disease, treatment and adverse events

(e.g., chemical or metabolic changes). This broad potential would undoubtedly raise awareness about the primary role of oral health in overall health, and could allay patients' fears about why their dentist is conducting salivary tests for systemic conditions.

Indeed, dentists and other health care providers will benefit from the development of rapid screening and diagnostic tests in venues such as community and public health settings, long-term care facilities, and hospitals. This would also strengthen interdisciplinary patient care. It's easy to see that, although dental practitioners work with saliva every day, oral fluid-based diagnostic tests will also be used in other health care settings as they become available. The effective integration of salivary diagnostic tests in clinical settings will also require that patients willingly provide test results with their physicians, In doing so, dentistry has an opportunity to be a primary provider and leader in applications of salivary diagnostic testing.

To address the needs of the profession as this technology develops, the ADA will closely monitor emerging research in this rapidly moving scientific field and continue to assess its roles in such areas as: helping to promote research funding; addressing reimbursement challenges and access-to-care issues; supporting academic education and training in salivary diagnostics, as well as appropriate continuing education programs. Education about the role of dentistry may need to include not only dentists and their staff members, but physicians, patients, government agencies and others.

For additional perspectives on salivary diagnostic research and technology, we invite readers to consult the **ADA Council on Scientific Affairs' Statement on Oral Fluid Diagnostics on ADA.org**,[13] and the resources included in the reference list to this primer.

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Additional Resources

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