



# Oral-Based Techniques for the Diagnosis of Infectious Diseases

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## ABSTRACT

Saliva and other types of oral samples can readily be used for noninvasive diagnosis of diseases of the oral cavity and systemic diseases. Following an introduction outlining the types of oral samples and the analytes that can be measured in these samples, a detailed description of a novel oral-based diagnostic system to detect multiple bacterial and/or viral pathogens is presented. A reasonably priced, portable, point-of-care diagnostic system should be available within five years.

The notion of using saliva for noninvasive diagnostic testing has been discussed and researched for more than 50 years.<sup>1</sup> While there were a few early successes, in general, the field produced many published reports but few approved commercial tests. A review of the literature from 1982-1992, published in conjunction with a New York Academy of Sciences meeting held in 1993, identified 7,500 publications. A more recent survey covering the period from 1966-2004 produced more than 20,000 citations. One of the earliest propo-

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nents of the field, Dr. Irwin Mandel from Columbia University, published a review titled, “Salivary Diagnosis: Promises, Promises.”<sup>2</sup>

Mandel pointed out that the potential utility of saliva-based diagnostic tools was obvious, but the challenges remaining were sensitivity and specificity of the diagnostic test.<sup>2</sup> Because the oral cavity is a complex structure with multiple sources of analytes, it was critical to establish what molecules could be measured in oral samples, how these molecules enter the oral cavity, and what methods are ideal for collecting and analyzing oral samples. The present review, based on a talk given in February 2005 at the American Association for the Advancement of Science annual meeting in Washington D.C., outlines the principles of oral-based diagnostics, and presents a review of the authors’ own work to design and develop a device capable of detecting multiple pathogens in oral samples.

### Overview of Oral-Based Diagnostics

Typically, when one thinks of saliva or oral-based diagnostics, the sample is considered to be whole saliva. Interestingly, many of the early publications comparing analysis of molecules present in blood and saliva samples never indicated the type of saliva collected, or how it was stored and prepared for analysis. Oral biologists recognize major differences between stimulated and unstimulated saliva, between whole vs. duct saliva, and a variety of types of oral samples that can be used for diagnostic purposes.<sup>3</sup> **Table 1** lists the major types of oral samples that have been used for oral diagnosis. The major advantages of collecting whole saliva are a) the ease of collection, b) the ability to obtain a sample without the use of

**Table 1**

#### Oral samples

- Whole saliva
- Duct saliva
- Gingival crevicular fluid
- Mucosal transudate
- Buccal swabs
- Plaque
- Volatiles

specialized collectors, and c) the realization that whole saliva accurately reflects the total salivary environment. The major disadvantages of using whole saliva as the diagnostic medium are that a) it contains other materials in addition to saliva (e.g., buccal cells, bacteria, gingival crevicular fluid, food debris, and contaminating blood), b) the biochemical components may be altered after secretion as a result of bacterial activity or selective binding to oral tissues, and c) there are esthetic drawbacks to “spitting into a tube” that may hamper commercial development of the diagnostic system.

Stimulation of salivary flow can be elicited in a variety of ways including chewing (parafilm, neutral gum base, rubber bands), repeated application of citric acid, or by sucking on hard candy. It should be noted that the differences in stimulated vs. unstimulated whole saliva include an increase in volume with stimulation, which reduces collecting time; however there is also an increase in the relative contribution of parotid vs. submandibular saliva when exogenous stimulation of flow is used. Also, it should be noted that while stimulation increases salivary flow, it decreases protein and drug concentra-

**Table 2**

#### Analytes detected in oral samples

- Ions
- Drugs
- Pathogens
- Hormones
- Antibodies
- Growth Factors
- DNA
- RNA

tions due to the dilution by water.

Duct saliva can be obtained using a variety of collectors designed to fit over the parotid gland opening, Stensen’s duct, or the submandibular/sublingual opening, Wharton’s duct.<sup>3</sup> Gingival crevicular fluid is typically collected on paper points or with small capillaries, and oral mucosal transudate is adsorbed onto collector pads. It is important to recognize that the molecular components of all of these fluids are different. Duct saliva contains, primarily, the products of the salivary glands, while the constituents in GCF and mucosal transudate more closely resemble serum. Parotid and submandibular/sublingual saliva qualitatively contain most of the same components, but there are quantitative differences in the amounts of specific proteins. Also, mucinous glycoproteins are absent from parotid saliva but form a major component of both submandibular and sublingual saliva.

In addition to fluid samples, the oral cavity is an excellent source of DNA, obtained by swabbing the buccal mucosal surface. DNA can be extracted immediately or alternatively, the swabs can be frozen for subsequent forensic analysis. Dental plaque is easily obtained from the tooth surface and is

**Table 3****Oral-based diagnostic challenges**

- Sensitivity
- Stability
- Quantitation
- Saliva/plasma ratio
- Marketing issues
  - Regulatory
  - Medical personnel
  - End-user

**Table 4****Overview of project**

- Convert existing technology for point-of-care detection of multiple pathogens
  - (Ag, Ab, RNA, DNA)
- Select/evaluate ideal collector
- Use *B. cereus* and HIV for proof of concept
- Design/develop novel microfluidic platform

routinely used for evaluating dentrifices and mouthwashes. Finally, the oral cavity can be analyzed for a wide range of volatile substances, useful in assessing halitosis or treatments thereof. In addition, oral volatiles have been used to diagnose a variety of metabolic and gastrointestinal conditions.

An abbreviated list of some of the analytes that can be monitored in oral samples is presented in **Table 2**. Some of these analytes have been detected in saliva (ions, certain drugs, steroid hormones, growth factors, RNA), others have been detected in GCF, and mucosal transudate (antibodies), swabs (DNA). Some of the challenges to be considered in developing new diagnostic tests are outlined in **Table 3**. Since many analytes are present at lower levels in saliva than in blood, detection sensitivity is clearly an issue. Fortunately, the advent of new amplification technologies such as ELISAs and PCR, make it feasible to accurately measure analytes even when oral concentrations are orders of magnitude lower than blood levels.

A major issue to consider for any potential diagnostic test is whether qualitative information will suffice or alternatively, if one needs a quantitative measurement. In the case of tests

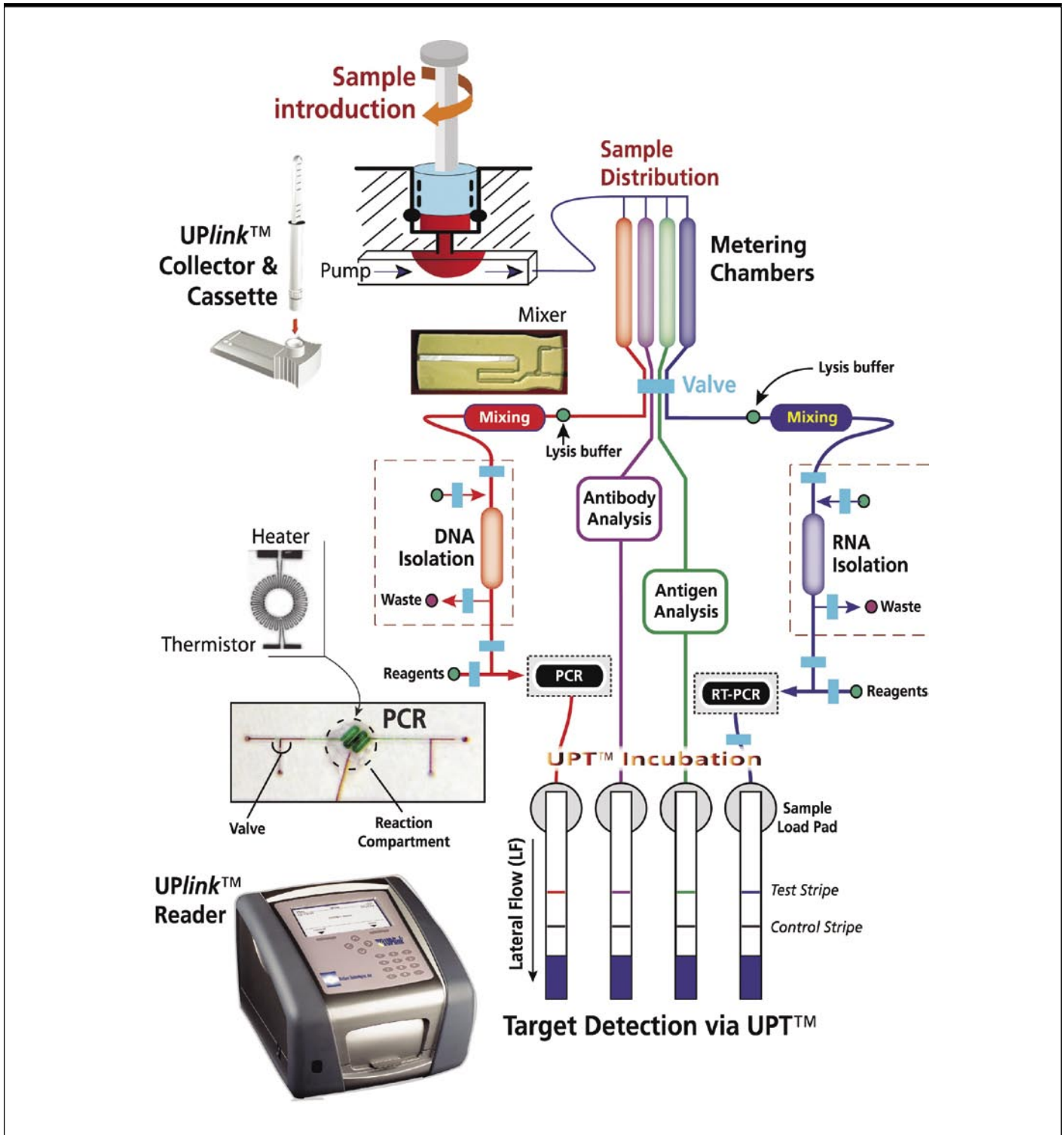
for HIV infection, a “yes/no” result is sufficient; while for monitoring glucose levels, it is necessary to have a precise quantitative value in order to know the blood glucose value. This issue presents as the ratio of salivary levels of the analyte being measured compared to blood levels of that analyte, referred to as the S/P ratio.<sup>4</sup> If salivary levels fluctuate directly with blood levels, as is the case with blood and oral alcohol levels, then a quantitative oral test is possible. If salivary levels are not correlated with blood levels, as is the case with glucose, then it is difficult, if not impossible, to develop a quantitative oral test.

In addition to the previously mentioned technical issues, in order for an oral test to replace or compete with an existing blood test, there are a series of regulatory and marketing considerations. Regulatory agencies tend to err on the conservative side, thus a novel oral-based diagnostic test must demonstrate robust comparisons with an existing blood-based test, and this requires extensive and expensive clinical trials. In the final analysis, the demand for a new diagnostic test will be determined by the end-user, and the perceived value of a noninvasive oral test as compared to a needle stick.

## Application of Oral-Based Diagnosis for Infectious Diseases

The authors' recent work in the field of oral-based diagnostics has focused on the development of a point-of-care device for the rapid identification of multiple bacteria and/or viruses, using several targets associated with each pathogen.<sup>5-7</sup> An overview of our project is shown in **Table 4**.

Initial studies were designed to select a collector that would be esthetically acceptable and be able to pick up and deliver fluid, bacteria, protein, and nucleic acid to the diagnostic platform.<sup>8</sup> The authors selected a group of nine commercially available collectors and compared their ability to transfer saliva, *B. cereus*, amylase, and DNA. The most effective devices were the OraSure and UPlink collectors. All of the subsequent studies have been carried out with the UPlink collector, which consists of a dried cellulose pad on the end of a plastic holder. When immersed in fluid, the sponge expands to collect ~300 µl of fluid. Fluid is extruded by pressure on the tip, which squeezes out the liquid. The UPlink collector is designed to deliver the fluid sample to a cassette. The existing cassette has a single nitrocellulose strip, the fluid flows by capillary action, and analytes are captured at specific zones by reagents designed to recognize those analytes. In a current project, the authors are designing a microfluidic platform that will fit into the footprint of the existing cassette. The overall plan involves dividing an oral sample into four pathways to detect: antibodies to the bacteria/virus, antigens present on the surface of the pathogen, and RNA or DNA present in the oral sample after lysis of the pathogens.<sup>6</sup> Each of these pathways leads to a nitrocellulose strip with the appropriate capture reagent immobilized in



**Figure 1.** Schematic diagram of the microfluidic system.

the target zone. The strength of this approach is that it monitors several targets from a single pathogen and it is also amenable to detecting multiple pathogens in an oral sample.

The project has been deconstructed into a series of steps. In each case, a protocol is worked out using bench top methodology and then modified, as needed, for the microfluidic platform. A summary of the authors' progress to date is shown in **Figure 1**, where the sample has been collected and distributed into a series of mixing chambers. Movement within the cassette is carried out using external pneumatic

detection with the new microfluidic system, the total time of assay from five to six hours can be reduced to less than one hour. This is critical for a point-of-care diagnostics system.

### Summary

Oral-based diagnostics is a rapidly growing field that will impact on the practicing dentist who will become a key player in the future. There are many types of oral samples that can be obtained for diagnostic purposes. The decision as to which type of sample to use will depend on several factors, including the analyte to be measured,

## ORAL BIOLOGISTS RECOGNIZE MAJOR DIFFERENCES BETWEEN STIMULATED AND UNSTIMULATED SALIVA, BETWEEN WHOLE VS. DUCT SALIVA, AND A VARIETY OF TYPES OF ORAL SAMPLES THAT CAN BE USED FOR DIAGNOSTIC PURPOSES.

pumping. Aliquots for PCR or RT-PCR enter a small chamber sealed with hydrogel valves, and the temperature of the reactor is cycled through 20 cycles to amplify the nucleic acids. Following DNA and RNA amplification, the valves are opened and the sample is moved to the detection strips containing the capture zones.<sup>9</sup> Samples for the detection of antibody and antigen proceed through simpler paths requiring only metering and mixing. The detection system relies on a process known as Up-converting Phosphor Technology (UPT).<sup>10</sup> In practice, UPT particles are coated with chemicals that bind the antibodies, antigens, or amplified nucleic acids (amplicons). With this system, the authors have been able to detect signals from a sample of saliva spiked with a mixture of bacteria and viruses. Comparing traditional bench top

the relative level of this analyte as compared to blood levels, and the requirement for quantitative vs. qualitative measurements. As noted in this paper, and in the other papers included in this issue, oral-based diagnostics have broad applicability to detect oral diseases (e.g. caries and periodontal disease), systemic diseases, including cancer and infectious diseases, and a variety of drugs including the drugs of abuse. The future for saliva and other oral based diagnostic systems appears very bright. **CDA**

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