

Saliva Testing in the Dental Practice

The current state of saliva-based diagnostics

by **Linda Douglas, RDH, BSc.**

Hygienetown clinical director **Linda M. Douglas, RDH, BSc.**, graduated as a dental hygienist from the Royal Dental Hospital in London in 1982. After graduation she worked in periodontology before moving to Toronto, where she has worked in private practice since 1990. Douglas' desire to support patients with xerostomia and eating disorders has instigated her in-depth study of their effect on oral health.



Saliva testing for oral and systemic disease has several advantages, which make it ideal for use in the dental office: It is noninvasive, painless and cost-effective, plus in many cases saliva samples can be tested at the point of care, for rapid results. In addition, saliva samples remain more stable than blood, and if laboratory analysis is required, they are easier to transport.

Dentists, physicians and patients have been shown to be quite receptive to chairside health screenings, as evidenced by the following survey results:

1. A national, random sample of U.S. general dentists' attitudes toward chairside screenings revealed that 87.7 percent of respondents were willing to collect oral fluids for salivary diagnostics, and 83 percent would be open to providing medical screenings that yield immediate results. Respondents were significantly more willing to collect saliva than blood via finger stick.
2. A survey of primary care physicians indicated most would welcome dental office screenings for hypertension, diabetes, HIV infection and cardiovascular disease, and would be willing to discuss results with dentists; they would also accept patient referrals from dentists.
3. Many patients also reported a willingness to have a dentist conduct screenings for these systemic diseases. In addition, most stated that their opinion of the dentist would improve regarding the dentist's professionalism, knowledge, competence and compassion if they conducted regular medical screenings.



Technologies used for saliva testing

Immunochromatography. Lateral flow immunochromatographic assays are used for home or point-of-care testing. They detect the presence (or absence) of a target analyte in a sample. The technology is based on capillary beds, which transport fluid spontaneously. As the sample migrates along the assay to the conjugate in the reaction zone, it encounters antibodies specific to the target analyte (Fig. 1). If the target analyte is detected within the sample, the antibodies will bind to it and subsequently reach the test window, where a line will appear.

Polymerase chain reaction analysis (PCR). PCR analyzes a short sequence of DNA or RNA by amplification of the minute quantities contained in samples; sections of DNA or RNA are reproduced, using the enzyme polymerase as a catalyst.

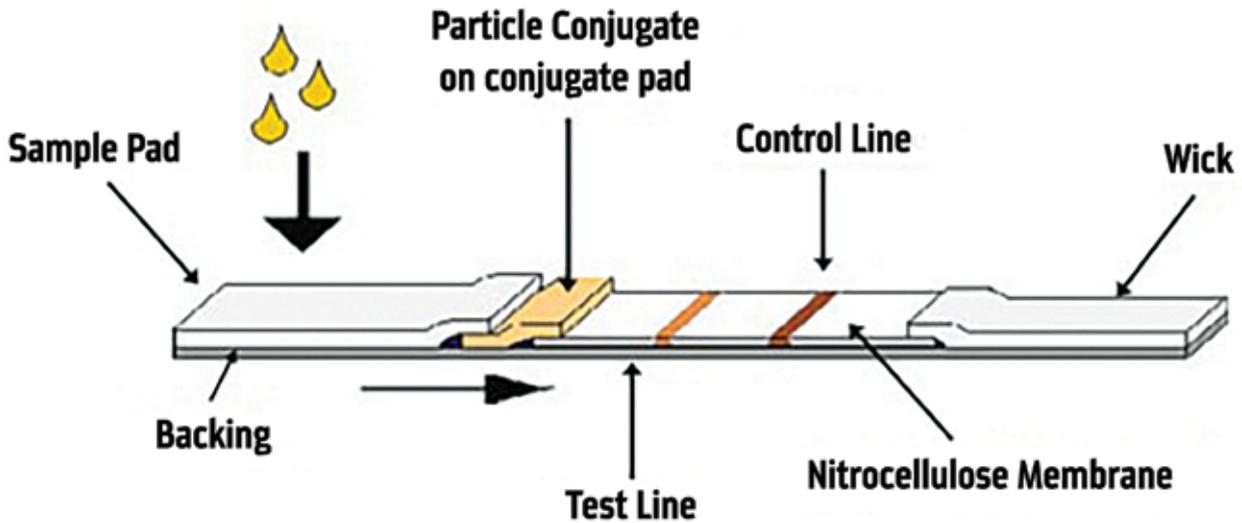
Oral fluidic nanosensor test. This is a point-of-care biosensor technology. Saliva samples are placed on a microarray platform and analyzed by mass spectrometry, which identifies molecules in a sample by determining their mass.

Electric field-induced release and measurement (EFIRM): EFIRM uses an electrochemical sensor with electrode chips to enable tumor-derived exosome vesicles in saliva to release DNA, RNA and proteins, while simultaneously detecting mutations. It requires just 40 microliters (μL) of saliva for testing. EFIRM has a sensitivity and specificity comparable to tissue-based testing such as biopsy (the gold standard), and the total detection time is less than 10 minutes.

Enzyme-linked immunosorbent assay (ELISA). This immunoassay uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein in a sample.

Sensing chronoamperometry. An electrochemical measurement technique, based on enzymatic oxidation of the target analyte in a fluid sample.

Fig. 1



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Dual-wavelength reflectance. Utilizes a test strip with reagents that change color when the sample is applied: the color change of the test strip pad after sample application is measured by the reflectance of light with wavelengths of 565nm, 635nm and 760nm. LEDs shine dual-wavelength light upon the test strip, and reflections are received at the detector.

Currently available chairside saliva tests

Saliva-Check Buffer. This is used to assess the buffering capacity of stimulated saliva (Fig. 2). The test pad has three sections; the acid level in each is different, so that the resistance of saliva against strong, medium and weak acid levels can be measured within two minutes.

Saliva-Check Mutans. GC America's Saliva-Check Mutans uses immunochromatography to detect *Streptococcus mutans* levels within 15 minutes.

OraRisk Caries by OralDNA examines cariogenic bacterial load and associated caries risk: It utilizes quantitative polymerase chain reaction analysis to test for *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus casei*.

Self-test for active periodontal disease. PerioSafe screens for the presence of matrix metalloproteinase-8 (aMMP-8), which is the key enzyme for tissue destruction in periodontitis. This is not a marker of inflammation; it is a destruction marker, and provides evidence of active periodontal disease and peri-implantitis, or stability. If aMMP-8 is elevated, this confirms the acute destruction of gingival, periodontal or peri-implant soft and hard tissues. A negative test result after periodontal therapy means the therapeutic measures were successful. Patients can use this screening device to test themselves and within 10 minutes determine whether they have active periodontitis.

MyPerioPath Salivary DNA-PCR analysis identifies the type and concentration of specific periodontal pathogens. The assessment is done at the initial diagnosis before treatment to measure the bacterial profile and individualize therapy, and then done to retest and monitor after therapy.

MyPerioID PST salivary DNA test measures risk of periodontitis associated with an exaggerated inflammatory response due to genetic variations of interleukin 1 and interleukin 6. Susceptible individuals release abnormally high levels of proinflammatory cytokines. Alert 2 combines these two tests from one saliva sample.

SILL-Ha by Arkray. This instrument assesses caries risk, markers for periodontal inflammation and biofilm by analyzing cariogenic bacteria, acidity, buffering capacity, blood, leukocytes, protein and ammonia in an oral rinse sample using dual-wavelength reflectance.

SaliMark OSCC salivary test by PeriRx. This is a test for oral squamous cell carcinoma. It is performed when a suspicious oral lesion is found, or if there is a history of high-risk behaviors such as smoking. This test identifies multiple biomarkers for the greatest accuracy; it uses messenger RNAs because they are more robust than the proteins, and easier to stabilize and transport. For a moderate- or high-risk test result, refer to a specialist for biopsy; for a low-risk result, schedule follow-up to ensure resolution.



The future of salivary diagnostics: Read online now!

Hygienetown clinical director Linda Douglas discusses how science is using salivary biomarkers for the detection of life-threatening diseases in Part II of her saliva diagnostics package, which is exclusively online. Head to dentaltown.com/salivary-research to read this article.



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OraRisk HPV identifies HPV status, which is a risk factor for oropharyngeal cancer, particularly HPV strains 16 and 18. This test measures the type of HPV and monitors whether the viral infection is active or dormant. HPV is the most common sexually transmitted infection globally; many people acquire HPV, but most clear the infection within one to two years. About 1 percent of the general population has HPV 16 or 18.

OraRisk HSV identifies herpes simplex virus 1 or 2 and detects active infections.

OraRisk Candida identifies all common species of candida. Some types of candida are resistant to standard antifungal treatments, so identification of candida species is key to treatment choice.

The OraRisk CT/NG test detects *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. A single sample can be used for more than one OraRisk test.

OraSure Technologies makes a take-home HIV screening test.

iQuickIt saliva analyzer glucose test. There is a proven correlation between saliva and blood glucose levels. This analyzer tests saliva for glucose; the strip is placed in the mouth for a few moments, then placed in the analyzer for testing. This might have possibilities for screening and monitoring in the dental office.

Celsus One salivary DNA tests assess for eight gene markers related to inflammatory response and provide insight into the genetic component of disease.

Saliva-based drug tests. Drug metabolism varies by individual, and OralDNA Labs DNA DrugMap tests help to personalize prescription and dosing based on individual genetics, instead of one-size-fits-all prescribing (Fig. 3). Most FDA-approved drugs are metabolized by the cytochrome P450 enzymes; these tests identify genetic variations that predict changes in CYP450 enzyme activity. A single saliva sample can be tested for various drugs. There are also tests that monitor saliva for cannabinoids, opioids,

Fig. 2



Saliva-Check BUFFER Testing Mat

(Results will be most accurate if patient avoids eating or drinking one hour prior to testing)

Step 1 - Resting Flow Rate:

Visually assess the lower lip labial secretion. Evert the lower lip, gently blot the labial mucosa with the small piece of gauze and observe the mucosa under good light. Droplets of saliva will form at the orifices of the minor glands. If the time taken for this to occur is greater than 60 seconds, the resting flow rate is below normal.

Greater than 60 seconds:	Resting flow rate	● Low
Between 30-60 seconds:	Resting flow rate	● Normal
Less than 30 seconds:	Resting flow rate	● High

Step 2 - Salivary Consistency:

Visually assess the resting salivary consistency in the oral cavity.

- Sticky frothy saliva: ● Residues
- Frothy bubbly saliva: ● Increased Viscosity
- Watery clear saliva: ● Normal Viscosity

Step 3 - Testing pH - Resting Saliva:

Instruct the patient to expectorate (spit) any pooled saliva into the collection cup. Take the enclosed pH strip, place one end of it into the sample of resting saliva for 10 seconds and then check the color of the strip. Be sure to save the other end of the pH Strip for step 5. Highly acidic saliva will be in the red section, pH 5.0 - 5.8. Moderately acidic saliva will be found in the yellow section, pH 6.0 - 6.6. Healthy saliva will be in the green section pH 6.8 - 7.8.

pH Paper

Dental Saliva pH Indicator:
pH 5.0 - 7.8

5.0	5.2	5.4	5.6	5.8	
6.0	6.2	6.4	6.6		
6.8	7.0	7.2	7.4	7.6	7.8

Results:
Compare the color of the test strip while the paper is still moist. Note the pH reading and record the results.

Note:
Discard saliva and keep cup for Step 4.

Step 4 - Testing Quantity - Stimulated Saliva:

Ask the patient to chew the supplied piece of wax. After 30 seconds, ask the patient to expectorate (spit) into the collection cup. They should then continue chewing the wax for an additional 5 minutes, expectorating every 15 - 20 seconds in the cup provided. It is preferred that you leave the patient alone in the room while they collect saliva. Measure the volume of liquid in the cup excluding froth and record the result. Keep saliva for Steps 5 and 6.

Volume of Saliva/Value

● <3.5mL Very Low	● 3.5- 5.0mL Low	● >5.0mL Normal
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Step 5 - Testing pH - Stimulated Saliva:

Take the pH test strip and place the unused end into the sample of saliva for 10 seconds and then check the color of the strip. This should be compared with the chart in Step 3. Highly acidic saliva will be in the red section, pH 5.0 - 5.8. Moderately acidic saliva will be found in the yellow section, pH 6.0 - 6.6. Healthy saliva will be in the green section, pH 6.8 - 7.8.

Step 6 - Testing Buffering - Stimulated Saliva:

Open the buffer test foil pack. Use the pipette to draw up some saliva from the cup. Dispense 1 drop from the cup onto each of the 3 test pads. Turn the test strip on its side to drain excess saliva onto a tissue. After 2 minutes, compare the color of each pad with the table below, total the 3 scores and record the results.

Green = 4 points	Green/Blue = 3 points	Blue = 2 points
		Blue/Red = 1 point
		Red = 0 points

Buffering Ability

● 0 - 5 Very Low	● 6 - 9 Low	● 10 - 12 Normal
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cocaine, nicotine, benzodiazepines, barbiturates, amphetamines and other drugs. These include the Saliva MultiDrug Screen test kit, QuickScreen and iScreen.

The Saliva-Check, PerioSafe SiLL-Ha and iQuickIt tests are point-of-care tests, which provide results chairside within minutes. For the OralDNA, OraRisk and SaliMark tests, a saliva sample is collected chairside in a specialized collection tube and sent by courier to a laboratory for analysis. The results are emailed to the clinician or accessed on a secure website.

New tests in the pipeline

Dr. David Wong of UCLA is collaborating with SaliMark to develop new saliva tests for early detection and screening of lung cancer, Sjögren's syndrome, prediabetes and type 2 diabetes.

Research is being conducted internationally on salivary biomarkers of oral and systemic disease; this specialty is termed *salivaomics*.



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Editor's note: Part 2 of this series, online at dentaltown.com, explores the latest research and developments in salivary diagnostics. ■

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