Salivary Diagnostics in Oral Diseases

Course Author(s): Balwant Rai, BDS, MS
CE Credits: 1 hour
Intended Audience: Dentists, Dental Hygienists, Dental Assistants, Dental Students, Dental Hygiene Students, Dental Assistant Students
Date Course Online: 03/01/2017    Last Revision Date: N/A    Course Expiration Date: 02/29/2020
Online Course: www.dentalcare.com/en-us/professional-education/ce-courses/ce521

Disclaimer: Participants must always be aware of the hazards of using limited knowledge in integrating new techniques or procedures into their practice. Only sound evidence-based dentistry should be used in patient therapy.

Introduction
Salivary biomarkers are useful in the early detection (diagnosis) and monitoring of oral diseases. The aim of this continuing education course is to offer information on the availability of salivary diagnostics relevant to the practice of dentistry.

Conflict of Interest Disclosure Statement
• The author reports no conflicts of interest associated with this course.

ADA CERP
The Procter & Gamble Company is an ADA CERP Recognized Provider.

ADA CERP is a service of the American Dental Association to assist dental professionals in identifying quality providers of continuing dental education. ADA CERP does not approve or endorse individual courses or instructors, nor does it imply acceptance of credit hours by boards of dentistry.

Concerns or complaints about a CE provider may be directed to the provider or to ADA CERP at: http://www.ada.org/cerp

Approved PACE Program Provider
The Procter & Gamble Company is designated as an Approved PACE Program Provider by the Academy of General Dentistry. The formal continuing education programs of this program provider are accepted by AGD for Fellowship, Mastership, and Membership Maintenance Credit. Approval does not imply acceptance by a state or provincial board of dentistry or AGD endorsement. The current term of approval extends from 8/1/2013 to 7/31/2017.

Provider ID# 211886
Course Contents
• Overview
• Learning Objectives
• Introduction
• Why Salivary Diagnostics?
• Dental Caries
• Salivary Biomarkers for Diagnosing Dental Caries
• Periodontal Disease
• Salivary Biomarkers for Diagnosing Periodontal Diseases
• Premalignant and Malignant Oral Lesions
• Salivary Biomarkers for Diagnosing Premalignant and Malignant Oral Lesions
• Oro-facial Pain and Salivary Biomarkers
• Saliva-based Drug Detection
• Saliva-based Detection of Infectious Diseases
• Conclusions
• Course Test
• References
• About the Author

Overview
This continuing education course discusses the basis of saliva-based technologies in the diagnosis of oral diseases such as dental caries, periodontal disease, oral pre-cancer, and cancer and oro-facial pain; and presents a brief overview of the availability of point-of-care saliva-based diagnostic technologies.

Learning Objectives
Upon completion of this course, the dental professional should be able to:
• Discuss the biochemistry and physiology of saliva in health and in diseases in general terms.
• Understand the diagnostic value of saliva-based technologies in the early detection of dental and oral diseases.
• Identify available saliva-based technologies for point-of-care use.

Introduction
Periodontal diseases, dental caries, oro-facial pain and oral cancer are main global oral health burdens. In recent years, saliva has been recognized as a promising diagnostic fluid both in clinical and research settings. The diagnosis of oral diseases at an early stage improves prognosis. Diagnosing and monitoring the progression of oral diseases by using non-invasive, point-of-care technologies would improve therapeutic outcomes and patients' quality of life.

Saliva acts as a medium to dissolve solids and it facilitates the digestion of starch, chewing, and bolus formation. It has a lubricating and cleansing effect on teeth and other oral tissues, it promotes the mineralization of teeth, and has antibacterial, antiviral, and antifungal properties. Saliva consists mainly of water (99%) and a variety of electrolytes and proteins (1%), e.g., ions, enzymes, protein complexes, and other organic molecules and bio-chemical agents.

Saliva may be considered either as gland-specific or as whole saliva. Gland-specific saliva is secreted by the parotid, submandibular, sublingual, and minor salivary glands. Whole saliva is a mixture of secretions from the salivary glands and constituents of non-salivary origin such as gingival crevicular fluid, blood and blood derivatives, desquamated epithelial cells and other cellular components, nasal and bronchial secretions, microorganisms, microbial enzymes, and many extrinsic substances (e.g., food debris, toothpaste and mouthwash components).

Changes in the concentration of enzymes or other components of saliva can be used as biomarkers or diagnostic indicators for oral and systemic diseases. Gland-specific saliva can be used to diagnose pathoses specific to one of the major salivary glands. Biomarkers in whole saliva may be used to diagnose or to identify patients at risk of several oral and systemic conditions, e.g., dental caries, periodontal diseases, hereditary disorders, autoimmune diseases, malignancies, certain infectious disease, and to monitor drug and hormone levels.
Why Salivary Diagnostics?
Salivary constituents include disease markers such as active peptides, proteins and enzymes; consequently, saliva is an ideal diagnostic fluid alternative to blood. Salivary biomarkers specific for head and neck carcinoma, oral cancer, breast cancers, gastric cancers, salivary gland dysfunction (e.g., Sjogren's syndrome), systemic sclerosis, dental and gingival pathology, psychiatric, neurological, and other diseases have been identified. Saliva sampling is noninvasive and saliva is easier to handle during diagnostic procedures than blood.

Dental Caries
Dental caries affects about two billion people worldwide. It is more prevalent in Latin America and the Middle East, and its prevalence is lowest in China. It is multifactorial, involving a combination of environmental and biological factors. Oral bacteria found in dental plaque release different enzymes (e.g., aminopeptidases, dipeptidyl peptidases, elastase etc.), which results in demineralization of susceptible dental hard tissues.

The initial stage of dental caries is asymptomatic. Advanced states are associated with symptoms of pulpitis. Caries may be detected visually, radiographically or in combination with other adjunctive methods such as quantitative light-induced fluorescence, laser fluorescence detection techniques, alternating current impedance spectroscopy, and computer-aided imaging. Furthermore, point-of-care salivary diagnostics can be used for caries-risk assessment.

Salivary Biomarkers for Diagnosing Dental Caries
Bacterial and host-derived proteins, enzymes, inflammatory mediators, ions and minerals in saliva may be used as salivary biomarkers for the diagnosis of dental caries. Statherin, proline-rich proteins and histatin-1 levels are considerably elevated with dental caries. Salivary calcium and phosphate concentrations are decreased. The concentration of salivary biomarkers associated with Streptococcus mutans and sobrinus, lactobacilli, and Actinomyces spp. are considerably higher in patients with dental caries and in those at risk of dental caries.

Point-of-care salivary diagnostics based on microbial biomarkers such as Dentocult Strip Mutans (Orion Diagnostica, Espoo, Finland), Ivoclar CRT (Ivoclar Vivadent, Amherent, NY), CariScreen caries susceptibility test (Oral BioTech, Albany, OR) are available for the detection of increased caries risk or caries activity. Since xerostomia also promotes demineralization, a determination of salivary flow rates may further contribute to the identification of patients at increased caries risk.

Periodontal Disease
Periodontal diseases affect about 11% of the general global population and about 75% of all adults. They are inflammatory conditions affecting both soft and hard structures supporting the teeth and are characterized by bleeding, swelling, increased pocket depth, and bone loss. Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola and Aggregatibacter actinomycetemcomitans are considered the main causative pathogens.

Periodontal diseases include gingivitis (i.e., a reversible stage of inflammation), initial stage periodontal disease (i.e., early bone loss with 3-5 mm pockets), followed by moderate periodontitis (i.e., bone loss with 5-7 mm pockets), and severe periodontal disease (i.e., with more than 7 mm bone loss). The clinical phase of gingival/periodontal problems is assessed visually, by physical examination, and radiographically.

The preclinical phase of periodontal diseases is detectable by using genetic, microbial, and protein biomarkers. Gingival crevicular fluid-based biomarkers indicate gingival and periodontal inflammation. However, it is invasive, expensive, time-consuming, requires training and needs laboratory processing. As compared to gingival crevicular fluid, whole saliva is more easily accessible and simple to collect.
Salivary Biomarkers for Diagnosing of Periodontal Diseases
Salivary proteins, enzymes, immunoglobulins, ions, DNA, and mRNA are useful biomarkers for the detection of periodontal diseases. Increased levels of salivary interleukin-1 beta (IL-1β), interleukin-6, tumor necrosis factor-alpha (TNF-α) and matrix metalloproteinases (MMP) have been associated with higher risk to or increased severity of existing periodontal diseases. Some biomarkers are related to host immune responses to microbial infection.  

Salivary MMP-2, -8, and MMP-9 levels are significantly elevated in periodontal diseases due to neutrophil collagenase. Salivary IgG, cathepsin (an enzyme that degrades proteins) and osteocalcin (a non-collagenous protein secreted by osteoblasts) are significantly elevated in patients with periodontal diseases. Currently available point-of-care salivary diagnostics include MyPerioID®, MyPerioPath®, and Electronic Taste Chips Technology.

MyPerioID® (OralDNA® Labs, Eden Prairie, MN) uses polymerase chain reaction (PCR) technology to help identify persons with genetic susceptibility to periodontal diseases. MyPerioPath® (OralDNA® Labs, Eden Prairie, MN) helps to identify the type and concentration of specific bacteria that are known to cause periodontal diseases. Electronic Taste Chips (Rice University, Houston, TX), a programmable Bio-Nano-Chip (p-BNC) technology, may be used to identify salivary biomarkers specific for periodontal disease.

Premalignant and Malignant Oral Lesions
Oral cancer is the sixth most common cancer in the world, about 90% of which are squamous cell carcinomas. Precancerous lesions and conditions include erythroplakia, leukoplakia, candidiasis, lichen planus, actinic cheilosis, and submucous fibrosis. Most common sites of oral cancer are the tongue, floor of the mouth, lips and gingivae. Tobacco smoking and chewing betel nut are major risk factors for oral pre-malignant and malignant squamous cell lesions.

The diagnosis is predicated on visual recognition of high-risk lesions and a confirmatory histological evaluation. However, the initial stages of pre-cancerous and cancerous lesion are almost always painless and often go undetected. Consequently, these lesions are not diagnosed until they have become symptomatic, i.e., they have reached advanced stages characterized by large ulcerations, pain, paresthesia, and lymphadenopathy.

Currently, radiotherapy and surgery are the primary treatment modalities. Surgical resection of large lesions may affect speech, swallowing, physical appearance, and the patient's quality of life. The rate of recurrence is also high (10-30%). Consequently, improved diagnostic strategies are needed to identify patients at risk of oral cancer and for early detection of pre-malignant and malignant lesions.

Currently available diagnostic technologies such as ViziLite®, OralCDx® Brush Biopsy, and VELscope® have their limitations. Biopsy, the gold standard for diagnosing premalignant and malignant lesions, is predicated on visual detection and is invasive. For these reasons, there is a need for specific point-of-care diagnostics for the early detection of pre-cancerous and cancerous lesions, which are non-invasive, easy to use, and are cost-effective.

Salivary Biomarkers for Diagnosing Premalignant and Malignant Oral Lesions
Saliva-based biomarkers for pre-malignant and malignant lesions include proteins, nucleic acids, vitamins, ions, genomic and proteomic targets.
such as enzymes, growth factors, cytokines, metalloproteinases, endothelin, cytokeratin, mRNA, DNA aberrations, and telomerase.\textsuperscript{40-42} MMPs have been studied as possible cancer biomarkers and have been found to be linked to tumor invasion and metastasis.\textsuperscript{43}

Other biomarkers include salivary IL-1β, and IL-8, methylation markers, actin, myosin and microRNAs (a small non-coding RNA molecule that regulates gene expression).\textsuperscript{44,45} The only currently available point-of-care technology to identify patients at risk of oral cancer is OralRisk\textsuperscript{®} HPV (OralDNA\textsuperscript{®} Labs, Eden Prairie, MN). It identifies biomarkers for oral human papilloma viruses, several strains of which are known to be associated with oral cancer.\textsuperscript{46}

Oro-facial Pain and Salivary Biomarkers
The prevalence of oro-facial pain is about 10% in the adult population.\textsuperscript{47} It may be a primary problem such as temporomandibular joint disorders (TMDs), neuropathic pain, and headaches; it may be a postoperative complication; or it may be connected with a malignancy. TMDs affect approximately 10 million people in the United States. Salivary cortisol and melatonin levels are significantly altered in TMDs, but point-of-care diagnostics are not available.\textsuperscript{48}

Saliva-based Drug Detection
Salivary levels of cannabinoids, opioids, cocaine, nicotine, benzodiazepines, barbiturates, lysergic acid, diethylamide, phencyclidine, and amphetamines can be measured or monitored.\textsuperscript{49} Available point-of-care diagnostics include Saliva MultiDrug Screen test kit\textsuperscript{®} (Medimpex Inc.), QuickScreen\textsuperscript{®} (Craig Medical Inc.), and iScreen\textsuperscript{®} (BioCheck). Point-of-care diagnostics to detect cotinine in smoking subjects, and methamphetamine and cocaine are not available.\textsuperscript{50,51}

Saliva-based Detection of Infectious Diseases
There are more than 1000 nonpathogenic and pathogenic organisms in the oral cavity. For several of the pathogenic organisms such as \textit{H. pylori}, \textit{M. tuberculosis}, \textit{B. burgdoferi}, \textit{Shigella}, \textit{T. solium}, and the human immunodeficiency virus (HIV) salivary biomarkers have been identified.\textsuperscript{52-56} Only OraQuick Advance\textsuperscript{®} Rapid HIV-1/2 Antibody Test (Orasure Diagnostics, Bethlehem, PA), which tests for HIV-specific antibodies, is available for point-of-care use.\textsuperscript{56}

Conclusions
Early diagnosis of oral and systemic diseases might improve treatment outcomes, reduce the cost of care, and have a salutary effect on the patients' quality of life. A few validated saliva-based diagnostic technologies are available for point-of-care use in dental settings; however, the technology for the early detection of most systemic diseases is still in its infancy.
Course Test Preview
To receive Continuing Education credit for this course, you must complete the online test. Please go to: www.dentalcare.com/en-us/professional-education/ce-courses/ce521/start-test

1. **Which of the following statements is correct with respect to saliva?**
   a. Saliva consists mainly of water (99%) and a variety of electrolytes and proteins (1%), e.g.,
      ions, enzymes, protein complexes, and other organic molecules and bio-chemical agents.
   b. Gland-specific saliva is secreted by the parotid, submandibular, sublingual, and minor
      salivary glands.
   c. Whole saliva is a mixture of secretions from the salivary glands and constituents of non-
      salivary origin.
   d. All of the above.

2. **Which of the following statements related to saliva or salivary biomarkers is correct?**
   a. Changes in the concentration of enzymes or other components of saliva can be used as
      biomarkers or diagnostic indicators.
   b. Gland-specific saliva can be used to diagnose pathoses specific to one of the major salivary
      glands.
   c. Biomarkers in whole saliva may be used to diagnose or to identify patients at risk of
      several oral and systemic conditions.
   d. All of the above.

3. **All of the following statements related to dental caries are correct EXCEPT which one?**
   a. Dental caries affects about two billion people worldwide and it is most prevalent in China.
   b. It is multifactorial, involving a combination of environmental and biological factors.
   c. Oral bacteria found in dental plaque release different enzymes (e.g., aminopeptidases,
      dipeptidyl peptidases, elastase etc.), which results in demineralization of susceptible dental
      hard tissues.
   d. Point-of-care salivary diagnostics can be used for caries-risk assessment.

4. **Which of the following are point-of-care salivary diagnostics based on microbial
   biomarkers available for the detection of increased caries risk or caries activity?**
   a. Dentocult Strip Mutans (Orion Diagnostica, Espoo, Finland).
   b. Ivoclar CRT (Ivoclar Vivadent, Amherst, NY).
   d. All of the above.

5. **Which of the following statements related to periodontal diseases is correct?**
   a. Periodontal diseases affect about 11% of the general global population.
   b. Periodontal diseases are inflammatory conditions affecting both soft and hard structures
      supporting the teeth and are characterized by bleeding, swelling, increased pocket depth,
      and bone loss.
   c. *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* and *Aggregatibacter
      actinomycetemcomitans* are considered the main pathogens involved in periodontitis.
   d. All of the above.
6. Which of the following statements is correct with respect to currently available point-of-care salivary diagnostics?
   a. MyPerioID® uses polymerase chain reaction (PCR) technology to help identify persons with genetic susceptibility to periodontal diseases.
   b. MyPerioPath® helps to identify the type and concentration of specific bacteria that are known to cause periodontal diseases.
   c. Electronic Taste Chips, a programmable Bio-Nano-Chip (p-BNC) technology, may be used to identify salivary biomarkers specific for periodontal disease.
   d. All of the above.

7. All of the following statements related to premalignant and malignant lesions are correct EXCEPT which one?
   a. Currently available diagnostic technologies such as ViziLite®, OralCDx® Brush Biopsy, and VELscope® have their limitations.
   b. Biopsy, the gold standard for diagnosing premalignant and malignant lesions, is predicated on visual detection and is invasive.
   c. The initial stages of pre-cancerous and cancerous lesion are almost always symptomatic and, therefore, they are easily undetected.
   d. Precancerous lesions and conditions include erythroplakia, leukoplakia, candidiasis, lichen planus, actinic cheilosis, and submucous fibrosis.

8. Which of the following statements related to salivary biomarkers for diagnosing premalignant and malignant oral lesions is correct?
   a. Potential saliva-based biomarkers for pre-malignant and malignant lesions include proteins, nucleic acids, ions, genomic and proteomic targets such as enzymes, growth factors, cytokines, metalloproteinases, endothelin, cytokeratin, mRNA, DNA aberrations, and telomerase.
   b. The only currently available point-of-care technology to identify patients at risk of oral cancer is OralRisk® HPV (OralDNA® Labs, Eden Prairie, MN), which identifies the DNA for oral human papilloma viruses, several strains of which are known to be associated with oral cancer.
   c. MMPs have been studied as possible cancer biomarkers and have been found to be linked to tumor invasion and metastasis.
   d. All of the above.

9. All of the following statements related to temporomandibular muscle and joint disorders (TMD) are correct EXCEPT which one?
   a. TMD affects about 10 million people in the United States.
   b. Salivary cortisol levels are significantly altered.
   c. Several point-of-care saliva-based tests are available to diagnose TMD.
   d. Salivary melatonin levels are significantly altered.

10. Which of the following statements related to saliva-based drug detection is correct?
    a. Salivary levels of cannabinoids, opioids, cocaine, nicotine, benzodiazepines, barbiturates, lysergic acid, diethylamide, phencyclidine, and amphetamines can be measured or monitored.
    b. Available point-of-care diagnostics include MultiDrug Screen test kit®, QuickScreen®, and iScreen® (BioCheck), and QuickScreen®.
    c. Point-of-care diagnostics to detect cotinine in smoking subjects, and methamphetamine and cocaine are not available.
    d. All of the above.
11. Which of the following statements related to saliva-based detection of infectious diseases is correct?
   a. There are more than 1000 nonpathogenic and pathogenic organisms in the oral cavity.
   b. For several oral pathogenic organisms such as *H. pylori*, *M. tuberculosis*, *B. burgdoferi*,
      *Shigella*, *T. solium*, and the human immunodeficiency virus (HIV) salivary biomarkers have
      been identified.
   c. Only OraQuick Advance® Rapid HIV-1/2 Antibody Test (Orasure Diagnostics. Bethlehem, PA),
      which tests for HIV-specific antibodies, is available for point-of-care use.
   d. All of the above.
References


About the Author

Balwant Rai, BDS, MS

Assoc. Professor (Dr.) Balwant Rai is founder of Aeronautic Dentistry and has written the curriculum and guidelines for the implementation of this new discipline. He is Program Director and Associate Professor of Aeronautic Dentistry. He is also the President and Founder of the JBR Institute of Health Education Research and Technology. Dr. Rai has multiple published articles in international and national journals, has written books, and is Editor-in-Chief of different international journals. He is also founder of the BR formula and BR regression equation used in forensic technology including forensic odontology. He wrote a book (Evidence based Forensic Odontology). His current work involves the effect of micro-gravity & simulated space analog environment on the oral cavity, human physiology and psychology and non-invasive biomarkers, including the elaboration of technologies to prevent the adverse effects of microgravity on the human physiology and psychology. His biography has been published in different books such as Who's Who in Health and Medicine and Who's Who in the World, USA, etc. He is invited Editor of Mars Quarterly. He is an invited reviewer to NRF, South Africa, reviewer of more than 10 different journals, and has different pending patents. He is an invited reviewer of many national and international indexed journals. He is consultant of different companies. He was selected as part of Crew 78 on the Mars Desert Research Station (MDRS) as Health and Safety Officer and appointed as Commander for 100 B crew on MDRS. He is principal investigator cum researcher on a project entitled “simulated space mission and human factors including oral cavity: non-invasive technology and herbal formulation.” Dr Rai is working on a number of space related research projects and is jury member for different space related programs. He has a strong belief in leaving a mark on space programs using non-invasive diagnostic technologies and herbal formulations. He is also a strong believer in leaving a mark on forensic odontology using novel and smart technologies.

Email: raibalwant29@gmail.com