WHITE PAPER

IMPROVING EARLY DETECTION OF ORAL CANCER WITH SALIVARY DIAGNOSTICS

VIGILANT BIOSCIENCES™
Fighting Cancer Every Step of the Way
BACKGROUND OF ORAL CANCER

Oral squamous cell carcinoma (OSCC) is a debilitating and deadly disease where both the treatment and the disease itself can often result in disfigurement and the impairment of speech and eating function when it does not otherwise result in death. This devastating type of cancer develops from the mucosal linings of the upper aerodigestive tract (UADT) comprising the nasal cavity and paranasal sinuses, the nasopharynx, the hypopharynx, trachea, oral cavity and oropharynx with areas at highest risk for developing cancer being the floor of the mouth, the lateroventral tongue and the soft palate. OSCC is the most frequent malignant tumor of the head and neck region. It is also an aggressive tumor with low response to chemotherapy and basic resistance to most standard of care anticancer drugs.

OSCC represents more than ninety percent of all head and neck cancers with a varied list of risk factors. For example, gender has a role with males having this type of cancer twice as often as females. Additionally, males in certain ethnic groups, such as African-Americans, have a higher incidence of oral and oropharyngeal cancers than do their Caucasian counterparts. Traditionally, tobacco products, in particular smokeless tobacco products, are known to be a primary cause of this disease. Excessive alcohol use is also considered a high risk factor, especially when combined with tobacco products since the two act synergistically. Recently, however, the human papilloma virus (HPV) infection commonly associated with sexual activity is now shown to be responsible for the rising proportion of OSCC cases independent of race, gender, tobacco use, alcohol consumption, or other risk factors. Many strains of HPV infection associated with oral cancer also overlap with the same strains associated with cervical cancer. Worldwide, HPV-16 prevalence accounts for 40.6% of oropharyngeal squamous cell carcinomas, 14.9% of oral cavity squamous cell carcinomas and 13.4% of laryngeal squamous cell carcinomas. HPV-18 accounts worldwide for 5.9% of oral cavity squamous cell carcinomas, 0.7% oropharyngeal squamous cell carcinomas and 1.6% of laryngeal squamous cell carcinomas. It is currently unclear if HPV alone is sufficient to cause oropharyngeal cancer, or if other risk factors (listed above) interact with HPV to cause these cancers. In addition to the risk factors mentioned, others include the Epstein-Barr virus, gastroesophageal reflux disease (GERD), and exposure to paint fumes, plastic by-products, wood dust, asbestos and gasoline fumes.
THE ORAL CANCER PROBLEM

According to the Oral Cancer Foundation, “close to 43,250 Americans will be diagnosed with oral or pharyngeal cancer this year,” resulting in over 8,000 deaths, equating to the killing of roughly 1 person per hour each day. Of those 43,250 newly diagnosed individuals, only 57% will be alive in 5 years. This number has not significantly improved in decades. When the definition of oral and oropharyngeal cancers is expanded to include cancer of the larynx, the numbers of diagnosed cases grow to approximately 54,000 individuals, and 13,500 deaths per year in the U.S. alone. Worldwide, the problem is much greater with over 600,000 new cases being found each year and 324,794 deaths from head and neck cancer (excluding nasopharyngeal cancer) each year. Survival rates 5 years from diagnosis had been stagnant for many decades at about 50%. Although the current 57% survival rate is an improvement over the last ten years, this improvement is due to the increase of HPV16 caused cancers which are more vulnerable to existing treatment modalities and can confer a significant survival advantage. Therefore, it is a change in etiology and not improved early discovery or treatments that are the sole cause for improvement. Additionally, certain ethnic groups also have poorer outcome with survival. For example, African-American males have mortality rates that are twice as high with a five-year survival rate being only 39.5%. With this understanding, the severity of this disease becomes even more glaring.

Studies also show that there is a 16-36% chance of oral cancer reoccurrence in addition to the probability of developing subsequent cancers elsewhere in the body due to metastasis. Metastasis occurs most commonly via the bloodstream or lymphatic system. Cancer cells once detached from the tumor can have access via these routes to every portion of the body. Oral cancer research has shown that saliva provides a good environment for metastasis. Recurrence of these cancers due to this metastasis factor is unfortunately very real, and continued follow-up care is vital to be sure that secondary cancers do not develop.

THE “GOLD STANDARD” FOR ORAL CANCER SCREENING

Oral cancer screening by definition is the process by which a practitioner evaluates an asymptomatic patient to determine if he or she is likely or unlikely to have a potentially malignant or malignant lesion. An important component of this screening is a thorough review of the patients’ health history.

Currently, the gold standard for screening is visual and tactile palpation during an extra and intra oral inspection by the health care professional during routine examination. This head and neck examination entails bimanual palpation of various external areas of: 1) the head and neck including the lower jaw, neck, glands and lymph nodes of this area, and 2) the oral cavity including the tongue, cheeks, floor and roof of the mouth, lips, back of the throat. During this examination, the frontline screeners traditionally look for clinical features of oral lesions that might raise suspicion of potential malignancy include sharp or distinct margins, a red component (color variation), a non-homogenous white component (surface irregularity), persistent ulceration and size larger than 1 centimeter. The clinician also should view with suspicion any persistent or progressive lesion of the ventrolateral tongue or the floor of the mouth (both of which are high-risk sites for oral squamous cell carcinoma). If these types of areas are present, the diagnostic gold standard of oral cancer diagnosis follows which requires the histopathological examination of surgical biopsy specimens.
When oral cancer is identified in Stage I or Stage II, before the cancer cells have been able to break through the basement membrane, the overall five-year survival rate is over 80%. All too often, however, the manifestations of this invasive and devastating disease are found in the late stage III or stage IV periods where the five-year survival rate falls to under 20%. Unfortunately, over 60% of oral cancer patients in the United States are identified with the advanced stage of disease.

The stages used to describe cancer of the lip and oral cavity

STAGE I
The cancer is less than 2 centimeters in size (about 1 inch), and has not spread to lymph nodes in the area (lymph nodes are small almond shaped structures that are found throughout the body which produce and store infection-fighting cells).

STAGE II
The cancer is more than 2 centimeters in size, but less than 4 centimeters (less than 2 inches), and has not spread to lymph nodes in the area.

STAGE III
Any of the following may be true: 1) The cancer is more than 4 centimeters in size, 2) The cancer is any size but has spread to only one lymph node on the same side of the neck as the cancer, or 3) The lymph node that contains cancer measures no more than 3 centimeters (just over one inch).

STAGE IV
Any of the following may be true: 1) The cancer has spread to tissues around the lip and oral cavity, 2) The lymph nodes in the area may or may not contain cancer, 3) The cancer is any size and has spread to more than one lymph node on the same side of the neck as the cancer, to lymph nodes on one or both sides of the neck, or to any lymph node that measures more than 6 centimeters (over 2 inches), or 4) The cancer has spread to other parts of the body.

RECURRENT
Recurrent disease means that the cancer has come back (recurred) after it has been treated. It may come back in the lip and oral cavity or in another part of the body.

Early-stage lesions unfortunately are often asymptomatic and may mimic other conditions, whereas other lesions may not be readily evident in routine examination. Also, because malignant and benign lesions may not be clinically distinguishable, the clinician cannot predict the biological relevance of lesions on the basis of their physical features alone. Our ability to identify this disease in its earliest stages with this screening modality therefore is not easy and has often eluded the medical and dental professions. The reason must be reiterated. Early oral cancers and precancerous lesions are often subtle and asymptomatic. This phenomenon is often labeled as occult or hidden from plain view, and although the tissue may appear normal it often hides the truth within the cells below the surface of the mucosa.
When oral cancer lesions are easily and visually identified and biopsied, they have likely progressed into Stage III or IV and have advanced so deeply that treatment requires radical surgical intervention and significant loss of the quality of life through impaired speech and eating function as well as disfigurement from the resulting surgery. Certainly by this stage, we have missed the opportunity attending to our patients at an earlier intervention when the five-year survival rates are best.

Thus, there is a need to identify these occult lesions as early as possible and reduce the need for aggressive treatment and the ramifications that ensue. Efforts over the last decade or so have been made to enable medical and dental clinicians to visualize early lesions using variety of techniques through adjunctive screening aids. However, we must understand these visualization modalities are not sensitive and specific (and therefore, not as accurate) for effective screening, diagnosis, or otherwise risk assessment of any type of abnormal lesion where only a definitive test can determine the biologic behavior of a lesion.31 While there are a variety of adjunctive screening products available, it remains unclear as to whether they improve early detection. To date, there have been no acceptable early detection, screening, or risk assessment tests for oral cancer.32 Many of the tests are very expensive and priced where payors (insurance and patients alike) are reluctant to pay for such a test particularly without demonstrated clinical utility. As a result, high costs to normal clinic practice implementing these adjuncts have served as an impediment for readily adoption of these types of adjuncts into the standard of care.

ANSWERING THE UNMET NEED

Dentistry has long been charged by groups like the US Preventive Services Task Force (USPSTF) to be at the forefront of oral cancer detection.33 Interestingly, the USPSTF recently concluded “the current evidence is insufficient to assess the balance of benefits and harms of screening for oral cancer in asymptomatic adults.” However, one would argue that it is because of the nature of the screening mechanisms available to date that led the USPSTF to this decision.

In 2004, National Institute of Dental and Craniofacial Research (NIDCR) funding encouraged the research community to comprehensively decipher, catalogue, and identify human salivary proteomes (i.e. proteins).34 This work has uncovered well over 1,100 proteins in human saliva. These proteins possess clues as to where and why they are being produced and hold the potential to help unlock underlying disease mechanisms, which are developed both locally in the oral cavity and elsewhere in the body. Saliva samples may provide the opportunity to solve the oral cancer challenge for earlier intervention.

Independent of the NIDCR initiative, however, researchers at the University of Miami (UM) had already undertaken studying key salivary protein biomarkers. Their initial discovery lead to a landmark publication that revealed a family of proteins (CD44) expressed from OSCC and thought to promote tumorigenesis.35 Based on this seminal work, further research was conducted over a period of 15 years. During this time, studies showed salivary solCD44 collected in 5mL of an oral rinse could effectively be used to reveal OSCC at all stages.36 Additionally, solCD44 was shown to be elevated in a majority of OSCC cases, and with different measures of elevated total protein, solCD44 could distinguish cancer from benign disease with high accuracy using traditional lab assays and equipment and thereby supporting an effective risk assessment test.37 Further studies demonstrated these protein marker levels were elevated regardless of the tumor size or stage, and thereby indicating these markers to be present early in carcinogenesis.38 They also discovered that that subjects with high solCD44 and protein levels in the oral rinse ELISA assay showed varying quantifiable risk levels including certain categories revealing a 25 times more likelihood of a patient to have cancer than those without these elevated levels.39

Patients with elevated solCD44 and protein levels are 25x more likely to have cancer than those without.
The data culminated from these and further ongoing studies revealed that a simple yet elegant oral rinse test can be an accurate, effective, and affordable method of detecting the risk of the onset of oral cancer and hold the promise to forever change the way clinicians identify and manage this horrific disease.

ELEVATING THE STANDARD OF CARE

In 2011, Vigilant Biosciences, Inc. ("Vigilant") licensed the intellectual property based on this research from University of Miami to commercialize this technology in the clinic. Since then, Vigilant began development of a two-step system for oral cancer risk assessment using the CD44 and total protein biomarkers. The system comprises of two assays: the OncAlert® POC (a rapid point-of-care oral cancer risk assessment test) kit and the OncAlert® LAB (a laboratory assay) with CE marks expected for both products early 2015 and a 510K FDA submission in late 2015.

The Vigilant OncAlert® system will overcome the unmet market need for an accurate, effective, and affordable early risk assessment process. The OncAlert® POC test comprises of a lateral flow test strip with results that can be read without any capital equipment and that will allow for determination of oral cancer risk detected in its earliest stages in a quick (within 10 minutes) and accurate assessment (95% specificity, 88% sensitivity). The test strip technology will allow the assay to be priced affordably such that the cost of the assay would not be an impediment to readily adoption into an elevated standard of care.

A side-by-side comparison of available test methods demonstrates the benefits of OncAlert Oral Cancer Risk Assessment system.

<table>
<thead>
<tr>
<th>DETECTION TECHNIQUE</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>REFERENCE</th>
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<tr>
<td>UNIVERSITY OF MIAMI CD44/PROTEIN TEST</td>
<td>0.88</td>
<td>0.95</td>
<td>PEREIRA ET AL., 2012, PRELIMINARY DATA</td>
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<td>STANDARD VISUAL / PHYSICAL ORAL EXAM</td>
<td>0.64</td>
<td>0.74</td>
<td>SANKARANARAYANAN ET AL., 2005</td>
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<td>TOLUIDINE (DYE)</td>
<td>0.40</td>
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<td>SU ET AL., 2010</td>
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<td>FLUORESCENCE (VELSCOPE / IDENTAFI / ORALID)</td>
<td>0.50</td>
<td>0.39</td>
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<td>BRUSH TEST (ORALCDX)</td>
<td>0.52</td>
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<td>MRNA (IL-8, IL-1B,SAT, OA21 - PERIRX)</td>
<td>0.45-0.79</td>
<td>0.72-0.77</td>
<td>ELASHOFF D., WONG, DT 2012</td>
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Improving Early Detection of Oral Cancer with Salivary Diagnostics

<table>
<thead>
<tr>
<th>ORAL CANCER RISK ASSESSMENT PRODUCT</th>
<th>FAST</th>
<th>SIMPLE TO USE</th>
<th>QUANTITATIVE</th>
<th>HIGH SPECIFICITY</th>
<th>COST TO CLINIC</th>
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<tr>
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<tr>
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No special training or steep learning curve is associated with the OncAlert™ tests, which will be of great benefit to the clinical offices by providing minimal disruption to the flow of the clinic while yet elevating the standard of care. During a routine dental prophylaxis appointment, OncAlert™ POC test is used after seating the patient and reviewing the patient’s medical history to ascertain whether they are any risk factors present as described earlier, such as smoking and alcohol consumption. The patient is then given the 5 ml of saline and uses it as follows:

**OncAlert™ POC TEST:**

**PROCEDURE:**

- Swish and gargle 5 ml saline in the mouth for 30 seconds.
- Spit into specimen cup.
- Insert test strip into specimen cup.
- Wait 10 minutes and get a colorimetric result indicating presence of CD44 with respect to pre-determined threshold levels for cutoffs and relative concentration of total protein within specimen.
Once the patient has provided the sample, the health care provider performs an external visual and bilateral manual palpation examination and an internal and external visual and bimanual palpation head and neck examination to determine if there are any visible or palpated abnormalities, as follows:

- Lips
- Floor of Mouth
- Roof of Mouth (Hard and Soft Palate)
- Buccal Mucosa
- Tongue (dorsal and ventral surfaces and lateral borders)
- Tonsil areas
- Palpate base of tongue, floor of mouth

The observations of this examination are noted in the patient record.

The test strip outcome is produced within 10 minutes and reveals if the patient is at risk due to an elevated level of the salivary markers for cancer. Together with the observations of the examination and the reading of the OncAlert POC test, the healthcare provider would then discuss the results with the patient during his/her examination. If no risk is indicated, then only annual OncAlert™ testing is needed.

If the test indicates they are at risk, the clinician will discuss this with the patient and prescribe a follow up regimen as outlined below:

**IF THE TEST IS POSITIVE:**

- Lifestyle changes to help reduce and possibly reverse disease progression (smoking cessation, oral hygiene and nutrition improvement).
- Schedule for a second OncAlert™ POC test in one to three months.
  - i. If second OncAlert™ test is negative, routine follow-up (per health care profession protocol) with repeat OncAlert™ POC test annually.
  - ii. If second OncAlert test is positive, refer to appropriate specialist (ENT, oral surgeon, otolaryngologist, oncologist).
    - Specialist can perform additional tests available for further examination.
    - Specialist obtains OncAlert™ LAB assay to assess risk more completely.

Anytime lesion is seen in dental or specialist office, biopsy performed. If lesion is of uncertain significance, OncAlert™ testing may be performed to guide decision-making. All patients encouraged in healthy lifestyle behaviors.

When the OncAlert™ POC test is used and indicates that the risk for the patient is high, then the OncAlert™ LAB assay would be utilized by the clinician to aid in categorizing the risk level for a patient so as to provide a more detailed assessment to determine the appropriate type of intervention for the patient. When used together, the OncAlert products will be the first “system” that will help health care workers with both a qualitative screening test and a quantitative lab test for oral cancer risk assessment.

With the OncAlert system, we are now on the threshold of a remarkable technology and product, which can finally provide the health care community with a useful tool to identify OSCC risk in its early stages and thereby aiding the clinician to provide the patient an earlier intervention in the hopes of successfully overcoming their battle with oral cancer.
Improving Early Detection of Oral Cancer with Salivary Diagnostics


2. ibid.


6. ibid.

7. ibid.

8. ibid.

9. ibid.

10. ibid.


12. ibid.

13. ibid.

14. ibid.

15. ibid.

16. ibid.

17. ibid.

18. ibid.

19. ibid.

20. ibid.

21. ibid.

22. ibid.

23. ibid.

24. ibid.

25. ibid.

26. ibid.

27. ibid.

28. ibid.

29. ibid.

30. ibid.

31. ibid.

32. ibid.

33. ibid.

34. ibid.

35. ibid.

36. ibid.

37. ibid.