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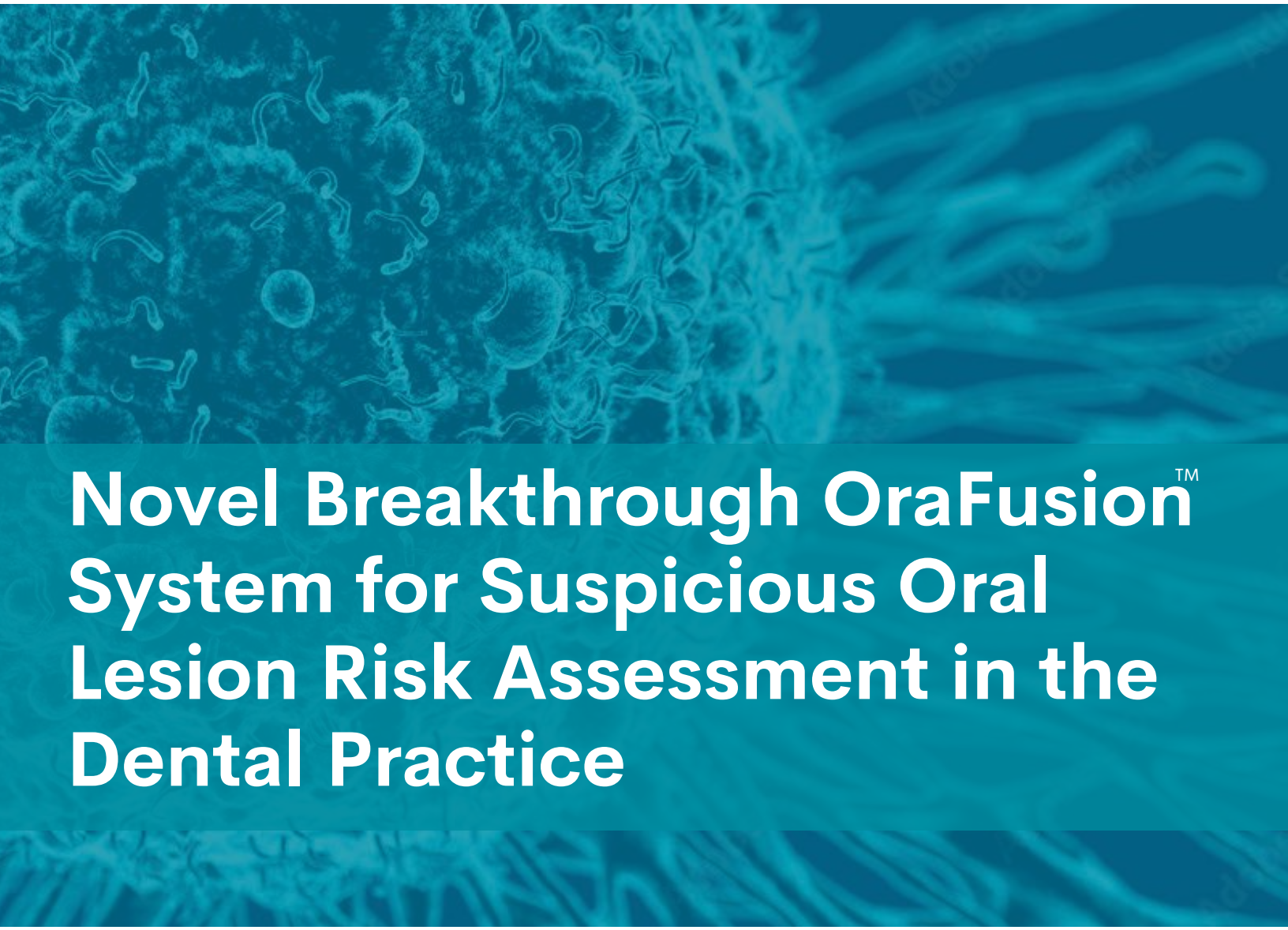
White Paper



Novel Breakthrough OraFusion™ System for Suspicious Oral Lesion Risk Assessment in the Dental Practice

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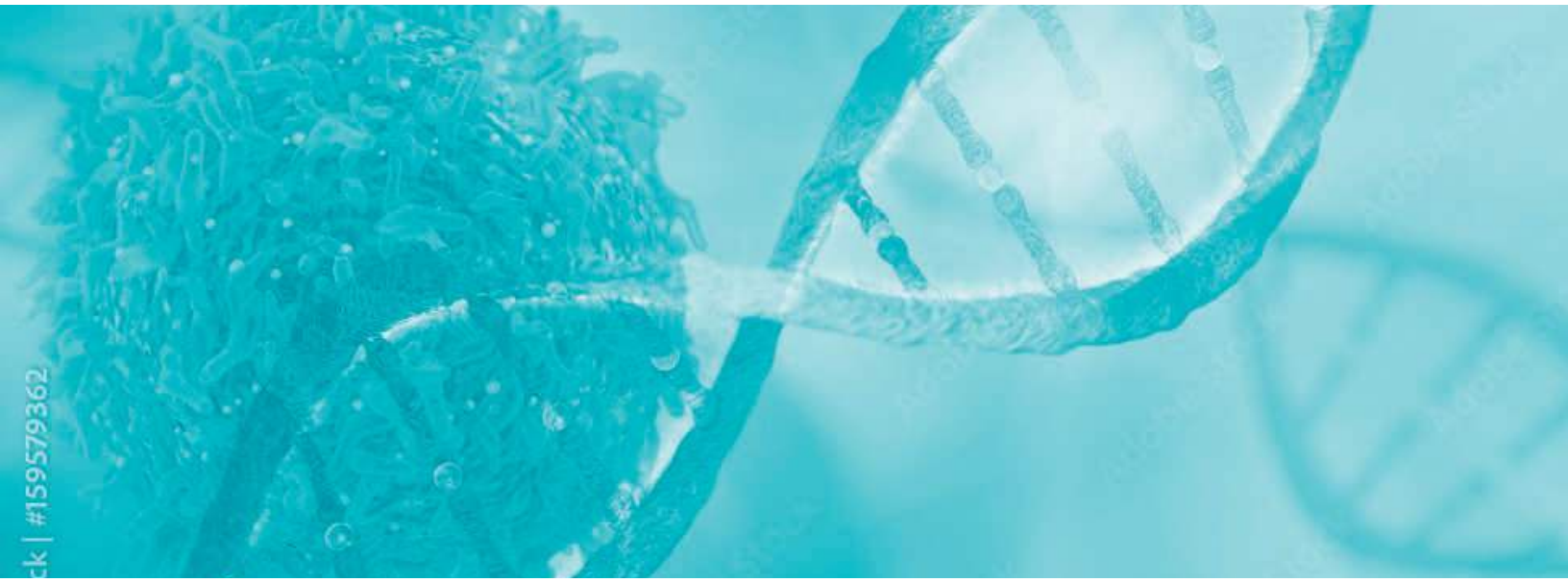


Abstract

Head and neck cancer, including oral cancer, is the sixth most common cancer in humans worldwide. More than 90% of oral cancers are squamous cell carcinomas. Current 5-year survival rates are less than 50% with over 70% diagnosed at stage III or stage IV.

Early detection at stage I and stage II improve 5 year survival rates to over 90%. The Vigilant Biosciences' OraFusion System is an adjunctive pre-diagnostic point-of-care (POC), multiplexed Lateral Flow Ora-3D LFD test, with the accompanied BeVigilant™ RAPID Reader device, used in healthcare setting (e.g., dental office) for a semi-quantitative oral cancer risk assessment.

Populations at increased risk for oral cancer would benefit from low-cost, easy-to-use medical devices enabling early diagnosis and improved outcomes. Conventional visual examinations achieve sensitivities of approximately 60%, with specificity over 98.5%, but are not routinely conducted and require visible lesions that persist over time, resulting in a 'watch and wait' approach potentially delaying a diagnosis.

Vigilant Biosciences developed the OraFusion System to provide semi-quantitative oral disease risk assessment to improve the evaluation of the oral cavity/ visible oropharynx (OCVO).

Introduction

The OraFusion System is intended to be used by qualified healthcare providers as a pre-diagnostic test on asymptomatic adult patients, with oral mucosal abnormalities visible during the oral cancer exam and risk assessment.

The OraFusion System is a point-of-care fluoroscopic and visual saliva biomarker-based semi-quantitative risk assessment tool intended as an adjunct to the standard of care (SOC) OCVO examination including mucosal abnormalities, and gross-appearing premalignant lesions, without the use of a tissue biopsy or cytologic procedure.

Vigilant Biosciences' OraFusion System is not intended as a substitute for standard medical care. Clinical judgment by an experienced clinician is required to evaluate and interpret the Vigilant Biosciences' OraFusion System and recommend next treatment steps.

Current Approach

Early detection and evaluation of the potential risk of malignant transformation for OPMD (oral potentially malignant disorders) is crucial for early diagnosis and subsequent intervention. OPMD is a heterogeneous group of oral mucosal lesions associated with an increased risk of malignant transformation (which includes dysplasia and cancer) and crucial for clinicians to identify, monitor and treat in order to improve survival rates.

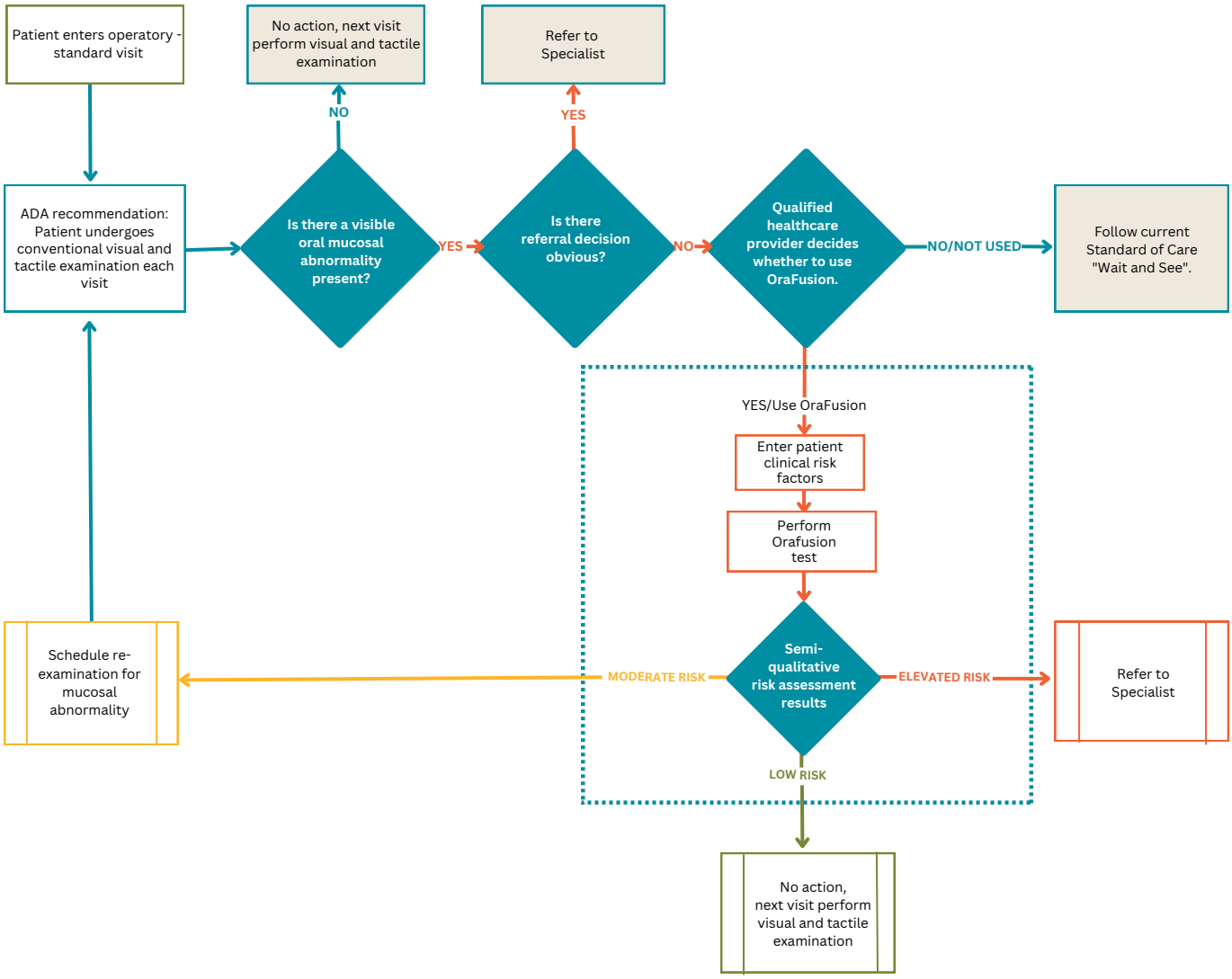
At present, a visual oral examination (VOE) with palpation is the routine screening method used to identify oral mucosal lesions. However, VOE depends heavily on the experience of the health care provider, owing to the fact that several OPMDs, such as white or red lesions (leukoplakia, erythroplakia), oral lichen planus, etc. and persistent palatal ulcers of reverse smoking, are often indistinguishable at its clinical presentation *(Mehrotra, R.; Gupta, D.K. Exciting new advances in oral cancer diagnosis: Avenues to early detection, Head Neck Oncol. 2011, 3, 33).*

Novel Approach

To enrich the current VOE with a point-of-care (POC) diagnostic requires that the test needs to be easy to administer, interpret, and miniaturized, to reduce the overall cost of materials, equipment, and personnel. Using saliva as the source material allows healthcare providers to perform both the test and a chairside exam. Of note, blood-based tests are not typically used by dentists in the dental office. Vigilant Biosciences' OraFusion System is developed to provide substantially improved pre-diagnostic capabilities to aid the VOE and other visual tools. Modified standard of care clinical use diagram which includes the OraFusion system is shown in (Figure 1).

Figure 1

Current standard of care with incorporation of the OraFusion Platform



The OraFusion platform utilizes a lateral flow multiplexed biomarker cartridge with a first-of-its- kind, digital semi-quantitative reader to provide a risk category of low, moderate or high to discriminate benign OCVO lesions from high grade dysplasia (including OPMD) and oral cancer. The assay measures the protein levels of two very well characterized, biologically independent biomarkers in the development and evolution of oral cancer: EGFR and p16. (*e.g. jawert et al., 2022; Devaraja et al., 2020*).

These two proteins are the foundation of the salivary lateral flow test platform developed by Vigilant Biosciences Inc. The test is performed and analyzed in a matter of minutes by Vigilant Biosciences BeVigilant™ RAPID Reader using the reader's sensor fusion algorithm. This would provide an integrated -semi-quantitative cancer risk assessment profile (Figure 2) during the visit in healthcare provider's office.

OraFusion System Risk Assessment Semi-Quantitative Result

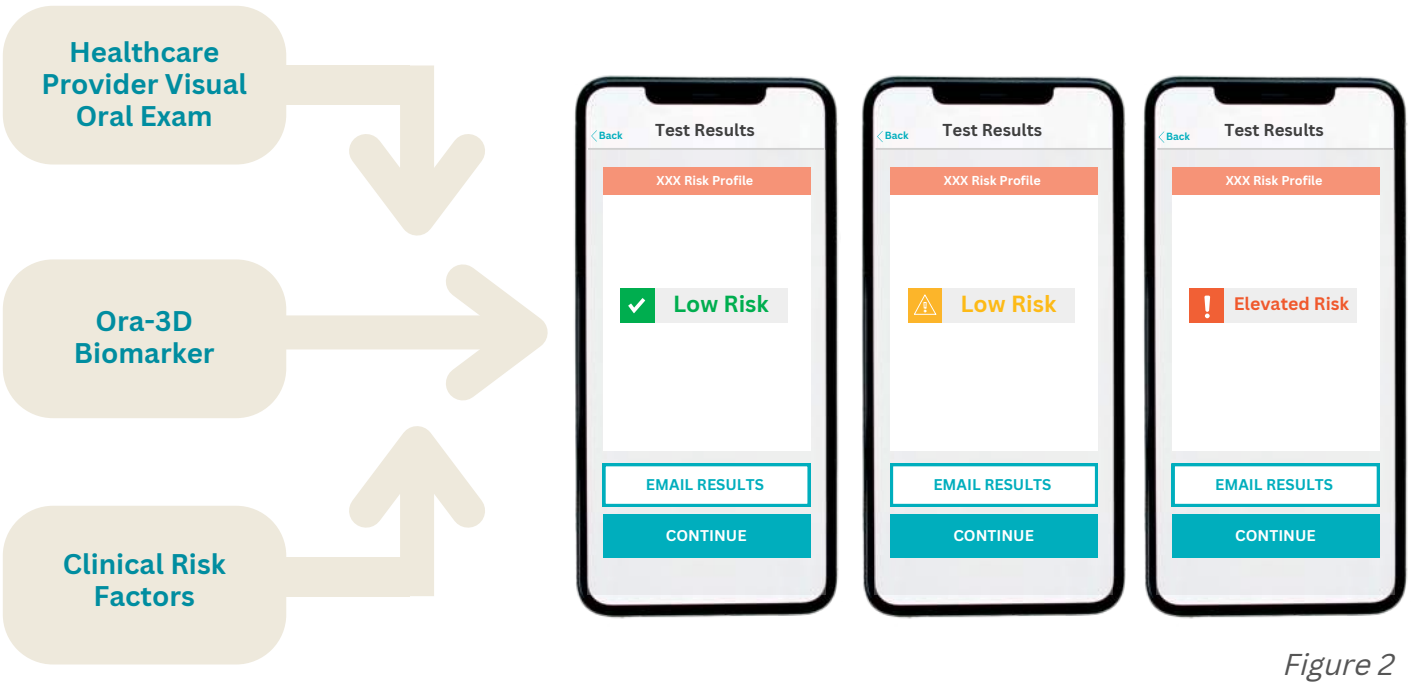


Figure 2

The OraFusion System is a multiplex lateral flow platform which (semi-quantitatively) measures two specific proteins in a single saliva sample. The values are differentially combined with clinical features (e.g. Age (yrs), sex (M/F), race (W/B/O), tobacco use, (Y/N), alcohol use (Y/N)) using an algorithm which provides a patient specific category of risk for high grade dysplasia or cancer.

The results are based on the collective inputs of the following features:

- Visual oral examination (VOE) by a healthcare provider
- An integrated risk assessment based on sensor fusion results (Ora-3D)
- Clinical risk factors (e.g., age, tobacco, alcohol, gender, other diagnosed cancers).

Our present specificity and sensitivity verification results are in the mid-90% range for both, using ELISA tests and lateral flow device.

OraFusion System Architecture

The Vigilant Biosciences' OraFusion System is a pre-diagnostic point-of-care (POC), multiplexed Lateral Flow Ora-3D test, with the accompanied BeVigilant™ RAPID Reader device, used in a healthcare setting (e.g., dental office) for a semi-quantitative oral cancer risk assessment. The Ora-3D is composed of Filter – OFCD-129 Porex Saletto™ Oral Fluid Collection Device and Test Cassette Assembly, Ora-3D. The OraFusion System is shown below *(Figure 3).*

Figure 3. OraFusion System

Vigilant Biosciences' Ora-3D is based on fluorescent technology and the sensor fusion of two biomarkers: P16 and EGFR. Combined with clinical risk factors, it is used to semi-quantitatively assess the risks of oral mucosal abnormalities in patients with visible lesions to support the visual examination by the healthcare provider (e.g., dentist). Ultimately, the primary objective is to support the healthcare provider decision regarding referral of the patient to a specialist.

The OraFusion System is designed to be used with small amounts (minimum 500ul (0.5ml) of whole fresh saliva (a complex solution that includes secretions from major and minor salivary glands, oral mucosa cells, microorganisms, and components from the plasma) collected into the Porex Saletto™ Oral Fluid Collection Device (*Figure 4. whole saliva disposable collection device*) in the dental office.

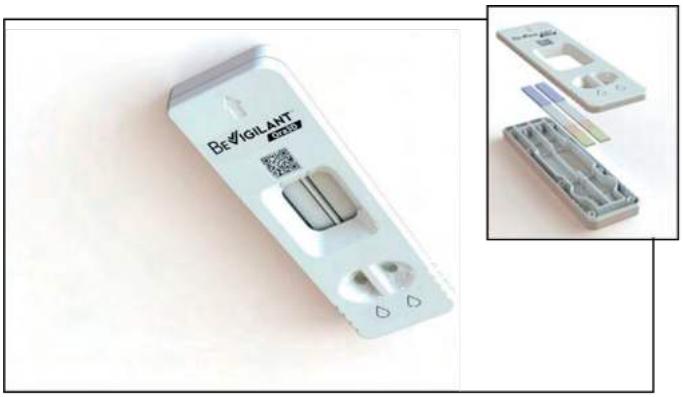
Porex Saletto™ Oral Fluid Collection Device, whole saliva disposable collection device

Figure 4.

After collection of the whole fresh saliva in the Porex Saletto™ Oral Fluid Collection Device, it is filtered and[md1] placed into the lateral flow device immediately *(Figure 5)*.

The lateral flow device is placed into the positioning tray of the BeVigilant™ RAPID Reader (*Figure 6. BeVigilant™ RAPID Reader*), where image processing is performed and

Ora-3D, Lateral Flow Immunoassay



after several minutes, the algorithm provides the semi-quantitative, pre- diagnostic oral cancer risk assessment unitless result (*Figure 2. OraFusion System Risk Assessment Result*). The oral visual exam (OVE) is supported with information from the Ora-3D digital platform based on the semi-quantitative assessment of two (2) biomarkers (which synergistically provide an oral cancer risk classification with performance metrics including specificity, sensitivity, NPV and PPV) combined with the clinical risk factors (age, sex, race, alcohol consumption, tobacco use). It is designed to support the healthcare provider in the decision process for referral to specialist.

Figure 6. BeVigilant™ RAPID Reader

This is the first fully integrated, multiplexed lateral flow device with p16 and EGFR biomarkers developed and manufactured for POC use. The BeVigilant™ RAPID Reader and algorithm are developed for real-time lateral flow response image analysis, clinical factor data entry, risk assessment and dentist visual exam aid.

Materials and Methods

Biomarker Selection for Ora-3D LFD

Early-stage diagnosis is a crucial step in reducing the mortalityrate in oral cancer cases [*Warnakulasuriya, 2009*]. GLOBOCON 2018 estimated 354,864 cases of lip and oral cancer in the world with annual death rate of 177,384 *(Bray et al., 2018*). Approximately 90% of the oral malignancies develop from the lining of the oral cavity or oral mucosa and are classified as oral squamous cell carcinoma (OSCC) *(Neville and Day, 2002)*. OSCC is an aggressive form of oral cancer, usually associated with a poor prognosis. The five-year survival rate of oral cancer patients is approximately 50% and can reach as low as 15%, depending on stage at diagnosis. (*McCullough et al., 2010*). The high rate of mortality is attributed to the delayed diagnosis, in a majority of cases, resulting in delayed treatment.

The biomarkers in the Ora-3D have shown a robust correlation with early detection of oral cancer and are the foundation of the OraFusion System.

Incorporating the use of optical immunosensor biomarker technology combined with fluorescence-based sensors yields a high sensitivity and very good specificity which is critically important when developing a clinical grade assay such as Ora 3D *(Borse et al, 2016b,2017b; Makkar et al., 2018).*

Therefore, our overall strategy is early cancer biomarker detection utilizing fluorescent and visible spectrum-based capability. Using this strategy our combined sensor fusion, sensitivity, specificity and NPV exceeded 90% which is a significant improvement over single detection biomarker assays such as salivary DNA for p16 [Biomarker 1] – Ora-3D

Oral squamous cell carcinoma is a devastating disease with high morbidity and mortality rates. Approximately 20-25%of OSCC are HPV-16 and HPV infection.

Of importance, p16 is a tumor suppressor protein, functioning as a cell cycle inhibitor (*Thanun Sritippho, Pareena Chotjumlong, Anak Iamaroon, 2015*). However, HPV-E7 protein in high- risk HPV subtypes can abrogate function of pRb protein resulting in aberrantly increased expression of p16 in HPV-related OSCC, hence, p16 is widely used as a surrogate biomarker for HPV infection (*Anshita Agarwala, Mala Kambojb, Balasundari Shreedhar, 2019*).

Various studies have concluded that the p16 protein was found immunohistochemically in 74% of HPV characterized tumors. However, low levels of the p16 protein biomarker has limitations based on its intracellular location hence a combination of biomarkers is needed for high specificity and sensitivity in OSCC and other oral lesions *(Alexander Grobe et al, 2013).*

EGFR/ErbB1/HER1 [Biomarker 2] – Ora-3D

Epidermal growth factor receptor (EGFR/ErbB1/HER1) overexpression is found in the majority of oral squamous cell carcinoma (OSCC) tumors, and association has been made between increased expression levels and an aggressive early phenotype, poor prognosis and chemo-resistance *(Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, Fu KK, Milas L., 2016)* Currently the monoclonal antibody cetuximab (Erbitux®) is approved for the treatment of advanced OSCC (*Loeffler-Ragg J, Schwentner I, Sprinzl GM and Zwierzina H)* EGFR inhibition as a therapy for head and neck squamous cell carcinoma. (*Expert Opin Investig Drugs 17: 1517- 1531, 2008*).

Because of the relationship between overexpression of EGFR and aggressive behavior of tumor cells, monoclonal antibodies directed against this receptor might prove to be effective therapeutic and detection agents (*Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J and Sato GH*). Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors *(Mol Biol Med 1: 511-529, 1983)*.

Some previous systematic reviews were published reporting the prevalence of EGFR mutation in patients with non-small cell lung cancer. These previously published works focused on patient subgroups such as smokers, adenocarcinomas or studies only in Chinese population *(Dearden S, Stevens J, Wu Y-L, Blowers D, 2013),* further emphasizing the importance of multiple biomarker combination for improved specificity and sensitivity to detect OSCC.

Novel lateral flow EGFR and P16 custom matched pair antibody development

The foundation of predictable and repeatable results was achieved by the development of custom antibody-antigen matched pairs that Vigilant Biosciences completed with our partner suppliers *(Leinco Technologies Inc, St. Louis, MO, USA)*. More than 20 antibody pairs were developed and tested in saliva. Following are the results of that development.

Antibody pair testing results 14H3.D10

Figure 7 shows a proprietary set of four EGFR candidates in the process of assessing the best candidates for the LFD matched pairs. The y-axis represents the highest optical absorbance of the EGFR and the x-axis represents the concentration from the highest to the lowest value. The optimal candidate shall be as flat as possible, which means that at any concentration yields a similar optical absorbance. In this example is 14H3.D10.

Figure 7. EGFR – Vigilant Biosciences Proprietary Novel Antibody pairs performance

Additional testing results

Figure 8 shows proprietary EGFR1 binding to the antibody. Similarly, C225 is in-vivo gold and it shows that it coats well to the bottom of the plate.

Following this experimental platform ELISA testing was performed with positive (confirmed oral cancer patient saliva) and negative (confirmed negative on oral cancer saliva).

Figure 9 shows confirmation of Level (?limit of detection) of Detection (LOD) using Vigilant proprietary antibody vs. commercially available. It shows favorable sensitivity at very low concentrations relative to the commercial antibody.

Figure 10 shows a proprietary set of six p16 candidates assessing the optimal LFD matched pair performers, similar to the EGFR selection process. The best performing reagents are used for LFD matched pairs, with the highest optical equivalent of the antibody concentration (see 14E8.H3).

Similar to the EGFR best matched pair selection Figure 11 shows the best candidates for p16 matched pairs as well. Based on the data shown, the 14e8.h3 was the best detector and the best conjugate is 5e10.c1 (coating antibody).

Figure 8. EGFR – Vigilant Biosciences EGFR Proprietary matched pairs

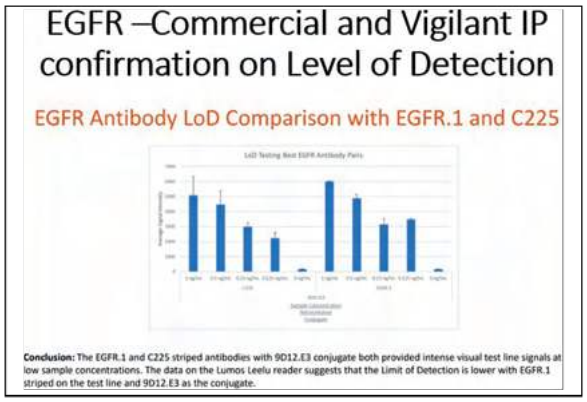


Figure 9 -Level of Detection(LOD), EGFR Vigilant Proprietary vs. Commercial

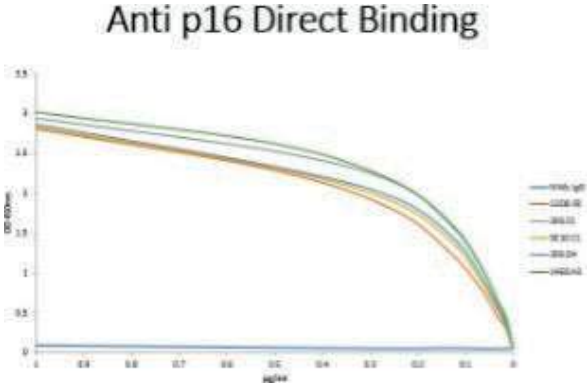


Figure 10 – Vigilant Biosciences Proprietary Novel p16 Antibody pairs performance

Elisa Results – Individual BioMarkers

The objective of the ELISA study was to characterize p16 and EGFR in four different cohorts with whole human saliva samples (positive -confirmed oral cancer, negative - presumably healthy patients, positive controls - confirmed positive from biobank subjects with OSCC , negative controls – confirmed negative post-biopsy biobank subjects with a non-cancer diagnosis) using an ELISA test.

This ELISA study was executed in accordance with PROTO-00025 Protocol *(Vigilant Biosciences internal protocol, "Phase I –ELISA Study Concentration Values")*. The protocol was successfully executed and identified a cut-off estimated sensitivity and specificity for biomarkers p16 and EGFR in whole human saliva.

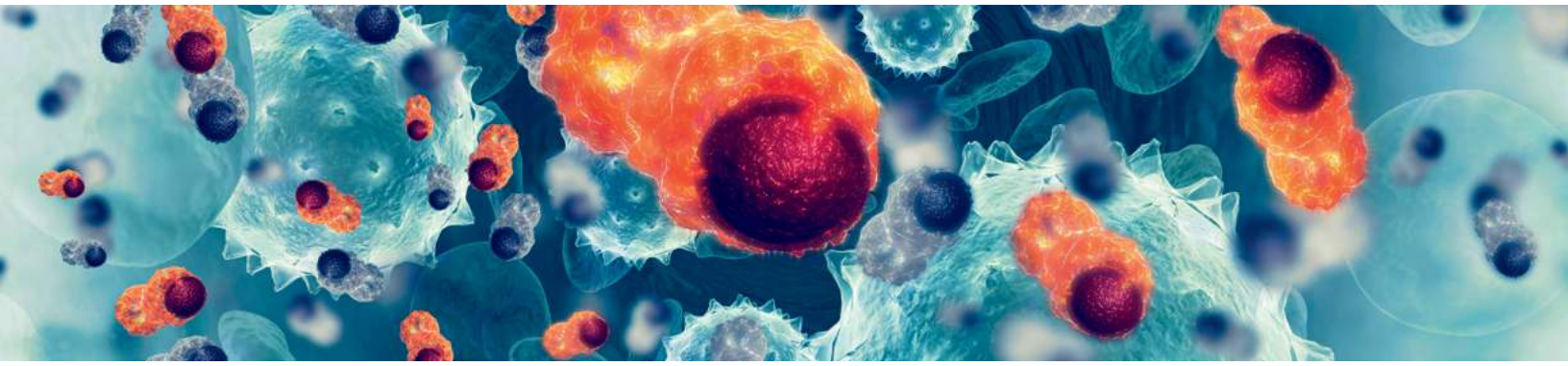
Samples and personnel were blinded in this study. Based on the results of positive and negative samples, a cutoff of 15ng/ml and 0.9ng/ml,respectively, for biomarkers p16 and EGFR, was identified. Estimated sensitivity and specificity were 88% and 92% for p16 and 92% and 100% for EGFR, respectively.

The results provide significant difference between saliva samples for positive and negative oral cancer patients. It also provided the concentration differences related to positive vs. negative OSCC subjects and the negative control samples samples. There was no significant overlap in the concentration range of negative and positive samples as presented in Table 1.

Table 1:
Concentration Values

Biomarker	Negative	Positive	Negative Control	Positive Control
P16-Mean Value	7.7 [ng/ml]	60.9 [ng/ml]	58.9 [ng/ml]	48.5 [ng/ml]
P16-Standard	5.5	42.4	32.1	30.7
Deviation Value				
EGFR-Mean Value	0.2 [ng/ml]	3.6 [ng/ml]	2.6 [ng/ml]	2.4 [ng/ml]
EGFR-Standard Deviation Value	0.2	4.1	1.5	1.5

Table 1. Concentration values for two biomarkers in four saliva cohorts



Based on this analysis the estimated sensitivity for p16 is 88% with a 15 ng/ml cut-off. An estimated sensitivity for EGFR is 92% with a 0.9 ng/ml cut-off. The sensitivity acceptance criteria for each biomarker sensitivity was >85%.

An estimated specificity for p16 is 92% with a 15 ng/ml cut-off and an estimated specificity for EGFR is 98% with an 0.9 ng/ml cut-off. The specificity acceptance criteria for each biomarker was >75%. The null hypothesis was **confirmed** and the results met the requirements for the lateral flow device.

Lateral Flow Results

For the purpose of this study, artificial saliva was selected as a stable substitute material for whole human saliva to continue the feasibility study of the full LFIA strips *(note: Vigilant Biosciences is in the process of performing various retrospective clinical validation studies using actual whole saliva with the University of Maryland School of Dentistry as well as the University of San Luis Potosi School of Dentistry, Mexico)*. Artificial saliva is formulated from known ingredients and is stable when kept refrigerated.

To develop the LFIA strips, artificial saliva was serially spiked with increasing concentrations of antigens to formulate reference solutions.

These reference solutions were used to construct dose-response curves to evaluate the performance of p16 and EGFR LFIA strips.

Previously described experimental studies using ELISA testing were performed to measure the concentrations of endogenous EGFR and P16 biomarkers in whole human saliva. This was necessary given the endogenous concentration of biomarkers.

The analytical performance of Ora 3D LFD to measure different concentrations of the p16 biomarkers was evaluated by building a standard curve with serially increasing concentrations of spiked antigen into artificial saliva. The antigen spiked artificial saliva (0, 0.5, 1, 2.0, 5, 10, 15, 30 ng/ml, n=8) was created from increasing concentrations of p16.

The analytical performance of Ora 3D LFD to measure different concentrations of the EGFR biomarkers was evaluated by building a standard curve with serially increasing concentrations of spiked antigen into artificial saliva.

The antigen spiked artificial saliva (0, 1, 5, 10, 20, 40, 75, and 150 ng/ml, n =8) was created from increasing concentrations of EGFR. The detection capabilities of the EGFR were evaluated in spiked artificial saliva. The limit of blank(LoB) was determined using 20 replicates.

An additional 7 concentrations (with 8 replicates) of spiked EGFR were evaluated to determine the dose response curve and the detectable range of the assay. The detectable range (2 – 30 ng/mL) of EGFR by the LFIA was in a similar physiologically detectable range for non-cancer and cancer patients. The limit of blank was calculated to be 438 Axxin units (commercially available LF reader).

From the LoB, the limit of detection (LoD) concentration was calculated that would yield test line (TL) units that were approximately 2 times the LoB. The LoD of the EGFR LFIA in the artificial saliva was determined to be 2 ng/mL, a concentration that yielded TL signals that were 2 times the LoB, without any replicates having TL signals below the Lob signals. Figure 11 shows the dose-response curve for EGFR constructed from increasing concentrations of antigen spiked artificial saliva.

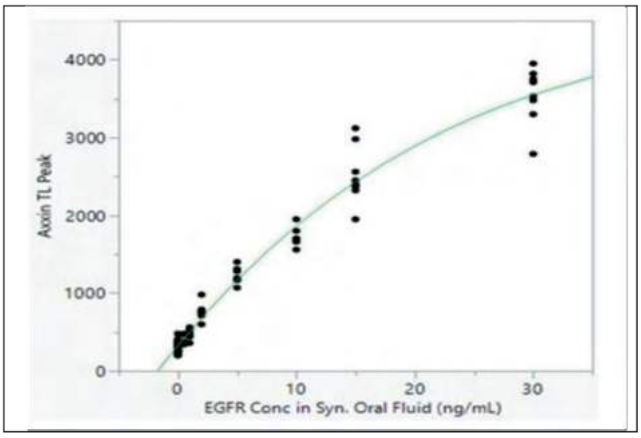


Figure 11. The dose-response for EGFR constructed from increasing concentrations of antigen spiked artificial saliva

Figure 12 is an example of lateral flow level of detection confirmation using Vigilant proprietary EGFR.11 antibodies compared to commercial C225.

This comparison was used for various LFD validation purposes during the development and it is an example of the selection process used during the development. Similar comparisons are done for p16 as well.

The detection capabilities of p16 were evaluated in artificial saliva. The limit of blank (LoB) was determined using 20 replicates. An additional 7 concentrations (8 replicates) of spiked p16 were evaluated to determine the dose response curve and the detectable range of the assay.

The detectable range of the assay was determined to be between 4 - 200 ng/ml. This is well within the physiological range of p16 in non-cancer patients and patients with cancer. The limit of blank was calculated to be 608 Axxin units. From the LoB, the LoD criteria was the concentration that would yield TL Axxin units that were approximately 2 times the LoB. The LoD of the p16 assay in the synthetic oral fluid was determined to be 4 ng/mL.

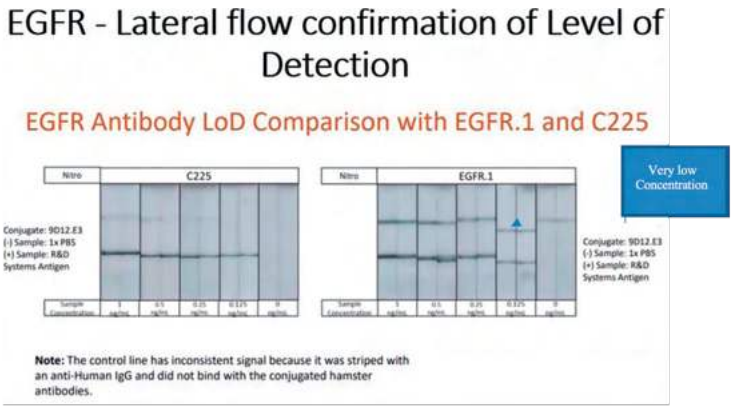


Figure 12. Example - lateral flow confirmation of Level of Detection (the lowest range)

This concentration yielded TL signals that were 2 times the LoB, without any replicates having TL signals below the LoB signals.

Figure 13 shows the dose-response curve for p16 constructed from increasing concentrations of antigen spiked artificial saliva.

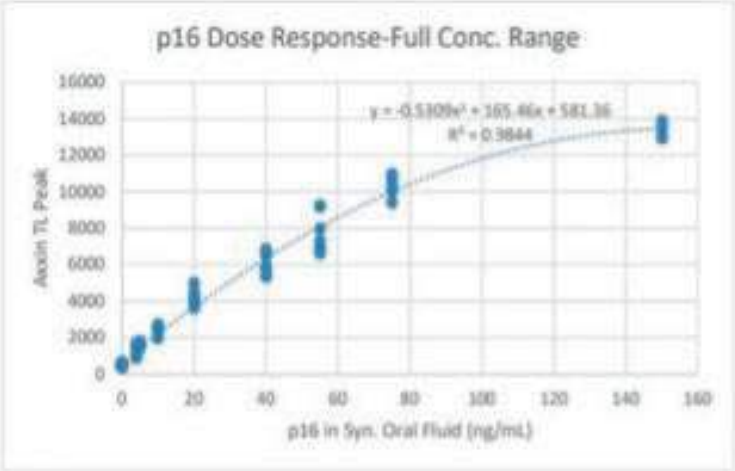


Figure 13. The dose-response for p16 constructed from increasing concentrations of antigen spiked artificial saliva

Biomarker Results Alignment

Based on above top-level findings for Vigilant Ora 3D LFD, it was shown that the device performs well in complex artificial saliva and similarly in pooled real human whole saliva matrix (separate report publication).

Following Tables 2 and 3 show summary results that the specific parameters of both biomarker sets match our results obtained by ELISA in four cohorts of saliva matrix as well as ELISA performed on artificial spiked saliva.

Table 2:
Limit of blank (LoB) for the ELISA (human saliva) and LFIA (artificial saliva)

	Limit of Detection (LoD)		Limit of Blank (LoB)	
Antigen	EGFR	P16	EGFR	P16
Value	Mean (ng/ml)	Mean (ng/ml)	Mean (ng/ml)	Mean (Axxin Units)
LFIA (a)	0.5 (a)	4-5	<0.1 (c)	1.26 (c)
ELISA (b)	0.3	1.3	0.1	0.75

Table 3:
Measuring Ranges for EGFR and p16 LFIA Strips vs. ELISA Values

Format	ELISA (a)		LFIA (b)	
Antigen	EGFR (ng/ml)	P16 (ng/ml)	EGFR (ng/ml)	P16 (ng/ml)
Range	0.3-20	1.3-150	0.5-30	(4-5) - 200 (a)

Semi-Quantitative OraFusion Algorithm

Clinical Factors

The OraFusion algorithm is based on two components:

- Clinical factors
- Sensor Fusion function from two biomarkers in LFD

Figure 14 shows the logic for clinical risk factors (CRF). The clinical factor vector will further evolve while training data set is being expanded to cover additional risk factors (e.g., betel nut, oral sex and etc.).

In addition, the current data set from 630 patients used for CRF model do have HPV status excluded throughout the given distribution

HPV clinical factor assessment will be the subject of further investigation. The present focus is on the visible oral cavity rather than throat and esophagus which would require tools typically possessed only by specialists such as the ENT. Figure 15 shows odds rations with 95% Wald confidence limits for major CRF categories used for this component of OraFusion algorithm (sample size 630 patients).

Self-Quantitative - Algorithm

Clinical Risk Factors - CRF

Model is using logistic regression method:

CRF(x)=A*Age(x)+B*Alcohol(x)+C*Tobacco(x)+D*Gender(x), for Other Cancers (x)=0, x=patient specific data

OR

If Other Cancers (x)=1, CRF(x)=Elevated

For x = 0, data not available sub CRF(x) (each individual component of CRF(X))= max value for given clinical factor

For x = 1, data available sub CRF(x,y) (each individual component of CRF (X,Y))= calculated with training set derived clinical factor number (A,B,C,D)

So model is of the following form:

CRF(x)=log (p/(1-p))

For training data set of 630 patients, the coefficient estimate of CRF relative to pathology outputs (no type of cancer and no stage of cancer data) are shown below:

A=5.54 B=7.0682 C=1.257 D=0.8006

Figure 14. Clinical Risk factors component of the algorithm and its logic

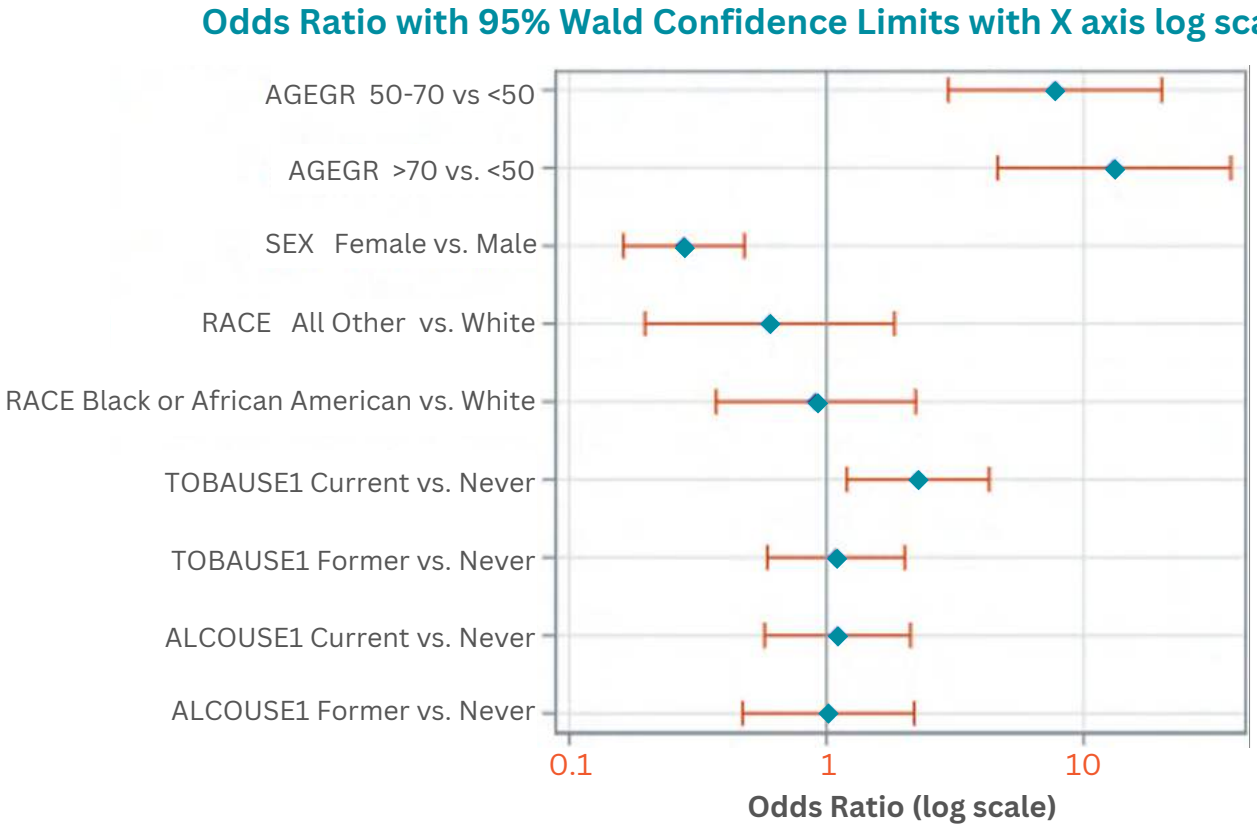


Figure 15. Odds ratios for key clinical risk factor categories (sample size 630 patients)

Biomarker Sensor Fusion

The algorithm sensor fusion component is defined by the two specific biomarkers and corresponding thresholds (LoB, LoD, C5, cutoff, C95 and Hook threshold). Figure 16 is shown the logic behind the two biomarkers (p16 and EGFR) sensor fusion.

Self-Quantitative - Algorithm

Sensor Fusion Function

S(p16,EGFR)=f(p16) (+) g(EGFR)

- f(p16) and g(EGFR) are determined as shown below
- Both biomarkers are independent oral disease biomarkers, so they are equally weighted in S(p16, EGFR)= f(p16)+g(EGFR), since they address different oral diseases from each other

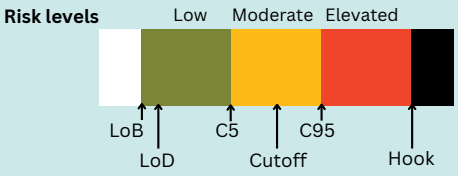


Figure 13. Risk level to biomarker decision points

Table 7 - Results summary

	LoB (ng/mL)	LoD (ng/mL	C5 (ng/mL	Cutoff (ng/mL	C95 (ng/mL	Hook (ng/mL
P16	8.3	10	23.4	41.0	54.7	>110
EGFR	0.75	1.50	1.68	2.5	3.32	>10.5

Figure 16. Sensor fusion component of the algorithm

Finally, Figure 17 shows combined logic between clinical factors as well as biomarker sensor fusion with the ultimate semi-quantitative output.

	CRF			EGFR			AND P16			SF (P16 & EGFR)			OUTPUT	REFERRAL
	LOW	MOD	ELEVATED	LOW	MOD	ELEVATED	LOW	MOD	ELEVATED	L	M	E		
	X			X			X			X			L	N
	X				X		X				X		M	Clinical Decision
	X					X	X					X	E	Y
	X			X				X			X		M	Clinical Decision
	X				X			X			X		M	Clinical Decision
	X					X		X				X	E	Y
	X			X					X			X	E	Y
E	X				X				X			X	E	Y
	X					X			X			X	E	Y
		X		X			X			X			M	Clinical Decision
		X			X		X				X		M	Clinical Decision
		X				X	X					X	E	Y
		X		X				X			X		M	Clinical Decision
		X			X			X			X		M	Clinical Decision
		X				X		X				X	E	Y
		X		X					X			X	E	Y
E		X			X				X			X	E	Y
		X				X	X					X	M	Y
			X	X			X			X			M	Clinical Decision
			X		X		X				X		M	Clinical Decision
			X			X					X		E	Clinical Decision
E			X	X				X			X		E	Y
			X		X			X				X	E	Y
			X			X		X				X	E	Y
			X	X					X			X	E	Y
			X		X				X			X	E	Y
			X			X			X			X	E	Y

H High
M Moderate
L Low

Y Y refer patient
CD Clinician Decision
N N no Referral - Clinician Decisions

Figure 17. OraFusion algorithm semi-quantitative output

Conclusion:

Vigilant Biosciences OraFusion system represents a state-of-the-art platform for semi-quantitative risk assessment and is a novel companion collaborative tool for dentists. It is a platform with the ability to incorporate and process a number of additional biomarkers currently under investigation within Vigilant Biosciences Inc.

A significant advantage of the Vigilant Biosciences OraFusion System over the standard visual oral examination is the introduction of an easy to use, accurate salivary biomarker assay which in combination with clinical features provides a risk of oral cancer or pre-malignant lesions in the oral cavity/visible oropharynx.

Based on preliminary data, sensitivity, specificity and NPV are in the low 90s percent range. When the biomarkers are combined with clinical risk factors accuracy improves to the mid-90s percent range. Large clinical study database results will be published in a follow up paper.

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Changing lives together through the transformation of early detection of oral cancer

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About Vigilant Biosciences:

Vigilant Biosciences is a leading innovator and developer of solutions that aid in the early detection of oral cancer. Our point–of–care solution is simple, accurate† and cost–effective–empowering clinicians to improve potential outcomes through earlier intervention.

Vigilant Biosciences' innovative BeVigilant™ RAPID Test is based on patented technology that detects specific protein markers clinically shown to be associated with early stage cancers.

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