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How to evaluate risk of oral cancer using ONCORAL testing procedure

INSTITUT CLINIDENT
Europole de l'Arbois
Domaine du Petit Arbois
Av Louis Philibert CS 80 465
13 592 Aix en Provence Cedex 3
France

www.institut-clinident.com
info@institut-clinident.com

Oral cancer disease is the consequence of prolonged presence of specific carcinogen molecules from alcohol/tabacco or cell integration of Human Papilloma Virus (HPV) into oral cavity.

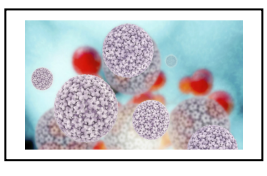
The prevalence of oral cancer is particularly high among men, the eighth most common cancer worldwide. Incidence rates for oral cancer vary in men from 1 to 10 cases per 100 000 population in many countries. In south-central Asia, cancer of the oral cavity ranks among the three most common types of cancer. In India, the age standardized incidence rate of oral cancer is 12.6 per 100 000 population. It is noteworthy that sharp increases in the incidence rates of oral/pharyngeal cancers have been reported for several countries and regions such as Denmark, France, Germany, Scotland, central and eastern Europe and to a lesser extent Australia, Japan, New Zealand and the USA (3, 4). The cancer epidemic in developed countries, and increasingly in developing countries, is due to the combined effect of the ageing of populations, and the high or increasing levels of prevalence of cancer risk factors. The evidence that tobacco causes oral cancer was confirmed by the International Agency for Research on Cancer . At the same time than cigarette smoking declined in developed countries, the number of oral cancer augmented. Human Papilloma Virus (HPV 16 & 18) is an identified new cause.

- ✓ **If detected at an early stage, survival from oral cancer is better than 90% at 5 years, whereas late stage disease survival is only 30%. Therefore, there is an obvious clinical utility for novel metabolic markers that help to diagnose oral cancer at an early stage and to monitor treatment response.**
- ✓ **About 50 to 70% of patients are diagnosed at an advanced stage depending on the countries.**
- ✓ **An increase of oral infected HPV patient has been observed in the past 20 years.**
- ✓ **7% of the US adults are infected**
- ✓ **HPV prevalence was identified in about 16% of tumor specimens collected between 1984 and 1989 versus about 72% of tumor specimens collected between 2000 and 2004, a trend affects younger populations**
- ✓ **It has now been established that the path that brings people to oral cancer contains at least two distinct etiologies; one through tobacco and alcohol and another via the HPV virus infection, particularly version 16 of the virus.**
- ✓ **In general it appears that HPV positive tumors occur most frequently in a younger group of individuals than tobacco related malignancies. (Tobacco oral cancers occur most frequently in the 5th through the 7th decade of life). HPV positive tumor also occur more in white males, and in non smokers.**
- ✓ **Classifying the HPV status of the cancer can offer eligible patients less intensive treatment with reduced side-effects. "get the most appropriate treatment for their cancer".**
- ✓ **HPV-positive oropharyngeal cancers have better outcomes and fewer relapses after treatment than HPV-negative cancers.**

A great number of cases could be early diagnosed according to the use of the biological risk assessment measures to guide clinical decision making.

Assessment tools definition:

- patient history (age, tobacco, alcohol, bad oral hygiene , sexual life, HPV statut)
- clinical examination (oral and noze)
- HPV and volatonomics analysis of saliva
- Biopsy if identified oral lesion



HPV

Human PapillomaVirus (also called HPV) is the most common sexually transmitted infection (STI) in developed countries. Most types of HPV are not harmful to people. There are more than 40 types of HPV that can infect the genital areas as well as the mouth and throat. Most people who become infected with HPV do not know that they are infected. HPV-induced cancers arise when viral sequences are accidentally integrated into the DNA of host cells. Some of the "early genes" carried by the HPV virus, such as genes E6 and E7, act as oncogenes that promote tumor growth and malignant transformation. Furthermore, HPV can induce a tumorigenic process through integration into a host genome which is associated with alterations in DNA copy number

What is oral HPV?
The same types of HPV that infect the genital areas can infect the mouth and throat. HPV found in the mouth and throat is called "oral HPV." Some types of oral HPV (known as "high risk types") can cause cancers of the head and neck area. Other types of oral HPV (known as "low risk types") can cause warts in the mouth or throat. In most cases, HPV infections of all types go away before they cause any health problems.

What head and neck cancers can be caused by HPV?
HPV can cause cancers in the back of the throat, most commonly in the base of the tongue and tonsils, in an area known as the "oropharynx." These cancers are called "oropharyngeal cancers."

What are the signs and symptoms of oropharyngeal cancer?
Signs and symptoms may include persistent sore throat, earaches, hoarseness, enlarged lymph nodes, pain when swallowing, and unexplained weight loss. Some persons have no signs or symptoms.

How common is oral HPV?
Studies in the U.S. have found that about 7% of people have oral HPV. But only 1% of people have the type of oral HPV that is found in oropharyngeal cancers (HPV type 16). Oral HPV is about three times more common in men than in women.

How common are cancers of the oropharynx?
Each year, in the U.S., about 9,000 people are diagnosed with cancers of the oropharynx that may be caused by HPV. Cancers of the oropharynx are about four times more common in men than women.

How do people get oral HPV?
Only a few studies have looked at how people get oral HPV, and some of these studies show conflicting results. Some studies suggest that oral HPV may be passed on during oral sex (from mouth-to-genital or mouth-to-anus contact) or open-mouthed ("French") kissing, others have not. The likelihood of getting HPV from kissing or having oral sex with someone who has HPV is not known. We do know that partners who have been together a long time tend to share genital HPV—meaning they both may have it. More research is needed to understand exactly how people get and give oral HPV infections.

Can the HPV vaccine prevent oral HPV and oropharyngeal cancers?
The HPV vaccine that is now on the market was developed to prevent cervical and other less common genital cancers. It is possible that the HPV vaccine might also prevent oropharyngeal cancers, since the vaccine prevents an initial infection with HPV types that can cause oropharyngeal cancers, but studies have not yet been done to determine if the HPV vaccine will prevent oropharyngeal cancers

What is Volatonomics analysis
Volatile organic compounds (VOCs) are organic chemicals that have a high vapor pressure at ordinary room temperature. Their high vapor pressure results from a low boiling point, which causes large numbers of molecules to evaporate or sublimate from the liquid or solid form of the compound and enter the surrounding air, a trait known as volatility. VOCs are numerous, varied, and ubiquitous. They include both human-made and naturally occurring chemical compounds. Most scents or odors are of VOCs. Analysis of specific VOCs profiling from specific sample (saliva, liquid biopsies, breath) is call Volatonomics. VOCs are detected by Mass Spectrometry analysis.

Oral cancer and Volatonomics
VOC pattern analysis in exhaled breath in patients with head and neck squamous cell carcinoma (HNSCC) have been reported in 2014. Logistic regression showed a significant difference ($P < 0.05$) in VOC resistance patterns between patients diagnosed with HNSCC and the control group, with a sensitivity of 90% and a corresponding specificity of 80%. VOCs are small molecules coming from human cell metabolism.

Oncoral ™
Institut clinident developed a new strategy for oral cancer risk measurement using stabilized saliva for HPV identification and saliva volatonomics analysis. The method and the volatonomics profil of saliva in relation with oral cancer has been patended by the laboratory.

Oral cancer symptoms and signs ?
- ✓ Persistent mouth sore: A sore in the mouth that does not heal is the most common symptom of oral cancer
- ✓ Pain: Persistent mouth pain is another common oral cancer sign
- ✓ A lump or thickening in the cheek
- ✓ A white or red patch on the gums, tongue, tonsil, or lining of the mouth
- ✓ A sore throat or feeling that something is caught in the throat that does not go away
- ✓ Difficulty swallowing or chewing
- ✓ Difficulty moving the jaw or tongue
- ✓ Numbness of the tongue or elsewhere in the mouth

- ✓ Jaw swelling that makes dentures hurt or fit poorly
- ✓ Loosening of the teeth
- ✓ Pain in the teeth or jaw
- ✓ Voice changes
- ✓ A lump in the neck
- ✓ Weight loss
- ✓ Persistent bad breath

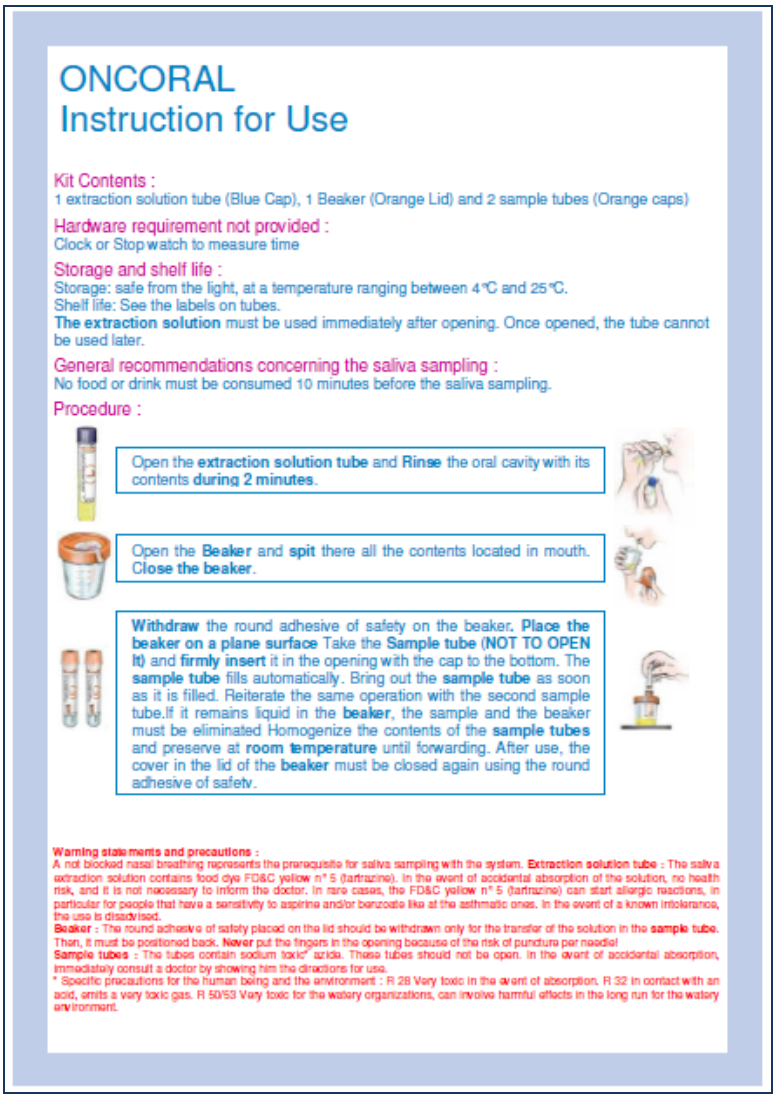
Technical recommendation and Oncoral™ objectives:

It is advised to carry out an analysis in the following situations:

- Patient with any symptoms or signs
- patient that smoke (more than 45 year old)
- patient that dring (more than 45 year old)
- patient with poor oral hygiene
- patient with genital HPV or other HPV infection
- 6 months after the first positive oral saliva test
- every year for all patients at risk

References: scientific publications could be provided to dentists under specific demand by email. Please contact info@institut-clinident.com or the institute clinident webpages (www.institut-clinident.com) and ask for publications.

Oncoral Instruction for use & kit contain : collected stabilized saliva should be send no more than 24H after collection via express mail.



ONCORAL
Instruction for Use

Kit Contents :
1 extraction solution tube (Blue Cap), 1 Beaker (Orange Lid) and 2 sample tubes (Orange caps)

Hardware requirement not provided :
Clock or Stop watch to measure time

Storage and shelf life :
Storage: safe from the light, at a temperature ranging between 4°C and 25°C.
Shelf life: See the labels on tubes.
The extraction solution must be used immediately after opening. Once opened, the tube cannot be used later.

General recommendations concerning the saliva sampling :
No food or drink must be consumed 10 minutes before the saliva sampling.

Procedure :

Open the **extraction solution tube** and **Rinse** the oral cavity with its contents **during 2 minutes**.

Open the **Beaker** and **spit** there all the contents located in mouth. **Close the beaker**.

Withdraw the round adhesive of safety on the beaker. **Place the beaker on a plane surface** Take the **Sample tube (NOT TO OPEN It)** and **firmly insert** it in the opening with the cap to the bottom. The **sample tube** fills automatically. Bring out the **sample tube** as soon as it is filled. Reiterate the same operation with the second sample tube.If it remains liquid in the **beaker**, the sample and the beaker must be eliminated Homogenize the contents of the **sample tubes** and preserve at **room temperature** until forwarding. After use, the cover in the lid of the **beaker** must be closed again using the round adhesive of safety.

Warning statements and precautions :
A not blocked nasal breathing represents the prerequisite for saliva sampling with the system. **Extraction solution tube :** The saliva extraction solution contains food dye FD&C yellow n° 5 (tartrazine). In the event of accidental absorption of the solution, no health risk, and it is not necessary to inform the doctor. In rare cases, the FD&C yellow n° 5 (tartrazine) can start allergic reactions, in particular for people that have a sensitivity to aspirine and/or benzoate like at the asthmatic ones. In the event of a known intolerance, the use is disadvised.
Beaker : The round adhesive of safety placed on the lid should be withdrawn only for the transfer of the solution in the **sample tube.** Then, it must be positioned back. **Never** put the fingers in the opening because of the risk of puncture per needle!
Sample tubes : The tubes contain sodium toxic* azida. These tubes should not be open. In the event of accidental absorption, immediately consult a doctor by showing him the directions for use.
* Specific precautions for the human being and the environment : R 28 Very toxic in the event of absorption. R 32 In contact with an acid, emits a very toxic gas. R 50/53 Very toxic for the watery organizations, can involve harmful effects in the long run for the watery environment.

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