

# The Practicality of Liquid Biopsies in the Diagnosis of Oral Cancer Compared to Other Techniques

Kamya Keerthivasan<sup>1</sup>, Jothsna Kethar<sup>#</sup> and Dr. Rajagopal Appavu, Ph.D.<sup>#</sup>

<sup>1</sup>Pomperaug High School

<sup>#</sup>Advisor

## ABSTRACT

In our current world, oral cancer is a significant health burden and is a relatively common diagnosis with an aggressive nature. Recently, oral cancer has had high mortality and morbidity rates as most cases were diagnosed in the later stages. Like many cancers, it is better to diagnose it early to prevent its growth. However, various diagnosis techniques have been in use and some are more practical than others. Whether it leaves the patient uncomfortable, causes pain, or costs too much, each technique has its disadvantages. With that, various patients have complained about the discomfort, cost, and accessibility, hoping for another technique that would be more effective in most aspects. In search of the practical technique, one in particular, the liquid biopsy, has shown room for growth and positive results in the aggressive oral cancer setting as it analyzes the various biomarkers present in a bodily fluid in a quick manner. As the need for a developing technique is continuously stressed on, this research paper discusses knowledge about the oral cancer detection factors and the liquid biopsy process. Primarily, it also determines whether the technique is a practical choice for the future of the diagnosis and detection of oral cancer.

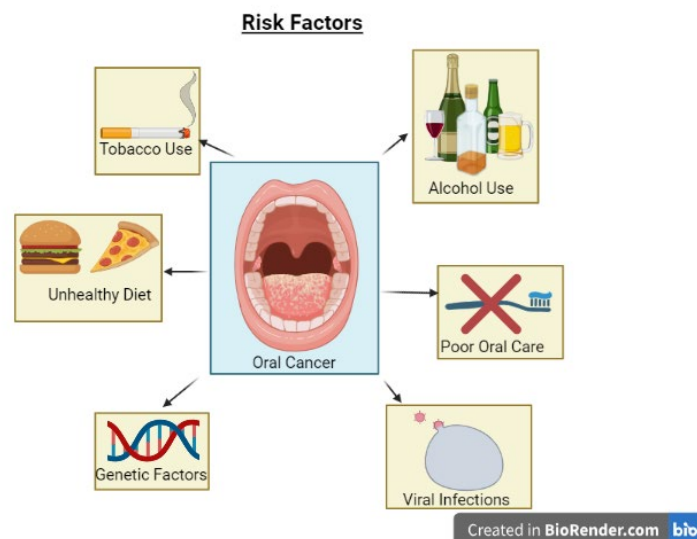
## Introduction

Imagine a scenario where a quick blood test or collection of saliva through a swab or spit tube has the ability to accurately identify and diagnose oral cancer. This would relieve the patients from the inconvenience of intrusive procedures and give physicians and clinicians enough time to deliberate. This vision is what scientists have aimed to reach, but in the past, this was too far-fetched. The golden standard, tissue biopsies, were conducted often to learn about the patient's conditions although they delayed the process and were limited. In the scientific world, however, our scientists have continued to do research, causing the previously imagined scenario to start to become a reality. The ability to diagnose cancer, which used to be painful, has changed as new technologies are researched, such as liquid biopsy. Since this type of biopsy can detect parts of tumors through the study of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and extracellular vesicles (EVs), this approach may be preferred once there is future research and validation. Due to this, it is critical for medical professionals or researchers to comprehend the new approach and its potential applications as the medicine shifts towards a targeted strategy. However, liquid biopsies come with various uncertainties with its ability to diagnose cancer due to its recent discovery. This brings up one question based on the research done on the topic: is the technique of a liquid biopsy for diagnosing oral cancer practical when compared to other common techniques?

## What Is Oral Cancer?

To understand the role of liquid biopsies in the diagnosis of oral cancer, the seriousness of the said disease needs to be discussed. Oral cancer is a form of malignancy that affects tissue within the oral cavity and oropharynx. This disease is recognized as a significant health concern as in 2023, around 54,540 adults in the United States will be

diagnosed with oral cancer, with its aggressive nature and damaging effect (Oral and Oropharyngeal Cancer - Statistics, 2023). Throughout history, researchers and scientists have investigated the risk factors for oral cancer. The most prominent of those being tobacco and alcohol use, faulty oral care, a substandard diet, viral infections, and genetic factors, as analyzed **Figure 1** below (Dhanuthai et al. 2017). Many of the risk factors discovered for oral cancer are ones that the general public do frequently, such as eating healthy and not maintaining a healthy oral microbiome. In order to prevent the development of oral cancer, these risk factors need to be kept in mind by both clinicians and the public.



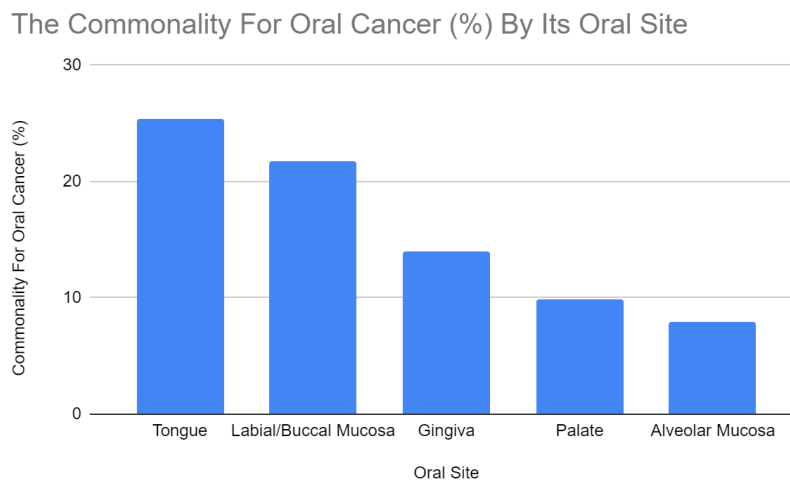
**Figure 1.** Risk Factors of Developing Oral Cancer for The General Public.

As noticed, there are various detrimental risk factors for developing oral cancer and if people continue to endanger their chances by not controlling the factors they can, it may become too late. This is because patients may only realize that they may have oral cancer once they find unusual sores or irritations that don't disappear after 3 weeks, irregular lumps, colored patches (particularly red or white), or rough spots (Mark, 2017). However, there are symptoms that patients may notice, such as unusual ear pain and tenderness or numbness with the throat (Mark, 2017). Letting these symptoms become more severe before seeing the dentist may worsen the condition and prevent an accurate cure.

Nevertheless, cancer is known to develop in various locations throughout the oral cavity and oropharynx as seen in Graph 1 (Dhanuthai et al., 2017). Since oral cancer is common in various anatomical areas, clinicians should not only focus on teeth, but in the oral mucosa, particularly where the data indicates high prevalence, such as the tongue and labial/buccal mucosa.

Oral cancer is known to be deadly as it can prevent one's ability to consume or speak in a normal manner and may result in death if not treated well in advance. Early detection was not common as many clinicians would diagnose oral cancer during the later stages as tissue biopsies took time to provide the necessary information. With that, the oral cancer survival rate hasn't improved significantly over the years as in most countries the 5-year rate is around 68% (Oral and Oropharyngeal Cancer - Statistics, 2023). Due to that, the clinician's ability to prevent the growth of cancer had significantly reduced as valuable time had dissipated.

Since the mouth is known as the pathway to many illnesses, whether it is a simple fever or cancer, treating and preventing oral cancer has become especially difficult. Similarly, with research, various scientists have reasoned that these tissue biopsies have failed to determine the tumor size sufficiently (Patel et al., 2022). Therefore, the alternative is a liquid biopsy, an new and easier technique due to its non-invasiveness/minimal invasiveness, rapidness, and ease of collection.



**Graph 1.** The Commonality for Oral Cancer In Various Oral Sites. Created By Kamyra K (Dhanuthai et al., 2017)

## A Comparison of Liquid Biopsies to The Alternatives

As stated, there are various techniques used by scientists and clinicians to detect oral cancer, some more practical than others. Although liquid biopsies and tissue biopsies are the techniques that are used more often, various common techniques are still used today. All of the following techniques have their advantages and disadvantages, some more prominent than the others. Since oral cancer is a significant illness, affecting 1 in 5 people under the age of 55, it is important to discover easy, quick, and feasible techniques that will provide accurate information to treat those affected (Oral Cavity & Oropharyngeal Cancer Key Statistics 2021, n.d.-b).

Table 1 is a list of common oral cancer detection techniques that are used and analyzed by doctors, scientists, and clinicians for diagnosis and shows their respective advantages and disadvantages.

The techniques of Tissue Biopsies, Visual Examinations, UGI Endoscopies, and Imaging Techniques have disadvantages that outweigh their advantages. With these techniques as they are, there are better alternatives to use for diagnosis.

However, though the technique of a liquid biopsy has both advantages and disadvantages, there is a clear future for the technique. Despite the disadvantages, this technique is developing and with more research and advancements, the technology can lead to a broader acceptance and accuracy in the future (Yang et al. 2022).

The techniques mentioned have various advantages and disadvantages, and when each technique is researched and improved, they will provide a practical technique used in the future to increase the accuracy of oral cancer diagnosis. In this research paper, the focus is on the technique of a liquid biopsy as it is currently new and developing. The discussed aspects of a liquid biopsy aid in the unique process of completing one, offering a non-invasive/minimally invasive and promising approach to diagnose and monitor oral cancer.

**Table 1.** Common Techniques For Detecting Oral Cancer and Their Advantages and Disadvantages

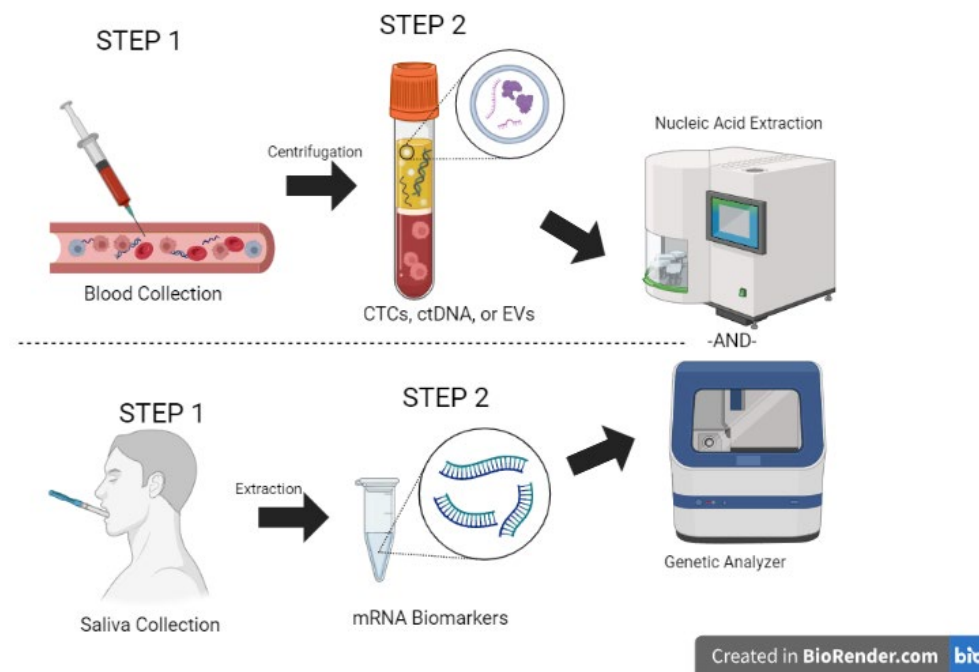
Technique Name	Description	Advantages	Disadvantages
Liquid Biopsy	Bodily fluid (blood, saliva, etc.) is analyzed for specific biomarkers or genetic material that can detect oral cancer (Yang et al. 2022).	<ol style="list-style-type: none"> <li>1. Non-invasive/ Minimally invasive</li> <li>2. Ease in collection</li> <li>3. Potential for early detection</li> <li>4. Ease in tracking changes through repetition (Yang et al. 2022)</li> </ol>	<ol style="list-style-type: none"> <li>1. Developing Technique</li> <li>2. Limited Availability</li> <li>3. Not As Accurate As Other Techniques</li> <li>4. Low Sensitivity (Yang et al. 2022)</li> </ol>
Tissue Biopsy	A small tissue is taken from the suspected area and examined through a microscope to identify the cancerous cells (Yang et al. 2022).	<ol style="list-style-type: none"> <li>1. Fairly Accurate</li> <li>2. Definitive Diagnosis</li> <li>3. Can Provide A Personalized Treatment Plan</li> <li>4. Only A Small Tissue In Necessary (Yang et al. 2022)</li> </ol>	<ol style="list-style-type: none"> <li>1. Invasive</li> <li>2. Causes Bleeding</li> <li>3. Delayed Results</li> <li>4. May Cause Infections (Yang et al. 2022)</li> </ol>
Visual Examination	A simple inspection of the oral cavity/oropharynx for abnormal or suspicious lesions (Walsh et al. 2013).	<ol style="list-style-type: none"> <li>1. Quick</li> <li>2. Cost-effective</li> <li>3. Non-invasive</li> <li>4. Easy once trained (Walsh et al. 2013)</li> </ol>	<ol style="list-style-type: none"> <li>1. Cannot determine malignancy</li> <li>2. May miss early/hidden lesions</li> <li>3. Limited to surface level findings</li> <li>4. False Negatives/Positives (Walsh et al. 2013)</li> </ol>
Upper Gastrointestinal (UGI) Endoscopy	A flexible tube with a camera is inserted in the mouth to examine the throat, vocal chords, and esophagus for abnormalities (Yang et al. 2022).	<ol style="list-style-type: none"> <li>1. Low Risk</li> <li>2. Direct Visualization</li> <li>3. Biopsy Capability</li> <li>4. Early Detection (Yang et al. 2022)</li> </ol>	<ol style="list-style-type: none"> <li>1. Invasive</li> <li>2. Requires Sedation</li> <li>3. Impractical Due to Discomfort</li> <li>4. Limited Scope (Yang et al. 2022)</li> </ol>
Imaging Techniques	Vital Staining, Virtual Staining, Narrow Band Imaging, Optical Coherence Tomography, and High Frequency Ultrasound are used to visualize the head and neck area for tumor detection (Romano et al. 2021).	<ol style="list-style-type: none"> <li>1. Early Detection</li> <li>2. Non-invasive</li> <li>3. Visualization</li> <li>4. Various Information Provided (Romano et al. 2021)</li> </ol>	<ol style="list-style-type: none"> <li>1. Specific Equipment</li> <li>2. Necessary Expertise</li> <li>3. Accessibility</li> <li>4. High Cost (Romano et al. 2021)</li> </ol>

## The Principles of Liquid Biopsy

Although in medicine, tissue biopsies and other techniques such as visual exams, endoscopies, and imaging techniques have been the status quo, it is important to realize that it may be a better alternative- possibly the liquid biopsy. A liquid biopsy is modern and non-invasive/minimally invasive for detecting diseases, especially cancer, using a simple blood test or spit tub/swab of saliva. Doctors can now learn about what’s going on inside our bodies by looking at the miniscule particles floating in our bodily fluids. These particles are biomarkers, and they give relevant information to the doctor about whether a disease is present or not (Lousada-Fernandez et al. 2018).

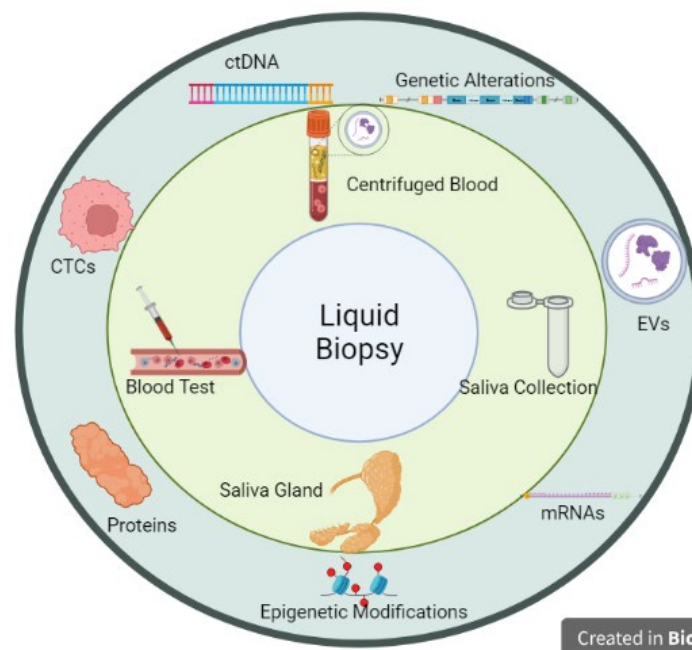
To distinguish between a healthy and cancerous individual, genetic material can tell the difference. In a healthy person’s sample, the genetic material appears stable, and the amount of cell-free DNA (cfDNA) released into the bloodstream is fairly constant. Healthy people may undergo some mutations and alterations but on a generally low level, not dealing with cancer. However, regarding a cancerous individual, when there are abnormal cells or a tumor in our body, they release genetic particles into our bodily fluids (blood, saliva and others). These cancer cells usually undergo genetic modifications that may change the number of copies of certain genes. These changes may release abnormal cfDNA, therefore detecting these mutations or alterations will provide insight as to what cancer a patient might have (Marrugo-Ramírez, et al, 2018).

When blood is drawn, the sample is sent for processing. In the lab, the blood is centrifuged, a process that separates the different components of blood, such as white blood cells, red blood cells, and plasma, as seen in **Figure 2**. The plasma is then sent for further analysis as it contains biomarkers. In plasma, the specific biomarkers associated with oral cancer need to be isolated if they are present. Depending on the target biomarker (CTCs, ctDNA, EVs, etc.), various extraction techniques can be used to isolate or concentrate it. In **Figure 2**, it is apparent that the nucleic acid extractor and genetic analyzer to grasp more information about the blood or saliva sample may be imperative to the diagnosis of oral cancer (Maass et al., 2021).



**Figure 2.** The Process Of A Liquid Biopsy With Various Bodily Fluids. (Maass et al., 2021)

The use of the extractors, such as the nucleic acid extractor and genetic analyzer, provides valuable information to clinicians and scientists studying the disease. Specifically, they provide information about genetic alterations and mutations. Identifying these mutations can help the scientists understand the molecular basis of the disease and may affect the personalized treatment (Marrugo-Ramírez et al. 2018). Similarly, information about the biomarkers are provided, as seen in **Figure 3**. What is meant by that is scientists will be able to analyze RNAs that act as biomarkers for oral cancer. Specific changes in the levels of the biomarkers will provide diagnostic information to the scientists, aiding in early detection (Marrugo Ramirez et al. 2018). Finally, the extractors can aid in providing more information regarding whether there is a viral infection linked to cancer development. In some cases, viral infections have been linked to oral cancer, like Human Papillomavirus (HPV). In this case, the extractors can identify the presence of viral DNA or RNA. This identification can help understand whether the infection associated with cancer aided in the development or progression of the said disease (Dhanuthai et al. 2017).



**Figure 3.** Information Provided from A Liquid Biopsy. (Lone et al. 2022)

The analysis of the samples collected aids scientists as a plethora of information can be discovered about the patient and their possible oral cancer condition. Specifically, the sample will provide information on the patient’s circulating tumor cells (CTCs), proteins, epigenetic modifications, mRNAs, extracellular vesicles (EVs), genetic alterations, circulating tumor DNA, and more, as seen in **Figure 3**. However, there is a possibility for inaccuracy. With the needed research, oral cancer diagnosis will be improved to the point where it will be able to be applied in the healthcare setting.

## The Clinical Application Of Liquid Biopsies For Oral Cancer Detection

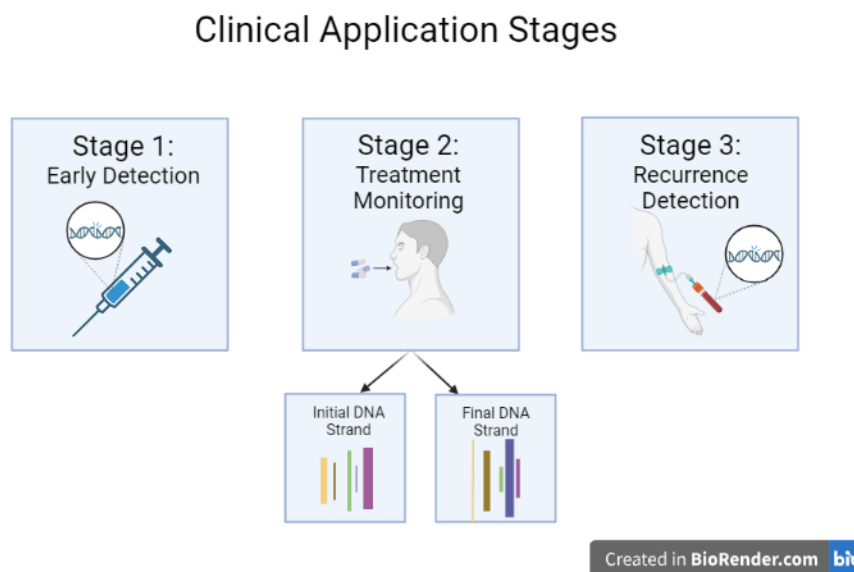
As a developing technique, the biopsy aids for a fairly early diagnosis as the ease of conducting a liquid biopsy and quick results pose for more time to form an accurate cure (Dhanuthai et al, 2017).

Similarly, these biopsies can monitor treatment response and progression. In a clinical trial by Dawson et al. (2013), a liquid biopsy was used to track the genetic alterations in patients who had oral squamous cell carcinoma

through the treatment. The study showed a dynamic change in tumor-cell mutations (Dawson et al. 2013). This provided insight to the physicians to adjust their strategies.

Next, these biopsies can be applied to detect minimal residual disease (MRD) and recurrence. For reference, MRD occurs when various cancer cells remain even after treatment that cannot be detected with scans (Boldt, 2020). In a study by Flach et al. 2022, the potential of liquid biopsy as a powerful tool for assessing recurrence in head and neck squamous cell carcinoma (HNSCC) patients was discovered. By analyzing the ctDNA through a personalized approach, LIONESS, a non-invasive method to detect MRD was used. This approach design aids for detecting early signs of recurrence, improving patient management and outcome (Flach et al. 2022).

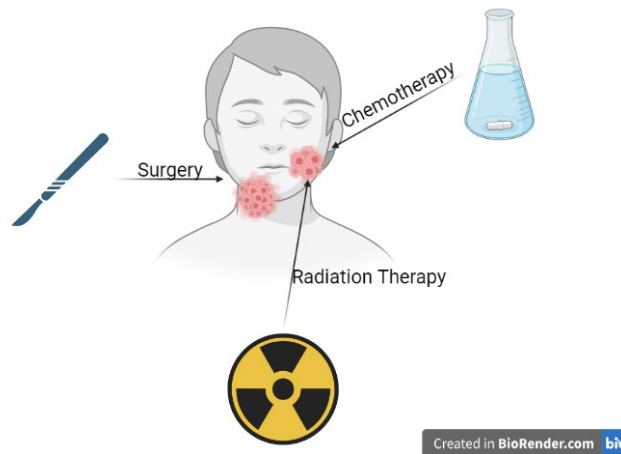
As seen in **Figure 4** below, these 3 stages are the basic clinical applications for liquid biopsies and can pose to be very helpful in the future. After these biopsies are completed, especially for early detection, the physicians start to diagnose and treat the patients for their given condition.



**Figure 4.** Various Clinical Applications for Oral Cancer Liquid Biopsies.

## The Treatment of Oral Cancer

Using a liquid biopsy can help physicians detect oral cancer in order to diagnose it and aid in personalized treatment plans. As a serious cancer, it requires various treatments to combat its aggressive growth. As seen in **Figure 5** below, the 3 common treatment methods for oral cancer are surgery, radiation therapy, and chemotherapy (Flach et al. 2022). Depending on the specific case, a combination of treatments may be used, depending on the stage and size of the cancer. The extent of the chemotherapy, radiation, and surgery can be determined from the specific biomarkers present in the patient, determined from the biopsy (Marrugo-Ramírez et al. 2018). The use of surgery as a treatment method involves the removal of the tumor and surrounding tissue to prevent it from spreading. However, if the cancer has spread too much, surgery is no longer an option. Radiation therapy, on the other hand, has high-energy beams directed at the tumor to destroy the cancer cells. This treatment method may not work if the tumor has grown to an extensive size and spread to various organs as it may not be able to control it. Similarly, if the location of the tumor is difficult to target, challenges may pose for an effective treatment. Finally, chemotherapy uses powerful drugs to slow the growth and attack the cancerous cells (Flach et al. 2022).



**Figure 5.** The Top 3 Treatments For Oral Cancer.

In all these cases, treatment becomes harder as the cancer develops. As a result, it is beneficial to detect it early on and start a treatment plan then, which can be possible with a liquid biopsy. With time and research, the technique may improve its accuracy and be used for its ease and early detection.

## The Challenges of Liquid Biopsies

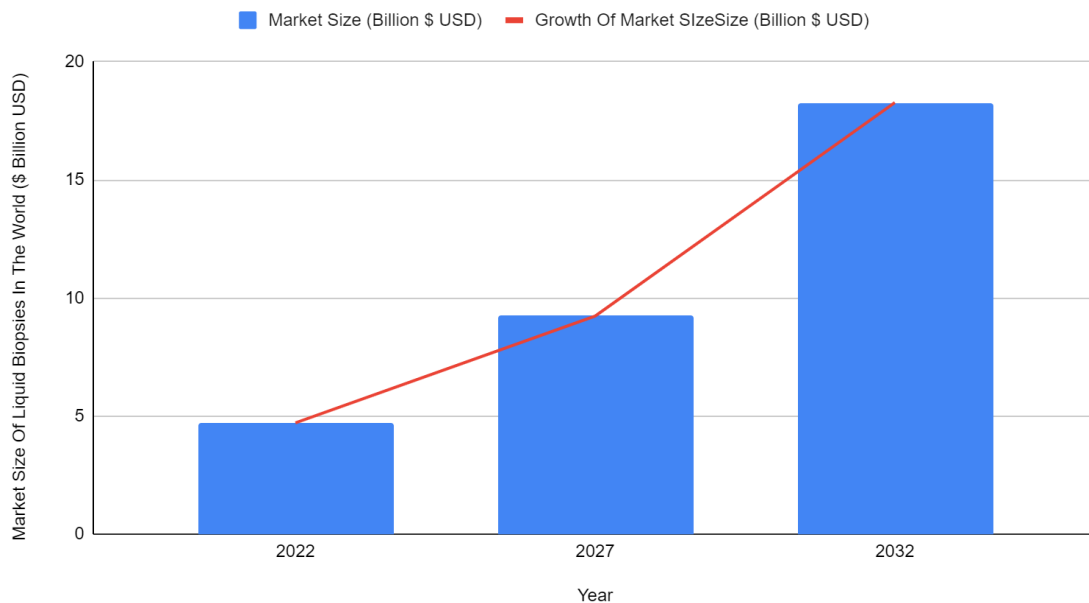
Although liquid biopsies have emerged as a prominent method for detecting and monitoring various aspects of oral cancer, there are several challenges that need to be addressed for clinical utility. One challenge in particular is the detection sensitivity of circulating tumor DNA (ctDNA). In a study by Newman et al. 2014, it was found that the ctDNA levels in patients can be low and vary between patients. Although a promising biomarker, the diagnostic methods have inadequate sensitivity. Since the sensitivity and ctDNA levels can vary, there is a challenge to accurately detect mutations, especially in the early stages (Newman et al. 2014). Another challenge posed is the complexity of the saliva. The composition contains various non-tumorigenic components which can affect the ability to detect the biomolecules of tumor origin (Patel et al. 2022). Along with this, the standard protocol for liquid biopsies hasn't been developed- the hurdle to create a set protocol will take time to implement and perfect for the most accuracy and ease. There are various challenges for a liquid biopsy, as shown earlier in **Table 1**. The cost and accessibility, tumor heterogeneity, and technical variability are other challenges that pose for this technique, but with the increase in demand over the next few years, there may be more research in the field to aid in more accuracy and depth.

## Future Direction Of Liquid Biopsies

Although there are many challenges for the implementation of liquid biopsies the future seems to be bright. As seen in **Graph 2** below, the market size in 2022 was \$ 4.71 billion USD. Over the span of the next couple of years until 2032, the market size grew to \$18.28 billion USD, as it is projected to grow about 4 times its size from 2022 (*Liquid Biopsy Market Size Growth USD 18.28 Billion by 2032 | CAGR of 14.5%, n.d.*). Due to this finding and the advancements of technology, money may be invested into research to address the current challenges as mentioned before, to propel this technique into the forefront of medicine.



Market Size Of Liquid Biopsies In The World (Billion \$ USD) by Year



**Graph 2.** The Growth of The Market for Liquid Biopsies in the World. Created by Kamyra K (*Liquid Biopsy Market Size Growth USD 18.28 Billion by 2032 | CAGR of 14.5%, n.d.*)

One focus for the future is the development of biomarkers beyond ctDNA, to ctRNA, exosomes, CTCs, and other molecules. These different biomarkers will provide complementary information about the tumor and possibly improve the sensitivity and specificity of the technique. Similarly, another future technique would be to integrate multi-omics approaches. This would entail combining genomic, epigenomic, proteomic, and metabolomic analyses. These approaches will provide a more comprehensive and holistic understanding of cancer. In a more clinical future stance, it is important to host multi-center clinical trials on a larger scale to validate and standardize the liquid biopsy process. This process will pave the way for an easier and broader implementation into the clinical world. Next, as artificial intelligence (AI) and machine learning continue to advance, the ability to analyze and interpret data for liquid biopsy accuracy capabilities can be refined (Armakolas et al., 2023). Although these methods have their share of challenges, incorporating them holds promise for transforming cancer care into a more precise and patient-centric approach.

## Conclusion

In today's society, scientists are striving to improve the timely intervention and accuracy of oral cancer diagnoses. This is critical because oral cancer becomes more aggressive as time goes on, meaning that it is harder to treat, the later it's found. This research paper identifies whether liquid biopsies were the practical method to diagnose oral cancer when compared to various common techniques. When compared to the other techniques, a liquid biopsy seems to be the best option as they show promise for non-invasive cancer detection through accessible bodily fluids. Similarly, comparative analysis highlights liquid biopsy practicality and evolving potential over traditional methods. The various other techniques do have advantages like those of liquid biopsies, however, their disadvantages outweigh them. However, the liquid biopsy process is not processed and has its own challenges. Since the liquid biopsy market

is growing exponentially at a compound annual growth rate (CAGR) of 14.5% from 2022 to 2032, research into this technique will increase the potential for higher accuracy and accessibility (Liquid Biopsy Market Size Growth USD 18.28 Billion by 2032 | CAGR of 14.5%, n.d.).

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