UCLA and Spectrum Solutions® Saliva Liquid Biopsy Proficiency Testing Uncovers IVD Device Advantage Demonstrating Unique Point-of-Collection Benefits to Deliver on Unmet Clinical Needs

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Author Note

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Table of Contents

UCLA and Spectrum Solutions® Saliva Liquid Biopsy Proficiency Testing Uncovers IVD Device Advantage Demonstrating Unique Point-of-Collection Benefits to Deliver on Unmet Clinical Needs

Executive Summary

Study efforts keenly focused on untreated plasma, untreated saliva, and preserved saliva specimens to demonstrate proof-of-concept viability in support of saliva's future mainstream liquid biopsy integration as well as other salivary diagnostic research opportunities. Successful detection of EGFR mutations was found in ultra-short circulating tumor DNA (usctDNA) associated with non-small cell lung cancer (NSCLC) in saliva using the EFIRM platform. Study insights exposed the critical need for superior innovation capable of helping magnify detection sensitivity and increase reproducibility.

The Spectrum Solutions SDNA-1000 saliva self-collection device with patented CV3 stabilization and preservation chemistry dramatically and significantly boosted the sensitivity of EGFR ctDNA testing. Considering the half-life of DNA in untreated (or neat) saliva at room temperature is about 4.2 hours, the study concluded that the effective protection of specific DNA targets was associated with the nature of the chaotropic anions found in Spectrum Solutions' proprietary CV3 buffer. Beyond stabilizing the EGFR targets, the study demonstrated an impressive 14-fold DNA amplification of detectable usctDNA from saliva collected and preserved using Spectrum's SDNA-1000 compared to neat saliva. Additionally, by diluting the signal down to 10 percent of the initial concentration, the team continued to see enhanced

amplification benefits from the Spectrum device, leading scientists to believe detection of a solitary ctDNA fragment in saliva is now possible.

Other important findings included: a significant decrease of the intra-assay coefficient of variability (COV) using the Spectrum preservative chemistry at a surprising low of **5%** compared to **26.8%** using raw or neat saliva. Likewise, this significant decrease in COV was repeated and observed in the diluted samples. Samples containing the Spectrum chemistry registered at a low **7.5%** and those diluted samples using raw or neat saliva registered at a high of **35.5%** COV. Generally, a value less than or equal to 20% is considered acceptable. The substantial decrease in observed intra-assay COV indicates a superior ability at the point-of-collection of the patented chemistry to precondition saliva sample and reduce the effects of saliva matrix on hybridization reactions and drastically improve assay repeatability and sensitivity.

The Background

According to a World Health Organization (WHO) February 2022 publication, cancer is the leading cause of death worldwide (1 in 6). In addition, the numbers show that lung cancer accounted for 1.8 million deaths and is the most common cause of cancer death.[1]

Tissue Biopsy

Cancer is a highly individualized disease. Successful cancer treatments depend on better, personalized therapies. Better therapies need more accessible access to real-time genetic disease data. A biopsy delivers this data opportunity by identifying specific cancer genetic biomarkers as well as current disease activity at the point and time of collection. More successful patient outcomes result from the combination of early

detection and a personalized treatment strategy using cancer-specific genetic biomarker data.

Most lung cancers (85%) fall into the category non-small cell lung cancer (NSCLC). Though this form of lung cancer progresses more slowly than small cell lung cancer (SCLC), 40% of those living with NSCLC will have it spread beyond the lungs by the time of detection.

A tissue biopsy is currently considered the gold standard in a cancer diagnosis. This type of traditional solid tumor biopsy has several drawbacks for the patient and the provider trying to identify the most successful treatment strategy. Some tumors are not easily accessible. Tumor biopsies can pose additional risks to the patient from the invasive nature as well as based on where the tumor is located.

In addition, studies have shown that biopsies are not always representative of the entire tumor. Because many tumors are heterogeneous, different sites within the tumor can have different levels of gene expression and metastatic potential, and a tissue biopsy may not be indicative of all tumor phenotypes. Cancer is also a dynamic disease set on change. During the course of a disease, cancers generally become more heterogeneous and divergent. As a result, the bulk of the tumor can harbor a diverse collection of cells with distinct and differing molecular signatures and different sensitivity levels to treatment. Heterogeneity provides the fuel for resistance; therefore, an accurate assessment of tumor activity and change is essential for developing effective therapies.[2]

With the understanding of just how valuable and crucial genetic disease data is to a successful outcome or the certain probability of ongoing cancer mutations changing

a patient's disease data over time–only then do we begin to realize a biopsy is not a once-and-done activity. Without the ability to easily harvest additional tissue samples, solid tissue biopsies create clinical obstacles for ongoing mutation analysis, the ability to monitor treatment sensitivity or resistance, and identifying new personalized treatment opportunities.

Liquid Biopsy

In recent years, several cancer-derived components that circulate in our body fluids, such as circulating tumor cells, circulating cell-free nucleic acids (RNA and DNA), extracellular vesicles/exosomes, and proteins, have been extensively investigated for cancer research. Isolation and molecular analysis of these tumor-derived components, including genomic, epigenomic, and proteomic assessments from liquid biopsy samples*, represent a new multimodal diagnostic tool*. With increasing knowledge of the pathways causing cancer and instrumental developments, liquid biopsy has begun to attract interest in implementation for clinical practice and routine molecular diagnostics.

Most common liquid biopsy technology requires the use of blood; however, research around the world is highly focused on other biofluids, like saliva. From blood, liquid biopsies have demonstrated the effectiveness of an altogether different and minimally invasive approach using free-floating cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA) in solid tumor cancers. A fraction of the total cfDNA in our body fluids is derived from tumor cells primarily undergoing apoptosis or necrosis called ctDNA. A liquid blood biopsy has quickly become an important part of the screening, diagnosis, and monitoring process for cancer patients with primary and secondary tumor sites. Additional blood draws can also be taken and reevaluated at different times

to offer a more well-rounded and complete tumor picture when tissue biopsies are more challenging, not easily accessible, or as a deciding factor weighted by disease data insights before moving toward a more complicated or problematic tissue biopsy decision.

Case Evaluation

The Organization

For the University of California at Los Angeles (UCLA) and head researcher Dr. David Wong, UCLA School of Dentistry, the journey to identify these tiny genetic NSCLC components within saliva started decades ago. One published study in 2015 by Dr. Wong and his team stressed that due to the invasive procedure of tissue biopsy and the progressive development of drug-resistant EGFR mutations, the effective initial detection and continuous monitoring of EGFR mutations were still unmet requirements.[3] According to Wong, the successful ability to detect EGFR mutations in saliva using ctDNA easily and safely would empower an ever-broadening range of clinical applications, including early detection of disease, prediction of treatment responses, and disease progression for the noninvasive assessment of an individual's lifelong cancer burden.

In a key study published in 2020, Dr. Wong and his team introduced a novel plate-based liquid biopsy platform capable of detecting circulating tumor DNA containing *EGFR* mutations directly from saliva and plasma in both early- and late-stage patients with NSCLC called EFIRM (Electric Field–Induced Release and Measurement).[4] EFIRM uses electric fields to enhance molecular hybridization

reactions with immobilized DNA probes followed by enzymatic signal amplification to detect ctDNA.[7]

Dr. Wong and his team used untreated plasma and saliva as well as preserved saliva inputs to demonstrate concept viability. Using the EFRIM platform, researchers were successfully able to detect the EGFR mutations from ctDNA fragments in saliva associated with NSCLC called 'ultra-short' ctDNA (usctDNA).[4] This significant step forward delivered saliva the valid proof-of-concept they had been looking for as well as additional research opportunities supporting saliva's promising ability for future mainstream liquid biopsy integration. With the primary focus on salivary diagnostics, study insights revealed the need to find a superior innovation capable of helping magnify detection sensitivity and increase reproducibility.

The Challenge

The sensitivity of liquid biopsy technologies for detecting ctDNA genomic alternations is limited by low concentration. The size of the usctDNA molecules also reflects the limitations of current liquid biopsy technologies, as well as standard DNA preservation and extraction methods deliver extremely poor yields of DNA fragments with such small sizes. Understanding the detailed biological properties of ctDNA is essential to developing ctDNA detection innovation that improves the performance of liquid biopsy platforms. Liquid biopsy that delivers detection sensitivity closest to tissue biopsy-based genotyping of tumor-specific ctDNA is an unmet clinical need. Although liquid biopsy and ctDNA analysis have quickly become a valuable, even lifesaving, and favored approach, the noninvasive use of saliva over blood still faces a big challenge. In saliva, the EGFR ctDNA is almost exclusively ultrashort. Knowing an EGFR mutation

status is critical to guide precision therapy and prognostic assessment of a patient's health.

The UCLA team and Dr. Wong identified low ctDNA content with a high background of cfDNA noise in saliva compounded by a high rate of degradation in detectable ctDNA. ctDNA fragments present a short half-life and achieving detectable concentrations of ctDNA in body fluids is not an easy task. Scattered mutations positionally in ctDNA add an additional degree of detection difficulty using PCR amplification-based techniques. Standard dsDNA-seq methodologies involve size selection resulting in the loss of library molecules with short inserts. Furthermore, traditional DNA extraction methods offer poor yields of these small usctDNA fragments. The usctDNA fragment associated with NSCLC is a third smaller in size than the average fragment. The degree of detection difficulty is further compounded by extremely low concentration levels that hinder any consistent or repeatable accuracy using current liquid biopsy approaches and compromise the sensitivities of assays to detect or underestimate ctDNA in clinical samples.

The discovery of sensitive methods to enrich ctDNA carries the potential to have a significant impact on any clinical applications of liquid biopsy.[5] The ability to achieve testing with the reproducibility and sensitivity required to improve mutation detection will further establish liquid biopsies as a tool for managing cancer, including earlier stage diagnostics, risk assessments, screening, ongoing treatment monitoring, personalized medicine, and detection of minimal residual disease.

Advances in targeted therapies for lung cancer are based on the ongoing and repeat evaluation of three EGFR gene mutations. Therefore, the noninvasive and self-

collection of saliva samples implementing sample preparation to reduce matrix effects would not only significantly impact laboratory test automation but also deliver an increase in opportunity and access for all populations to modernized care for in-clinic and direct-to-patient at-home use worldwide.

> *"Understanding how many face the likelihood of treatment resistance, it is actually life-changing to know the results from this research collaboration with Dr. Wong and UCLA has real power to positively impact patient outcomes," said Steve Fanning, CEO of Spectrum Solutions. "For patients, the ability to use the self-collection of saliva empowers a new, safer, easier and pain-free era of detection and treatment innovation with real at-home remote care possibilities."*

The Solution

To increase detection sensitivity, the research team at UCLA integrated the Spectrum Solutions SDNA-1000 saliva collection device with patented CV3 DNA stabilizing and preservation chemistry as a core material within their research study. Two groups of saliva samples were procured. The first group was collected with Spectrum SDNA-1000 collector following the device's instructions for use (IFU). Then a second control saliva sample was collected from each donor without adding the preservative solution. Three synthetic oligos harboring one of three EGFR mutations were spiked into each saliva sample with the same 100pM concentration. Additionally, to test the linearity of each assay with and without the preservation chemistry, the mixture of all three oligos was diluted down to 10% of the original 100pM concentration.

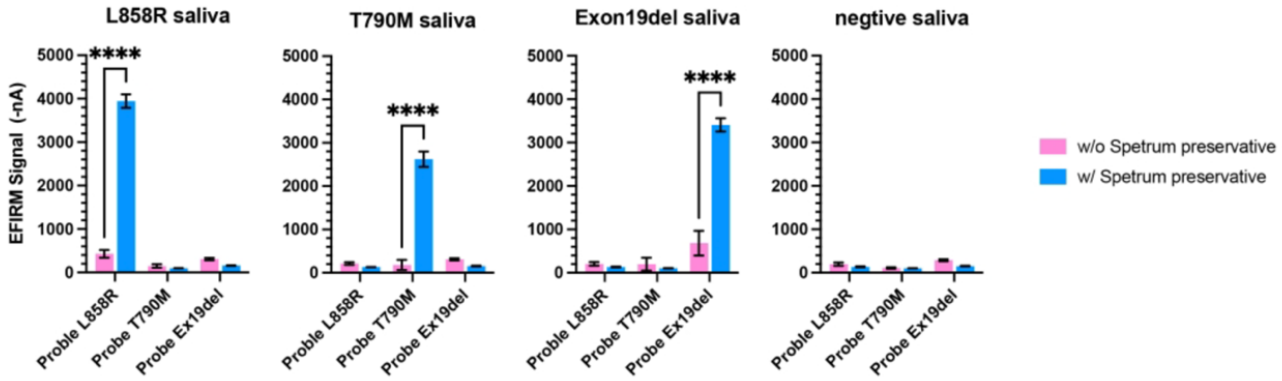
The Conclusion

The Spectrum Solutions SDNA-1000 saliva self-collection device with patented CV3 stabilization and preservation chemistry significantly boosted the sensitivity and detection of EGFR ctDNA oligos in saliva on the EFIRM liquid biopsy platform. Study findings demonstrated an impressive 14-fold increase of detectable usctDNA from saliva collected and stabilized using Spectrum's SDNA-1000 compared to raw or neat saliva (see figure 1). It also improved the reproducibility of the assay by decreasing the variability with saliva collected between individuals. These attributes are critical and foundational utilities for early cancer assessment and treatment monitoring using the liquid biopsy method.

> *"The ability to detect EGFR mutations using ctDNA enables a broad range of clinical applications such as the early detection of disease, practical applications of personalized treatments, predicting treatment response, and monitoring resistance," said Rohit Gupta, Chief Medical Officer for Spectrum Solutions. "Proving it can be done using saliva is groundbreaking."*

Figure 1

Comparison of EGFR mutation detection with mutation harboring oligos spiked-in saliva samples with or without Spectrum preservative solution.



*Note: Saliva samples collected from 4 different healthy subjects with or without spectrum preservative solution. Three EFIRM assays for L858R, T790M and exon19 del detection were tested. The p value was determined by two-way ANOVA and the error bar represents the standard error in duplicates of 4 samples. **** means p < 0.0001.*

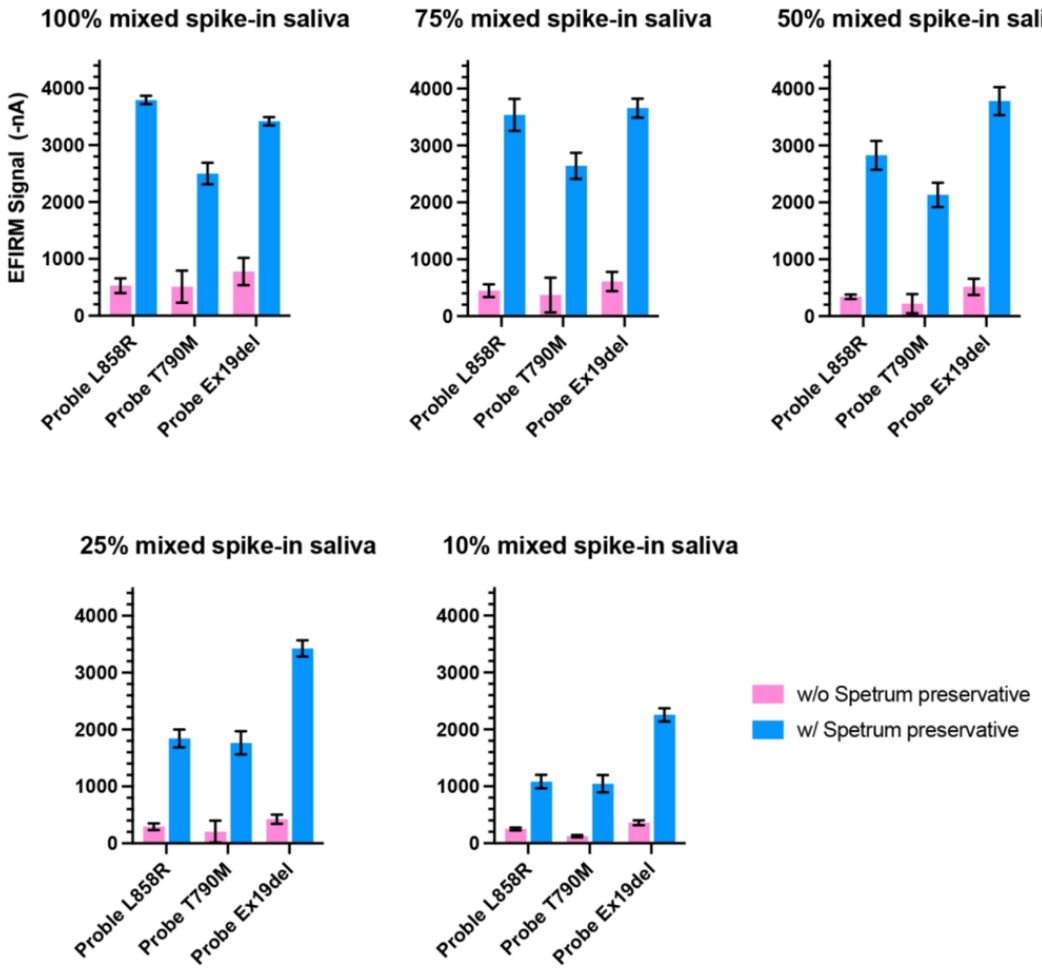
Additionally, after diluting the signal down to 10 percent of the initial concentration, the team continued to see enhanced amplification benefits from the Spectrum device, leading scientists to believe detection of a solitary ctDNA fragment may now be possible. Results illustrated each EFIRM assay with preserved saliva (blue bars) returning much higher signals than those with non-preserved saliva (purple bars) at different target concentrations (see figure 2). The average fold increases of the EFIRM signal with saliva from the Spectrum device are 6.8, 7.7, and 6.4 with L858R, T790M, and Exon19del assay, respectively.

A significant decrease in the intra-assay coefficient of variability (COV) using the Spectrum preservative chemistry was also observed at a surprising low of 5% compared to 26.8% using raw or neat saliva. Likewise, this significant decrease in COV was repeated and observed in the diluted samples. Samples containing the Spectrum chemistry registered at a low 7.5% and those diluted samples using raw or neat saliva registered at a high of 35.5% COV. The COV makes it easier to compare the overall precision of two analytical systems. The COV is a more accurate comparison than the standard deviation, as the standard deviation typically increases as the concentration of the analyte increases. Typically, a value less than or equal to 20% is considered an acceptable working range. The substantial decrease in observed intra-assay COV indicates a superior ability at the point-of-collection of the patented chemistry to

precondition saliva sample and reduce the effects of saliva matrix on hybridization reactions and drastically improve assay repeatability and sensitivity.

Figure 2

Comparison of EGFR mutation detection with different concentrations of mixed spike-in oligos in saliva samples with or without Spectrum preservative solution.



Note: Saliva samples were collected from 4 different healthy subjects with or without spectrum preservative solution. The mixed spike-in oligos harboring EGFR L858R,

T790M and exon19 del, respectively, were diluted with each preserved or non-preserved saliva sample.

Results in this study highlight the limitations of neat saliva. The significant decrease of intra-assay COV also indicates proactive preparation of the sample by the Spectrum patented chemistry at the point of collection, reducing the effects of saliva matrix on hybridization reactions to increase assay reproducibility and sensitivity significantly.

Considering the half-life of DNA in saliva at room temperature is about 4.2 hours,[6] it was concluded that the signal amplification was not solely attributed to the efficient ability of the Spectrum chemistry to protect specific DNA targets but also the additional amplification enhancement in detection and sensitivity for DNA oligo hybridization lays within the proprietary Spectrum CV3 chemistry itself.

> *"Achieving detectable concentrations of ctDNA in body fluids is not an easy task. The significance of successfully demonstrating this capability with saliva is the holy grail of liquid biopsy research," said Dr. David Wong, UCLA School of Dentistry. "This discovery delivers, for the first time, a noninvasive opportunity for detection and the ongoing 'real-time' understanding of somatic mutation activity to direct and redirect successful treatment strategies."*

Acknowledgments

The published manuscript of the UCLA and Spectrum Solutions research study findings reported in this case study can be found at the Biopreservation and Biobanking Journal. Manuscript ID# BIO-2022-0093 Published August 2022.

https://www.liebertpub.com/doi/epdf/10.1089/bio.2022.0093

Published findings for the liquid biopsy platform comparison and the proficiency demonstration of EFIRM over ddPCR to detect ctDNA EGFR L858R and exon 19 deletion mutations in plasma and saliva of NSCLC patients can be found

Cancers 2020, *12*(8), 2041; https://doi.org/10.3390/cancers12082041

About Spectrum Solutions®

Headquartered in Salt Lake City, Utah, Spectrum Solutions is a medical science ideation and clinical test commercialization partner with focused industry expertise in laboratory medicine to drive noninvasive molecular diagnostic and screening IVD innovation. Our stand-alone and fully integrated clinical laboratory services, pioneering research, award-winning products, direct-to-consumer specialized test kit development, and compounded patient-specific therapy capabilities are setting new standards of modernized care in the testing, monitoring, and treatment of disease. We believe when you bridge the gap between science and real direct-to-patient medical solutions you deliver innovation with the practical power to impact and change outcomes. For more information, please visit spectrum**solution**.com.

For questions regarding this case study or for additional information on Spectrum's SDNA-1000 Saliva Collection device for your clinical test validation, please email Leslie Titus-Bryant at Spectrum Solutions.

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