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Optimal DNA Isolation of Liquid Biopsy

White Paper

Executive Summary

When cell apoptosis or necrosis occurs, DNA is released into the cell environment – referred to as **cell-free DNA** (cfDNA). Cell-free DNA can be released from healthy, cancerous, or fetal tissue and transplanted organs. This cfDNA can be extracted from whole blood samples (liquid biopsies), and recent scientific developments have made it more possible and effective to use these extractions in an array of crucial methods to detect and track tumors through **circulating tumor DNA** (ctDNA). Although there are reservations regarding the inconsistent test sensitivities and high costs, the capabilities these liquid biopsies offer sharply contrast with the high risk and invasiveness of tissue biopsies. Cell-free DNA can be extracted using automated or manual methods.

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Discovery and Development of cfDNA Extraction

Freely circulating DNA outside of cells was first discovered in 1948, but clinical application did not occur until 1997[1], when the presence of Y-chromosomal DNA was detected in the plasma of women pregnant with male fetuses. This discovery allowed for earlier detection of a fetus's gender and any potential chromosomal aberrations. These findings garnered interest in cfDNA's applications for diagnosis and monitoring of cancer progression.

Circulating tumor DNA[2] specifically was first found in 1977. An initial hurdle was the difficulty in detecting the extremely low concentration of ctDNA within the cfDNA, resulting in unreliable test results. However, significant developments have been made in recent years to accurately detect and identify ctDNA in cfDNA extractions with highly developed sensitivity.

Cancer patients exhibit higher levels of cfDNA as compared to healthy individuals because tumors have a greater rate of cell turnover and higher presence of necrotic cells. Studies have found that the median concentration of cfDNA in plasma from those with solid tumors is three times as high as it is in healthy individuals[1]. The rate at which ctDNA is released into the bloodstream is dependent on the tumor burden and site – this also enables correlation of the level of ctDNA concentration with the tumor burden (i.e. there is a higher concentration as the tumor burden increases, and a lower one in response to treatment)[1].

Advantages and Benefits of Liquid Biopsies vs. Traditional Biopsies

Among the various methods to detect cancer (tissue biopsies, medical imaging, and liquid biopsies), liquid biopsies lend themselves to easy sample procurement and obtainment of information crucial to cancer identification and progression. With the increasing interest and development in this area, it seems highly likely that liquid biopsies will become price-competitive with standard methods.

The primary tumor sample can only be obtained during surgical procedures, which requires specific instruments, venues, and faculty. In the case of insufficiently obtained material, patients face repeat procedures which are risky and dangerous.

Obtaining liquid biopsies is a minimally invasive method because liquid samples are easier and quicker to access and collect. This easier handling does not require surgical equipment and personnel and is also significantly less stressful and painful for patients – especially beneficial for patients who are frail, have damage to the tissue of the tumor site, have inoperable tumors, or are at risk of infection. Liquid biopsies in these cases are optimal. Accordingly, they also have fewer potential side effects because no invasive surgery is necessary. With liquid biopsies, doctors can obtain repeat samples to ensure continual monitoring of the tumor. Currently, liquid biopsies are most frequently taken through whole blood or urine samples, but as test sensitivities improve, it is likely that samples will be taken from body fluids closest to the tumor sites (e.g. saliva, pleural fluid, bronchoalveolar washings, etc.).

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Below is a table that compares the characteristics of a liquid biopsy vs. a tissue biopsy:

Parameters	Biopsy	(FFPE) Tissue Biopsy
Accessibility	Ease of access	Surgical procedure
Degree of Risk	Low risk	Risk for infections and complications
Availability	Multiple sampling	Only through surgical procedure
Equipment	Blood collection, stabilization, and DNA extraction tools	Surgical venue and tools, sterile surrounding, and subsequent DNA extraction tools
Time for DNA Extraction on XTRACT 16+	1.2 hr	2.7 hr
Downstream	qPCR, ddPCR, NGS, DNA methylation analysis	qPCR, ddPCR, NGS, DNA methylation analysis
Possible Applications	- Early detection of ctDNA for high risk patients - Comparison of mutation in primary tumor vs. liquid biopsy followed by disease progression monitoring and therapy monitoring for best possible therapy adjustment	- Imaging followed by diagnosis and obtaining biopsy sample - Analysis of primary tumor, detection of mutations, and therapy adjustments

One of the greatest benefits of cfDNA extraction is that it enables temporal identification and tracking of the tumor's progression. The implications of this capability are far-reaching. With easy accessibility to multiple samples and cfDNA's half-life of a few hours, it is a much more dynamic and timely way to monitor the progression of the tumor and monitor therapy response. Higher sensitivity also allows for earlier detection of cancerous cells, which results in more timely treatment. This can potentially contribute to a higher survival rate. Not only does this granular sensitivity improve detection capabilities in the preliminary stages and support risk stratification and choice of treatment, but this also helps uncover residual disease and the regression of dormant cells post-treatment by identifying tumor-specific and new somatic mutations in the cfDNA[1].

Challenges of cfDNA Extraction

Because this is a newer field, every step (from sample collection to analysis to storage methodologies) needs to be standardized to obtain routine, consistent analyses and results. Larger clinical trials also need to be held according to standardized procedures, and guidelines for defining biomarkers for tumor types will also need to be developed.

Cell-free DNA requires careful, precise handling because of its small fragment sizes and half-life. Material stabilization after sample obtainment is crucial – since the circulating cfDNA is approximately only 170bp and has a short half-life of a few hours, it can be degraded easily by DNase present in the plasma. Plasma preparation needs to be done as quickly as possible to avoid exposure to room temperature for a long period of time.

As congruency between biopsy samples and cfDNA mutational patterns is not 100% perfect, analyses will require certain guidelines for defining tumor variants. This may require multiple samples from the same patient to account for variation, and there is currently a limited number of commercially available biomarker detection systems. In addition, the upfront costs of liquid biopsies for clinical use should be carefully considered (training and potentially hiring personnel, and procuring equipment).

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Automated vs. Manual cfDNA Extraction

There are both automated and manual extraction options that differ further with various starting volumes of plasma, urine, serum, or saliva. Aside from an extraction robot or a manual kit, equipment for obtaining blood samples (EDTA/STRECK tubes) and preparation, stabilization, and storage of these samples are indispensable. After the samples are properly prepared, extraction is performed either on an automated robotic platform or manually using the appropriate kit.

The benefits of automated cfDNA extraction are many:

- Can be implemented in future routine diagnostics as automation is crucial for standardization
- Reduces human error, resulting in higher reliability and consistency
- Increases daily throughput and produces comparable total yields
- Automation spares hands-on-time, freeing up technicians' time so that efficiency and productivity in the lab increases

On the other hand, manual extraction does allow for more flexibility in minimizing elution volume to potentially increase the concentration of cfDNA.

AutoGen, a provider of fully automated workflow solutions, assessed an array of competing products with its flagship XTRACT 16+.

Company	Kit	Hands-on Time* (approx.)	Sample Number	Instrument	Procedure	Time	Volume (µl)	FFPE Protocol	UV Light
AutoGen	XTRACT Liquid Biopsy Kit (4ml)	10 min*	16/run	XTRACT 16+	Fully Automated	1.2h	4000	yes	yes
Qiagen	QIAsymphony DSP Circulating DNA Kit	10 min*	96/run	QiaSymphony	Fully Automated	6h	2000 or 4000	no	yes
Qiagen	EZ1 ccfDNA Midi Kit	5 min* 20 min**	14/run	EZI Advanced XL	Semi-Automated	1h	5000**	yes	no
Perkin Elmer	NextPrep-Mag™ cfDNA Kit	20 min*	24/run	chemagicTM 360 instrument	Semi-Automated	1.5h	5000	no	no
Promega	Maxwell® RSC ccfDNA Plasma Kit	10 min*	16/run	Maxwell 16 RSC	Semi-Automated	1h	1000***	yes	no
Roche	cobas® cfDNA Sample Preparation Kit	60 min	24 per kit		Manual	1.5h	1000	yes	n/a
Bioo	NextPrep-Mag™ cfDNA Isolation Kit	15 min	50 per kit		Manual	0.5h	3-5 ml	yes	n/a
Promega	cfPure® Cell Free DNA Extraction Kit	50 min	25 per kit for 5 ml		Manual	1.25h	5 ml	yes	n/a
Roche	QIAamp® Circulation Nucleic Acid DNA Kit	60 min	50 per kit		Manual	1.5h	1-5 ml	yes	n/a

*instrument loading (buffer, plastic)
** manual enrichment
***additional protocol with 4000µl available

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Overall, these instruments partly offer semi-automated processes with lower volumes of starting materials. In comparison, the XTRACT 16+ offers full automation, is highly effective and reliable, and shows competitive acquisition and operating costs. When the process of DNA extraction from tissue biopsies is compared to the automated extraction of cfDNA extraction from liquid biopsies, the difference in resources and time is evident.

In order to standardize and control cfDNA extraction, laboratories can control their cfDNA extraction by micro-capillary electrophoresis methods such as TapeStation or Agilent Bioanalyzer, control concentrations, and do spike-in controls for PCR-based downstream applications.

Conclusion and Looking Forward

It is clear that with the continuing development and decreasing costs of utilizing cfDNA extraction to detect, treat, and monitor cancer progression and therapy, liquid biopsies will soon become a standard method in clinical use. Its advantages of higher sensitivity to contribute to earlier detection and dynamic reflection of the tumor's progression has great potential to result in more accurate and effective therapy choices and higher survival rates. In order to take full advantage of this more effective method for cancer diagnosis, monitoring and screening methods for high-risk patients, it will be crucial to acquire the most optimal equipment that is automated, efficient, precise, and ensures an effective standardization.

Sources:
1. Krishnamurthy et al. 2017, Liquid Biopsies for Cancer: Coming to a Patient near You, J Clin Med., Jan; 6(1): 3, doi: 10.3390/jcm6010003
2. Li G, Sun Y 2017, Liquid Biopsy: Advances, Limitations and Clinical Applications. JSM Biotechnol Bioeng 4(2): 1078

About AutoGen

AutoGen is a leading provider of automated nucleic acid extraction workflows that allows lab professionals to produce premier quality and value-added extraction results. Our workflows provide solutions that are the best fit for our customers' laboratory needs and budget, and our customers include biorepositories, contract research organizations, academic research laboratories, pharmaceutical companies, clinical diagnostic laboratories, and government institutions all over the world. We strive to provide quality instrumentation and chemistries, as well as dedicated technical support – all with a level of post-sale service that is truly unmatched. Visit www.AutoGen.com to learn more.

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