CASE STUDY

Driving precision oncology with chemagic technology.

Liquid biopsies present a game-changing option for cancer detection and monitoring, providing real-time insights into the disease state in a non-invasive manner. Although the concept of liquid biopsies is far from new, only recently have advances in technology enabled researchers to reliably detect and measure key blood-bound biomarkers such as cell-free DNA (cfDNA). Molecular biomarkers are becoming increasingly important in oncology for early detection, prognosis, identification of relevant genomic alterations, selection of targeted therapies and treatment monitoring[1].

Despite its great value as a biomarker, extracting and purifying cfDNA has traditionally posed challenges due to its low concentration and high level of fragmentation within the bloodstream. Revvity's chemagic™ technology offers a robust solution to address these challenges. Leveraging M-PVA magnetic bead technology[2] to extract cfDNA from large plasma volumes, the DNA extraction and purification platform is designed to maximize yield and purity. When combined with advanced quantification technology such as Droplet Digital™ PCR (ddPCR), the workflow provides unparalleled precision and repeatability in early cancer biomarker research.

In a recent webinar hosted by Revvity, Professor Niels Pallisgaard, a seasoned clinical molecular oncologist from Zealand University Hospital, Denmark, shared his invaluable insights in the arena of liquid biopsies. Read on to explore his experiences, and how chemagic DNA extraction and purification technology is helping to optimize results.

What is cfDNA?

cfDNA consists of short DNA fragments that are released into the bloodstream from cells throughout the body. These fragments are typically produced during processes such as apoptosis and necrosis. cfDNA can originate from both normal and pathological tissues, including tumors, presenting a valuable source of genetic material for non-invasive diagnostic testing. The abundance and genetic composition of cfDNA can provide real-time insights into tumor dynamics, allowing clinicians to monitor disease progression, treatment response, and detect early signs of relapse.

cfDNA includes circulating tumor DNA (ctDNA), which is especially valuable in the detection of heterogenous cancers, since it expresses genetic mutations and alterations specific to an individual's cancer cells. By analyzing ctDNA, clinicians can identify disease-relevant mutations and tailor personalized treatment strategies. Plasma-bound DNA biomarkers are also critical for early disease detection – elevated levels of cfDNA or the presence of specific ctDNA mutations can indicate the presence of cancer before it is detectable through imaging or symptomatic presentation, facilitating early intervention and optimal patient outcomes.

Prof. Niels Pallisgaard
Zealand University Hospital

Making waves in the world of liquid biopsies

With over 25 years of experience in oncology, Prof. Pallisgaard has made significant contributions to cancer research, particularly in the field of molecular diagnostics. His extensive research has primarily focused on the application of liquid biopsy techniques to improve cancer detection and monitoring. Prof. Pallisgaard is especially interested in leveraging cfDNA for cancer diagnosis and monitoring.

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A valuable biomarker for a variety of cancer types

Prof. Pallisgaard and his team have harnessed ctDNA analysis to detect and monitor a broad range of cancer types: The team's recent research has demonstrated that ctDNA monitoring can swiftly and reliably detect when lung cancer treatment is failing, often before it becomes evident through radiologic assessments[3]. Meanwhile, in the arena of anal cancer, Prof. Pallisgaard's research has highlighted the importance of cfDNA monitoring during chemoradiotherapy. By assessing human papillomavirus (HPV) cfDNA, Prof. Pallisgaard's team can stratify anal cancer patients based on their risk of treatment failure, allowing for more personalized and effective treatment plans[4].

What are the challenges in cfDNA extraction?

Throughout his time researching liquid biopsies, Prof. Pallisgaard has employed a range of kit suppliers and technologies for cfDNA extraction and purification. Traditionally, extracting and purifying cfDNA has been challenging due to several factors:

- **Low concentration:** cfDNA is typically present at very low concentrations in the bloodstream, particularly in early-stage cancer patients.
- **Contamination:** DNA from lysed blood cells can contaminate the sample, making it challenging to isolate the tumor-specific cfDNA accurately.
- **High fragmentation:** cfDNA is highly fragmented, generally around 150-200 base pairs in length. This fragmentation makes extraction and purification more challenging, and may affect the accuracy of downstream DNA quantification.
- **Compatibility with automation:** Many cfDNA isolation platforms suffer a drop in performance when moving from a manual to an automated workflow. Compatibility with automation is key to achieving accurate and consistent results across a large number of clinical samples.

Optimizing cfDNA extraction with chemagic technology

When seeking the optimal purification platform for their cfDNA workflow, Prof. Pallisgaard's team appraised several options. Identical plasma samples were purified across 8 different labs using a variety of platforms and

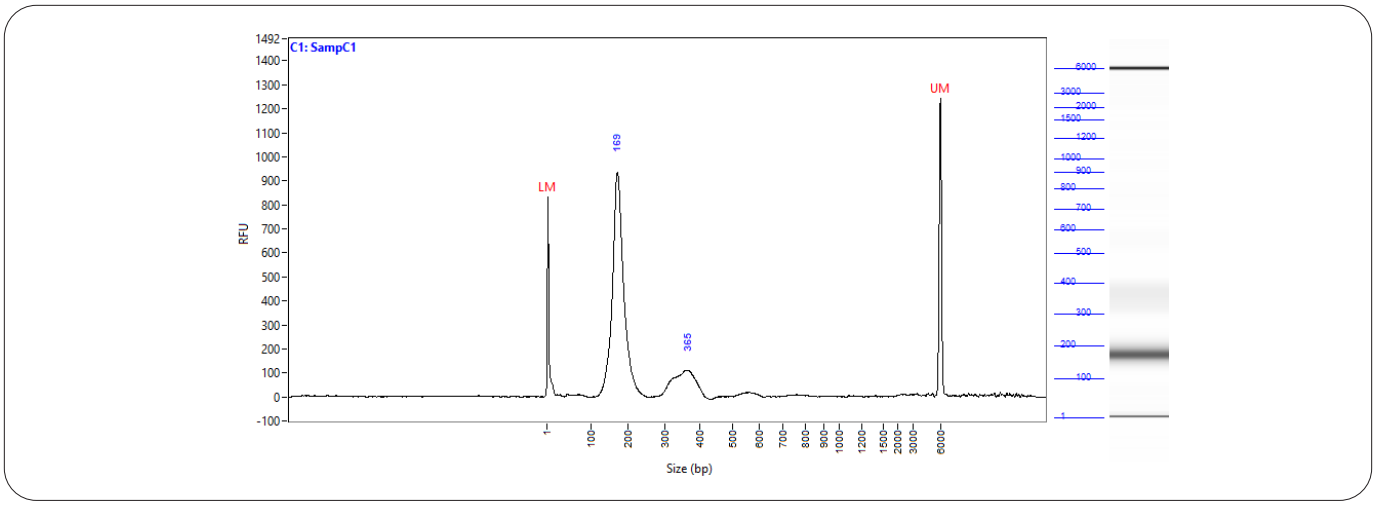
The chemagic 360 instrument used by Prof. Pallisgaard's team for automated cfDNA purification.

purification kits. A strong cfDNA yield, measured by ddPCR, was achieved by Revvity's chemagic cfDNA kits on the chemagic 360 instrument - encouraging the team to adopt the technology into their clinical workflow.

At the core of chemagic technology lies its use of M-PVA magnetic beads. These beads have a high binding affinity for nucleic acids, allowing for efficient binding and isolation of cfDNA from plasma. One of the key capabilities of chemagic technology over other nucleic extraction methods, is its ability to handle large sample volumes, processing up to 18 mL of plasma at a time. This capability significantly contributes to its ability to extract high yields of cfDNA, as larger sample volumes increase the likelihood of capturing sufficient cfDNA fragments, even from low-abundance sources.

In addition to the excellent cfDNA yield, Prof. Pallisgaard made the decision to adopt the chemagic technology due to its consistency and scalability. The technology's automation capability and lack of bias towards single-stranded/double stranded DNA was also favorable – significantly enhancing the reliability of downstream assays. Its compatibility with various upstream conditions and a broad range of downstream analysis methods, including NGS and PCR, made it a robust and versatile choice for cfDNA extraction and purification.

Implementing Revvity's chemagic technology into Zealand University Hospital's existing laboratory was swift and straightforward. With support from Revvity's dedicated support team, and thanks to the platform's user-friendly design, labs seeking to transition from a manual workflow to an automated one can do so seamlessly. Furthermore, the system's compatibility with Laboratory Information Management Systems (LIMS) ensures full traceability and integration with existing lab infrastructure.



cfDNA was isolated from 4 mL plasma with the chemagic cfDNA 5k Kit H24 on a chemagic 360 instrument. The cfDNA eluate was analyzed on an Agilent Femto Pulse system with the Agilent Ultra Sensitivity NGS kit. A clear mononucleosomal cfDNA peak was detected at 169 bp, a dinucleosomal peak at 365 bp and a small trinucleosomal fraction was observed between 500 – 600 bp.

Prof. Pallisgaard's cfDNA purification workflow

Now established and validated, chemagic technology is a critical component of Prof. Pallisgaard's cfDNA workflow. Here's an overview of the cfDNA extraction and purification steps used by the team at Zealand University Hospital:

1. Sample preparation

Blood samples are collected in EDTA or Streck tubes to ensure the integrity of cfDNA. Blood samples are centrifuged to separate plasma from blood cells.

2. Magnetic bead-based extraction

Extraction of cfDNA from 5 ml plasma is performed using the chemagic cfDNA 5k Kit H24 (CMG-1304) on the chemagic 360 instrument. The M-PVA magnetic beads bind to the cfDNA fragments, capturing them from the plasma. A magnetic field is then applied, which causes the M-PVA magnetic beads (now bound with cfDNA) to separate from the rest of the plasma lysate.

3. Wash step

The beads are washed multiple times to remove impurities and contaminants, ensuring that only cfDNA remains bound to the beads. The chemagic 360 instrument utilizes an advanced magnetic separation technology that enhances the washing and resuspension processes. Metal rods are transiently magnetized by an electromagnet to attract M-PVA magnetic beads bound with cfDNA. During washing steps, the electromagnet is deactivated, and the rods are rotated to resuspend the beads thoroughly. This efficient resuspension ensures that impurities are effectively removed without transferring any liquid between steps, resulting in high yields and purity of cfDNA.

4. Elution

The bound cfDNA is then eluted from the M-PVA magnetic beads using a dedicated buffer. This step releases the cfDNA into a clean solution, ready for downstream applications. Typically, an elution volume between 75 uL and 100 uL is optimal when purifying cfDNA from 5 mL of plasma.

Quality control

Ensuring the integrity and accuracy of cfDNA analysis is paramount in Prof. Pallisgaard's research at Zealand University Hospital. To achieve this, a comprehensive set of quality control (QC) measures are integrated into the plasma sampling and cfDNA isolation workflow[5]. Spike-in controls are implemented using a synthetic DNA fragment from the soybean gene CPP1, added to each sample before extraction. Since CPP1 isn't naturally present in human samples, the team can monitor the entire workflow by measuring its recovery rate, ensuring the extraction process yields the expected amount of cfDNA.

Fragmentation control is another QC measure that ensures the extracted cfDNA is of the right fragment size, typically around 150-200 base pairs, and therefore distinguished from the high molecular weight DNA found in blood cells. The CPP1 spike-in control is also used here to analyze

the size distribution, confirming the cfDNA's suitability for downstream applications.

Mimix™ reference standards from Revvity offer an alternative to CPP1 spike-in controls, and can also be leveraged to validate end-to-end cfDNA workflows. Blended from cell lines containing disease-relevant variants in key cancer genes, these reference standards offer an off-the-shelf solution for a broad range of oncology research applications.

Downstream analysis

Following DNA extraction and purification with chemagic technology, Prof. Pallisgaard's team leverage Droplet Digital™ PCR technology from Bio-Rad Laboratories for DNA detection and quantification. ddPCR is a highly sensitive and precise method of quantitative PCR that enables the absolute quantification of nucleic acids. The technique detects very low levels of target DNA with high precision. This is crucial in cancer research, where ctDNA is often present at very low concentrations.

By partitioning the sample into thousands of droplets, ddPCR effectively isolates and amplifies rare target mutations associated with cancer or pre-cancer. This reduces the background noise and enhances the detection of low-abundance ctDNA fragments. By combining chemagic cfDNA extraction with ddPCR, Prof. Pallisgaard has developed a robust workflow that enhances the precision and reproducibility of ctDNA detection.

At Zealand University Hospital, Prof. Pallisgaard's team currently processes hundreds of plasma samples each month from across Scandinavia. Now that their automated chemagic technology and ddPCR workflow has been established, the team can achieve a rapid sample-to-result turnaround time—from cfDNA extraction to analytical results—in just 24 hours. This efficient process produces the precise, reliable ctDNA analysis results they require to advance their clinical research.

> "We use cfDNA extracted with chemagic technology in droplet digital PCR assays to support research of colorectal cancer, lung cancer and anal cancer (based on HPV cfDNA detection) with a sample to result turnaround time down to 24 hours"
>
> - *Prof. Niels Pallisgaard*

The magic touch in precision oncology

Revvity's chemagic technology, combined with ddPCR, is revolutionizing cfDNA extraction and cancer biomarker research. By enabling high yields of pure cfDNA from large plasma volumes and integrating seamlessly into existing lab workflows, chemagic technology ensures precise, reliable ctDNA analysis. For Prof. Niels Pallisgaard and his team, the chemagic 360 instrument has become an indispensable tool, transforming their approach to precision oncology research and helping drive better patient outcomes in future.

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