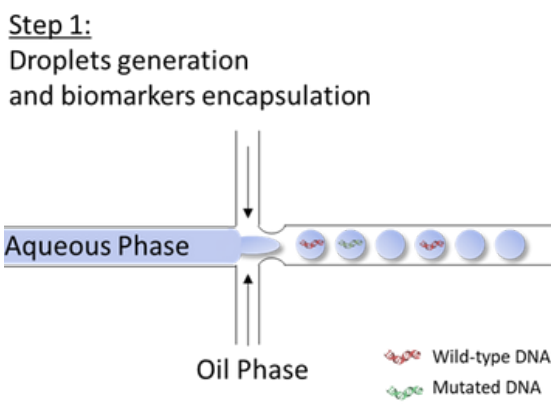
WORLD CANCER DAY, FEBRUARY 4

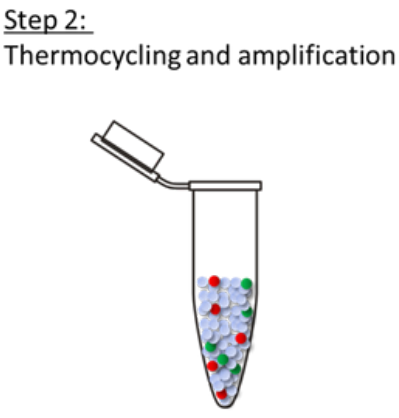
Emulseo takes part actively to the race against cancer by providing chemical formulations for droplet-based microfluidic applications enabling early cancer detection, patient follow-up and personalized cancer treatment. The last decade, huge breakthroughs in cancer diagnosis have been accomplished with the emergence of liquid biopsy. Droplet-based microfluidic technology has been instrumental in these progresses.

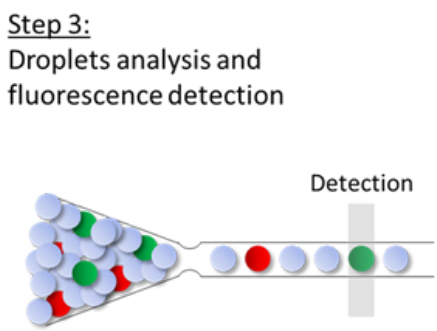
Liquid biopsy consists of the analysis of cancer-specific components within blood, urine, or other bodily effluents. As opposed to traditional tissue biopsy, this approach ensures non-invasive, regular and rapid quantitative detection of cancer-specific components. The use of liquid biopsy has shown great applications in screening and early detection of cancers; once diagnosed, it also permits for dynamic monitoring of cancer evolution.

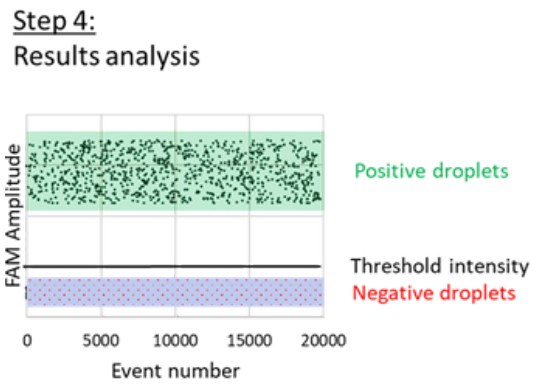
Today liquid biopsy is mainly based on the analysis of blood-circulating biomarkers including circulating tumor cells (CTC), cell-free circulating tumor DNA (ctDNA), and extracellular vesicles (such as exosomes). In particular, ctDNA has recently attracted high interest for cancer diagnosis, patient follow-up and treatment monitoring. Cancer specific genetic or epigenetic alterations can be detected in ctDNA. However, cancer biomarkers, such as ctDNA, are present in very low quantities in blood and their analysis thus requires highly sensitive and specific technologies.

Droplet-based microfluidic technology allows the encapsulation of biomarkers and their detection in droplets. Each droplet acts as an isolated reaction chamber where the high local concentration of sample allows for high-sensitivity and high throughput detection of the rare biomarkers of interest. The droplet-based microfluidic approach gives the ability to ensure quantitative, precise, fast, and low-cost detection of biomarkers that could be hardly detectable with most other approach. One of the most commonly used droplet-based microfluidic technique for cancer detection is the digital droplet Polymerase Chain Reaction (ddPCR).









ddPCR requires four steps: the encapsulation of the biomarkers in the droplets with PCR reagents , the DNA amplification through PCR, the detection of the ctDNA alterations via analysis of the droplet fluorescence, and the analysis of the results.

In the first step, the droplets are generated by using two immiscible phases, a continuous phase, and a dispersed phase. The continuous phase is commonly oil while the dispersed phase consists in an aqueous solution in which the biomarkers are soluble. The genetic material is therefore in the aqueous droplet dispersed in oil. In ddPCR, the droplets must be stable from their generation to their detection. To ensure the stability of the droplets through all the process, the use of a surfactant, such as FluoSurf™, is required.

After encapsulation, the droplets contain either wild-type ctDNA (healthy ctDNA), ctDNA with cancer specific alteration (cancer characteristic) or no genetic material. Following the partitioning of the biomarkers in the droplets, the droplets are collected and generally submitted to thermal-cycling, leading to the polymerase chain reaction (PCR) amplification of the ctDNA. During this step, the concentration of markers in the droplets is increased. The genetic alterations specific to cancer become detectable. Commonly, the detection of mutation of the DNA is made by fluorescence.

All the droplets contain a fluorogenic agent. During the incubation, the fluorogenic probe reacts only in the droplets containing ctDNA. It is converted into a fluorescent signal.
The empty droplets stay almost non-fluorescent and are considered as negative droplets whereas the droplets containing ctDNA become fluorescent and are defined as positive droplets.
Thanks to a wise choice of the fluorescent probes, the droplets containing altered DNA and the droplets containing wild-type DNA do not emit at the same wavelength and are distinguishable. The data are analyzed by plotting the fluorescence intensity versus the droplet event number to determine to concentration of cancer-specific (ie. mutated) DNA in the original sample giving access to highly precisequantification of biomarkers of interest.

To conclude, droplet-based microfluidics and ddPCR have demonstrated to be a highly sensitive and resolutive techniques for the analysis of ctDNA as a tool for early cancer detection through liquid biopsy.

To learn more about surfactants and other formulation products for droplet microfluidics, please visit www.emulseo.com

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