Opinion

Cancerous tissue is highly heterogeneous and cancer biomarkers vary across types of disease and stages of disease’s progression, this complicates cancer detection and identification at early stages [1]. The cancer identification of mutations present in an individual tumor often rely on analysis of biopsy or cytology samples, where only a small fraction of the tumor is analyzed, and may not provide a complete representation of tumor heterogeneity; these has significant disadvantages, because low abundance mutations relative to wild type DNA [2]. In addition, the sub clonal populations of cells within a tumor may contain a mutation that differs from the primary mutation, and the sub clonal mutation could be correlated to a prognosis and/or a response to personalized treatments [1]. Sequencing is still the gold standard for mutations identification, its cost is steadily decreasing as much effort is made to reduce the cost and also to improve the data interpretation in downstream analysis of next-generation sequencing [3], despite this, its use remains limited for the diagnostic in a routine laboratory. The detection of mutations is performed mainly by real-time quantitative PCR (qPCR) [4], but this technique presents limitations including preferential amplification of small fragments, production of chimeric sequences, the amplify all alleles with approximately equal efficiency comparable to their initial concentrations, and difficulty in detecting low abundance or poorly represented sequences [5].

In the last years, a technology called digital PCR (dPCR) has become commercialized. As in qPCR, fluorescent dyes are included in the DNA amplification reaction. However, unlike qPCR the amplification reaction in dPCR is divided into thousands of individual reactions prior to amplification. These partitioning can be achieved by using microwell plates, capillaries, oil emulsions, or arrays. Ideally, partitioning occurs such that each individual reaction mixture contains either a single target molecule or none at all [4]. The acquisition of data at reaction end point, and the number of positive (fluorescent) and negative partitions iscounted, the target copy number in the sample is calculated based on the number of positive and negative partitions [6]. The Poisson’s Law is used to accurately calculate the number of DNA targets per partition and the copy number in the original sample. These offers the advantage of quantify directly the absolute concentration of targets present in a DNA sample without the need for external calibrators [7], it is less susceptible to PCR inhibition and high background DNA levels in samples such as DNA isolated from FFPE biopsies [8]. The sensitivity is significantly higher than qPCR, the accuracy and precision of the assay improves by counting larger numbers of molecules individually.

These detection limits facilitate the detection of minor alleles, such as in circulating tumor DNA, with a relatively simple and non-invasive approach to monitoring disease recurrence, which requires a high sensitivity of mutation detection to provide effective therapies at the earliest stage of progression. Cases such as the detection of the BRAF V600E mutation as well as for follow-up monitoring to determine the treatment response in patients with malignant melanomas [9,10], the detection of mutated genes in liquid biopsies for metastatic colorectal cancer [11,12], the detection of minimal residual disease with
BCR-ABL translocations for lymphoproliferative disorders [14], all of them reflect the need tools to mutation detection highly sensitive, the which is relevant to for determine the treatment response. The dPCR technology, being promissory for the detection of mutations in the range of 0.001% of occurrence and maximized to enable transformational advances in cancer research, could be this such sought tool. However, the implementation of dPCR assays should be undertaken after some consideration, although dPCR has several specific advantages over qPCR, dPCR is not likely to replace all qPCR assays in the clinical laboratory. Also requires a precise standardization that varies from one platform to another.

References

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