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# Detection of the EGFR G719S mutation in Non-Small Cell Lung Cancer by non-invasive blood-based analyses using droplet digital PCR

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**Introduction:** Lung cancer (LC) is the most frequent malignant tumor and the most common cause of cancer death in the world today (1 out of 4 deaths). 87% of all cases of lung cancer are non-small cell lung cancers (NSCLC). In this group of tumors, several recurrent mutations have been reported, among which *EGFR* mutations appear in 15-20% of cases. Tumor biopsies are the gold standard method for detecting these mutations. However, they are spatially and temporary limited, as well as being an invasive technique often difficult to perform. In this context, liquid biopsy is a non-invasive alternative technique for the genetic study of the tumor. Due to its simplicity, it is possible to use liquid biopsies both to implement the most appropriate treatment and to monitor the patient, in order to detect the appearance of new mutations during cancer treatment. G719S mutation in the *EGFR* gene is a rare mutation (less than 4%) which produces a gain of function of the protein. The aim of this study was to develop a digital droplet PCR-based assay to accurately detect this mutation in liquid biopsy with a high sensitivity.

**Objectives:** The main objectives of the study were 1) to set-up a droplet digital PCR (ddPCR) assay for the non-invasive detection of G719S *EGFR* mutation in NSCLC patients; 2) to determine the limits of detection of the ddPCR assay for G719S mutation and 3) to compare COBAS® and ddPCR System for G719S quantification in plasma.

**Methods:** Blood samples were collected from 19 patients diagnosed with clinical stage IV A, B NSCLC according to the TNM Classification of Malignant Tumors. Then, plasma cfDNA was extracted with the Qiagen Circulating Nucleic Acids kit and quantified by QuantiFluor® dsDNA System. Finally, the mutational study of *EGFR* was carried out by digital droplet PCR (ddPCR) with the QX200 Droplet Digital PCR System with specific probes and primers. Statistical analysis was performed with IBM SPPS Statistics 22 and Graphpad Prism 5 software.

**Results:** We found that ddPCR detects mutation presence at 0.03% with a sensitivity of 30 mutant copies in a background of 10,000 wild-type copies. In the specificity analysis, low levels of G719S mutation were detected in healthy volunteers with a peak of 14,95 mutant copies per milliliter of plasma. In those patients whose tissue biopsy was positive for G719S mutation, mutant alleles could also be detected in cfDNA mutational analysis using both ddPCR and COBAS® System. Finally, when mutational status was studied using both genotyping techniques, higher mutant copies/ml in ddPCR correlated with higher Semiquantitative Index obtained by COBAS®.

**Conclusions:** Although tissue biopsies cannot be replaced due to the large amount of information they provide regarding tumor type and structure, liquid biopsy and ddPCR represent a new promising strategy for genetic analysis of tumors from plasma samples. In the present study, G719S mutation was detected in a highly sensitive manner, allowing its monitorization with a non-invasive technique.