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Title: Diagnostic utility of PNA-LNA PCR clamp method for detection of EGFR exon 19 deletions and exon 21 codon L858 mutations in NSCLC samples with low tumor cells content

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Body: PNA-LNA PCR clamp method demonstrates potent accuracy and ability to detect mutant alleles even if present in low fraction of cells, while direct sequencing has limited sensitivity. Our aim was to compare PNA-LNA PCR clamp diagnostic utility for detection of most common EGFR mutations in clinical specimen characterized by different tumor cell content. DNA samples were isolated from fresh frozen NSCLC resected tissues - (n=84), FFPE samples (n=51) or biopsy - FFPE (n=53) and cytology (n=23) materials. Samples were analyzed by PNA-LNA PCR clamp method and/or direct sequencing. In all analyzed samples exon 19 deletions (n=8), exon 21 L858R (n=14), L858P (n=1) and L858M+L861Q double mutation (n=1) were detected. Distribution of total n=24 detected mutations among groups characterized by different tumor cells content was: In samples with >50% tumor cells - 14/24 mutations were detected by both PNA-LNA PCR clamp and direct sequencing, in samples containing 10-50% tumor cells - 5/24 mutations by PNA-LNA PCR clamp while only 2/24 by direct sequencing, in materials with less than 10% - 2/24 mutations were detected by PNA-LNA PCR clamp only. Further analysis proved that sensitivity of mutation detection depended only on tumor cells content, regardless sample type or tissue formalin fixation. PNA-LNA PCR clamp method as compared to direct sequencing enables considerably more sensitive and reliable detection of EGFR exon 19 and 21 mutant alleles in resected, FFPE and cytology specimen with low cancer cell content.