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Title: Evaluation of PNA-LNA PCR clamp method sensitivity for detection of key EGFR gene exon 19 and exon 21 mutations

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Body: Highly-sensitive, robust and reliable diagnostic method is crucial for clinically useful detection of EGFR mutations in NSCLC. Due to low sensitivity, direct sequencing is restricted for samples containing at least 50% of tumor cells. PNA-LNA PCR clamp represents allele-specific approach to gene analysis and demonstrates high accuracy and ability to detect mutant alleles present in low fraction of tumor cells. Aim: To compare the in vitro sensitivity of PNA-LNA PCR clamp method versus direct Sanger sequencing in detecting EGFR exon 19 deletions and exon 21 L858R mutation. Methods: Mutated DNA was isolated from cell lines harboring endogenous exon 19 deletion or L858R mutation. Sensitivity of direct Sanger sequencing and PNA-LNA PCR clamp method was analyzed in serial dilutions of mutant allele intermixed with wild-type DNA. Results: PNA-LNA PCR clamp method reliably detected both exon 19 deletion and L858R down to 1% DNA admixture level. Direct sequencing presented considerably lower sensitivity detecting only down to 50% of mutated exon 19 allele in DNA mixture and down to 5% in samples with L858R mutant allele. Conclusions: PNA-LNA PCR clamp method is the highly sensitive tool for detection of EGFR activating mutations. It might be particularly useful in heterogenous samples with low content of mutant allelle, like biopsy material. Direct sequencing presents lower sensitivity, limited by type of mutation.