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Title: Validation of PNA-LNA PCR clamp assay for detection of EGFR exon 19 and 21 mutations in various types of clinical non-small cell lung cancer specimens

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Body: The aim of the study was to assess diagnostic reliability of PNA-LNA PCR clamp assay in EGFR mutations detection in different NSCLC samples. Evaluation was performed: (i) in reference NSCLC tissue FFPE samples (n=10), (ii) in comparison to direct sequencing in resected NSCLC tissue (n=199) and biopsy material specimens (n=179) characterized by different tumor cells content (TCC) and fixation [Table 1].

NSCLC samples characteristic.

Resected tissue		Biopsy material	
fresh-frozen	FFPE	FFPE	Cytology smear
84	115	115	64

[Table 1]

(i) PNA-LNA PCR clamp correctly detected all exon 19 deletions and L858R mutations in the reference FFPE materials, including those with meager TCC (5% and 10%). (ii) PNA-LNA PCR clamp method and direct sequencing presented high conformity (overall percent agreement, OPA=99%; Cohen's Kappa score of 0.94 (95% CI=0.9, 0.99) in n=100 samples with >50% TCC analyzed. (iii) In total of 378 samples

analyzed with PNA-LNA PCR clamp method, EGFR mutations were detected in 36 (9.5%). (iv) Only in 24 out of 36 (67%) mutation positive samples reevaluation with direct sequencing proved positive [Table 2]. PNA-LNA PCR clamp presented higher sensitivity in samples with TCC <50% (p=0.004).

Comparison of PNA-LNA PCR clamp vs direct sequencing EGFR mutation detection sensitivity in materials with different TCC.

	PNA-LNA PCR clamp	direct sequencing
≥50%	25/25 (100%)	22/25 (88%)
20<50%	6/6 (100%)	2/6 (33%)
≤20%	5/5 (100%)	0/5 (0%)
total	36/36 (100%)	24/36 (67%)

[Table 2]

PNA-LNA PCR clamp method proved its diagnostic utility and high sensitivity of EGFR exon 19 and 21 mutations detection, especially in samples with TCC lower than 50%.