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Title: Feasibility of EGFR mutation testing on EBUS-TBNA and bronchial biopsy samples obtained during routine practice

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Body: Introduction. Approximately 80% of NSCLC present with advanced disease, in whom the diagnosis is often based on small samples obtained during bronchoscopy. Aims. We aimed to evaluate the performance of bronchoscopic small tissue samples: (1) the percentage of tumour cells and quantity of DNA extracted; (2) the performance of EGFR mutation testing; (3) their feasibility compared to surgical samples. Methods. Between Sep 2010 and Dec 2012, we screened advanced stage non-squamous histology for EGFR mutations using Therascreen. All diagnostic bronchial biopsies (n=130; 4 biopsies per patient) and EBUS-TBNA samples (n=81; 4 needle aspirations brought in cell block), as well as surgical samples (n=67) were retrieved. Results. The median percentage tumour cells and quantity of DNA extracted was significantly higher in surgical vs bronchoscopic samples (80% vs 30% and 2.3µg vs 1.6µg, P<0.0001); no statistically significant difference was observed between EBUS-TBNA and bronchial biopsies.

Table 1. Tissue sample performance characteristics

	Surgical biopsy (n=67)	Bronchial biopsy (n=130)	EBUS-TBNA (n=81)
Area of Tumour			
Mean (SD)	66% (±31%)	32% (±22%)	36% (±31%)
Median (range)	80% (5-100)	28% (5-90)	30% (2-100)
Total amount of DNA			
Median (range)	2330ng (560-6240)	1700ng (250-7880)	1390ng (130-7520)
DNA control curve (Cp)			
Median Cp	29.4	29.1	29.0
Cp <32	96%	96%	88%

Cp >35	3%	1.5%	1%
EGFR mutation rate	12.3%	10.9%	12.3%

Although 25% of bronchoscopic samples had <10% tumour cells, an amount of DNA extracted <200ng and poor DNA quality (Cp>35) were observed in 1%. Conclusions. Bronchoscopic samples result in accurate EGFR mutation analysis in routine practice, provided a sufficient samples are taken.