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Original article

Validation of a next generation sequencing panel for detection of hotspot cancer mutations in a clinical laboratory

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ABSTRACT

Recent advances in sequencing technologies have enabled us to scrutinize the versatile underlying mechanisms of cancer more precisely. However, adopting these new sophisticated technologies is challenging for clinical labs as it involves complex workflows, and requires validation for diagnostic purposes. The aim of this work is towards the analytical validation of a next generation sequencing (NGS) panel for cancer hotspot mutation analysis.

Characterized formalin-fixed paraffin-embedded (FFPE) samples including biopsy specimens and cell-lines were examined by NGS methods utilizing the Ion Torrent™ Oncomine™ Focus DNA Assay and the PGM™ platform. Important parameters for somatic mutations including the threshold for differentiation of a positive and a negative result, coverage, sensitivity, specificity, and limit of detection (LoD) were analyzed.

Variant calls with coverage of <100x were found to be inaccurate. The limit of detection for identifying hotspot mutations was determined to be 4.3%. The sensitivity and specificity of the method were 96.1% and 97.8% respectively. No statistically significant difference was found between different gene targets in terms of performance of hotspot frequency measurement for the subset tested. In every validation study, the number of samples, the manner of sample selection, and the number and type of variants play a role in the outcome. Therefore, these parameters should be assessed according to the clinical needs of each laboratory undertaking the validation.

1. Introduction

A major goal in tumor pathology is to predict the behavior of neoplasms and their response to different treatment modalities [1]. However, tumors defy prediction to some extent because of the complexity and versatility of underlying mechanisms leading to evasion, plasticity and heterogeneity [2–4]. Advances in molecular and immunophenotyping techniques have enabled us to glean more data to add to clinical and morphological information to come to a more precise classification. These methods though will soon be deemed inadequate especially in the era of modern oncology, targeted therapy, and personalized cancer care [5]. During the past decade, advances in technology have allowed for next generation sequencing (NGS) platforms to become commonly available for cancer genome analysis.

Massive parallel sequencing of numerous small DNA fragments (targets) is the cornerstone of most NGS systems [6]. Overlapping sequences are later aligned by software to assemble the sequences of larger regions, with the added capability of quantification of target variations [6]. Variants that are detected by NGS include: single and multiple nucleotide variation (SNV/MNV); copy number variation (CNV); deletions-insertions (indels); fusions, and epigenetic abnormalities [7,8]. Moreover, NGS is capable of reading large genomic regions with only small amounts of sample [7,8]. These capabilities are attractive to molecular pathology laboratories and are quickly being exploited.

The utility of NGS in clinical labs in the field of cancer pathology falls into four categories according to the size of the target region to be sequenced in increasing order: 1) hotspot variations; 2) cancer genes; 3) whole exome; and 4) whole genome [9,10]. Other advantages of NGS platforms include having a high capacity for

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Table 1
Analytical controls with variant frequencies.

GENE	EGFR					KRAS						NRAS			BRAF		PIK3CA			KIT	CTNNB1		IDH1	JAK2	MAP2K1
VARIANT TARGET	T790M	L585R	ΔE746 – A750	L861Q	G719S	G12C	G12R	G12A	G12D	G13D	Q61H	G12V	G12D	Q61K	V600K	V600E	H1047R	E453K	E545K	D816V	S33Y	S45del	R132C	V617F	P124L
SAMPLE ID																									
HDx FFPE EGFR 5% Multiplex Control (HD300)	5%	5%	5%	5%	5%											66.7%	50%								
HDx FFPE KRAS 5% Multiplex Control (HD301)					33.3%				5%	5%	5%	5%		5%							50%				
HDx FFPE Quant Multiplex Control (HD200)	1%	3%	2%		24.5%				6%	15%				12.5%		10.5%	17.5%		9%	10%	33%	10%			
HDx FFPE KRAS G12C control (HD256)					ND	50%																			
HDx FFPE KRAS G12D control (HD204)					ND				50%																
HDx FFPE NRAS G12D control (HD745)					ND								50%												
HDx gDNA Tru-Q Ref Std 1 5% (HD7258)	4.2%				16.7%		5%	5%		5%				5%	4%	8%	30%						5%	5%	5%
HDx gDNA Tru-Q 0 Ref Std (HD752)	0	0	0	0	16.70%	0	0	0	0	25%	0	0	0	0	0	8%	30%	0	0	0	0	0	0	0	0
HDx FFPE EGFR WT (HD141)	0	0	0	0	0											ND	ND								
HDx FFPE KRAS WT (HD135)					33%	0	0	0	0	0	0										ND	ND			

ND = variant present, but frequency not determined.