Comparison between two different next generation sequencing platforms for clinical relevant gene mutation test in solid tumours

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ABSTRACT

In the present study, we analysed 44 formalin fixed paraffin embedded (FFPE) from different solid tumours by adopting two different next generation sequencing platforms: GeneReader (QIAGEN, Hilden, Germany) and Ion Torrent (Thermo Fisher Scientific, Waltham, Massachusetts, USA). We highlighted a 100% concordance between the platforms. In addition, focusing on variant detection, we evaluated a very good agreement between the two tests (Cohen's kappa=0.84) and, when taking into account variant allele fraction value for each variant, a very high concordance was obtained (Pearson's r=0.94). Our results underlined the high performance rate of GeneReader on FFPE samples and its suitability in routine molecular predictive practice.

INTRODUCTION

In the last decade knowledge on cancer's development had considerable moved steps forward, helping the improvement of clinical decision making in the patient management. Cancer is a complex and heterogeneous disease characterised by genomic aberrations involving a subset of genes. In this setting, predictive molecular pathology may allow physician to choose the best treatment option for any single cancer patient by analysing predictive biomarkers, in order to employ a 'tailored therapy' approach.[1 2] Nonetheless, a not negligible percentage of advanced stage patients do not undergo molecular analysis due to the limited amount of tissue available.[3] In this scenario a high number of biomarkers has been approved in clinical practice or under investigation. The latest international guidelines suggested in order to cover the largest number of clinical relevant biomarkers the adoption of next generation sequencing (NGS) platforms with expanded panels.[4–7] NGS allows the detection of multiple genetic alterations in different patients, simultaneously.[8] Different NGS platforms are commercially available.[8]

In this study, we compare the results obtained by two different NGS platforms, GeneReader (QIAGEN, Hilden, Germany) and Ion Torrent (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in different solid tumours, in order to introduce GeneReader NGS platform in the clinical diagnostic molecular laboratory at S. Stefano Hospital-Prato, integrated in the Complex Unit of Pathological Anatomy Empoli-Prato, Azienda USL Toscana Centro, Italy.

METHODS

Study design

NGS analysis was performed on 44 formalin fixed paraffin embedded (FFPE) samples (19 metastatic colorectal cancer, 24 non-small cell lung cancer, one skin cancer). These samples were previously analysed with the laboratory developed and validated SiRe panel on Ion Torrent platform (from here named 'reference test') at the University of Naples Federico II, and subsequently analysed by the QIAact Actionable Insights Tumor Panel V2 on GeneReader (QIAGEN, from here named 'index test') at the Complex Unit of Pathological Anatomy Empoli-Prato, S. Stefano Hospital-Prato, Azienda USL Toscana Centro. In order to evaluate intrarun and inter-run reproducibility, four samples were tested twice, for a total of 48 evaluations.

Written informed consent was obtained from all patients and documented in accordance with the general authorisation to process personal data for scientific research purposes from 'The Italian Data Protection Authority' (http://www.garanteprivacy.it/web/guest/home/docweb/-/docwebdisplay/export/2485392). All information regarding human material was managed using anonymous numerical codes, and all samples were handled in compliance with the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/).

Sample preparation and DNA extraction

First of all, an experienced pathologist selected representative tumour tissue areas (>10% neoplastic cells) on H&E stained slides. To enrich for tumour cells, manual microdissection of the tumour tissue selection was carried out from up to four unstained sections by using a sterile scalpel. DNA extraction was performed with QIAamp DNA Mini Kit (Qiagen, Crawley, West Sussex, UK), following the manufacturer's instructions, resuspending the DNA in 30 µL of RNAsi/DNAsi free water (Ambion, Thermo Fisher Scientific). In order to assess DNA quantity (ng/µL) and quality (in term of DNA integrity number), 1 µL of resuspended DNA for each sample was analysed by using genomic DNA screentape assay on the 4200 TapeStation system (Agilent Technologies, Santa Clara, California, USA) with a proprietary software, prior to proceed to NGS library preparation.

NGS assays

The reference test is able to detect actionable mutations in seven different genes: Kirsten rat

Table 1 Sequencing run parameters

Samples	Average reads target regions	Average median coverage regions of interest	Base positions coverage ≥200× (%)	Base positions coverage ≥500× (%)
44	1 602 540	8931	99.78	98.96

sarcoma viral oncogene homolog (*KRAS*), neuroblastoma RAS viral oncogene homolog (*NRAS*), V-raf murine sarcoma viral oncogene homolog B (*BRAF*), epidermal growth factor receptor (*EGFR*), KIT proto-oncogene, receptor tyrosine kinase (*KIT*), platelet derived growth factor receptor alpha (*PDGFRA*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*).[9] Ion Torrent analysis was carried out as previously described.[10–12]

The index test allows the analysis of hotspot regions of 12 genes (*NRAS*, anaplastic lymphoma kinase (*ALK*), raf-1 proto-oncogene, serine/threonine kinase (*RAF1*), *PIK3CA*, *PDGFRA*, *KIT*, oestrogen receptor 1 (*ESR1*), *EGFR*, *BRAF*, *KRAS*, Erb-B2 receptor tyrosine kinase 2 (*ERBB2*), Erb-B2 receptor tyrosine kinase 3 (*ERBB3*)), simultaneously.[13 14] GeneReader analysis was carried out as previously described.[13 14]

Index test sequencing performance parameters

Index test run parameters were evaluated for each FFPE sample analysed. In particular, we focused the attention on the average number of reads in target regions, the average of median coverage in regions of interest, the average of percentage of base positions in regions of interest with coverage ≥200× and ≥500×.

Comparison between reference and index test

The set of samples analysed was divided into two groups by using the reference test as the gold standard, based on the absence/presence of at least one significant variant (variant allele fraction (VAF) >5%) in at least one of the genes shared by both panels. After the concordance rate between the two tests was assessed. Subsequently, we analysed the variants detected by both assays, and the level of concordance was evaluated. In addition, sensitivity, specificity and overall agreement (OA) were reported.

Statistical analysis

The Cohen's kappa coefficient (k) is used to measure the degree of agreement between reference and index results.

The Pearson correlation coefficient (r) was used to measure the linear correlation between two independent variables (in this study to correlate the allelic frequencies percentage between two variants).

RESULTS

GeneReader NGS performance

For the 44 analysed samples, an average number of reads in target regions of 1 602 540 was observed, with an average of median coverage in regions of interest of 8931. The average of percentage of base positions in regions of interest with coverage ≥200× and ≥500× was 99.78% and 98.96%, respectively (table 1).

Comparison between reference and index test

Based on the results obtained with the reference test, six wild type and 38 mutated samples were reported (table 2). No false positive results emerged with the index test among the wild type group. Similarly, in the remaining 38 positive samples the presence of at least one gene alteration in the shared genes was reported. We highlighted a 100% concordance between reference and index test to correct classify the samples in wild type or positive categories.

As far as number of variants detected was concerned, the reference test identified a total of 52 significant gene alterations among the 38 positive samples (table 3). Interestingly, the index test was able to correct recognise 51 (98%) out of 52 mutations. Moreover, the index test identified an additional *EGFR* exon 19 p.D761Y resistance mutation that was missed by the reference test. On the overall, sensitivity, specificity and OA were 98.0% (95% CI 89.88 to 99.66), 85.7% (95% CI 48.70 to 97.43) and 96.6%, respectively (table 3).

The agreement between the two tests was classified as very good (Cohen's kappa=0.84). We collected 50/51 (98%) VAF values and the concordance for each variant was very high (Pearson's r=0.94, figure 1).

DISCUSSION

Our data support the validity of GeneReader analysis on routine FFPE samples. By comparing the results with those obtained with Ion Torrent platform using a custom panel as the gold standard, a 100% concordance was obtained. In particular, no false positive results were reported with the index test among the six wild type samples. In addition, in all 38 positive instances the presence of at least one gene alteration in the shared genes was confirmed. Similarly, focusing on variant detection, the index test showed a sensitivity, specificity and OA of 98.0% (95% CI 89.88 to 99.66), 85.7% (95% CI 48.70 to 97.43) and 96.6%, respectively. Interestingly, only one variant was not identified by the index test, whereas an additional *EGFR* exon 19 p.D761Y resistance mutation was detected by the index test.

Due to the increasing number of tested genes for predictive purposes in solid tumours, NGS plays a key role in molecular predictive pathology laboratories. NGS allows the simultaneously analysis of multiple gene targets for different patients with

Table 2 Concordance of reference and index tests referring to MUT+ and MUT– samples

	REF test MUT+	REF test MUT–	Total
IND test MUT+	38	0	38
IND test MUT–	0	6	6
Total	38	6	44

IND test, QIAact Actionable Insights Tumor Panel V2 on GeneReader (QIAGEN) platform; MUT+, mutated cases; MUT–, wild-type cases; REF test, SiRe panel on Ion Torrent (Thermo Fisher Scientific) platform.

Table 3 Concordance of reference and index tests in relation to different gene variants

	REF test+	REF test–	Total
IND test+	51	1	52
IND test–	1	6	7
Total	52	7	59

IND test, QIAact Actionable Insights Tumor Panel V2 on GeneReader (QIAGEN) platform; REF test, SiRe panel on Ion Torrent (Thermo Fisher Scientific) platform.

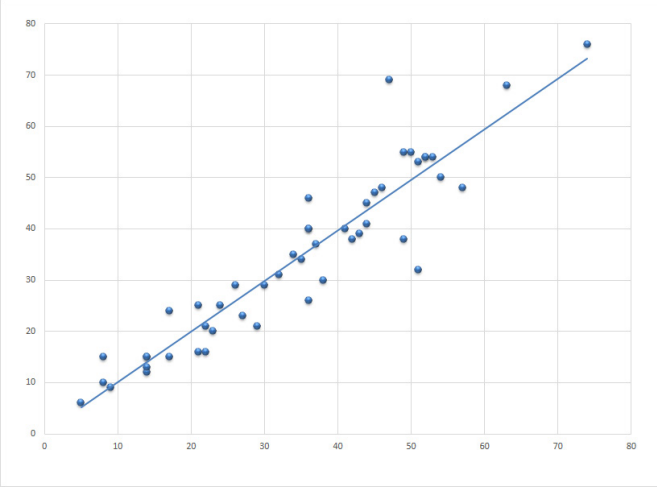

Figure 1 Variant allele frequency (VAF) correlation between index and reference results.

a reduction of costs and turnaround time.[15] However, a critical point in particular when considering these high sensitivity technologies is represented by a careful in-house validation.[6] For this reason we compared the results obtained by QIAGEN platform with those obtained with a consolidated laboratory test based on the use of a custom panel (SiRe) on Ion Torrent (Thermo Fisher Scientific) system. We identified a very good agreement between the two tests (Cohen's kappa=0.84) and, when taking into account VAF for each variant a very high concordance was obtained (Pearson's r=0.94).

In conclusion, all these results underlined the high-performance rate of GeneReader on FFPE samples and its suitability in routine molecular predictive practice. In addition, we strongly evidenced the high degree of concordance of these two different NGS systems, and underlined the necessity of orthogonal techniques in order to confirm doubtful results.

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