Background  An important aspect of ctDNA NGS assay validation is comparison of variants detected in plasma vs those found in tissue. Here we present critical examination of plasma to tissue concordance by cancer type using Illumina’s cell-free ctTSO500 commercial liquid biopsy assay. In order to present an expected solid-to-liquid concordance we followed data from Prestinger et al., Verma et al., LoCoco et al., and O’Rourke et al.1–4

Methods  We examined 124 cases of colon, gastric, uterine, lung, prostate, bladder, larynx, ovary, cervix, breast and skin/melanoma (in descending order of prevalence). We labeled oncogenic/likely oncogenic variants using GenomOncology Knowledge Management System and we filtered to 1% variant allele fraction for both SNV’s and indels, using Illumina’s LocalApp2.1 on Dragen pipeline.

Results  We found in uterine cancers we detected 65.9% of the SNV/indel variants in plasma that were also in FFPE, 47% in colon cancer, 67% in gastric cancer, 56% in bladder cancer, 51% in larynx cancer, and 41% in prostate. This tracks generally with patients who have stage III/IV cancer, pre-treatment, with biopsy and blood taken within 15 days.

To establish gold-standard to trust this performance, we require another certified lab to conduct an identical analysis using the ctTSO500 assay. We selected 2 orthogonal labs, and sent 18 samples to lab1 and 24 samples to lab2, to compare our in-house results for TMB, MSI, CNV, SNV and Indel sensitivity. Upon comparison of CNV and TMB the concordance between in-house results and lab2 were >90% across the board, while concordance between lab2 and lab1 for a single sample was <80% for TMB, MSI and CNV, and <90% for the raw SNV and indels at 0.5% VAF. When comparing the 18 lab1 samples to in-house data, and the 24 lab2 samples to in-house data, we found that lab1 had consistently lower concordance to in-house results, while lab2 had near-perfect concordance. When using orthogonal data, it is imperative that the lab performing validation using orthogonal data create a fixed standard against which data quality is compared.

Conclusions  We set our quality metrics to a level that we felt best achieved the expected performance advertised by Illumina. Quality management and regulatory issues should be linked using data provided by bioinformatics groups and the sequencing lab. We found that the ctTSO500 liquid biopsy from Illumina was reproducible, sensitive and of practical importance to patients who cannot or do not wish to provide a solid biopsy for cancer screening.

References


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