xF+ Validation

The non-invasive Tempus xF+ liquid biopsy assay detects cell-free DNA (cfDNA) in blood specimens of advanced solid tumor patients. The assay covers clinically relevant exons and select non-coding regions in 523 genes (including 14 genes for insight into HRR gene-mutated mCRPC) spanning ~1.8 Mb of genomic space and is capable of detecting mutations in four variant classes: single nucleotide variants (SNVs) and insertion-deletions (INDELs) in 523 genes; copy number variants (CNVs): gains in 7 genes & losses in 2 genes; and translocations (gene rearrangements) in 10 genes (including NTRK 1/2/3 and FGFR 1/2/3). 114 genes will be sequenced with enhanced coverage and lower limit of detection (0.25% VAF for SNVs). Select variants may be reported at VAFs lower than 0.25% at pathologist discretion[1]. Blood Tumor Mutational Burden (bTMB) as well as detected Microsatellite Instability High (MSI-H) will be reported. Tempus xF+ is designed to capture clinically relevant biomarkers for solid tumors.

CAP/CLIA validation of the Tempus xF+ panel focused on the detection of actionable oncologic variants including resistance mutations in plasma. The assay requires two 8.5 mL Streck tubes of peripheral blood. Clinical sequencing is performed to >5000x and >1500x unique coverage for enhanced and additional regions, respectively.

When comparing clinical samples run on an orthogonally validated assays, xF+ has 90% sensitivity for single nucleotide variants (SNV) in the enhanced region at or above 0.25% variant allele fraction (VAF), ≥95% sensitivity for INDELs in the enhanced region at or above 1% VAF, 84% sensitivity for copy number variants (CNVs), >99% sensitivity for translocations (gene rearrangements), 90% sensitivity for MSI-H detection, and 79% sensitivity for bTMB when median TMB VAF is at or above 2.5% (Table 1).

Not intended for:

- Hematologic malignancies
- Early stage (stage I/II) cancers
- Primary CNS malignancies

TABLE 1

Biomarker	Sensitivity	Specificity
Enhanced SNVs (≥ 0.25% VAF)	90%	>99%
Nonenhanced SNVs (≥ 1% VAF)	>99%	>99%
Enhanced INDELs (≥ 1% VAF)	≥95%	>99%
Nonenhanced INDELs (≥ 2% VAF)	88%	>99%
CNVs	84%	92%
Translocations	100%	100%
MSI-H	90%	100%
bTMB	79%	100%

The sensitivity of xF+ at different variant types and different VAFs is shown in Table 2, and was determined by comparing positive control samples with ddPCR validated variants.

TABLE 2

Variant Type[2]	0.25% VAF	0.50% VAF	1% VAF	5% VAF
Enhanced SNV	99%	100%	100%	100%
Enhanced INDEL	85%	95%	100%	100%
CNV	NA	NA	100%	100%
Translocations	NA	79%	94%	100%

1 Sensitivity of detecting variants with VAFs lower than 0.25% may be lower than values shown in Table 2.

2 Sensitivity established using reference materials