

TEMPUS xT (v3) VALIDATION

The Tempus xT next generation sequencing assay is designed to detect actionable oncologic targets by sequencing tumor samples with matched normal saliva or blood samples, when available. The third version of the xT assay (v3) covers 648 genes spanning ~3.6 Mb of genomic space. From DNA sequencing, somatic and incidentally detected germline single nucleotide variants (SNVs), insertions and deletions (indels), copy number variants (CNVs) and translocations in 23 genes are detected, along with two promoter regions (PMS2 and TERT) and 239 sites to determine microsatellite instability status. From RNA-seq, gene fusions (translocations) are detected in an unbiased and comprehensive manner, which allows matching to all fusion targeting FDA approved drugs and drugs in clinical trials. Tumor mutational burden (TMB) and Microsatellite Instability (MSI) status are also reported. Some viral sequences, such as HPV and EBV, may be reported as a diagnostic or prognostic insight when deemed appropriate by our Pathologists.

CAP/CLIA validation of the Tempus xT panel focused on actionable oncologic variants. The assay requires specimens with a tumor content of 20% post macrodissection (30% for MSI status). For solid tumors, an FFPE tumor sample is sequenced along with a matched normal blood or saliva sample. For circulating hematologic malignancies, a blood or bone marrow sample is sequenced. Clinical sequencing is performed at an average of 500x depth of coverage for tumor specimens and 150x for normal specimens. Performance specifications are listed in Table 1 below. These results establish high sensitivity and specificity for the Tempus xT assay (v3).

The xT assay is used across a diverse set of clinical settings including leading academic centers, NCI designated cancer centers, hospital networks and community hospitals.

TABLE 1: PERFORMANCE SPECIFICATIONS

Variant Class	Limit of Detection	Sensitivity (%)	Specificity (%)
Single Nucleotide Variants	5% VAF	96.6	99.95
Insertions and Deletions	10% VAF	93.4	99.99
Copy Number Alterations	30% tumor purity; loss—0 copies; gain—8 copies	94.7	99.99
Rearrangements/Fusions*	10% tumor purity	99.9	99.99
Microsatellite Instability Status	30% tumor	99.9	99.9

* Utilizing both DNA and RNA sequencing