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 fourth version of the xT assay (v4) 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 22 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 association with fusion targeting FDA-approved therapies and investigational therapies in clinical trials. Tumor mutational burden (TMB) and Microsatellite Instability (MSI) status are reported. Whole transcriptome RNA expression counts are analytically validated. 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 (minimum 30% for MSI status). For solid tumors, an FFPE tumor sample is sequenced along with a matched normal blood or saliva sample (when available). For circulating hematologic malignancies, a blood or bone marrow sample is sequenced. Clinical sequencing is performed to 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 (v4) assay.

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	98.2	99.95
Insertions and Deletions	5% VAF	91.8	99.99
Copy Number Alterations	Gain—30% tumor purity; loss—40% tumor purity; gain—8 copies; loss—0 copies	92.0	99.99
Rearrangements/Fusions*	30% tumor purity	91.7	99.9
Microsatellite Instability Status	30% tumor purity	99.9	99.9

** Utilizing both DNA and RNA sequencing*