Tempus xE is a whole exome next-generation sequencing assay that analyzes the entire coding region (exome) of the patient's genome, combined with whole transcriptome RNA sequencing. This is done as a tumor:normal matched assay with a tumor and normal specimens sequenced to an average depth of 250x and 150x, respectively. Whole transcriptome RNA-seq is 50 million reads. It encompasses 19,396 genes covering ~39 Mb of genomic space and is optimized for formalin fixed paraffin embedded (FFPE) tissue samples. The FFPE tumor tissue is matched to a normal blood or saliva sample to ensure fidelity of somatic variant calling. The xE assay identifies actionable oncogenic variants. From DNA sequencing, somatic and incidentally detected germline single nucleotide variants (SNVs), insertions and deletions (indels), and copy number variants (CNVs) are reported. From RNA-seq, gene fusions (translocations) are detected in an unbiased and comprehensive manner. In addition, tumor mutational burden (TMB) is reported. Microsatellite Instability (MSI) status has not been clinically validated with the Tempus xE assay, but is available in the portal as a research use only metric.

CAP/CLIA validation of the Tempus xE assay focused on actionable oncogenic variants. The assay requires specimens with tumor content of at least 40% post macrodissection. Performance specifications are listed in Table 1 below as compared to internally developed orthogonal tests. These results establish high sensitivity and specificity for the Tempus xE assay.

TABLE 1: PERFORMANCE SPECIFICATIONS

Variant Class	Limit of Detection	Sensitivity (%)	Specificity (%)
Single Nucleotide Variants	10% VAF	98.8	>99.9
Insertions and Deletions	10% VAF	97.9	99.9
Copy Number Alterations	30% tumor purity; loss—0 copies; gain—8 copies	87.0	>99.9
Rearrangements/Fusions*	N/A	>99.9	>99.9

** From RNA sequencing only*