HRD Validation

Tempus HRD is a laboratory developed test available as an additional test for patients who are tested with Tempus xT or Tempus xR. For ovarian and breast cancer, the Tempus HRD test (version 2) provides a result based on DNA genome-wide loss-of-heterozygosity (GWLOH) or evidence of biallelic BRCA1 or BRCA2 loss from the xT test. For patients with other cancers in which there is no established or accepted method for HRD measurement, Tempus HRD provides a whole transcriptome RNA expression score using data from the xR test. The RNA version uses mRNA expression to predict the probability that a tumor's gene expression profile correlates with well characterized benchmarks of the HRD phenotype, such as biallelic BRCA loss.

The DNA-based method (HRD-DNA) uses the GWLOH biomarker threshold and biallelic loss-of-function alterations in *BRCA1* or *BRCA2* to determine HRD status. GWLOH is determined by the length-weighted percentage of probed regions with LOH by the Tempus copy number calling algorithm (CONA). The GWLOH threshold was determined as the threshold that best distinguished the BRCA-biallelic samples from the HRR-WT samples, measured by F1-score*. GWLOH is considered positive for HRD at > 21% for breast cancer and > 17% for ovarian cancer. The performance of the HRD-DNA method for breast and ovarian cancers are detailed in Table 1 below.

TABLE 1: HRD-DNA MODEL PERFORMANCE

	Breast Cancer	Ovarian Cancer
Sensitivity	100.0%	92.1%
Specificity	96.3%	100.0%
Positive predictive value	96.7%	100.0%
Negative predictive value	100.0%	85.7%

The RNA-based method (HRD-RNA) uses a logistic regression model, trained on normalized RNA-seq gene counts (~20,000 genes) and BRCA status from tens of thousands of patients. Similar to the approach taken with the GWLOH-based HRD-DNA assay discussed above, for HRD-RNA, a pan-cancer threshold of HRD based on analytical performance (maximum F1-score*) was trained and validated to distinguish biallelic BRCA-positive from BRCA-negative samples. The Tempus HRD-RNA test outputs an HRD score between 0 to 100, with ≥50 being the cutoff for HRD positivity, while samples with RNA-HRD scores below 50 will receive a result of HRD-Not Detected.

HRD-RNA was evaluated on its ability to predict BRCA1/2 status. BRCA-positive status was defined by a biallelic loss of BRCA1 or BRCA2; BRCA wild-type was defined as no evidence of any alteration in BRCA1 or BRCA2. The HRD-RNA model was evaluated for cancer types that included at least 3 BRCA-biallelic samples in the evaluation set, and achieved the performance detailed in Table 2 below.

TABLE 2: HRD-RNA MODEL PERFORMANCE

	CRC	NSCLC	Pancreatic	Prostate
Sensitivity	20.0%	56.0%	53.0%	85.0%
Specificity	100.0%	99.0%	100.0%	98.0%
PPV	100.0%	71.0%	100.0%	92.0%
NPV	97.0%	98.0%	93.0%	96.0%

TABLE 3: HRD MODEL SCORING METHODS AND THRESHOLDS

Feature	DNA-Based Version	RNA-Based Version
Calculated Score	Genome Wide LOH **OR** Evidence of biallelic loss of BRCA1 or BRCA2	HRD score, a logistic equation calculated using all gene expressions in the transcriptome
Threshold values	>21% for breast cancer; >17% for ovarian cancer	≥50 for all other cancers
Thresholds optimized for	F1 Scores*	

For more information on the validation and performance of the Tempus HRD algorithm, please refer to the validation manuscript (Leibowitz BD, Dougherty BV, Bell JSK, et al. Validation of genomic and transcriptomic models of homologous recombination deficiency in a real-world pan-cancer cohort. *BMC Cancer.* 2022;22(1):587).

**F1 is the harmonic mean of precision and recall, which weighs both sensitivity and specificity equally.*

Tests run since January 2022 used the HRD V2 test.