Tempus HRD is a laboratory developed test available in conjunction with Tempus xT Tumor + Normal Match or Tempus xR. For ovarian and breast cancer, the Tempus HRD test 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.

HRD-DNA

GWLOH is determined by the number of basepairs with LOH (excluding regions of aneuploidy with LOH that span > 80% of a chromosome arm) divided by the total number of basepairs among DNA segments across the genome inferred by the Tempus copy number calling algorithm (excluding X and Y chromosomes and filtered regions).

The HRD-DNA model was trained on a cohort that is representative of patients eligible for testing using an expanded logic for the HRR wild-type (WT) group than was described previously in Leibowitz et al[1] (see training labels for details) and performs in close alignment with population frequency in the literature.[2-6] The GWLOH threshold was established as the threshold determined to best distinguish the BRCA-biallelic loss samples from the HRR WT samples in addition to other clinically relevant metrics (as defined in Table 1). GWLOH is considered positive for HRD at > 8.5% for breast cancer and > 8.0% for ovarian cancer; all samples with BRCA biallelic loss are considered positive regardless of GWLOH. The sensitivity of the HRD-DNA method for Breast and Ovarian cancers at predicting BRCA biallelic loss are detailed in Table 1 below.

TABLE 1: HRD-DNA MODEL PERFORMANCE

	Breast Cancer	Ovarian Cancer
HRD score definition	Genome Wide LOH	Genome Wide LOH
Threshold values	8.5%	8.0%
Thresholds tuned on	Sensitivity, specificity, HRD prevalence among Ovarian and Breast cancers[2-6] and among CCNE1 amplified Ovarian cancer[7-9]	
Sensitivity	83.3%	90.0%
Training labels		
HRR WT: Samples with no detected pathogenic mutations, fusions, or biallelic loss in BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, and RAD54L and no gene-level LOH + low gene expression in BRCA1 or RAD51C.		
BRCA1/2 Biallelic loss: Samples with (a) homozygous deletion, (b) a pathogenic germline or pathogenic somatic mutation with overlapping LOH of the other allele, or (c) a co-occuring pathogenic germline and pathogenic somatic mutation in BRCA1 or BRCA2.		

For more information regarding the HRD-DNA model please reach out to **support@tempus.com**.

HRD-RNA

The RNA-based method (HRD-RNA) uses a logistic regression model, trained on mRNA expression (~20,000 genes) and BRCA status from tens of thousands of patients. A pan-cancer threshold of HRD based on analytical performance (maximum F1-score*) was trained and validated to distinguish biallelic BRCA-positive from HRR WT (as defined in Table 2) 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 HRD-RNA scores below 50 will receive a result of HRD-Not Detected.

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
HRD score definition	HRD-RNA score is a logistic equation calculated using all gene expressions in the transcriptome			
Threshold value	≥50 for solid cancers outside of Breast and Ovarian			
Threshold tuned on	F1 score: harmonic mean of precision and recall, which weighs both sensitivity and specificity equally.			
Sensitivity	20.0%	56.0%	53.0%	85.0%
Training labels				
HRR WT: Samples with no detected pathogenic mutations, including variants of unknown significance (VUS), fusions, copy loss, or LOH in BRCA1, BRCA2, CDK12, PALB2, RAD51B, RAD51C, or RAD51D.				
BRCA1/2 Biallelic loss: Samples with (a) homozygous deletion, (b) a pathogenic germline or pathogenic somatic mutation with overlapping LOH of the other allele, or (c) a co-occuring pathogenic germline and pathogenic somatic mutation in BRCA1 or BRCA2.				

For more information on the validation and performance of the Tempus HRD-RNA algorithm, please refer to the validation manuscript.[1]

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