Tempus HRD is a laboratory developed test available in conjunction with Tempus xT CDx 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 an 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 (see training labels for details) and performs in close alignment with population frequency in the literature.[1-5] 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[1-5] and among CCNE1 amplified Ovarian cancer[6-8]	
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 data using samples with training labels defined with DNA data from xT on the same sample (as defined in Table 2) from tens of thousands of patients. The Tempus HRD-RNA test is based on 1660 genes and outputs an HRD score between 0 to 100, with cancer cohort specific cutoffs for HRD positivity.

Status may be reported as Positive, Not Detected, or Indeterminate. The algorithm classifies tumors as 'Positive' if the gene expression profile resembles the transcriptomic profile of tumors with BRCA1/2 biallelic loss (HRD). The algorithm classifies tumors as 'Not Detected' if the gene expression profile resembles the transcriptomic profile of tumors that are homologous recombination proficient (HRP) based on wild-type status of multiple homologous recombination repair genes.

The HRD-RNA model was evaluated in a held-out validation set in Pancreatic, Prostate, and 35 other solid cancers and performs in close alignment with population frequency in the literature,[2,9] as detailed in Table 2 below.

TABLE 2: HRD - RNA MODEL PERFORMANCE

	Pancreatic	Prostate	All other cancers
HRD score definition	The HRD-RNA score is calculated by processing gene expression values through a 1,660 gene logistic regression model trained to predict biallelic *BRCA1/2* loss.		
Threshold value	The thresholds used to determine HRD status are cancer cohort specific. An Indeterminate zone was created to account for test result variability near the threshold.		
Threshold tuned on	Sensitivity, specificity, HRD prevalence among individual cancer types[2,9]		
Sensitivity	82%	82%	54%
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 on the validation and performance of the Tempus HRD-RNA algorithm, please refer to the validation abstract.[10]

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