Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K241868

B Applicant

Tempus AI, Inc.

C Proprietary and Established Names

xR IVD

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PZM	Class II	21 CFR 866.6080 - Next Generation Sequencing Based Tumor Profiling Test	Pathology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Rearrangements in BRAF and RET using RNA isolated from formalin-fixed paraffin embedded (FFPE) tissue specimens from previously diagnosed cancer patients with solid malignant neoplasms.

C Type of Test:

Next-generation sequencing tumor profiling test

III Intended Use/Indications for Use:

A Intended Use(s):

The Tempus xR IVD assay is a qualitative next generation sequencing-based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of rearrangements in two genes, using RNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens from patients with solid malignant neoplasms. Information provided by xR IVD is intended to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with previously diagnosed solid malignant neoplasms. Results from xR IVD are not intended to be prescriptive or conclusive for labeled use of any specific therapeutic product.

B Indication(s) for Use:

Same as above

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For *in vitro* diagnostic use Only

D Special Instrument Requirements:

Illumina® NovaSeq 6000 platform (qualified by Tempus)

IV Device/System Characteristics:

A Device Description:

xR IVD is a next generation sequencing (NGS)-based assay for the detection of alterations from RNA that has been extracted from routinely obtained FFPE tumor samples. Extracted RNA undergoes conversion to double stranded cDNA and library construction, followed by hybridization-based capture using a whole-exome targeting probe set with supplemental custom Tempus-designed probes. Using the Illumina NovaSeq 6000 platform, qualified by Tempus, hybrid-capture–selected libraries are sequenced, targeting > 6 million unique deduplicated reads. Sequencing data is processed and analyzed by a bioinformatics pipeline to detect gene rearrangements, including rearrangements in BRAF and RET.

Alterations are classified for purposes of reporting on the clinical report as Level 2 or Level 3 alterations in accordance with the FDA Fact Sheet describing the CDRH's Approach to Tumor Profiling for Next Generation Sequencing Tests[1] and as follows:

- Level 2: Genomic Findings with Evidence of Clinical Significance
- Level 3: Genomic Findings with Potential Clinical Significance

xR IVD is intended to be performed with the following key components, each qualified and controlled by Tempus under its Quality Management System (QMS):

- Reagents
- Specimen Collection Box
- Software
- Sequencing Instrumentation

All reagents used with respect to the operation of xR IVD are qualified by Tempus and are compliant with the medical device Quality System (QS) regulation. xR IVD includes a specimen collection and shipping box (the Specimen Box). The Specimen Box contains the following components:

- Informational Brochure with Specimen Requirements
- Collection Box Sleeve
- Collection Box Tray
- Seal Sticker
- ISO Label

The proprietary xR IVD bioinformatics pipeline comprises data analysis software necessary for the xR IVD. The software is used with sequence data generated from NovaSeq 6000 instruments qualified by Tempus. Data generated from the pipeline is saved to a cloud infrastructure.

xR IVD uses the *Illumina NovaSeq 6000 Sequencer,* a high throughput sequencing system employing sequencing-by-synthesis chemistry. All instruments are qualified by Tempus utilizing the Tempus QMS.

1. **Sample Preparation**:

   FFPE (Formalin-Fixed Paraffin Embedded) tumor specimens are received either as unstained tissue sections on slides or as an FFPE block using materials supplied in the Specimen Box and prepared following standard pathology practices. Preparation and review of a Hematoxylin and Eosin (H&E) slide is performed prior to initiation of the xR IVD assay. H&E-stained slides are reviewed by a board-certified pathologist to

[1]FDA Fact Sheet. CDRH's Approach to Tumor Profiling Next Generation Sequencing Tests. Accessed April 2, 2025. https://www.fda.gov/media/109050/download.

ensure that adequate tissue, tumor content and sufficient nucleated cells are present to satisfy minimum tumor content (tumor purity).

Specifically, the minimum recommended tumor purity for detection of alterations by xR IVD is 20%, with macrodissection required for specimens with tumor purity lower than 20%. The recommended tumor size and minimum tumor content needed for testing are shown in Table 1, below.

Table 1: Tumor Volume and Minimum Tumor Content

Tissue Type	Recommended Size	Minimum Tumor Content	Macro-Dissection Requirements*	Limitations	Storage
FFPE blocks or 5 µm slides	1mm3 of total tissue is recommended	20%	Macro-dissection must be done if the tumor content/purity is less than 20%	Archival paraffin embedded material subjected to acid decalcification is unsuitable for analysis. Samples decalcified in EDTA are accepted.	Room temperature

**These requirements are based on the specimen's tumor content*

2. **RNA Extraction**:

Nucleic acids are extracted from tissue specimens using a magnetic bead-based automated methodology followed by DNAse treatment. The remaining RNA is assessed for quantity and quality (sizing) at RNA QC1, which is a quality check (QC) to ensure adequate RNA extraction. The minimum amount of RNA required to perform the test is 50 ng. RNA is fragmented using heat and magnesium, with variable parameters, to yield similar sized fragments from RNA inputs with different starting size distributions.

3. **Library Preparation**:

Strand-specific RNA library preparation is performed by synthesizing the first-strand cDNA using a reverse transcriptase (RT) enzyme followed by second-strand synthesis using a DNA polymerase to create double stranded cDNA. Adapters are ligated to the cDNA and the adapter-ligated libraries are cleaned using a magnetic bead-based method. The libraries are amplified with high fidelity, low-bias PCR using primers complementary to adapter sequences. Amplified libraries are subjected to a 1X magnetic bead-based clean-up to eliminate unused primers, and quantity is assessed (QC2) to ensure that pre-captured libraries were successfully prepared. Each amplified sample library contains a minimum of 150 ng of cDNA to proceed to hybridization.

4. **Hybrid Capture**:

   After library preparation and amplification, the adapter-ligated library targets are captured by hybridization, clean-up of hybridized targets is performed, and unbound fragments are washed away. The captured targets are enriched by PCR amplification followed by a magnetic bead-based clean-up to remove primer dimers and residual reagents. To reduce non-specific binding of untargeted regions, human COT DNA and blockers are included in the hybridization step. Each post-capture library pool must satisfy a minimum calculated molarity (≥2.7 nM) to proceed to sequencing (QC3). The molarity is used to load the appropriate concentration of library pools onto sequencing flow cells.

5. **Sequencing**:

   The amplified target-captured libraries are sequenced with a 2x76 read length to an average of 50 million total reads on an Illumina NovaSeq 6000 System using patterned flowcells (SP/S1, S2, or S4). Pooled sample libraries are fluorometrically quantified and normalized into a sequencing pool of up to 28 samples (SP flowcell), 56 samples (S1 flowcell), 140 samples (S2 flowcell), 336 samples (S4 flowcell) with each flowcell including 2 external controls. Partial batches are supported using a set threshold of loading capacity down to a defined percentage. Pooled sample libraries are loaded on a sequencing flow cell and sequenced.

6. **Data Analysis**:

   a. Data Management System (DMS): Sequence data is automatically processed using software that tracks sample names, sample metadata processing status from sequencing through to analysis and reporting. Reports of identified alterations are available in a web-based user interface for download. Sequencing and sample metrics are available in run and case reports, including sample and sequencing quality.

   b. Demultiplexing and FASTQ Generation: Demultiplexing software generates FASTQ files containing sequence reads and quality scores for each of the samples on a sequencing run. The FASTQ formatted data files are used for subsequent processing of samples.

   c. Indexing QC Check: Samples are checked for an expected yield of sequence reads identified to detect mistakes in pooling samples. Samples outside the expected range are marked as failed.

   d. Read Alignment and BAM Generation: Genome alignment is performed to map sequence reads for each sample to the human reference genome (hg19). Alignments are saved as Binary Alignment Map (BAM) formatted files, which contain read

placement information relative to the reference genome with quality scores. Aligned BAM files are further processed in a pipeline to identify genomic alterations.

e. Sample QC check: A sample QC check (QC4) evaluates the quality of the samples processed through the bioinformatics pipeline (sample level metrics in Table 2). Samples are evaluated for contamination by evaluating the percent of a tumor sample contaminated with foreign nucleic acid with a threshold below 5%. Sample sequencing coverage is assessed through RNA gene-ids expressed which counts all genes raw expression abundance (>12,000) and RNA GC-distribution (45-59%). The sample mapping rate (>80%), RNA strand % sense (>88%) and RNA strand % failed (≤ 10%) metrics provide confidence in the sample quality.

f. Alteration calling: A fully automated pipeline for bioinformatic analysis is used to identify gene rearrangements. The assay is validated to report specific gene rearrangements. Gene rearrangements are identified based on observations of reads supporting gene rearrangements in genomic alignments of discordantly mapped or split read pairs.

7. Controls:

a. Negative control: A no template control (NTC) is processed to serve as a negative control to validate the acceptability of all the test samples processed through extraction, library preparation and hybridization and capture steps by testing for sample or reagent contamination. The NTC is not included on the sequencing run.

b. Positive control: xR IVD uses multiple external controls consisting of contrived material with synthetically derived alterations or a pool of multiple cell lines. A positive control sample containing known gene rearrangements will be included with each sequencing run. The external controls are processed from library preparation through sequencing to serve as an end-to-end control to demonstrate assay performance. The external controls are checked during library preparation and after sequencing. Failure of the external control to meet the pre-defined quality metrics will result in all test samples on the run being reported as Quality Control (QC) failure.

8. Result Reporting:

xR IVD reports oncologically relevant gene rearrangements in BRAF and RET as genomic findings with evidence of clinical significance or with potential clinical significance. Gene rearrangements are assessed as oncogenic based on required genomic regions specified in a Tempus-developed curated database. Gene rearrangements that retain the genomic region(s) required for oncogenicity are assigned a level of clinical significance consistent with FDA's Fact Sheet and reported. Gene rearrangements that do not retain the region(s) required for oncogenicity are not reported.

9. **Quality Metrics**:

Reporting takes into account the quality metrics outlined in **Table 2**. Quality metrics are assessed across the following categories:

- Batch-level: Metrics that are quantified per sequencing run; if the positive control fails these criteria, no results are reported for the entire batch of samples.
- Sample-level: Metrics that are quantified per sample; no device results are generated for samples failing these metrics. These metrics are also referred to as sequencing quality control (QC4).
- Analyte-level: Metrics that are quantified for individual alteration types. Alterations passing analyte-level metrics (threshold) are reported.

Table 2: Summary of xR IVD Post-Sequencing Key Quality Metrics at Batch, Sample (QC4), and Analyte Levels

Quality Metric	Batch/Sample/Analyte	Required Value
Positive Control	Batch level	Known sequence mutations are detected
Expression Positive Control	Batch level	≥0.9 r2
RNA gene IDs expressed	Sample level	>12,000
RNA GC distribution	Sample level	45-59%
Mapping rate	Sample level	>80%
RNA strand percent sense	Sample level	>88%
RNA strand percent failed	Sample level	≤10%
Unique deduplicated reads	Sample level	>6,000,000
Tumor RNA junction saturation 50_100	Sample level	>1%
Contamination fraction	Sample level	<5%

Quality Metric	Batch/Sample/Analyte	Required Value
Gene Rearrangements (BRAF, RET)	Analyte level	≥4 reads

B Principle of Operation:

xR IVD consists of a series of steps from nucleic acid extraction to bioinformatics data analysis that enable the reporting of select gene rearrangements.
The end-to-end workflow of the xR IVD assay consists of the steps listed below.

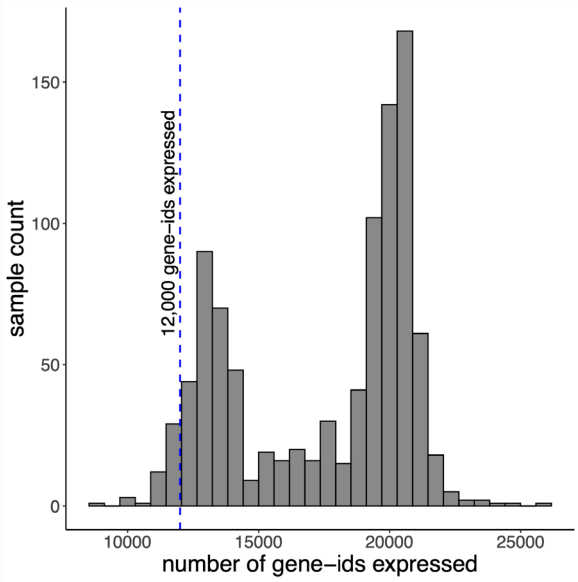
xR IVD Workflow Steps:

1. Specimen Accessioning and Pathology
2. Total Nucleic Acid (TNA) and RNA Extraction
3. Library Preparation
4. Hybridization Capture
5. Sequencing (generates sequencing data)
6. Data Analysis (alteration calling and classification)
7. Report Generation

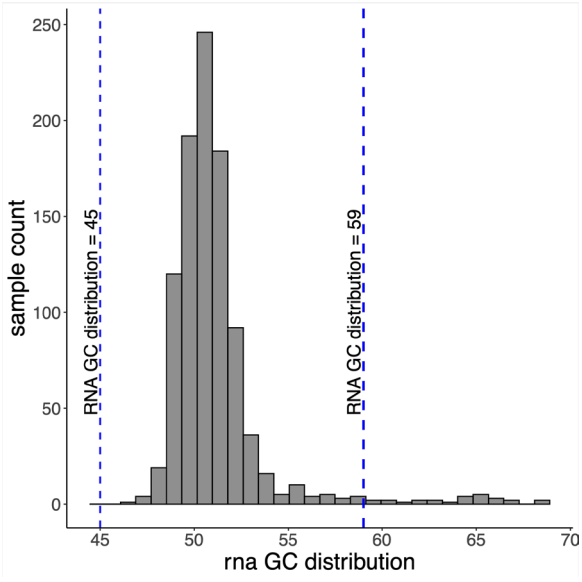
C Determination of Assay Thresholds:

xR IVD thresholds were set based on literature and analyses of existing solid tumor data. A total of 11,000 clinical samples were evaluated to establish thresholds at which there was robust detection of gene rearrangements while minimizing the failure rate for the pre-determined metrics to less than 1%. The graphs below represent the distribution of samples per quality control metric with the threshold indicated by the dotted line. Results from these analyses demonstrate that each sample is sequenced with adequate read depth and ensures the uniformity of coverage across the exome and ensures adequate coverage.

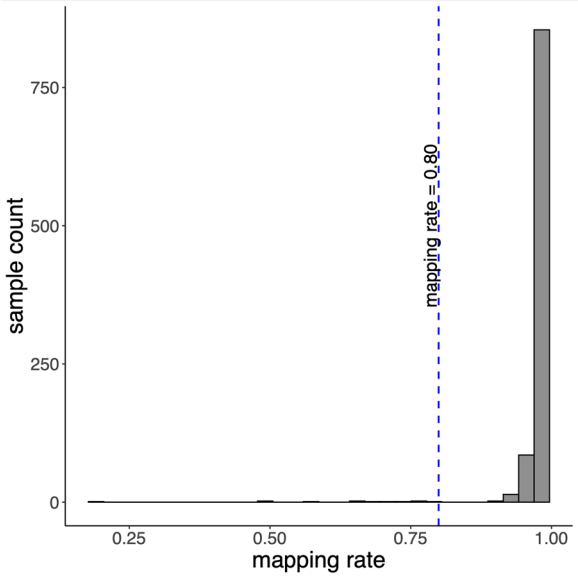
A.


sample count
number of gene−ids expressed
12,000 gene−ids expressed
0
50
100
150
10000
15000
20000
25000

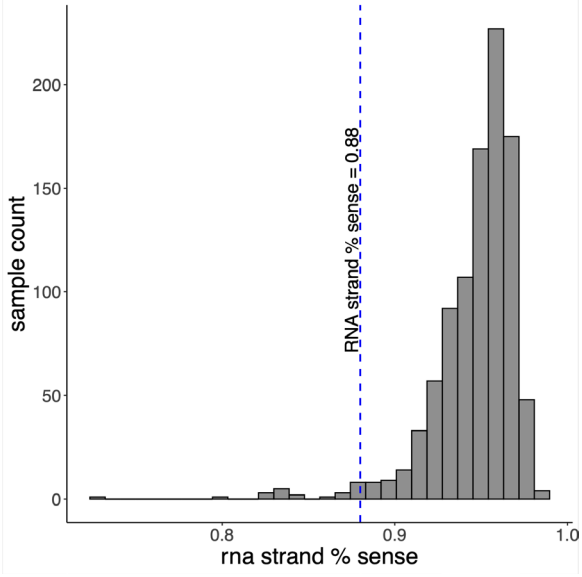
B.


sample count
rna GC distribution
RNA GC distribution = 45
RNA GC distribution = 59
0
50
100
150
200
250
45
50
55
60
65
70

C.


sample count
mapping rate
mapping rate = 0.80
0
250
500
750
0.25
0.50
0.75
1.00

D.


sample count
rna strand % sense
RNA strand % sense = 0.88
0
50
100
150
200
0.8
0.9
1.0

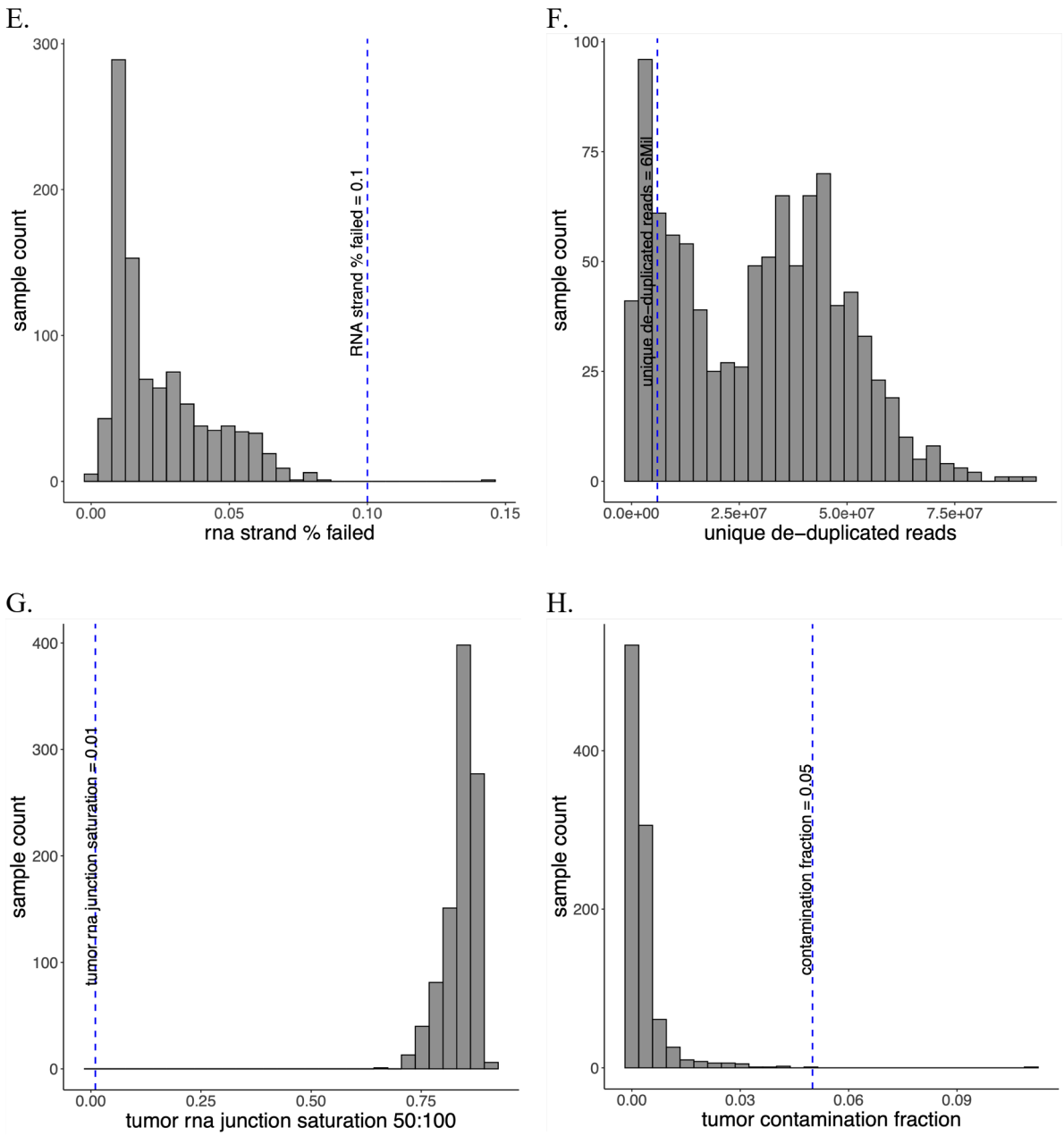


Figure 1: Distribution of quality control metrics in 1000 samples that were run through xR IVD. The blue, dotted line shows the threshold cutoff for each of the following metrics: A. gene-ids expressed, **B.** RNA-GC distribution, **C.** mapping rate, **D.** RNA strand percent sense, **E.** RNA strand percent failed, **F.** unique-deduplicated reads, **G.** tumor RNA junction Saturation 50:100, and **H.** tumor contamination fraction.

Thresholds were established at the point that balances robust detection of alterations with less than 1% failure rate for all QC4 metrics (dotted line in the graphs above). Thresholds were established as follows:

- Gene-ids expressed: >12,000

- RNA-GC distribution: 45-59%
- Mapping rate: >80%
- RNA strand percent sense: >88%
- RNA strand percent failed: ≤10%
- Unique-deduplicated reads: >6,000,000
- Tumor RNA junction Saturation 50:100: >1%
- Tumor contamination fraction: <5%

D Substantial Equivalence Information:

1. Predicate Device Name(s):

MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets)

2. Predicate 510(k) Number(s):

DEN170058

3. Comparison with Predicate(s):

	Predicate (MSK-IMPACT)	Candidate (Tempus xR IVD)
	Similarities	
Intended Use	The MSK-IMPACT assay is a qualitative in vitro diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded tumor tissue matched with normal specimens from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi gene panel. The test is intended to provide information on somatic mutations (point mutations and small insertions and deletions) and microsatellite instability for use by qualified health care professionals in accordance with professional guidelines and is not conclusive or prescriptive for labeled use of any specific therapeutic product. MSK-IMPACT is a single-site assay performed at Memorial Sloan Kettering Cancer Center.	The Tempus xR IVD assay is a qualitative next generation sequencing-based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of rearrangements in two genes, using RNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens from patients with solid malignant neoplasms. Information provided by xR IVD is intended to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with previously diagnosed solid malignant neoplasms. Results from xR IVD are not intended to be prescriptive or conclusive for labeled use of any specific therapeutic product.

	Predicate (MSK-IMPACT)	Candidate (Tempus xR IVD)
Intended Patient Population	Previously diagnosed cancer patients with solid malignant neoplasms	Same
Rx / OTC	Rx Only	Same
Technology	Next Generation Sequencing (hybrid capture methodology)	Same
Controls	• Matched normal • Positive control • Negative control • No template control (NTC)	• Positive control • No template control (NTC)
Result Report Format	Oncopanel results are reported under one of these two categories: • "Cancer Mutations with Evidence of Clinical Significance" or • "Cancer Mutations with Potential Clinical Significance."	xR IVD results are reported under one of these two categories: • "Genomic Findings with Evidence of Clinical Significance" or • "Genomic Findings with Potential Clinical Significance."
Clinical Evidence Curation	Classification criteria were developed by MSK using the in-house OncoKB database. OncoKB undergoes periodic updates through the review of new information by a panel of experts.	Classification criteria were developed by Tempus using an in-house reference set database. The reference set database undergoes periodic updates through the review of new information by a panel of experts.
	Differences	
Specimen Type	DNA isolated from FFPE tumor tissue from cancer patients with solid malignant neoplasms	RNA isolated from FFPE tumor tissue from cancer patients with solid malignant neoplasms
Instrument	HiSeq 2500 Sequencer (qualified by MSK)	NovaSeq 6000 Sequencer (qualified by Tempus)
Average Target Coverage	>200x target coverage	Unique deduplicated reads, >6,000,000
Variant Types	SNVs, indels, and MSI	Translocations (RET, BRAF)

	Predicate (MSK-IMPACT)	Candidate (Tempus xR IVD)
Assay cut-off	MSK-IMPACT does not report mutations below 2% for known hotspot mutations and 5% for non-hotspot mutations.	xR-IVD does not report gene rearrangements with <4 reads.

E Standards/Guidance Documents Referenced:

The following FDA guidance documents were consulted:

- ISO 13485:2016, Medical devices - QMS - Requirements for Regulatory Purposes (2016-03)
- ISO 14971:2019, Medical devices - Application of Risk Management to Medical Devices (2019-12)
- ISO 15223-1:2021, Medical Devices - Symbols to be used with Information to be Supplied by the Manufacturer Fourth Edition (2021-07)
- ISO 20417:2021, Medical Devices - Information to be Supplied by the Manufacturer, (2021-12)
- IEC 62366-1:2015, Medical devices - Part 1: Application of usability engineering to medical devices, (2020-06)
- IEC 62304:2006 + A1:2015, Medical device software - Software Life Cycle Processes (2015-06)
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition (2012-06)
- AAMI TIR 45:2012, Guidance On The Use Of Agile Practices In The Development Of Medical Device Software (2012)
- Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions (2023-09)
- Postmarket Management of Cybersecurity in Medical Devices (2016-12)

F Performance Characteristics:

1. Analytical Performance- General:

The xR IVD assay is a next generation sequencing in vitro diagnostic test that detects rearrangements in BRAF and RET genes. These gene rearrangements were validated at the gene level in key analytical studies.

a) Invalid Rates

The invalid rates across multiple tumor types obtained from historical data were evaluated with 59451 FFPE clinical specimens from 39 tumor types. The data shows

the separate invalid rates for the different steps involved in the assay workflow including the percentage of specimens with insufficient tumor (rejected at specimen qualification), insufficient TNA or RNA integrity and yield after TNA extraction and RNA isolation (QC1), the percentage with failed library construction (QC2), the percentage with failed hybridization capture (QC3) and the percentage that failed the sequencing run (QC4) per cancer type (Table 3).

xR IVD supports repeat testing if key in-process or automated data quality metrics are not met. The overall invalid rate for xR IVD after repeat testing is 10.7%, where 4612 samples failed of the 43186 total number of samples that started the workflow (Table 4). Samples failed primarily due to insufficient TNA/RNA yield related to the specimen source. The data shows that the invalid rates across all assay steps are comparable across tumor types supporting the performance of pan-tumor profiling.

Table 3: Invalid Rate Per Tumor Type

Tumor Type	Percent Rejected at Specimen Qualification	Percent Invalid - Assay Steps					Total Assay Invalid Rate
		TNA Extraction (TNA QC1)	RNA Isolation (RNA QC1)	Library Construction (QC2)	Hybridization Capture (QC3)	Sequencing (QC4)	
Adrenal Cancer	3.5% (3/86)	3.1% (2/64)	8.9% (5/56)	0% (0/51)	0% (0/50)	0% (0/45)	10.9% (7/64)
Basal Cell Carcinoma	10.8% (4/37)	0% (0/26)	0% (0/21)	0% (0/21)	0% (0/21)	0% (0/20)	0% (0/26)
Biliary Cancer	8.8% (142/1617)	6.2% (70/1138)	6.0% (55/915)	0.5% (4/857)	0% (0/849)	1.7% (13/798)	12.5% (142/1138)
Bladder Cancer	4.7% (80/1689)	3.0% (37/1243)	3.2% (34/1068)	1.1% (11/1033)	0% (0/1018)	1.2% (12/962)	7.6% (94/1243)
Brain Cancer	3.5% (4/114)	4.9% (4/81)	6.6% (4/61)	0% (0/56)	0% (0/55)	1.8% (1/52)	11.1% (9/81)
Breast Cancer	7.7% (393/5089)	4.5% (163/3643)	3.4% (98/2874)	0.8% (23/2763)	0.11% (3/2722)	1.9% (48/2538)	10.9% (335/3643)
Cervical Cancer	2.1% (7/333)	3.3% (8/239)	5.1% (11/214)	2.0% (4/204)	0% (0/197)	0.51% (1/186)	10.0% (24/239)
Chromophobe Renal Cell Carcinoma	0% (0/21)	5.6% (1/18)	0% (0/16)	0% (0/16)	0% (0/16)	7.1% (1/14)	11.1% (2/18)
Clear Cell Renal Cell Carcinoma	5.8% (37/638)	3.8% (18/472)	3.8% (15/400)	1.3% (5/381)	0% (0/374)	1.0% (4/357)	8.9% (42/472)
Colorectal Cancer	4.8% (353/7282)	2.7% (146/5332)	2.2% (102/4694)	0.9% (40/4581)	0.07% (3/4527)	0.66% (30/4307)	6.0% (321/5332)
Endocrine	5.7%	7.0%	4.8%	0.4%	0% (0/274)	1.4%	12.2%

Tumor Type	Percent Rejected at Specimen Qualification	Percent Invalid - Assay Steps					Total Assay Invalid Rate
		TNA Extraction (TNA QC1)	RNA Isolation (RNA QC1)	Library Construction (QC2)	Hybridization Capture (QC3)	Sequencing (QC4)	
Tumor	(28/494)	(26/369)	(14/292)	(1/277)		(4/257)	(45/369)
Endometrial Cancer	3.2% (36/1124)	2.0% (17/845)	1.9% (15/770)	0.8% (6/755)	0% (0/742)	0.80% (5/705)	5.1% (43/845)
Esophageal Cancer	3.9% (78/2005)	2.0% (30/1494)	2.5% (32/1275)	0.6% (8/1245)	0.16% (2/1235)	0.41% (5/1174)	5.2% (77/1494)
Gastric Cancer	7.2% (77/1076)	3.0% (23/756)	1.9% (12/642)	0.6% (4/626)	0% (0/621)	2.1% (13/581)	6.9% (52/756)
Gastrointestinal Stromal Tumor	3.4% (14/409)	4.6% (14/303)	3.7% (9/245)	0.9% (2/235)	0.43% (1/232)	1.7% (4/222)	9.9% (30/303)
Glioblastoma	0.3% (2/611)	4.5% (21/470)	3.8% (15/401)	0.3% (1/385)	0% (0/382)	0.51% (2/363)	8.3% (39/470)
Head and Neck Cancer	3.1% (11/360)	2.7% (7/255)	2.8% (6/217)	1.9% (4/208)	0% (0/203)	0.96% (2/195)	7.5% (19/255)
Head and Neck Squamous Cell Carcinoma	4.0% (58/1465)	3.7% (41/1107)	3.4% (33/971)	1.4% (13/935)	0% (0/921)	1.5% (14/871)	9.1% (101/1107)
Kidney Cancer	7.7% (33/430)	7.1% (23/325)	6.9% (17/246)	0.4% (1/229)	0% (0/224)	1.8% (4/212)	13.8% (45/325)
Liver Cancer	5.7% (26/459)	6.9% (24/350)	3.4% (9/261)	1.6% (4/253)	0% (0/248)	0.40% (1/237)	10.9% (38/350)
Low Grade Glioma	2.8% (3/106)	8.1% (7/86)	2.9% (2/63)	0% (0/61)	0% (0/60)	1.6% (1/56)	11.6% (10/86)
Medulloblastoma	0% (0/9)	0% (0/8)	0% (0/8)	0% (0/8)	0% (0/8)	0% (0/8)	0% (0/8)
Melanoma	7.2% (130/1799)	2.8% (38/1337)	3.2% (35/1101)	1.0% (11/1064)	0.10% (1/1048)	0.85% (9/992)	7.0% (94/1337)
Meningioma	0.6% (1/178)	2.2% (3/136)	1.6% (2/127)	0% (0/125)	0% (0/125)	0.8% (1/119)	4.4% (6/136)
Mesothelioma	5.0% (9/180)	3.6% (5/140)	2.6% (3/114)	2.7% (3/111)	0% (0/107)	1.9% (2/100)	9.3% (13/140)
Neuroblastoma	0% (0/6)	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/3)	0% (0/5)
Non-Small Cell Lung Cancer	7.5% (990/13219)	4.7% (454/9691)	9.4% (760/8102)	0.8% (59/7336)	0.15% (11/7250)	3.3% (225/6793)	15.6% (1509/9691)
Oropharyngea	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)

Tumor Type	Percent Rejected at Specimen Qualification	Percent Invalid - Assay Steps					Total Assay Invalid Rate
		TNA Extraction (TNA QC1)	RNA Isolation (RNA QC1)	Library Construction (QC2)	Hybridization Capture (QC3)	Sequencing (QC4)	
l Cancer							
Ovarian Cancer	5.7% (88/1533)	1.9% (20/1079)	2.0% (19/954)	1.5% (14/933)	0% (0/911)	1.2% (10/864)	5.8% (63/1079)
Pancreatic Cancer	8.9% (400/4492)	3.7% (121/3238)	7.5% (194/2597)	0.4% (9/2388)	0.04% (1/2367)	2.6% (59/2230)	11.9% (384/3238)
Peritoneal Cancer	11.5% (6/52)	8.1% (3/37)	3.7% (1/27)	0% (0/26)	0% (0/26)	0% (0/26)	10.8% (4/37)
Prostate Cancer	8.4% (463/5503)	6.3% (229/3649)	5.7% (152/2653)	1.4% (35/2482)	0.04% (1/2437)	0.6% (15/2284)	11.8% (432/3649)
Skin Cancer	4.5% (26/584)	2.0% (9/451)	3.3% (13/393)	0.5% (2/379)	0% (0/376)	0% (0/348)	5.3% (24/451)
Small Cell Lung Cancer	4.4% (24/549)	2.6% (11/423)	11.6% (43/372)	0% (0/327)	0% (0/326)	3.4% (11/308)	15.4% (65/423)
Testicular cancer	2.4% (1/42)	3.3% (1/30)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)	3.3% (1/30)
Thymoma	6.3% (4/63)	10.6% (5/46)	0% (0/35)	0% (0/35)	0% (0/35)	2.9% (1/33)	13.0% (6/46)
Thyroid Cancer	6.3% (44/699)	4.3% (23/536)	2.4% (11/454)	3.6% (16/442)	0% (0/421)	2.2% (9/400)	11.0% (59/536)
Tumor of Unknown Origin	7.2% (365/5045)	5.6% (207/3725)	6.5% (193/2977)	0.5% (14/2770)	0.07% (2/2748)	2.0% (54/2593)	12.6% (470/3725)
Uveal Melanoma	4.1% (2/49)	2.6% (1/38)	11.1% (4/36)	3.1% (1/32)	0% (0/31)	3.3% (1/29)	18.4% (7/38)
Total (All Cohorts)	6.6% (3944/59451)	4.2% (1812/43186)	5.4% (1918/35682)	0.9% (295/33660)	0.08% (25/33217)	1.8% (562/31307)	10.7% (4612/43186)

Table 4: Overall Assay Invalid Rate

	Failing Samples	Overall Invalid Rate (2-sided 95% CI)
First Testing	9847/43186	22.8% (0.2241, 0.2320
After Repeat Testing	4612/43186	10.7% (0.1039, 0.1097)

2. Precision/Reproducibility:

Two studies were conducted to evaluate the precision for xR IVD. Study 1 evaluated precision of multiple gene rearrangements to provide totality of supporting data for xR IVD panel and Study 2 evaluated gene rearrangements in two select genes, RET and BRAF.

Study 1

Study 1 evaluated general inter-run and intra-run variability for the xR IVD panel. Inter-run precision evaluated variability from lot-to-lot, operator, instruments, and days while intra-run precision evaluated replicates of the same sample within a single batch. Inter- and intra-run precision was evaluated using 25 FFPE clinical tumor samples and 2 commercially available control materials. A total of 29 gene rearrangements were evaluated.

The overall Positive Percent Agreement (PPA) for gene rearrangement calls between runs (inter-run) was 98.3% (95% CI 0.9795, 0.9984) and 99.0% (95% CI 0.9700, 0.9965) between replicates within the same run.

Overall (across all runs) Negative Percent Agreement (NPA) was 99.9% (95% CI 0.9989, 0.9997) for gene rearrangements and while the NPA between tested replicates was 100% (95% CI 0.9991, 0.9998) for gene rearrangements.

Study 2

a) Precision for RET and BRAF Gene Rearrangements:

Precision was evaluated for RET and BRAF fusions in 12 FFPE samples from 7 tumor types, including glioblastoma, bladder cancer, non-small cell lung cancer, thyroid and colorectal cancer. Specimens evaluated for reproducibility were run using 3 different library preparation reagent lot combinations, 2 different operators, and 3 different instrument (sequencer) combinations in duplicate on non-consecutive days, for a total of 36 replicates per sample. Specimens were evaluated for within or intra-run precision by running replicates using a single reagent lot and operator throughout the workflow.

Precision for RET rearrangements was determined by calculating the percent agreements, PPA and NPA, at the gene level and the sample level and across all measurements against the majority call or the most frequently occurring observation. The PPA for RET gene rearrangement detection was 98.61% (Table 5). Similarly, the PPA at the sample level was 100% for 3 of the 4 RET-positive samples evaluated (Table 6). A single sample expected to be a RET-positive sample had 2 false negative replicate results (2 of 36 total) for a PPA of 94.44%. The NPA for all conditions was 100% since there were no false positives observed.

Table 5: PPA and NPA at the Gene Level for RET

Driver Gene	Total	PPA	Two-Sided 95% CI	NPA	Two-Sided 95% CI
RET	432	98.61%	[0.951-0.998]	100%	[0.987-1]

Table 6: PPA and NPA at the Sample Level for RET

Sample ID	Fusion	FDA Level*	Total Number of Replicates	Positive Replicates Observed	Negative Replicates Observed	Relative LOD	PPA (Two-Sided 95% CI)	NPA (Two-Sided 95% CI)
Sample 01	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 02	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 03	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 04	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 05	CCDC6_ RET	Level 2	36	34	2	1.98	94.44% (81.34%, 99.32%)	-
Sample 06	CCDC6_ RET	Level 2	36	36	0	4.06	100% (90.26%, 100%)	-
Sample 07	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 08	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 09	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 10	RET Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)

Sample 11	KIF5B_ RET	Level 2	36	36	0	3.92	100% (90.26%, 100%)	-
Sample 12	KIF5B_ RET	Level 2	36	36	0	3.42	100% (90.26%, 100%)	-

* N/A = criteria are not applicable for negative samples.

Precision for BRAF rearrangements was also determined by calculating the percent agreements, PPA and NPA, at the gene level and the sample level and across all measurements against the majority call or the most frequently occurring observation. The PPA was 100% for BRAF gene rearrangement detection at the gene (Table 7) and sample level (Table 8). The NPA for all conditions was 100% since there were no false positives observed.

Table 7: PPA and NPA at the Gene Level for BRAF

Driver Gene	Total	PPA	Two Sided 95% CI	NPA	Two Sided 95% CI
BRAF	432	100%	[0.975-1]	100%	[0.987-1]

Table 8: PPA and NPA at the Sample Level for BRAF

Sample ID	Fusion	FDA Level*	Total Number of Replicates	Positive Replicates Observed	Negative Replicates Observed	Relative LOD	PPA (Two Sided 95% CI)	NPA (Two Sided 95% CI)
Sample 01	AGAP3_ BRAF	Level 3	36	36	0	1.71	100% (90.26%, 100%)	-
Sample 02	RRBP1_ BRAF	Level 3	36	36	0	6.13	100% (90.26%, 100%)	-
Sample 03	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 04	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 05	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)

Sample 06	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 07	KDM7A_ BRAF	Level 3	36	36	0	1.86	100% (90.26%, 100%)	-
Sample 08	KIAA154 9_BRAF	Level 2	36	36	0	2.32	100% (90.26%, 100%)	-
Sample 09	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 10	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 11	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)
Sample 12	BRAF Negative	N/A	36	0	36	0	-	100% (90.26%, 100%)

* N/A = criteria are not applicable for negative samples.

Wild type precision was evaluated by reporting the NPA for target gene fusions across all replicates of all samples. There were no false positives detected across the study for the genes evaluated for an NPA of 100% (288/288 concordant wild-type measurements).

b) Analysis of Sources of Variability

The PPA and NPA was assessed to analyze the imprecision caused by different sources of variance – sample replicates, reagent lots, operators/days, and instruments. Data analysis is presented stratified by sample.

Table 9: Precision Analysis based on Sources of Variability per Gene.

Gene	Metric	Overall (95% CI)	Inter-reagent lot (95% CI)	Inter-Operator/Day (95% CI)	Inter-Instrument (95% CI)	Repeatability (Within-Run) (95% CI)
BRAF	PPA	100% (97.47%, 100%)	100% (97.47%, 100%)	100% (97.47%, 100%)	100% (97.47%, 100%)	100% (95.01%, 100%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)

RET	PPA	98.61% (95.07%, 99.83%)	98.61% (95.07%, 99.83%)	98.61% (95.07%, 99.83%)	98.61% (95.07%, 99.83%)	97.22% (90.32%, 99.66%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)

Table 10: Precision Analysis based on Sources of Variability per Gene Rearrangement.

Gene Rearrangement	Metric	Overall (95% CI)	Inter-reagent lot (95% CI)	Inter-Operator/Day (95% CI)	Inter-Instrument (95% CI)	Repeatability (Within-Run) (95% CI)
AGAP3_BRAF	PPA	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (81.47%, 100%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)
RRBP1_BRAF	PPA	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (81.47%, 100%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)
KDM7A_BRAF	PPA	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (81.47%, 100%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)
KIAA1549_BRAF	PPA	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (90.26%, 100%)	100% (81.47%, 100%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)
CCDC6_RET	PPA	97.22% (90.32%, 99.66%)	97.22% (90.32%, 99.66%)	97.22% (90.32%, 99.66%)	97.22% (90.32%, 99.66%)	94.44% (81.34%, 99.32%)
	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)
KIF5B_RET	PPA	100% (95.01%, 100%)	100% (95.01%, 100%)	100% (95.01%, 100%)	100% (95.01%, 100%)	100% (90.26%, 100%)

	NPA	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (98.73%, 100%)	100% (97.47%, 100%)

3. Analytical Sensitivity - Limit of Detection (LoD):

The sensitivity of the assay for the detection of gene rearrangements was evaluated by determining the minimum tumor purity or limit of detection (LoD), which was subsequently confirmed using additional samples at the established LoD level.

The initial LoD for gene rearrangements was estimated by testing 12 FFPE clinical specimens (from 8 different cancer types, including bladder, breast, low grade glioma, pancreatic, prostate, and thyroid cancer, among others) containing 12 gene rearrangements at five dilution levels ranging from 5% to ≥40% tumor purities and an undiluted sample. A commercially available control material was also tested containing an additional 15 gene rearrangements. LoD for gene rearrangements was confirmed by testing an additional 21 FFPE clinical specimens (representing 11 tumor types: basal cell, biliary, brain, esophageal, non-small cell lung, pancreatic, prostate and thyroid cancer and glioblastoma, low grade glioma, and melanoma) containing 58 gene rearrangements. Results from duplicate testing of the specimens, using 2 reagent lots were used to confirm the LoD by comparing the testing results to the expected positive results. A PPA of 94.8% (95% CI 0.8586, 0.9823) confirmed that the LoD for gene rearrangement detection is 20% tumor purity.

A read-based LoD for RET gene rearrangements was determined using a probit regression model by pooling results from 3 FFPE clinical specimens (from thyroid, non-small cell lung, and colorectal cancer), with starting tumor purity ranging between 40-60%, tested at 6 dilution levels. The dilutions levels ranged from 1.875 - 30 reads and each dilution level had a minimum of 12 replicates (or observations). Dilutions were evaluated by 2 different operators, with at least 6 replicates per dilution level per operator, on different days using different manufacturer, reagent lots, and sequencing instruments.

For RET, 3 unique FFPE samples, each containing 1 RET gene rearrangement, were evaluated in this study and were run at 6 different dilution levels each with 12 replicates per dilution level. Samples originated from 3 different tumor types, including, Colorectal, Thyroid, and Non-Small Cell Lung Carcinoma.

Using the probit regression model, the LoD estimate was 6.67 reads for CCDC6-RET, with a P-value of 1. Table 11 shows the read support LoD from the probit model in the second to last column. A chi-square goodness of fit test was used to assess probit model fit to the data, results for which are shown in the last column. The RET LoD per sample

and tumor type was determined to be 5-7 reads based on a sample-level probit analysis as shown in Table 12.

Table 11: Probit Regression Model for LoD Estimation per RET Rearrangement

Gene Rearrangement	Dilution Level (reads)	N of observations	Hit-rate	Hit-rate 95% CI	LoD Estimates (reads)	Goodness of fit P-Value
CCDC6-RET	1.875	37	0.541	0.369 - 0.705	6.667	1
	3.75	38	0.868	0.719 - 0.956		
	5.63	24	0.958	0.789 - 0.999		
	7.5	36	1	0.903 - 1		
	15	36	1	0.903 - 1		
	30	36	1	0.903 - 1		

Table 12: xR IVD LoD for RET per Sample and Tumor Type

Gene	Sample ID - Tumor Type	Fusion	Sample Level LoD (reads)	LoD Range (reads)
RET	LOD 06 - Thyroid Cancer	CCDC6-RET	5	5 - 7
	LOD - 08 Non-Small Cell Lung Cancer	CCDC6-RET	5	
	LOD - 02 Colorectal Cancer	CCDC6-RET	7	

The LoD for BRAF gene rearrangements was determined using a probit regression model by pooling results from 3 clinical specimens from breast, prostate, and pancreatic cancer, with starting tumor purity ranging between 50-70%, tested at 6 dilution levels (Table 13). The dilution levels ranged from 1.875 to 30 reads. Dilutions were evaluated by 2 different operators, with at least 6 replicates per dilution level per operator, on different days using different manufacturer reagent lots and sequencing instruments. Using the probit regression model, the LoD estimate ranged from 6.54 reads to 8.91 reads when evaluating BRAF with

2 different rearrangement partners, SND1-BRAF and CCNY-BRAF, respectively. The P-value for each of the rearrangements was 0.987 for CCNY-BRAF and 1 for SND1-BRAF.

The probit regression model at the sample level in Table 14 evaluated the LoD for BRAF in the different tumor types. The analysis confirmed the LoD to be between 6-9 reads for BRAF rearrangements, depending on fusion partner and/or tumor type.

Table 13: Probit Regression Model for LoD Estimation per BRAF Rearrangement

Gene Rearrangement	Dilution Levels (Reads)	N of observations	Hit-rate	Hit-rate 95% CI	LoD Estimates (reads)	Goodness of fit P-Value
CCNY-BRAF	1.875	12	0.333	0.099 - 0.651	8.91	0.987
	3.75	12	0.583	0.277 - 0.848		
	5.63	12	1	0.735 - 1		
	7.5	12	0.917	0.615 - 0.998		
	15	12	1	0.735 - 1		
	30	12	1	0.735 - 1		
SND1-BRAF	1.875	24	0.542	0.328 - 0.744	6.54	1
	3.75	24	0.917	0.73 - 0.99		
	5.63	12	0.917	0.615 - 0.998		
	7.5	23	1	0.852 - 1		
	15	24	1	0.858 - 1		
	30	24	1	0.858 - 1		

Table 14: xR IVD LoD for BRAF per Sample and Tumor Type

Gene	Sample ID - Tumor Type	Fusion	Sample Level LoD (reads)	LoD Range (reads)
BRAF	LOD 01 - Breast Cancer	SND1-BRAF	8	6 - 9

Gene	Sample ID - Tumor Type	Fusion	Sample Level LoD (reads)	LoD Range (reads)
	LOD 07 - Prostate Cancer	SND1-BRAF	6	
	LOD 09 - Pancreatic Cancer	CCNY-BRAF	9	

4. Linearity/assay reportable range

Not applicable

5. Traceability, Stability, Expected Values (controls, calibrators, or methods)

a) Traceability:

The xR IVD assay is not traceable to any known standard. Controls and quality metrics are described in the device description section.

b) *Stability/Shelf life*:

Reagent stability is initially established based on manufacturer expiration dating and supported by verification testing. It is extended through long term stability testing of the reagents, which are monitored through the use of consistent controls.

Reagent Stability:

The stability of RNA isolation, library preparation, and hybridization capture reagents was evaluated over a period of 180 days of storage under manufacturer-recommended storage conditions. Stability was evaluated by comparing the agreement in variant calling (positive and negative percent agreements, PPA and NPA) between aged and baseline conditions. 38 unique samples were run with 3 different RNA isolation, library preparation, and hybridization capture reagent lots over 3 time points. Timepoints evaluated were T0, baseline (first use); T1, T = 95 +/- 2 days; T2, T = 187 +/- 5 days. The PPA and NPA for BRAF and RET detection at both the time points compared to T0 were 100%. The stability of RNA isolation, library preparation, and hybrid capture reagents used in the xR IVD was determined to be up to 180 days when stored under manufacturer recommended conditions.

c) Expected values (controls, calibrators or methods)

xR IVD uses multiple external controls consisting of contrived material with synthetically derived alterations or a pool of multiple cell lines. A positive control sample containing known gene rearrangements is included with each sequencing run. The external controls are processed from library preparation through sequencing to serve as an end-to-end control for assay performance. The external controls are checked during library preparation and after sequencing. Failure of the external control to meet the pre-defined quality metrics results in all test samples on the run being reported as Quality Control (QC) failure. In addition, several quality metrics are established as thresholds for reporting results to provide for high confidence data.

6. Analytical Specificity:

a) Cut-off/False positive rate (Limit of Blank)

The cut-off for calling alterations was based on the limit of blank (LoB) of xR IVD. The LoB was determined by establishing the threshold of total reads (supporting reads for gene rearrangements) at which a negative call is confidently called. The LoB was established by assessing the frequency of false-positive calls in clinical samples known to be wild-type (alteration-negative) for gene rearrangements. 24 samples representing 12 different tumor types were tested in duplicate (at the maximum RNA input of 300 ng) and using 2 lots of reagents. The false positive rate for gene rearrangements was 1.04% which was used to set LoB threshold of 3 total supporting reads. xR IVD does not report gene rearrangements below 4 total supporting reads; ≥4 total reads will be required to call a positive gene rearrangement.

b) Interfering Substances

The impact of Axygen MAG PCR Clean-Up Beads (Axygen), ethanol, melanin, gDNA, UMI, RNA XP Clean Beads (XP), Proteinase K, xylene, and tissue necrosis as potentially interfering substances was evaluated across 2 separate studies. The impact of these interfering substances was assessed by processing RNA from FFPE clinical specimens tested in the presence of each interfering substance at varying amounts added at the applicable steps during the xR IVD workflow. The specimens were evaluated for concordance of variant calls when compared to samples processed without the interfering substances.

Between 3-7 clinical samples and 0-2 control materials were evaluated per interferent and tested with no interferent (control), and two levels per each interferent - low level interferent and high level interferent (Table 15). Study results are shown in Table 16.

Table 15: Final Reaction Concentrations of Interfering Substances Assessed

Interfering Substance	Step Added	Low Concentration	High Concentration
Axygen MAG PCR Clean-Up Beads (Axygen)	Hybridization	0.5%	1%
Ethanol	Library Preparation	5%	10%
Melanin	Library Preparation	0.05 µg/mL	0.1 µg/mL
gDNA	Library Preparation	0.1 ng/µL	2.5 ng/µL
Universal Molecular Identifier (UMI) Adapters	Library Preparation	+15%	+30%
RNA XP Clean Beads (XP)	Library Preparation	5%	10%
Proteinase K	RNA Isolation	0.002 mg/mL	0.02 mg/mL
Xylene	RNA Isolation	0.000025%	0.000050%
Tissue Necrosis	TNA Extraction	10% - 30%	40% - 60%

Table 16. Agreement between each interferent condition compared to the untreated condition.

Interferent	Interferent Concentration	PPA	Two-Sided 95% CI	NPA	Two-Sided 95% CI
Axygen	Low	100%	(0.6756, 1)	100%	(0.9595, 1)
	Low	100%	(0.6756, 1)	100%	(0.9595, 1)
	High	100%	(0.6756, 1)	100%	(0.9595, 1)
	High	100%	(0.6756, 1)	100%	(0.9595, 1)
Ethanol	Low	100%	(0.7225, 1)	100%	(0.9615, 1)
	Low	100%	(0.7225, 1)	100%	(0.9615, 1)
	High	100%	(0.7225, 1)	100%	(0.9558, 1)
	High	100%	(0.7225, 1)	100%	(0.9615, 1)
gDNA	Low	100%	(0.6457, 1)	100%	(0.9586, 1)

Interferent	Interferent Concentration	PPA	Two-Sided 95% CI	NPA	Two-Sided 95% CI
	Low	100%	(0.6756, 1)	100%	(0.9607, 1)
	High	100%	(0.5655, 1)	100%	(0.9582, 1)
	High	100%	(0.5655, 1)	100%	(0.9582, 1)
Melanin	Low	100%	(0.7575, 1)	100%	(0.9591, 1)
	Low	100%	(0.7575, 1)	99%	(0.9404, 0.9994)
	High	100%	(0.7575, 1)	100%	(0.9591, 1)
	High	100%	(0.7412, 1)	99%	(0.9404, 0.9994)
UMI	Low	100%	(0.7225, 1)	100%	(0.8389, 1)
	Low	100%	(0.7225, 1)	100%	(0.8389, 1)
	High	100%	(0.7225, 1)	100%	(0.8389, 1)
	High	100%	(0.7225, 1)	100%	(0.8389, 1)
XP	Low	100%	(0.7412, 1)	100%	(0.9519, 1)
	Low	100%	(0.7412, 1)	100%	(0.9519, 1)
	High	100%	(0.7412, 1)	100%	(0.9519, 1)
	High	100%	(0.6756, 1)	100%	(0.9519, 1
ProK	Low	100%	(0.5101, 1)	100%	(0.9143, 1)
	Low	100%	(0.5101, 1)	100%	(0.9143, 1)
	High	100%	(0.5101, 1)	100%	(0.9143, 1)
	High	100%	(0.5101, 1)	100%	(0.9143, 1)
Xylene	Low	100%	(0.6097, 1)	100%	(0.9103, 1)
	Low	100%	(0.5655, 1)	100%	(0.9124, 1)
	High	100%	(0.6097, 1)	100%	(0.9103, 1)
	High	100%	(0.5655, 1)	98%	(0.8712, 0.9987)
Necrosis	Low	100%	(0.0513, 1)	100%	(0.7847, 1)

Interferent	Interferent Concentration	PPA	Two-Sided 95% CI	NPA	Two-Sided 95% CI
	Low	100%	(0.0513, 1)	100%	(0.7847, 1)
	High	100%	(0.0513, 1)	100%	(0.7412, 1)
	High	NA*	(NaN, NaN)*	100%	(0.7009, 1)

c) Sample Carryover and Cross-Contamination:

During sample processing: RNA sample cross-contamination (within plates) and carryover (between plates) during sample processing in library preparation was assessed by calculating the false positive rate of gene rearrangements in expected negative samples. Positive and negative samples were prepared in a checkerboard pattern within one run and in an inverse checkerboard pattern relative to the previous run to assess between-run carryover, respectively. Samples used in this study included replicates of SeraSeq v4 (positive for gene rearrangements), Universal Human Reference RNA (negative), and a 50:50 mixture as a detection control. Cross-contamination was considered true if gene rearrangement variants from a positive sample were observed in the sequencing output of a neighboring well. Carryover between library preparation runs was considered true by identifying the presence of positive calls expected in the first run in the second run (as false positives). No false positive results were detected within Run 1 or within Run 2 with adapters specific to each plate, meaning no cross-contamination was detected in either run for gene rearrangements during library preparation. No false positive results were detected in Run 2 with adapters specific to Run 1, meaning no carryover was detected for gene rearrangements during library preparation on the same instrument over consecutive runs.

During Sequencing: This study evaluated carry over between two consecutive NovaSeq 6000 sequencing runs. The runs alternated 13 variant-positive samples followed by 13 negative samples at the same well both with the same adapter. Samples used in this study included replicates of SeraSeq v4 (positive for gene rearrangements) and Universal Human Reference RNA (negative). Carryover was evaluated by determining the false positive rate of variant detection in expected negative samples in the second run from the first. No false positive results were detected in the second run with adapters specific to first run indicating that there is no carryover during sequencing on the same instrument over consecutive runs.

7. Robustness Studies

a) FFPE Slide Stability:

Slide stability was determined by evaluating the xR IVD positive and negative percent agreement (PPA and NPA) in gene rearrangement calls between freshly prepared and slides aged up to 377 days. Freshly prepared slides were stored at ambient temperature at Tempus and mimicked the expected conditions for clinical slide storage. Testing occurred at different timepoints (T0, T = 0; T1, T = 32 days +/- 2 days; T2, T = 187 days +/- 5 days; T3, T = 377 days +/- 10 days). Testing included 3 clinical specimens and 1 control material. The PPA and NPA for gene rearrangement detection between T = 0 and all other timepoints was 100%. These data demonstrated that FFPE slides are stable for use with xR IVD up to 365 days when stored under expected conditions for clinical slide storage (ambient temperature).

b) FFPE Scroll Stability:

The stability of RNA within FFPE scrolls over a period of 30 days under standard storage conditions within the Tempus laboratory environment was evaluated. Testing included 2 clinical samples and 2 control materials. Across all samples tested in this study, the PPA and NPA for gene rearrangement detection between T = 0 and all other timepoints was 100%. The stability of FFPE scrolls as material used for testing with xR IVD for gene rearrangements was determined to be 30 days when stored at 4°C.

c) FFPE Block Stability:

Stability was assessed by testing FFPE blocks after 0-2 years, 2-5 years, and 5-10 years of storage since block preparation. 94 total samples representing 18 different tumor types were tested as part of the 3 storage ranges (or bins): 21 samples at 0-2 years, 37 samples at 2-5 years, and 36 samples at 5-10 years. 97% of the blocks in the 0-2 age group passed quality metrics (3% invalid rate) demonstrating that blocks that have been stored for up to 2 years are stable for use with xR IVD. The invalid rate of the blocks in the 2-5 years bin and the 5–10-year bins was 16% and 43%, respectively at the library preparation step. For all passing samples, the invalid rate at subsequent steps of the xR IVD workflow dropped to 0%.

Stability was also evaluated by comparing the performance of xR IVD in fresh blocks vs. blocks that had been stored under Tempus laboratory conditions for 0 (baseline condition) to 1 year. 7 FFPE clinical samples and 1 control material were tested that included rearrangements in BRAF and RET. Rearrangements in these genes were consistently detected in 1 year old blocks for a PPA of 100%.

d) Real-time and freeze-thaw TNA stability:

Stability of extracted TNA and RNA was evaluated by assessing the agreement for gene rearrangement detection between freshly extracted nucleic acid and after storage at -70ºC at different timepoints and up to 377 days or after storage at -70ºC at different freeze-thaw cycles and up to 15 cycles.

TNA real-time stability and freeze-thaw stability was established by testing 3 clinical specimens and 2 control materials. PPA and NPA across all samples for the real-time stability was found to be 100%. Thus, TNA stability for use with xR IVD was determined to be up to 365 days when stored at < -70°C. PPA and NPA across all samples for freeze-thaw stability was found to be 100%. Thus, the stability of TNA as material used for testing with xR IVD for gene rearrangements was determined to be 13.5 freeze-thaw cycles.

RNA real-time stability and freeze-thaw stability was established by testing 8 clinical specimens and 2 control materials. PPA and NPA across all samples for the real-time stability was found to be 100%. Thus, RNA stability for use with xR IVD was determined to be up to 365 days when stored at < -70°C. PPA and NPA across all samples for freeze-thaw stability was found to be 100%. Thus, the stability of RNA as material used for testing with xR IVD for gene rearrangements was determined to be 13.5 freeze-thaw cycles.

e) RNA Input

RNA input tolerance range at the library preparation, hybridization capture, and sequencing steps of the xR IVD workflow were evaluated by determining the number of samples passing quality metrics when varying the RNA input at each step of the workflow. The input ranges evaluated were 10% - 50% above or below the intended range of operation. The intended range of operation at each step of the workflow is in Table 17, in bold text.

4 FFPE clinical specimens and 1 commercially available control material were evaluated at the library preparation step. 9 FFPE clinical specimens were evaluated at the hybridization capture step and 4 clinical FFPE samples and 2 commercially available control materials were evaluated at the sequencing step.

Across all samples tested in this study, the PPA and NPA for gene rearrangement detection between the intended input level and all other mass input levels was found to be 100%.

Table 17: Workflow steps and RNA input levels evaluated.

Workflow Step	Input Level
Library Capture	ng 40 - 0.8x minimum (-20%)

Workflow Step	Input Level
	ng 45 - 0.9x minimum (-10%)
	ng 50 - 1x minimum
	ng 300 - 1x maximum
	ng 360 - 1.2x maximum (+20%)
Hybridization Capture	ng 120 - 0.8x minimum (-20%)
	ng 135 - 0.9x minimum (-10%)
	ng 150 - 1x minimum
	ng 200 - 1x maximum
	ng 220 - 1.1x maximum (+10%)
	ng 240 - 1.2x maximum (+20%)
Sequencing	0.5x minimum (-50%)
	0.75x minimum (-25%)
	1x minimum
	1x maximum
	1.25x maximum (+25%)
	1.5x maximum (+50%)

f) ***Nucleic acid extraction***: The ability of xR IVD to extract TNA (total nucleic acid) containing amplifiable RNA from FFPE material derived from varying tissues of origin was evaluated by evaluating invalid rates at each sample quality check step. Blocks were grouped into 3 different age ranges: 0-2 years, >2-5 years, and >5-10 years. 104 total samples over 19 cancer types were used in this study (21 samples of 0-2 years of age, 47 samples >2-5 years of age, and 36 samples >5-10 years of age). Over the course of this study, 3 lots of extraction reagents and 2 extraction instruments were used.

The invalid rate for specimens with 0-2 years of age was $\leq$4.76%. The invalid rates for specimens >2-5 years of age and >5-10 years were 14.89% and 42.86%, respectively. These invalid rates are within the predetermined rates for aged specimens. These data demonstrated that xR IVD is capable of successfully extracting TNA/RNA from FFPE specimens derived from varying tissues of origin across different ages.

8. Comparison Studies:

a) Method Comparison (Accuracy)

The accuracy of the xR IVD assay for detecting oncologically relevant gene rearrangements in patients with solid tumors was evaluated by assessing the concordance of gene rearrangement detection results between the xR IVD and an externally validated NGS-based comparator method. Positive percent agreement (PPA), negative percent agreement (NPA), and the associated two-sided 95% confidence intervals (CIs) were calculated. After testing with the orthogonal method, there were a total of 290 samples with valid results that were included in PPA and NPA analyses representing a total of 30 different tumor types.
Of all samples evaluated, there were 13 samples from 4 tumor types (endocrine tumor, gastric cancer, non-small cell lung cancer, thyroid cancer) containing RET gene rearrangements and 277 RET gene rearrangement-negative samples. xR IVD correctly identified all 13 RET rearrangement-positive samples for a PPA of 100% (95% CI 0.7719, 1) and 277 of 277 negative events for an NPA of 100% (95% CI 0.9863, 1) (Table 18 and Table 19).

Table 18: Contingency matrix summarizing agreement at the variant level between xR IVD and the orthogonal method for RET gene rearrangements.

	Orthogonal Method		
xR IVD	RET Positive	RET Negative	Total
RET Positive	13	0	13
RET Negative	0	277	277
Total	13	277	290

Table 19: Agreement at the variant level between xR IVD and the orthogonal method by gene for RET rearrangements.

Gene	**Total Samples**	**PPA (95% CI)**	**NPA (95% CI)**
RET	290	100% (0.7719, 1)	100% (0.9863,1)

Of all samples evaluated, there were 13 samples from 8 tumor types (including biliary cancer, colorectal cancer, low grade glioma, melanoma, non-small cell lung cancer, pancreatic cancer, prostate cancer) containing BRAF gene rearrangements. xR IVD correctly identified 12 of the 13 BRAF rearrangement-positive samples for a PPA 92.3% and an NPA of 100% (Table 20 and Table 21).

Table 20: Contingency matrix summarizing agreement at the variant level between xR IVD and the orthogonal method for BRAF gene rearrangements.

	Orthogonal Method		
xR IVD	BRAF Positive	BRAF Negative	Total
BRAF Positive	12	0	12
BRAF Negative	1	277	278
Total	13	277	290

Table 21: Agreement at the variant level between xR IVD and the orthogonal method by gene for BRAF rearrangements.

Gene	Total Samples	PPA (95% CI)	NPA (95% CI)
BRAF	290	92.3% (0.6669, 0.9961)	100% (0.9863, 1)

b) Wild-Type Calls

There were 171 gene rearrangements detected across the 290 clinical samples, with 168 samples containing 1 or more gene rearrangements and 122 gene rearrangement-negative samples. xR IVD successfully detected 14909 of 14910 negative events.

V Instrument Name:

Illumina NovaSeq 6000 platform (qualified by Tempus)

VI System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device? Yes ____✓____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission? Yes _____ or No ____✓____

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types: Yes ____✓____ or No _____

3. Level of Concern:

   Moderate

4. Specimen Handling

   Refer to Device Description section above.

5. Calibration and Quality controls

   Refer to Device Description section above.

VII Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

VIII Proposed Labeling:

The labeling is sufficient, and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

IX Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

X Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence conclusion.