September 19, 2025

Tempus AI, Inc.
Sarah Piloto, Ph.D.
Director of Regulatory Affairs
600 W Chicago Ave.
Ste. 510
Chicago, Illinois 60654

Re: K241868
Trade/Device Name: xR IVD
Regulation Number: 21 CFR 866.6080
Regulation Name: Next Generation Sequencing Based Tumor Profiling Test
Regulatory Class: Class II
Product Code: PZM
Dated: June 27, 2024
Received: June 27, 2024

Dear Dr. Sarah Piloto:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Zivana Tezak-fragale -S

Zivana Tezak, Ph.D.
Branch Chief
Division of Molecular Genetics and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K241868

Device Name
xR IVD

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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TEMPUS

510(k) Summary

xR IVD

Sponsor Name:	Tempus AI, Inc. 600 W Chicago Ave Ste #510, Chicago, IL 60654 Phone: (833) 514-4187
Contact Person:	Sarah Piloto, Ph.D Director, Regulatory Affairs Tempus AI, Inc. sarah.piloto@tempus.com
510(k) Number:	K241868
Device Trade Name:	xR IVD
Common Name:	Next-generation sequencing tumor profiling test
Classification Name:	Class II
Regulation Number:	21 CFR § 866.6080
Product Code:	PZM
Predicate Device:	MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets)
Submission Number:	DEN170058
Product Code:	PZM

A. Intended Use

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. 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

1. Reagents
All reagents used with respect to the operation of xR IVD are qualified by Tempus.

2. Test Kit Contents
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

[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.

3. Software

The proprietary xR IVD bioinformatics pipeline comprises data analysis software necessary for the xR IVD assay (software version is displayed on the xR IVD clinical report). 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.

4. Instrument

xR IVD uses the *Illumina NovaSeq 6000 Sequencer,* a high throughput sequencing system employing sequencing-by-synthesis chemistry. The xR IVD device is intended to be performed with serial number-controlled instruments. All instruments are qualified by Tempus utilizing the Tempus Quality Management System (QMS).

5. 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 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*

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. Result reporting

xR IVD reports oncologically relevant gene rearrangements 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[2] and reported. Gene rearrangements that do not retain the region(s) required for oncogenicity are not reported.

13. 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

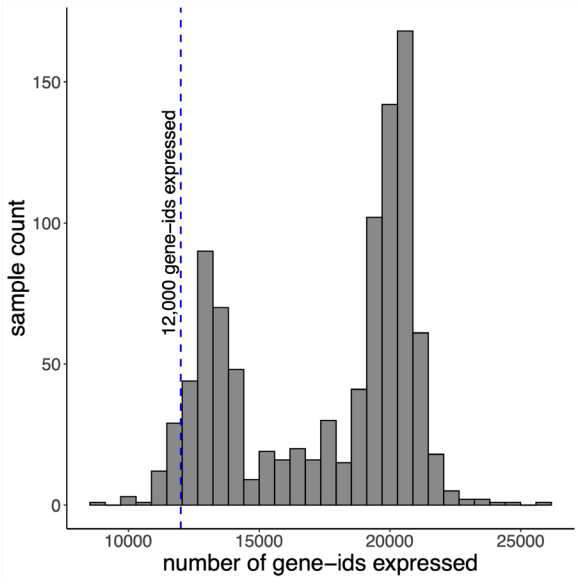
Quality Metric	Batch/Sample/Analyte	Required Value
Positive Control	Batch level	Known sequence mutations are detected
Expression Positive Control	Batch level	≥0.9 r2
RNA gene IDs expressed	Sample level	>12,000
RNA GC distribution	Sample level	45-59%
Mapping rate	Sample level	>80%

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

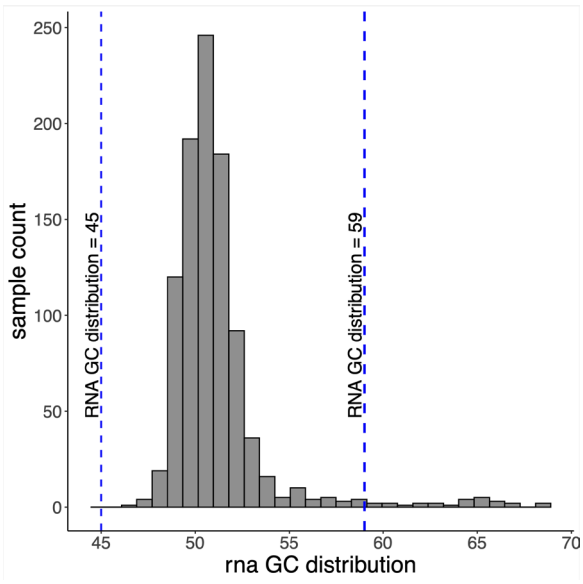
Quality Metric	Batch/Sample/Analyte	Required Value
RNA strand percent sense	Sample level	>88%
RNA strand percent failed	Sample level	≤10%
Unique deduplicated reads	Sample level	>6,000,000
Tumor RNA junction saturation 50_100	Sample level	>1%
Contamination fraction	Sample level	<5%
Gene Rearrangements (BRAF, RET)	Analyte level	≥4 reads

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.


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

A.


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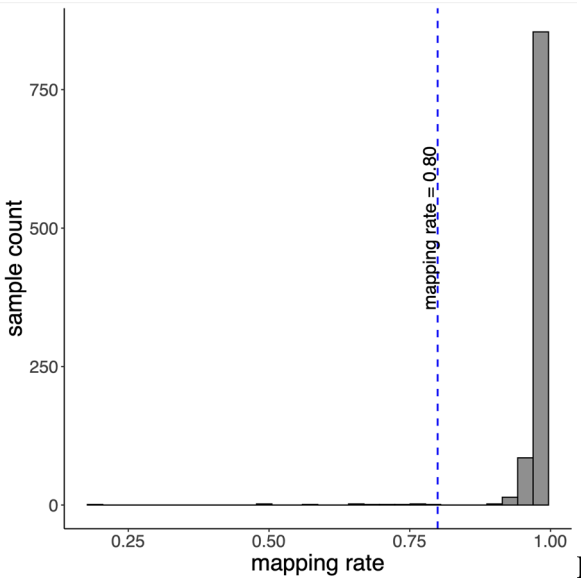
50
100
150
200
250
45
50
55
60
65
70

B.


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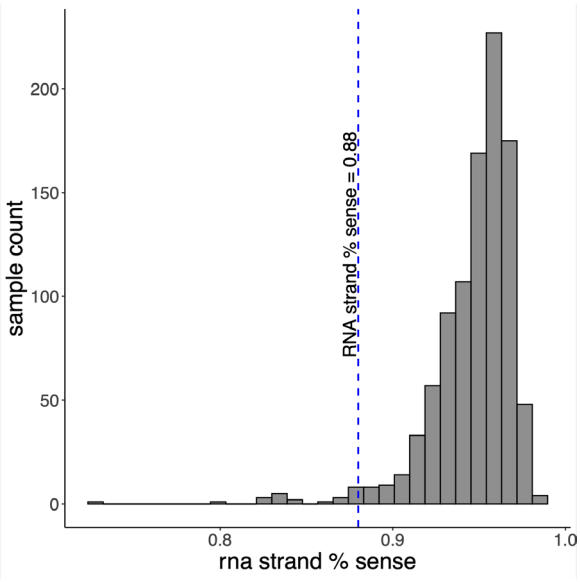
250
500
750
0.25
0.50
0.75
1.00

C.


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

D.

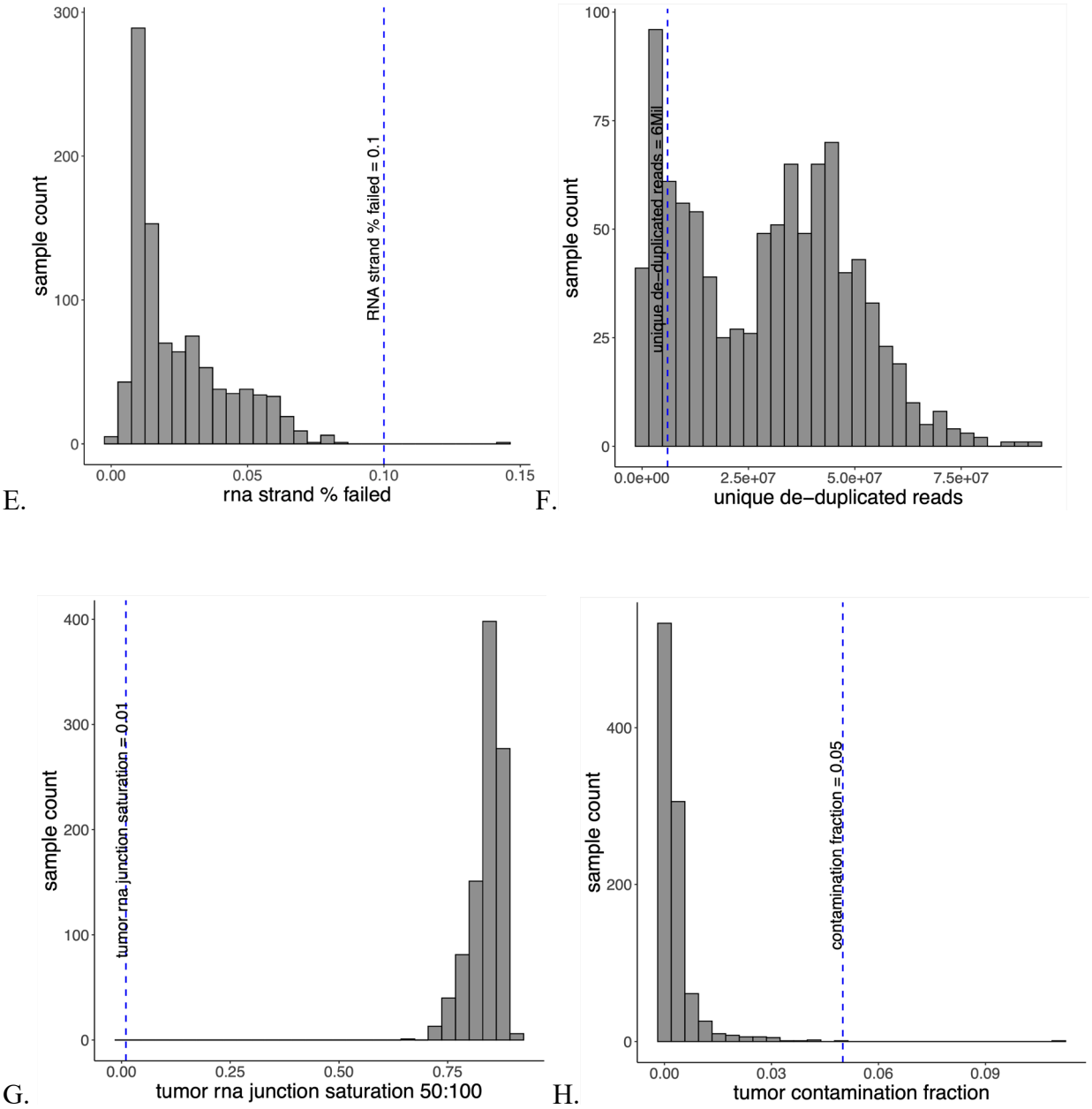


Figure 1: Distribution of quality control metrics in 1000 samples that were run through xR IVD. The blue, dotted line shows the threshold cutoff for each of the following metrics: A. gene-ids expressed, **B.** RNA-GC distribution, **C.** mapping rate, **D.** RNA strand percent sense, **E.** RNA strand 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. 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)

E. Intended Use and Technological Characteristics Comparison

	Predicate (MSK IMPACT)	Candidate (Tempus xR IVD)
Similarities		

	Predicate (MSK IMPACT)	Candidate (Tempus xR IVD)
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.
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.”

	Predicate (MSK IMPACT)	Candidate (Tempus xR IVD)
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)
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.

The primary technological characteristics and indications for use of xR IVD are substantially equivalent to MSK-IMPACT. Both assays are intended for previously diagnosed cancer patients with solid malignant neoplasms to provide tumor profiling information for use by a qualified healthcare professional and in accordance with professional guidelines. Same as the predicate, results are reported in two levels based on clinical significance determined by clinical evidence curation databases. xR IVD and the predicate also have similar technological characteristics given that both assays are qualitative and use hybrid capture next generation sequencing (NGS) for the detection of alterations. Same as the predicate, xR IVD is intended to be performed using a qualified sequencer and it is intended for prescription use only.

Both assays use nucleic acids (RNA for xR IVD and DNA for the predicate) isolated from formalin-fixed paraffin embedded (FFPE) tissue specimens. Similarly, both assays employ controls and cut-offs to ensure accuracy and robustness of the performance and as applicable to the nucleic acid used for the assay. For example, while the predicate has a >200x target coverage, xR IVD uses > 6 000 000 deduplicated reads as an equivalent metric. These differences do not raise any issue regarding the intended use or technological characteristics of the assay and do not constitute a new Indications for Use nor raise different issues of safety and effectiveness of the device.

F. Performance Data

The performance of xR IVD was evaluated based on the following non-clinical studies and following special controls outlined for next generation sequencing based tumor profiling tests (21 CFR 866.6080).

1. Precision

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 select genes included in this submission, 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) NPA was 99.9% (95% CI 0.9989, 0.9997) for gene rearrangements while the NPA between tested replicates was 100% (95% CI 0.9991, 0.9998) for gene rearrangements.

Study 2

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 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%. Similarly, the PPA at the sample level was 100% for 3 of the 4 RET-positive samples evaluated (**Table 3**). A single sample expected to be a RET-positive sample had 2 false negative replicate results (of 36 total) for a PPA of 94.44%. The PPA for BRAF gene rearrangement detection for all samples evaluated was 100% (**Table 4**). The NPA for all conditions was 100% since there were no false positives observed. 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).

Table 3: 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 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 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.

Table 4: 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	KIAA1549_	Level 2	36	36	0	2.32	100%	-

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)
	BRAF						(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.

2. Sensitivity

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 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. Using the probit regression model, the LoD estimate was 6.67 reads for CCDC6-RET, with a P-value of 1 (**Table 5**).

Probit analysis at the sample level was conducted to evaluate the LoD across the different sample types. The LoD from the probit regression model at the sample level was confirmed to be 5-7 reads for RET, depending on the cancer type (**Table 6**).

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 7**). 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 8** 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 5: 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 6: 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	CCDC6-RET	5	5 - 7

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

Table 7: 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 8: 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
	LOD 07 - Prostate Cancer	SND1-BRAF	6	
	LOD 09 - Pancreatic Cancer	CCNY-BRAF	9	

3. Specificity

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.

Samples were evaluated per interferent and tested with no interferent (control), low level interferent and high level interferent (**Table 9**). Study results showed a high PPA (100%) and NPA (≥98%) for gene rearrangement detection between the control condition and both the levels of interferents (**Table 10**).

Table 9: 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%

Interfering Substance	Step Added	Low Concentration	High Concentration
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 10: PPA and NPA by Interferent

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)
	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)

Interferent	Interferent Concentration	PPA	Two-Sided 95% CI	NPA	Two-Sided 95% CI
	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)
	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)

*The samples in this condition consisted of true negative samples only, as such, PPA is not evaluable.

4. Assay Cut-Off

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 were

tested in duplicate (at the maximum RNA input of 300 ng) and using 2 lots of reagents. A total of 24 FFPE clinical specimens representing 12 different tumor types were evaluated. The false positive rate for gene rearrangements was 1.04% which was used to set an LoB threshold of 3 total supporting reads. ≥4 total reads will be required to call a positive gene rearrangement.

6. Accuracy / Method Comparison

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. xR IVD correctly identified all 13 RET rearrangement-positive samples for a PPA and NPA of 100% (**Table 11** and **Table 12**).

Table 11: 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 12: 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 13** and **Table 14**).

Table 13: 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 14: 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)

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.

7. 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 15**).

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 16**). 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 15: 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 Tumor	5.7% (28/494)	7.0% (26/369)	4.8% (14/292)	0.4% (1/277)	0% (0/274)	1.4% (4/257)	12.2% (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)

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)	
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)
Oropharyngeal Cancer	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)
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 16: 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)

8. Linearity/Assay Reportable Range
Not applicable

9. 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.

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.

H. Clinical Performance

xR IVD is a molecular profiling test that uses next generation sequencing to detect gene rearrangements in tumor specimens. xR IVD reports mutations under two categories: “Genomic findings with evidence of clinical significance” and “Genomic findings with potential clinical significance” consistent with the intended use in clinical settings. Alterations with evidence of clinical significance are represented in professional guidelines as established by consensus opinion of experts in the health care community.

I. Conclusions

The candidate device, xR IVD, is substantially equivalent to the predicate device MSK IMPACT (DEN170058).