MyChoice® HRD Plus Technical Information

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Intended Use

Myriad Genetics MyChoice® HRD Plus is used to detect Homologous Recombination Deficiency (HRD) by assessing the GIS Status and the Tumor Mutation *BRCA1/BRCA2* Status in genomic DNA extracted from tumor specimens. This test may aid in identifying patients with a positive HRD status and should be used in accordance with the approved therapeutic product labeling.

NOTE: *The analytical assay used for MyChoice HRD Plus has been developed and validated in accordance with FDA Quality System Requirements (QSR) and is the same analytical assay used with the FDA approved myChoice CDx test.*

Sequencing and large rearrangement analyses are also performed on all analyzable regions of the following genes that have been analytically validated using multiple cancer types: *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, and *RAD54L*. Results from these genes are provided for informational purposes only. Follow-up germline testing may be appropriate for mutations in genes associated with hereditary cancer risk.

Contraindication

- There are no known contraindications.

Warnings and Precautions

- There are no known warnings or precautions.

Limitations

- For *in vitro* diagnostic use
- For professional use only
- For prescription use only
- This test identifies germline and somatic variants in the tumor but does not distinguish between the two.
- Reduced hybridization efficiency of DNA fragments spanning long insertions and deletions (indels) or rearrangements may result in under-representation of mutant DNA molecules in the final sequencing library. This will result in a reduction in the observed frequency of sequence reads spanning the mutation.
- Indels > 25 bp in length can be detected by this assay. However, the ability to detect any particular indel may be impacted by the location and nature of the mutation, the local sequence context, the DNA quality, and the non-tumor DNA content in the sample provided.

- This test has been designed to detect genomic rearrangements including large rearrangements (LRs) involving the promoter and coding exons of the analyzed genes, however, this test does not assess the promoter region for the following genes: *CDK12, CHEK1, FANCL, PPP2R2A, RAD51B,* and *RAD54L*. The detection of large rearrangement deletions and duplications is dependent on the quality of the submitted specimen.
- Whole gene duplications and deletions may not be detected by this assay.
- Other terminal duplications are reported as variants of uncertain significance.
- This analysis is believed to rule out the majority of abnormalities in the genes analyzed. There may be uncommon genetic abnormalities such as specific insertions, inversions, and certain regulatory mutations that will not be detected by this assay.
- Samples with a DNA yield below 30 ng can be tested, however, samples with a low DNA yield (<30ng) may exhibit reduced read coverage, potentially affecting the assay's sensitivity in detecting variants with low allele frequencies (<10%). In addition, samples with a low DNA yield (<30ng) are more likely to result in a failure of GIS analysis.
- Alterations at allele frequencies below the established limit of detection may not be detected consistently.
- The limit of detection of the myChoice CDx test for BRCA1/2 large rearrangements has not been confirmed using ovarian cancer clinical specimens due to sample limitations.

Test Principle

The MyChoice HRD Plus test determines a patient's Myriad HRD Status by detecting single nucleotide variants (SNVs), variants in homopolymer stretches, indels, and LRs in the *BRCA1* and *BRCA2* genes and determining a genomic instability score using DNA obtained from ovarian and breast tumor tissue. A positive Myriad HRD Status result is due to either the presence of a pathogenic mutation in *BRCA1* and/or *BRCA2* (sequencing and/or LR) [Tumor Mutation *BRCA1/BRCA2* Status] and/or a genomic instability score above a defined threshold [Genomic Instability Status].

The assay employs a single DNA extraction method from fixed tissue specimens, 7.5-200 ng of which undergoes multiple steps including fragmentation, end repair and adenylation, adapter ligation, library construction/amplification, hybridization and capture, sequencing, and data analysis.

The MyChoice HRD Plus test is composed of the following major processes:

- Tumor Sample Collection and Shipping
- Tumor genomic DNA Extraction
- DNA Processing using the following assays:
  - MyChoice HRD Plus next generation sequencing is used to detect sequence variants and genomic rearrangements (i.e., large deletions and duplications) in *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, and RAD54L,* and genomic instability analysis.
- Variant Classification

- Genomic Instability Score (GIS) Calculation
- Results Reporting

Summary and Explanation

The MyChoice HRD Plus test is a companion diagnostic for certain poly ADP-ribose polymerase (PARP) inhibitors. Patients whose tumors have a positive Genomic Instability Status and/or pathogenic variants in *BRCA1* or *BRCA2* may show improved progression free survival when treated with certain PARP inhibitors compared to placebo in the treatment setting. The test should be used in accordance with the approved therapeutic product labeling.

The analytical assay used for MyChoice HRD Plus has been developed and validated in accordance with FDA Quality System Requirements (QSR) and is the same analytical assay used with the FDA approved myChoice CDx test.

Test Kit Contents

A sample collection kit provided by Myriad is used by the ordering laboratories/physicians. The collection kits contain the following components:

- Instructions for Use
- Test Request Form
- Specimen Bag
- Bar Coded Labels x4
- Slide/Specimen Holder
- Air Waybill
- Bag Overwrap

Sample Collection and Test Ordering

To order MyChoice HRD Plus testing, the Test Request Form (TRF) included in the test kit must be fully completed.

Please refer to the Collection Instructions and Mailing Instructions for further details about collecting fixed tissue samples and mailing the samples to Myriad.

Test Results and Interpretation

Upon completion of testing at Myriad, a test report will be sent to the designated physician. The results of each test component are provided. If multiple variants are detected, the overall test interpretation most relevant to patient management is based on the most severe variant identified. Standard interpretative information included in test reports is listed below. Note that variants determined to have a classification of favor polymorphism or polymorphism are not included on the test report.

MyChoice HRD Plus Components:

The overall results are composed of two major components, namely Genomic Instability Status, and Tumor Mutation *BRCA1/2* Status. The combined results form the basis of an overall interpretation of the Myriad HRD Status. Potential results for these two components are

described below:

"Genomic Instability Status: Positive"

The test results demonstrate homologous recombination deficiency based on the genomic instability score alone.

"Genomic Instability Status: Negative"

The test results demonstrate homologous recombination proficiency based on the genomic instability score alone.

"Tumor Mutation BRCA1/BRCA2 Status (t*BRCA1/2* Status***): Positive for a Clinically Significant Mutation"***

The test results demonstrate the presence of a deleterious or suspected deleterious sequencing mutation or large rearrangement.

"Tumor Mutation BRCA1/BRCA2 Status (t*BRCA1/2* Status***): Negative for a Clinically Significant Mutation"***

The test results do not demonstrate the presence of a deleterious or suspected deleterious sequencing mutation or large rearrangement in *BRCA1* or *BRCA2*. This category includes genetic variants for which published data demonstrate absence of substantial clinical significance and truncating mutations in *BRCA2* that occur at and distal to amino acid 3.326 (Mazoyer S et al., *Nature Genetics* 1996, 14:253-254). It also includes variants in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of the gene that have been demonstrated to have no pathogenic effect on the length or stability of the mRNA transcript. There may be uncommon genetic abnormalities that will not be detected by MyChoice HRD Plus testing (see **Limitations**).

Clinically significant mutations detected in *ATM, BARD1, BRCA1,BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, or RAD54L* are provided separately for informational purposes only and have not been clinically validated for use with Poly-ADP Ribose Polymerase (PARP) inhibitors.

Interpretive Criteria

A positive Myriad-HRD tumor status is defined as the presence of deleterious or suspected deleterious mutation(s) in the *BRCA1* and/or *BRCA2* genes and/or a positive Genomic Instability Score. The interpretive criteria are based on the following classification categories:

"Deleterious": Includes most nonsense and frameshift mutations that occur at/or before the last known deleterious amino acid position of the affected gene. In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high-risk families, functional assays, biochemical evidence, statistical evidence, and/or demonstration of abnormal mRNA transcript processing. Deletions and duplications of an entire exon(s) identified by the MyChoice HRD Plus test may also be interpreted to be deleterious. Deleterious large genomic rearrangements

include single exon and multi-exonic deletions that are out-of-frame. Out-of-frame single or multi-exonic duplications are classified as deleterious if the orientation is determined to be in tandem and head-to-tail. In- frame deletions/duplications are interpreted on an individual basis and the specific evidence supporting the classification of these mutations is included in the individual patient report.

"Suspected Deleterious": Includes genetic variants for which the available evidence indicates a high likelihood, but not definitive proof, that the mutation is deleterious. The specific evidence supporting an interpretation will be summarized for individual variants in the patient report.

"Genetic Variant of Uncertain Significance": Includes missense variants and variants that occur in analyzed intronic regions whose functional significance has not yet been determined, as well as nonsense and frameshift mutations that occur distal to the last known deleterious amino acid positions of the affected genes.

"Favor Polymorphism" and "Polymorphism": Includes genetic variants for which available evidence indicates that the variant is highly unlikely to alter protein production and/or function. Variants of this type are not reported.

Change of interpretation and issuance of amended reports

The classification and interpretation of all variants identified in the assay reflect the current state of scientific understanding at the time the report is issued. In some instances, the classification and interpretation of such variants may change as new scientific information becomes available.

Whenever there is a change in the classification of a variant that affects the overall interpretation of a patient's test result, an amended report will be provided by Myriad.

PERFORMANCE CHARACTERISTICS

Summary of Non-clinical Laboratory Studies

The specific performance characteristics of the MyChoice HRD Plus assay (MyChoice) were determined by studies using FFPE tumor samples. Samples were selected to evaluate a range of representative tumor *BRCA1* and *BRCA2* sequence variants (e.g., single nucleotide variants, insertions or deletions, and variants in homopolymers) and LRs (e.g., deletions and duplications affecting single and multiple exons) detected by MyChoice, as well as a representative range of Genomic Instability Scores, as reflected in the device labeling.

1. Correlation with Orthogonal Reference Method (Accuracy)

The accuracy of the Myriad HRD Status determined by the test was demonstrated using a validated Next Generation Sequencing (NGS)-based assay with a combination of non-clinical samples and FFPE clinical specimens from cancer patients enrolled in clinical trials from whom sufficient quantity and quality of DNA was available for testing with the NGS comparator assay. A total of 209 FFPE tumor specimen-derived DNA samples were tested with both the assays. Samples representing the following subgroups were tested in the study: 5 t*BRCA1/2* Status positive / Genomic Instability Status negative, 71 t*BRCA1/2* Status negative / Genomic Instability Status negative, 66 t*BRCA1/2* Status positive / Genomic

Instability Status positive, 61 t*BRCA1/2* Status negative / Genomic Instability Status positive.

a. t*BRCA1/2* Sequence Variant and LR Analytical Calls

A total of 1,733/1,733 valid *BRCA1/2* sequence variant test calls were observed compared to the valid reference (comparator) *BRCA1/2* sequence variant calls with ≥ 10% allele frequencies across all samples evaluated. This corresponds to a positive percent agreement (PPA) of 100%. Including variants with < 10% allele frequency, a total of 1,733/1,734 valid *BRCA1/2* sequence variant calls were observed, corresponding to a PPA of 99.94% with a 95% lower confidence limit of 99.7267%. In addition, a total of 3,605,951/3,605,951 valid *BRCA1/2* sequence non-variant base calls were observed, corresponding to a negative percent agreement (NPA) of 100% with a 95% lower confidence limit of 99.9999%. A total of 402/402 concordant valid *BRCA1/2* LR calls were observed compared to the valid reference (comparator) *BRCA1/2* LR calls across all samples evaluated. This corresponds to an overall percent agreement (OPA) of 100% for LR calls.

The results of the accuracy study were evaluated for three patient outcomes: (i) the t*BRCA1/2* Status based on *BRCA1* and *BRCA2* sequence and LR analyses; (ii) the Genomic Instability Status based on the genomic instability score; and (iii) the overall Myriad HRD Status based on the combined results of the t*BRCA1/2* Status and Genomic Instability Status. The agreement between the test and the comparator (reference) assay is summarized below.

b. t*BRCA1/2* Status Results

Concordance analysis of all 200 valid patient results produced by both the test and Comparator assays resulted in an OPA of 100%.

c. Genomic Instability Status Results

Concordance analysis of all 206 valid patient results produced by both MyChoice and the Comparator assays revealed a PPA of 98.5%, a NPA of 97.4%, and an OPA of 98.1%.

d. Myriad HRD Status Results

Concordance analysis of all 206 valid patient results from both MyChoice and Comparator assay revealed a PPA of 98.5%, a NPA of 98.6%, and an OPA of 98.5%.

2. Analytical Sensitivity

a. Limit of Blank (LoB)

Twenty-six (26) FFPE normal tissue samples were tested wherein all low frequency variants were expected to be spurious technical artifacts rather than true biological events. The distribution of allele frequencies was plotted. The frequencies of spurious variants were typically very low with the distribution decreasing very rapidly from 1% to 5%. There were no spurious variants with frequency above 5%.

One hundred thirty-six (136) FFPE t*BRCA1/2* Status negative (wildtype) tissue samples were analyzed. These samples produced 2,357,861/2,357,861 (100%) concordant non-variant base calls, resulting in a false positive rate of 0% with a 95% upper confidence

limit of 0.00013%. Of the 136 samples, 70 samples were both t*BRCA1/2* Status negative / Genomic Instability Status negative. These 70 samples produced 1,213,621/1,213,621 (100%) concordant non-variant base calls, resulting in a false positive rate of 0% with a 95% upper confidence limit of 0.00025%. This retrospective analysis provides empirical data for setting a minimum allele frequency threshold of 5% to differentiate spurious background noise from real variants.

b. Limit of Detection (LoD)

i. t*BRCA1/2* Sequence Variants

DNAs from four FFPE samples with known *BRCA1/2* pathogenic sequence variants [*BRCA1* c.181T>G (p.Cys61Gly), a single nucleotide variant, *BRCA1* c.1961del, a < 10 bp deletion in an 8 bp homopolymer sequence, *BRCA1* c.5266dupC, a < 10 bp insertion and *BRCA2* c.9117_9117+11del, a ≥ 10 bp deletion] and FFPE tumor DNA samples without these *BRCA1/2* variants (WT) were used to create simulated tumor:normal DNA samples with 10%, 8%, 6.5%, 5% and 2% allele frequencies of these mutations. Twenty replicates of each of the various DNA mixes were run and *BRCA1/2* sequencing analytical calls were analyzed using CLSI's EP17-A2 probit analysis methods to determine the LoD of these mutations.

The results of this study show that the LoDs of the four pathogenic sequence variants have different ranges of allele frequencies. The LoD of a single bp substitution was at 7.23% allele frequency. The LoD of a < 10 bp deletion in an 8 bp homopolymer sequence was 6.66%. The LoD for a < 10 bp insertion was 6.36%, and the LoD for a ≥ 10 bp deletion was 5.98%.

ii. **t*BRCA1/2* Large Rearrangements**

Two FFPE tumor DNA samples, each carrying a different large rearrangement (LR): [*BRCA1* del exon 8 LR exon and *BRCA2* del exons 19–21 LR], were each mixed with a FFPE DNA with no detectable *BRCA1/2* LRs (WT) to create tumor : normal samples with 50%, 40%, 30% and 10% allele frequencies of each LR. Ten replicates of each of the various DNA mixes were run and the concordance between the *BRCA1/2* LR test calls and reference calls from each of the undiluted tumor samples was analyzed. The LoD for each LR was defined based on CLSI's EP17-A2 guidance that recommends ≥95% concordant, positive LR calls.

The t*BRCA1/2* LR portion of MyChoice LoD for the ≥ 3 exons LR is at 30% allele frequency, while the LoD for the 1-2 exons LR is at 50% allele frequency.

iii. Genomic Instability Status

Four FFPE matched tumor-normal samples were evaluated in this LoD study. DNAs extracted from each pair of FFPE matched tumor and normal samples were mixed to create five different tumor:normal DNA mixes at 40%, 30%, 20%, 10% and 0% tumor DNA content. Ten replicates of each of the tumor:normal mixes were run along with replicates of the undiluted tumor and normal DNAs. All the tumor:normal DNA mixes were tested using MyChoice and the final Genomic

Instability Status results were analyzed to assess the LoD of this assay.

The results from this LoD study show that MyChoice yields highly valid genomic instability score results and the Genomic Instability Status results, for all samples at all tumor DNA content levels that produced valid results. Based on this study, the LoD of the genomic instability score portion of MyChoice is at ~ 30% tumor DNA content.

3. Analytical Specificity

a. Interference (t*BRCA1/2* Sequence Variant and LR Analytical Calls)

To evaluate the potential impact of three classes of substances (endogenous [hemoglobin, triglycerides], exogenous [tissue marking dye, paraffin wax], and method-specific interferents [ethanol, NaOH]) that can potentially interfere with the assay, this study evaluated seven FFPE specimens (5 t*BRCA1/2* Status positive / Genomic Instability Status positive and 2 t*BRCA1/2* Status negative / Genomic Instability Status negative) representing single nucleotide variants, insertions or deletions of < 10 bp in length, insertions or deletions of ≥ 10 bp in length, homopolymer variants (5+ bp), and large rearrangements affecting ≥ 3 exons. The effects of potential interfering substances were tested at one or two replicates to determine if they would impact the results of MyChoice, and the results were compared to the control (no additional interferents) condition.

All treated samples across all six substances at the high test levels passed the acceptance criteria, with the exception of method-specific NaOH at the high test level (1.0 N), which failed as 10/14 (71.4%) tests successfully generated valid *BRCA1/2* sequencing and LR results. However, 14/14 (100%) of the tests at the low 0.4 N NaOH level passed. All samples run under each condition produced valid positive or negative patient calls. The positive and negative patient calls were 100% concordant when compared to samples without additional interferents.

b. Interference (Genomic Instability Score)

All treated samples produced valid genomic instability scores for each potential interfering substance tested. In addition, all samples run under each condition produced valid positive or negative patient calls showing 100% concordance when compared to samples without additional interferents.

Taken together, these results demonstrate that the t*BRCA1/2* sequencing and genomic instability score portions of the assay are minimally impacted or not impacted by the presence of any of the substances tested in this study.

Necrosis of ≥ 10% of the tumor area was observed in 11% (n = 66) of tumor samples in an ovarian study with niraparib (NCT NCT02354586). Only 3% of samples had necrosis in > 10% of the tumor area, and no samples were identified with necrosis involving > 60% of the tumor area. The standard procedure in Myriad's Anatomic Pathology laboratory is to macro-dissect the fixed tumor tissues on slides to maximize tumor content, i.e., minimize the inclusion of non-tumor content including necrotic tissue. Thus, based on the above analysis, it was concluded that necrosis of ≤ 60% does not impact the MyChoice results.

c. Carryover

The purpose of this study was to demonstrate that the MyChoice test minimizes carryover across samples. Fourteen FFPE specimens (11 t*BRCA1/2* Status positive / Genomic Instability Status positive, 1 t*BRCA1/2* Status positive / Genomic Instability Status positive and 2 t*BRCA1/2* Status negative / Genomic Instability Status negative) were processed consecutively through DNA extraction. The DNA from these 14 FFPE samples were then set up in a checkerboard pattern, alternating between low (50 ng) and high (200 ng) inputs. Two consecutive batches were set up in this pattern, with one checkboard pattern inversed, to assess intra-run (1st batch) and inter-run (2nd batch) carryover. An additional reference batch was run with all samples at 200 ng to compare for concordance. For both the intra-run and inter-run batches, all 14/14 samples produced complete, valid analytical calls that were 100% concordant for *BRCA1* and *BRCA2* sequence and LR calls, and all genomic instability scores were valid. In addition, the patient results of all samples run in both batches were 100% concordant.

Additionally, using the SNVs analyzed in the assay, carryover was quantified within and between batches. The highest intra-run carryover observed was 0.2% and the highest inter-run carryover observed was 0.1%. The average intra-run carryover observed was 0.14% and average inter-run carryover was 0.10%**.** The overall analytical results show that MyChoice has very low intra-run and inter-run sample carryover and poses minimal risk to patient results.

d. Cross-Reactivity

Cross-reactivity studies for sequence-based assays are intended to differentiate between target analyte sequences and sequences generated from other sources. Three types of spurious sequences that could potentially be mistaken for target sequences, e.g., pseudogenes or other genomic regions that are highly homologous to targeted genes and regions, off-target regions that hybridize to hybridization baits or DNA sequences that carry-through the process, and process artifacts and low quality sequences. The purpose of this study is to demonstrate that captured sequences not originating from target regions do not materially affect the MyChoice test.

Sequencing data for 7 FFPE tissue samples (4 t*BRCA1/2* Status positive / Genomic Instability Status positive, 1 t*BRCA1/2* Status negative / Genomic Instability Status negative, 1 t*BRCA1/2* Status negative / Genomic Instability Status positive, and 1 t*BRCA1/2* Status unknown / Genomic Instability Status positive) were processed through MyChoice in quadruplicate, yielding 28 total tests results that were mapped to the human genome sequence to reveal off-target sequences due to pseudogene and cross-reactivity of hybridization baits (capture probes).

The MyChoice test has two methods for mitigating the impact of pseudogenes. The capture baits were designed to minimize the capture of pseudogene regions, and the analysis algorithm identifies and excludes pseudogene-derived sequences as part of *BRCA1/2* variant detection and LR calling.

No off-target sequences were found to affect the tumor *BRCA1* and *BRCA2* component of the device. Only 1.2% of SNVs used to calculate the genomic instability score were affected by off-target sequences with a minimal effect on the reported score. This retrospective analysis suggests that cross-reactivity poses a minimal risk to the MyChoice test.

4. Repeatability and Reproducibility

The repeatability and reproducibility of MyChoice was investigated by testing DNA extracted from FFPE clinical specimens. The purpose of these studies was to demonstrate that the assay generates highly reproducible *BRCA1* and *BRCA2* sequencing and LR analytical calls and genomic instability scores on the tested samples from five different studies over five periods of time. In the first three studies (Studies 1–3), a total of 18 unique samples were tested. All but two of these samples were tested at the DNA input amount of 200 ng per assay. The fourth study (Study 4) evaluated 7 FFPE tumor specimens from the first three studies at 50 ng DNA input. The samples were run in duplicate per run, over 6 runs, using 3 lots of reagents, 3 different sets of instruments, 6 different operators, and 3 different data reviewers. An additional study (Study 5) was performed using 5 unique FFPE samples with 18 replicates per sample divided across 9 independent runs over multiple days using 3 different sequencers and 3 lots of critical reagents. Thus, a total of 23 FFPE samples were evaluated (10 t*BRCA1/2* Status positive / Genomic Instability Status positive, 2 t*BRCA1/2* Status positive / Genomic Instability Status negative, 6 t*BRCA1/2* Status negative / Genomic Instability Status positive, 3 t*BRCA1/2* Status negative / Genomic Instability Status negative, 2 t*BRCA1/2* Status unknown / Genomic Instability Status positive). The samples contained single nucleotide variants, insertions or deletions of < 10 bp in length, insertions or deletions of ≥ 10 bp in length, homopolymer variants (5+ bp), large rearrangements affecting 1-2 exons, and large rearrangements affecting ≥ 3 exons. The tested samples had a wide genomic instability score range.

a. *tBRCA1/2* Sequence Variant and LR Analytical Calls

All 228/228 samples and replicates tested from Studies 1–3 produced complete, valid *BRCA1* and *BRCA2* sequencing and LR analytical calls. Overall, 2,220/2,220 *BRCA1* and *BRCA2* sequence variant calls, 3,951,603/3,951,603 non-variant bases and 456/456 LR calls across all samples/replicates tested were 100% concordant.

Study 4 analyzed 7 FFPE tumor samples at 50 ng DNA inputs, which had previously been evaluated at 200 ng DNA input levels in Studies 1-3. All samples and replicates run generated complete (100%) valid *BRCA1* and *BRCA2* sequence and LR calls. Altogether, all 708/708 *BRCA1* and *BRCA2* sequence variant calls, 1,450,393/1,450,393 non-variant bases and 168/168 LR calls across all samples/replicates tested at the 50 ng DNA input level were 100% concordant. All *BRCA1* and *BRCA2* sequence variant and LR calls from the 7 samples run at 50 ng DNA inputs were 100% concordant with analytical calls from the same samples run at 200 ng DNA inputs.

Study 5 tested five new additional ovarian tumor specimens that were t*BRCA1/2* Status negative / Genomic Instability Status positive. The specimens were tested at the lowest

DNA input of 30 ng with Genomic Instability Status being low positives. The MyChoice test produced 700/702 concordant *BRCA1/2* sequence variant calls, at mean allele frequency (MAF) ≥ 10%, resulting in a 99.7% PPA. There were two false negative calls in one sample in a single nucleotide variant with a MAF of 11.2%. When all valid *BRCA1/2* sequence variants were analyzed, including those below 10% MAF, MyChoice produced 788/792 concordant sequence variant calls resulting in a 99.5% PPA and 99.99% (1,553,331/1,553,332) concordance for non-variant base calls. For these five samples, *BRCA1/2* LR concordance was 100% (165/165).

b. t*BRCA1/2* Status Results

All samples and replicates run for all five studies produced valid positive or negative patient results, except for two samples, which were inconclusive for the t*BRCA1/2* Status result. However, both of these samples had a positive Genomic Instability Status and were therefore both Myriad HRD Status positive. All valid positive and negative patient calls were 100% concordant, resulting in 100% PPA and NPA. In addition, valid patient results for all samples run at 50 ng DNA input level were 100% concordant with their corresponding sample run at the 200 ng DNA input level.

c. Genomic Instability Score

For the genomic instability score portion of the assay, all 23 samples and replicates at all DNA inputs (200 ng, 50 ng, and 30 ng) from the five studies produced valid genomic instability scores. The overall 95% confidence interval for the true proportion of majority calls across samples correspond to 98.8% - 100% for the first four studies and 81.5% - 100% for the fifth study.

d. Genomic Instability Status and Myriad HRD Status Results

All samples and replicates run for all five studies produced complete, valid, positive or negative patient calls in both sets of patient results. All positive and negative patient calls were 100% concordant, resulting in 100% PPA and NPA. In addition, all 7 samples run at the 50 ng and 200 ng DNA input levels were 100% concordant.

These results demonstrate that *BRCA1/2* sequence variant and LR calls, genomic instability score, and patient results of MyChoice are highly reproducible across the different process and reagent variabilities introduced in these studies.

5. Guardbanding

The MyChoice guardband/robustness studies challenged the performance of the assay across three key parameters: (i) amount of FFPE tumor tissue-derived DNA input into the assay, (ii) hybridization temperature for probe capture, and (iii) library input onto the HiSeq instrument.

For the DNA input guardband study, 28 FFPE tumor samples (13 t*BRCA1/2* Status positive / Genomic Instability Status positive, 1 t*BRCA1/2* Status positive / Genomic Instability Status negative, 4 t*BRCA1/2* Status negative / Genomic Instability Status positive, 10 t*BRCA1/2* Status negative / Genomic Instability Status negative) were run. For the hybridization temperature and library input guardband studies, seven FFPE tumor samples (3 t*BRCA1/2* Status positive / Genomic Instability Status positive, 2 t*BRCA1/2* Status positive / Genomic Instability Status negative, 2 t*BRCA1/2* Status negative / Genomic Instability Status negative) were run in triplicate at standard assay conditions for the generation of reference results for each guardband condition. All analytical calls (i.e., *BRCA1/2* sequence variants, LR calls, and genomic instability score) and patient results for the reference and test samples and replicates were reported.

a. DNA Input Guardband/Robustness

Two studies were performed to evaluate the range of FFPE tumor extracted DNA input into the assay. In the first study, 14 samples were run in triplicate at 200 ng (used as the reference), 100 ng and 50 ng, and in singlet at 300 ng, 40 ng, 30 ng, 20 ng, and 10 ng. The second study ran a different set of 14 samples in triplicate at 200 ng (used as the reference) and 30 ng. The results from samples with different DNA input and samples near LoD of the t*BRCA1/2* portion (< 10% and ≥ 10% MAF) were reported.

For the t*BRCA1/2* portion of the assay, all valid *BRCA1* and *BRCA2* sequence variant calls from 300 ng to 10 ng DNA input levels were 99.8% concordant and all valid *BRCA1* and *BRCA2* non-variant base calls were 99.99% concordant. All valid *BRCA1* and *BRCA2* LR calls produced from 300 ng to 20 ng DNA input levels were 100% concordant (no valid LR calls were produced at the 10 ng input level). For the genomic instability score portion of the assay, the total allowable error (TAE) analysis of valid genomic instability scores displayed acceptable amounts of bias and variation by passing pre-defined acceptance criteria from 300 ng to 30 ng input levels. In addition, the valid patient calls of all tests run were 100% concordant, except for a single false negative Genomic Instability Status patient call in one out of three replicates of one sample tested at 30 ng. All replicates of this sample had t*BRCA1/2* Status positive patient results, and as such, all patient results were Myriad HRD Status positive.

b. Hybridization Temperature for Probe Capture

The hybridization temperature for probe capture was evaluated by varying the temperature by ± 1ºC and ± 2ºC from the 65ºC standard condition. Seven FFPE samples were run at standard hybridization temperature to generate reference results. The results of the 7 samples from each guardband test condition were compared to the results obtained at the standard hybridization temperature. All samples across all guardband test conditions generated complete, valid *BRCA1* and *BRCA2* sequencing and LR results, as well as valid genomic instability scores. All *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. Additionally, all samples tested at all guardband conditions produced valid genomic instability score analytical results and all patient results were 100% concordant.

c. Library Input onto the HiSeq Instrument

The library input onto the HiSeq instrument was evaluated by varying the library concentration by ± 2 pM and ± 4 pM from the 6 pM standard condition. Seven FFPE samples were run at the standard library input amounts to generate reference results. The results of the 7 samples from each guardband test condition were compared to the results obtained at the standard library input amounts. Samples across all guardband test conditions generated complete, valid *BRCA1* and *BRCA2* sequencing and LR results, as well as genomic instability score. All *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. All genomic instability scores generated at the different test conditions were valid and passed the pre-defined acceptance criteria. In addition, all patient results were 100% concordant.

These results show that MyChoice is robust and is not affected by these process variations.

6. Stability Studies

a. Stability of FFPE Clinical Specimens

This study evaluated the real-time stability of FFPE tumor blocks and FFPE tumor sections on slides, stored at laboratory temperature. Reference results were defined from replicates of each specimen run at the initial (earliest) time point. The analytical and patient results of aged specimens, run in singlet, were compared to the reference calls for concordance.

i. FFPE Tumor Blocks

Stability data was analyzed for 16 unique FFPE tumor blocks for up to the 5.5-year time point. Analytical calls from aged blocks at each time-point were compared to those from the initial (T0), reference time point. Analytical results have been obtained for 12, 7 and 6 samples at the 3.5, 5.0, and 5.5 year time points, respectively. For *BRCA1* and *BRCA2* sequencing, a single false positive *BRCA2* sequence variant was called at an allele frequency of 8.3% at the 3.5 year time point. All other *BRCA1* and *BRCA2* sequence variant calls were 100% concordant across all aged FFPE tumor blocks tested at each of the time points. All *BRCA1* and *BRCA2* LR calls were 100% concordant. In addition, the genomic instability scores were valid and patient results were 100% concordant across all aged blocks tested at the different time points. The stability study has been confirmed up to 5.5 years.

ii. FFPE Tumor Sections

The stability of unique FFPE tumor sections on slides is being evaluated at the following time points: 0, 1, 3, 5 and 5.5 years. Analytical calls from aged tumor sections at each testing time-point were compared to those from the initial (T0), reference time point. At the 1 year time point, all 10/10 samples generated complete, valid analytical calls and patient results. All *BRCA1* and *BRCA2*sequence and LR calls were 100% concordant. In addition, all genomic instability scores were valid and all patient results were 100% concordant across all specimens tested. The stability study has thus far been confirmed up to 1-year

time point.

b. FFPE Tumor Extracted DNA Stability

The stability of DNAs extracted from 9 FFPE tumor specimens and stored at -20 ºC was evaluated at 0 (T0), 30 days, 60 days, 90 days and 6 months. Analytical calls from aged DNAs at each time-point were compared to those from the initial (T0), reference time point. All tests performed from 30 days to 6 months produced complete, valid analytical calls and patient results. For each of the stability testing time points, all *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. In addition, the genomic instability scores were valid and patient results were 100% concordant across all aged FFPE tumor extracted DNAs. The claimed stability for the extracted DNA is 5 months at -20°C.

c. Reagents Stability

The real-time stability of critical reagents used in the device was evaluated. Three lots of each of these reagents were stored at specified conditions and run at 0, 1 month, and 4 months. Each reagent lot was tested with 6 FFPE tumor DNAs in duplicate at each aged reagents time point. Analytical calls from aged reagents at each time-point were compared to those from the initial (T0) reference time point. All tests performed at 1 month and 4 months produced complete, valid analytical calls and patient results. For each of the stability testing time points, all *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant, the genomic instability scores generated were valid, and all the patient results were 100% concordant across all lots of aged reagents.