

BRACAnalysis CDx™

Technical Information Summary

Intended Use

BRACAnalysis CDx™ is an *in vitro* diagnostic device intended for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes using genomic DNA obtained from whole blood specimens collected in EDTA. Single nucleotide variants and small insertions and deletions (indels) are identified by polymerase chain reaction (PCR) and Sanger sequencing. Large deletions and duplications in *BRCA1* and *BRCA2* are detected using multiplex PCR. Results of the test are used as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious germline BRCA variants eligible for treatment with Lynparza™ (olaparib). This assay is for professional use only and is to be performed only at Myriad Genetic Laboratories, a single laboratory site located at 320 Wakara Way, Salt Lake City, UT 84108.

Contraindication

- Patients who have undergone a previous allogeneic bone marrow transplant should not be tested with the BRACAnalysis CDx™.

Warnings and Precautions

- When drawing blood for the BRACAnalysis CDx™ test, universal precautions for bloodborne pathogens should be observed.
- Patients under consideration for testing who have been diagnosed with a hematologic malignancy, such as leukemia, could generate a positive (deleterious or suspected deleterious) result that is somatic, and not germline, due to chromosome instability.
- The classification and interpretation of all variants identified reflects the current state of scientific understanding at the time the result report is issued. In some instances, the classification and interpretation of variants may change as scientific information becomes available.

Limitations

- For *in vitro* diagnostic use
- For professional use only
- For prescription use only
- Performance of the BRACAnalysis CDx™ test was not evaluated for deletions larger than 5 nucleotides and small insertions using the BRACAnalysis CDx™ Sanger Sequencing test.
- The test is designed to detect germline *BRCA1* and *BRCA2* variants within specific regions of the genes. Consistent with a genetic test, additional variants outside of the assessed regions that may impact patient care will not be detected.
- The test has been designed to detect genomic rearrangements (i.e., deletions or duplications) involving the promoter and coding exons of *BRCA1* and *BRCA2*, but the test will not detect some types of errors in RNA transcript processing. Insertions that do not result in duplications will generally not be detected. Also, the test may not accurately differentiate between duplications and triplications.
- Unequal allele amplification may result from rare polymorphisms under primer sites and lead to false negative results.
- There are limited portions of either *BRCA1* or *BRCA2* for which sequence determination can be performed only in the forward or reverse direction.
- The test is intended to be performed on specific serial number-controlled instruments at Myriad Genetic Laboratories, Inc.

Test Principle

BRACAnalysis CDx™ is performed by a single laboratory, Myriad Genetic Laboratories, Inc. (Myriad), located in Salt Lake City, UT. The test is intended to detect germline *BRCA1* and *BRCA2* variants and provide a clinical interpretation of the identified variants. Results of the test are used as an aid in the identification of ovarian cancer patients who may be considered for treatment with Lynparza™ (olaparib).

The BRACAnalysis CDx™ test is composed of the following major processes:

- Whole Blood Collection and Shipping
- Genomic DNA Extraction
- DNA Processing using the following assays:
 - BRACAnalysis CDx™ Sanger Sequencing Test - used to detect sequence variants
 - BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx) - used to identify genomic rearrangements (i.e., large deletions and duplications)
- Variant Classification
- Results Reporting

Reportable variants are confirmed by repeat analysis or by confirmatory testing. Approximately 98% of all variants detected by the BRACAnalysis CDx™ are confirmed by repeat testing; the remaining variants (about 2%) require confirmatory analysis by the following tests:

- Alternate Primer Sequencing (APS) - used to identify potential heterozygous base changes under the primers used in the BRACAnalysis CDx™ Sanger Sequencing test or the BART® CDx test
- Confirmatory PCR Analysis (CPA) - used to confirm *BRCA1* and *BRCA2* large rearrangements detected initially by the BART® CDx test

Summary and Explanation

The BRACAnalysis CDx™ device is a companion diagnostic for AstraZeneca's drug Lynparza™ (olaparib), a poly ADP-ribose polymerase (PARP) inhibitor. Cells that possess at least one normal *BRCA1* and *BRCA2* allele are relatively resistant to PARP inhibition. *BRCA1* or *BRCA2* dysfunction, defined as mutant cells lacking wild-type *BRCA1* or *BRCA2*, sensitizes cells to PARP inhibition leading to chromosomal instability, cell cycle arrest and apoptosis (Bryant et al. 2005*, Farmer et al. 2005**).

Test Kit Contents

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

- Monoject™ Blood Collection Tube, Silicone Coated Lavender Stopper, buffered EDTA(K3) 0.10 mL 15.0% Solution, or equivalent
 - The shelf life is printed on each individual tube. Prior to using a tube for blood collection, check the expiration date.
- Test Request Form (TRF)
- Example TRF
- Collection Instructions
- Mailing Instructions
- Technical Information Summary

Sample Collection and Test Ordering

To order the BRACAnalysis CDx™ test, the Test Request Form (TRF) included in the test kit must be fully completed.

Please refer to the BRACAnalysis CDx™ Collection Instructions and Mailing Instructions for further details about collecting blood samples and mailing samples to Myriad.

For more detailed information including Performance Characteristics, please find the complete Technical Information at: https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

References

- *Nature. 2005 Apr 14;434(7035):913-7. "Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase." Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T.
- **Nature. 2005 Apr 14;434(7035):917-21. "Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy." Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A.

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

Myriad Genetics Laboratories, Inc.
320 Wakara Way, Salt Lake City, UT 84108
Phone: 1-800-469-7423
www.myriad.com

Note: Test results should be communicated to the patient in a setting that includes appropriate counseling

Intended Use

BRACAnalysis CDx™ is an *in vitro* diagnostic device intended for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes using genomic DNA obtained from whole blood specimens collected in EDTA. Single nucleotide variants and small insertions and deletions (indels) are identified by polymerase chain reaction (PCR) and Sanger sequencing. Large deletions and duplications in *BRCA1* and *BRCA2* are detected using multiplex PCR. Results of the test are used as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious germline *BRCA* variants eligible for treatment with Lynparza™ (olaparib). This assay is for professional use only and is to be performed only at Myriad Genetic Laboratories, a single laboratory site located at 320 Wakara Way, Salt Lake City, UT 84108.

Contraindication

- Patients who have undergone a previous allogeneic bone marrow transplant should not be tested with the BRACAnalysis CDx™ test.

Warnings and Precautions

- When drawing blood for the BRACAnalysis CDx™ test, universal precautions for bloodborne pathogens should be observed.
- Patients under consideration for testing who have been diagnosed with a hematologic malignancy, such as leukemia, could generate a positive (deleterious or suspected deleterious) result that is somatic, and not germline, due to chromosome instability.
- The classification and interpretation of all variants identified reflects the current state of scientific understanding at the time the result report is issued. In some instances, the classification and interpretation of variants may change as scientific information becomes available.

Limitations

- For *in vitro* diagnostic use
- For professional use only
- For prescription use only
- Performance of BRACAnalysis CDx™ test was not evaluated for deletions larger than 5 nucleotides and small insertions using the BRACAnalysis CDx™ Sanger Sequencing test.
- The test is designed to detect germline *BRCA1* and *BRCA2* variants within specific regions of the genes. Consistent with a genetic test, additional variants outside of the assessed regions that may impact patient care will not be detected.
- The test has been designed to detect genomic rearrangements (i.e., deletions or duplications) involving the promoter and coding exons of *BRCA1* and *BRCA2*, but the

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

test will not detect some types of errors in RNA transcript processing. Insertions that do not result in duplications will generally not be detected. Also, the test may not accurately differentiate between duplications and triplications.

- Unequal allele amplification may result from rare polymorphisms under primer sites and lead to false negative results.
- There are limited portions of either *BRCA1* or *BRCA2* for which sequence determination can be performed only in the forward or reverse direction.
- The test is intended to be performed on specific serial number-controlled instruments at Myriad Genetic Laboratories, Inc.

Test Principle

BRACAnalysis CDx™ is performed by a single laboratory, Myriad Genetic Laboratories, Inc. (Myriad), located in Salt Lake City, UT. The test is intended to detect germline *BRCA1* and *BRCA2* variants and provide a clinical interpretation of the identified variants. Results of the test are used as an aid in the identification of ovarian cancer patients who may be considered for treatment with Lynparza™ (olaparib).

The BRACAnalysis CDx™ test is composed of the following major processes:

- Whole Blood Collection and Shipping
- Genomic DNA Extraction
- DNA Processing using the following assays:
 - BRACAnalysis CDx™ Sanger Sequencing - used to detect sequence variants
 - BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx) - used to identify genomic rearrangements (i.e., large deletions and duplications)
- Variant Classification
- Results Reporting

Reportable variants are confirmed by repeat analysis or by confirmatory testing. Approximately 98% of all variants detected by the BRACAnalysis CDx™ are confirmed by repeat testing; the remaining variants (about 2%) require confirmatory analysis by the following tests:

- Alternate Primer Sequencing (APS) - used to identify potential heterozygous base changes under the primers used in the BRACAnalysis CDx™ Sanger Sequencing test or the BART® CDx test
- Confirmatory PCR Analysis (CPA) - used to confirm *BRCA1* and *BRCA2* large rearrangements detected initially by the BART® CDx test

Summary and Explanation

The BRACAnalysis CDx™ device is a companion diagnostic for AstraZeneca's drug Lynparza™ (olaparib), a poly ADP-ribose polymerase (PARP) inhibitor. Cells that possess at least one normal *BRCA1* and *BRCA2* allele are relatively resistant to PARP inhibition. *BRCA1* or *BRCA2* dysfunction, defined as mutant cells lacking wild-type *BRCA1* or *BRCA2*, sensitizes cells to PARP inhibition leading to chromosomal instability, cell cycle arrest and apoptosis. [Bryant et al. Specific killing of *BRCA2*-deficient tumours with inhibitors of poly(ADP-ribose) polymerase.

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

Nature 2005;434(7035):913-7., Farmer et al. Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy. *Nature* 2005;434(7035):917-21.]

Test Kit Contents

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

- Monoject™ Blood Collection Tube, Silicone Coated Lavender Stopper, buffered EDTA(K3) 0.10 mL 15.0% Solution, or equivalent
 - The shelf life is printed on each individual tube. Prior to using a tube for blood collection, check the expiration date.
- Test Request Form (TRF)
- Example TRF
- Collection Instructions
- Mailing Instructions
- Technical Information Summary

Instruments

The BRACAnalysis CDx™ device is intended to be performed with the following instruments, as identified by specific serial numbers:

- QIASymphony SP
- Tecan Freedom Evo 150
- Tecan Infinite F200 Pro Platerreader
- MasterCycler EP & MasterCycler Pro 384 & 96 well
- ABI 3730xl
- E-Gel iBase
- E-Gel Imaging System InGenius3
- E-Gel Safe Imager Real-time Transilluminator

Sample Collection and Test Ordering

To order BRACAnalysis CDx™ testing, the Test Request Form (TRF) included in the test kit must be fully completed.

Please refer to the BRACAnalysis CDx™ Collection Instructions and Mailing Instructions for further details about collecting blood samples and mailing the samples to Myriad.

Test Results and Interpretation

Patients evaluated with the BRACAnalysis CDx™ test that are determined to carry a deleterious or suspected deleterious germline *BRCA1* or *BRCA2* mutation can be considered for treatment with Lynparza™ (olaparib) under the supervision of a physician.

Upon completion of testing at Myriad, a test report will be sent to the designated physician. The results of each test component, along with the interpretation of the variant(s) identified, are provided. If multiple variants are detected, the overall test interpretation most relevant to patient management is based on the most severe variant identified (as reported in the Test Results and

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

Interpretation section of the result report). Standard interpretative information included in test reports is listed below. Note that variants determined to have a classification of polymorphism are not included on the test report.

- **Positive for a deleterious mutation:** All mutations (nonsense, insertions, deletions) that prematurely terminate the protein product before the last documented deleterious mutation of the gene. In addition, some specific missense mutations and non-coding intervening sequence mutations are recognized as deleterious on the basis of compelling scientific data derived from linkage analysis of high risk families, functional assays, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.
- **Genetic variant, suspected deleterious:** Genetic variants for which available evidence indicates a strong likelihood, but not definitive proof, that the mutation is deleterious.
- **Genetic variant, favor polymorphism:** Genetic variants for which available evidence indicates that the variant is highly unlikely to contribute substantially to compromised protein function.
- **Genetic variant of uncertain significance:** Genetic variants whose clinical significance has not yet been determined. These can include certain missense variants, variants that occur in analyzed intronic regions, as well as terminating variants that truncate the gene distal to the last known deleterious mutation.
- **No mutation detected:** This includes results with no variants differing from the wildtype sequence, or polymorphic genetic variants. Polymorphisms include variant 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 the non-coding portions of the gene that have no deleterious effect on the mRNA transcript. These also include genetic variants for which published data demonstrate absence of clinical significance.

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

All mutations and genetic variants are named according to the convention of Beaudet and Tsui. (Beaudet AL, Tsui LC. A suggested nomenclature for designating mutations. *Hum Mut* 1993; 2:245-248). Nucleotide numbering starts at the first transcribed base of *BRCA1* and *BRCA2* according to GenBank entries U14680 and U43746, respectively. (Under these conventions, the two mutations commonly referred to as "185delAG" and "5382insC" are named 187delAG and 5385insC, respectively.)

Performance Characteristics

1. Accuracy

a. BRACAnalysis CDx™ Sanger Sequencing Test Accuracy

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

The accuracy of the BRACAnalysis CDx™ Sanger Sequencing assay was evaluated in this study by comparing its sequencing results with those of a validated NGS assay on a set of 100 blinded, patient blood-derived DNA samples.

After variant and non-variant calls (relative to wild-type sequences) were made for each test, the results were compared. From the set of samples tested, a total of 796 variant bases (representing 790 variants) and 1,732,907 non-variant bases were identified by the NGS-based test. For each sample tested with the BRACAnalysis CDx™ Sanger Sequencing test, successful calls were made for all amplicons that are part of the assay, and the no call rate was 0%. All variant and non-variant base calls between the two tests were concordant. The agreement analysis between the results from both tests demonstrated a positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement of 100%. The lower bounds of the 95% confidence intervals for PPA and NPA were 99.62% and 99.99%, respectively, which met the pre-specified acceptance criteria for the study.

Overall, these results demonstrate that results from the BRACAnalysis CDx™ Sanger Sequencing assay are highly concordant with those from a validated NGS assay.

b. BART® CDx Test Accuracy

The accuracy of the BRACAnalysis CDx™ Large Rearrangement (BART® CDx) assay was evaluated in this study by comparing its large rearrangement results with those of a validated microarray assay on a set of 100 blinded, blood-derived DNA samples.

Accuracy of the results from the BART® CDx test was demonstrated by comparison against the positive and negative calls from the microarray test. Based on the microarray results, 26 samples were positive for a large rearrangement in *BRCA* genes, and 74 samples were negative. For the BART® CDx test, 95 samples yielded valid results and 5 samples yielded results requiring further investigation. Among the 95 samples with callable results, 94 samples had results that matched those from the microarray assay, while one did not. The miscalled, or discordant, variant was identified as a multi-exon duplication by the BART® CDx test and a multi-exon triplication by the microarray test. Although both tests detected an increase in dosage of the same region, the BART test is not designed to differentiate between duplications and triplications, and therefore, this is a limitation of the BART® CDx test.

Overall, the results demonstrate that the BART® CDx test generates results that are highly concordant with the results from a validated microarray assay, for the detection of *BRCA1* and *BRCA2* large rearrangements.

2. Analytical Sensitivity – DNA Input

a. BRACAnalysis CDx™ Sanger Sequencing Test

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

PCR Amplification is the critical step in the BRACAnalysis CDx™ Sanger Sequencing test for generating high levels of specific amplicons for the sequencing reactions. To assess the acceptable range of genomic DNA input to achieve the PCR performance requirements of the test, DNA extracted from 5 specimens were each diluted to evaluate 6 DNA input concentrations (0.2 ng, 1 ng, 4 ng, 20 ng, 40 ng, and 100 ng) per PCR reaction. The rate of successful calls at each DNA input level was assessed, in addition to the concordance between the replicates per amplicon. The optimal DNA input concentration for PCR amplification is 20 ng, as specified in the protocol for the BRACAnalysis CDx™ test. At this level, all of the results for each sample met the quality criteria, and the duplicate results for each amplicon were fully concordant for all of the variant and non-variant calls. The performance of the BRACAnalysis CDx™ Sanger Sequencing test was not significantly affected by DNA input levels from 1 ng to 100 ng.

b. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

The BART® CDx test is a multiplex PCR assay that amplifies specific regions in the *BRCA1* and *BRCA2* genes. To evaluate the DNA input range for the PCR step, DNA concentrations higher and lower than the optimal DNA input amount specified in the assay protocol were evaluated. The rate of successful calls for each sample per DNA input level was assessed, as well as the concordance across DNA concentrations. The results demonstrate that DNA input levels from 2 ng to 12 ng may generate results identical to those at 8ng, which is the DNA concentration specified for the BART® CDx test.

3. Analytical Specificity – Cross Reactivity

a. BRACAnalysis CDx™ Sanger Sequencing Test

The ability of the BRACAnalysis CDx™ Sanger Sequencing test to detect sequence variants is highly dependent upon the specificity of the primers for PCR amplification. To assess the potential for amplification of non-specific products from human genomic DNA, *in silico* analysis of the PCR primers used in the assay was performed. No non-standard primer combinations were evaluated since the assay consists of only singleplex PCR reactions. Non-specific products were not predicted for any of primer pair combinations.

b. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

A specificity analysis was conducted to determine if the PCR primers used in the BART® CDx test have the potential to amplify non-target sequences in the human genome. A bioinformatics program was used to align primer pairs against genomic sequence to predict if there may be any non-specific amplicons. Every possible primer pair combination per multiplex reaction was evaluated. In total, 3,016 combinations were assessed. No non-specific products were predicted for any of the potentially cross-reactive primer pairs, in any of the BART® multiplex PCR reactions.

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

4. Interference

To evaluate how potential interfering substances may impact the performance of the BRACAnalysis CDx™ test, the effects of three classes of substances were assessed: 1) endogenous substances normally present in human whole blood (i.e., hemoglobin, Albumin, IgG, and bilirubin); 2) an exogenous substance (i.e., K₃EDTA); and 3) substances used in the standard process of the device (i.e., ethanol and bleach). Three whole blood samples from healthy subjects carrying a total of 30 different *BRCA* variants were evaluated. All of the samples were processed with the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test.

The variant and non-variant calls were compared across spiked and unspiked samples to determine if the potential interferents may lead to alterations in the test results. All three non-spiked blood specimens yielded results that passed the acceptance criteria for both the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test. With the exception of IgG at 60 g/L, treatment with each potentially interfering substance at the maximum concentration tested did not affect the performance of either test (i.e., hemoglobin at 20 g/dL, albumin at 50 g/L, conjugated bilirubin at 5 mg/dL, K₃EDTA at 5%, ethanol at 12.75%, and 10% bleach at 0.5%). The condition with IgG at 60 g/L failed to meet the acceptance criteria for the BART® CDx test, so a lower concentration of 9.5 g/L was tested. When the three samples were spiked with an IgG concentration near the average concentration typically detected in blood (i.e., 9.5 g/L), all samples met the quality criteria for each test, and generated callable results matching those of the corresponding unspiked samples.

5. Reproducibility and Repeatability

a. Combined Reproducibility

Reproducibility of the BRACAnalysis CDx™ test was assessed by testing a set of 20 samples over 3 independent runs using 2-3 instruments of each type, 3 reagent lots, and 3 operators for each manual processing step. The 3 runs were conducted over non-consecutive days using the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test. The confirmatory assays were also performed, in accordance with the standard protocols.

In total, 37 sequencing variants (single nucleotide variants and deletions up to 5 bp) and 3 large rearrangements (single exon deletion, single exon duplication, and multi-exon deletion) were identified. For both tests, the no call rate was 0%, since all runs produced successful calls for all samples. For each sample, all calls were consistent across all runs. The positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement were all 100%, which met the acceptance criteria for the study.

b. Intra-Run Repeatability

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

i. BRACAnalysis CDx™ Sanger Sequencing Test

To determine if the BRACAnalysis CDx™ Sanger Sequencing test is reproducible across replicates of the same sample within a single batch run, 3 blood samples were tested in triplicate using 10 amplicons of the test. The amplicons were selected to represent the range of genomic regions that are evaluated in the BRACAnalysis CDx™ Sanger Sequencing test. These include the longest amplicon, the shortest amplicon, the most GC-rich amplicon, and the least GC-rich amplicon. Regions for which the presence of a homopolymer track renders only one sequencing direction readable were also included. The amplicons were tested in two batches, such that 9 amplicons were run in one batch and 1 amplicon in another. For the batch with 9 amplicons, all of the replicates for each sample produced callable and concordant results. For the batch with only one amplicon, 8 of 9 reactions were callable and concordant; one reaction yielded no call.

ii. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

To demonstrate that the BART® CDx test can generate repeatable results across replicates of the same samples within a single batch run, 10 specimens were processed in triplicate within one run. Variant types, such as a single exon duplication and a multi-exon deletion were represented in the study. The BART® CDx test was performed in accordance with the assay protocol. All replicates for each of the samples passed the quality criteria and produced callable results, which were fully concordant.

6. Guardbanding

a. BRACAnalysis CDx™ Sanger Sequencing Test

Guardbanding studies were performed to evaluate if the performance of the BRACAnalysis CDx™ Sanger Sequencing test is robust to withstand process variations around two key parameters: PCR annealing temperature, and sequencing annealing temperature. Five samples were tested in duplicate per tested condition, and variant types such as single nucleotide variants and small deletions (up to 5 bp) were represented.

i. PCR Annealing Temperature

The thermal cycling profile was guardbanded by varying the PCR annealing temperature by $\pm 1^\circ\text{C}$, $\pm 2^\circ\text{C}$ and $\pm 3^\circ\text{C}$. For three test conditions (+ 1°C , - 2°C and - 3°C), all replicates for each amplicon tested per sample yielded successful results that matched the expected call. Similar results were observed for the other test conditions (- 1°C , + 2°C and + 3°C), with the exception that only one

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

replicate of one of the tested amplicons for one sample generated no call. In all cases, the acceptance criteria were met, and all test conditions were tolerated.

ii. Sequencing Reaction Annealing Temperature

The annealing temperature for the sequencing reaction was challenged by varying the temperature by $\pm 1^{\circ}\text{C}$, $\pm 2^{\circ}\text{C}$ and $\pm 3^{\circ}\text{C}$. For three test conditions ($- 1^{\circ}\text{C}$, $+ 2^{\circ}\text{C}$ and $- 3^{\circ}\text{C}$), all replicates for each amplicon tested per sample yielded successful results that were in agreement with the expected call. For the other test conditions ($+ 1^{\circ}\text{C}$, $- 2^{\circ}\text{C}$ and $+ 3^{\circ}\text{C}$), one replicate for one of the tested amplicons for one sample generated no call, while all other replicates generated results that matched the expected call. Thus, all of the tested temperature variations did not appear to affect the performance of the sequencing reactions.

b. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

The robustness of two critical parameters of the BART® CDx test was assessed: PCR annealing temperature and injection time of the PCR product input for capillary electrophoresis. In both cases, the same set of 28 unique samples was assessed and analyzed, of which two were run in duplicate. Two samples were positive for *BRCA* multi-exon deletions.

i. PCR Annealing Temperature

The PCR annealing temperature was varied by $\pm 1^{\circ}\text{C}$, $\pm 2^{\circ}\text{C}$, and $\pm 3^{\circ}\text{C}$. Four test conditions ($\pm 1^{\circ}\text{C}$, $- 2^{\circ}\text{C}$, and $- 3^{\circ}\text{C}$) yielded successful and concordant calls for all samples. At the two other conditions ($+ 2^{\circ}\text{C}$ and $+ 3^{\circ}\text{C}$), one sample yielded miscalled deletion results due to decreased primer binding efficiency caused by an identified variant affecting one BART® PCR primer annealing site.

ii. Injection Time

Different levels of PCR product injected onto the ABI 3730xl platform were assessed by altering the injection time of the PCR product. The injection time was set at 2, 4, 5, 6, 7, 10, or 20 seconds, while the voltage was held constant (2 kV), resulting in 4, 8, 10, 12, 14, 20 or 40 kV·s, respectively. All conditions, except 40 kV·s, resulted in successful, concordant results for all samples. At 40 kV·s, calls of acceptable quality were not obtained for any sample, indicating that this setting falls outside of the linear detection range of the capillary electrophoresis instrument. Thus, the optimal condition of 12 kV·s was within the acceptable PCR input injection conditions from 4 kV·s to 20 kV·s.

7. Cross Contamination

The potential for crossover contamination within a run and between runs was evaluated at 3 processes of BRACAnalysis CDx™ test: 1) DNA extraction from whole blood specimens, 2) the BRACAnalysis CDx™ Sanger Sequencing test, and 3) the BART® CDx test.

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

Specimens with different *BRCA* genotypes (for sequence variants and large rearrangements) were processed adjacent to each other in microtiter plate formats to maximize the potential for carryover between wells within a plate and between plates in separate batch runs. Two sequential batches were evaluated for inter-run carryover, and each run was evaluated separately for intra-run carryover.

a. DNA Extraction

DNA extraction from whole blood samples is an automated process on the QIASymphony robotic platform. For all samples in all batches, successful results were generated. All replicates were fully concordant within each run and between runs. Thus, sample crossover events were not detected.

b. BRACAnalysis CDx™ Sanger Sequencing test

Two samples with unique *BRCA* sequence variants were set up within one PCR plate in a checkerboard pattern at alternating high (20ng for the first sample) and low (4 ng for the second sample) DNA input levels. In the first run, there were 84/90 (93%) successful calls and 6/90 no calls for the two samples tested. All callable results were concordant. In the second run, there were 89/90 (99%) successful calls, all of which were concordant. No miscalled results were generated.

c. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

For the BART® CDx test, a total of 10 samples were evaluated. The samples were arranged such that 8 unique *BRCA* large rearrangement-negative samples, along with two samples positive for *BRCA* large rearrangements, were tested in each batch. For all replicates of all samples in each batch, the results were of acceptable data quality and were fully concordant with the expected results. Carryover events, resulting in miscalls, were not observed.

8. Stability

Validation studies were performed to evaluate the stability of whole blood specimens, reagents, standards and controls. Testing supports the following expiration dating:

a. Specimen Stability

Whole Blood Specimens – up to 30 days at 4°C and up to 5 days at 30°C

b. Reagent Stability

Sanger Sequencing PCR Reagent Plates – up to 24 hours at 30°C

Sanger Sequencing Oligo Reagent Plates – up to 7 days at 30°C

Sanger Sequencing CAPSeq Reagent Plates – ongoing

BART® PCR Reagent Plates – ongoing

Quantification Standards – up to 30 days at 4°C and up to 48 hours at 30°C

c. Control Stability

Sanger M13 F+R Negative Controls – ongoing

BART® Cell Line Positive Controls – up to 2 months at 4°C

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

BART® Alternate Positive Controls – less than 1 month at 4°C

BART® Amplicon Negative Controls – ongoing

CPA PCR Amplification Controls – up to 2 months at 4°C

CPA No Genomic DNA Controls – ongoing

Stability testing of whole blood specimens, reagents, standards and controls supports the performance of the device under the specified storage conditions and stability/expiration times for the listed device components.

9. Variant Classification Study

To evaluate the robustness and reliability of the variant classification process, a set of 262 unique *BRCA* variants was subjected to classification as if they were new variant observations. The variants were classified in a blinded manner according to defined classification criteria. The resulting classifications for each variant were compared to the existing classifications in Myriad's database, and the concordance rate was determined. One variant that was not previously observed at Myriad, and therefore was not previously classified, was excluded from the study. The majority of variants (185/262) were identified from clinical studies for Lynparza™ (olaparib), and the remaining variants (77/262) were selected for inclusion into the study to adequately cover the spectrum of variant types for classification. The results are summarized in the tables below.

Classification Results

Classification	Previous Classification	New Classification
Deleterious (DM)	130	126
Suspected Deleterious (SD)	7	11
Uncertain Significance (VUS)	34	32
Favor Polymorphism (FP)	9	17
Polymorphism (PM)	81	75
Excluded from study	1	1
TOTAL	262	262

Discordant Results by Classification

Previous Classification	New Classification	Discordant Results	
		Classification	Treatment
DM	SD	5	0
SD	DM	1	0
SD	VUS	1	1
VUS	SD	1	1
VUS	FP	2	0
PM	FP	6	0
TOTAL		16	2

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

Comparison of the new classifications to the previous classifications resulted in agreement for 245 variants (93.9%; 95% CI: 90.2% to 96.5%). The criteria and current evidence provided opportunity to update the classification for 16 of the 262 variants. Of the 16 variants with inconsistent results, 14 would not affect treatment eligibility for Lynparza™ (olaparib). Of the remaining two, one is a missense variant classified in the study as suspected deleterious (SD), but the previous classification was a variant of uncertain significance (VUS). The change in classification resulted from new structural and functional evidence, which recently became available, to support the SD classification. The other was an intronic splicing variant classified as VUS in this study, but previously was SD. The classification changed due to available evidence. This variant has only been observed twice in the population tested at Myriad since 1996. Thus, by comparing results from two separate and independent variant classifications, the concordance rate - as defined as leading to the same eligibility status for treatment with Lynparza™ (olaparib) - is 99.2% (95% CI: 97.2% to 99.9%).

Summary of Clinical Studies

1. Summary Of Primary Clinical Study

The clinical benefit of BRACAnalysis CDx™ test was demonstrated in a retrospective analysis of efficacy and safety data obtained from the open-label, non-randomized study to assess the safety and efficacy of olaparib treatment in patients with ovarian cancer who have a deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*) and who have been previously treated with at least 3 lines of prior chemotherapy. Patients were enrolled from 13 centers in six countries, including the United States. Local test results for *BRCA* status were used to assess patient eligibility for the trial. Samples from a subset of enrolled patients were retrospectively evaluated at one laboratory, Myriad (Salt Lake City, UT) using the BRACAnalysis CDx™ test. The clinical utility of the BRACAnalysis CDx™ test was established by comparing the mutation results and the associated clinical outcomes for the overall population to those for the subset of patients with confirmed *gBRCA* status upon retrospective testing with the BRACAnalysis CDx™ test. Lynparza™ (olaparib) demonstrated a robust overall response rate with a clinically meaningful duration of response in *gBRCAm* patients with ovarian cancer who had received three or more prior lines of chemotherapy. The magnitude of response in the population tested with the BRACAnalysis CDx™ test was comparable to that in the overall population. Data from this bridging study were used to support PMA approval.

a. Accountability of PMA Cohort

Based on local test results, a total of 317 patients with advanced cancers were enrolled in the study. There were 193 patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer, among whom 137 had measurable disease and had received three or more lines of prior chemotherapy. Out of the 137 patients, specimens from 61 patients were available for retrospective testing with the BRACAnalysis CDx™ test in the clinical bridging study.

i. Effectiveness Results

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

The analysis of efficacy analysis was based on objective response rate (ORR) and duration of response (DoR) observed in 137 patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received three or more prior lines of chemotherapy and who had measurable disease. In this cohort, the ORR was 34% (95% CI: 26% - 42%) with a median DoR of 7.9 months. The results are listed in the table below. The observed ORR represents an improvement over existing therapies and is reasonably likely to predict clinical benefit in the indicated population. Confirmatory studies are in progress.

The effectiveness analysis for the BRACAnalysis CDx™ test was based on a subset of 61 *gBRCAm* patients with ovarian cancer who had received three or more prior lines of chemotherapy, who had measurable disease, and for whom specimens were available for retesting with BRACAnalysis CDx™. The level of concordance between the local test results, as reported in the Case Report Form, and the results from the BRACAnalysis CDx™ test was determined to be 96.7% (59/61). Among the discordant results, one sample did not yield a callable result with BRACAnalysis CDx™ test, and another sample had different classification results between the local test and the BRACAnalysis CDx™ test (deleterious vs. variant of unknown significance, respectively), although the specific variant that was detected by both tests matched. In addition, the clinical outcome data for the 59 patients with confirmed *gBRCAm* status was as follows: ORR was 41% (95% CI: 28% - 54%), and median DoR was 8.0 months. Taken together, the results in the subset of *gBRCAm* patients tested with the BRACAnalysis CDx™ test were comparable to those observed in the cohort of 137 patients, which supports effectiveness of the device. The results are summarized in the table below.

Clinical Study Results

Subset*	Subjects with response n (%)	ORR	95% CI	Progressed n (%)	Median DoR (months)	95% CI
All	46 (33.6)	0.34	(0.26, 0.42)	30 (65.2)	7.9	(5.6, 9.6)
With BRACAnalysis CDx™ test result	24 (40.7)	0.41	(0.28, 0.54)	14 (58.3)	8.0	(3.8, NC)
Without BRACAnalysis CDx™ test result	22 (28.2)	0.28	(0.19, 0.40)	16 (72.7)	7.9	(6.0, 9.6)

*Ovarian cancer patients with measureable disease who had received at least 3 lines of prior chemotherapy

b. Robustness Analyses

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with a positive BRACAnalysis CDx™ test result, but who may have been negative by the local test. Patients with such test results are part of the intended use population of the BRACAnalysis CDx™ test; however, they were excluded from the clinical trial due to negative results upon local test screening. To account for this missing data, the efficacy of olaparib treatment (based on ORR) in

BRACAnalysis CDx™ Technical Information

https://myriad-library.s3.amazonaws.com/technical-specifications/BRACAnalysis_CDx_Tech_Specs.pdf

patients with positive results from the BRACAnalysis CDx™ test was estimated assuming different combinations for multiple parameters.

The confidence intervals were calculated based on the imputed ORR from the subset of 137 patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received 3 or more prior lines of chemotherapy and who had measurable disease in the study. The smallest estimated ORR value estimated for the BRACAnalysis CDx™ test-positive population is 34% (95% CI: 26% - 43%), which is not significantly different from that observed for the overall subpopulation of patients (n=137) who had measurable disease and who had received 3 or more lines of prior chemotherapy (34%, 95% CI: 26% - 42%). These results support the finding that the observed improvement in ORR in the indicated population is robust.

The data describing the performance characteristics above, as well as the clinical study endpoints, support the clinical utility of BRACAnalysis CDx™ as a companion diagnostic to Lynparza™ (olaparib).