clonoSEQ is available as an FDA-cleared in vitro diagnostic (IVD) test service provided by Adaptive Biotechnologies to detect measurable residual disease (MRD) in bone marrow from patients with multiple myeloma or B-cell acute lymphoblastic leukemia (B-ALL) and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). clonoSEQ is also available for use in other lymphoid cancers as a CLIA-validated laboratory developed test (LDT) service.
The Clonality (ID) Test is used to identify dominant DNA sequence(s) in a high disease load diagnostic sample. Identification of at least one dominant DNA sequence is a prerequisite to future monitoring of MRD.

After the dominant DNA sequence(s) has been identified utilizing the Clonality (ID) Test, subsequent monitoring of the associated clone(s) can be completed by ordering Tracking (MRD) Tests.

clonoSEQ is only available by prescription from a licensed healthcare professional. clonoSEQ results should always be used in combination with clinical examination, patient medical history, and other findings. Results may vary based on sample type, body site/location sampled, and other factors. False positive or false negative results may occur for reasons including, but not limited to: contamination, technical, and/or biological factors.
Clonality (ID) Report with Dominant Sequences Identified

This is an example B-cell Clonality (ID) Report. The clonoSEQ B-cell Clonality (ID) Report provides results based on analysis of the IgH, IgK and IgL loci as well as Bcl1 and Bcl2 translocations.

Page 1 of the report shows that dominant DNA sequences were identified from the submitted sample (1). A more detailed description of the results for this sample can be found in the “Results Summary” section (2). Additional observations provided by a licensed medical professional relating to the report result may be included in an “Additional Comments” section (not pictured). A summary of the criteria used to determine which DNA sequences are dominant and thus can be followed as markers of malignancy is provided at the bottom of the page for reference (3).
Clonality (ID) Report with Dominant Sequences Identified (continued)

Page 2 of the report shows detailed information relating to the sample (4) including the actual rearranged DNA nucleotide sequence or sequences identified, the sample collection date, the receptor locus in which each dominant sequence was found, the specimen type analyzed, the frequency of the dominant sequence as a fraction of the total nucleated cells assessed, and the total number of cells carrying the rearranged DNA sequence.
Clonality (ID) Report with Dominant Sequences Identified (continued)

Page 3 of the report provides more details on the immune repertoire of the analyzed sample, including the sample clonality, the number of sequences assessed for each locus, and the number of unique sequences assessed (5).
Clonality (ID) Report with No Dominant Sequence Identified

This is an example B-cell Clonality (ID) Report. The clonoSEQ B-cell Clonality (ID) Report provides results based on analysis of the IgH, IgK and IgL loci as well as Bcl1 and Bcl2 translocations.

In this sample report, no dominant DNA sequences were identified from the submitted sample so the result is described as “Polyclonality” (1). This result is often encountered when a sample of insufficient disease load is supplied for testing, so it is important to ensure that samples sent for Clonality (ID) testing are high disease load diagnostic samples. For reference, information about the criteria for defining a sequence as dominant is also provided at the bottom of the page (2).

A ‘Polyclonality’ result is reported when no dominant sequences are identified in the supplied sample

Criteria for defining a dominant sequence

1. **RESULTS SUMMARY**
   - Genomic DNA was extracted from a bone marrow aspirate slide sample.
   - There were no sequences that met the criteria for a “dominant” sequence.
   - The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

2. **CRITERIA FOR DEFINING “DOMINANT” SEQUENCES**
   - The sequence must comprise at least 3% of all like sequences (IGH-involved, IGK, and IGL are considered independently).
   - The sequence must comprise at least 0.2% of the total nucleated cells in the sample.
   - The sequence must be discontinuously distributed (≤5 sequences in the next decade of sequences when ranked by frequency).
   - The sequence must be carried by at least 40 estimated genome equivalents in the analyzed sample.

3. **ASSAY DESCRIPTION**
   - The clonoSEQ® Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgG and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL).

4. **CLONALITY RESULT**
   - **No Dominant Sequence Identified (Polyclonality)**
   - Clone tracking (e.g. MRD determination) is not enabled by this sample

5. **ASSAY METHODS AND LIMITATIONS**
   - METHOD
     - The clonoSEQ Assay utilizes NGS to determine the level of remaining presumptive disease-associated cells in patients with previously diagnosed lymphoid malignancies. The patient-specific sequence(s) carried by the presumed transformed clone is first identified in a diagnostic sample using a set of multiplexed, locus-specific primer sets for the immunoglobulin heavy-chain locus (IGH), including both complete (IGH-VDJ) and incomplete (IGH-DJ) rearrangements, the immunoglobulin k locus (IGK), the immunoglobulin λ locus (IGL) and IGH-BCL1/2 translocations. The assay is then applied in one or more follow-up samples to detect the level of the patient-specific sequence(s) corresponding to the prevalence of the sequence carrying clone.
   - ASSAY LIMITATIONS
     - False positive or false negative results may occur for reasons including, but not limited to: sample mix up, misidentification, and/or contamination; technical and/or biological factors. Results may vary by sample type or body site/location sampled. The assay may overestimate MRD frequencies near the limit of detection.
Clonality (ID) Report with No Dominant Sequence Identified (continued)

Since no dominant sequences were identified in the Clonality (ID) test, a sequences table is not shown on Page 2 of the report. Instead, Page 2 provides more details on the immune repertoire of the analyzed sample, including sample clonality, the number of sequences assessed for each locus, and the number of unique sequences assessed (3).

**APPENDIX**

**SUPPLEMENTAL SAMPLE INFORMATION**

<table>
<thead>
<tr>
<th>SAMPLE CLONALITY</th>
<th>TOTAL NUCLEATED CELLS</th>
<th>LOCI</th>
<th>TOTAL SEQUENCES</th>
<th>TOTAL UNIQUE SEQUENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.13</td>
<td>82,497</td>
<td>IGH</td>
<td>158</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IGK</td>
<td>&gt;=43</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IGL</td>
<td>&gt;=13</td>
<td>13</td>
</tr>
</tbody>
</table>

1. Sample Clonality: A measure of the lymphocyte population diversity (distinct lymphocyte clonal sub-populations or "clones") comprising the immune repertoire in a given biological sample. Values for clonality vary from 0 to 1. Values close to 1 represent samples with one or a few predominant clones. Values near zero represent a more polyclonal sample.

2. Total Nucleated Cells: The total number of nucleated cells calculated within the sample, based on quantitation of non-immune receptor loci contained in the reaction and the assumption that the DNA content per cell is diploid.

3. Total Sequences: A measure of the number of nucleotide sequences detected in the sample for each defined immune receptor locus.

4. Total Unique Sequences: A measure of the number of unique nucleotide sequences detected in the sample for each defined immune receptor locus.

**REFERENCES**


**REPORT APPROVAL**

Reviewed and Released by
Maria C Santos, MD

Signature

Date & Time
03/30/2018 12:22 PM

This report has been approved by the Clinical Laboratory Director, Stephanie Hallam, PhD, DABMG. The clonoSEQ Assay is a laboratory service performed at Adaptive Biotechnologies’ single site located at 1551 Eastlake Ave E, Seattle WA 98102. This test was developed and its performance characteristics determined by Adaptive Biotechnologies Corporation. The laboratory is regulated under CLIA (WA-MTS CLIA# 50D2046518) as qualified to perform high complexity clinical testing.
MRD Detection and Monitoring

After the dominant DNA sequence(s) has been identified utilizing the Clonality (ID) Test, subsequent monitoring of the associated clone(s) can be completed by ordering Tracking (MRD) Tests throughout treatment.
ASSAY DESCRIPTION
The clonoSEQ® Assay is an *in vitro* diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL).

SAMPLE-LEVEL MRD RESULT

Result indicating whether residual sequences were detected and quantifying MRD level as a fraction per 1 million cells

Residual Sequences Detected

ESTIMATED MRD VALUE:

8 residual clonal cells per million nucleated cells (Range: 3 - 14)

Sequence determining MRD result: IGL Sequence B

The MRD range presented above represents the 95% confidence interval for the measured number of residual clonal sequences per million nucleated cells. Details for each identified dominant sequence from this sample are provided on subsequent pages of this report.

RESULTS SUMMARY

- Genomic DNA was extracted from a bone marrow aspirate slide sample.
- 2 of the 2 dominant sequences identified in a diagnostic sample from this patient were still present in this current sample.
- 15 copies of the dominant sequence determining the MRD result were observed out of 1,933,098 total nucleated cells evaluated from this sample.

The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

SAMPLE-LEVEL MRD TRACKING (shows only the sequence determining the MRD results for each time point)
In the bottom third of the page, a chart (3) provides a longitudinal view of the sample-level MRD result for the current sample as well as for past samples sent for clonoSEQ testing for this patient. Each result is shown as a point on the chart with a corresponding test date; each MRD time point also has an associated confidence interval displayed. Note that the results shown in this chart are “sample level” results, meaning that they reflect the MRD result for the highest frequency dominant sequence in each tested sample. For sequence-level MRD results, see the table on page 2 and/or the chart on page 3 of the report.
Page 2 of the report shows detailed information relating to the current and previous samples (4) including the actual rearranged DNA nucleotide sequence or sequences identified, sample collection dates the receptor locus which each dominant DNA sequence was found, the specimen type analyzed, the estimated sequence abundance (i.e., the number of residual clonal cells per million nucleated cells), and the 95% confidence interval for each MRD result.

A blue bar (5) will be placed next to one of the sequences listed on this page to indicate that it is the sequence determining the MRD result for the current sample. The sequence(s) that determined the MRD result for previous samples are noted with a blue check mark.

Any MRD result which falls below the limit of detection (LOD) for a particular sequence is indicated with a double-cross which will be displayed in the estimated sequence abundance column next to the relevant result(s).
Page 3 of the report displays the sequence-level information from Page 2 in a chart format (7). This "sequence-level MRD" chart provides a longitudinal view of results for each individual tracked sequence, for the current sample as well as for past samples sent for clonoSEQ testing for this patient. Similar to the "sample-level" chart on Page 1, the sequence-level chart includes a point on the chart for each test with a corresponding test date, but on this chart, each individual sequence is displayed separately.

In addition to the sequence-level chart, this page lists the criteria used by the clonoSEQ Assay to define dominant sequences, as well as a summary of the assay method and limitations (8).

The appendix provides more details on the immune repertoire of the analyzed sample, including the sample clonality, the number of sequences assessed for each locus, and the number of unique sequences assessed (9).
Page 4 of the report continues details on the immune repertoire of the analyzed sample, noting the limit of detection and limit of quantification for each sequence tracked (10). The limit of detection (LOD) and limit of quantitation (LOQ) are independently calculated for each trackable sequence and hence can vary by sequence based on factors including the amount of input DNA, the uniqueness of the sequence, and/or relative amplification due to nucleotide sequence polymorphism or mutation.

A glossary of terms and references relevant to the report is also provided on this page (11).

Within the glossary, the assay’s limit of blank, which is zero, is stated and defined (12). A limit of blank equal to zero indicates that in tests of assay performance on samples that were known to have zero residual disease, the clonoSEQ Assay did not generate any false-positive results.

Note: False positive or false negative results may still occur, for reasons including contamination, technical and/or biological factors.
Understanding the clonoSEQ® Assay TRACKING (MRD) REPORT

ASSAY DESCRIPTION
The clonoSEQ® Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL).

SAMPLE-LEVEL MRD RESULT
No Residual Sequences Detected
ESTIMATED MRD VALUE:
0 residual clonal cells (Range: 0 - 2) **

Sequence determining MRD result: IGH Sequence A

The MRD range presented above represents the 95% confidence interval for the measured number of residual clonal sequences per million nucleated cells. Details for each identified dominant sequence from this sample are provided on subsequent pages of this report.

RESULTS SUMMARY
Genomic DNA was extracted from a bone marrow aspirate slide sample.
The 3 dominant sequences identified in a diagnostic sample from this patient were not detected in this current sample.
The sensitivity of this assay is directly related to the total number of cells (or cellular equivalents of genomic DNA) analyzed. There were 1,331,826 total nucleated cells evaluated from this sample.

Further down the page, the Results Summary states the actual number of sequences observed by the assay and the total number of nucleated cells assessed in the sample.

MRD DETECTION AND MONITORING
Tracking (MRD) Report With No Residual Sequences Detected
This is an example B-cell Tracking (MRD) Report. The clonoSEQ B-cell Tracking (MRD) Report provides results based on analysis of the IgH, IgK and IgL loci as well as Bcl1 and Bcl2 translocations.

In this sample, residual disease was NOT detected by the clonoSEQ Assay. This is indicated by the language “Residual Sequence(s) Not Detected” in the blue box on page 1 of the report. Also in the blue box, the report provides a quantitative assessment of the number of detected residual cells containing that sequence, displayed as a number per 1 million cells in the sample. In this example, the number of detected residual cells is zero.

Note that a range is also included to the right of the quantitative MRD value. This range represents the 95% confidence interval for the measured number of residual clonal sequences per million nucleated cells. The size of the range varies depending on the total number of input cells assessed and the limit of detection of the sequence determining the MRD result. For a test in which residual disease was not detected, the range reflects the fact that there was zero disease in the tested sample, but that some disease could still be present in the patient (due to sampling bias).

Further down the page, the Results Summary states the actual number of sequences observed by the assay and the total number of nucleated cells assessed in the sample.
In the bottom third of the page, a chart (3) provides a longitudinal view of the sample-level MRD result for the current sample as well as for past samples sent for clonoSEQ testing for this patient. Each result is shown as a point on the chart with a corresponding test date; each MRD time point also has an associated confidence interval displayed. Note that the results shown in this chart are “sample level” results, meaning that they reflect the MRD result for the highest frequency dominant sequence in each tested sample. For sequence-level MRD results, see the table on page 2 and/or the chart on page 3 of the report.
### Identifying Dominant Sequences

The number of clonal cells may vary by sample type. As such, changes in clonal cell values over time are best compared using the same sample type.

<table>
<thead>
<tr>
<th>IGH - Sequence A</th>
<th>Collection Date</th>
<th>Sample ID</th>
<th>Specimen Type</th>
<th>Estimated Sequence Abundance (Residual Clonal Cells per Million Nucleated Cells)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10/15/2019</td>
<td>SP-237584</td>
<td>Bone Marrow Aspirate Slides</td>
<td>not detected</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
<td>05/15/2019</td>
<td>SP-546732</td>
<td>Bone Marrow Aspirate Slides</td>
<td>not detected</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
<td>12/15/2018</td>
<td>SP-974563</td>
<td>Fresh Bone Marrow</td>
<td>62,222</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IGH - Sequence B</th>
<th>Collection Date</th>
<th>Sample ID</th>
<th>Specimen Type</th>
<th>Estimated Sequence Abundance (Residual Clonal Cells per Million Nucleated Cells)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10/15/2019</td>
<td>SP-237584</td>
<td>Bone Marrow Aspirate Slides</td>
<td>not detected</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
<td>05/15/2019</td>
<td>SP-546732</td>
<td>Bone Marrow Aspirate Slides</td>
<td>not detected</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
<td>12/15/2018</td>
<td>SP-974563</td>
<td>Fresh Bone Marrow</td>
<td>106,978</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IGL - Sequence C</th>
<th>Collection Date</th>
<th>Sample ID</th>
<th>Specimen Type</th>
<th>Estimated Sequence Abundance (Residual Clonal Cells per Million Nucleated Cells)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10/15/2019</td>
<td>SP-237584</td>
<td>Bone Marrow Aspirate Slides</td>
<td>not detected</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
<td>05/15/2019</td>
<td>SP-546732</td>
<td>Bone Marrow Aspirate Slides</td>
<td>not detected</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
<td>12/15/2018</td>
<td>SP-974563</td>
<td>Fresh Bone Marrow</td>
<td>138,763</td>
<td></td>
</tr>
</tbody>
</table>

Since no residual sequences were detected in the current sample, the estimated sequence abundance is zero.

For some patients, the report may indicate that one or more of the dominant DNA sequences being tracked may have been identified using a prior version of the clonoSEQ Assay (5). Strong concordance data support the use of this/these sequence(s) for continued MRD tracking with clonoSEQ. MRD results from a prior version of the clonoSEQ Assay will not display on this report, but are available via the Diagnostic Portal. Please note that quantitative MRD values may not be directly comparable across assays, particularly for small differences in values.

MRD DETECTION AND MONITORING

Tracking (MRD) Report With No Residual Sequences Detected (continued)

Page 3 of the report displays the sequence-level information from Page 2 in a chart format (6). This “sequence-level MRD” chart provides a longitudinal view of results for each individual tracked sequence, for the current sample as well as for past samples sent for clonoSEQ testing for this patient. Similar to the “sample-level” chart on Page 1, the sequence-level chart includes a point on the chart for each test with a corresponding test date, but on this chart, each individual sequence is displayed separately.

In addition to the sequence-level chart, this page lists the criteria used by the clonoSEQ Assay to define dominant sequences, as well as a summary of the assay method and limitations (7).

The appendix provides more details on the immune repertoire of the analyzed sample, including the sample clonality, the number of sequences assessed for each locus, and the number of unique sequences assessed (8).
MRD DETECTION AND MONITORING

Tracking (MRD) Report With No Residual Sequences Detected (continued)

Page 4 of the report continues details on the immune repertoire of the analyzed sample, noting the limit of detection and limit of quantification for each sequence tracked (9). The limit of detection (LOD) and limit of quantitation (LOQ) are independently calculated for each trackable sequence and hence can vary by sequence based on factors including the amount of input DNA, the uniqueness of the sequence, and/or relative amplification due to nucleotide sequence polymorphism or mutation.

A glossary of terms and references relevant to the report is also provided on this page (10).

Within the glossary, the assay’s limit of blank, which is zero, is stated and defined (11). A limit of blank equal to zero indicates that in tests of assay performance on samples that were known to have zero residual disease, the clonoSEQ Assay did not generate any false-positive results.

Note: False positive or false negative results may still occur, for reasons including contamination, technical and/or biological factors.
clonoSEQ® is available as an FDA-cleared in vitro diagnostic (IVD) test service provided by Adaptive Biotechnologies to detect measurable residual disease (MRD) in bone marrow from patients with multiple myeloma or B-cell acute lymphoblastic leukemia (B-ALL) and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). clonoSEQ is also available for use in other lymphoid cancers as a CLIA-validated laboratory developed test (LDT) service. For important information about the FDA-cleared uses of clonoSEQ including test limitations, please visit clonoSEQ.com/technical-summary.

Test Limitations

ALL, MM and CLL: MRD values obtained with different assay methods may not be interchangeable due to differences in assay methods and reagent specificity. The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings. The clonoSEQ Assay is for use with specimens collected in EDTA tubes. Results may vary according to sample time within the course of disease or by sampling site location. The assay may overestimate MRD frequencies near the limit of detection (LoD). The MRD frequency LoD varies based on the amount of gDNA that is tested and using lower gDNA input may prevent MRD detection at low frequencies. Sample processing and cell enrichment strategies may affect the measured MRD frequency. The volume and cellularity of sampled input material may affect the ability to detect low levels of disease. False positive or false negative results may occur for reasons including, but not limited to: contamination; technical and/or biological factors such as the type of rearrangement or the size of the junction region. The assay has been validated with the Illumina NextSeq500 and 550.

For CLL: MRD is based on measurements of tumor cells detected in peripheral blood and/or bone marrow. However, patients may have significant residual disease in unassessed compartments and U-MRD in one compartment cannot fully rule out the presence of disease in the other compartment; for example, U-MRD in blood may not be the same in bone marrow. Therefore assessment of MRD in CLL should employ a multimodal approach including clinical examination, patient medical history, and other findings. Outcome for patients with MRD detectable in bone marrow but not in peripheral blood (PB-/BM+) may differ according to type of therapy. This assay is capable of monitoring specific tumor clonotypes. The association between MRD assessments and patient clinical status for the purpose of monitoring changes in disease (e.g., relapse, remission, stable disease) has not been demonstrated. The value of MRD in CLL for previously untreated or “watch and wait” patients is not established. CLL is a heterogeneous disease. MRD values and expectations for outcome may not be generalizable across treatments. Changes in MRD should be interpreted with caution when used to evaluate disease burden in therapies that have not been validated. Regardless of MRD status, cytogenetics play an independent role in patient risk status and its impact on PFS/OS.

clonoSEQ Clinical Services

P: 888 552 8988 | F: 866 623 4408 | clinicalservices@adaptivebiotech.com