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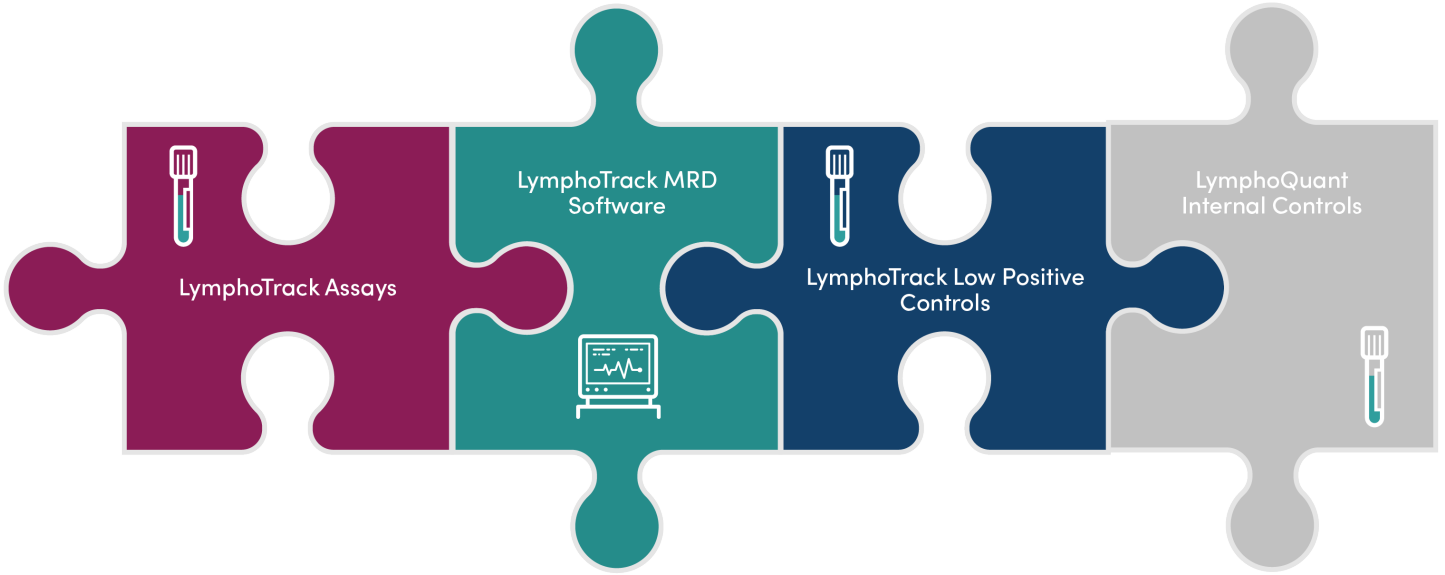
Trend MRD in Longitudinal Studies

Accelerate your Minimal Residual Disease Testing with the LymphoTrack® MRD Software

LymphoTrack®
MRD Software

For Research Use Only. Not for use in diagnostic procedures.

THE MRD SOLUTION



The LymphoTrack MRD Solution can be used in your own laboratory to track levels of MRD in subjects exhibiting *IGH* or T-cell clonality. The LymphoTrack MRD Solution is used to assess levels of MRD in subjects following therapy and throughout remission in your own laboratory. The National Comprehensive Cancer Network (NCCN) guidelines now recommend MRD testing for several lymphoid cancers, including multiple myeloma (MM), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL).[1,2,3,4] European LeukemiaNet (ELN) guidelines recommend MRD testing following induction and consolidation courses to assess remission status and determine kinetics of disease response, and sequentially beyond consolidation to detect impending morphologic relapse.[5]

LymphoTrack MRD Software	Catalog # 7-500-0008	
LymphoTrack Assay	Low Positive Control	Internal Control
IGHV Leader, *IGH* FR1/2/3	LymphoTrack® B-Cell Low Positive Control Catalog # 4-088-0098	LymphoQuant® B-Cell Internal Control Catalog # 4-088-0118
TRG, TRB	LymphoTrack® T-Cell Low Positive Control Catalog # 4-088-0108	LymphoQuant® T-Cell Internal Control Catalog # 4-088-0128

1. NCCN Clinical Practice Guidelines in Oncology: Multiple Myeloma. Version 2.2020.
2. NCCN Clinical Practice Guidelines in Oncology: Acute Lymphoblastic Leukemia. Version 2.2019.
3. NCCN Clinical Practice Guidelines in Oncology: Pediatric Acute Lymphoblastic Leukemia. Version 1.2020.
4. NCCN Clinical Practice Guidelines in Oncology: Chronic Lymphocytic Leukemia. Version 1.2020.
5. Dohner et al. Blood. 2017 Jan 26; 129(4): 424–447.

LymphoTrack®
MRD Software

Assessment of Minimal Residual Disease using v2.0.2+

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ADVANTAGES OF NGS-BASED MRD TESTING

Sensitive

Track clonal sequences in subsequent samples with up to $1x10^{-6}$ sensitivity

Accurate

Calculate estimated cell equivalents for accurate MRD assessment and tracking over time

Efficient

Simultaneously track up to 5 gene rearrangements

Standardized

Offer concordant, objective testing worldwide by tracking sequence specific DNA targets

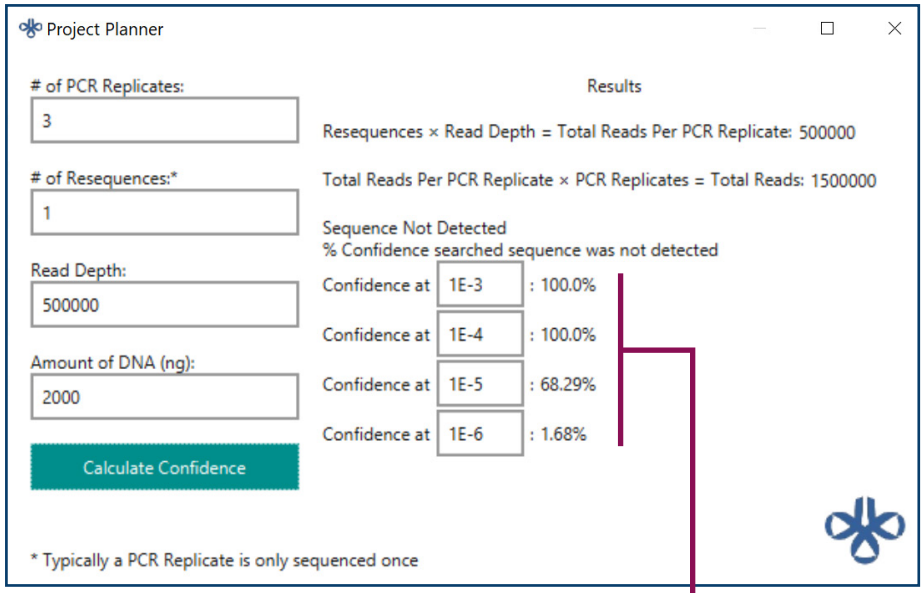
Proactive

Detect refractory and relapsed disease earlier in oncology studies

Vigilant

Monitor and assess the effectiveness of therapies and/or remission status in oncology studies

PLAN YOUR EXPERIMENT

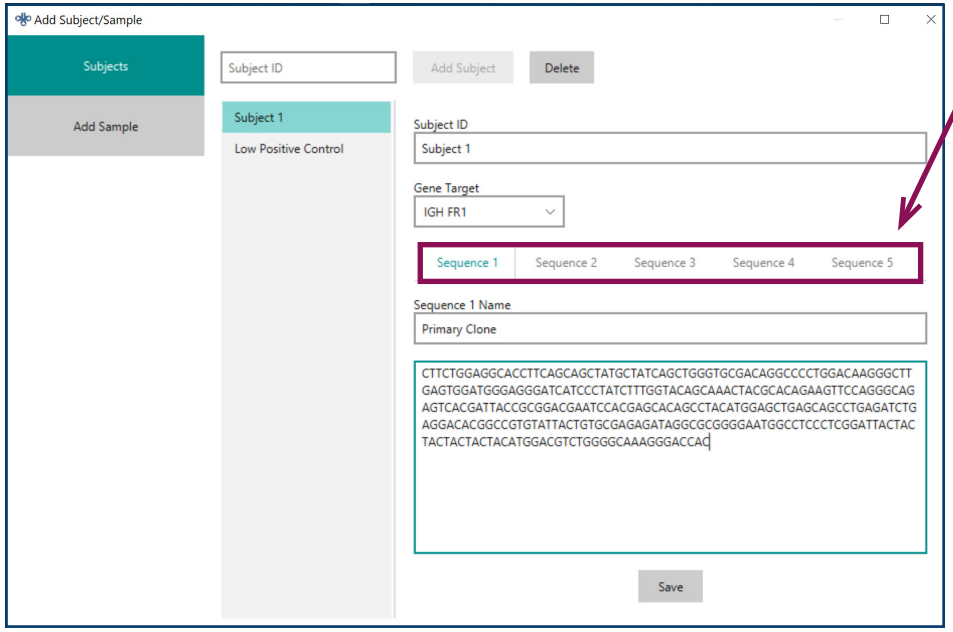


Gain Confidence in your Results

The Project Planner is a tool that is integrated in the software to aid in experimental design. The user may use default levels or customize the confidence levels. The tool allows the user to calculate the level of confidence for a specific sensitivity by manipulating experiment parameters:

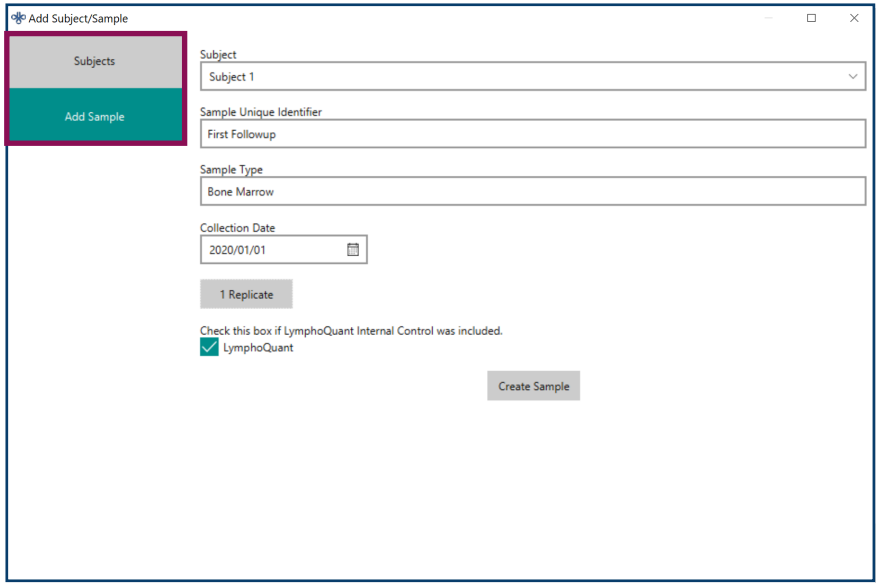
- # of PCR Replicates
- # of Resequences
- Read Depth
- Amount of DNA (ng)

ADD A SUBJECT



New Subject Setup

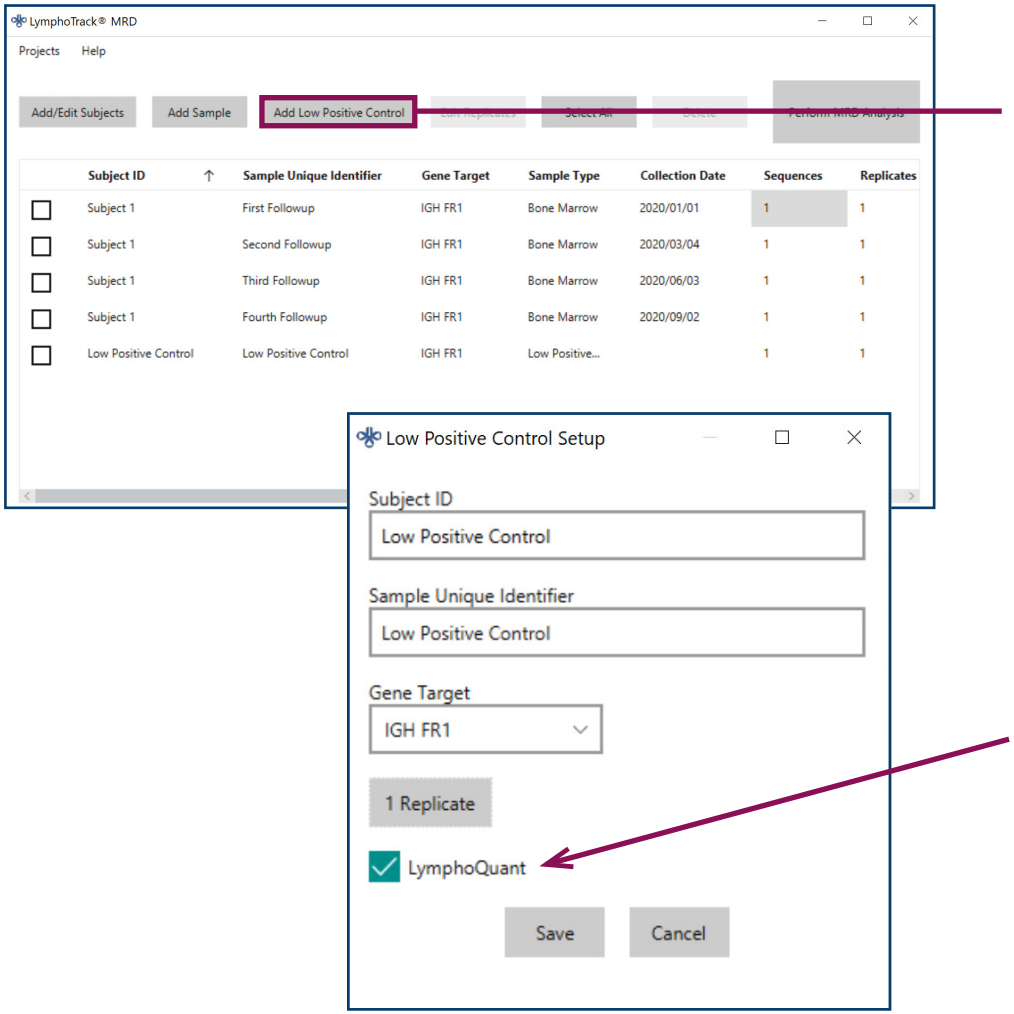
Once clonal sequences are identified using the LymphoTrack (Dx) Software, up to 5 clonal sequences may be simultaneously monitored in longitudinal studies.



Associate Sequence(s) with Subject Samples

The Add Subject/Sample window is used to add new Samples to a Subject and associate the Sample information with respective data files.

LOW POSITIVE AND INTERNAL CONTROL SETUP



Low Positive Controls

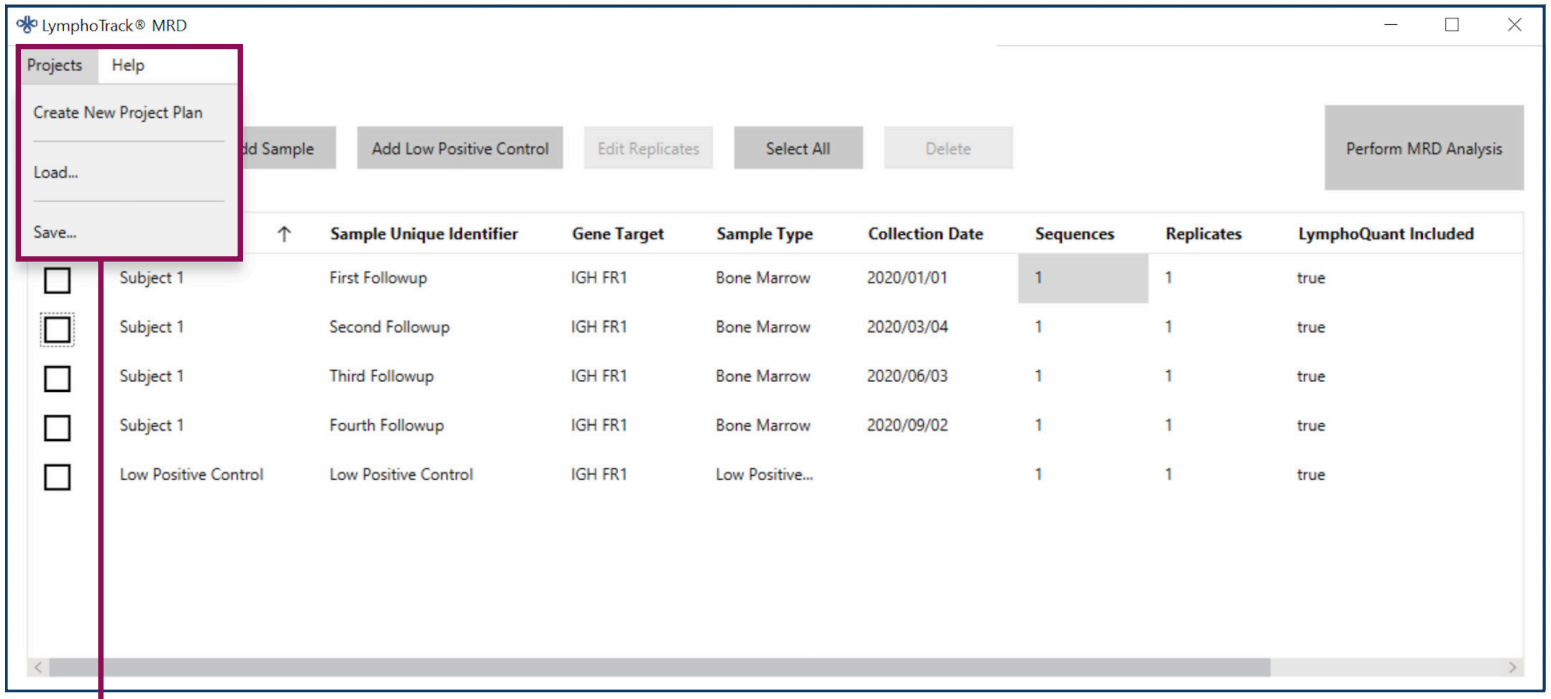
Designed specifically for MRD testing, the LymphoTrack Low Positive Controls* are optimized to work in concert with the LymphoQuant Internal Controls. B-Cell and T-Cell Low Positive Controls may be run in lieu of the positive controls provided in the LymphoTrack *IGHV* Leader, *IGH* FR1, FR2, FR3, *TRG* and *TRB* kits to ensure that MRD levels of sensitivity are being confidently interrogated in samples.

LymphoQuant Internal Controls

B-cell or T-cell LymphoQuant Internal Control may be spiked into the PCR to estimate the respective number of clonotype equivalents and percent clonotype present. Consistent use of a LymphoQuant Internal Control enables clinicians to objectively monitor the disease over time with a highly standardized, sensitive method.

* *IGK* is poorly suited for MRD analyses due to the complexity and low genetic diversity of this locus. If you choose to use *IGK* for MRD testing, verify the sequence of interest is not detected in a polyclonal negative control and carefully select a low positive control appropriate for *IGK*.

MRD PROJECTS



Create, Load, and Save MRD Subject Projects

Once clonal sequences are associated with a Subject and Samples, a Project can be Saved for future use.

Saved Projects can be loaded when additional time points are added to a study.

LymphoTrack® MRD Reports

Understanding the LymphoTrack MRD Reports

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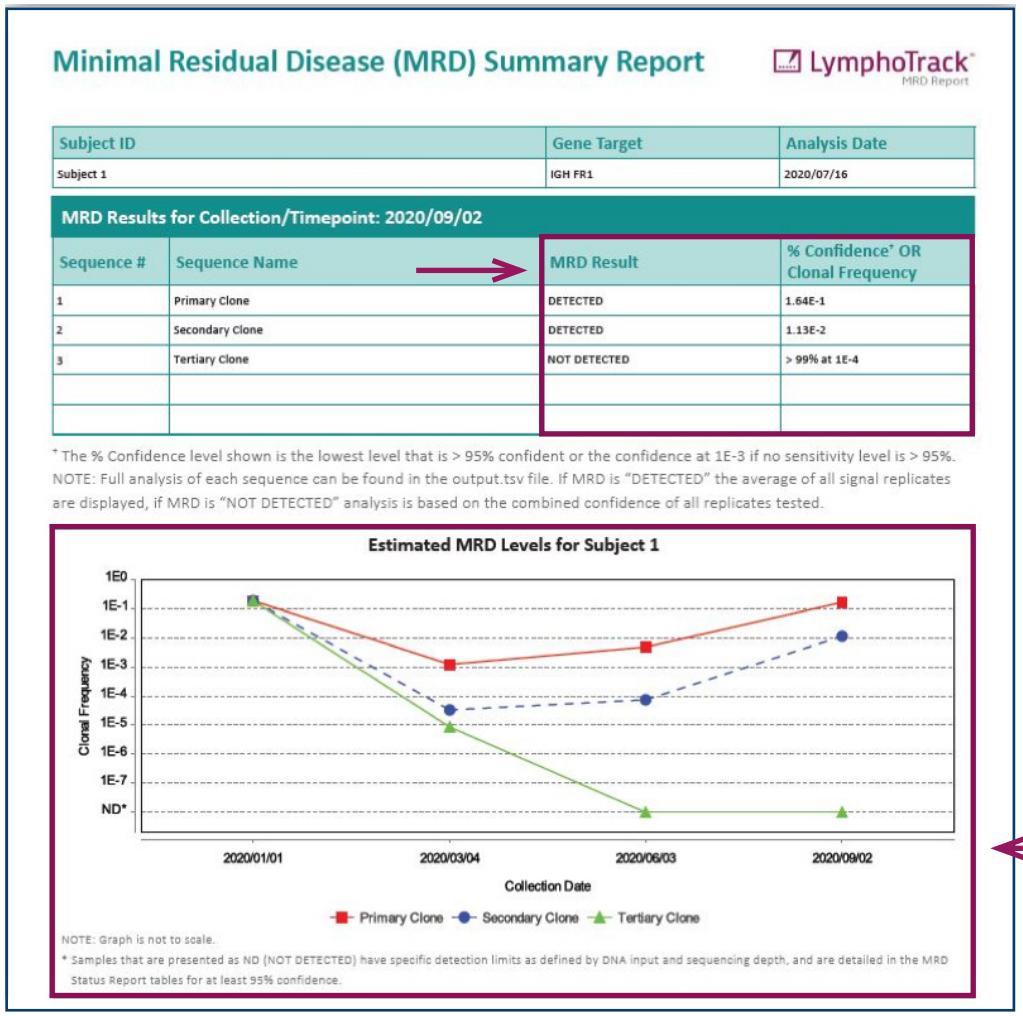


Minimal Residual Disease (MRD) Summary Report

LymphoTrack MRD Report

Subject ID	Gene Target	Analysis Date
Subject 1	IGH FR1	2020/07/16

MRD Results for Collection/Timepoint: 2020/09/02

Sequence #	Sequence Name	MRD Result	% Confidence* OR Clonal Frequency
1	Primary Clone	DETECTED	1.64E-1
2	Secondary Clone	DETECTED	1.13E-2
3	Tertiary Clone	NOT DETECTED	> 99% at 1E-4

* The % Confidence level shown is the lowest level that is > 95% confident or the confidence at 1E-3 if no sensitivity level is > 95%. NOTE: Full analysis of each sequence can be found in the output.tsv file. If MRD is "DETECTED" the average of all signal replicates are displayed, if MRD is "NOT DETECTED" analysis is based on the combined confidence of all replicates tested.

NOTE: Graph is not to scale.

* Samples that are presented as ND (NOT DETECTED) have specific detection limits as defined by DNA input and sequencing depth, and are detailed in the MRD Status Report tables for at least 95% confidence.

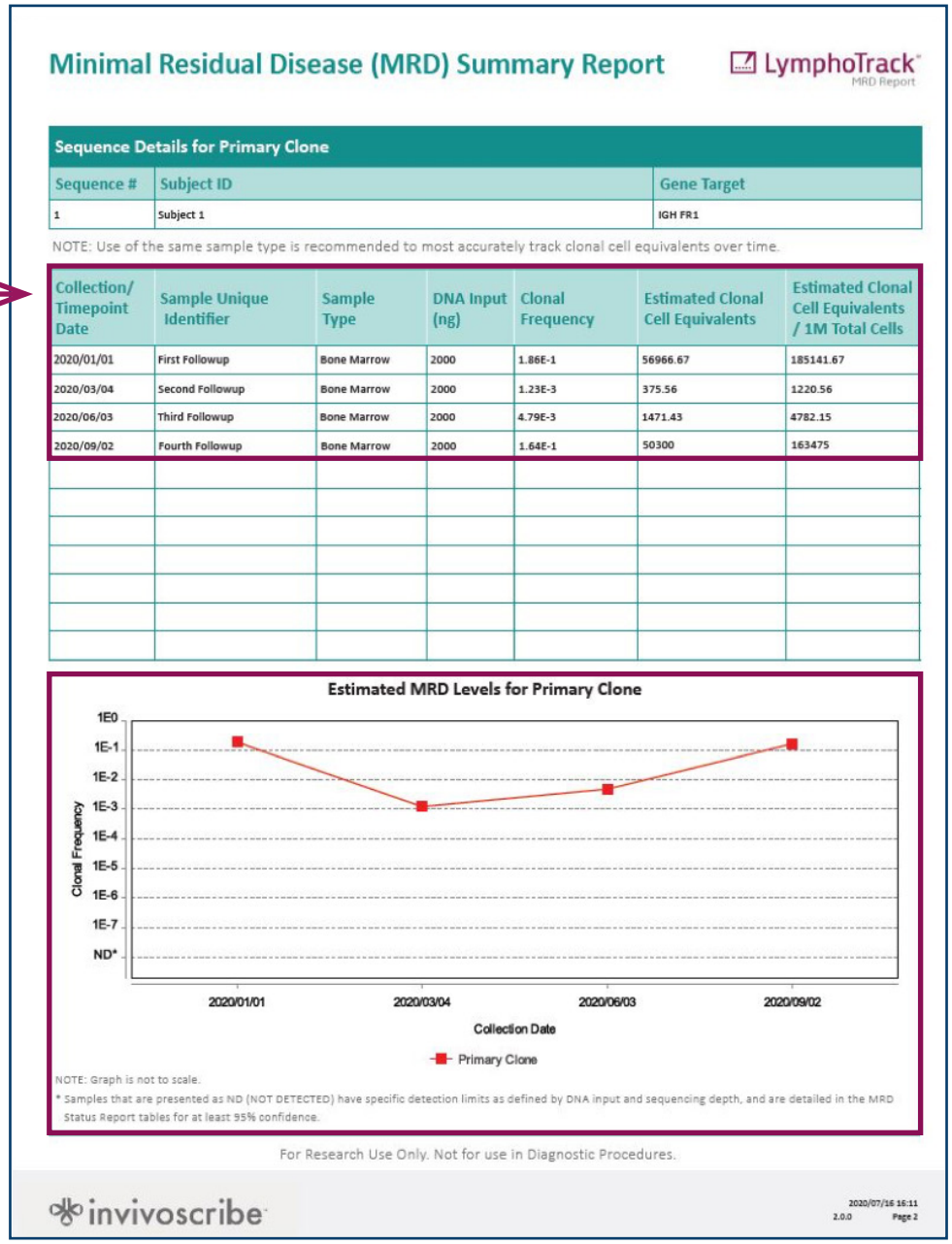
MRD SUMMARY REPORT

The MRD Summary Report provides the status of the clonal sequences that are being tracked. Detailed information is provided for the most recent time point. In this sample, MRD was detected by the software for 2 of the 3 interrogated clonal sequences. If "DETECTED" an estimated Clonal Frequency is displayed. If the sequence is NOT DETECTED (e.g. Sequence #3) the last column of the table displays a quantitative assessment of the % Confidence that the result is a true negative.

Estimated MRD Levels for Subject

This chart provides a longitudinal view of the sample-level MRD results for all sequences queried at each collection date/timepoint. Each result is shown as a Clonal Frequency level (estimated clonal cells per total cells analyzed). When MRD is not identified for a clonal sequence, it is graphed as NOT DETECTED (ND).

Note that this chart provides visual representation of the results; please see the Sample Report for specific detection limits.



Minimal Residual Disease (MRD) Summary Report

LymphoTrack MRD Report

Sequence Details for Primary Clone

Sequence #	Subject ID	Gene Target
1	Subject 1	IGH FR1

NOTE: Use of the same sample type is recommended to most accurately track clonal cell equivalents over time.

Collection/ Timepoint Date	Sample Unique Identifier	Sample Type	DNA Input (ng)	Clonal Frequency	Estimated Clonal Cell Equivalents	Estimated Clonal Cell Equivalents / 1M Total Cells
2020/01/01	First Followup	Bone Marrow	2000	1.86E-1	56966.67	185141.67
2020/03/04	Second Followup	Bone Marrow	2000	1.23E-3	375.56	1220.56
2020/06/03	Third Followup	Bone Marrow	2000	4.79E-3	1471.43	4782.15
2020/09/02	Fourth Followup	Bone Marrow	2000	1.64E-1	50300	163475

Estimated MRD Levels for Primary Clone

NOTE: Graph is not to scale.

* Samples that are presented as ND (NOT DETECTED) have specific detection limits as defined by DNA input and sequencing depth, and are detailed in the MRD Status Report tables for at least 95% confidence.

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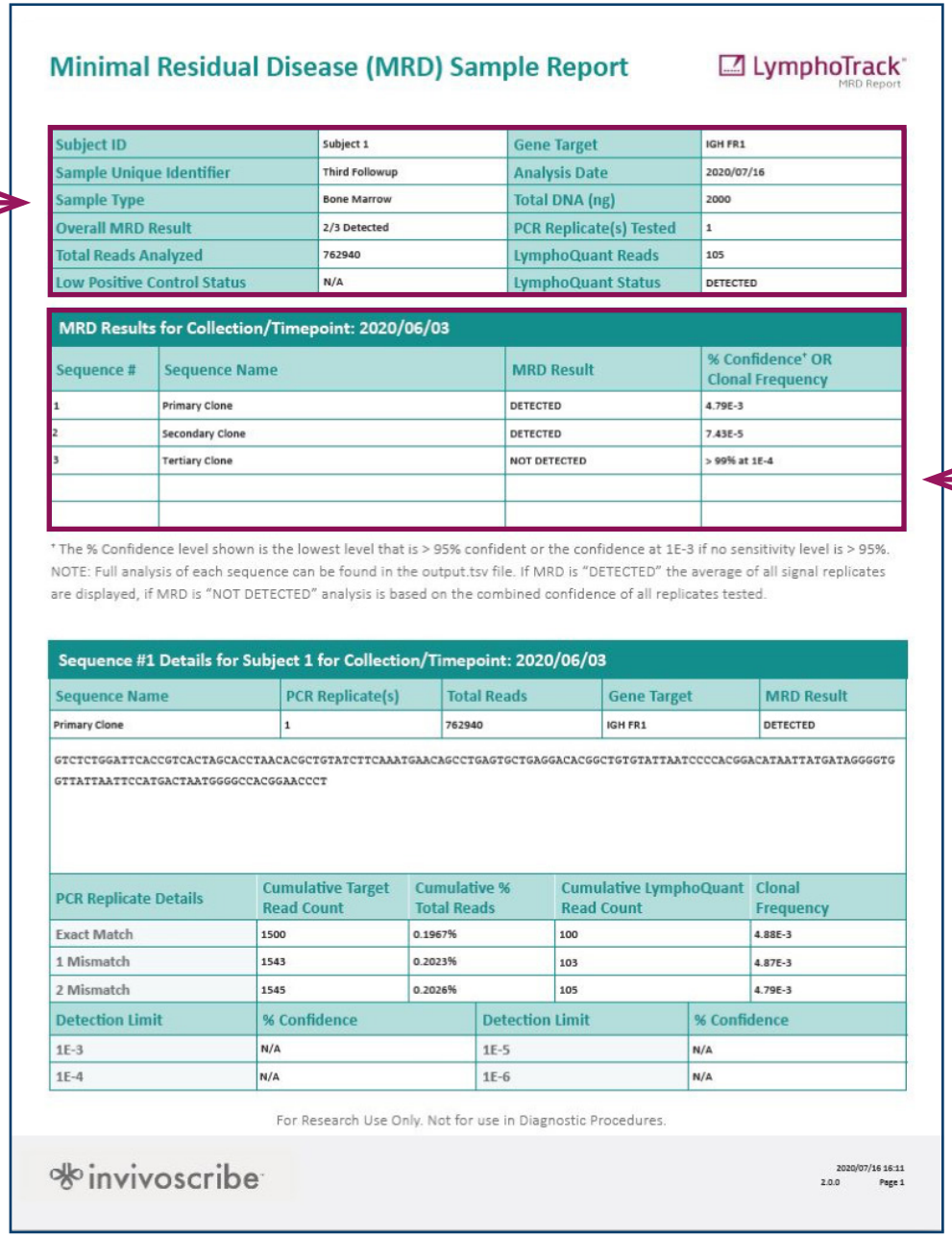
MRD SUMMARY REPORT (PAGE 2)

Details for each Sequence

Subsequent pages of the MRD Summary Report provide details for each of the clonal sequences being tracked. For example, this table provides the following information for sequence #1 (e.g. Primary Clone):

- Collection/Timepoint Date
- Sample Unique Identifier (i.e. First Followup)
- Sample Type (Peripheral Blood, Bone Marrow, etc.)
- DNA Input (in nanograms) per PCR
- Clonal Frequency
- Estimate Clonal Cell Equivalents
- Estimated Clonal cell Equivalents per 1 Million Total Cells

This chart provides a longitudinal view of the sample-level MRD results for individual sequences queried at each collection date/timepoint.



Minimal Residual Disease (MRD) Sample Report

LymphoTrack MRD Report

Subject ID	Subject 1	Gene Target	IGH FR1
Sample Unique Identifier	Third Followup	Analysis Date	2020/07/16
Sample Type	Bone Marrow	Total DNA (ng)	2000
Overall MRD Result	2/3 Detected	PCR Replicate(s) Tested	1
Total Reads Analyzed	762940	LymphoQuant Reads	105
Low Positive Control Status	N/A	LymphoQuant Status	DETECTED

MRD Results for Collection/Timepoint: 2020/06/03

Sequence #	Sequence Name	MRD Result	% Confidence* OR Clonal Frequency
1	Primary Clone	DETECTED	4.79E-3
2	Secondary Clone	DETECTED	7.43E-5
3	Tertiary Clone	NOT DETECTED	> 99% at 1E-4

* The % Confidence level shown is the lowest level that is > 95% confident or the confidence at 1E-3 if no sensitivity level is > 95%. NOTE: Full analysis of each sequence can be found in the output.tsv file. If MRD is "DETECTED" the average of all signal replicates are displayed, if MRD is "NOT DETECTED" analysis is based on the combined confidence of all replicates tested.

Sequence #1 Details for Subject 1 for Collection/Timepoint: 2020/06/03

Sequence Name	PCR Replicate(s)	Total Reads	Gene Target	MRD Result
Primary Clone	1	762940	IGH FR1	DETECTED

GTCTCTGGATTCACCGTCACTAGCACCTAACACGCTGTATCTTCAAATGAACAGCCTGAGTGCTGAGGACACGGCTGTGTATTAATCCCCACGGACATAATTATGATAGGGGTG
GTTATTAATTCCATGACTAATGGGGCCACGGAACCCT

PCR Replicate Details	Cumulative Target Read Count	Cumulative % Total Reads	Cumulative LymphoQuant Read Count	Clonal Frequency
Exact Match	1500	0.1967%	100	4.88E-3
1 Mismatch	1543	0.2023%	103	4.87E-3
2 Mismatch	1545	0.2026%	105	4.79E-3

Detection Limit	% Confidence	Detection Limit	% Confidence
1E-3	N/A	1E-5	N/A
1E-4	N/A	1E-6	N/A

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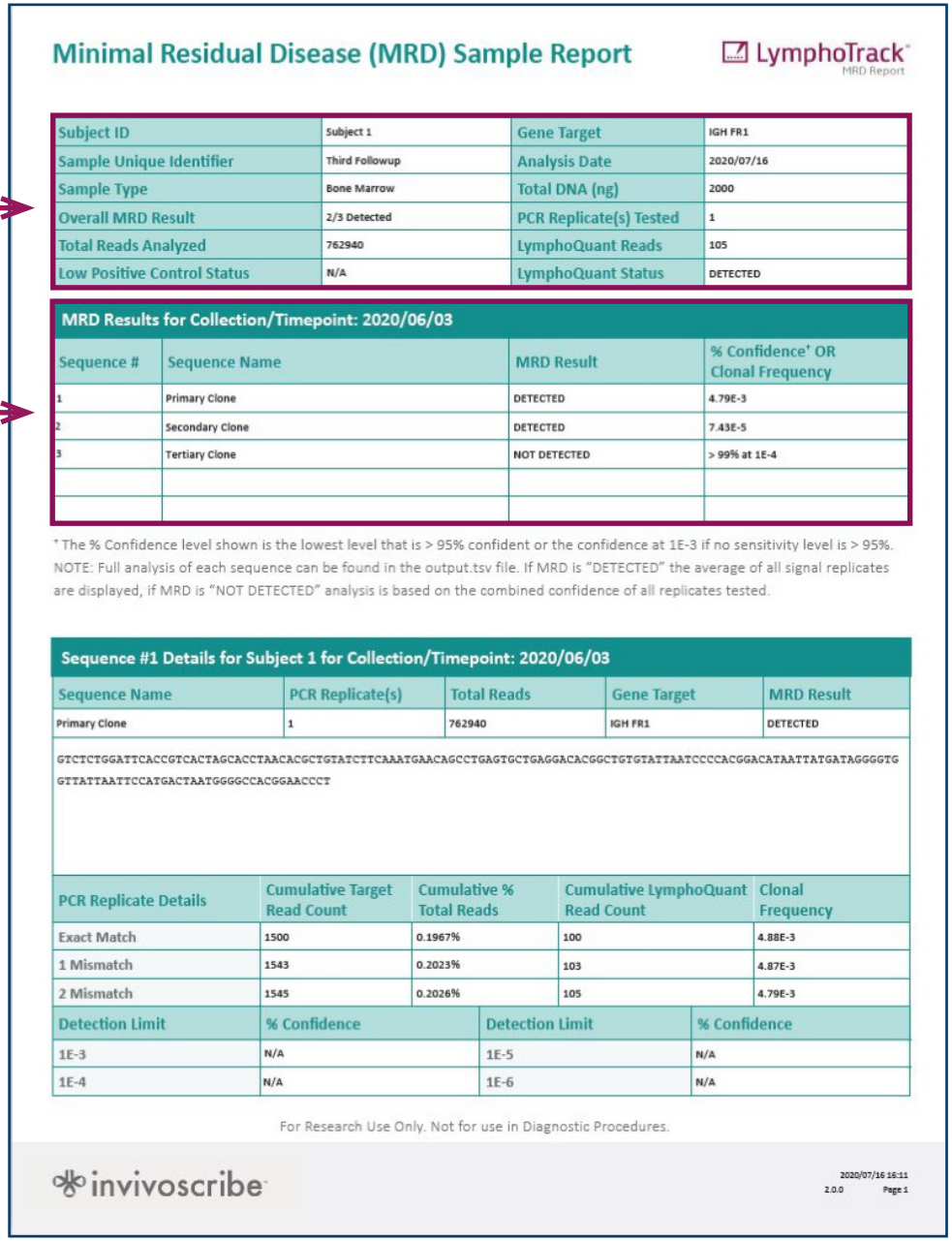
MRD SAMPLE REPORT

Details for each Subject

The Sample Report begins with the Subject ID and Sample specific information. The overall MRD Result displays how many sequences were DETECTED in the total number of sequences analyzed. This table shows the total number of reads that include the B- or T-cell LymphoQuant Internal Control. The LymphoQuant Status will display whether or not sufficient reads were detected.

Details for each Collection Date or Timepoint

This table provides a birds eye view of the MRD results for the Sample Collection Date or Timepoint. Details are summarized for each sequence analyzed including the Clonal Frequency if DETECTED and the % Confidence if NOT DETECTED.



Minimal Residual Disease (MRD) Sample Report

LymphoTrack MRD Report

Subject ID	Subject 1	Gene Target	IGH FR1
Sample Unique Identifier	Third Followup	Analysis Date	2020/07/16
Sample Type	Bone Marrow	Total DNA (ng)	2000
Overall MRD Result	2/3 Detected	PCR Replicate(s) Tested	1
Total Reads Analyzed	762940	LymphoQuant Reads	105
Low Positive Control Status	N/A	LymphoQuant Status	DETECTED

MRD Results for Collection/Timepoint: 2020/06/03

Sequence #	Sequence Name	MRD Result	% Confidence* OR Clonal Frequency
1	Primary Clone	DETECTED	4.79E-3
2	Secondary Clone	DETECTED	7.43E-5
3	Tertiary Clone	NOT DETECTED	> 99% at 1E-4

* The % Confidence level shown is the lowest level that is > 95% confident or the confidence at 1E-3 if no sensitivity level is > 95%. NOTE: Full analysis of each sequence can be found in the output.tsv file. If MRD is "DETECTED" the average of all signal replicates are displayed, if MRD is "NOT DETECTED" analysis is based on the combined confidence of all replicates tested.

Sequence #1 Details for Subject 1 for Collection/Timepoint: 2020/06/03

Sequence Name	PCR Replicate(s)	Total Reads	Gene Target	MRD Result
Primary Clone	1	762940	IGH FR1	DETECTED

GTCTCTGGATTCACCGTCACTAGCACCTAACACGCTGTATCTTCAAATGAACAGCCTGAGTGCTGAGGACACGGCTGTGTATTAATCCCCACGGACATAATTATGATAGGGGTG
GTTATTAATTCCATGACTAATGGGGCCACGGAACCCT

PCR Replicate Details	Cumulative Target Read Count	Cumulative % Total Reads	Cumulative LymphoQuant Read Count	Clonal Frequency
Exact Match	1500	0.1967%	100	4.88E-3
1 Mismatch	1543	0.2023%	103	4.87E-3
2 Mismatch	1545	0.2026%	105	4.79E-3

Detection Limit	% Confidence	Detection Limit	% Confidence
1E-3	N/A	1E-5	N/A
1E-4	N/A	1E-6	N/A

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MRD SAMPLE REPORT FOR THE LOW POSITIVE CONTROL

Details for Low Positive Control

A Sample Report is generated for the Low Positive Control and contains the same information as the Subject Sample Report. The only difference is that the Low Positive Control Status will indicate "Passed" if a sufficient number of reads were identified, "Failed" if an insufficient number of reads were identified, or "N/A" if Low Positive Control was not sequenced.

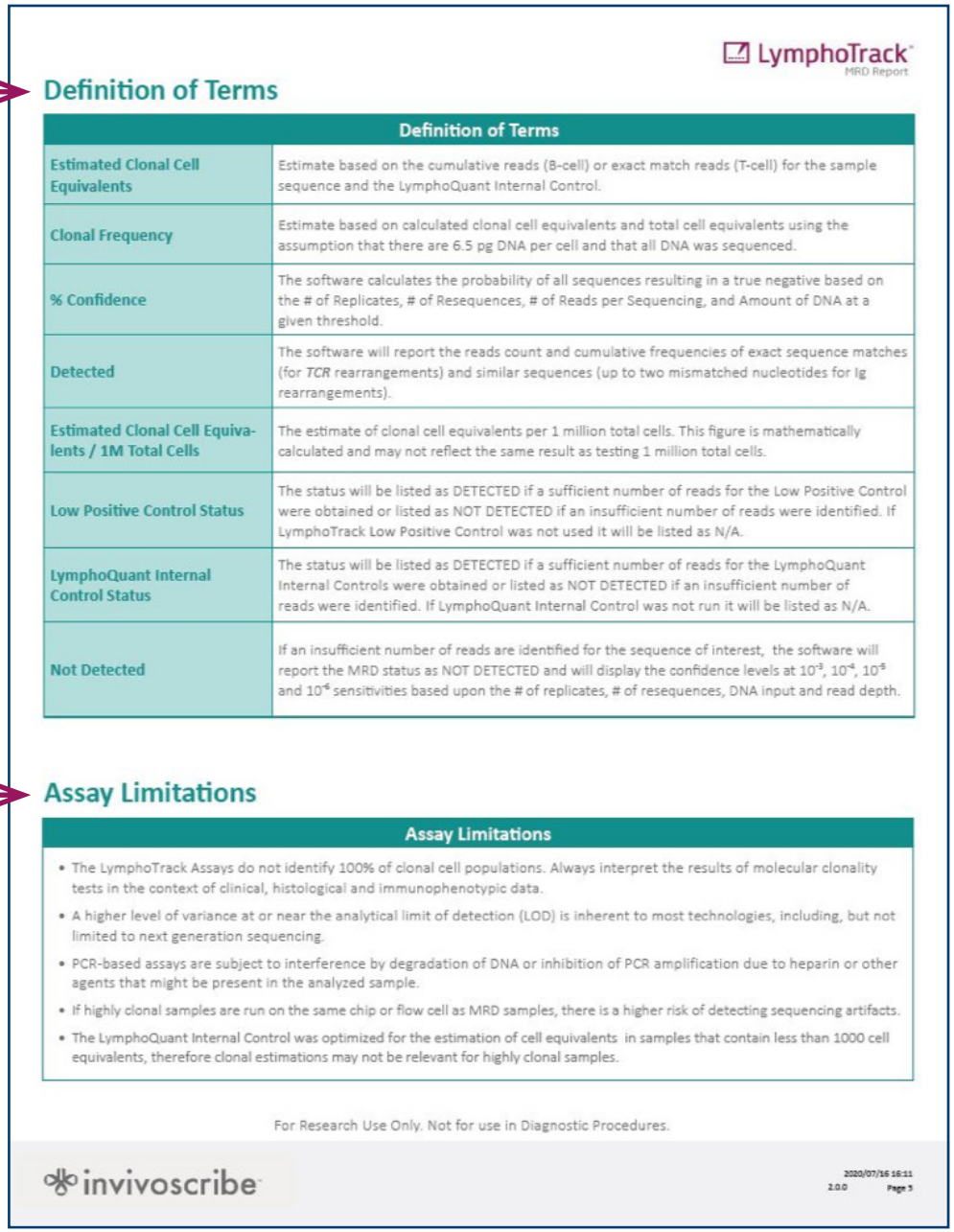
MRD SUMMARY REPORT CONTINUED

Sequence Details

This table provides details about each sequence analyzed.

- The interrogated sequence
- PCR replicate details
- The Cumulative Read
- The Cumulative % Total Reads
- The LymphoQuant Internal Control Cumulative Reads

If the sequence was DETECTED, Clonal Frequencies are shown. If the sequence was NOT DETECTED the % Confidence at 1E-3, 1E-4, 1E-5 and 1E-6 are given.at 1E-3, 1E-4, 1E-5 and 1E-6 are given.



LymphoTrack® MRD Report

Definition of Terms

Definition of Terms	
Estimated Clonal Cell Equivalents	Estimate based on the cumulative reads (B-cell) or exact match reads (T-cell) for the sample sequence and the LymphoQuant Internal Control.
Clonal Frequency	Estimate based on calculated clonal cell equivalents and total cell equivalents using the assumption that there are 6.5 pg DNA per cell and that all DNA was sequenced.
% Confidence	The software calculates the probability of all sequences resulting in a true negative based on the # of Replicates, # of Resequences, # of Reads per Sequencing, and Amount of DNA at a given threshold.
Detected	The software will report the reads count and cumulative frequencies of exact sequence matches (for *TCR* rearrangements) and similar sequences (up to two mismatched nucleotides for Ig rearrangements).
Estimated Clonal Cell Equivalents / 1M Total Cells	The estimate of clonal cell equivalents per 1 million total cells. This figure is mathematically calculated and may not reflect the same result as testing 1 million total cells.
Low Positive Control Status	The status will be listed as DETECTED if a sufficient number of reads for the Low Positive Control were obtained or listed as NOT DETECTED if an insufficient number of reads were identified. If LymphoTrack Low Positive Control was not used it will be listed as N/A.
LymphoQuant Internal Control Status	The status will be listed as DETECTED if a sufficient number of reads for the LymphoQuant Internal Controls were obtained or listed as NOT DETECTED if an insufficient number of reads were identified. If LymphoQuant Internal Control was not run it will be listed as N/A.
Not Detected	If an insufficient number of reads are identified for the sequence of interest, the software will report the MRD status as NOT DETECTED and will display the confidence levels at 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} sensitivities based upon the # of replicates, # of resequences, DNA input and read depth.

Assay Limitations

Assay Limitations
• The LymphoTrack Assays do not identify 100% of clonal cell populations. Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data. • A higher level of variance at or near the analytical limit of detection (LOD) is inherent to most technologies, including, but not limited to next generation sequencing. • PCR-based assays are subject to interference by degradation of DNA or inhibition of PCR amplification due to heparin or other agents that might be present in the analyzed sample. • If highly clonal samples are run on the same chip or flow cell as MRD samples, there is a higher risk of detecting sequencing artifacts. • The LymphoQuant Internal Control was optimized for the estimation of cell equivalents in samples that contain less than 1000 cell equivalents, therefore clonal estimations may not be relevant for highly clonal samples.

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EXPLANATION OF DEFINITION OF TERMS

Definitions for many of the terms used in the MRD Software and Reports.

ASSAY LIMITATIONS

Additional information regarding the capabilities of the LymphoTrack Assays and Software, assay variance, potential for interference, degradation and inhibition, sequencing artifacts and estimations of clonal cell equivalents.

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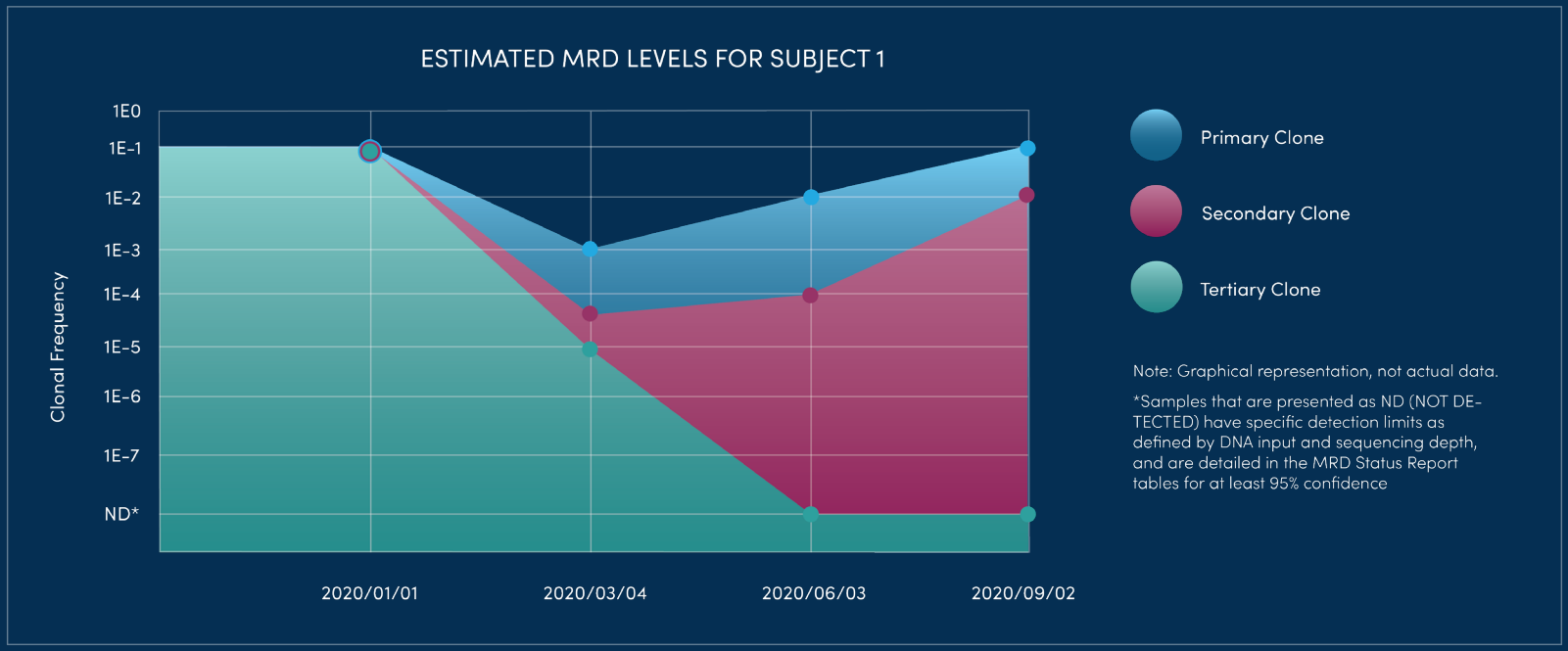
Ask How to Accelerate Your MRD Testing

The LymphoTrack® MRD Solution



For Additional Information

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