Personalized
Molecular Medicine®

2021

Services Catalog

invivoscribe.com

LabPMM®

an invivoscribe company

Invivoscribe's wholly-owned Laboratories for Personalized Molecular Medicine® (LabPMM) is a network of international reference laboratories that provide the medical and pharmaceutical communities with worldwide access to harmonized and standardized clinical testing services. We view internationally reproducible and concordant testing as a requirement for consistent stratification of patients for enrollment in clinical trials, and the foundation for establishing optimized treatment schedules linked to patient's individual profile.

LabPMM provides reliable patient stratification at diagnosis and monitoring, throughout the entire course of treatment in support of Personalized Molecular Medicine® and Personalized Molecular Diagnostics®.

Invivoscribe currently operates four clinical laboratories to serve partners in the USA (San Diego, CA), Europe (Munich, Germany), and Asia (Tokyo, Japan and Shanghai, China). These laboratories use the same critical reagents and software which are developed consistently with ISO 13485 design control. Our cGMP reagents, rigorous standards for assay development & validation, and testing performed consistently under ISO 15189 requirements help ensure LabPMM generates standardized and concordant test results worldwide.

LabPMM is an international network of PersonalMed Laboratories® focused on molecular oncology biomarker studies.

LabPMM LLC

Located in San Diego, California, USA, it holds the following accreditations and certifications: ISO 15189, CAP, and CLIA, and is licensed to provide diagnostic laboratory services in the states of California, Florida, Maryland, New York, Pennsylvania, and Rhode Island.

LabPMM GmbH

Based in Martinsried (Munich), Germany. It is an ISO 15189 accredited international reference laboratory. CLIA/CAP accreditation is planned.

LabPMM 合同会社

Located in Kawasaki (Tokyo), Japan and a licensed clinical lab.

Invivoscribe Diagnostic Technologies (Shanghai) Co., Ltd.

Located in Shangai, China. It is the newest international reference lab, supporting clinical trial work in China.

LabPMM Laboratories are wholly-owned subsidiaries of Invivoscribe.

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LabPMM Testing Services

CRO Services

Global Stratification & enrollment of patients in clinical trials

Diagnostic testing, Research & Development

Worldwide experts in precision medicine and companion diagnostics (CDx) of hematological malignancies.

LABPMM OFFERS EFFICIENT AND RELIABLE STANDARDIZED TESTS THAT MEET THE HIGHEST QUALITY AND SERVICE STANDARDS.

The Laboratories for Personalized Molecular Medicine (LabPMM) is the Invivoscribe® global network of clinical reference labs located in the USA, Europe and Asia, which provides international access to harmonized molecular testing.

LabPMM specializes in oncology with an emphasis on leukemias and lymphomas. We offer an ever-expanding menu of molecular assays to support medical diagnosis, research testing, stratification & enrollment of patients in clinical trials, optimization of treatments and development of companion diagnostics (CDx).

The comprehensive test menu is focused on biomarkers that demonstrate clinical utility, providing data and information that is clinically actionable and critical to making informed treatment decisions. Our CE-IVD assays and bioinformatics are further developed and manufactured consistently under ISO 13485 in our FDA-registered facility, making them eligible to be submitted to worldwide regulatory authorities for registration.

LabPMM is one of the only international laboratory networks that offers both flow cytometry and NGS-based MRD testing for coherent comparisons for a number of clinically significant biomarkers. Accordingly, test results generated in any of our laboratories in Europe, Asia, or the USA are internationally concordant and reproducible. The harmonized testing provided by LabPMM assists healthcare providers in offering optimized and consistent care for their patients, as the test results accurately and reproducibly stratify patients for international clinical trials, thus ensuring that patients receive optimal treatment and that drugs are approved quickly.

LabPMM Locations

LABPMM SPECIALIZES IN PERSONALIZED MOLECULAR TESTING SERVICES FOR ONCOLOGY, INCLUDING LEUKEMIA AND LYMPHOMA. WE ARE COMMITTED TO PROVIDING HIGH-QUALITY TESTING IN SUPPORT OF PERSONALIZED MOLECULAR MEDICINE®.

Our diagnostic and research portfolio includes a full range of oncology services, such as single gene assays for *FLT3, NPM1,* clonality testing of B- and T-cells, minimal residual disease (MRD) assessments and comprehensive next-generation sequencing (NGS) gene panels for AML and other hematologic malignancies.

Rapid turnaround times are vital to ensure that the physician can make timely informed treatment decisions. Our turnaround times for individual gene tests are in the range of 1 to 3 days following sample receipt, while turnaround times for our NGS assays and gene panels are 5 to 14 days. When using LabPMM, physicians receive results faster which expedites patient care and streamlines clinical trials. The reason is simple: all of our LabPMM sites initiate tests the day of clinical sample receipt, avoiding delays caused by batching of samples for testing.

Customer support is an important aspect of our services. We provide responsive, timely support both via email and telephone. We are also bound by strict privacy laws and use only secure proven methods to communicate patient-related data and results.

Available services include:

- CDx *FLT3*
- NGS Gene Panels
- Clonality testing (*IGH, IGK, TRG & TRB*)
- MRD assays
- Custom assays
- Multiparametric Flow Cytometry

SAN DIEGO, CA, UNITED STATES

MARTINSRIED (MUNICH), GERMANY

KAWASAKI (TOKYO), JAPAN

SHANGHAI, CHINA

How to Order a Test

PLEASE CONTACT YOUR LOCAL LABPMM SITE IN ORDER TO RECEIVE THE NECESSARY FORMS TO INITIATE A SERVICES ORDERING ACCOUNT.

Americas: support@labpmm.com | Europe: info@labpmm.de

Japan: services@labpmm.co.jp | China: sales@invivoscribe.com

Specimen Collection and Shipment

We advise our customers to send all specimens through an overnight delivery service.

Please notify your local LabPMM site of urgent samples so we know when to expect the specimens and can investigate any shipping issues if needed.

Specimens for DNA and RNA Assays

Collect blood and bone marrow specimens in sodium heparin, EDTA, or an ACD (acid citrate dextrose) tube. Specifically for *FLT3* ITD MRD and *NPM1* MRD, only samples collected in EDTA and ACD are accepted. Blood and bone marrow may be stored at 2-8 °C for up to 7 days.

Please ship blood and bone marrow at ambient temperature or with cool packs, do not freeze. Please ship previously isolated DNA at ambient temperature, with cool packs, or on dry ice, as applicable. Previously isolated DNA may be stored indefinitely at -65°C to -85°C.

Specimens for Flow Cytometry Panels

Collect blood or bone marrow specimens in sodium heparin or EDTA tubes. Blood and bone marrow may be stored at 2-8 °C for up to 7 days. Please ship blood and bone marrow at ambient temperature or with cool packs, do not freeze.

CDx *FLT3* Mutation Assay

Peripheral blood or bone marrow aspirate samples collected in sodium heparin or EDTA tubes are accepted. Preserved specimens may be stored at 2-8°C for up to 7 days. ACD specimen collection not accepted.

Accredited Test Menu

CDx *FLT3*
NPM1

NGS B-cell Clonality
NGS T-cell Clonality

NPM1 MRD
FLT3 ITD MRD

MyAML®
MyMRD®

Multiparametric Flow Cytometry
- » Hematolymphoid Screening Panel
- » AML MRD Assay

Patient Consent and Confidentiality

Patient Consent

- LabPMM will only process routine diagnostic samples submitted by medical institutions, whereby consent for diagnostic testing is obtained by the submitting physician.
- No data is forwarded to outside organizations without specific prior consent.
- Samples for the MyAML and MyMRD assays must have a completed patient consent form, signed by the patient and the submitting physician to confirm that the patient has understood and given consent for the testing requested.

Patient Confidentiality

- By sending samples to LabPMM for routine diagnostic testing, patients will be protected by the strict data protection laws.
- Patient samples will only be reused for quality control of the assays requested on the original requisition form.
- At LabPMM GmbH, unless prior consent is given, primary samples or DNA from patient samples is retained for 12 months for quality control purposes only. Thereafter patient samples are de-identified and destroyed.
- Our data servers are located in a facility in the USA. Data is encrypted prior to transfer and the transferred data is subject to the same safeguards as data held in Germany.

Partner with Us

CONTACT US FOR MORE INFORMATION: businessdevelopment@invivoscribe.com

Your Ideal Partner for Laboratory Services, Clinical Trial Testing, and Companion Diagnostic Development

LabPMM (an Invivoscribe® company) is your partner of choice for diagnostic, research, and clinical trial services. Our network of laboratories located in the USA, Europe and Asia specialize in internationally harmonized molecular testing, and flow cytometry, and collectively have CLIA and ISO 15189 certifications via CAP and DAkkS. We also offer contract research organization (CRO) services, and are a comprehensive companion diagnostics (CDx) and custom assay development partner, providing ISO 13485-compliant biomarker development, cGMP manufacturing, regulatory capability, global laboratory services and commercialization.

We offer an ever expanding menu of molecular assays, including NGS gene and MRD panels, *FLT3* and *NPM1* mutation assays, and B- and T-cell clonality and MRD assessment. Last year LabPMM has expanded its testing capabilities to include MRD flow cytometry. Our comprehensive test menu will now eliminate the need for partners to split primary specimens, dramatically decreasing turnaround times and allowing for coherent comparison of flow-based and NGS-based MRD test results.

State of the Art

We thrive in international cooperation and in continuous investment in the advancement of precision medicine. We work with a full range of collaborators: key opinion leaders, leading healthcare institutions, and top-tier pharmaceutical companies. We work on the premise that all those reliant on data and results from clinical testing (healthcare providers, pharmaceutical companies and most importantly, patients) will benefit from better standardization and more consistent performance of molecular diagnostic tests.

Quality

Internationally-harmonized diagnostics through our global network of laboratories. We follow full QSR design control for assay and software development. Products are manufactured under cGMP and ISO 13485.

Partnership

We support partnerships worldwide to develop, validate, and commercialize custom biomarker assays and reagents. Our global distribution network operates in more than 700 laboratories in 160 countries.

Expertise

With over 25 years of experience we are the foremost experts in providing molecular products and services for oncologic testing. We offer dedicated support in design and development, manufacturing, software and bioinformatics, technical support, quality assurance, and global regulatory affairs.

An Ideal CDx Partner for Drug Approvals

Partner with Us

IVD Product Development

- Over 25 years of assay development experience
- Biomarker assays & software development under full ISO 13485 design controls
- Comprehensive NGS gene panels that identify actionable biomarkers
- Custom biomarker assay and CDx development

Global Regulatory, Quality and Commercial Expertise

- Experienced staff & proven Quality Management System
- Full adherence to FDA 21 CFR part 820 and ISO13485 standards
- Registered Medical Device Establishment with the US FDA, KFDA, Saudi Arabia, and the PMDA
- Multiple CDx approvals supporting various drugs: by the FDA (US), PMDA/MHLW (Japan), and TGA (Australia). CDx CE-marks in the EU
- 50+ CE-marked IVDs available in the EU and select ROW markets;
- 60 tests registered with the ARTG in Australia
- Marketing Authorization Holder (MAH) and National reimbursement for CDx in Japan. CDx submitted for reimbursement in the US
- Supporting ongoing clinical drug trials in the US, EU, Japan, China and ROW.

Clinical Testing

Global Clinical Reference Laboratory Network

- A dozen years of clinical reference lab experience
- Internationally standardized CDx and biomarker testing with labs serving the US, Europe, and Asia
- Comprehensive LymphoTrack® clonality/MRD assays and CAP and CLIA-certified NGS MyGene™ panels identify clinically actionable biomarkers
- Complementary MRD assays for all biomarkers – potential for surrogate endpoints per agency inputs
- Testing services have supported hundreds of enrollment sites worldwide

Manufacturing

- FDA/CDRH-registered and ISO 13485-certified cGMP manufacturing facility based in San Diego
- Comprehensive Dx and CDx Manufacturing
  - » CDx for USA (PMA), Japan, EU, Australia, and ROW markets
  - » 50+ CE-IVDs (NGS assays + bioinformatics software) IUO & RUO assays, ASRs & GPRs
- DNA / RNA controls, MRD controls & proficiency panels

Partner with Us

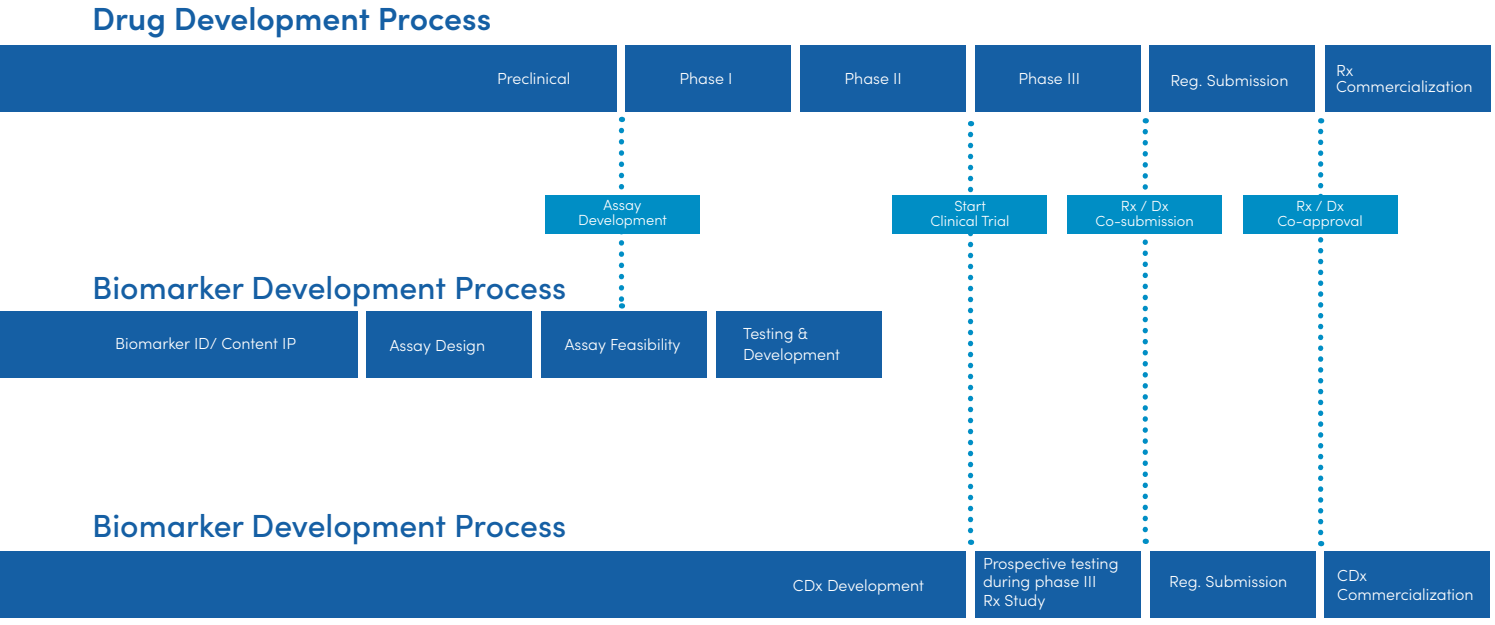
Join the 60+ Pharma Companies Worldwide who are Working with Invivoscribe on Comprehensive Projects

CONTACT US FOR MORE INFORMATION: businessdevelopment@invivoscribe.com

Integrated Approach to CDx Development

We provide efficient handling of all stages of CDx development, from the biomarker discovery process to commercialization, including:

- Supportive, collaborative relationship with our pharmaceutical partners
- Dedicated CDx development team with extensive expertise in program management, feasibility studies, product development, quality control, regulatory, and commercialization
- Sense of urgency and commitment to partner's success



CDx Commercialization Phasing

Planning	Pre-Launch	Launch	Post-Launch
Develop plan in collaboration with partner	Identify and nurture key opinion leaders (KOLs)	Establish lab network	Expand lab network and physician awareness after reimburement established and guidelines updated
Share market insights and collective knowledge	Align CDx development studies with commercialization studies	Create physician awareness	Continue to nurture KOLs
Align Rx / Dx commercial objectives	Voice of customer to drive product requirements and commercialization considerations	Activate KOL network to start commercialization studies	Monitor and adjust activities to achieve market adoption
Define commercialization strategy and country level prioritization/timing	Prepare marketing message for PMA submission	Complete HEOR studies, prepare HTAs, request reimbursement coverage	Transition to maintenance campaigns
Determine activities requiring partner support		Initiate PR and marketing campaigns	

Examples of *FLT3* CDx Partnerships:

NOVARTIS

- *FLT3* CDx for RYDAPT® (midostaurin)
- Breakthrough designation in the USA
- FDA, Swissmedic & EU approval in 2017
- ARTG-inclusion in AUS

ASTELLAS

- *FLT3* CDx for XOSPATA® (gilteritinib fumarate)
- >140 international collection sites
- PMDA approval in Japan in 2018
- Reimbursement approved in Japan
- FDA approval in USA in 2018
- EU approval in 2019

DAIICHI-SANKYO

- *FLT3* CDx for VANFLYTA® (quizartinib hydrochloride)
- Bridging study for multiple labs in the USA, Europe and Asia
- PMDA approval in Japan in 2019

COMPANION DIAGNOSTIC TESTING

Companion Diagnostic Testing

According to the U.S. FDA definition, a companion diagnostic is a medical device, often an *in vitro* device (IVD), which provides information that is essential for the safe and effective use of a corresponding drug or biological product. The use of a companion diagnostic will therefore help clinicians and healthcare providers determine whether a patient is likely to benefit from the drug in question and monitor the response. In the European Union under the new IVDR, the definition of a companion diagnostic expands to require patient screening before and/or during treatment for those likely to experience benefit and/or increased risk as a result of treatment with the corresponding medicinal products.

The use of assays that have not been specifically validated for the safety and effectiveness of a drug or biological product may deliver inaccurate results that could harm the patient. For instance, a false positive result could lead to treatment with a drug without the proven benefits, exposing the patient to potential toxic side effects. Likewise, a false negative test result could withhold or delay a potentially beneficial treatment, putting the patient at risk.

Companion diagnostics help demonstrate drug efficacy and accelerate approval. They have become an important tool for improving individual patient treatment.

LabPMM embraces international harmonization and partnering. We work with key opinion leaders to standardize molecular diagnostic testing and we are also partnered with pharmaceutical companies to develop companion diagnostic tests. Our proud history of partnerships have led to outstanding work towards internationally standardized testing, exeplified by FDA and PMDA approval of the first companion diagnostic for acute myeloid leukemia.

LeukoStrat® CDx *FLT3* Mutation Assay – USA

PREDICTIVE TEST FOR THE EFFICACY OF RYDAPT® (MIDOSTAURIN) AND XOSPATA® (GILTERITINIB FUMARATE)

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA® (gilteritinib) treatment is being considered.

The test is for use on the 3500xL Dx Genetic Analyzer.

1. **Mutations in the *FLT3* gene are the most common mutations found in AML**
2. **Presence of a *FLT3* activation mutation in patients with AML may be prognostic and clinically actionable.**
3. **The LeukoStrat CDx *FLT3* Mutation Assay is used as aid in the selection of AML patients for whom midostaurin or gilteritinib fumarate are being considered**
4. **NCCN, ELN and CAP Guidelines recommend *FLT3* testing to inform patient treatment decisions.**
5. **Gilteritinib fumarate was approved by the FDA for the treatment of adult patients with relapsed/ refractory *FLT3*mut+ AML**
6. **Midostaurin was approved by the FDA for the treatment of adult patients with newly diagnosed AML who are *FLT3* mutation positive**
7. **First-to-market, this CDx is FDA approved (PMA#P160040) as a predictive test for the efficacy of midostaurin therapy in all AML patients, regardless of cytogenetics.**

FDA APPROVED

Test Name

LeukoStrat® CDx *FLT3* Mutation Assay

Assay Type

Capillary Electrophoresis

Method Description

The LeukoStrat® CDx *FLT3* Mutation Assay is designed to detect ITD and TKD mutations in the *FLT3* gene.

The assay is performed on DNA isolated from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with AML.

Primers targeting both in and around the juxtamembrane region for ITD testing and kinase domain of the *FLT3* gene are used to amplify DNA extracted from a patient sample.

The TKD PCR product is further digested with a restriction enzyme. The ITD PCR products and the digested TKD PCR products are analyzed on a capillary electrophoresis instrument.

FLT3 ITDs are detected by a change in the expected size of a wild type fragment. An amplicon larger than the wild type fragment indicates the presence of *FLT3* ITD. The TKD digestion pattern identifies loss of the normal gene sequences and ensures that digestion occurred.

Indications for Testing

- At initial diagnosis or relapse of AML
- As an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered.
- As a tool to identify AML patients eligible for treatment with XOSPATA® (gilteritinib fumarate).

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued, indicating whether the patient is eligible for midostaurin and/or gilteritinib fumarate treatment	2 to 3 business days >95% patient samples are reported within 48 hours of receipt	**Recommended Specimen Volume (Preservative)** • 2 mL of peripheral blood in Sodium Heparin or EDTA • 0.5mL of bone marrow in Sodium Heparin or EDTA **Minimum Specimen Volume (Preservative)** • 1mL of peripheral blood in Sodium Heparin or EDTA • 0.25 mL of bone marrow in Sodium Heparin or EDTA	2°C to 8°C up to 72 hours; do not freeze.	2°C to 8°C up to 7 days

LeukoStrat® CDx *FLT3* Mutation Assay (CE-marked)

PREDICTIVE TEST FOR THE EFFICACY OF RYDAPT® (MIDOSTAURIN) AND XOSPATA® (GILTERITINIB FUMARATE)

Intended Use

The LeukoStrat® CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplications (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

In regions where midostaurin is available, the LeukoStrat® CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered.

In regions where gilteritinib fumarate is available, the LeukoStrat® CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA® (gilteritinib fumarate) treatment is being considered.

1. **Mutations in the *FLT3* gene are the most common mutations found in AML**
2. **Presence of a *FLT3* activation mutation in patients with AML may be prognostic and clinically actionable.**
3. **NCCN, ELN and CAP Guidelines recommend *FLT3* testing to inform patient treatment decisions.**
4. **The LeukoStrat® CDx *FLT3* Mutation Assay is used as an aid in the assessment of AML patients for whom midostaurin and/or gilteritinib treatment is being considered**
5. **Midostaurin was approved by the Swissmedic and European Commission for the treatment of adult patients with newly diagnosed AML who are *FLT3* mutation positive**
6. **Gilteritinib fumarate was approved by the European Commission for the treatment of relapsed/ refractory *FLT3*mut+ AML**

EUROPEAN COMMISSION & SWISSMEDIC APPROVED

Test Name

LeukoStrat® CDx *FLT3* Mutation Assay

Assay Type

Capillary Electrophoresis

Method Description

The LeukoStrat® CDx *FLT3* Mutation Assay is designed to detect ITD and TKD mutations in the *FLT3* gene.

The assay is performed on DNA isolated from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with AML.

Primers targeting both in and around the juxtamembrane region for ITD testing and kinase domain of the *FLT3* gene are used to amplify DNA extracted from a patient sample. The TKD PCR product is further digested with a restriction enzyme. The ITD PCR products and the digested TKD PCR products are analyzed on a capillary electrophoresis instrument.

FLT3 ITDs are detected by a change in the expected size of a wild type fragment. An amplicon larger than the wild type fragment indicates the presence of *FLT3* ITD. The TKD digestion pattern identifies loss of the normal gene sequences and ensures that digestion occurred.

Indications for Testing

- At initial diagnosis or relapse of AML
- In regions where midostaurin is available, the LeukoStrat CDx *FLT3* mutation assay is used as an aid in the assessment of AML patients for whom midostaurin treatment is being considered.
- In regions where gilteritinib fumarate is available, the LeukoStrat CDx *FLT3* mutation assay is used as an aid in the assessment of AML patients for whom gilteritinib fumarate treatment is being considered.

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating the absence or presence of a *FLT3* mutation and its corresponding signal ratio. The report will further indicate whether the patient is eligible for a therapy with midostaurin or gilteritinib hydrochloride.	2 to 3 business days >95% of patient samples are reported within 48 hours of receipt	**Recommended Specimen Volume (Preservative)** • 2 mL of peripheral blood in Sodium Heparin or EDTA • 0.5mL of bone marrow in Sodium Heparin or EDTA **Minimum Specimen Volume (Preservative)** • 1mL of peripheral blood in Sodium Heparin or EDTA • 0.25 mL of bone marrow in Sodium Heparin or EDTA	2°C to 8°C up to 72 hours; do not freeze.	2°C to 8°C up to 7 days

LeukoStrat® CDx *FLT3* Mutation Assay – Japan

PREDICTIVE TEST FOR THE EFFICACY OF XOSPATA® (GILTERITINIB FUMARATE) AND VANFLYTA™ (QUIZARTINIB HYDROCHLORIDE)

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based, *in vitro* diagnostic test designed to detect internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom Gilteritinib Fumarate treatment is being considered.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom Quizartinib Hydrochloride treatment is being considered.

1. **Mutations in the *FLT3* gene are the most common mutations found in AML**
2. **Presence of a *FLT3* activation mutation in patients with AML may be prognostic and clinically actionable.**
3. **The LeukoStrat® CDx *FLT3* Mutation Assay is the first PMDA approved test for assessment of AML patients eligible for treatment with gilteritinib fumarate or quizartinib hydrochloride**
4. **Gilteritinib fumarate received manufacturing and marketing approval for the treatment of *FLT3* mutation-positive relapse or refractory AML in Japan**
5. **Quizartinib hydrochloride is MHLW/PMDA approved for the treatment of relapsed/ refractory *FLT3*-ITD⁺ AML**

MHLW/PMDA APPROVED

Test Name

LeukoStrat® CDx *FLT3* Mutation Assay

Assay Type

Capillary Electrophoresis

Method Description

The LeukoStrat® CDx *FLT3* Mutation Assay is designed to detect ITD and TKD mutations in the *FLT3* gene.

The assay is performed on DNA isolated from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with AML.

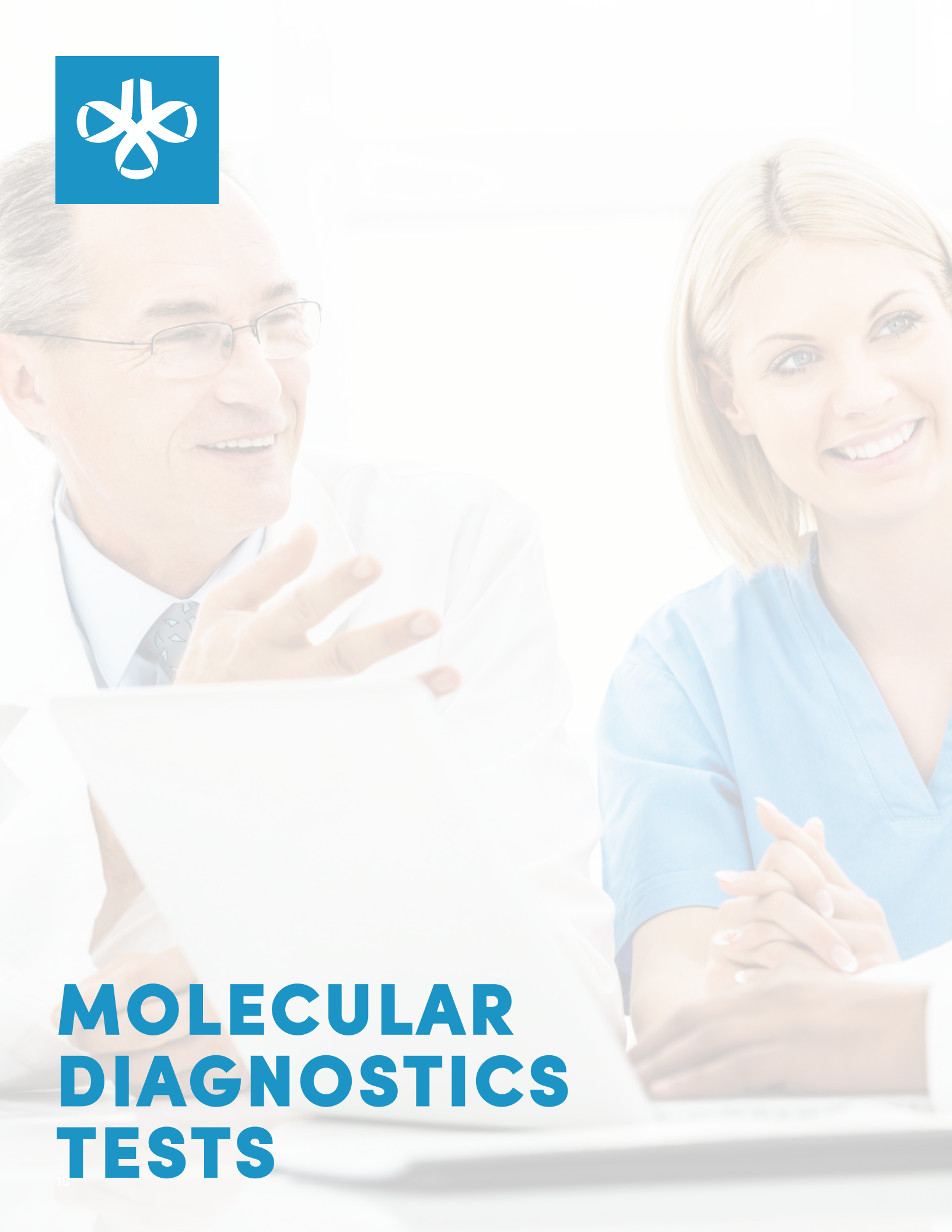
Primers targeting both in and around the juxtamembrane region for ITD testing and kinase domain of the *FLT3* gene are used to amplify DNA extracted from a patient sample. The TKD PCR product is further digested with a restriction enzyme. The ITD PCR products and the digested TKD PCR products are analyzed on a capillary electrophoresis instrument.

FLT3 ITDs are detected by a change in the expected size of a wild type fragment. An amplicon larger than the wild type fragment indicates the presence of *FLT3* ITD. The TKD digestion pattern identifies loss of the normal gene sequences and ensures that digestion occurred.

Indications for Testing

- At initial diagnosis or relapse of AML
- As a tool for the assessment of AML patients for whom gilteritinib fumarate treatment is being considered
- As a tool for the assessment of AML patients for whom quizartinib hydrochloride treatment is being considered

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Reimbursement Points
An interpretive report will be issued, indicating whether the patient is eligible for gilteritinib fumarate treatment or quizartinib hydrochloride	2 to 3 business days > 95% of patient samples reported within 48 hours of receipt	**Recommended Specimen Volume (Preservative)** • 2 mL of peripheral blood in Sodium Heparin or EDTA • 0.5mL of bone marrow in Sodium Heparin or EDTA **Minimum Specimen Volume (Preservative)** • 1mL of peripheral blood in Sodium Heparin or EDTA • 0.25 mL of bone marrow in Sodium Heparin or EDTA	2°C to 8°C up to 72 hours; do not freeze. **Specimen Stability** 2°C to 8°C up to 7 days	4200 points



MOLECULAR DIAGNOSTICS TESTS

Molecular Diagnostics Tests

Somatic mutations play an increasingly important role in the risk stratification and management of leukemia and lymphoma patients. Traditionally, classification and risk stratification have relied on cytogenetic studies; however, molecular detection of gene mutations and gene rearrangements are now central in the classification, risk stratification, and management of lymphoproliferative diseases. Molecular testing also complements cytogenetic testing results, which helps further refine stratification and prognosis, especially within specific disease subgroups.

All of LabPMM's molecular tests conform to the Standard of Care as defined by the World Health Organization (WHO) and are recommended by members of the National Comprehensive Cancer Network, LeukemiaNet, and other world opinion leaders in hematology.

NPM1

Clinical Information

The Nucleophosmin (*NPM1*) gene is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurring in about 35% of AML patients at diagnosis.[1] The vast majority of *NPM1* mutations are insertions in exon 12 occurring near the C-terminus of the protein that result in cytoplasmic localization.[2] Currently there are over 40 known *NPM1* mutations, most of which will be detected with our assay.

Clinical studies have found that *NPM1* mutations are associated with increased blast counts, higher extramedullary involvement and increased platelet counts in AML.[3] Furthermore, in the absence of a *FLT3* ITD mutation (or *FLT3* ITD with a low ratio), *NPM1* mutations are associated with a favorable prognosis.[4]

It has been suggested that the identification of mutations in both *NPM1* and *FLT3* genes allows for the stratification of the AML patients into three different prognostic groups:

- Favorable prognosis: *NPM1* mutation without *FLT3* ITD or with *FLT3* ITD^{low}
- Intermediate prognosis: *NPM1* mutation and *FLT3* ITD^{high}; *NPM1*$^{-}$ without *FLT3* ITD or with *FLT3* ITD^{low} (without adverse-risk genetic lesions)
- Poor prognosis: *NPM1* wild-type and *FLT3* ITD^{high}

It is recommended that AML patients be screened for *NPM1* mutations as an effort to assess prognosis and aid in treatment decisions. Results from *NPM1* and *FLT3* mutational screening should be available within 48 to 72 hours (at least in patients eligible for intensive chemotherapy). Utilizing both *NPM1* and *FLT3* (mutant:wild-type ratio) mutation status is the most common molecular method for stratification of the AML population.

References

1. Thiede C, et al. (2006) Prevalence and prognostic impact of *NPM1* mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 107:4011–4020.
2. Falini B. et al. (2007) Translocations and mutations involving the nucleophosmin (*NPM1*) gene in lymphomas and leukemias. Haematologica 92(4):519–532.
3. Döhner K, et al. (2005) Mutant nucleophosmin (*NPM1*) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 106(12):3740–3746.
4. Döhner H, et al. (2017) Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129:424–447.

Test Name

NPM1 mutation analysis (qualitative)

Assay Type

Capillary Electrophoresis

Method Description

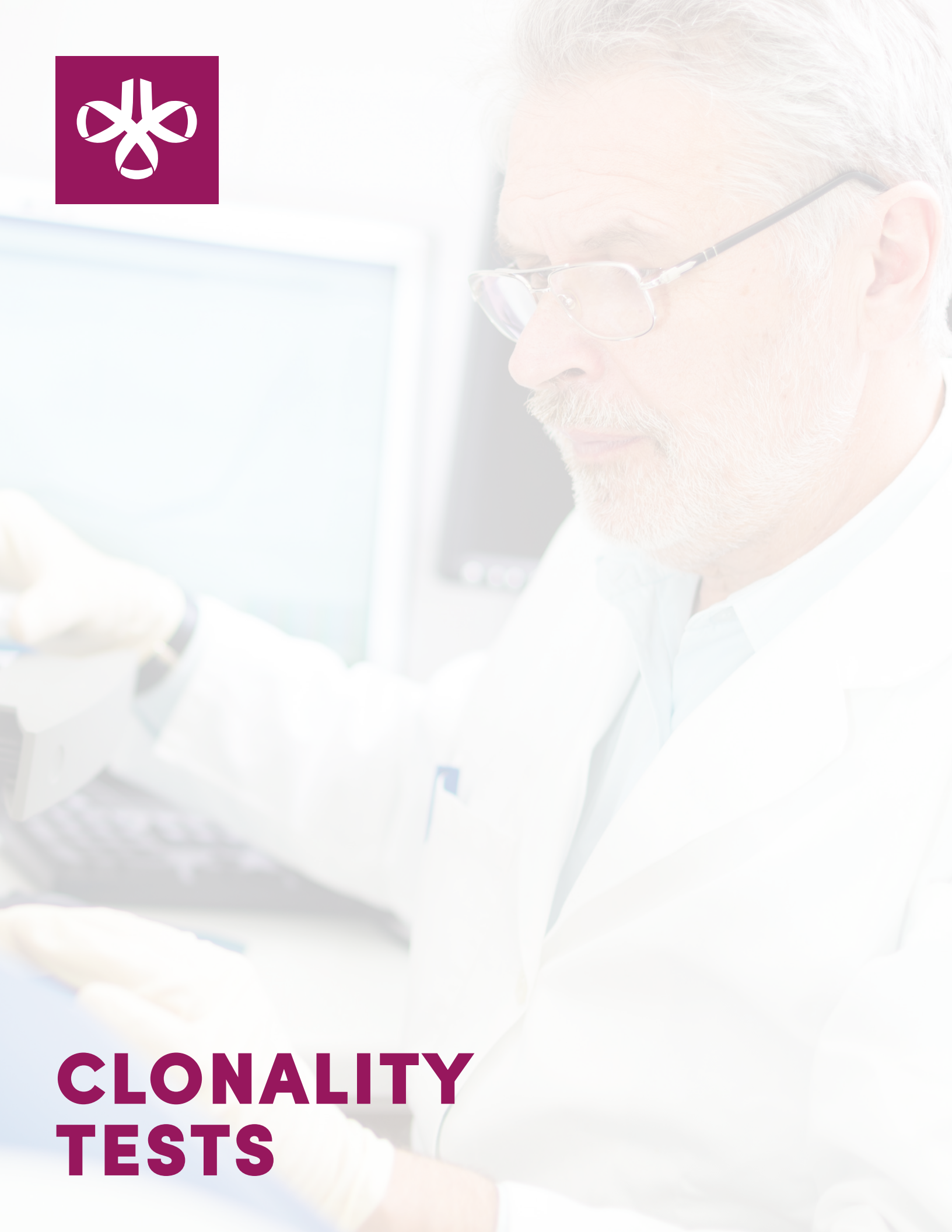
Primers targeting exon 12 on the *NPM1* gene are used to amplify the patient's DNA. The size of the *NPM1* PCR product is determined by capillary electrophoresis.

LabPMM offers the only internationally harmonized assay for *NPM1* mutations and testing is performed pursuant to patents licensed from TrovaGene, Inc. of San Diego, CA.

Indications for Testing

- At initial diagnosis of AML
- Stratification high and low risk AML
- Recurrence of leukemia after induction therapy on patients not initially screened for *NPM1* mutations

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether a *NPM1* mutation was detected	1-3 business days	• 1-3 mL peripheral blood in EDTA, ACD or Heparin • 0.25-1 mL bone marrow in EDTA, ACD or Heparin • 250 ng of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	• Room Temp up to 72 hours • 2-8 °C up to 7 days



CLONALITY TESTS

Clonality Tests

The unique process of genetic rearrangements in the immunoglobulin (Ig) and T-cell receptor (*TCR*) gene loci during immune cell development and maturation generates a vast pool of genetically distinct cells.

During early lymphoid differentiation, genes encoding the Ig and *TCR* molecules are formed by stepwise rearrangement of variable (V), diversity (D), and joining (J) gene segments. During this V-D-J recombination process, nucleotides are deleted and randomly inserted at the joining sites, resulting in an enormous diversity of unique antigen receptors. As Ig/*TCR* gene rearrangements occur sequentially in the earliest stages of lymphoid differentiation, they are present in almost all immature and mature lymphoid cells.

Since lymphoma is a cancer of the lymphatic or the immune system, the vast majority of lymphomas exhibit rearrangements in Ig and/or *TCR* genes. Lymphoid malignancies are characterized by the reduced population diversity of these gene loci originating from the proliferative transformation of an individual lymphoid cell. The associated cellular population typically shares one or more cell-specific or "clonal" antigen-receptor gene rearrangements. The detection of these clonal cells provides the basis for clonality assessment in leukemia, lymphoma, and hematologic disease diagnosis.

Invivoscribe (LabPMM's parent company) is an industry pioneer with 25 years of experience in providing clonality test solutions. Our expertise in clonality testing assures the highest rates of detection of clonal populations as well as international standardization of results.

IGH Clonality Assays

Clinical Information

Lymphoid cells are different from the other somatic cells in the body as during development, the antigen receptor genes of these cells undergo somatic gene rearrangement.[1]

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 non-functional variable (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) gene segments spread over 1250 kilobases. During B-cell development, genes encoding the *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection. These gene rearrangements of the variable, diversity and joining segments generate V_H-D_H-J_H combinations of unique length and sequence for each cell.[2,3]

Since leukemia and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Clonality does not always imply malignancy; all results must be interpreted in the context of all of the other available diagnostic criteria. Tests that detect *IGH* clonal rearrangements are useful in the characterization, monitoring, and treatment of B- and T-cell malignancies.

References

1. Tonegawa S (1983) Somatic Generation of Antibody Diversity. Nature 302:575-581.
2. Trainor KJ et al. (1990). Monoclonality in B-lymphoproliferative disorders detected at the DNA level. Blood 75:2220-2222.
3. JE Miller et al., Molecular Genetic Pathology (2013, 2nd ed.) Springer Science & Business Media 302.2.7.13 and 30.2.7.18.

Test Name

IGH FR1 Clonality Assay
IGH FR2 Clonality Assay
IGH FR3 Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)

This test is performed by using the LymphoTrack® or LymphoTrack® Dx Assay from Invivoscribe

Method Description

For detection of the vast majority of clonal *IGH* V_H-J_H rearrangements, including the associated V_H-J_H region DNA sequences, a multiplex master mix targeting the conserved framework region 1, framework region 2, or framework region 3, as well as the joining region, is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to clonal gene rearrangements. Bioinformatics tools facilitate the characterization of sequences present at greater than 2.5% of the population. These sequences can be used to track specific clonal populations.

Indications for Testing

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether evidence of clonality was detected. The report further provides a summary of the top 5 merged sequences, including the % total reads, the rearrangement class and the sequence.	5 to 10 business days	• 1-3 mL of peripheral blood in Heparin, EDTA or ACD • 0.25-1 mL of bone marrow in Heparin, EDTA or ACD • 500 ng of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	• Room Temp up to 72 hours • 2-8 °C up to 7 days prior to testing

IGH Somatic Hypermutation

Clinical Information

Lymphoid cells are different from the other somatic cells in the body as during development, the antigen receptor genes in these cells undergo somatic gene rearrangement.[1] During B-cell development, genes encoding the human immunoglobulin heavy chain (*IGH*) proteins are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating V_H-D_H-J_H combinations that are unique in both length and sequence for each cell.[2-3] An additional level of diversity is generated by point mutations in the variable regions, also known as somatic hypermutations (SHM).

Leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, which means that all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal rearrangements can be useful in the study of B-cell malignancies.

Immunoglobulin variable heavy chain gene hypermutation status provides important prognostic information for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). The presence of *IGH* SHM is defined as greater or equal to 2% difference from the germline VH gene sequence, whereas less than 2% difference is considered evidence of no SHM. The status of SHM for clone(s) has clinical relevance, as there is a clear distinction in the median survival of patients with and without SHM. Hypermutation of the *IGH* variable region is strongly predictive of a good prognosis, while lack of mutation predicts a poor prognosis.[4] In addition, this assay identifies clonal rearrangements involving the V3-21 gene, which has been associated with a poor prognosis in CLL independent of SHM status. This assay has been shown to further stratify CLL patients.[5]

References:

1. Tonegawa S (1983). Somatic Generation of Antibody Diversity. Nature 302:575-581.
2. Trainor KJ et al. (1990). Monoclonality in B-lymphoproliferative disorders detected at the DNA level. Blood 75:2220-2222.
3. JE Miller et al., Molecular Genetic Pathology (2013, 2nd ed.) Springer Science & Business Media 302.2.7.13 and 30.2.7.18.
4. P. Ghia, et al. (2007). ERIC recommendations on *IGHV* gene mutational status in chronic lymphocytic leukemia. Leukemia 21:1-3.
5. Stamatopoulos, B et al. (2017). Targeted deep sequencing reveals clinically relevant subclonal IgHV rearrangements in chronic lymphocytic leukemia. Leukemia 31(4):837-845.

Test Name

IGHV Leader Somatic Hypermutation Assay

Assay Type

Next-Generation Sequencing (NGS)

This test is performed by using the LymphoTrack® or LymphoTrack® Dx Assay from Invivoscribe.

Method Description

For detection of the vast majority of clonal *IGH* V_H-J_H rearrangements, including the associated V_H-J_H region DNA sequences, a multiplex master mix targeting the conserved framework region 1 (FR1) or leader and the joining region is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify the frequency distribution of V_H region and J_H region segment utilization, as well as for the definition of the extent of somatic hypermutation present in the *IGH* gene. Bioinformatics tools facilitate the characterization of sequences present at greater than 2.5% of the population and the level of somatic hypermutation present in the dominant clone. Bioinformatics also identify clonal rearrangements that involve the V3-21 gene, which has been associated with a poor prognosis in CLL, independent of SHM status.

Indications for Testing

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating the level of *IGH* SHM along with the rearrangement class for the dominant clones and the specific sequence for the dominant clone.	5 to 10 business days	• 1-3 mL Peripheral Blood in EDTA, ACD or Heparin • 0.25-1 mL of bone marrow in Heparin, EDTA or ACD • 500 ng of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

IGK Clonality Assay

Clinical Information

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements.[1]

The human immunoglobulin kappa (*IGK*) locus on chromosome 2 (2p11.2) includes 7 variable (V_k) region gene segments and 5 joining (J_k) gene segments upstream of the constante (C_k) region. The kappa deleting element (K_{de}), approximately 24 kb downstream of the J_k-C_k region, can also rearrange with V_k gene segments and the isolated recombination signal sequence in the J_k-C_k intronic region.[2] Specifically during B-cell development, genes encoding *IGK* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating gene receptors unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, which means that all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

References

1. Tonegawa S (1983) Somatic Generation of Antibody Diversity. Nature 302:575-581.
2. JE Miller et al., Molecular Genetic Pathology (2013, 2nd ed.) Springer Science & Business Media 302.2.7.13 and 30.2.7.18.

Test Name

IGK Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)

This test is performed by using the LymphoTrack® or LymphoTrack® Dx Assay from Invivoscribe.

Method Description

For detection of the vast majority of *IGK* gene rearrangements, a multiplex master mix targeting the conserved V_k, J_k, C_k ,and kappa deleting element (K_{de}) regions is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to clonal gene rearrangements. Bioinformatics tools facilitate the characterization of sequences present at greater than 5% of the population. These sequences can be used to track specific clonal populations.

Indications for Testing

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether evidence of clonality was detected. The report further provides a summary of the top 5 merged sequences, including the % total reads, the rearrangement class and the sequence.	5 to 10 business days	• 1-3 mL of peripheral blood in Heparin, EDTA or ACD • 0.25-1 mL of bone marrow in Heparin, EDTA or ACD • 500 ng of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

TRB Clonality Assay

Clinical Information

The human T-cell receptor beta (*TRB*) gene locus on chromosome 7 (7q34) includes 64-67 variable (V_B) gene segments (belonging to 30 subgroups), 2 diversity (D_B) gene segments, and 13 joining (J_B) gene segments, spread over 685 kilobases, making this locus far more complex than others. Nevertheless, accurate molecular analysis of the *TRB* genes is an important tool for the assessment of clonality in suspected T-cell and some B-cell proliferations, as *TRB* gene rearrangements occur not only in almost all mature T-cell malignancies, but also in about one-third of precursor B-acute lymphoblastic leukemias (B-ALL).[1]

Lymphoid cells are different from the other somatic cells in the body, as during development the antigen receptor genes in lymphoid cells (including gene segments within the *TRB* locus), undergo somatic gene rearrangement.[2]

These developmentally regulated, programmed gene rearrangements generate combinations that are unique for each cell.[1] Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Clonality does not always imply malignancy; all results must be interpreted in the context of all of the other available diagnostic criteria. Tests that detect *TRB* clonal rearrangements can be used to help identify T-cell and certain B-cell malignancies.

References

1. JE Miller et al., Molecular Genetic Pathology (2013, 2nd ed.) Springer Science & Business Media 302.2.7.13 and 30.2.7.18.
2. Tonegawa S (1983) Somatic Generation of Antibody Diversity. Nature 302:575-581

Test Name

TRB Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)

This test is performed by using the LymphoTrack® or LymphoTrack® Dx Assay from Invivoscribe.

Method Description

For detection of the vast majority of *TRB* gene rearrangements, a multiplex master mix targeting the V_{β}, J_{β} and D_{β} regions is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to clonal gene rearrangements. Bioinformatics tools facilitate the characterization of sequences present at greater than 2.5% of the population. These sequences can be used to track specific clonal populations.

Indications for Testing

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether evidence of clonality was detected. The report further provides a summary of the top 5 merged sequences, including the % total reads, the rearrangement class and the sequence.	5 to 10 business days	• 1-3 mL of peripheral blood in Heparin, EDTA or ACD • 0.25-1 mL of bone marrow in Heparin, EDTA or ACD • 500 ng of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

TRG Clonality Assay

Clinical Information

The human T-Cell Receptor Gamma (*TRG*) locus on chromosome 7 (7q14) includes 14 variable (Vγ) genes (Group I, II, III, and IV), 5 joining (Jγ) gene segments, and 2 constant (Cγ) genes spread over 200 kilobases.[1]

Lymphoid cells are different from the other somatic cells in the body, as during development the antigen receptor genes in lymphoid cells (including gene segments within the *TRG* locus), undergo somatic gene rearrangement.[2] These developmentally regulated, programmed gene rearrangements generate Vγ-Jγ combinations that are unique for each cell.[3]

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, which means that all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Clonality does not always imply malignancy; all results must be interpreted in the context of all of the other available diagnostic criteria. Tests that detect *TRG* clonal rearrangements can be used to help identify T-cell and certain B-cell malignancies.

Note: During T-cell ontogeny, rearrangement of the *TRG* locus occurs before rearrangement of the alpha beta loci. Therefore, clonal rearrangements of *TRG* are often present, commonly detected, and can be tracked in T-cell malignancies involving alpha-beta T-cells. This makes *TRG* a powerful tool for both clonal and MRD analysis of T-cell and some B-cell tumors.

References

1. LC Lawnickie, et al. (2003). The distribution of gene segments in T-cell receptor gamma gene rearrangements demonstrates the need for multiple primer sets. J Mol Diagn. 5:82-87.
2. Tonegawa S (1983) Somatic Generation of Antibody Diversity. Nature 302:575-581.
3. JE Miller et al., Molecular Genetic Pathology (2013, 2nd ed.) Springer Science & Business Media 302.2.7.13 and 30.2.7.18.

Test Name

TRG Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)

This test is performed by using the LymphoTrack® or LymphoTrack® Dx Assay from Invivoscribe.

Method Description

For detection of the vast majority of *TRG* gene rearrangements, a multiplex master mix targeting the Vγ and Jγ regions are used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to clonal gene rearrangements. Bioinformatics tools facilitate the characterization of sequences present at greater than 2.5% of the population. These sequences can be used to track specific clonal populations.

Indications for Testing

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether evidence of clonality was detected. The report further provides a summary of the top 5 merged sequences, including the % total reads, the rearrangement class and the sequence.	5 to 10 business days	• 1-3 mL of peripheral blood in Heparin, EDTA or ACD • 0.25-1 mL of bone marrow in Heparin, EDTA or ACD • 500 ng of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

MINIMAL RESIDUAL DISEASE TESTS

Minimal Residual Disease Tests

Minimal Residual Diseases (MRD) testing has shown strong potential for the optimization of therapeutic management of lymphoproliferative diseases. Currently, MRD tests complement and leverage the information obtained at diagnosis. Due to their increased sensitivity, these measurements are most useful at time points where they are compared and contrasted with more traditional methods. An example of this is before transplant, when MRD levels have been shown to be predictive of transplantation success.

Several patient-specific PCR-based (e.g. ASO-PCR) and flow cytometric technologies have been developed by regional test centers in order to routinely assess MRD levels during the course of therapy. However, ASO-PCR requires patient- and tumor-specific primer and probe sets, making it cost prohibitive and impossible to offer as a standardized method. Flow cytometry – even more sensitive multiparameter flow cytometry protocols – are difficult to standardize between testing centers. Neither of these methods meet the internationally recognized criteria for a standardized, quantitative measure of residual disease and therefore do not meet the standards required to take them through the regulatory agencies.

Next-Generation Sequencing (NGS) methods have recently been developed for the detection and monitoring of MRD. These forefront technologies use regulatory-compliant chemistries, run on regulatory-compliant instruments, and can be interpreted using regulatory compliant, and design-controlled bioinformatics software. Due to the read depth of this non-biased patient agnostic testing approach, ultra deep sequencing overcomes virtually all of the shortcomings of other MRD technologies, providing internationally harmonized MRD testing for virtually any targeted biomarker.

LabPMM's MRD tests are NGS-based assays that can be used to detect clonal gene rearrangements identified at diagnosis within virtually all of the antigen receptor loci (B- and T-cells). Once a specific rearrangement sequence (the clonotype) has been identified in a primary sample, bioinformatics tools allow for objective longitudinal tracking of clonal populations with a sensitivity up to 1×10^{-6}, provided sufficient DNA is tested. Sensitivity (limit of detection) is determined by the number of cell equivalents of DNA that are interrogated and the number of sequencing reads generated per sample.

LabPMM also offers *FLT3* ITD and *NPM1* MRD assays, which are used for the detection of targeted mutations. These sensitive NGS-based assays reliably detect sequences present at 5×10^{-5}.

AML – *FLT3* ITD MRD Assay

Clinical Information

Minimal residual disease (MRD) detection in patients with leukemia has proven to be useful in the clinical management of disease and can facilitate the development of new therapies. Mutations in the fms-like tyrosine kinase 3 (*FLT3*) gene are the most prevalent mutations found in acute myeloid leukemia (AML)[1] and are characterized by an aggressive phenotype with a high prevalence of relapse. Internal tandem duplication (ITD) mutations within the juxtamembrane domain are the most common mutations of *FLT3*.[2] The development of a sensitive and specific assay for *FLT3* ITD mutations represents a significant advancement in guiding treatment decisions.

LabPMM's *FLT3* ITD MRD test is an NGS-based, targeted, deep-sequencing assay that detects ITDs ranging from 3 bp to over 200 bp in size. Once a specific ITD (length and sequence) has been identified in a primary sample, it can easily be tracked in subsequent samples at a sensitivity of $5x10^{-5}$, provided sufficient DNA quantity is tested.

The treatment of AML has become a paradigm for precision medicine. This MRD assay is at least two orders of magnitude more sensitive than other commercially available *FLT3* assays. It detects the persistence of a driver mutation, *FLT3* ITD, in patients with no overt evidence of disease, allowing clinicians to identify those patients that can benefit from continuation or modification of treatment.[3]

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

References

1. The Cancer Genome Atlas Research Network (2013) Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. N Engl J Med. 368: 2059–2074.
2. Konig H. et al. (2015) Targeting *FLT3* to treat leukemia. Expert Opin Ther Targets 19:37-54.
3. Levis, M. J. et al (2018) A next-generation sequencing–based assay for minimal residual disease assessment in AML patients with *FLT3*-ITD mutations. Blood Advances, 2: 825-831

Test Name

FLT3 ITD MRD Assay

Assay Type

Next-Generation Sequencing (NGS)

CLIA-validated assay

Method Description

To track and identify previously detected *FLT3* ITD mutations in post-treatment follow-up samples, a multiplex master mix targeting the juxtamembrane domain of the *FLT3* gene is used to amplify DNA extracted from a patient sample.

Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified mutations detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at an allelic sensitivity level of $5x10^{-5}$.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether *FLT3* ITD MRD was detected	7 to 10 business days	• 1-3 mL of peripheral blood in EDTA, • 0.25-1 mL of bone marrow in EDTA • 1 µg of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

AML – *NPM1* MRD Assay

Clinical Information

Minimal residual disease (MRD) detection in patients with leukemia is useful for the clinical management of disease, and can facilitate the development of new therapies.

Mutations in the nucleophosmin (*NPM1*) gene represent some of the most prevalent gene mutations in AML.[1] *NPM1* mutations predominantly occur in AML with normal cytogenetics and are of prognostic value, especially within the context of *FLT3* ITD mutations. Furthermore, because *NPM1* displays a homogeneous mutation pattern, this gene represents an attractive target for MRD monitoring.[2]

LabPMM's *NPM1* MRD test is a NGS-based, targeted, deep-sequencing assay that can be used to detect *NPM1* mutations that were previously identified in a primary sample. The sensitive assay reliably detects sequences present at 5×10^{-5}.

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

References

1. Falini B. et al. (2005) Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 352:254–266.
2. Krönke J. et al. (2011) Monitoring of minimal residual disease in *NPM1*-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol 29:2709-2716.

Test Name

NPM1 MRD Assay

Assay Type

Next-Generation Sequencing (NGS)

CLIA-validated assay

Method Description

To track and identify previously detected *NPM1* mutations in post-treatment follow-up samples, a multiplex master mix targeting exon 12 on the *NPM1* gene is used to amplify DNA extracted from a patient sample. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified mutations detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at an allelic sensitivity level of $5x10^{-5}$.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether *NPM1* MRD was detected	7 to 10 business days	• 1-3 mL of peripheral blood in EDTA, • 0.25-1 mL of bone marrow in EDTA • 1 µg of previously isolated DNA	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

IGH MRD Clonality Assays

Background

Combinations of chemotherapy, radiation therapy and bone marrow transplantation are potentially curative for several hematologic malignancies. However, in some patients, occult tumor cells exist and are thought to increase the patient's risk of relapse.[1] These subclinical levels of residual leukemia are termed minimal residual disease (MRD) and can be evaluated using more sensitive assays.

The tracking of antigen-receptor gene rearrangements for clonality analyses and MRD monitoring can be applied to virtually all patients. During early B-cell development, the germline variable (V_H), diverse (D_H), and joining (J_H) fragments of the human immunoglobulin heavy chain (*IGH*) locus become rearranged through the random deletion or insertion of nucleotides within the junctional region, generating specific and unique sequences within each lymphocyte. Cancer cells that arise from alterations in single lymphoid precursors acquire clonal *IGH* junctional regions, which can be used as tumor-specific markers.[2-3]

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

References

1. Rezuke WN et al. (1997) Molecular diagnosis of B- and T-cell lymphomas: fundamental principles and clinical applications. Clin Chem 43:1814-23.
2. Gazzola A et al. (2014) The evolution of clonality testing in the diagnosis and monitoring of hematological malignancies. Ther Adv Hematol. 5:35-47.
3. González D et al. (2007) Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood 110:3112-21.

Test Name

IGH FR1 MRD Clonality Assay
IGH FR2 MRD Clonality Assay
IGH FR3 MRD Clonality Assay
IGHV Leader MRD Somatic Hypermutation Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)
For Research Use Only

This test is performed by using the LymphoTrack® Assay from Invivoscribe. Data is analyzed using the LymphoTrack® MRD Software (RUO).

Method Description

To track and identify previously detected *IGH* clonal sequences in post-treatment follow-up samples, a multiplex master mix targeting the conserved framework region 1, framework region 2, or framework region 3, and the joining region is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified clonal rearrangements detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at MRD levels up to 1 x 10^{-6} with sufficient DNA input.

The assay typically requires a sample taken at diagnosis as well as the post-treatment follow-up samples. If the patient has previously been tested by LabPMM for *IGH* clonality, no diagnostic sample is needed.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether *IGH* MRD was detected	5 to 14 business days	• 1-3 mL of peripheral blood in EDTA • 0.25-1 mL of bone marrow in EDTA • 700-3500 ng of previously isolated DNA depending on level of sensitivity required	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

IGK MRD Clonality Assay

Background

Combinations of chemotherapy, radiation therapy and bone marrow transplantation are potentially curative for several hematologic malignancies. However, in some patients, occult tumor cells exist and are thought to increase the patient's risk of relapse.[1] These subclinical levels of residual leukemia are known as minimal residual disease (MRD), and can be evaluated using sensitive assays.

The tracking of antigen-receptor gene rearrangements for clonality analyses and MRD monitoring can be applied to virtually all patients. During early B-cell development, the germline variable (V_κ), constant (C_κ), and joining (J_κ) fragments of the immunoglobulin kappa (*IGK*) locus become rearranged through the random deletion or insertion of nucleotides within the junctional region, generating specific and unique sequences within each lymphocyte. Cancer cells that arise from alterations in single lymphoid precursors acquire clonal *IGK* junctional regions which can be used as tumor-specific markers.[2-3]

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

References

1. Rezuke WN et al. (1997) Molecular diagnosis of B- and T-cell lymphomas: fundamental principles and clinical applications. Clin Chem 43:1814-23.
2. Gazzola A et al. (2014) The evolution of clonality testing in the diagnosis and monitoring of hematological malignancies. Ther Adv Hematol. 5:35-47.
3. González D et al. (2007) Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood 110:3112-21.

Test Name

IGK MRD Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)

For Research Use Only

This test is performed by using the LymphoTrack® Assay from Invivoscribe. Data is analyzed using the LymphoTrack® MRD Software (RUO).

Method Description

To track and identify previously detected *IGK* clonal sequences in post-treatment follow-up samples, a multiplex master mix targeting the conserved V_k, J_k, C_k and kappa deleting element (K_{de}) regions is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified clonal rearrangements detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at MRD levels up to 1×10^{-6} with sufficient DNA input.

The assay typically requires a sample taken at diagnosis as well as the post-treatment follow-up samples. If the patient has previously been tested by LabPMM for *IGK* clonality, no diagnostic sample is needed.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether *IGK* MRD was detected	5 to 14 business days	• 1-3 mL of peripheral blood in EDTA • 0.25-1 mL of bone marrow in EDTA • 700-3500 ng of previously isolated DNA depending on level of sensitivity required	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

TRB MRD Clonality Assay

Background

Combinations of chemotherapy, radiation therapy and bone marrow transplantation are potentially curative for several hematologic malignancies. However, in some patients, occult tumor cells exist and are thought to increase the patient's risk of relapse.[1] These subclinical levels of residual leukemia are termed minimal residual disease (MRD) and can be evaluated using sensitive assays.

The tracking of antigen-receptor gene rearrangements for clonality analyses and MRD monitoring can be applied to virtually all patients. During early T-cell development, the germline variable (V_β), diversity (D_β), and joining (J_β) fragments of the T-cell receptor beta (*TRB*) locus become rearranged through the random deletion or insertion of nucleotides within the junctional region, generating specific and unique sequences within each lymphocyte. Cancer cells that arise from alterations in single lymphoid precursors acquire clonal *TRB* junctional regions which can be used as tumor-specific markers.[2,3]

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

References

1. Rezuke, W.N. et al. (1997) Molecular diagnosis of B- and T-cell lymphomas: fundamental principles and clinical applications. Clin Chem 43:1814-23.
2. Gazzola, A. et al. (2014) The evolution of clonality testing in the diagnosis and monitoring of hematological malignancies. Ther Adv Hematol. 5:35-47.
3. González, D. et al. (2007) Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood 110:3112-21

Test Name

TRB MRD Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)
For Research Use Only

This test is performed by using the LymphoTrack® Assay from Invivoscribe. Data is analyzed using the LymphoTrack® MRD Software (RUO).

Method Description

To track and identify previously detected *TRB* clonal sequences in post-treatment follow-up samples, a multiplex master mix targeting the $V_ß$, $J_ß$ and $D_ß$ regions is used for PCR amplification. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified clonal rearrangements detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at MRD levels up to 1×10^{-6} with sufficient DNA input.

The assay typically requires a sample taken at diagnosis as well as the post-treatment follow-up samples. If the patient has previously been tested by LabPMM for *TRB* clonality, no diagnostic sample is needed.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether *TRB* MRD was detected	5 to 14 business days	• 1-3 mL of peripheral blood in EDTA • 0.25-1 mL of bone marrow in EDTA • 700-3500 ng of previously isolated DNA depending on level of sensitivity required	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

TRG MRD Clonality Assay

Background

Combinations of chemotherapy, radiation therapy and bone marrow transplantation are potentially curative for several hematologic malignancies. However, in some patients, occult tumor cells exist and are thought to increase the patient's risk of relapse.[1] These subclinical levels of residual leukemia are termed minimal residual disease (MRD) and can be evaluated using sensitive assays.

The tracking of antigen-receptor gene rearrangements for clonality analyses and MRD monitoring can be applied to virtually all patients. During early T-cell development, the germline variable (Vγ), constant (Cγ), and joining (Jγ) fragments of the T Cell Receptor Gamma (*TRG*) locus become rearranged through the random deletion or insertion of nucleotides within the junctional region, generating specific and unique sequences within each lymphocyte. Cancer cells that arise from alterations in single lymphoid precursors acquire clonal *TRG* junctional regions which can be used as tumor-specific markers.[2,3]

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

References

1. Rezuke WN et al. (1997) Molecular diagnosis of B- and T-cell lymphomas: fundamental principles and clinical applications. Clin Chem 43:1814-23.
2. Gazzola A et al. (2014) The evolution of clonality testing in the diagnosis and monitoring of hematological malignancies. Ther Adv Hematol. 5:35-47.
3. González D et al. (2007) Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. Blood 110:3112-21.

Test Name

TRG MRD Clonality Assay

Assay Type

Next-Generation Sequencing (NGS)

For Research Use Only

This test is performed by using the LymphoTrack® Assay from Invivoscribe. Data is analyzed using the LymphoTrack® MRD Software (RUO).

Method Description

To track and identify previously detected *TRG* clonal sequences in post-treatment follow-up samples, a multiplex master mix targeting the Vγ and the Jγ region is used for PCR amplification.

Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified clonal rearrangements detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at MRD levels up to 1×10^{-6} with sufficient DNA input.

The assay typically requires a sample taken at diagnosis as well as the post-treatment follow-up samples. If the patient has previously been tested by LabPMM for *TRG* clonality, no diagnostic sample is needed.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating whether *TRG* MRD was detected	5 to 14 business days	• 1-3 mL of peripheral blood in EDTA • 0.25-1 mL of bone marrow in EDTA • 700-3500 ng of previously isolated DNA depending on level of sensitivity required	Ambient or Cool; do not freeze (peripheral blood or bone marrow) Ambient or frozen on dry ice (isolated DNA)	2-8 °C up to 7 days prior to testing

NGS CANCER PANELS

NGS Cancer Panels

Cytogenetic identification of chromosome abnormalities has become essential for the clinical management of patients with leukemia, and it is currently used to help classify patients into risk groups. With the development of novel genomics technologies, such as Next-Generation Sequencing, numerous new mutations and gene expression signatures have been identified. These breakthroughs allow us to better understand the molecular heterogeneity of hematologic diseases and to better stratify and assess risk for cancer patients.

Using these molecular tools, it has become evident that leukemias, lymphomas, and hematologic diseases are characterized by a remarkable amount of genetic heterogeneity, with individual patients presenting distinct and almost unique combinations of chromosome changes and somatically-acquired gene mutations.

LabPMM offers comprehensive NGS gene panels for AML and other hematological malignancies. Our MyAML® cancer panel is designed to analyze and interpret sequence information in genes known or suspected to be involved in AML and other hematologic diseases. This comprehensive assay is capable of detecting single nucleotide substitutions, insertions, deletions, and gene rearrangements.

Our MyMRD® panel was designed to sensitively capture all classes of variants identified in a precisely defined set of targets that commonly drive myeloid malignancies including AML, MPN, and MDS .

Our MyAML® and MyMRD® cancer panels are aimed at promoting a broader understanding of patients' clinical responses and outcomes. Panels run at the time of diagnosis identify both clinically-actionable driver mutations associated with the primary tumor, as well as the subclonal architecture that may be present. Temporal specimens collected and tested during the course of treatment identify the loss or elimination of driver mutations, as well as emergence or re-emergence of new clones and new potential therapeutic targets.

MyAML®

Clinical Information

Understanding the clonal architecture of AML patients is vital for successful treatments.[1] Many different mutations, epigenetic aberrations, or downstream abnormalities can generate the same clinical treatment plan. However, these differences are responsible for the variable responses observed with therapy, which is a major feature in patients with AML.[2] Therefore, since varied somatic mutations affect patient outcomes, conventional genotyping is no longer the most suitable method for screening patients.

MyAML is a CLIA-validated assay that identifies clinically actionable, pathogenic, and potentially pathogenic mutations in 194 genes associated with AML. Using the latest version in Next-Generation Sequencing chemistry, MyAML identifies all somatic mutations, large and small insertions/deletion, and translocations under NCCN/ELN guidelines, as well as novel somatic variants that may have prognostic significance for AML.

Screening with MyAML allows informed treatment decisions to be made once all the relevant mutations are known, both in the prevalent clones, as well as the 'secondary' or 'tertiary' clones, which could become the new prominent clones leading to refractory and relapsed disease.

List of Genes on the MyAML Panel

Structural Rearrangements

Inv(16) t(16;16) t(8;21) t(15;17) t(9;11) inv(3) t(3;3) t(6;9) t(9;22)

Genes

CEBPA DNMT3A FLT3 IDH1 IDH2 KIT NPM1

Other Fusions and Gene Rearrangements

ABL1 ADGRG7 AFF1 BCR CBFB CREBBP DEK EIF4E2 ELL ETV6 GAS6 GAS7 KAT6A KAT6B KMT2A MECOM MKL1 MLLT10 MLLT1 MLLT3 MLLT4 MYH11 NSD1 NUP214 NUP98 PICALM PML RARA RBM15 RPN1 RUNX1 RUNX1T1 SEPT5 SET TFG TMEM255B

Other Genes

ABCC1 ACVR2B ADRBK1 AKAP13 ANKRD24 ARID2 ARID4B ASXL1 ASXL2 ASXL3 BCOR BCORL1 BRINP3 BRPF1 BUB1 CACNA1E CBL CBX5 CBX7 CDC73 CEP164 CPNE3 CSF1R CSTF2T CTCF CYLD DCLK1 DDX1 DDX23 DHX32 DIS3 DNAH9 DNMT1 DNMT3B DYRK4 EED EGFR EP300 EPHA2 EPHA3 ETV3 EZH2 FANCC GATA1 GATA2 GFI1 GLI1 HDAC2 HDAC3 HNRNPK HRAS IKZF1 JAK1 JAK2 JAK3 JMJD1C KDM2B KDM3B KDM6A KDM6B KMT2B KMT2C KRAS MAPK1 METTL3 MST1R MTA2 MTOR MXRA5 MYB MYC MYLK2 MYO3A NF1 NOTCH1 NOTCH2 NRAS NRK OBSCN PAPD5 PAX5 PDGFRA PDGFRB PDS5B PDSS2 PHF6 PKD1L2 PLRG1 POLR2A PRDM16 PRDM9 PRKCG PRPF3 PRPF40B PRPF8 PTEN PTPN11 PTPN14 PTPRT RAD21 RBBP4 RBMX RPS6KA6 SAP130 SCML2 SETBP1 SETD2 SF1 SF3A1 SF3B1 SMC1A SMC3 SMC5 SMG1 SNRNP200 SOS1 SPEN SRRM2 SRSF2 SRSF6 STAG2 STK32A STK33 STK36 SUDS3 SUMO2 SUPT5H SUZ12 TCF4 TET1 TET2 THRB TP53 TRA2B TRIO TTBK1 TYK2 TYW1 U2AF1 U2AF1L4 U2AF2 UBA3 WAC WAPAL WEE1 WNK3 WNK4 WT1 ZBTB33 ZBTB7B ZRSR2

References

1. Döhner K et al. (2014) Intermediate-risk acute myeloid leukemia therapy: current and future. Hematology Am Soc Hematol Educ Program 1,34-43.
2. Estey EH (2014) Acute myeloid leukemia: 2014 update on risk-stratification and management. Am J Hematol 89:1063-1081.

Test Name

MyAML - NGS Gene Panel Assay

Assay Type

Next-Generation Sequencing (NGS)

CLIA-validated assay

Method Description

Using proprietary design, the coding regions and potential genomic breakpoints within known somatic gene fusions are sequenced to an average depth of coverage of 1000x. By utilizing long read lengths, the assay accurately detects and characterizes the breakpoints of structural variants and gene fusions, often with single base-pair precision. In addition, these long reads enhance the ability to identify both the insertion site and DNA content of large internal tandem duplications. Coupling comprehensive gene coverage with enhanced depth of coverage, long read lengths, and the power of our robust annotation software and bioinformatics database, MyAML identifies the underlying somatic mutations that are present as low as 5% allelic frequency. The data and report include single base resolution of the genomic breakpoint and sequences of mutations, facilitating optimized treatment plans and longitudinal tracking of minimal residual disease.

A completed patient consent form must be submitted for each sample sent to LabPMM.

Indications for Testing

- At initial diagnosis of AML
- Stratifying risk for AML
- Monitor and evaluate for refractory and relapsed disease

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating the SNVs, indels, inversions and translocations identified	14 to 21 business days	• 3 mL of peripheral blood in Heparin, EDTA or ACD • 1 mL of bone marrow in Heparin, EDTA or ACD • Cell Pellets in cell culture media or buffered solutions without fixatives • 1 µg of purified, high quality genomic DNA	Ambient or Cool; do not freeze	• Room Temp up to 72 hours • 2-8 °C up to 7 days

MyMRD®

Clinical Information

Minimal residual disease (MRD) detection has proven to be useful in the clinical management of patients with leukemia and can facilitate the development of new therapies.

Patients with myeloid neoplasms are typically divided into different prognostic groups based upon both cytogenetics and traditional molecular profiles;[1] however, this may not reflect the heterogeneity of disease[2] that can be exploited using MRD assessment. Moreover, multiple sampling is not feasible for many patients and thus the development of a sensitive and reliable assay to detect several mutations within one sample represents a significant advancement in guiding treatment decisions.

The MyMRD is a hotspot panel that detects all classes of variants identified in a precisely defined set of targets that commonly drive myeloid malignancies including AML, MPN and MDS. It can detect SNVs, indels and translocations to the genomic base pair, yielding unparalleled precision and detection of low level mutations in patients. The MyMRD assay, detects at least one driver mutation in 90%–95% of all AMLs. This gene panel is validated to a $5x10^{-3}$ level of detection for all targeted sites.

List of Genes on the MyMRD Panel

SNV and Indel Targets in Genes (Exons) (23 genes)

ASXL1 BRAF CALR CEBPA CSF3R DNMT3A FLT3 IDH1 IDH2 JAK2 KIT KMT2A KRAS MPL MYH11 NPM1 NRAS PTPN11 RUNX1 SF3B1 SRSF2 TP53 ZRSR2

Structural Variants (Translocations and Partial Tandem Duplications in Intronic Structures)

CBFB–MYH11 KMT2A RUNX1–RUNX1T1

References

1. Arber, DA et al. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, 127(20):2391–2405.
2. Sperling, AS et al. (2017). The genetics of myelodysplastic syndrome: from clonal hematopoiesis to secondary leukemia. Nature Reviews. Cancer, 17(1):5–19.

Test Name

MyMRD - NGS Gene Panel Assay

Assay Type

Next-Generation Sequencing (NGS)

CLIA-validated assay

Method Description

Indexed whole-genome libraries are hybridized with MyMRD probes targeting mutation hotspots in a total of 23 genes (*ASXL1 BRAF CALR CEBPA CSF3R DNMT3A FLT3 IDH1 IDH2 JAK2 KIT KRAS MPL NPM1 NRAS PTPN11 RUNX1 SF3B1 SRSF2 TP53 ZRSR2 CBFB-MYH11 KMT2A RUNX1-RUNX1T1*). In addition to targeting single nucleotide variants (SNVs) and indels in the first 21 genes, 5 structural variant breakpoints within the final 3 genes are also targeted. Coupling comprehensive gene coverage with enhanced depth of coverage, long read lengths, and the power of our robust MyInformatics® annotation software and bioinformatics database, MyMRD confidently and reproducibly detects mutations with a mutant allele frequency of $5x10^{-3}$, while some mutations, such as *FLT3* ITDs, are detected at mutation allele frequencies as low at $1x10^{-3}$.

A completed patient consent form must be submitted for each sample sent to LabPMM.

Indications for Testing

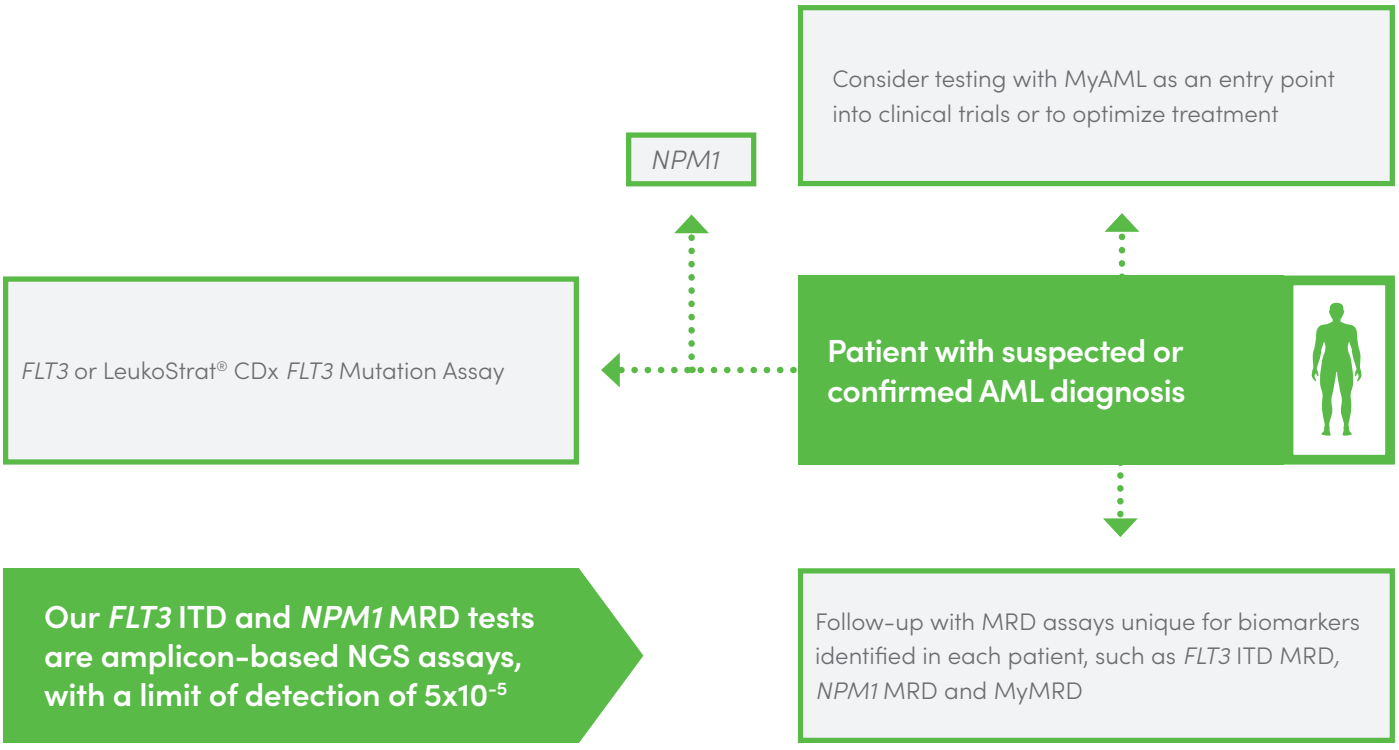
- Identify tumor-specific markers for post-treatment monitoring
- Monitor and evaluate for refractory and relapsed disease

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued indicating the detected pathogenic mutations and their frequencies in the interrogated sample.	14 to 21 business days	• 3 mL of peripheral blood in Heparin, EDTA or ACD • 1 mL of bone marrow in Heparin, EDTA or ACD • 1 µg of purified, high quality genomic DNA	Ambient or Cool; do not freeze	• Room Temp up to 72 hours • 2-8 °C up to 7 days

LabPMM Assay Guidelines

MyAML®

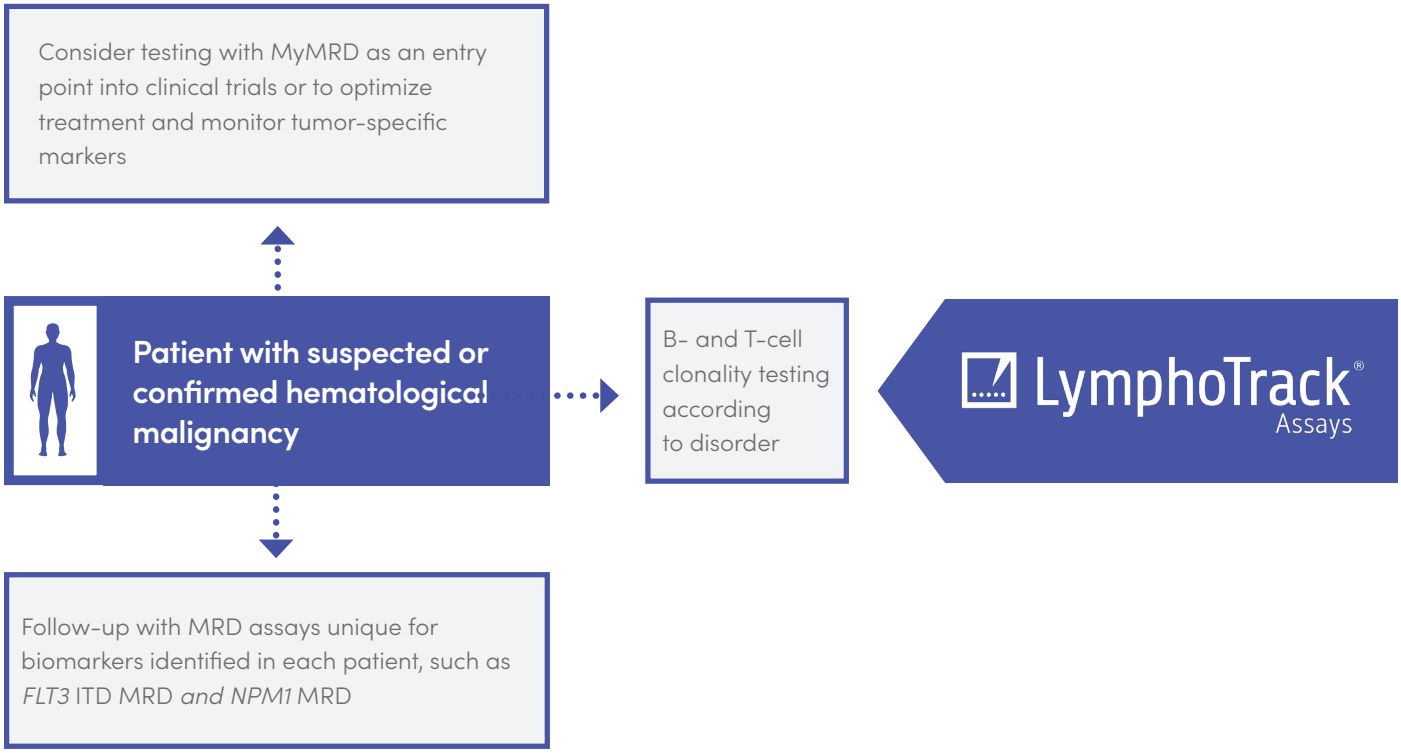
MyAML is a targeted gene panel that analyzes the coding and non-coding exons of nearly 200 genes, as well as the breakpoint hotspots within 36 genes. MyAML combines long read chemistry and deep sequencing with an optimized and validated custom bioinformatics pipeline, MyInformatics®, to specifically examine genomic variants in AML patients.



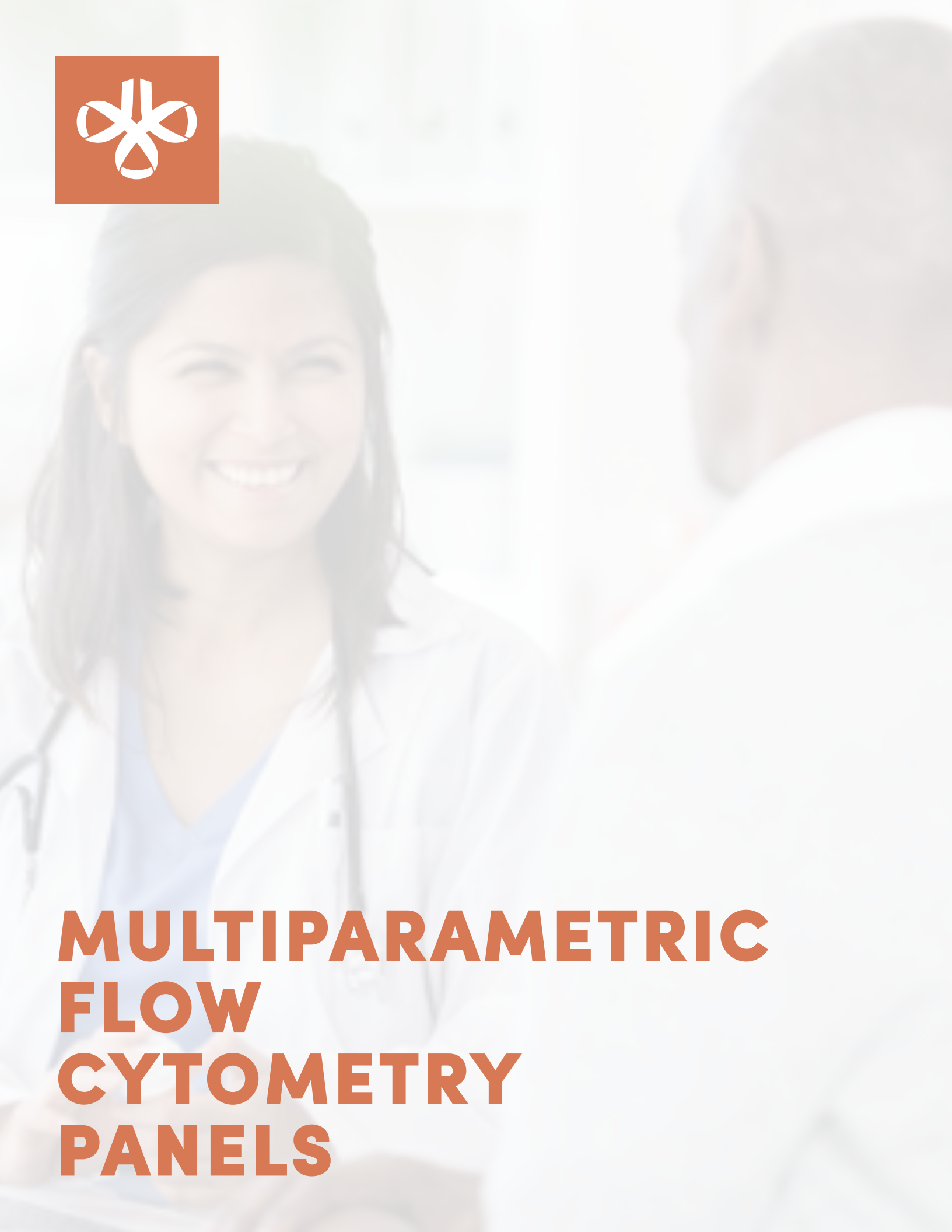
MyAML and MyMRD are CLIA validated assays.

MyMRD®

The MyMRD is a NGS-based panel that assesses variants in hotspots from 23 genes that commonly drive myeloid malignancies, including AML, MPN and MDS. It can detect SNVs, indels, and translocations to the genomic basepair, offering unparalleled precision and detection of low level mutations in patients. The MyMRD assay detects at least one driver mutation in 90%-95% of all AMLs.



MyMRD is a CLIA validated assay.



MULTIPARAMETRIC FLOW CYTOMETRY PANELS

Multiparametric Flow Cytometry Panels

Multiparametric Flow Cytometry (MFC) is a diverse technology platform with many clinical applications. One application of MFC is the ability to evaluate individual cells in suspension for the presence and absence of specific antigens and can be used to characterize disease in a clinical setting. The technology plays a critical role in the evaluation of peripheral blood and bone marrow samples for screening, diagnosis, prognosis and classification of many hematolymphoid neoplasia, including AML. Immunophenotyping white blood cells allows for the identification of normal and abnormal cell phenotypes which can identify normal cell populations, lymphoid neoplasia, plasma cell dyscrasia, acute leukemia and other disease of various cell lineages. MFC in the clinical setting now allows for >12 parameters to be simultaneously measured and analyzed and can provide rapid and actionable results for clinical decision making.

Another application of MFC includes measurable residual disease (MRD) testing. MRD testing by MFC can detect disease levels down below 10^{-4} and can provide information about remission status, outcome prediction, early identification of relapse and as a potential surrogate end point to accelerate drug testing and approvals.

Hematolymphoid Screening Panel - Flow Cytometry

Clinical Information

The 10-color Hematolymphoid Screening Panel provides a comprehensive approach for evaluating bone marrow and peripheral blood samples for the presence or absence of hematolymphoid malignancies. The panel characterizes and identifies all major white blood cell lineages and identifies all major types of hematopoietic neoplasia. Biomarker selection follows the 2006 Bethesda Consensus[1] for immunophenotypic analysis of hematolymphoid neoplasia with additions chosen by our internal hematopathologist based on recent literature including the 2017 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.[2-8]

This panel not only provides evaluation and monitoring of patients with hematological malignancy but provides a wide array of applications due to its comprehensive biomarker selection.

Biomarkers in the Screening Panel

CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD23, CD33, CD34, CD38, CD45, CD56, CD57, CD64, CD71, CD117, CD123, HLA-DR, Kappa, Lambda, and *TCR* Gamma/Delta.

References

1. BL Wood et al., *Cytometry B Clin Cytom.* 72:S14-S22 (2007).
2. FE Craig et al., *Blood.* 111(8):3941-67 (2008).
3. BL Wood. *Arch Pathol Lab Med.* 130:680-90 (2006).
4. SH Swerdlow et al., WHO Classification of Tumours, Revised 4th Edition, Volume 2 (2017)
5. CLSI H43: Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells, 2nd Edition
6. JJ Van Dongen et al., *Leukemia.* 26(9):1908-75 (2012).
7. A Porwit and A Rajab. *Int. Jnl. Lab. Hem.,* 37:133-143 (2015).
8. Sheikholeslami et al., *Methods in Molecular Biology.* 378. 53-63. 10.1007/978-1-59745-323-3_4 (2007).

Test Name

Hematolymphoid Screening Panel

Assay Type

Multiparametric Flow Cytometry (10-color)

CAP/CLIA-validated

Method Description

Immunophenotyping-Lyse/Wash/Stain

Indications for Testing

- Classifying Acute Leukemia
- Diagnosing and Classifying B cell Disorders
- Evaluating T cell and NK disorders
- Evaluating Plasma Cell Dyscrasias

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will be issued for the interrogated sample indicating presence/absence of normal and abnormal cell populations and their associated immunophenotypic profile.	24-48 hours	2-4 mL of bone marrow or peripheral blood in EDTA or Sodium Heparin	Ambient or Cool; Do not freeze	Specimens should be stored at 2-8°C and must be received by the lab within 48 hours after draw

AML MRD Assay by MFC

Clinical Information

Measurable residual disease, also called "minimal residual disease" (MRD) refers to persistent leukemic cells in the blood or bone marrow of cancer patients during or after treatment. Undetected or untreated MRD is a main cause of cancer recurrence hence sensitive MRD tests are necessary for guiding optimal treatment programs and providing a prognostic indicator for risk stratification of treated patients.

Multi-parameter Flow Cytometry (MFC) is a widely accepted platform for assessing MRD in patients with Acute Myeloid Leukemia (AML) and can be performed within a short period of time. In our lab we utilize a comprehensive panel of antibody markers to characterize potential AML blast cells using a LAIP based different from normal (DfN) approach. This approach takes into account information from diagnosis, if available but also identifies aberrant cells that have differentiated from normal maturation without previous patient history.

Using a comprehensive selection of antibodies and a standardized panel across all testing points, MRD populations can be characterized and tracked down to 0.01% sensitivity. Utilizing up to 12 biomarkers per tube allows for the identification of more LAIPS with less sample than previously was available before.

This panel not only provides evaluation and monitoring of patients with hematological malignancy but provides a wide array of applications due to its comprehensive biomarker selection.

Biomarkers in AML MRD Panel

CD2, CD4, CD5, CD7, CD11b, CD13, CD14, CD15, CD16, CD19, CD33, CD34, CD36, CD38, CD45, CD 56, CD64, CD117, CD123, HLADR, 7AAD

References

1. Schuurhuis, Gerrit J et al. "Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party." *Blood* vol. 131,12 (2018): 1275-1291. doi:10.1182/blood-2017-09-801498NCCN

2. Wood BL. Acute Myeloid Leukemia Minimal Residual Disease Detection: The Difference from Normal Approach.Curr Protoc Cytom. 2020 Jun;93(1):e73. doi: 10.1002/cpcy.73.PMID: 32311834

3. Cloos J, Harris JR, Janssen JJWM, Kelder A, Huang F, Sijm G, Vonk M, Snel AN, Scheick JR, Scholten WJ, Carbaat-Ham J, Veldhuizen D, Hanekamp D, Oussoren-Brockhoff YJM, Kaspers GJL, Schuurhuis GJ, Sasser AK, Ossenkoppele G. Comprehensive Protocol to Sample and Process Bone Marrow for Measuring Measurable Residual Disease and Leukemic Stem Cells in Acute Myeloid Leukemia. J Vis Exp. 2018 Mar 5;(133):56386. doi: 10.3791/56386.PMID: 29553571

4. Short NJ, Ravandi F. How close are we to incorporating measurable residual disease into clinical practice for acute myeloid leukemia? Haematologica. 2019 Aug;104(8):1532-1541. doi: 10.3324/haematol.2018.208454. Epub 2019 Jul 4. PMID: 31273094; PMCID: PMC6669140.

5. Zeijlemaker W, Kelder A, Cloos J, Schuurhuis GJ. Immunophenotypic Detection of Measurable Residual (Stem Cell) Disease Using LAIP Approach in Acute Myeloid Leukemia.Curr Protoc Cytom. 2019 Dec;91(1):e66. doi: 10.1002/cpcy.66.PMID: 31763792 .

6. Dix, C.; Lo, T.-H.; Clark, G.; Abadir, E. Measurable Residual Disease in Acute Myeloid Leukemia Using Flow Cytometry: A Review of Where We Are and Where We Are Going. J. Clin. Med. 2020, 9, 1714

Test Name

AML MRD Assay by MFC

Assay Type

Multiparametric Flow Cytometry (12-color)

CAP/CLIA-validated

Method Description

12 color Multiparametric Flow Cytometry with a sensitivity of $1x10^{-4}$.

Indications for Testing

- Identify tumor-specific markers for post-treatment monitoring
- Monitor response to therapy
- Monitor and evaluate for disease relapse and recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Specimen Stability
An interpretive report will indicate the presence/absence of AML cell populations, level of detection in relation to the clinical cutoff, percent and number of aberrant myeloblasts and their associated immunophenotypic profile.	24-48 hours	3-5 mL of bone marrow in EDTA or Sodium Heparin	Ambient or Cool; Do not freeze	Specimens should be stored at 2-8°C and must be received by the lab within 48 hours after draw

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