Personalized Molecular Medicine®

2020 Services Catalog

25th Anniversary Edition

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 three clinical laboratories and one clinical trial support office 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 generate standardized and concordant test results worldwide.

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

LabPMM Laboratories are wholly-owned subsidiaries of Invivoscribe.

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 Munich, Germany. It is an ISO 15189 accredited international reference laboratory. CLIA/CAP accreditation is planned.

LabPMM 合同会社

Located in Tokyo, Japan and a licensed clinical lab.

Invivoscribe Diagnostic Technologies (Shanghai) Co., Ltd.

Located in Shangai, China. It is the newest Invivoscribe subsidiary, supporting clinical trial work in China.

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LabPMM Testing Services

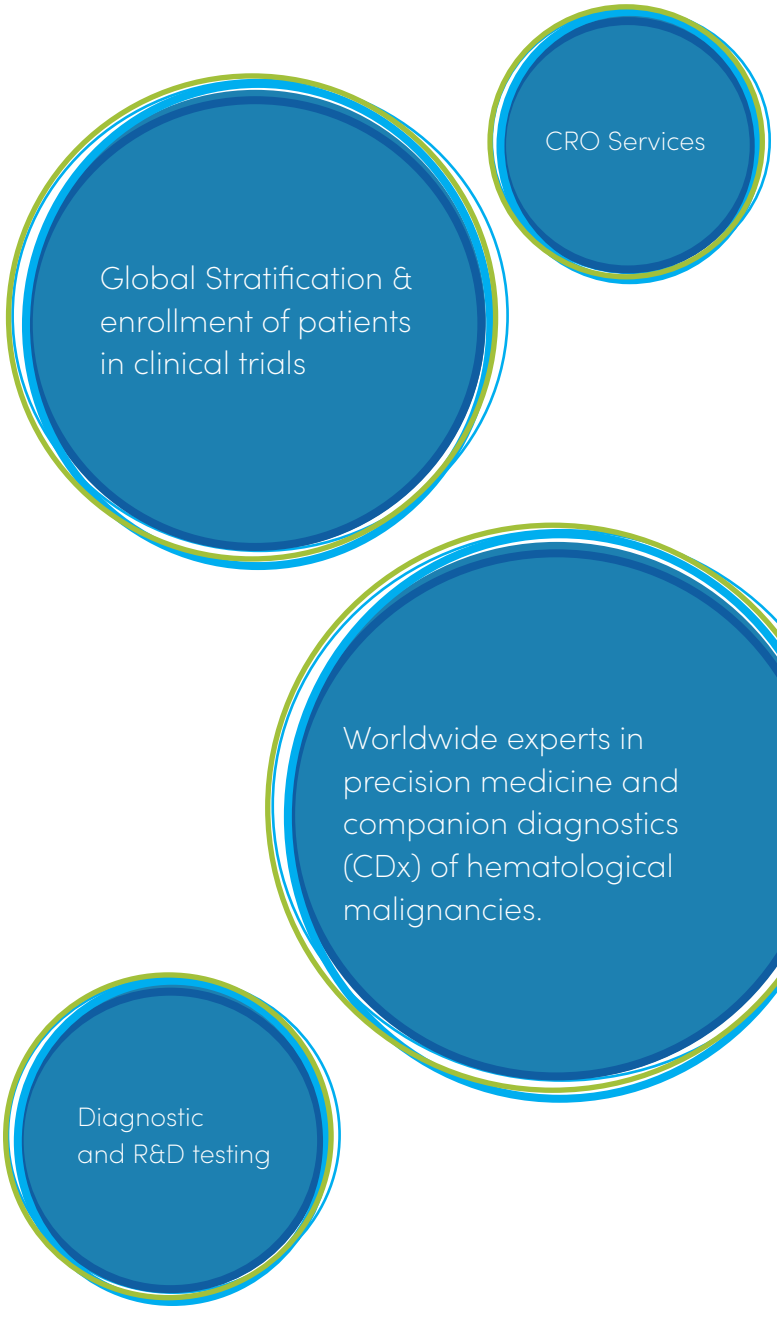
LabPMM offers efficient and reliable standardized tests that meet the highest quality and service standards.

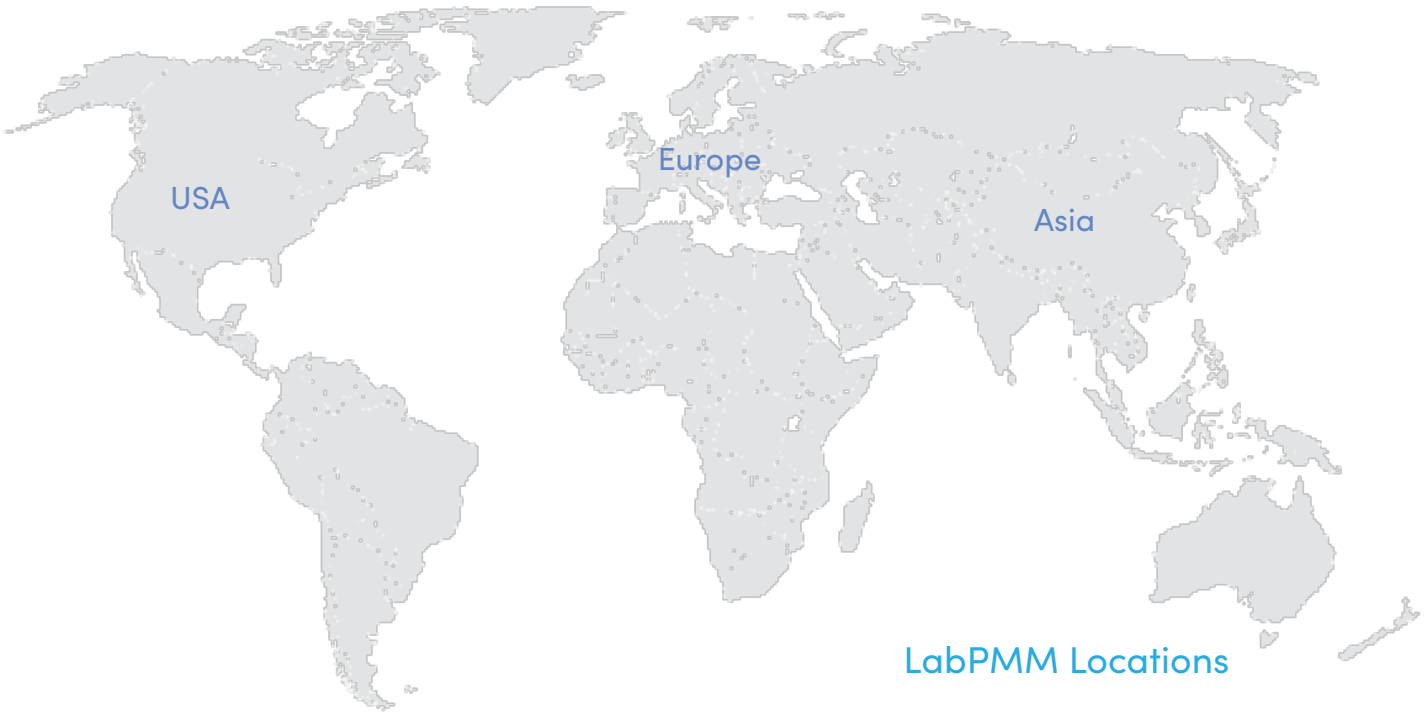
The Laboratories for Personalized Molecular Medicine (LabPMM) is the Invivoscribe® global network of clinical reference labs located in the USA, Europe and Asia, which provides international access to harmonized molecular testing.

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).

LabPMM 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 the only laboratory that offers internationally harmonized testing 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®.

Available services include:

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

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

Rapid turnaround times are vital to ensure that all testing results and information are in the hands of the physician so that timely treatment decisions can be made. 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. Testing at LabPMM expedites and streamlines patient care and clinical trial studies, as physicians receive results more quickly. 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.

How to Order a Test

Please contact your local LabPMM site in order to receive the necessary forms to initiate a services ordering account.

- Europe: info@labpmm.de
- Americas: support@labpmm.com
- Asia: services@labpmm.co.jp

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.

Blood and Bone Marrow Specimens for DNA and RNA Assays

Collect specimens in a Heparin, EDTA, or an ACD (acid citrate dextrose) tube. Specifically for *FLT3* ITD MRD and *NPM1* MRD, only samples collected EDTA and ACD are accepted. Minimum Volumes are 5 mL of peripheral blood or 3 mL of bone marrow. Blood and bone marrow may be stored at room temperature for up to 72 hours, or 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.

Previously Isolated DNA

Please ship previously isolated DNA at ambient temperature or with cool packs. Previously isolated DNA may be stored indefinitely at -65°C to -85°C.

CDx *FLT3* Mutation Assay

Only blood or bone marrow aspirate samples collected in sodium heparin tubes are accepted.

Japan Only: blood or bone marrow aspirate samples collected in EDTA tubes or Sodium heparin tubes are accepted.

Accredited Test Menu by Site Location

LabPMM GmbH
Europe/Middle East/Africa

DAkkS-accredited tests to ISO 15189

- CDx *FLT3*
- *FLT3* ITD
- *FLT3* ITD SR
- *FLT3* TKD
- *NPM1*
- *IGH*
- *IGK*
- *TRG*
- *TRB*

LabPMM LLC
Americas

CAP ISO 15189-accredited and CLIA-certified:

- CDx *FLT3*
- *FLT3* ITD MRD
- *NPM1*
- *NPM1* MRD
- MyAML
- MyMRD
- *IGH*
- *IGK*
- *TRG*
- *TRB*

New York State Licensed Tests:

- CDx *FLT3*
- *NPM1*

LabPMM GK
Asia/Pacific

Licensed Clinical Lab

- CDx *FLT3*

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, MyHEME 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. An U.S.-EU privacy shield certification is available. 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 collectively have CLIA, CAP, DAkkS, and ISO 15189 certifications. We also offer contract research organization (CRO) services, and are a comprehensive companion diagnostics (CDx) 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 molecular assays and reagents. Our global distribution network operates in more than 700 laboratories in 160 countries.

Expertise

With 25 years of experience we are the foremost experts in providing molecular products and services for leukemia and lymphoma 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

IVD Product Development

- 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 development

Quality/Regulatory/Commercialization
Global Regulatory 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 ISO 15189 labs serving the US, Europe, a locally accredited lab in Asia, including clinical trial management in China
- 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
  - PMA CDx for US, 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

Contact us for more information: businessdevelopment@invivoscribe.com

Streamlined CDx®

NOVARTIS

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

ASTELLAS

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

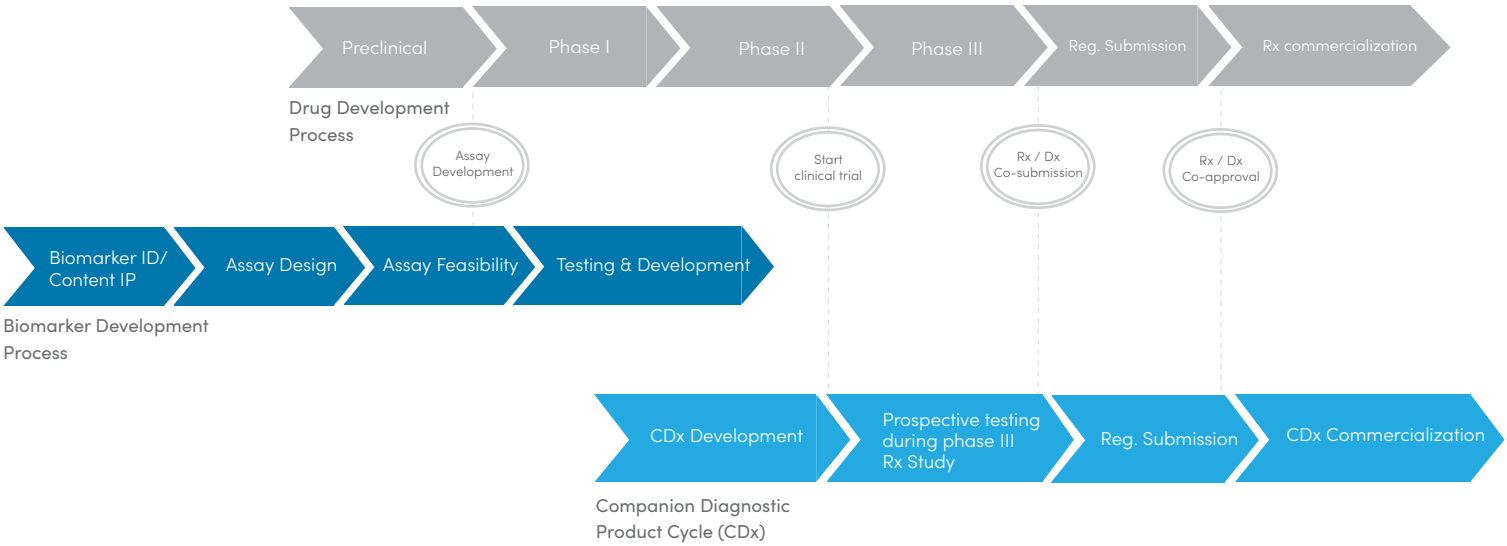
DAIICHI-SANKYO

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

Integrated Approach to CDx Development

We provide efficient handling of all stages of CDx development, from the biomarker discovery process and 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

- Develop plan in collaboration with partner
- Share market insights and collective knowledge
- Align Rx / Dx commercial objectives
- Define commercialization strategy and country level prioritization/timing
- Determine activities requiring partner support

Pre-Launch

- Identify and nurture key opinion leaders (KOLs)
- Align CDx development studies with commercialization studies
- Voice of customer to drive product requirements and commercialization considerations
- Prepare marketing message for PMA submission

Launch

- Establish lab network
- Create physician awareness
- Activate KOL network to start commercialization studies
- Complete HEOR studies, prepare HTAs, request reimbursement coverage
- Initiate PR and marketing campaigns

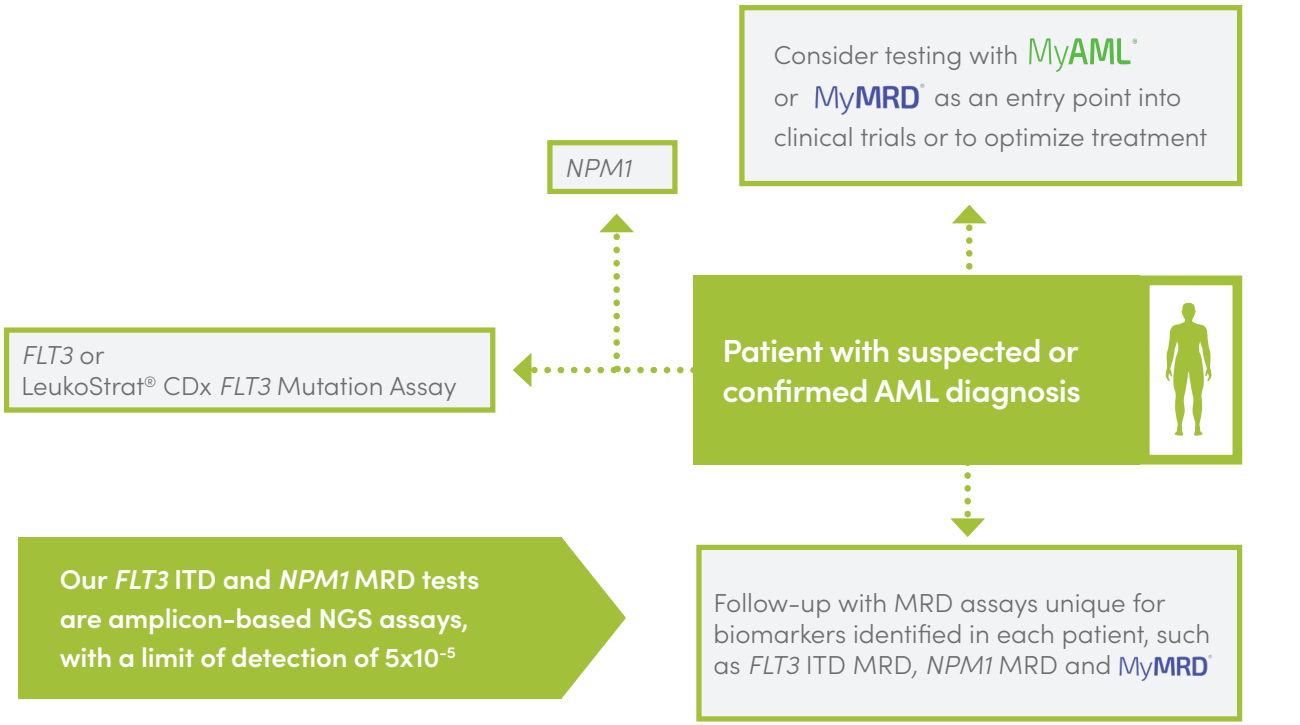
Post-Launch

- Expand lab network and physician awareness after reimburement established and guidelines updated
- Continue to nurture KOLs
- Monitor and adjust activities to achieve market adoption
- Transition to maintenance campaigns

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.



MyMRD®

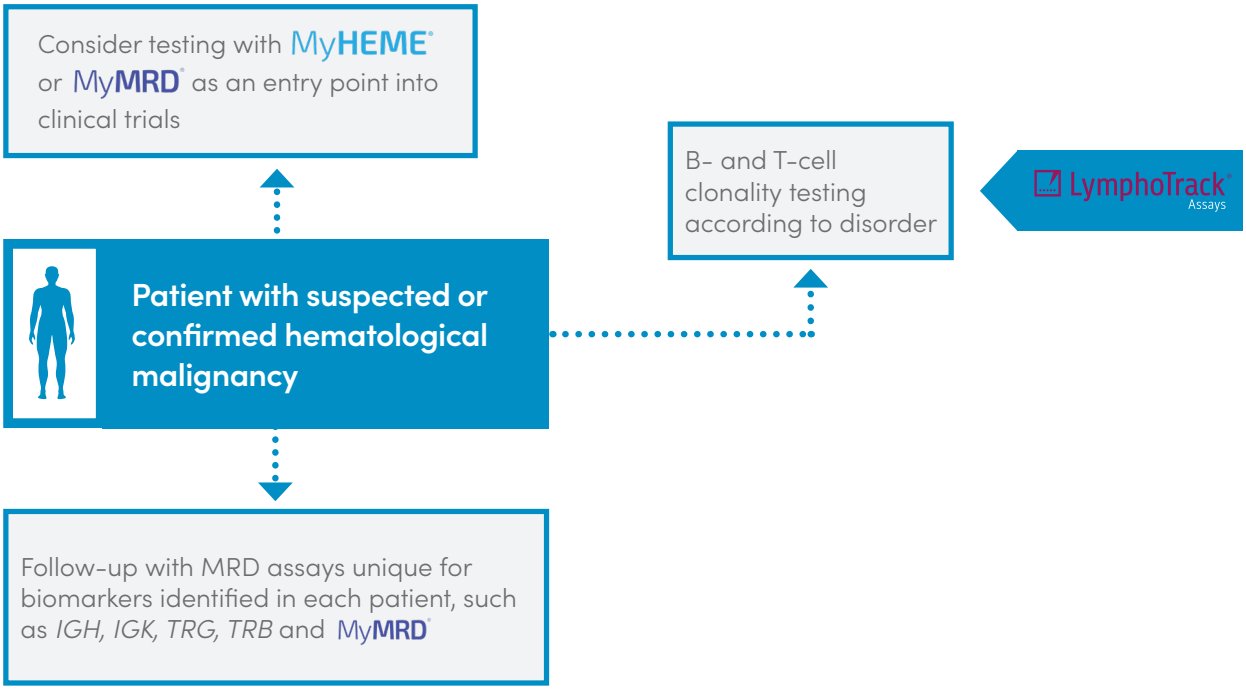
The MyMRD is a NGS-based panel that assesses variants in hotspots from 23 genes that commonly drive myeloid malignancies, including AML, MPN and MDS. It can detect SNVs, indels, and translocations to the genomic basepair, offering unparalleled precision and detection of low level mutations in patients.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

MyAML and MyMRD are CLIA validated assays.

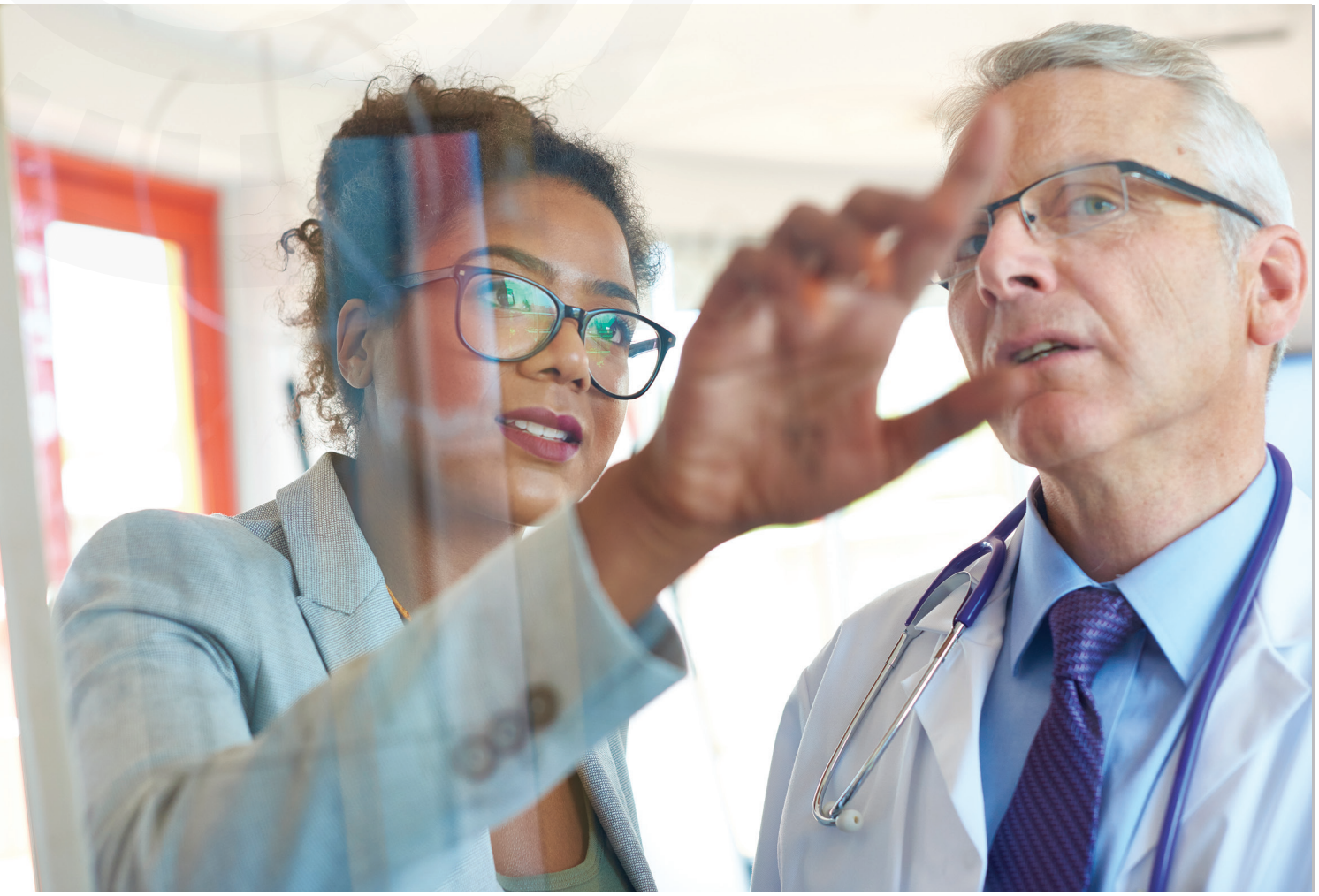
MyHEME®

For blood cancers other than AML, consider MyHEME.

The MyHEME NGS panel identifies clinically actionable, pathogenic, and potentially pathogenic mutations in more than 700 genes associated with hematologic malignancies.



MyHEME is Research Use Only (RUO).



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 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).

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

The LeukoStrat CDx *FLT3* Mutation Assay is to be performed only at Laboratory for Personalized Molecular Medicine (LabPMM) LLC, a single laboratory site, located at 10222 Barnes Canyon Rd., Bldg. 1, San Diego, CA 92121.

1. **Mutations in the *FLT3* gene are the most common mutations found in AML**
2. **Presence of a *FLT3* mutation in patients with AML is both highly prognostic and clinically actionable**
3. **Midostaurin was approved by the FDA for the treatment of adult patients with newly diagnosed AML who are *FLT3* mutation positive**
4. **The LeukoStrat® CDx *FLT3* Mutation Assay is the first FDA approved (PMA# P160040) predictive test for the efficacy of midostaurin therapy in all AML patients, regardless of cytogenetics**
5. **Gilteritinib fumarate was approved by the FDA for the treatment of adult patients with relapsed/refractory *FLT3*mut+ AML**
6. **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**

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 of AML
- As an aid in the selection 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	Storage Conditions
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	• 3 mL of peripheral blood in sodium heparin tubes only • 1 mL of bone marrow in sodium heparin tubes only	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* mutation in patients with AML is both highly prognostic and clinically actionable**
3. **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**
4. **Midostaurin was approved by the Swissmedic and European Commission for the treatment of adult patients with newly diagnosed AML who are *FLT3* mutation positive**
5. **Gilteritinib fumarate was approved by the European Commission for the treatment of relapsed/ refractory *FLT3*mut+ AML**

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

- 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	Storage Conditions
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	• 3 mL of peripheral blood in sodium heparin tubes only • 1 mL of bone marrow in sodium heparin tubes only	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* mutation in patients with AML is both highly 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**

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 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	Storage Conditions
An interpretive report will be issued, indicating whether the patient is eligible for gilterinib fumarate treatment or quizartinib hydrochloride	2 to 3 business days > 95% of patient samples reported within 48 hours of receipt	• 3 mL of peripheral blood in sodium heparin or EDTA tubes only • 1 mL of bone marrow in sodium heparin or EDTA tubes only	2°C to 8°C up to 72 hours; do not freeze.	2°C to 8°C up to 7 days

Molecular Diagnostic 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	Storage Conditions
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

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
IGHV Leader Somatic Hypermutation 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	Storage Conditions
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

IGH somatic hypermutation assay
IGHV leader 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	Storage Conditions
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	Storage Conditions
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_β) gene segments (belonging to 30 subgroups), 2 diversity (D_β) gene segments, and 13 joining (J_β) 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	Storage Conditions
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	Storage Conditions
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 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. Both of these methods do not generate results that meet the internationally recognized criteria for harmonization for a quantitative measure of residual disease, and neither 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 easy tracking of clonal populations at greater sensitivity, 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-enabled 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	Storage Conditions
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, Natteparin, or ACD • 0.25-1 mL of bone marrow in EDTA, Natteparin, or ACD • 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	Storage Conditions
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 Data Analysis Tool (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 10^{-6}.

The assay 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 diagnosic 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	Storage Conditions
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 Data Analysis Tool (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_{κ}, J_{κ}, C_{κ} 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 10^{-6}.

The assay 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 diagnosic 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	Storage Conditions
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 Data Analysis Tool (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_{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 previously identified clonal rearrangements detected at diagnosis. Bioinformatics tools facilitate the detection of these specific sequences present at MRD levels up to 10^{-6}.

The assay 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 diagnosic 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	Storage Conditions
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 Data Analysis Tool (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 10^{-6}.

The assay 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 diagnosic 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	Storage Conditions
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

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, respectively. 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®, MyMRD® and MyHEME® 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 recurrence.

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 temporal 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
- Recurrence of leukemia

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Storage Conditions
An interpretive report will be issued indicating the SNVs, indels, inversions and translocations identified	7 to 14 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 disease recurrence

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Storage Conditions
An interpretive report will be issued indicating the detected pathogenic mutations and their frequencies in the interrogated sample.	7 to 14 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

MyHEME®

Background

Hematologic diseases are characterized by aberrant gene expression often resulting from specific genetic translocations or mutations that lead to unregulated signal transduction. These types of alterations allow classification of leukemias and lymphomas into specific subgroups and frequently suggest treatment strategies[1].

The MyHEME identifies clinically actionable, pathogenic, and potentially pathogenic mutations in more than 700 unique genes associated with hematological malignancies. Using the latest version in Next-Generation Sequencing chemistry, MyHEME identifies all somatic mutations, large and small insertions/deletions and translocations under NCCN/ELN guidelines, as well as novel somatic variants that may have prognostic significance for hematologic diseases.

Screening with MyHEME allows for 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 recurrence.

List of Genes on the MyHEME Panel:

DNA Targets (571 genes)

ABI1 ABL1 ABL2 ACSL6 ACVR1C ACVR2B ADGRG7 ADNP ADRBK1 AFF1 AFF3 AFF4 AKAP13 AKT1 ALK ANKHD1 ANKRD26 ARAF ARHGAP26 ARHGEF12 ARID1A ARID1B ARID2 ARID4B ARID5B ARNT ASXL1 ASXL2 ASXL3 ATG2B ATIC ATM ATP2B3 ATR ATRX AXL B2M BAP1 BAX BAZ2A BCL10 BCL11A BCL11B BCL2 BCL3 BCL6 BCL7A BCL9 BCOR BCORL1 BCR BIRC3 BLM BMI1 BRAF BRCA1 BRCA2 BRD4 BRIP1 BRPF1 BTG1 BTK BUB1 C15orf65 CACNA1E CALR CAMK1G CAMKK1 CAMTA1 CARD11 CARS CASC5 CBFA2T3 CBFB CBL CBLB CBLC CBX5 CBX7 CCDC6 CCND1 CCND2 CCND3 CD274 CD70 CD79A CD79B CDC42EP1 CDH23 CDK11B CDK16 CDK6 CDKN1B CDKN2A CDKN2B CDKN2C CDX2 CEBPA CEBPE CHD2 CHIC2 CIITA CLP1 CLTC CLTCL1 CNOT3 CPNE3 CREBBP CRLF2 CSF1R CSF3R CSMD1 CSTF2T CTNNA1 CTNNB1 CUL1 CUX1 CXCR4 CYP1A1 DCLK1 DDR2 DDX1 DDX10 DDX11 DDX23 DDX3X DDX41 DDX6 DEK DHX15 DHX29 DHX32 DICER1 DIS3 DNAJC11 DNM2 DNMT3A DNMT3B DOT1L DTX1 DUSP2 DYRK4 ECT2L EED EGFR EGR1 EGR2 EIF4A2 EIF4E2 ELF4 ELL ELN EP300 EPHA10 EPHA2 EPHA3 EPOR EPS15 ERBB2 ERBB3 ERG ESCO2 ETNK1 ETV3 ETV6 EWSR1 EZH2 EZR FAM175A FAM46C FANCA FANCC FANCD2 FANCE FANCF FANCG FAS FAT3 FBXO11 FBXW7 FCGR2B FCRL4 FEV FGFR1 FGFR1OP FGFR2 FGFR3 FGFR4 FH FHIT FIP1L1 FLI1 FLT1 FLT3 FNBP1 FOXO1 FOXO3 FOXO4 FOXP1 FSIP2 FSTL3 FUBP1 FUS GAS6 GAS7 GATA1 GATA2 GATA3 GLI1 GMPS GNA13 GNAS GNB1 GPHN GSKIP HDAC1 HDAC2 HDAC3 HFE HIP1 HIST1H1B HIST1H1E HIST1H3B HIST1H4I HLF HLX HNRNPK HOXA11 HOXA13 HOXA9 HOXC11 HOXC13 HOXD11 HOXD13 HRAS HSP90AA1 HSP90AB1 ICE1 IDH1 IDH2 IKBKB IKZF1 IKZF2 IKZF3 IKZF4 IL15 IL2 IL21R IL7R INPP5D INTS12 IRAK1 IRF1 IRF4 IRF8 ITK ITPKB JAK1 JAK2 JAK3 JMJD1C KAT6A KAT6B KDM2B KDM3B KDM5A KDM6A KDM8 KDR KDSR KIT KLHDC8B KLHL6 KMT2A KMT2B KMT2C KMT2D KRAS LASP1 LCK LCP1 LMO1 LMO2 LPP LRIG3 LRP1B LTB LUC7L2 LYL1 MAF MAFB MALT1 MAP2K1 MAP3K1 MAP3K13 MAPK1 MAU2 MDM2 MDM4 MDS2 MECOM MED1 MED12 MEF2B MET METTL3 MIR142 MIR155 MKL1 MLF1 MLLT1 MLLT10 MLLT11 MLLT3 MLLT4 MLLT6 MN1 MNX1 MPL MSI2 MSN MST1R MTA2 MTCP1 MTOR MUC1 MUTYH MYB MYC MYD88 MYH11 MYH9 MYLK2 MYO3A NACA NBN NCKIPSD NCOA2 NF1 NF2 NFKB2 NFKBIE NIN NIPBL NKAP NLRP2 NOTCH1 NOTCH2 NPM1 NRAS NRG1 NRK NSD1 NT5C2 NUMA1 NUP214 NUP98 NXF1 OLIG2 P2RY8 PAFAH1B2 PAK1 PALB2 PAX5 PBRM1 PBX1 PCLO PCM1 PCSK7 PDCD1LG2 PDE4B PDE4DIP PDGFB PDGFRA PDGFRB PDS5A PDS5B PEG3 PER1 PHF6 PICALM PIGA PIK3CA PIK3CD PIM1 PIP4K2A PLCG1 PLRG1 PML POLR2A POLR3B POT1 POU2AF1 POU2F2 PRDM1 PRDM16 PRDM9 PRF1 PRKCG PRKD3 PRPF3 PRPF8 PRRX1 PSIP1 PTCH1 PTEN PTPN11 PTPN14 PTPN2 PTPN5 PTPN6 PTPRC PTPRF PTPRT PYGL RABEP1 RAD21 RALGDS RANBP17 RAP1GDS1 RARA RASA2 RBBP4 RBM15 RBMX REC8 REL RELN RET RHOA RHOH RMI2 RNF213 RNF217-AS1 ROBO1 RPL10 RPL22 RPL5 RPN1 RPS14 RPS15 RPS2 RPS6KA6 RPS6KB2 RUNX1 RUNX1T1 S1PR2 SAMHD1 SAP130 SBDS SCML2 SEPT5 SEPT6 SEPT9 SET SETBP1 SETD1A SETD2 SETDB1 SF3B1 SH2B3 SH2D1A SH3GL1 SKIV2L2 SMARCA2 SMARCA4 SMARCB1 SMC1A SMC3 SMC5 SMG1 SMO SNRNP200 SNX29 SNX7 SOCS1 SOS1 SP140 SPECC1 SPEN SPI1 SPOP SRP72 SRRM2 SRSF2 SRSF3 SRSF6 STAG1 STAG2 STAT3 STAT5B STAT6 STIL STK11 STK32A STK33 STK36 SUDS3 SUPT5H SUZ12 SYK SYNE1 TAF15 TAL1 TAL2 TBL1XR1 TCF3 TCL1A TCL6 TERC TERT TET1 TET2 TFG TFPT TFRC TGDS TLR2 TLX1 TLX3 TMEM255B TNF TNFAIP3 TNFRSF14 TNFRSF17 TOP1 TP53 TP63 TPM3 TPM4 TPMT TRA2B TRAF3 TRIM24 TRIO TRIP11 TSC1 TSC2 TTBK1 TTL TYK2 TYRO3 U2AF1 U2AF1L4 U2AF2 UBR5 VHL WAC WAPL WAS WEE1 WHSC1 WHSC1L1 WNK1 WNK3 WNK4 WT1 WWTR1 XPO1 XRCC1 ZBTB16 ZBTB33 ZBTB7B ZC3H18 ZMYM2 ZMYM3 ZNF292 ZNF384 ZNF471 ZNF521 ZRSR2

RNA Targets and Gene Fusions (371 fusions)

ABI1 ABL1 ABL2 ACER1 ACSL6 ADD3 ADGRG7 AFF1 AFF3 AFF4 AHI1 ALK ANKRD28 AP2A2 ARHGAP20 ARHGAP26 ARHGEF12 ARHGEF17 ARNT ASXL1 ATF7IP ATIC AUTS2 BAALC BACH2 BAZ2A BCL10 BCL11A BCL11B BCL2 BCL2L1 BCL3 BCL5 BCL6 BCL7A BCL9 BCOR BCR BIRC3 BRD1 BRWD3 BTBD18 BTG1 C15orf65 CAPRIN1 CARS CASC5 CBFA2T3 CBFB CBL CCDC6 CCDC88C CCND1 CCND2 CCND3 CD274 CDK5RAP2 CDK6 CDX2 CEBPA CEBPB CEBPD CEBPE CEP170B CEP85L CHD6 CHIC2 CHST15 CIITA CLCA2 CLP1 CLTC CLTCL1 CNTRL CPSF6 CREBBP CRLF2 CUX1 DAB2IP DACH1 DACH2 DDX10 DDX6 DEK DMRT1 DTD1 DUSP22 EEFSEC EIF4A2 ELF4 ELL ELN EML1 ENAH EP300 EPOR EPS15 ERC1 ERG ERVK-6 ERVW-1 ETS1 ETV6 EWSR1 FAM46C FCGR2B FCRL4 FEN1 FGFR1 FGFR1OP FGFR1OP2 FGFR3 FIP1L1 FLT3 FNBP1 FOXO3 FOXO4 FOXP1 FRA7H FRYL FSTL3 FUS GAPDH GAS5 GAS6 GAS7 GATA1 GIT2 GLIS2 GMPS GOLGA4 GOLGA6A GOT1 GPHN GPR34 GRHPR HIP1 HIPK1 HIST1H4I HLF HMGA2 HOXA10 HOXA11 HOXA13 HOXA9 HOXC11 HOXC13 HOXD11 HOXD13 HRASLS5 HSP90AA1 HSP90AB1 ID4 IGF2BP1 IGH IGK IGL IKZF1 IL2 IL21R IL3 IQCG IRF4 IRS4 ITK JAK2 JAK3 KANK1 KAT6A KAT6B KDM5A KDSR KIAA1524 KIAA1549L KIF5B KMT2A KRAS KRT18P6 LASP1 LCK LCP1 LHX2 LHX4 LMBRD1 LMO1 LMO2 LNP1 LOC100289656 LPP LPXN LRMP LYL1 LYN MACROD1 MAF MAFB MALT1 MAML2 MAP3K9 MAPRE1 MBNL1 MBTD1 MDS2 MECOM MIR29A MKL1 MLF1 MLLT1 MLLT10 MLLT11 MLLT3 MLLT4 MLLT6 MN1 MNX1 MSI2 MSN MTCP1 MUC1 MYB MYC MYH11 MYH9 MYO18A NACA NAPA NBEAP1 NCKIPSD NCOA2 NCOA3 NCOR1 NDE1 NEBL NF1 NFKB2 NID2 NIN NIPBL NKX2-5 NOP2 NOTCH1 NPM1 NSD1 NTRK3 NUMA1 NUP214 NUP98 OLIG2 P2RY8 PAFAH1B2 PAK1 PAX5 PBX1 PCM1 PCSK7 PDCD1LG2 PDE4DIP PDGFB PDGFRA PDGFRB PER1 PHF21B PHF23 PICALM PIM1 PLAG1 PML POM121 POU2AF1 PPP1CB PRDM1 PRDM16 PRKAR1A PRKG2 PRRX1 PRRX2 PSIP1 PSMD2 PTPRR PVT1 RABEP1 RALGDS RANBP17 RANBP2 RAP1GDS1 RARA RBM15 RCSD1 RHOA RHOH RMI2 RNF213 RNF217-AS1 RPL22 RPN1 RUNX1 RUNX1T1 SARNP SART3 SEC31A SEPT2 SEPT5 SEPT6 SEPT9 SET SETBP1 SFPQ SH3D19 SH3GL1 SLCO1B3 SNHG5 SNX29 SORBS2 SPECC1 SPTBN1 SQSTM1 SRSF3 SSBP2 ST6GAL1 STAT5B STIL STRN SYK TAF15 TAL1 TAL2 TAOK1 TCF3 TCL1A TCL6 TCTA TET1 TFG TFPT TFRC THADA TLX1 TLX3 TMEM255B TNFRSF17 TOP1 TP53BP1 TP63 TPM3 TPM4 TRA TRAF1 TRB TRD TRIM24 TRIP11 TRPS1 TTL TYK2 USP16 USP42 WHSC1 WHSC1L1 XBP1 YPEL5 YTHDF2 ZBTB16 ZFP64 ZFPM2 ZFYVE19 ZMIZ1 ZMYM2 ZMYND11 ZNF384 ZNF521 ZNF687

References

1. Gabrilove JL et al. (2001) Hematologic Malignancies: An Opportunity for Targeted Drug Therapy. Oncologist 6 Suppl 5:1-3.

Test Name

MyHEME - NGS gene panel assay

Assay Type

Next-Generation Sequencing (NGS)

For Research Use Only

Method Description

Using customized design, the coding and non-coding exons of 571 genes are sequenced to an average depth of coverage of 1000x. Long reads enhance the ability to identify indels, including large internal tandem duplications (ITDs). RNA sequencing of 371 genes identifies gene fusions and rearrangements, as well as provides gene expression information. Coupling comprehensive gene coverage with enhanced depth of coverage, long read lengths, and the power of our robust annotation software and bioinformatics database, MyHEME identifies the underlying somatic mutations that are present in as low as 5% allelic frequency. The data and report include sequences of mutations, which facilitates both minimal residual disease testing and temporal and longitudinal studies.

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

Indications for Testing

- Stratifying patients with hematologic disease
- Recurrence of leukemia or lymphoma

Interpretation	Turnaround Time	Specimen Requirements	Shipping Conditions	Storage Conditions
An interpretive report will be issued indicating the SNVs, indels, inversions and translocations identified	10 to 14 business days	**DNA** • 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 • Purified, high quality genomic DNA **RNA** • PAXgene tube for BMA or PBMC with 5 mL minimum blood volume	Ambient or Cool; do not freeze	• Room Temp up to 72 hours • 2-8 °C up to 7 days

Notes

2020 Services Catalog

25TH ANNIVERSARY EDITION

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