Dear Colleagues,

We are excited to offer you an innovative test for a clinical diagnosis of plasma cell neoplasms. The Pittsburgh Cytogenetic laboratory has completed the validation of a microarray-based technology that enables whole genome profiling of chromosome alterations in a population of purified CD138-positive plasma cells.

The aim of the Center for Clinical Genetics and Genomics is to advance clinical diagnosis and genetic practice for our patients by the application of the latest genomic methods. The launch of this new test is a step toward to the personalized medicine where genomics may offer new scope for preventing disease, offering targeted therapy, and promoting health.

As always, we welcome your thoughts, comments, and ideas for laboratory services and improvements.

Dr. Aleksandar Rajkovic
Director, Center for Clinical Genetics and Genomics

Background

Plasma cell myeloma is the most common type of plasma cell neoplasm characterized by specific chromosomal aberrations of diagnostic and prognostic significance. However, in routine bone marrow aspirate samples, plasma cells constitute from 1% to 15% of total cells in up to 70% of patients, particularly in the early disease stages. The low percentage of neoplastic cells is the major drawback in the application of traditional cytogenetic techniques such as classical karyotyping and fluorescence in situ hybridization (FISH) for the evaluation of plasma cell neoplasms. Therefore, it is important to use a purified population of plasma cell for cytogenetic studies.

Current cytogenetic testing

In 2016, the Pittsburgh Cytogenetics Laboratory implemented FISH testing on purified plasma cell samples that were separated from bone marrow samples using CD138+ magnetic beads. FISH on plasma cell enriched samples resulted in a significant improvement, raising the detection rate of chromosomal abnormalities from 36% to 68% in patients with plasma cell myeloma. Despite this improvement, there are a number of patients for whom a complete FISH panel analysis is problematic due to a limited number of isolated plasma cells. To overcome this problem, we developed a new array comparative genomic hybridization approach for the detection of chromosomal imbalances using samples containing as few as 10-20 plasma cells, while the balanced IGH and MYC gene rearrangements can be tested by FISH technique.

Microarray analysis in combination with IGH FISH

The new microarray analysis performed on DNA from CD138+ plasma cells will provide additional knowledge regarding chromosomal alterations helping in disease classification, risk stratification, and treatment selection. Microarray is both high-throughput and highly accurate and therefore, a superb new tool for evaluating cancer genomes. The directors and personnel of the Pittsburgh Cytogenetics Laboratory will provide you with a concise report and interpretation on microarray findings as well as results of concurrent FISH analysis for the detection of balanced IGH gene rearrangements. This microarray test will replace the former FISH panel and results will also be considered in light of clonal cytogenetics findings.

We are proud of our ongoing collaboration with the Division of Hematopathology that has resulted in the development of this innovative test. We look forward to integrating these new genomic technologies into routine clinical care for the benefit of our patients.

Steven Swerdlow, MD
Director, Division of Hematopathology, UPMC

Svetlana Yatsenko, MD
Director, Pittsburgh Cytogenetics Lab, UPMC

Dr. Aleksandar Rajkovic
Director, Center for Clinical Genetics and Genomics

OUR WEBSITE: HTTP://PITTGENOMICS.ORG/
### FISH PANEL ON PLASMA CELLS

<table>
<thead>
<tr>
<th>Hyperdiploidy</th>
<th>IGH rearrangement</th>
<th>IGH/CCND1</th>
<th>13q loss</th>
<th>1q gain</th>
<th>TP53 loss</th>
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<tbody>
<tr>
<td>Sample type:</td>
<td>CD138 positive plasma cells isolated from a bone marrow sample</td>
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<tr>
<td>Indications:</td>
<td>Plasma Cell Myeloma, MGUS</td>
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<tr>
<td>Regions tested:</td>
<td>9 loci - chr 5, 7, 9 for hyperdiploidy, IGH translocations, IGH/CCND gene fusion, 13q deletion/monosomy, 1p loss (CDKN2C), 1q gain (CKS1B), TP53 gene deletion</td>
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<td>Limitations:</td>
<td>Insufficient number of cells to complete all experiments (100-300 cells are required per locus)</td>
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### MICROARRAY ON CD138+ PLASMA CELLS + IGH FISH

| Sample type: | CD138+ purified plasma cells isolated from a bone marrow sample, 10-20 cells are sufficient for microarray analysis |
| Indications: | Suspected or definite diagnosis of Plasma Cell Myeloma, MGUS, Plasma Cell Leukemia |
| Regions tested: | Unbalanced alterations of all chromosomes |
| Advantages: | Requires very few plasma cells in a sample Detection of additional genomic alterations of prognostic and diagnostic significance |
| Limitations: | Balanced rearrangements involving IGH will require concurrent FISH studies |
| FISH studies: | Complementary IGH/CCND1, IGH/FGFR3, IGH/MAF, IGH/CCND3, IGH/MAFB fusion analysis in cases positive for IGH rearrangement, MYC rearrangements |
How to order Microarray testing on CD138+ plasma cells

1. Download the Oncology Cytogenetics Study Requisition form from our website www.pitgenetics.org/cytogenetics requisition forms/oncology analysis (http://www.pittgenetics.com/PDFfiles/Oncology%20Cytogenetics%20Requisition%20form.pdf).

2. Provide diagnosis such as plasma cell myeloma (MM) or other plasma cell neoplasms (specify).
   - It is crucial to indicate a suspected or previously diagnosed plasma cell myeloma for an optimal separation of CD138+ cells, which can be successfully accomplished within 72 hours upon a bone marrow sample collection.

3. Request “MM microarray with IGH & FISH” test.
   - FISH study to detect IGH rearrangement will be completed in parallel with microarray analysis.

3. Individual FISH testing can be ordered from a list of probes for a follow up study.

Cyto Lab Website: pittgenetics.org
MESSAGE FROM THE DIRECTOR OF CYTOGENETIC LABORATORY

I am very grateful to the people who played a crucial role in the development and implementation of this innovative test into clinical practice in our laboratory. Development of this test was started by our University of Pittsburgh undergraduate students Jonathan Ghobrial, Jocelyn George, and Shawn Griffiths who participated in this clinical research project through the First Experiences in Research Program. The promising results obtained by the students were expanded and validated by a group of talented colleagues and laboratory personnel. Our team embraced this project with extraordinary enthusiasm and professionalism, providing invaluable support, technical expertise, and insights at every stage of development. A project of this scale and complexity could not have been accomplished without a team of outstanding individuals working in the Pittsburgh Cytogenetics Laboratory; those who were directly involved in test development and those who by their daily work provided an opportunity for their colleagues to work on the implementation of this test.

I am proud of the success our team has achieved and the expertise we can offer to our clinicians and patients.

Svetlana Yatsenko, MD