LMP’s Second Anniversary: Looking Back and Looking Ahead

By: William A. Muller, MD, PhD, Magerstadt Professor and Chair of Pathology

This issue marks the end of the second year of Laboratory Medicine Pulse. This quarterly e-publication of the Department of Pathology is more than a newsletter. A newsletter reports recent events that are probably of not much interest to anyone outside the group that is writing it (and their mothers). In contrast, Laboratory Medicine Pulse is a compendium of articles that are meant to be interesting and instructive to our clinical colleagues. Pathology is at the interface of basic science and clinical medicine. Our articles describe the scientific basis and rationale behind the laboratory tests you order all the time, advice on which are the most appropriate for a given situation, and latest molecular genetic tests. For example the Spring issue of LMP included articles on screening for hepatitis C infection, appropriate testing for maternofetal hemorrhage, how to work up a thyroid nodule, and the pros and cons of using fasting plasma glucose vs. hemoglobin A1c for the diagnosis of diabetes. These are all things every practitioner should know, but may have forgotten. Links to LMP are published in Medical Staff Minute and are archived on the NM Interactive on the Medical Staff Minute site.

I want to thank the first two Editors-in-Chief, Drs. Ritu Nayar and Kalliopi Siziopikou, and the Editorial Board, whose names appear on the masthead, and for their dedication to this project and their hard work over the past two years. I want to thank all of the faculty, fellows, and residents who contributed such outstanding articles. And I especially want to thank Dr. Greg Retzinger, whose vision this was and who was instrumental in getting it off the ground. We are looking forward to many more informative issues in the coming years.
LMP’s Second Anniversary: Looking Back and Looking Ahead (contd.)

At the risk of making this read like a real newsletter, I would like to list a few of the many highlights that the Department of Pathology has celebrated over the past two years: We have grown our Diagnostic Molecular Pathology Lab. We have expanded our Cytogenetics Laboratory. We have successfully transitioned to subspecialty surgical pathology signout. We have mutually benefitted from the opening of Lurie Children’s Hospital. Members of the Department of Pathology have won too many grants and national awards to list here.

Looking forward, the future is bright. By the time you read this, the Diagnostic Molecular Pathology Lab will be offering test panels by Next Generation Sequencing. We hope to be testing Nanopore sequencing technology soon. The Laboratory Utilization Committee, which was recently reorganized in partnership with our clinical colleagues will work hard to ensure that we are performing the most appropriate and most cost-effective lab testing. We look forward to further expanding our outreach program to become a Reference Laboratory for the region in Anatomic as well as Clinical Pathology. We look forward to making the journey with you.

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Tumor Markers

By: Pawel Mroz, MD, PhD, Resident in Pathology

Tumor markers, which are substances detectable in body fluids and/or tissues of persons who have cancer, serve as surrogate indicators of malignant disease. Many are proteins or glycoproteins. Tumor markers can be used clinically to evaluate cancer risk, screen for early cancers, establish diagnosis, estimate prognosis, predict response to therapy, and/or monitor disease recurrence or progression. Some tumor markers facilitate more efficient use of a therapy by identifying those patients most likely to respond to that therapy. Alternatively, the same markers might be used to reduce therapy-related toxicities in patients who are less likely to benefit from a particular treatment.

In this era of molecular genetics, the definition of tumor marker has been broadened to include nucleic acids, a consequence of advances in molecular techniques that allow precise evaluation of genetic changes that occur in different neoplasms. Specific mutations, chromosomal translocations and fusion gene expressions have been identified in certain cancers. These genetic changes are being used for diagnosis, prognosis and treatment monitoring in the same manner as the more ‘traditional’ tumor markers, described above.
Tumor Markers (contd.)

To enhance the clinical reliability and utility of tumor markers, the following considerations are apropos:

1. The results of marker assays should be used specifically for a predetermined application, e.g., risk assessment, screening, diagnosis, prognosis, prediction, or post-treatment monitoring.
2. The results of marker assays should facilitate randomization of patients into separate groups/populations, the clinical prognosis/treatment outcomes of which are significantly different. (Such a difference allows a caregiver to change patient management accordingly.)
3. The estimate of the separation of outcomes for marker positive and marker negative individuals should be reliable.

Unfortunately, there is, as yet, no universal tumor marker that permits diagnosis/screening of all cancers. Many tumor markers, however, are shared by different tumor types and even normal tissues. Currently, over 20 clinically-applicable tumor markers have been identified and are used routinely in day-to-day oncology practice. These are summarized in the table, below (adapted from 1–4).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cancer type</th>
<th>Tissue</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK gene rearrangements</td>
<td>Non-small cell lung carcinoma</td>
<td>Tumor</td>
<td>Treatment, Prognosis</td>
</tr>
<tr>
<td></td>
<td>Anaplastic large cell lymphoma</td>
<td></td>
<td></td>
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<tr>
<td>α-fetoprotein</td>
<td>Hepatocellular carcinoma</td>
<td>Blood</td>
<td>Diagnosis, Prognosis, Treatment response</td>
</tr>
<tr>
<td></td>
<td>Germ cell tumors</td>
<td></td>
<td></td>
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<tr>
<td>β2-microglobulin</td>
<td>Multiple myeloma</td>
<td>Blood, Urine</td>
<td>Prognosis, Treatment response</td>
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<tr>
<td></td>
<td>Chronic lymphocytic leukemia</td>
<td>CSF</td>
<td></td>
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<tr>
<td></td>
<td>Lymphomas</td>
<td></td>
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<tr>
<td>β-human chorionic gonadotropin</td>
<td>Choriocarcinoma</td>
<td>Urine, Blood</td>
<td>Staging, Prognosis, Treatment response</td>
</tr>
<tr>
<td></td>
<td>Testicular tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCR-ABL fusion gene</td>
<td>Chronic myeloid leukemia</td>
<td>Blood, Bone marrow</td>
<td>Diagnosis, Disease monitoring</td>
</tr>
<tr>
<td>BRAF mutation V600E</td>
<td>Melanoma</td>
<td>Tumor tissue</td>
<td>Prognosis, Treatment response</td>
</tr>
<tr>
<td></td>
<td>Colorectal carcinoma</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Hairy cell leukemia</td>
<td></td>
<td></td>
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<tr>
<td>CA15-3/CA27.29</td>
<td>Breast carcinoma</td>
<td>Blood</td>
<td>Treatment response, Recurrence</td>
</tr>
<tr>
<td>CA19-9</td>
<td>Gastrointestinal tumors</td>
<td>Blood</td>
<td>Treatment response</td>
</tr>
<tr>
<td>CA-125</td>
<td>Ovarian carcinoma</td>
<td>Blood</td>
<td>Diagnosis, Treatment response, Recurrence</td>
</tr>
</tbody>
</table>
## Tumor Markers (contd.)

<table>
<thead>
<tr>
<th>Tumor Marker</th>
<th>Source Tissue</th>
<th>Type of Sample</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcitonin</td>
<td>Madullary thyroid carcinoma</td>
<td>Blood</td>
<td>Diagnosis Treatment response Recurrence</td>
</tr>
<tr>
<td>Carcinoma antigen</td>
<td>Colorectal carcinoma Breast carcinoma</td>
<td>Blood</td>
<td>Metastatic spread Treatment response</td>
</tr>
<tr>
<td>CD20</td>
<td>Non-Hodgkin lymphoma</td>
<td>Blood</td>
<td>Targeted therapy</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>Neuroendocrine tumors Blood Tumor tissue</td>
<td>Diagnosis Treatment response Recurrence</td>
<td></td>
</tr>
<tr>
<td>Chromosomes 3, 7, 17, 9p21</td>
<td>Bladder carcinoma Urine</td>
<td>Recurrence</td>
<td></td>
</tr>
<tr>
<td>EGFR mutation analysis</td>
<td>Lung adenocarcinoma Tumor tissue</td>
<td>Treatment Prognosis</td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor/ progesterone receptor</td>
<td>Breast carcinoma Tumor tissue</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Fibrin/fibrinogen</td>
<td>Bladder carcinoma Urine</td>
<td>Disease progression Treatment response</td>
<td></td>
</tr>
<tr>
<td>HE4</td>
<td>Ovarian carcinoma Blood</td>
<td>Progression Recurrence</td>
<td></td>
</tr>
<tr>
<td>HER2/neu</td>
<td>Breast carcinoma Gastric carcinoma Esophageal carcinoma Tumor tissue</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Multiple myeloma Waldenstrom macroglobulinemia Blood Urine</td>
<td>Diagnosis Treatment response Recurrence</td>
<td></td>
</tr>
<tr>
<td>cKIT/CD117</td>
<td>Gastrointestinal stromal tumor Tumor tissue</td>
<td>Diagnosis Treatment</td>
<td></td>
</tr>
<tr>
<td>KRAS mutation analysis</td>
<td>Lung carcinoma Colorectal carcinoma Tumor tissue</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Germ cell tumors Blood</td>
<td>Staging Prognosis Treatment response</td>
<td></td>
</tr>
<tr>
<td>Nuclear matrix protein 22</td>
<td>Bladder carcinoma Urine</td>
<td>Treatment response</td>
<td></td>
</tr>
<tr>
<td>Prostate-specific antigen</td>
<td>Prostate carcinoma Blood</td>
<td>Diagnosis Treatment response Recurrence</td>
<td></td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>Thyroid carcinoma Tumor tissue</td>
<td>Treatment response Recurrence</td>
<td></td>
</tr>
<tr>
<td>Urokinase plasminogen activator and plasminogen activator inhibitor-1</td>
<td>Breast carcinoma Tumor tissue</td>
<td>Prognosis Treatment</td>
<td></td>
</tr>
<tr>
<td>5-Protein signature (Ova1)</td>
<td>Ovarian carcinoma Blood</td>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>21-Gene signature (Oncotype DX)</td>
<td>Breast carcinoma Tumor tissue</td>
<td>Recurrence risk</td>
<td></td>
</tr>
<tr>
<td>70-Gene signature (Mammaprint)</td>
<td>Breast carcinoma Tumor tissue</td>
<td>Recurrence risk</td>
<td></td>
</tr>
</tbody>
</table>
Tumor Markers (contd.)


Acknowledgement: I would like to thank Dr. G. Retzinger, Professor of Pathology, Director of Anatomic and Clinical Pathology, Vice-Chair Department of Pathology, for his advice and help in the preparation of this article.

Specimen Collection and Turnaround Time

By: Celina Villa, MD Resident in Pathology
Gregory Retzinger, MD, PhD, Professor and Vice Chair of Pathology

The timeliness of the reporting of test results, i.e., turnaround time (TAT), is often used as a key indicator of laboratory performance. There is a difference, however, in the way TAT is perceived by clinicians and laboratory professionals (1–5). The 1998 CAP Q-Probes Program Survey revealed that many clinicians feel TAT begins when a test is ordered and ends when the test is resulted. Laboratory professionals, on the other hand, feel TAT begins when a sample is actually received by the lab and ends when the associated test is resulted (4). The difference in TAT perception stems from the challenge of monitoring the many steps involved in the generation of laboratory data, including ones not under the control of the laboratory. Nevertheless, the perceptual incongruence often leads to laboratory dissatisfaction by clinicians.

In order to address TAT goals, all aspects of the testing process need to be considered. Lundberg (6) defined the ‘total testing cycle’ as a series of steps including: ordering, collection, identification, transportation, preparation, analysis, reporting, interpretation, and action. The use of lab-associated phlebotomists initiates the laboratory phase of the testing cycle at the collection step. This phase can be easily tracked because lab-associated phlebotomists routinely label the specimen with both their initials and the time of
Specimen Collection and Turnaround Time (contd.)

sample collection (along with necessary patient identifiers). Specimens collected by non-phlebotomists, however, are not usually similarly labeled and, in addition, are often transported to the lab by non-lab couriers. Importantly, data for extra-laboratory phases of activities are not generally available. Thus, monitoring the time it takes to collect and transport specimens by non-phlebotomists is often exceedingly problematic, confounding TAT as perceived.

The NMH Clinical Laboratory advocates the proper labeling of specimens, to include the collection time and the initials of the person drawing the blood, along with necessary patient identifiers. These measures facilitate assessment of the actual time it takes to collect and transport specimens, and they also help identify unnecessary lag times that may lead to increased TAT. Inasmuch as long periods between obtaining and processing specimens are associated with deterioration of analytical integrity, improving specimen transport and delivery not only improves TAT but also decreases pre-analytical variation.


Acknowledgement: I would like to thank Dr. G. Retzinger, Professor of Pathology, Director of Anatomic and Clinical Pathology, Vice-Chair Department of Pathology, for his advice and help in the preparation of this article.

CALR analysis: Further insight into the mutational repertoire of myeloproliferative neoplasms

By: Rachel Mariani, MD, Fellow in Hematopathology
Juehua Gao, MD, PhD, Assistant Professor of Pathology

Chronic myeloproliferative neoplasms (MPN) are a heterogenous group of clonal stem cell disorders characterized by proliferation of ≥1 myeloid lineages. Dyserythropoiesis or granulocyte dysplasia is usually not observed. Patients are at risk for thrombotic and hemorrhagic events, as well as for progression to acute myeloid leukemia. MPN are usually diagnosed by blood and bone marrow examination as well as by the presence of well-defined cytogenetic and molecular abnormalities.
**CALR analysis: Further insight into the mutational repertoire of myeloproliferative neoplasms (contd)**

The association of certain MPN and Janus kinase 2 (JAK2) mutations has been well-established. A great majority of patients with polycythemia vera demonstrate JAK2 V617F or exon 12 mutations. However, in cases of primary myelofibrosis (PMF) and essential thrombocythemia (ET) the frequency of JAK2 mutations is at most 50–60%. Additionally, mutations of the thrombopoietin receptor gene MPL are present in 5 to 10% of patients with ET or PMF with wildtype JAK2. Recently, mutations of the calreticulin gene CALR on chromosome 19 have been demonstrated in PMF and ET with wildtype JAK2. CALR and JAK2 mutations are essentially mutually exclusive. CALR mutations are heterogeneous yet all mutations reported involve insertions or deletions in exon 9. Either a 52–bp deletion or a 5–bp insertion account for the great majority (≥ 80%) of mutations. Overexpression of the most frequent CALR deletion causes cytokine-independent growth owing to the activation of signal transducer and activator of transcription 5 (STAT5) by means of an unknown mechanism.

CALR mutation analysis by polymerase chain reaction fragment analysis is offered in the Diagnostic Molecular Biology Laboratory for whole blood or bone marrow specimens. Specifically, the test interrogates CALR exon 9 and detects a frameshift caused by one of the two most common mutations (see images below).

*A 293bp peak corresponds with wildtype CALR. Detection of additional peaks either at 242bp (Image 1, red arrow) or 298bp (Image 2, red arrow) correspond with the presence of the 52-bp deletion or the 5-bp insertion, respectively. A negative and positive control is run with each specimen (not shown).*

Image 1. 52-bp deletion.

![Image 1](image1.png)

Image 2. 5-bp insertion.

![Image 2](image2.png)
**CALR analysis: Further insight into the mutational repertoire of myeloproliferative neoplasms (contd.)**

After excluding reactive causes of thrombocytosis (e.g., iron deficiency anemia, surgical or functional asplenia, metastatic cancer, trauma, acute bleeding or hemolysis, various infectious/inflammatory processes) and/or in the setting of thrombocytosis with wildtype JAK2, one of the main clinical utilities of the CALR mutation analysis is to assist the detection of PMF or ET. Among patients with ET or PMF with wildtype JAK2 or MPL, CALR mutations are detected in up to 67% of those with ET and as much as 88% of those with PMF. Additionally, testing provides important prognostic information, as it has been shown that cases with CALR mutation are associated with lower risk of thrombosis and longer overall survival than patients with mutated JAK2.

**References:**


**Toxicological Testing at Northwestern Memorial Hospital**

By: Joseph Peevey, MD, Resident in Pathology

Technological advances within the field of toxicology have transformed drug screening and confirmation assays from time-consuming, labor-intensive, qualitative analyses to more automated, quantitative, sensitive, and specific tests. Despite these advances, the number of prescribed and non-prescribed compounds has grown rapidly, and the development of toxicological assays that can accurately and reliably detect the presence of these compounds and their metabolites has lagged. This is particularly evident with designer synthetic street drugs, including spice and bath salts.

Within the emergency department setting of acute toxicity, a standard urine immunoassay [NMH order: Drugs of Abuse] provides a fast (1–2 hour turnaround time), reliable method for quickly assessing the presence of certain drugs. The screening assay detects amphetamines, barbiturates, benzodiazepines, cocaine, cannabinoids, opiates and phencyclidine (PCP). The strengths of the immunoassay include low cost, speed,
and reliability. Weaknesses include relatively poor specificity, limited scope of screened compounds and lack of detection of many compounds within a single drug class. For example, the urine immunoassay detects natural opiates, including codeine and morphine, but has low sensitivity for nearly all semi-synthetic and synthetic opiates, including oxycodone (OxyContin), meperidine (Demerol) and fentanyl. Although specific immunoassays for individual synthetic and semi-synthetic opiates exist, they are not suitable for large-scale screening because they do not detect unknown compounds or other classes of commonly abused drugs. A limited screening assay remains appropriate because rapid drug identification using a more comprehensive screen would, in most instances, have little influence on immediate treatment of acute poisoning.

For non-emergency toxicological analyses (e.g., psychiatry, pain and addiction treatment settings), a variety of drug screening modalities are available. Thin-layer chromatography (TLC) is one such modality that has certain advantages (e.g., methodological ease); however, it is qualitative, relatively time-consuming (1–2 day turnaround time), subject to variability in manual technique, and dependent on judgment for interpretation. TLC use at NMH was recently discontinued in favor of liquid chromatography–tandem mass spectrometry (LC/MSMS), the current gold standard for toxicological analysis. Presently, LC/MSMS is available at NMH as both send–out and in–house options. As a send–out option [NMH order: Comprehensive Urine Drug Screen], LC/MSMS is used to assess approximately 75 known drugs and metabolites, and has a 2–3 day turnaround time. It detects benzodiazepines, salicylates, cannabinoids, and tricyclic antidepressants as class screenings rather than as individual compounds. As an in–house option, NMH has validated an opiate screen [NMH order: Opiate Confirmation] that detects 10 natural, semi–synthetic and synthetic opiates and metabolites. Additional in–house testing and validation are ongoing, and will include common psychiatric compounds, psychoactive substances, tobacco alkaloids, antihistaminics, stimulants, barbiturates, and comprehensive panels similar to the current send–out panel. Mass spectrometry is able to definitively identify and quantify large numbers of compounds, with high sensitivity and specificity. Developing LC/MSMS for in–house analysis requires a large capital investment, specialized technical support and individual standardization and validation for all screened compounds. Implementation of this analytical modality will provide more focused, accurate, actionable and timely results for clinicians.

Acknowledgement: I would like to thank Dr. G. Retzinger, Professor of Pathology, Director of Anatomic and Clinical Pathology, Vice–Chair Department of Pathology, for his advice and help in the preparation of this article.
Announcements

United States and Canadian Academy of Pathology (USCAP) Annual Meeting 2015

The 104th Annual Meeting of the United States and Canadian Academy of Pathology (USCAP) took place at the Hynes Convention Center in Boston, MA, March 21–27, 2015. Our pathology residents, fellows and faculty gave seven (7) proffered paper platform presentations and sixteen (16) poster presentations during the main academic conference and companion meetings this year. Congratulations to all!

Congratulations Dr. Nayar!

Dr. Ritu Nayar was elected to be a Trustee of the American Board of Pathology (ABP) for a term beginning in 2016. “The American Board of Pathology (ABP), a member of the American Board of Medical Specialties, was established in 1936. The mission of ABP is to promote the health of the public and advance the practice and science of pathology by establishing certification standards and assess the qualifications for physicians seeking to practice the specialty of pathology”. Congratulations!

Congratulations Dr. Rao!

Dr. Sam Rao, Professor of Pathology was chosen to receive the 2015 Gary A. Mecklenburg Distinguished Physician Award. “This prestigious award is presented annually to the practicing NM physician who embodies both professionalism and humanism and who demonstrates a strong commitment to enriching the lives of others through either research, teaching, patient care or community outreach”. Congratulations!

Congratulations Dr. Engman!

Dr. David Engman, Professor of Pathology and Microbiology–Immunology was selected by the Medical Faculty Council (MFC) as one of the two 2015 Mentors of the Year. The MFC stated that “mentoring is a key contributor to the academic growth of the Northwestern faculty and is fundamental to the successful development of the next generation of leading medical educators. Mentors, such as Dr. Engman, set an example of service to future doctors, researchers and educators”. Congratulations!

Congratulations Dr. Blanco!

Dr. Luis Blanco, Jr, Assistant Professor of Pathology received the Women Faculty Organization (WFO) first place award for Women’s Health Research at the Annual Northwestern University Lewis Landsberg Research Day in the Clinical/Public Health category. Dr. Blanco’s work was titled “Increased CD68-positive macrophages and CD4-positive lymphocytes in tumor associated inflammation in pregnancy associated breast cancer may contribute to a poor prognosis”. Congratulations!
Clinical and Anatomical Pathology Services at NMH

Northwestern Memorial Hospital’s medical staff in the Department of Pathology, are led by William Muller, MD, PhD (Chair) and Gregory Retzinger, MD, PhD (Associate chair). The department offers full-service clinical and anatomic pathology services to patients and physician offices. With 42 pathologists and 300 staff members conducting over 9 million reportable tests every year, our department is structured to offer the very highest level of customer service.

Clinical Pathology Services
- Blood Bank - Glenn E. Ramsey, MD, Director
- Chemistry - Gregory Retzinger, MD, PhD, Director
- Cytogenetics - Yanming Zhang, MD, PhD, Director
- Flow Cytometry - Kristy L Wolniak, MD, PhD, Director
- Hematology & Hematopathology - Yi-Hua Chen, MD, Director
- Hemostasis - Paul F. Lindholm, MD, Director
- Immunology - Yashpal Kanwar, MD, PhD, Director
- Microbiology - Chao Qi, PhD, Director
- Molecular Diagnostics - Nike Beaubier, MD Director
- Virology - Yijun Zhu, MD, Director

Anatomic Pathology Services
- Autopsy - Jon Lomasney, MD, Director
- Cytopathology - Ritu Nayar, MD, Director
- Surgical Pathology (includes Cardiac Pathology, Gastrointestinal/ Hepatic Pathology, Genitourinary Pathology, Gynecologic/ Reproductive Pathology, Thoracic/ Soft Tissue/Endocrine Pathology) - Guang-Yu Yang, MD, PhD, Director
- Breast Pathology - Kalliopi Sizopikou, MD, PhD, Director
- Neuropathology - Eileen Bigio, MD, Director
- Perinatal Pathology - Linda Ernst, MD, Director
- Renal Pathology - Yashpal Kanwar, MD, PhD, Director
- Immunohistochemistry - Ximing Yang, MD, PhD Director

Pathology Residency and Fellowship Programs
The residency program in anatomic and clinical pathology and fellowship training programs in breast pathology, cytopathology, hematopathology, neuropathology, surgical pathology, genitourinary pathology and gastrointestinal pathology seek to train tomorrow’s anatomic and clinical pathologists in the professional practice of pathology.

Contact Us
The Department of Pathology’s staff may be contacted by using the Physician Access Line at 1-800-638-3737