# "Molecular" is not your traditional lab

## Where disease management meets acute care

By Marilyn R. Carlson, DMD, MD, RAC

Molecular diagnostic (dx) testing has changed the practice of medicine and has introduced a new type of laboratory to the industry. Like traditional laboratories, molecular diagnostic laboratories perform a range of tests, employ skilled laboratorians, issue test reports, and provide patient information for use by clinicians. In almost every other aspect, molecular diagnostic laboratories differ from traditional labs and, based on the types of molecular testing performed, may also differ from one another. This article describes the growing importance of molecular testing, provides examples of tests used to guide the treatment of patients with cancer, and contrasts aspects of the molecular diagnostic lab with characteristics of the traditional lab.

### Molecular Dx helps individualize medicine

Molecular diagnostics is a new discipline that uses cutting-edge technologies, such as gene chips and microarrays, to obtain information about the activity patterns of genes and proteins in normal cells and in cancer cells.[1] Researchers use this information to design better treatments and to identify subgroups of patients with cancer types that make them likely to respond to specific therapies. Contrast this approach with the practice of medicine before molecular diagnostics when cancer cells were examined under a microscope and categorized by their appearance.

As more tests are validated in the clinic, there is an increased demand for patient-specific information and an increased acceptance of the value of molecular diagnostics by regulatory agencies as well as clinicians. It is not surprising that molecular diagnostic testing is the fastest growing segment of the *in vitro* diagnostics (IVD) market with an estimated annual growth rate of 11%. In 2007, the molecular diagnostics market totaled $3.21 billion in revenues and is projected to reach $5.42 billion by 2012.[2]

This ability to diagnose the molecular basis or the genetic components of a disease has advanced the concept of "individualized medicine" beyond finding the right drug at the right dose for the right patient. Today, clinicians rely on molecular test results to diagnose disease, and choose therapies most likely to be effective for a specific tumor and a specific patient.

### Molecular Dx improves the management of cancer

In 2008, cancer specialists used the information provided by molecular diagnostic tests to identify individuals at increased risk, to enhance the accuracy of diagnoses, and to individualize treatment decisions for a variety of cancers including two of the most common forms: breast cancer and colorectal cancer.

Meanwhile, other uses of these tests are being validated in the clinic. One important use, particularly for oncologists, is the ability to use the information to predict the course of a disease making these tests "prognostics" as well as diagnostics.

***Breast cancer:*** Approximately 5% to 10% of the more than 192,000 American women diagnosed each year with breast cancer have a hereditary form of the disease. Inherited alterations in the genes called BRCA1 and BRCA2 are involved in many cases of hereditary breast and ovarian cancer. Women with an altered BRCA1 or BRCA2 gene are three to seven times more likely to develop breast cancer than women without alterations in those genes. This information enables patients from high-risk families to intervene before cancer occurs.

Of the women diagnosed with breast cancer, 25% have tumors that overexpress human epidermal growth factor receptor 2 (HER-2). These tumors are particularly aggressive and have a high risk of recurrence. A monoclonal antibody, trastuzumab (Herceptin), was found to be most effective in treating patients with HER-2 positive tumors.[3] Fluorescence *in situ* hybridization (FISH) is a molecular test used to determine the tumor's HER-2 status. In fact, the Food and Drug Administration (FDA)-approved use of Herceptin is the adjuvant or combination treatment of women with HER-2 overexpressing breast cancer.[3]

***Colorectal cancer:*** Colorectal cancer is the second most common cancer in North America. Following surgery, accurate tumor staging is the most important tool for determining treatment and prognosis.[4,5] Patients with disease limited to the colon or rectum may have no further treatment; but once cancer has spread to the adjacent lymph nodes, additional treatment will be recommended.

When traditional histopathology fails to visualize cancer cells in regional lymph nodes, it is possible the tumor has not metastasized — but it is also possible cancer cells were not seen in the section of the lymph node examined. In fact, colorectal cancer will recur in 25% to 30% of patients thought to have negative lymph nodes based on examination by traditional histopathology.[4]

In 2008, a molecular test became commercially available, which is used to thoroughly examine lymph nodes for the presence of guanylyl cyclase C (GCC), a transmembrane receptor protein specifically expressed in the lumen of the gastrointestinal tract[6] using a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR).

In patients with negative lymph

nodes by traditional histopathology, the detection of GCC in a regional lymph node is consistent with spread of cancer outside the colon or rectum.[7] This information enables clinicians to predict which patients are likely to experience a recurrence and should receive additional therapy.

Once it is determined patients with colorectal cancer require additional therapy, molecular diagnostic testing of their tumor tissues provides important information to optimize the choice of chemotherapeutic agents.

ResponseDX: Colon (Response Genetics Inc., Los Angeles, CA), includes three molecular tests used together to "personalize" cancer chemotherapy for an individual patient with colorectal cancer. The patient's own tumor tissue is tested for ERCC1 and for TS (thymidylate synthetase) expression using qRT-PCR and PCR is used to analyze the DNA to determine K-ras gene (*KRAS*) mutational status.

A better response to 5-FU and oxiplatin (FOLFOX) is seen with low expression of ERCC1 and with low expression of TS. *KRAS* mutations occur in approximately 40% of colorectal cancers and confer resistance to treatment with epidermal growth factor receptor (EGFR)-targeted therapies such as Erbitux (cetuximab) and Vectibix (panitumumab).[8] The National Comprehensive Cancer Network (NCCN) amended its guidelines this year to recommend *KRAS* mutation status be determined for all patients diagnosed with metastatic colorectal cancer. Since the presence of *KRAS* mutation strongly predicts a lack of response as well as shorter survival from EGFR-directed chemotherapy, only patients with metastatic colorectal cancer who have normal *KRAS* status should receive anti-EGFR therapies.

## Traditional lab vs. molecular lab: Acute care meets disease management

Due to the growing importance of molecular testing, it is of interest to compare and contrast a traditional lab with a molecular diagnostic lab. In simplistic terms, a traditional lab provides information needed by clinicians in an acute care setting while molecular diagnostic labs provide genomic and proteomic information to guide the management of chronic diseases like cancer. Key characteristics of each type of lab are summarized here and in Table 1 (see next page).

***Accreditation required:*** The United States Congress enacted the Clinical Laboratory Improvement Amendments (CLIA) of 1988 to ensure the accuracy and reliability of all laboratory testing. The CLIA statute is based on the complexity of tests performed and includes all types of testing sites. Simple tests with a small chance of error or risk, may be exempt from CLIA rules and are referred to as "waived test." Depending on the types of testing offered, a traditional lab may be required to secure CLIA certification. For all molecular diagnostic testing sites, CLIA certification is mandatory.

***Tests offered and test methods:*** In a traditional laboratory, automated analyzers perform the frequently requested chemistry and hematology tests. Reference ranges and standards are based on populations. In contrast, molecular testing is highly complex and includes fluorescence *in situ* hybridization, polymerase chain reaction (PCR), reverse transcription-PCR (RT-PCR), DNA chip technology, and gene microarrays. The genetic information from an individual is analyzed and the results are unique to each patient. Unlike some of the tests performed at the traditional labs, molecular diagnostic tests are not routinely used for surveillance nor are they repeated every few weeks or months.

***Sensitivity and specificity:*** In contrast to the tests performed in the traditional laboratory, molecular diagnostic tests need to be both highly sensitive and highly specific because patients are diagnosed and treatments are administered based on the results of these tests. A false positive or a false negative might result in a patient not getting the appropriate treatment. Although no tumor marker is present in all tumors and none is 100% specific to a tumor, PCR is more sensitive and specific than protein-based tests such as immunohistochemistry (IHC) and enzyme-linked immunosorbent as-

say (ELISA) offered in traditional labs.

***Samples analyzed:*** A traditional laboratory receives samples of fresh or frozen human body fluids for testing. In comparison, a molecular diagnostic lab may receive fresh tissues or fixed specimens obtained from bone marrow, lymph nodes, and tumors. New tests are performed using formalin-fixed, paraffin-imbedded (FFPE) lymph nodes and tumor tissues, respectively.

***Turnaround time:*** Traditional labs that perform routine analyses (e.g., chemistry and hematology) can often provide results in less than one hour. This is due to automation in response to sample volume, price competition, and demand for rapid turnaround. For complex multistep molecular tests, there is currently less demand for rapid turnaround, however, most molecular tests can be completed within several days.

***Test reports:*** The traditional lab report includes patient information as well as test results and normal ranges with minimal, if any, information on the interpretation or the clinical significance of the values reported. Due to the complexity of molecular diagnostic tests, interpretation within the context of the disease or disorder as well as the test methodology for each individual patient is provided for the ordering clinician. This information may be provided with graphics as well as in narrative form.

**Table 1.** Comparison of key characteristics of laboratories

| Lab features | Traditional lab | Molecular diagnostic lab |
|---|---|---|
| **CLIA certification** | May be waived for simple tests | Required |
| **Types of tests offered** | Chemistry<br>Hematology<br>Urinalysis<br>Microbiology<br>Blood gases | BRCA<br>HER-2<br>GCC<br>KRAS<br>ERCC1<br>TS |
| **Test methods** | Automated analyzers<br>Culture and sensitivity<br>IHC<br>ELISA | Gene microarray<br>Fluorescence in situ hybridization<br>Polymerase chain reaction (PCR)<br>Reverse transcriptase-PCR<br>DNA chip technology |
| **Sensitivity & specificity** | Low | High |
| **Samples analyzed** | Venous blood<br>Arterial blood<br>Urine<br>Cerebrospinal fluid | Blood<br>Bone marrow<br>Tumor tissues<br>Lymph nodes |
| **Turnaround time** | Less than 24 hours | Several days |
| **Test report information** | Patient identification<br>Specimen type<br>Dates obtained, received, processed, reported.<br>Test results<br>Reference ranges | Same plus;<br>Graphs<br>Diagrams<br>Narrative (interpretation) |
| **Reimbursement** | Covered by most third-party payers | Reimbursement varies by test and third-party payer |

***Reimbursement:*** The tests provided by traditional labs are covered by most third-party payers although price competition has led some insurers to restrict patients' choice of covered laboratories. For molecular diagnostics, reimbursement must be secured from third-party payers for each individual test. Molecular labs must work with individual insurance plans to provide the types of information required.

## A new frontier for laboratories

Cutting-edge technologies based on genomics and proteomics have given rise to a new discipline of molecular diagnostics which, in turn, has changed the landscape of the traditional laboratory. Molecular diagnostic labs offer complex tests with results based on the genetic make-up of the tissues from an individual patient and provide information in their test reports to aid in the interpretation of the results to assist clinicians.

Clinicians rely on information from molecular diagnostic tests to guide treatment decisions for individual patients, while the FDA uses information from these tests to approve new treatments for patients most likely to benefit.

The high complexity of the tests performed in the molecular diagnostic laboratory, the need for interpretation of the test results for each individual patient, and the disease setting in which the information is used are some of the key differences in comparison to traditional laboratory. □

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## Contest offers organizational help for the messy office

Again this year, Lab Quality Confab and Ascendium Consulting are sponsoring "The (Dis)Organization 'LEAN' Contest" to help one lab professional straighten up that messy desk once and for all. The winner receives a day-long date with LEAN expert Caroline Ambrose, managing consultant for Ascendium, which provides an office-space makeover, based on the lucky winner's organizational type, and ideas to help sustain an orderly workspace long-term. While lab supervisors might do a great job implementing LEAN principles on the workbench, their desk areas often are not up to par, says Ambrose. LEAN means "Everything has a place." An organized office is not just for show, she notes. Time lost trying to locate papers is wasting a valuable resource. "With unannounced inspections, how much time would staff waste looking for a piece of paper if it was needed for an inspection when you are absent?"

Last year's contest winner, Ina Aiazzone, manager of laboratory quality management and POCT at St. Joseph's Healthcare System, Patterson, NJ, is a "piler."

"Every time someone needed a document, it took me 15 minutes to find it." she says. Aiazzone maintains desktop order by now using that 15 minutes to sort and discard unneeded materials on her desk. An out-basket collects everything that belongs somewhere else, which Aiazzone delivers to their destinations at the end of the day. She uses five project boxes with lids to collect everything related to current projects. Her lab is currently going through a state licensing process, she says. "Now, rather than piles of paper, anything pertaining to the state license is in a container where I can always find it."

Anyone *attending* 2009 Lab Quality Confab, September 29-30 in Atlanta is eligible to enter this contest. A contestants should e-mail name, organization, job title, mailing address, and phone number, along with a picture of the desktop in question, and explain in 50 words or less why he/she deserves to win. Entries must be *received before midnight*. Thursday, Sept. 11. The winner will be announced at 2009 Lab Quality Confab.