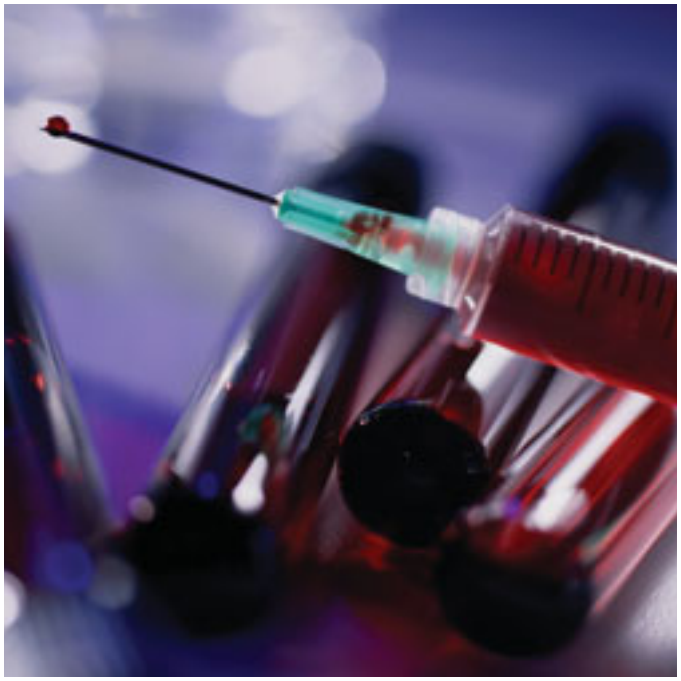
# Pushing the frontiers of diagnosis

## Molecular tests are improving medical odds.

BY RANDALL C. WILLIS



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An aging Western population, economic improvements in the developing world, and technological advances in health care are combining to push the clinical diagnostics industry through a period of significant growth over the next few years, according to *The U.S. Clinical Diagnostic Equipment Market,* a 2003 report by Business Communications Co. Already accounting for $13.6 billion in sales in 2002, the clinical diagnostics market will continue to expand, BCC consultants predict, at almost a 7% average annual growth rate through 2007.

One company that has seen the market shift and has made corresponding moves is Ciphergen Biosystems. Looking to expand on the company's expertise in biomarker discovery using its SELDI ProteinChip technology, company president and CEO William Rich announced the appointment of Gail Page as president of a newly formed diagnostics division in January.

"The major focus of the company is really proteomic-based translational medicine," Rich says. "We want to translate biomarkers into high-value, multimarker clinical diagnostic tests for diagnosis, prognosis, and treatment monitoring (theranostics) of disease. The 200+ clinical researchers who own our systems and are doing biomarker discovery represent a 'farm club' for us, and we hope to be licensing multimarkers and multimarker assays from them for commercialization in the future as well."

Looking to do for biomarker analysis what Affymetrix has done for gene expression studies, Ciphergen plans to take its platform before the FDA for approval and believes that the system will be the first really new and powerful protein clinical diagnostic platform in decades.

Market growth will likely be pushed by new and upcoming advances in molecular methods designed to replace or enhance existing diagnostic technologies, allowing clinicians to look at medical samples of all types and at all stages of disease.

### Inborn errors

Some diseases are caused by massive chromosomal abnormalities that can be visualized with standard cytogenetic methods, such as chromosome spreads (karyotypes). These assays can even be performed on children in utero. By staining cell samples with dyes that bind chromosomal DNA, clinicians can look for translocations (swapping material from one chromosome to another) or aneuploidy (extra copies of one or more chromosomes). For example, researchers can diagnose chronic myelogenous leukemia by looking for evidence of the Philadelphia chromosome, a translocation between chromosomes 9 and 22 that results in the formation of a *bcr–abl* gene fusion. Likewise, clinicians can diagnose Down's syndrome by looking for chromosome 21 trisomy (three copies instead of two).

The problem with standard karyotyping methods, however, is that they can generally only be used to detect large chromosomal alterations, whereas most disease-causing errors occur within gene segments rather than throughout a chromosomal arm. To address this problem, clinicians and researchers have developed methods such as fluorescence in situ hybridization (FISH), which uses short, labeled oligonucleotide fragments to locate and identify subtle chromosomal alterations. For example, technicians at

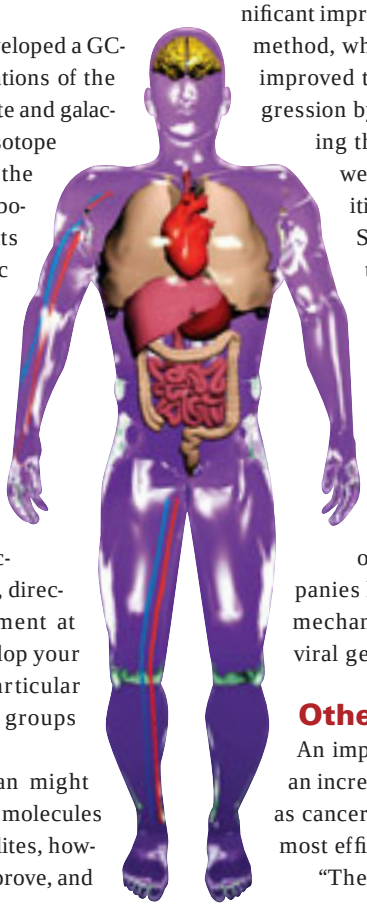
the Kleberg Cytogenetics Laboratory of the Baylor College of Medicine use FISH on blood samples to determine if an individual carries a genetic duplication that indicates Charcot-Marie-Tooth disease, an inherited form of peripheral neuropathy.

Of course, not all inborn errors are the result of massive chromosomal rearrangement; rather, many develop from smaller mutations, which may not be easily discernible. For this reason, clinicians often have to rely on infant symptomology for the initial clues. Alternatively, there has been a strong move in the medical community over the past few years to make newborn screening for various metabolic disorders, such as phenylketonuria, mandatory. To rapidly screen for several disorders, however, clinicians require assays that are straightforward to perform.

Peter Schadewaldt and colleagues at Heinrich-Heine-Universität Düsseldorf have described a method of determining another inborn disorder, galactosemia. In this disease, deficiencies in one or more enzymes involved in dietary galactose metabolism lead to the toxic buildup of metabolites. Clinicians have traditionally diagnosed galactosemia on the basis of fluorimetric or radioisotopic enzyme assays, but these methods are not sensitive enough to identify mild cases.

"All these methods are of comparable performance and are well suited for monitoring the enhanced concentrations of galactose 1-phosphate in severe classical galactosemia," Schadewaldt reports. "Due to a limited sensitivity, however, the methods frequently fail to reliably quantitate low metabolite levels that may occur in, for example, healthy subjects and postabsorptive patients with mild galactosemia."

To address this problem, the researchers developed a GC-MS method to determine the relative concentrations of the main galactose metabolites—galactose 1-phosphate and galactitol—in human red blood cells using a stable isotope dilution technique. The investigators found that the new method was linear over a wide range of metabolite concentrations and offered detection limits more than 10-fold better than the fluorimetric assay (<0.1 µmol/L vs >2 µmol/L).



One of the problems with the LC- and GC-MS-based methods, however, is that they test for only one condition. To be cost-effective and labor-saving, newborn screening programs will have to cast as wide a diagnostic net as possible. To address this challenge, several institutions have turned to MS or MS/MS methods.

"It used to be the case that we had a particular test for a particular analyte," says Blas Cerda, director of R&D for tandem MS product development at PerkinElmer Life Sciences, "so you would develop your extraction or sample prep procedure for that particular instrument. Now, with MS, we can screen for groups of analytes."

Using current MS/MS methods, a clinician might achieve femtomole-level sensitivity in detecting molecules such as fatty acids. To look at a variety of metabolites, however, sample preparation methods will need to improve, and efforts are under way to effect these changes.

"Tandem MS has really revolutionized the way we do newborn screening analysis," Cerda adds. "We can now help many more people in many more ways than we could before. We can now screen for more than 20 disorders that we couldn't screen for before, so the impact on the community once this technology gets more established will be great."

## Infections

Another area of intense assay development is the diagnosis and identification of infections and infectious agents. Whereas a clinician might have days or weeks to discern an inborn metabolic condition before problems arise, this timeline might be reduced to hours or days with an infection. Nowhere was this better shown than in the 2003 outbreak of severe acute respiratory syndrome (SARS).

Researchers at Hong Kong's Princess Margaret Hospital and Public Health Laboratory Centre relate that by May 28, 2003, 745 patients worldwide had died of SARS and another 8240 people were infected. One of the problems in diagnosing the condition arises from its nonspecific symptoms, which overlap with those of other respiratory conditions such as pneumonia. With the discovery of coronavirus as the causative agent of SARS, however, researchers have developed various laboratory tests for the syndrome. One such test relies on identifying viral RNA molecules in nasal or stool samples using reverse-transcriptase PCR (RT-PCR).

With RT-PCR, the researchers detected coronavirus in 71.6% of samples from patients at the onset of SARS symptoms, a significant improvement over earlier experiments using the same method, which achieved only 32–50% success. This number improved to >90% in 8–10 days after onset, but disease progression by this point precluded the researchers from waiting this long to begin treatment. Although the results were suboptimal, the investigators believe that a positive RT-PCR result "should raise the possibility of SARS in appropriate clinical settings and should alert the clinician of the possible clinical deterioration of the patient."

Sometimes, simply identifying the infectious agent is insufficient for the clinician to begin treatment. This problem is best exemplified by HIV and its multiple-drug-resistant phenotypes. It is not enough to know that a patient is infected with the virus; rather, a clinician needs to know the viral strain(s) to decide what combinations of drugs will help. For this reason, several companies have developed HIV genotyping kits that, by one mechanism or another, identify the sequences of various viral genes.

## Other diseases

An important aspect of the aging Western population is an increased prevalence of chronic wasting diseases such as cancer and Alzheimer's disease (AD). In each case, the most efficacious treatments are those that start early.

"The increasing awareness of the possibilities for drug

treatment of AD has made people with memory disturbances seek medical advice very early," says Kaj Blennow, a physician at Sahlgren's University Hospital in Sweden. "In this phase of the disease, there is no clinical method to determine which of these patients will progress to AD with dementia and which will not."

Trying to correct this situation, Blennow worked with scientists at Ciphergen to identify biomarkers in cerebrospinal fluid that would help clinicians diagnose early-stage AD and distinguish AD from other dementias. They presented their findings at the Society of Neuroscience meeting last November. The researchers identified a pattern of 4 biomarkers that correctly classified 29 of 30 AD patient samples and 33 of 35 age-matched control samples.

"The AD profile discovered in this study using the SELDI ProteinChip technology may be a major breakthrough as a method to help clinicians identify AD very early in the disease process," Blennow says.

Like AD, cancer has few specific early-stage symptoms and therefore is not typically diagnosed until late in its progression. Thus, methods must be developed that will allow patients to be diagnosed early. Pascale Macgregor and colleagues at Toronto's University Health Network Microarray Centre used cDNA microarrays carrying 19,200 known genes and expressed sequence tags to examine the gene expression profiles of tissues from healthy individuals and those diagnosed with ovarian cancer, the fifth-most-common cause of cancer-related death in North America. They also looked at differences in gene expression patterns between patients with good or bad prognoses for a long disease-free interval (DFI) after surgery.

On the basis of the gene expression patterns, the researchers clustered the tissue samples into distinct groups and observed clear down- and upregulation of several genes in the cancer samples. They then compared the gene expression profiles of tissues from patients with a good (DFI > 12 months) and bad (DFI < 6 months) prognosis. Although the differences between the two samples were small, the researchers were able to identify specific genes that could help them determine prognosis.

"The accuracy of the classifier was only around 70%, and that was not high enough to consider using those genes as a diagnostic tool," Macgregor says. "For microarrays—or any test—to be used as a clinical diagnostic tool, they need to achieve specificity and sensitivity on the order of 99.5%. I haven't seen any example of cancer prognostication based on microarrays that achieves these levels yet."

"There is, however, great promise in using microarrays in prognostication of patient outcome and/or response to treatment using microarray profiling," she adds. "Right now, we haven't seen any application in the clinic because the technology is still new, and we all need to gain confidence in the technique. I suspect that five years from now, we will see things very differently."

By combining newer molecular methods with tried-and-true clinical techniques, a new generation of diagnostic tools is on the horizon. The challenge remains, however, to make these methods sufficiently sensitive, inexpensive, and diverse to screen the maximum number of people for the widest spectrum of diseases and disorders with the smallest amount of sample. ■