Clinical applications of microarray-based diagnostic tests

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Nearly 15 years have passed since the possibility of analyzing nucleic acid analytes in a massively parallel fashion was proposed using the then new concept of microarrays. A decade ago, proof of principle demonstration projects established the use of high density microarrays to genotype multiple polymorphisms within a large gene (cystic fibrosis transmembrane regulator [CFTR]), to rapidly analyze DNA sequences by hybridization and to ascertain differential gene expression of the entire genome of an organism. The use of microarrays has had an explosive influence on the rate at which new biological information can be learned, including in a nonhypothesis driven manner. The past decade has also seen these research tools applied increasingly to questions of clinical and medical relevance. Genotyping drug metabolizing enzyme genes, resequencing important tumor suppressor genes, and classifying neoplastic disease by differential gene expression profiles are but a few of the many possibilities to provide clinically useful information using microarray-based diagnostic tests.

INTRODUCTION

Ten years ago, Cronin and colleagues demonstrated that oligonucleotide microarrays could effectively genotype a large number of polymorphic sites within the cystic fibrosis transmembrane regulator (CFTR) gene that are associated with the development of cystic fibrosis (1,2). Coupling multiplex PCR amplification to isolate the genomic regions of interest, these investigators developed highly redundant probe tiling approaches that yielded highly reproducible and accurate CFTR genotypes. This success led scientists at Affymetrix (Santa Clara, CA, USA) to develop other research tests using similar approaches, including the p53 resequencing test for detecting p53 mutations in cancer samples, and the genotyping test for the CYP2D6 and CYP2C19 genes. This latter application was further developed to become the first Food and Drug Administration (FDA)-approved microarray-based in vitro diagnostic test, the AmpliChip CYP450 test by Roche Molecular Systems (Pleasanton, CA, USA). In conjunction with knowledge gained from sequencing the human genome, many microarray assays were developed to allow profiling of the expression of thousands of genes (3). Microarray analysis of entire transcriptomes of clinical samples is extremely useful for understanding the molecular biology of disease, providing opportunities for molecular classification of disease, the identification of novel molecular target for intervention of disease, and the prediction of therapeutic response. This article will discuss clinical applications of microarray-based tests by using the AmpliChip CYP450 test, the p53 GeneChip Assay (Affymetrix), and various examples of gene expression profiling to illustrate the unique opportunities and challenges associated with each procedure.

MICROARRAY-BASED GENOTYPING: THE AMPLICHIP CYP450 TEST

It has long been known that significant proportions of human populations lack either CYP2D6 or CYP2C19 enzyme activity due to inheritance of two nonfunctional alleles (4). Poor metabolizer status, the complete lack of CYP2D6 enzyme activity, disproportionately affects 7%–8% of Caucasians, while genetically determined CYP2C19 poor metabolizers occur with a frequency of 15%–20% among people of Asian geographical origin (5). These two enzymes affect the metabolic clearance or activation of approximately 15% of drugs currently in use and therefore may lead to aberrant pharmacokinetic responses in those individuals who lack enzyme activity. Major classes of therapeutics including antidepressants, antipsychotics, antiarrhythmics, antihypertensives, antimalarials, antianxiolytics, and many others can be profoundly influenced by these polymorphic genes (6).

The promise of individualized medicine has, in part, rested upon the notion that identification of individuals who exhibit genetically determined aberrant drug metabolism could lead to more effective treatment by individualizing drug doses to compensate for enzyme activity and pharmacokinetic differences (7). While these Mendelian traits clearly do lead to exaggerated drug responses, or failure to respond in the case of prodrugs, they explain only a portion of the individual variation observed in drug responses. Factors such as age, gender, diet, co-medication, and disease co-morbidity also influence drug responsiveness and are routinely considered in therapeutic decision making (8). Thus genotyping polymorphic drug metabolizing enzyme genes has the potential to provide physicians with additional clinically relevant information to guide therapy choices.

The AmpliChip CYP450 Test, based on a long multiplex PCR coupled with oligonucleotide microarray analysis, provides detailed genotypic and predictive phenotypic information about the intrinsic drug metabolizing activity of individuals, regardless of race or ancestry. The test has been designed to be comprehensive in reporting the most common allelic variants of both CYP2D6 and CYP2C19. In the latter case, two allelic variants (*2 and *3), each leading to nonfunctional CYP2C19 enzyme activity, account for the virtually 100% of Asian poor metabolizers, and between 85%–90% of Caucasian and African poor metabolizers (5). In contrast, the CYP2D6 locus is far more polymorphic and has unique challenges in terms of the breadth of genetic variation that underlies inherited enzyme activity aberrancies, including complete gene deletion (*5 allele), various types of point mutations, in-frame deletions and gene conversion events, as well as gene duplication of up to nine different allelic variants, including null alleles (www.imm.ki.se/CYPalleles). Gene duplication of functional alleles can lead to profound excesses in enzyme activity due to gene dosage effects, with 2–13 tandem functional copies of CYP2D6 known to occur (9). Thus it becomes important to be able to distinguish not only the presence of gene duplications, but which allele has been duplicated. The AmpliChip CYP450 microarray (Figure 1A) contains over 15,000 oligonucleotide sequencings querying for over 30 genetic alterations of the CYP2D6 gene and 2 single nucleotide polymorphisms within the CYP2C19 gene, reporting 27 variant alleles, including 7 duplicated alleles of CYP2D6 and 3 alleles of CYP2C19. The genotyping accuracy of the AmpliChip CYP450 Test was excellent when tested against direct sequencing analysis of over 400 clinical samples with a sensitivity of 99.2% and a specificity of 100% for CYP2D6 and a sensitivity of and specificity of 100% for CYP2C19 (Table 1). This accuracy is due primarily from the use of redundant probe tiling on the microarray, which in turn allows one to set very robust cutoffs to
discriminate between homozygous wild-type or mutants or heterozygotes. The tiling strategy of a single block with 40 oligonucleotide sequences is showed in Figure 1B. Up to six such blocks with a total of 240 oligonucleotide probes are used for the determination of each genetic alteration, and over 15,000 probes are used on the Amplichip CYP450 microarray.

From an assay development point of view, the human CYP2D6 locus provides many of the challenges seen in other parts of the genome, including the presence of highly conserved nonfunctional pseudogenes (10), and a degree of global polymorphic variability that challenges test robustness due to variation near or under PCR primer and probe sites that can lead to artificial genotyping results in a very small proportion of samples tested worldwide. It is likely that many, if not most, multiplex genotyping assays developed in the future will face these challenges, many of which do not become obvious until an assay is tested in a large number of individuals representative of the genetic diversity that exists around the globe. Thus, unless large databases of DNA sequence are generated for clinically relevant genes across large populations of differing geographical origin or ethnicity/race, these issues will almost certainly lead to the need for second generation test development for many genetic and pharmacogenetic tests.

Figure 1. Amplichip CYP450 Test. (A) Pictures of Amplichip CYP450 microarray and a scanned chip image. (B) Probe tiling for genotyping test. For each known mutation site, there is a box containing five columns of probes complementary to wild-type sequence (W) and five columns of probes complementary to mutant sequence (M). The five columns interrogating wild-type sequence are interdigitated with the five columns interrogating mutant sequence, such that each pair of columns of probes interrogates the same nucleotide position in the target sequence. Each pair of columns successfully interrogates the target nucleotide sequences from two bases upstream to the mutation site (M-2) to two bases downstream to the mutation site (M+2). Each probe in a column contains a specific mismatch position called the “substitution” position, where each of the four possible nucleotides (A, C, G, T) are substituted into the probe sequence. There are a total of 40 probes for a block of columns. Probes in columns for interrogating wild-type sequence are perfectly complementary to wild-type except at the substitution position, and the same is true for probes interrogating mutant sequence. Optimized hybridization condition facilitates the annealing of DNA target to the probes that best match its sequence. The patterns in the hybridization image reliably indicate whether wild-type and/or mutant sequences are present. WT, wild-type; HET, heterozygotes; MT, mutants.

From a clinical and regulatory perspective, analytical and clinical accuracy, as well as clinical utility, are the benchmarks against which the value of in vitro diagnostic test procedures are measured. Although highly accurate, genotype-phenotype correlations for CYP2D6 are imperfect for the following reasons: (i) unsuspected drug-drug inhibition effects can influence enzyme activity; (ii) examples of substrate-specific differences for certain classes of drugs with certain low activity, but not null, allelic variants (11); and (iii) there are yet to be discovered allelic variants that may or may not be detected as larger populations around the globe are tested. For physicians to make ready use of drug metabolizing enzyme (DME) genotype information, they must be provided phenotype predictions based on genotype, and suggested dose adjustments for the classes of drugs most affected; such data have recently been provided in the area of antidepressants (12). For drugs with extremely narrow therapeutic indices, dose adjustment, or a switch to another therapeutic unencumbered by these liabilities, may be a prudent therapeutic decision.

Dozens of studies report associations between CYP2D6 or CYP2C19 genotype and drug responses, and the genes are considered valid biomarkers by the FDA in their Guidance for Industry Pharmacogenomic Data Submissions, yet the medical community is not well versed in the use of such genetic information in making treatment decisions. Thus, even when robust FDA-approved in vitro diagnostics such as the Amplichip CYP450 Test are available, further clinical research will be needed to establish where such genotype information will provide the greatest benefit to maximize drug efficacy and enhance drug safety (13).

MICROARRAY-BASED RESEQUENCING: THE p53 GeneChip ASSAY

It has been almost 10 years since the p53 GeneChip assay, a microarray-based resequencing test, was first used for detecting p53 mutations in cancer (14). The tumor suppressor gene p53 plays a key role in multiple cellular pathways and functions as a transcription factor by regulating genes that control cell proliferation, cell survival, and genomic integrity (15). Disrupting its function promotes checkpoint defects, genomic instability, and inappropriate survival, leading to the uncontrolled proliferation of damaged cells. In fact, p53 mutations are the most common genetic alterations found in cancer. Between 30% and 70% of human cancers of almost every organ contain a point mutation in one of the two p53 gene copies. Mutations of the p53 gene have been associated with poor prognosis in numerous human cancers and are a major determinant of patient outcome for response to adjuvant chemotherapy or radiotherapy (16).

The human p53 protein, consisting of 393 amino acids, is encoded by exons 2–11 of the p53 gene. The nature and distribution of mutations varies among cancer types; most p53 mutations (74%) are missense mutations and are due to single base changes at the central DNA binding region of the p53 gene, resulting in mutant p53 proteins that likely have lost transcription factor activity. Over the years, p53 mutations in cancer
have been widely studied and collected in two independent p53 databases. One is maintained and developed at the International Agency for Research on Cancer (IARC p53 database; www-p53.iarc.fr). The latest version (R9) contains 19,806 somatic mutations, 264 germline mutations, and functional data on 423 mutant proteins. Another database is maintained by T. Soussi (p53.free.fr) and contains information about 21,717 p53 mutations. Since 1989, most p53 mutations have been identified by methods such as single-stranded conformational polymorphism (SSCP) followed by DNA sequencing analyses. Other methods such as denaturing gradient gel electrophoresis (DGGE), dideoxyfingering (ddF), heteroduplex analysis, and various mutation cleavage methods have also been used. In general, these traditional gel-based sequencing methods are relatively time-consuming and labor-intensive, especially for analyzing all 10 exon coding regions of the p53 gene, which are spread across 8 kb. In addition, the heterogeneity of tumor samples, which is often a mixture of normal cells and tumor cells, presents another challenge for mutation detection. Therefore, a high-throughput, sensitive, and accurate method is needed for detecting p53 mutations for large number of clinical samples.

Ahrendt and colleagues (14) first published the results of p53 resequencing for 100 fresh frozen lung cancers with an oligonucleotide microarray assay (p53 GeneChip) and demonstrated that the GeneChip assay is a rapid and reasonably accurate approach for detecting p53 mutations when compared to direct DNA sequencing. Later, the p53 GeneChip was used for detecting p53 mutations in 108 ovarian cancers (17), 140 fresh frozen bladder cancers (18), and 20 colon cancers (19). All of these studies showed a high sensitivity of the oligonucleotide microarray analysis for detection of single-base pair substitution when compared to the conventional manual DNA sequencing method as shown in Table 2. In some cases, the p53 GeneChip assay could detect certain base substitution mutations with an abundance of as little as 1% mutant DNA (20). In contrast, the microarray assay has not proven as sensitive for the detection of deletion or insertion mutations, as evidenced by reduced accuracy for frameshift detection in all studies reported to date. According to the IARC data base, the occurrence of p53 deletion or insertion frameshift mutations is about 12%, whereas the occurrence of missense mutation, nonsense mutation, and splice site mutations due to single base changes is about 87%. Therefore, oligonucleotide microarray analysis is a rapid and still quite accurate approach for detecting p53 mutations for a large number of clinical samples.

Similar to the AmpliChip CYP450 Test, the GeneChip p53 assay is also based on the hybridization of labeled target nucleic acids to a high density oligonucleotide microarray followed by fluorescence detection. It allows rapid sequence variation analysis of approximately 1300 bp of exons 2–11 of human p53 gene, including the flanking intron sequence of splice junctions. Probes on the array are arranged with both a “standard” tiling, as well as redundant, alternate tiling arrangements for increased robustness. Mutations are detected based on differences in hybridization pattern and intensities between a reference DNA microarray and a target DNA microarray using a proprietary mixture detection algorithm. For the p53 GeneChip assay, a GeneChip score is assigned for each position where a mutation is detected. This GeneChip score is based on the sum of probe sets for both sense and antisense strands and all tiling formats. The higher the score for a probe set contributing to a given base position, the higher the likelihood for this base to be mutated. Mutations that occur in codons with redundantly tiled oligonucleotides in addition to the standard tiling, usually have the highest confidence scores. Because the p53 GeneChip array was a first generation product of Affymetrix with oligonucleotide feature size of 50 µm² and approximately 65,000 oligonucleotides in total, redundant tiling was provided for only the most frequently mutated positions of the p53 gene (approximately 350 of 1300 positions). Therefore, the possible confidence score and the accuracy of calling mutations varies for each position, depending on the total number of probes tiled for that position. An arbitrary cutoff score of 10–13 was used for all sites which might cause false positive calls at certain positions, or false negative calls at other positions (Table 2). To increase the accuracy of overall mutation detection, a uniform highly redundant tiling strategy is needed for all positions, where more sets of probes for each position could increase the statistical power associated with a more precise cutoff. Improvements of microarray manufacturing over the years, has led to a great increase in the number of probes that can be synthesized in a given array surface area. The ever smaller size of unique oligonucleotide features, now allows the possibility of using over 200,000 probes to query these approximately 1300 positions in a future generation oligonucleotide microarray-based p53 resequencing test.

Previously published studies of microarray-based p53 sequencing (Table 2) have demonstrated a higher sensitivity and specificity for detecting single base substitution changes as compared to conventional DNA sequencing methods. However, all of these studies also showed that the array-based assay did not detect frameshift mutations due to multibase deletions or insertions. This was not surprising, since the p53 GeneChip was not designed to detect frameshift mutations other than single base pair deletions. There are only two probe sets containing single base deletion probes in the p53 GeneChip array, thus the highest possible GeneChip score of for these deletions cannot be higher than the cutoff of 10–13. Hence single base deletions were also missed by the microarray studies due to the cutoff value chosen (14,17,20). In a future generation of p53 resequencing microarrays, more single base deletion probes will need to be used to increase the probability of achieving a good discrimination ratio of perfect matched and mismatched probes for detecting single base deletion mutations, which represent about 30%–40% of all deletion mutations. Because of low prevalence of insertion mutations and large deletion mutations in cancer, they represent less than 10% of all p53 mutations; thus microarray sequence analysis will still be an effective sequencing method.

Another challenge for any tumor tissue-based p53 resequencing test is the sample source. Most cancer samples are formalin-fixed paraffin-embedded tissues (FPET), which could result in a high failure rate of 30%–40% for PCR amplification of p53 exons, especially the longer exon 4 (21). A robust genomic DNA extraction method for paraffin-embedded tissues is highly desirable, and methods have been developed that improve the efficiency of PCR amplification from FPET (22). Tailoring the assay procedure to accommodate the genomic DNA extracted from FPET is thus another challenge and requirement for the next generation of p53 resequencing test.

A microarray-based resequencing test can provide rapid and accurate sequencing analysis of the tumor p53 suppressor gene in a format that is readily adaptable to a clinical laboratory setting and permits the analysis of a large volume of clinical samples. Recently, the p53 GeneChip has been used to detect p53 mutations in over 250 head and neck tumors (23),188 non-small cell lung cancers

<table>
<thead>
<tr>
<th>Table 1. Performance of AmpliChip CYP450 Test*</th>
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<tbody>
<tr>
<td>Samples</td>
</tr>
<tr>
<td>2D6</td>
</tr>
<tr>
<td>Specificity (normal alleles)</td>
</tr>
<tr>
<td>Sensitivity (mutant alleles)</td>
</tr>
</tbody>
</table>

*Data were quoted from the package insert of AmpliChip CYP450 Test.
(NSCLC) (24), and 60 sarcomas (25) in studies directed towards establishing clinical indications for p53 mutations. Interestingly, analysis of NSCLCs revealed that not only the presence or absence of p53 mutations, but also the type and location of mutations is important for the prognosis of cancer patients (24). An improved, accurate, robust, and user-friendly p53 resequencing test will thus allow clinicians to efficiently analyze p53 mutation status in clinical trials and help to define its clinical utility.

**MICROARRAY-BASED EXPRESSION PROFILING DIAGNOSTIC TESTS: PROMISING FUTURE APPLICATIONS**

In conjunction with knowledge gained from sequencing the human genome, microarray-based expression profiling assays have given scientists powerful new tools for understanding the molecular biology of disease and the potential for differential diagnosis heretofore not possible with conventional tools. Microarrays permit the analysis of entire transcriptomes during biological state changes, adding new insight into functionality of genes and their networks of regulation in disease causation and progression. The expression of genes clearly changes as biological states change in response to stimuli or to reflect the molecular function of a particular cell or tissue type and as disease alters specific cells and tissues.

Early fundamental microarray studies helped define the appropriate types of questions, study designs, and data analysis methods that are essential to successful application of this powerful technology. While study design is critical to the validity and value of data generated, it is clear the data analysis methods are also pivotal to data integrity. Numerous published studies offer examples of the types of information that can be obtained in well-designed gene expression experiments (26–29).

During the past decade, thousands of examples of microarray-based gene expression studies that address the molecular underpinnings of clinical disease have been published. These studies can be broadly divided into the following four groups: (i) the use of microarrays for molecular classification (stratification of disease); (ii) the search of novel molecular targets of disease; (iii) the study of molecular prognosis of disease; and (iv) the study of molecular patterns that correlate with therapeutic response (e.g., pharmacogenomics).

Golub and colleagues (26) were the first group to demonstrate how gene expression signatures in blood cells of leukemia patients could define subgroups of this disease. The authors made the point the gene expression signatures could not only aid in cancer classification but also serve as a new tool to identify new classes of disease. Subsequent gene expression profiling for molecular classification and prognosis was demonstrated in pediatric and adult leukemias (30–33).

This approach has also been employed to demonstrate that several molecular subgroups exist among breast cancers (34). Others have utilized gene expression profiles from multiple types of tumors to identify tumor-specific markers (28,35). Further, such profiles apparently can be useful for determining the source of tumor (i.e., is it a primary tumor arising within the tissue or a distant metastasis from another site). This type of information would greatly aid in selection of the appropriate therapy. Furthermore, gene expression profiles have lead to the identification of gene expression signatures associated with metastatic potential (36). Interestingly, this signature was consistent across many different types of solid tumors. Because some primary tumors harbor this signature, such a profile might suggest that these tumors are poised for metastasis and an associated poor clinical outcome (36). These examples and others demonstrate the potential utility of gene expression patterns in stratifying neoplastic disease.

Researchers have utilized microarray technology to study disease states, in human disease and model systems, in order to gain insight into new therapeutic targets. Interestingly, the breast cancer analyses suggest that two of the five distinct molecular subgroups of breast cancer (luminal A and luminal B) contain the estrogen receptor (ER) positive tumors and that these patients are candidates for standard antihormone therapies. Another breast cancer subtype identified by gene expression profile exhibits Her-2 overexpression, and these are candidates for Her-2-targeted therapies such as Herceptin®, while yet another group described as “basal-like” contains a subset of patients that exhibit overexpression of epidermal growth factor receptor (EGFR) and may thus be candidates for EGFR therapies [tyrosine kinase inhibitors or monoclonal antibody (MAb) therapies]. These results provide encouragement for the notion that gene expression profiles of cancer and other diseases may help guide targeted therapies in the future.

Gene expression profiles have also been used to develop a molecular staging algorithm of colorectal cancer (37). The data provided suggest that expression-based molecular staging of colorectal cancer is more accurate than the currently accepted Duke's Staging system.

A recent investigation by Sorlie and colleagues points out that four separate studies, which attempted to identify expression profiles that correlated with patient outcome, had little to no overlap in the results (38). Perhaps this result is not surprising given that each study was conducted with different populations and different microarray technologies. However, a reexamination of these studies showed concordance when the data was examined in light of the “Sorlie” breast cancer subgroups. This finding verifies the value of

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**Table 2. Comparison of p53 GeneChip to DNA Sequencing Analysis**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Samples (No.)</th>
<th>p53 Mutations</th>
<th>p53 Mutations Identified by Both Methods</th>
<th>Mutations Missed by Chip (No.)</th>
<th>Mutations Missed by DNA (No.)</th>
<th>Cutoff Score</th>
<th>Sensitivity (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>100</td>
<td>57</td>
<td>31</td>
<td>11 (5 deletions, 6 substitutions)</td>
<td>15 (15 substitutions)</td>
<td>13</td>
<td>81</td>
<td>14</td>
</tr>
<tr>
<td>Ovary</td>
<td>108</td>
<td>77</td>
<td>57</td>
<td>6 (5 deletions, 1 substitutions)</td>
<td>14 (14 substitutions)</td>
<td>10</td>
<td>92</td>
<td>17</td>
</tr>
<tr>
<td>Bladder</td>
<td>140</td>
<td>79</td>
<td>62</td>
<td>9 (4 deletions, 1 insertion, 4 substitutions)</td>
<td>8 (8 substitutions)</td>
<td>Calculated</td>
<td>78</td>
<td>18, 20</td>
</tr>
<tr>
<td>Colon</td>
<td>20</td>
<td>13</td>
<td>13</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>100</td>
</tr>
</tbody>
</table>
utilizing gene expression profiles to identify molecular subgroups of disease, but cautious to nonstandardized attempts to study disease subtypes by this approach can lead to confusion. Another use of gene expression microarrays is demonstrated with the examination of ex vivo cultures of patient’s leukemia cells to ascertain drug response (27). This manuscript determined a gene panel that correlates with therapy response and accurately predicted the drug response of prospective patients.

It is likely that gene expression profiling will continue to play a central role in understanding the molecular biology of disease, identification of new disease therapeutic targets, prognosis of disease outcome, and therapy response. Continual improvements to the technology and data analysis methods will also allow these approaches to be developed as in vitro diagnostic tests. Although to date virtually all microarray tests based on differential gene expression have been applied in pure research settings, development of this promising technology into robust diagnostic applications will require much additional effort.

Validation of diagnostic applications of gene expression microarray technology will require well-designed, controlled clinical studies. In contrast to most research studies reported to date, much larger sample sizes are required. Genome-wide expression studies are susceptible to false positive results when many genes are assayed with minimal samples. Therefore robust statistical testing must be applied to clinical studies during validation. Methods to minimize false discovery errors are often employed to alleviate this issue, but studies must be powered by sufficient sample numbers.

Microarray technology is complex and requires many steps throughout the process from obtaining the clinical sample to data analysis. In order to develop assays that exhibit robust performance acceptable for a diagnostic test, it is imperative to define well documented and standardized assay protocols. Ideally many quality control steps will be embedded in the process that will serve as a gatekeeper preventing diagnostic error.

Specific methods of sample collection, preprocessing, and processing need to be part of clinical assay validation. Clinical specimens provide a challenge; many variables exist that may impact sample integrity and may thus effect gene expression profiles. For example, complex tissues, which give rise to tumors, offer the potential for sample to sample heterogeneity. Time and conditions that occur prior to nucleic acid extraction may add variability to assay precision. Diagnostic assay development and validation should address these issues in order to understand the tolerance of the assay and identify acceptable methods.

Diagnostic assays will require validated instruments, microarrays, reagents, and protocols for RNA amplification and labeling. Additionally, the software that operates the instruments, collects the data, and performs the data analysis will need appropriate validation. Even such details as heating devices used for enzyme reactions must be examined for influence on the diagnostic results. The method of classification and the actual genes that are selected for diagnostic classification will have an influence on how much these factors will impact overall assay performance.

Process controls and well-defined stop-go parameters need to be in place to verify the end results are reportable. External RNA controls are one source of such process controls. Placed in known quantities into clinical sample RNA, they can control for all steps from cDNA synthesis, amplification, labeling, and hybridization. High density microarrays that can examine thousands of genes simultaneously can accommodate multiple such external RNA controls. Hence, the use of multiple different spikes can also add dynamic range and quantitative discrimination controls.

This is just a very brief overview of the types of effort that will be required to bring this powerful research technology into routine diagnostic use. Certainly the effort is worth the potential payoff. The future holds the promise that individualized medicine will impact patients and society as a whole. If molecular tests can identify which drugs are more likely to prove efficacious, patients and health costs will all be beneficiaries.

COMPETING INTERESTS STATEMENT

The authors are employed by Roche Molecular Systems, an in vitro diagnostic manufacturer that develops microarray and PCR-based molecular diagnostic test kits similar to, or such as, those described in this article.

REFERENCES


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