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DNA STAT: the ability to perform clinical analysis in real time

'...STAT testing will need to be available if the true benefits of pharmacogenomic testing are to be realized...'

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The maturing field of molecular pathology has rapidly evolved from the daily investigations of the basic science laboratory to the most highly complex interrogations of the clinical diagnostic laboratory. Diagnostic applications driven by advancing technologies, and technologies driven by advancing applications, have created a paradigm of unprecedented demand on the clinical laboratory. As such, modifications to traditional laboratory medicine practices and performance criteria, including quality assurance, to accommodate these new technologies and applications continue to be developed.

One parameter that has been addressed by rapid developments in technology is turnaround time (TAT). The monitoring of TAT is typically performed by documenting the time of specimen collection, receipt in the laboratory and reporting of test results. TAT has been a black cloud for molecular diagnostic testing due, in part, to the detection chemistries used and the labor intensity of most molecular procedures. For example, Southern blot was once a method of choice for molecular diagnostic laboratories, albeit a very labor intensive one. This method includes: DNA extraction, restriction endonuclease digestion, gel electrophoresis, transfer, hybridization and detection. While the first five steps could be performed within 3–4 days, it was the radioisotopic detection step that required up to several weeks for results to be obtained. More recently, detection steps have been performed with chemiluminescent reagents that decrease the time for detection to just several hours. Thus, in this example, a single reagent change decreased TAT from several weeks to approximately 4 days.

However, the expansion of molecular diagnostic applications to more critical clinical dilemmas resulted in the need for still more rapid TAT. The introduction of PCR resulted in rapid replacement of many blotting techniques for various clinical applications, and TAT improved to a point where physicians could actually make clinical management decisions based on molecular test results. PCR testing includes: DNA or RNA extraction, amplification and detection. While this could be performed within several days, the detection step once again became the limiting factor with respect to TAT. Most recently, both amplified and nonamplified technologies, as well as automated extraction methods, have been developed that allow the laboratory to perform molecular diagnostic testing within 1 day of sample receipt. For example, whole blood can be extracted using the

BioRobot® EZ1 (Qiagen, Inc.) in less than 20 min, with subsequent real-time amplification results available within several hours or less. Thus, more rapid TATs are technically possible for most molecular-based testing.

Many would agree that the need for a traditional, same-day TAT for most molecular tests is not clinically necessary. However, with maturing efforts in infectious disease testing and new developments in oncology and pharmacogenomics, the clinical management of the patient will best be served by such rapid clinical testing. To this end, it is not inconceivable to think of molecular diagnostic testing on a STAT basis. STAT, from the Latin *statim*, meaning 'immediately', indicates the need for an immediate test result. In more developed laboratory medicine disciplines, this typically

means providing a test result within 1 h of receipt of the specimen. Clearly, molecular technologies and instruments are available to provide STAT testing.

Of the amplified technologies, none has revolutionized the molecular diagnostics laboratory more than real-time PCR. This method, using various chemistries for probe labeling or intercalating dyes, allows for the simultaneous amplification and detection of both DNA and RNA targets. Several real-time PCR instruments are commercially available for the clinical laboratory and most can perform reactions within 2 h. One disadvantage of many of these instruments is the requirement to run in batch mode; thus, a test designated as STAT would either wait to be run with other samples for the same target or a single patient specimen would occupy the instrument for the entire time needed to complete the run.

This disadvantage has been overcome by the recent ability to provide random-access real-time PCR testing for numerous applications using the SmartCyclerII[®] (Cepheid). This instrument includes 16 modules that can be programmed to run under the same conditions or they can each be programmed to run independently. Thus, multiple targets can be assayed simultaneously within 30–60 min in a random-access fashion. Random-access STAT testing can also be performed using the GeneXpert[®] (Cepheid). This instrument, using a proprietary cartridge, performs nucleic acid extraction and real-time PCR analysis.

The development of molecular infectious disease testing applications and automated instrumentation has resulted in the evolution of traditional quality assessment and performance characteristics that have been well established in other clinical laboratory disciplines. The ability to perform qualitative, quantitative and genotypic resistance testing has helped establish many of the traditional practices of analytical validation and quality assurance needed as automated instrumentation becomes more readily available for routine testing. In addition, the application of random-access STAT testing for molecular infectious disease targets offers the laboratory a unique opportunity to provide clinically relevant test results in real time, and thus have a major impact on patient management. Many of the current viral tests performed can be concluded in real time (<1 h) and US Food and Drug Administration-approved assays for group B streptococcus and methicillin-resistant *Staphylococcus aureus* are commercially available and completed within 1 h or less when run on the GeneXpert.

Paralleling molecular efforts in infectious disease testing, molecular oncology testing faces many of the same challenges. From optimizing specimen type and extraction methods to performing qualitative, quantitative and mutational analysis, the analytical criteria remain the same, as do many of the clinical criteria. Unlike molecular infectious disease testing where many pathogens and their target genes have been identified, much of this remains in development for oncology applications. Nonetheless, qualitative testing will offer diagnostic capabilities with unprecedented performance characteristics, as will our ability to

accurately quantify tumor cell populations. For example, the ability to monitor minimal residual disease in real time will allow for STAT testing whereby management of the cancer patient based on molecular testing could result in changes to therapeutic dosages or treatment regimens. In addition to real-time PCR, other nonamplified technologies such as Invader[®] (Third Wave Technologies, Inc.) interrogations and nanoparticle arrays (Nanosphere, Inc.) will allow clinical laboratories to perform mutation analyses associated with prognosis and therapeutic management options within clinically reasonable TATs.

The ability to provide such tailored or personalized drug therapy has led to an explosion in the field of pharmacogenomics. Understanding drug metabolism, transport and distribution with respect to polymorphisms in various genes can better determine which patients may be poor responders to a particular therapeutic, who may have an adverse reaction, and which dose may be optimal for a given patient. Many of the efforts in this field have focused on the cytochrome P450 super gene family that is responsible for the majority of drug metabolism. In addition, with many targeted small-molecule therapeutics becoming available for treating various cancers, it becomes important to identify and potentially quantify the target in a given patient's tumor. The hallmark example has become the identification of HER-2/neu gene amplification in breast cancer patients to determine eligibility for trastuzumab treatment. While TAT is somewhat of an issue, the STAT designation may not always apply to such situations. However, one can imagine a time when a patient is being seen in the physician's office or clinic that has been diagnosed with disease 'X'. Prior to prescribing a therapy, the physician will obtain a pharmacogenomic test to help identify the proper medication and dosage for this patient. It is at this point that STAT testing will need to be available if the true benefits of pharmacogenomic testing are to be realized.

Following in the footsteps of other, more established clinical diagnostic technologies and disciplines, molecular pathology is becoming more automated and routine for the evaluation of numerous disease types. A major disadvantage to the more traditional molecular techniques has been the inability for clinically useful TATs. Hence, a STAT designation was typically unbecoming of nucleic acid-based tests. More recent methods for performing automated DNA/RNA extraction and real-time amplification and detection have dramatically reduced the TATs for these assays. Hence, the ability to perform STAT molecular diagnostic testing has become available and will certainly change the way many patients are managed clinically.

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