# *Automation of Molecular Diagnostics*

In the 1970s and '80s a new technology emerged, known by the names of "Molecular Biology" or "Recombinant DNA." This new technology allows the detailed study of nucleic acids and has created a new dimension of our understanding of disease processes. Until 20–30 years ago, many diseases were evaluated biochemically at the level of protein or metabolite or with functional studies. For example, cystic fibrosis was known to involve a pathological process associated with abnormal regulation of electrolytes, and one of the major diagnostic tests was based on the analysis of sweat chloride. The protein involved, presumably an ion-pump, was not known. When the cystic fibrosis gene was cloned, the abnormality was precisely defined at the level of DNA, the tremendous clinical heterogeneity of the disease was explained, and the protein and its function became known. Moreover, reliable and simple testing has been devised that allows identification of the most frequent mutations within a day or two. The new testing can identify heterozygotes as well as homozygotes and can classify patients according to the precise location of their mutation. Similar examples can be cited for many other diseases.

Although recombinant DNA technology has been known for many years and its capabilities for routine diagnosis have long been recognized, it has also been associated with some disadvantages. Diagnostic laboratory scientists were not familiar with or trained in the general principles of recombinant DNA and were intimidated by such terms as plasmids, cosmids, bacteriophage, restriction enzymes, ligation, Southern transfer, sequencing, and cloning. Moreover, the techniques were intrinsically very slow, labor intensive, and qualitative.

The current scene is quite different. The younger clinical chemists now receive intensive training and are quite familiar with the new technologies of molecular diagnostics. Moreover, a single spectacular discovery has transformed the way molecular biology is performed. This discovery, the polymerase chain reaction (PCR), characterized by awesome amplification power and amazing simplicity, enabled scientists to change essentially everything that was done with conventional methods to the new technology. PCR is so simple that it requires only a few pipetting steps to mix the necessary reagents and samples. PCR is also powerful, in that it allows the study of any sequence of DNA and answers the following and many other crucial questions: (*a*) Is the DNA of interest present in the sample? This allows for diagnosis of infectious disease. (*b*) Is the DNA of interest normal or mutated? This allows diagnosis of genetic disease and study of malignant disease. (*c*) Is a particular gene expressed, and at what level? This allows study of mRNA species and cell growth and differentiation.

Over the last 5 years PCR has been optimized and become more quantitative, and some of its major limitations, including contamination, have been addressed and mostly solved. Currently, many laboratories are carrying out routine molecular diagnosis of genetic, infectious, and malignant disease, using molecular techniques. As the technology disseminates, scientists and companies are addressing vigorously the question of automation of such technologies. A few such instruments already exist, and other companies have such instrumentation under development. Automation can offer speed, improved precision and accuracy, better handing of contamination, and more productivity at a lower cost.

In this issue of *Clinical Chemistry*, Walker et al. (*1*) describe a method for detecting *Mycobacterium tuberculosis* DNA, based on strand displacement amplification (SDA) and transient-state fluorescence polarization (FP). Their method is unique, being based on a homogeneous assay format and having a few other interesting aspects.

What is SDA? One of many alternatives to PCR (*2*), SDA was first published in 1992 (*3*). It is suitable for exponential amplification of DNA targets but has some unique features that distinguish it from PCR. First of all, it is an isothermal technique, working at ~40 °C. This is an advantage over PCR because the instrumentation is simpler and cheaper than thermocyclers (essentially, a good water bath will work). SDA uses four primers instead of two for PCR, and utilizes two enzymes, a DNA polymerase and a restriction enzyme. SDA also requires the presence of a modified nucleotide, dATPaS. Operationally, SDA can achieve similar amplification factors as PCR ($10^6$- to $10^8$- fold), but the length of the product is restricted to <200 nucleotides, whereas PCR can now amplify targets a few thousand nucleotides long.

The mechanism of SDA amplification is relatively complicated. In general, the major features of the technique are as follows: (*a*) Instead of using temperature cycling to dissociate and reanneal primers, SDA uses two primers per target end (four in total), which are both extended. During the extension, the externally bound primer (bumper primer) displaces into solution the extended internal primer. (*b*) The restriction enzyme used can create a nick in the double-stranded extended product. The use of dATPaS ensures that the nick occurs on only one strand of the product. Once the nick is created, the DNA polymerase will initiate DNA synthesis, starting from the nick, and at the same time displace the already existing strand, which will serve as template for further exponential amplification.

The noteworthy aspect of SDA is that, although the assay principle is complex and requires the concerted action of two enzymes and four primers, the execution of the protocol is very simple. One need only mix the target with the four primers, deoxynucleoside triphosphates, with dATPaS and buffers. After denaturation once by heating and adding the two enzymes after cooling at 40 °C, the reaction proceeds efficiently so that in 2–3 h the target is amplified $10^6$- to $10^8$- fold.

How could the amplified product be detected? Conventionally, one can use a radiolabeled detector primer that binds to the SDA product and then extended once, at the end of the protocol, by the Klenow DNA polymerase enzyme. The labeled product can then be resolved on polyacrylamide gels and detected by autoradiography (*2*, *3*). Alternatively, one can use sandwich hybridization and chemiluminescence (*4*). However, in the present paper Walker et al. used an oligonucleotide labeled with the fluorescent probe La Jolla Blue and measured fluorescence polarization (FP). During SDA, the probe, originally single-stranded (generating low FP signal) becomes double-stranded (resulting in increased FP signal), thus offering a simple means to monitor the amplification by continuous FP measurements.

What are the capabilities of this technique? It can detect one or a few copies of the target sequence in a few hours by using a simple temperature incubation. This is a most impressive achievement. The automated protocol further ensures produc-

tivity, and the closed system approach should minimize the chance of contamination, an issue of paramount importance when using any exponentially amplifying technique for routine diagnostics.

What are the limitations of the technique? Walker et al. used two different protocols to perform SDA coupled to FP. The endpoint protocol requires SDA to be performed first and then FP measured afterwards. This method has limited potential because the final signal is generally not related to the initial target concentration. In most samples, the detector probe is fully consumed and becomes double-stranded (saturation), generating the same signal. Apparently, the two-step SDA/FP protocol was used because of the current unavailability of a suitable instrument that could perform a single-step protocol. The limited data presented with the single-step protocol are more encouraging and show that the rate of change of FP during SDA is somehow related to the amount of initial target. The authors recognize limitations in this procedure as well. For example, one problem is that, even with complete conversion of probe, the FP value will change by only twofold. Thus, one must be able to measure very small changes in FP with excellent precision to distinguish two different target concentrations. Second, the exponential phase of the amplification is not monitored efficiently, because most of the FP change is observed at the plateau phase, making the method semiquantitative at best. Third, if the clinical samples contain SDA inhibitors that change the kinetics of amplification, the observed change in FP may not accurately represent the initial target concentration. Such inhibitors do exist in some samples.

The report by Walker et al., based on target amplification and nonisotopic detection, is a valuable addition to existing automated and nonautomated molecular diagnostic techniques. Clearly, we are witnessing the growth of another major diagnostic technology that will undoubtedly expand further to supplement already successful technologies such as immunodiagnostics. The outstanding feature of the new technology is its surprisingly simple execution despite the complexity of the concepts involved. Indeed, clinical chemists should be encouraged with this report, which seems to place this new technology in the category of the automated analyses used in the biochemistry laboratory for some 10–15 years. Fluorescence polarization is one of the most successful homogeneous immunoassay formats used for therapeutic drug monitoring and hormone analysis. Interestingly, other automated molecular diagnostic methods are using the principles of enzyme immunoassay for detection. These approaches allow one to speculate that the future automated molecular diagnostics machinery may be operated by the same technologists and the results interpreted by the same professionals currently responsible for immunodiagnostics and therapeutic drug monitoring.

*Note added in proof.* Since the preparation of this editorial, Walker et al. have reported a more specific, sensitive, and efficient SDA protocol based on thermostable enzymes. This new protocol is applied with detection systems based on $^{32}$P *[5]* and fluorescence polarization *[6]*.

**References**

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