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Developing a fully automated instrument for molecular diagnostic assays

Second-generation nucleic acid amplification testing systems present unique challenges to IVD manufacturers.

By: Matthew C. Friedenberg, Jerzy Macioszek, Robert Scalese, Craig S. Hill, and Valerie M. Day

In recent years, nucleic acid amplification tests (NAAT) for infectious diseases have come into widespread use in many clinical microbiology and blood-screening laboratories. A number of these tests have been approved by FDA and then commercialized for detecting infectious disease organisms in clinical samples. Such tests have had a major impact on clinical laboratory diagnostics. This is due to the higher sensitivity of NAATs compared with conventional tests and their ability to detect organisms that are difficult or impossible to culture.

Blood-screening laboratories have already been using NAATs to detect pathogens in blood donations while a donor is in the preseroconversion window phase, is infected with immunovariant viruses, or is a nonseroconverting chronic carrier. Direct, sensitive detection of viral nucleic acids has decreased the incidence of transfusion-associated infections.

Although first-generation NAATs have demonstrated excellent performance, many laboratories have been slow to adopt the tests because of increased reagent costs and labor requirements, and concerns about carryover contamination. Larger laboratories have had difficulties with NAATs due to the low throughput of many of the test systems and the requirements for multiple instruments to handle large daily test volumes. Some of the current first-generation NAATs have been partially automated in an attempt to reduce the tests' complexities and labor requirements. While most of the semiautomated instruments have automated the amplification and detection steps of the assay, the sample-processing step has not been automated, which is typically the most troublesome step.

In addition, first-generation NAAT sample-processing procedures are labor intensive, often requiring centrifugation and numerous manual pipetting steps. They are not effective in removing interfering substances from clinical specimens, which can inhibit the amplification reaction and produce false-negative results.

The manual, complex nature of first-generation NAATs also has a direct impact on laboratory costs. Such costs include the need for more experienced and higher-paid technologists to run the tests. The ongoing shortage of laboratory personnel has made it difficult for laboratories to dedicate additional resources to perform NAATs. Run failure, contamination, and inhibition problems can lead to higher costs due to repeat testing of samples with questionable results. This reduces efficiency and causes delays in reporting results. Repetitive-motion injuries, such as carpal tunnel syndrome, can occur from manual pipetting and result in higher costs to the laboratory due to absenteeism and workers compensation settlements.

The Tigris System

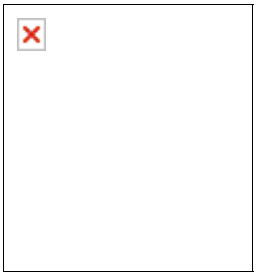
The Tigris system by **Gen-Probe Inc.** (San Diego) addresses the laboratory needs described above by providing full automation of NAATs and processing of up to 1000 tests per day (see Figure 1). As the nucleic acid extraction process has been integrated into the design of the system and standardized for each assay, primary sample tubes can be placed directly on the system for analysis. Multiple specimen types (e.g., urogenital swabs and urine) can be processed within the same work list.

Figure 1. The Tigris system by Gen-Probe Inc. (San Diego).

To minimize sample handling, the Tigris system provides cap-piercing functionality to process capped samples directly from collection sites. This eliminates any need for sample preparation and cap removal for such specimens. The system also offers the ability to set up reagents for up to four different assay kits at one time and allows work lists of different assays to be run in a back-to-back mode.

Hardware and Software Design

The Tigris system is divided into the upper and lower bay sections. The upper bay consists of a specimen queue, a multitube unit (MTU) input station, an MTU loading station, and MTU mixers, incubators, magnetic separation stations, and reagent pipettors, a refrigerated reagent bay, an oil addition station, a luminometer, an MTU deactivation station, and other modules (see Figure 2). The lower bay contains common fluids that are shared between different assays, and storage areas for liquid and solid waste.



[5]

Figure 2. CAD drawing of the upper deck of the Tigris System, which contains the sample processing and assay processing modules (click to enlarge).

The Tigris system's software architecture includes the following elements (see Figure 3). The master controller software is responsible for the overall control and operation of the instrument and consists of a user interface component that provides a graphical user interface for the operator (see Figure 4). The assay definition module contains all of the information that is specific to a given assay and provides flexibility in adding menu options to the system. The embedded controller manages all of the individual devices on the instrument.

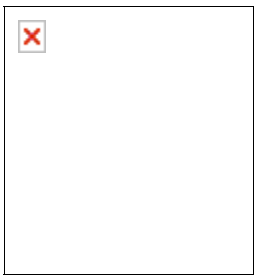


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Figure 3. Overview of Tigris software architecture (click to enlarge).

Development Approaches

Requirements Management. In IVD product development, requirements management is critical to a successful program. A complex instrumentation program such as an automated NAAT instrument has thousands of requirements that must be traced from customer input to design requirements, assigned to appropriate functions for implementation and verification, and finally traced to appropriate verification evidence. A development team should implement a requirements management database that enables the team to sort requirements by varied categories and status, and provides the requirement trace matrices to link design output to design input.

Design Reviews and Decision Tools. As part of any design control and as a sound business practice, cross-functional design reviews are used to evaluate designs, balance trade-offs, and make recommendations. Concept-screening methods are used to identify and weight design criteria and score options.[1-3]

Risk Management. Risk management is a fundamental component of automated NAAT instrument development efforts. A top-down, system-level approach ensures human factors, hardware, software, samples, reagents, consumables, and ancillaries are all considered relative to potential risks to patients, operators, bystanders, and the environment. A fault tree analysis provides a graphical representation of the hazards and their relationships to one another. A risk analysis identifies the risks associated with each hazard. It is supplemented by a human factors hazards analysis, software hazards analysis, and failure modes and effects analysis that provide detailed analyses of all potential sources of risk.

Engineering Solutions. One major challenge in designing an automated NAAT instrument is achieving a high instrument throughput while controlling carryover contamination, a risk that is intrinsic to all amplification technologies. NAATs are more complex than the conventional immunoassays that are widely employed nowadays to test for a variety of infectious diseases. A typical NAAT requires isolating nucleic acids from human specimens by removing all inhibitory substances, amplifying target nucleic acids, detecting amplified products, and inactivating or containing the amplicon. Each of these steps could be as complex as a stand-alone immunoassay. As a result, the time that is required to process a single NAAT is approximately 3.5 hours, whereas automated immunoassays can be completed in 45 minutes or less.

[7]

Figure 4. The Tigris system software provides a graphical user interface to facilitate interaction between the operator and the instrument. An example of the inventory status screen, used for managing reagents and consumables, is shown here (click to enlarge).

The throughput goal for the Tigris system was 1000 samples processed in 14 hours or less. This goal required designing a system that is capable of processing more than 100 samples per hour through numerous assay steps. This level of throughput would be difficult to achieve by processing a single sample at a time. This led to the MTU concept that combines five separate test tubes into a single disposable unit. Running an assay in minibatches of five tests minimizes the required number of movement steps and simplifies reagent addition by allowing a delivery of reagents to five tests with a single aspiration step.

Fully automated NAAT instruments with a continuous mode of operation require the full harmonization of individual processing steps. As a result, the maximum number of tests that can be processed within an hour is a function of the duration of the longest step. It requires identification and then timing optimization of all assay steps that are critical from the throughput perspective. In some cases, multiple individual modules have to be provided to execute a given step in a parallel mode. This was the case for the magnetic separation station: five identical modules were installed on the instrument to accommodate the 12-minute interval that is required to process one MTU through this step.

Another major challenge facing automated NAAT instrument developers is the target carryover and overall contamination control. A typical carryover requirement for immunoanalyzers is in the range of one part per million. Because of its ability to detect target levels as low as a few copies, this level of carryover is unacceptable for a fully automated NAAT system. At the same time, the amplification process generates as many as 1012 copies of amplified target sequences, or amplicon. Any amplified product that is carried over to a preamplification step can generate a false-positive result. As a result, the carryover requirement is reduced to one part per billion.

One element of the Tigris design is that all amplicon is inactivated at the conclusion of assay processing by adding a sodium hypochlorite–based deactivation reagent into the MTU. A

deactivator fluid dispense verification system was developed to monitor this critical instrument function.

Although inactivation of amplicon is an effective mitigator for carryover contamination, the following additional precautions were built into the system to further reduce the contamination risk:

• Transcription-mediated amplification (TMA) technology is designed so that in all processing steps following the target capture step, when assay contamination risk is the highest, assay reagents are added directly into the MTU tubes and no transfer of amplicon is required. This minimizes the risk of carryover contamination during pipetting.
• Oil is used as an overlay in each tube, reducing the contamination risk through aerosol creation.
• Mixing steps have been optimized to eliminate splashing or aerosol creation.
• Dedicated pipettor probes and wash cups are used for reagent dispensing during the pre- and postamplification processing steps.
• During the dispensing of reagents, pipettor probes are positioned above the interior tube surface, isolating them from areas where potential contamination can occur during MTU mixing.

Product Validation and Commercialization

Clinical Diagnostics. In December 2003, the Tigris system was cleared by FDA for use with the Aptima Combo 2 (AC2) assay. Several studies have compared the AC2 assay on the Tigris system with semiautomated NAAT procedures. One group of researchers tested 1991 specimens from 1061 patients on the Tigris and semiautomated instruments.[4] The study showed good positive and negative agreement between the two systems for urine and urogenital swab samples. Positive agreements ranged from 91.4 to 98.8% for chlamydia and gonorrhea. Negative agreements for both organisms ranged from 99.8 to 100%.

At the same time, 26 discordant results were observed from the 1991 specimens tested. Most of these discordant results were positive with the semiautomated procedure and negative on the Tigris system. Retesting these samples with the AC2 assay and with separate chlamydia and gonorrhea TMA assays that target alternate rRNA regions showed that 75% or more of the discordant results were resolved in favor of the Tigris system. The researchers suggested that this discordance was the result of operator-related errors on the semiautomated instruments, which were avoided by using a fully automated NAAT system.

A similar comparison study was conducted at a large clinical diagnostic reference laboratory.[5,6] This laboratory's testing volume was approximately 1100 chlamydia and gonorrhea samples per day, which included endocervical swab samples and urine samples. Positive and negative agreement between the instruments for chlamydia and gonorrhea ranged from 98.8 to 100%. Of the 8000 samples run, there were only 10 discordant chlamydia samples and 1 discordant gonorrhea sample. Retesting these samples with separate chlamydia and gonorrhea TMA assays that target alternate rRNA regions resolved all but one of the chlamydia samples in favor of the Tigris system. This study further supports the idea that a fully automated NAAT instrument reduces the chances of operator error, and suggests that adverse events such as carryover contamination are minimized when full automation is employed for NAAT assays.

Blood Screening. Current semiautomated methods that are used for blood screening do not have sufficient throughput to support testing of individual donations. As a result, current procedures involve pooling plasma specimens prior to screening. If the sample pool is reactive, the individual samples that comprise the pool are tested to identify the reactive donations. As a result of this pooling, there is an intrinsic risk, although low, that viral nucleic acids are diluted below the detection limit. Employing fully automated, high-throughput NAAT systems enables routine screening of individual blood donations.

The Procleix Tigris system is the instrument that is used in the blood screening market. The system and blood screening assays are distributed by **Chiron Corp.** (Emeryville, CA). Gen-Probe has submitted this system for FDA review using the Procleix Ultrio and West Nile virus (WNV) assays. In addition, the Ultrio assay has been granted a CE mark in accordance with the European Union Directive 98/79/EC (the IVD Directive), and it is used overseas with the Tigris system.

During the clinical trials, the performance of the Ultrio assay and HIV-1, hepatitis C virus (HCV), and hepatitis B virus (HBV) discriminatory assays on the Procleix Tigris system were evaluated. For the study, a total of 6006 plasma specimens that had tested negative with licensed HIV, HCV, and HBV assays were tested on the Tigris system with the Ultrio assay and the three discriminatory assays. Ten of the specimens (0.17%) were initially reactive. None of the initially reactive specimens were reactive upon repeat testing, demonstrating the high clinical specificity of the system.

The sensitivity of the Ultrio assay was evaluated in separate HIV-1, HCV, and HBV seroconversion panels using both the semiautomated and Procleix Tigris system platforms. The testing on the semiautomated system showed that the assay can detect HIV-1 infection in individual seroconversion panels from 4 to 22 days earlier than the most sensitive HIV-1 p24 antigen test. The Ultrio assay was also able to detect HCV infection from 0 to 52 days earlier than the HCV antibody test. For HBV, the Ultrio assay was able to detect the infection from 10 to 29 days earlier than the HBV surface antigen tests.[7]

While data on the Procleix Tigris system have not yet been published, they were similar. As with the original Procleix HIV-1/HCV assay approved by FDA, the data indicated that applying fully automated NAATs for blood testing on individual donations will further decrease the risk of infection through transfusion from donors during the preseroconversion window period.

Clinical trials have also been completed for the WNV assay. In addition, since 2003, more than 19 million units of donated blood have been screened for West Nile virus on the semiautomated and Tigris platforms under an investigational new drug (IND) application. This testing has intercepted more than 1200 WNV-positive donations, preventing transfusion of contaminated blood into as many as 3600 blood recipients.[8]

Conclusion

Fully automated nucleic acid amplification testing has the potential to revolutionize not only infectious-disease testing and blood screening, but also many other areas of clinical testing in genetics, cancer, and other chronic human diseases. However, although new FDA-approved NAATs are becoming available, many laboratories have difficulties implementing the tests due to the high labor requirements, contamination concerns, and inhibition problems of the first-generation tests. Larger laboratories are particularly hindered by the high labor requirements and low throughput of many NAAT systems. New, fully automated instruments alleviate such constraints by increasing test throughput, reducing operator errors, and decreasing labor requirements, thereby reducing the overall costs of NAATs. Second-generation, fully automated technologies are making NAATs a reality for more laboratories, leading to improvements in healthcare through more-accurate, rapid, and cost-efficient diagnostic tests.

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