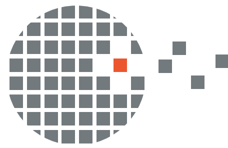

Real-Time Detection of Clinically Relevant Bacterial Pathogens: Description and Preliminary Performances of a New System

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Revised Abstract

Real-time detection of pathogens in clinical microbiology is still an exception. It requires both very sensitive and specific methods combined with characteristics of ease of use, robustness and cost effectiveness. The system described hereafter carries all these features. Sensitivity and specificity are provided by the system concept based on the real-time nucleic acid testing (NAT). Ease of use, robustness and cost effectiveness are provided by the system design which combines ready-to-use room-temperature stable reagents, reduced hands-on time, random access instrument (Smart Cycler®) and dedicated software. The first applications target three important pathogens which require real-time detection: Group B streptococci (Group B strep), methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

A unique standard protocol applies to the detection of these pathogens: (i) the specimen is collected on a swab proven to be NAT compatible – (ii) the swab is eluted into a lysis buffer – (iii) the eluted bacterial cells are lysed through mechanical and chemical steps– (iv) an aliquot of the lysate is transfered into the Smart Cycler reaction tube where the NAT occurs; total procedure takes less than 1 hour: 15 minutes for (i) to (iii) and 35 to 40 minutes for (iv). Specific targets have been designed for the three above pathogens either from literature or from new genetic analysis: *cfb* for Group B strep, the *SSCmec* for MRSA and the *van A* and *van B* genes for VRE. Primers and detection probes (beacons) have been designed and validated through demonstration of sensitivity and specificity.

Analytical sensitivity of the detection ranged from 5 to 20 copies of genomic DNA on pure models. Analytical sensitivity on matrix of clinical specimen spiked with known concentrations of bacteria demonstrated respective sensitivities of 2000 CFU/specimen for Group B strep, and VRE and of 200 CFU/specimen for MRSA. Preliminary data collected on real clinical specimens exhibited clinical sensitivities of 97.2% and 100%, for Group B strep and VRE respectively. Sensitivity of MRSA could significantly vary according to the standard method of comparison.

The use of external controls (positive and negative) and of an internal control indicated less than 5% of unresolved results were due to inhibition of NAT. The conclusions of these studies demonstrated that this system should offer the performances required to provide clinically relevant diagnostic information rapidly and reliably.

Introduction

Timely diagnostic of pathogens in clinical microbiology is still not routinely done. Indeed the vast majority of testing is performed through culture-based methods which take at least two days before the availability of the final result. Even though automation brought significant progress, time to result has not changed significantly.

The real breakthrough will come from direct testing of the clinical specimen which will enable the physician to support his clinical decision.

Molecular technologies are making this direct detection possible. Thanks to the progress occured during the past few years: real-time detection of amplicons, miniaturization of disposables, integration of optoelec-tronic devices.

Moreover, in order to fulfill the routine requirements, these new methods must be both very sensitive and specific as well as exhibiting characteristics for ease of use, robustness and cost effectiveness.

We describe hereafter a diagnostic system currently in development which carries all these features.

Description of the System

The system is composed of (i) a real-time PCR instrument, (ii) ready-to-use reagents for specimen preparation, target amplification and detection and (iii) a dedicated interpretation software.

The instrument used is the Smart Cycler (Figure 1) which uses the I-CORE® technology (Figure 2). (Cepheid, Sunnyvale, California). The main features are:

- Multiplex capability through four optoelectronics channels
- Random access capability provided by the I-CORE modules
- Robustness offered through independent modules

The reagents are composed of two parts:

- Specimen preparation allowing rapid extraction of nucleic acids.
- Reaction tube containing the amplification and detection reagents including the internal control (Figure 3). Reaction tubes containing external positive and negative controls.

The software includes:

- The interpretation algorithms
- A graphical user interface

The protocol, from a specimen collected on a swab (Figure 4) is described as follow:

- Elution in a sample buffer
- Transfer into the lysis reagent for chemical/mechanical/thermal lysis
- Transfer of extracted nucleic acids into the reaction tube with a diluent added
- Transfer of reaction tube into the I-CORE for amplification/detection run

Figure 1: The Smart Cycler System.

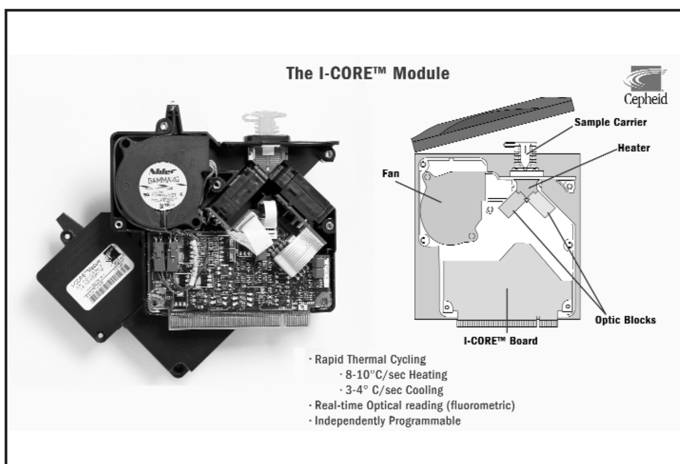

Figure 2: The I-Core Module.

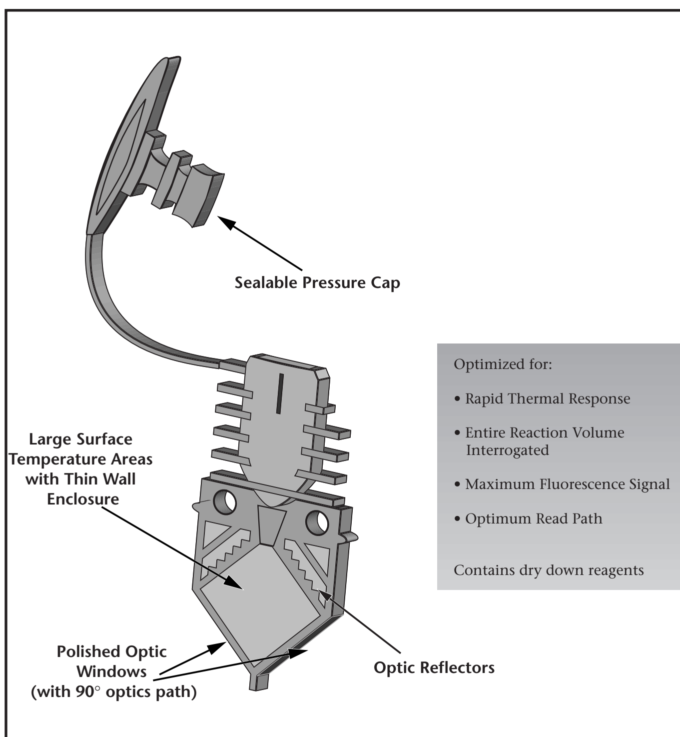

Figure 3: The Reaction Tube.

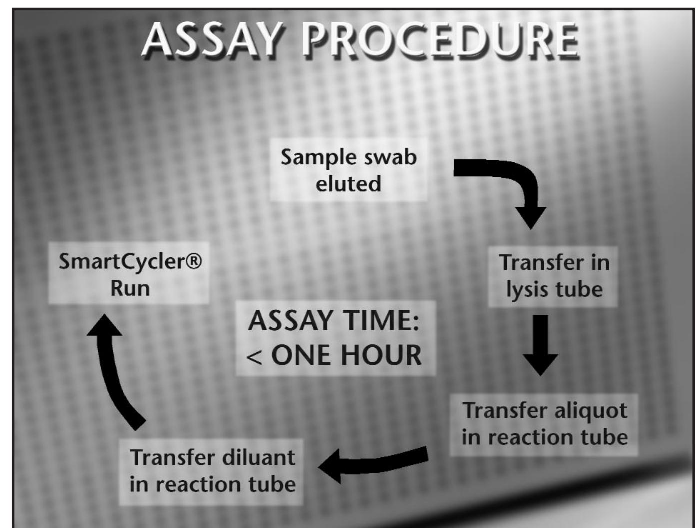

Figure 4: Assay Procedure.

Description of Assays

Description of assays in development

Examples are given for three pathogens: Group B streptococci (Group B strep), methicillin-resistant *Staphylococcus aureus* (MRSA),and vancomycin-resistant enterococci (VRE).

Molecular targets have been defined for these pathogens either from literature or new genetic analysis: *cfb* for Group B strep, *SSCmec* for MRSA, *vanA* and *vanB* genes for VRE.

Primers and detection probes (beacons) have been designed and validated through demonstration of sensitivity and specificity.

Preliminary performance

Analytical sensitivity of the detection ranged from 5 to 20 copies of genomic DNA on pure models.

Analytical sensitivity on matrix of clinical specimen spiked with known concentrations of bacteria demonstrated respective sensitivities of 2000 CFU/specimen for Group B strep and VRE and of 200 CFU/specimen for MRSA.

Preliminary data collected on real clinical specimens exhibited clinical sensitivities of 97.2% and 100%, for Group B strep and VRE, respectively. Sensitivity for MRSA could significantly vary according to the standard method used for comparison.

The use of external controls (positive and negative) and of an internal control indicated less than 5% of unresolved results due to inhibition of NAT.

Compliance to the needs for routine use

The hands-on time is reduced :

- The nucleic acid extraction takes approximately 15 minutes and may vary according to the assays.
- The PCR assay on the Smart Cycler takes approximately 40 minutes upon completion.

The practicability is optimized :

- The number of reagents is limited to four ready-to-use tubes and two external positive and negative controls.
- Reagents are packaged in unit dose thus reducing handling and preventing risk of cross-contamination.
- The closed nature of this system would also significantly decrease the risk of amplicon contamination in the testing area.
- Storage is done at room temperature or between 2° to 8°C.

Time to result is minimized :

- Total assay time takes one hour or less. The operational time for specimen transportation and registration has to be added to that time.
- The random access characteristic of the instrument and the unit dose format of the reagents enable STAT analysis to be performed.

Conclusions

These studies demonstrated that this system should offer the performances expected for the routine diagnostic in clinical microbiology.

- Robustness
- Ease of use
- Random access
- Analytical performance
- Time to result ≤ 1 hour
- Reduced risk of contamination

Additional performance evaluations are on-going for confirmation of these initial data.