Multiple Applications and Business Impact of a Novel Rapid PCR-Based Bacterial Identification Technology

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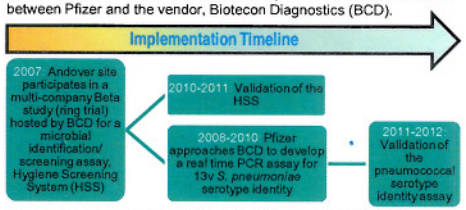
ABSTRACT

Polymerase Chain Reaction (PCR) is widely used for various laboratory analyses in the pharmaceutical industry. An emerging novel application of this technique is rapid microbial identification, where PCR kits designed for detection of specific microorganisms are used in conjunction with an instrument that has the capability to simultaneously execute PCR cycling and amplicon detection at multiple fluorescent wavelengths. The technology employs multiplex fluorescence-based Real-time PCR using primers and probes specific to unique, conserved microbial gene sequences. In addition to a real-time PCR microbial identification assay (Hygiene Screening System, or HSS) presently employed in the Pfizer Andover Quality Control Microbiology laboratory, we are implementing three applications of a proprietary assay designed for confirmation of the serotype identity of *Streptococcus pneumoniae* culture used in the manufacturing process for a pneumococcal vaccine intermediate, replacing an antibody-based assay (agglutination). Implementation of these assays has reduced the amount of bench test reagent consumption, analysis time, and has eliminated a complex standard/control preparation, qualification, and maintenance scheme for antibodies and controls (for agglutination). Through the use of one novel platform technology for multiple applications, assay objectivity is enhanced and results are obtained in hours instead of days; a direct impact to manufacturing cycle time.

INTRODUCTION

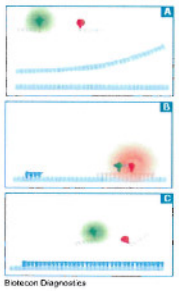
Implementation of the real time PCR assays has been a collaboration between Pfizer and the vendor, Biotecon Diagnostics (BCD).



The BCD assays are inexpensive compared to other microbial identification assays, easy to use, and are performed on the same instrument (LightCycler) using experiment-specific programs designed for each assay. Each program is established as a software macro so no user configuration is required for routine use.

This poster provides a technology overview of the BCD/Roche technology, a summary of the BCD methods employed by Pfizer, and comparison between the agglutination and pneumococcal serotype identity methods to demonstrate the business impact of this technology.

FLUORESCENCE-COUPLED PCR FORMAT



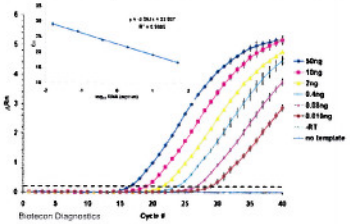
A: Fluorescence-coupled PCR technology consists of two sequence-specific oligonucleotide hybridization probes labeled with different fluorescent dyes, added into a PCR mixture with primers.

B: The probes hybridize to the target sequences on the amplified DNA fragment during the annealing phase, bringing the two dyes (donor and acceptor) into close proximity. FRET (Fluorescence Resonance Energy Transfer) occurs and the acceptor dye emits fluorescent light at a certain wavelength, which is directly proportional to the amount of target DNA generated during the PCR process.

C: The probes are displaced during elongation and denaturation steps when temperature is raised.

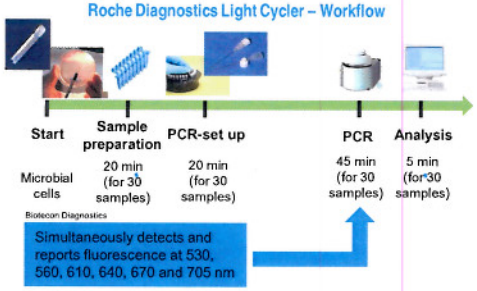
REVIEW OF REAL-TIME PCR

In all Real-time PCR assays, the amount of fluorescence detection is correlated with PCR amplification. Only the presence of a specific amplification product causes an increase in fluorescence.



As PCR progresses, the amount of PCR product is monitored for each cycle by fluorescence. If fluorescence is plotted against cycle number, the accumulation of PCR products can be visualized on a PCR "growth" curve.

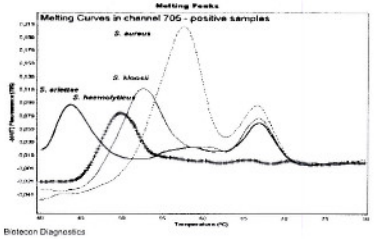
Roche Diagnostics Light Cycler – Workflow



MELTING CURVE ANALYSIS

A unique feature of fluorescence-coupled RT-PCR is the melting curve analysis, which is used for further differentiation (e.g., species and strain/serotype level). Melting curve analysis determines the temperature at which the probes and target DNA melt/separate when heated. While multiple species/serotypes may have the same gene target, the affinity of the probes to the target can differ. The more stable the hybridization between the probe and the target sequence, the higher the melting temperature.

Melting Curve Analysis for HSS



HSS FOR ROUTINE QC MICROBIAL IDENTIFICATION

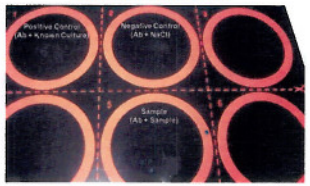
The HSS is a valuable tool for fast and reliable detection of *Staphylococcus* (including *S. aureus*), *Micrococcus*, and *Corynebacterium* in the Andover laboratory These three genera comprise the majority of the flora recovered in the Andover Manufacturing facilities. The implementation of the HSS technology has transformed the microbial identification program by replacing classic phenotypic methods for isolates that require a genus level identification based on area classification and microbial count.

Traditional phenotypic methods may require additional incubation time based on age of culture, specialized media, may produce false positive results and require the use of controls. The HSS is not media dependent, isolates can be run upon determination of a pure culture, and the results are unambiguous as outlined in the melting curve chart example.

PFIZER 13v PNEUMOCOCCAL SEROTYPE IDENTITY TESTING

The agglutination assay is performed to verify the serotype identity of in-process samples from a pneumococcal vaccine intermediate.

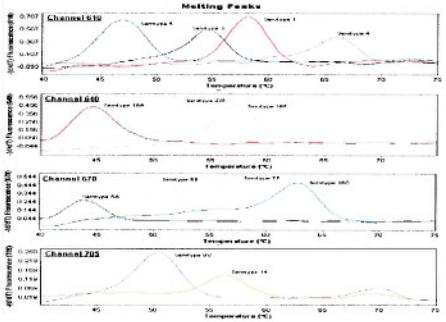
Antibody Agglutination



An aliquot of serotype-specific antibodies are combined with *S. pneumoniae* cells. Agglutination (clumping) occurs when the antibodies bind to the polysaccharide antigen present on the cells. The clumping is subject to analyst interpretation.

The pneumococcal serotype identity assay, which is proprietary to Pfizer, employs multiple primer/probe sets within a single PCR mixture to target specific gene regions of the *S. pneumoniae* DNA. The 13v serotypes are further differentiated through melting curve analysis; each of the 13v serotypes has a distinct melting temperature. Multiplex PCR and melting curve analysis allow for detection of any of the Pfizer 13v *S. pneumoniae* serotypes using just one kit.

Melting Curve Analysis for Pneumococcal Serotype Identity



COMPARISON OF AGGLUTINATION AND PFIZER 13v PNEUMOCOCCAL SEROTYPE IDENTITY

There are many advantages of the Pfizer 13v pneumococcal serotype identity method as compared to the agglutination method as detailed in the table below:

Rationale	Agglutination Method	Pfizer 13v Pneumococcal Serotype Identity Method
Labor	Very laborious to maintain antibodies (~3.5 hours 2x/year) and positive controls (~12 hours 2x/year), antibody production/sourcing is issue, extensive time spent on inventory control	No antibody sourcing or maintenance, ready-to-use kits, inventory can be readily managed
Materials	Two reagents (antibodies and NaCl), antibodies expire in 7 days at 2-8°C once thawed	One reagent (DNA extraction buffer), longer expiry and ambient storage
Analysis	Subjective, up to 2-4 day assay, phenotypic, results in <1 hour	Objective, genotypic (serotype-specific probes), results in 1.5 hours

The 13v Pfizer pneumococcal serotype identity assay has been successfully validated in the Andover, MA Quality Control laboratory as an identity method for pneumococcal vaccine intermediate, plated cultures, and frozen cultures (e.g., cell bank vials). The assay is in the process of being filed with regulatory agencies as an alternative method for serotype identity testing of pnuemococcal vaccine intermediate samples.

CONCLUSIONS

The real time PCR-based rapid identification platform developed by BCD/Roche Diagnostics has proven to be a valuable technology for routine use in the QC laboratories within pharmaceutical environments. The HSS has eliminated the need for time-consuming phenotypic methods to identify common flora recovered in the manufacturing environment. The Pfizer 13v pneumococcal serotype identity application will reduce the subjectivity of the serological based agglutination assay, will remove the labor required to maintain the antibodies and positive controls, and the time to result will be reduced to hours vs. days. Such PCR applications are simple to perform, the assays are specific to target DNA, and the result interpretation is objective.

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