ApplicationForum

Antibiotic Resistance Monitoring and Detection (ARM-D™) PCR Kits: ARM-D™ for β -Lactamase ID – Technical Note

Detection of ESBLs, MBLs, KPCs, and plasmid-mediated AmpCs, using the β -Lactamase ID kit to identify antibiotic resistance in Gram-negative pathogens

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Overview

β -Lactam resistant Gram-negative organisms, producing multiple β -lactamases, are difficult to identify phenotypically and necessitate more specific detection methods to identify clinically important β -lactamases. Genetic identification of these resistance mechanisms is critical for infection control and can guide selection of the most efficacious antimicrobial therapies. PCR-based molecular tests are useful for detecting these resistance mechanisms but often require several hours to complete the assay. The purpose of this study was to demonstrate the utility of an optimized 18-minute multiplex PCR assay kit that detects 9 different β -lactamase gene families using the Philisa® Thermal Cycler, Streck's rapid PCR platform. The ARM-D for β -Lactamase ID kit provides two control reactions that detect genes encoding members of the following β -lactamase families: IMP-1, NDM, OXA-48, CTX-M-14, CTX-M-15, CMY-2, DHA, VIM, and KPC. An endogenous internal control is also included to reduce false negatives; it targets a conserved region common in Gram-negative bacteria. The β -Lactamase ID kit is designed to facilitate fast and accurate genetic detection with a user-friendly protocol. As such, the kit also includes a single tube containing all primers in a 10X Primer Mix and a custom DNA polymerase supermix. The ARM-D 2X Supermix, developed in collaboration with New England BioLabs®, is optimized for amplification of purified DNA from clinical isolates harboring the kit-specific resistance mechanisms. The ARM-D 2X Supermix includes DNA polymerase, PCR reaction buffer, dNTPs, and agarose gel loading dye. Samples can be pipetted directly from the PCR tube to an agarose gel for resolution of PCR products associated with clinical isolates and compared against the kit's DNA controls.

The β -Lactamase ID kit has been validated by extensive testing using previously characterized clinical isolates with the Philisa Thermal Cycler. Total PCR run time for this kit is 18 minutes, including hold times; the β -Lactamase ID kit can rapidly screen test samples for the indicated gene families associated with antibiotic resistance. Optimization and validation studies demonstrated the β -Lactamase ID kit's reliability when paired with the Philisa Thermal Cycler. However, the β -Lactamase ID kit can be optimized for compatibility with most DNA polymerases and thermal cycler platforms.

Materials and Methods:

Testing of the β -Lactamase ID kit was done at Creighton University School of Medicine, Department of Medical Microbiology and Immunology, Center for Research in Anti-Infectives and Biotechnology (C.R.A.B). Data was provided with permission by Dr. Nancy Hanson.

PCR Amplification:

The ARM-D for β -Lactamase ID kit (Catalog No.: 250029), Philisa Thermal Cycler (Catalog No.: 250000), and associated reagents were obtained from Streck, Inc. (Omaha, NE USA).

PCR was carried out as per the manufacturer's instructions for the ARM-D for β -Lactamase ID kit: Hot start of 98 °C for 30 seconds, followed by 25 cycles [98 °C for 5 seconds, 60 °C for 10 seconds, and 72 °C for 20 seconds], and a final extension of 72 °C for

30 seconds. Rapid thermal cycling was carried out on the Philisa Thermal Cycler and the Bio-Rad C1000 Touch™. PCR amplicons were stained with ethidium bromide and resolved on a 2.5% agarose gel. A Molecular Imager Gel Doc EZ System with Image Lab Software was used for PCR amplicon detection and imaging.

Results and Discussion:

To validate the β -Lactamase ID kit, 60 previously characterized clinical isolates have been tested to date with the Philisa Thermal Cycler. Purified isolate DNA was tested in this study, which included samples originating from *E. coli*, *Klebsiella pneumoniae*, *Enterobacter sp.*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. As a demonstration of the optimized kit, Figure 1A illustrates agarose gel data from typical control PCRs (see control #1 and control #2) using the β -Lactamase ID kit. When tested with the indicated clinical isolates per Streck's recommended instructions for use, the PCR results from this assay clearly indicate amplification and successful resolution of amplicons for the indicated β -Lactamase gene families and the internal control (Figure 1B). To identify samples testing positive for their respective β -Lactamase gene family, the resolved amplicon sizes of the test isolates are compared to the matching control amplicon on the same gel. Using the key provided in the β -Lactamase ID kit instructions for use, a match to the specified β -lactamase gene families can be determined. Furthermore, β -Lactamase ID kit testing of the same sample set on a different thermal cycler platform, the Bio-Rad C1000 Touch, demonstrated similar results (Figure 1C). However, the Philisa Thermal Cycler had the shortest PCR cycling time, producing results in 18 minutes compared to the 33-minute assay time for use of the β -Lactamase ID kit with the Bio-Rad C1000 Touch. Although the β -Lactamase ID kit performs optimally with Streck's thermal cycler and DNA polymerase, our collective studies demonstrate the kit can be used with alternative PCR platforms and DNA polymerases. Validation testing of the β -Lactamase ID kit using the parameters described above indicated 92% sensitivity and 100% specificity.

Conclusions:

When coupled with the Philisa Thermal Cycler, the ARM-D for β -Lactamase ID kit facilitates sensitive and specific detection of antibiotic resistance in β -lactamase-producing Gram-negative bacteria and decreases the overall time-to-results for the laboratory. Appropriate use of the newest ARM-D kit can provide valuable information for active surveillance of antimicrobial resistance, epidemiologic studies of resistance patterns important to public health in the prevention of outbreaks, and guiding the selection of the most effective antibiotic therapy.

Figure 1: Detection of ESBLs, MBLs, KPCs, and plasmid-mediated AmpCs using the ARM-D for β -Lactamase ID kit. Gel image depicts PCR products from the β -Lactamase ID kit controls (A) as well as previously characterized test isolates (B and C). (A) Agarose gel picture of β -Lactamase ID kit controls as a representative example. (B) PCR amplification using the manufacturer's recommended protocol for rapid PCR with the Philisa Thermal Cycler. (C) PCR amplification using the manufacturer's recommended PCR cycling protocol for rapid PCR with the Bio-Rad C1000 Touch. As indicated, control #1 PCR products include CMY-2, CTX-M-14, NDM, and IMP-1 gene families with an internal control. Control #2 PCR products include OXA-48, DHA, VIM, KPC, and CTX-M-15 gene families.