Technical Note

Evaluation of the Philisa® *amp*C ID kit using conventional and fast PCR platforms with Philisa*FAST*™ and Platinum®*Taq* DNA Polymerase

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Overview

Gram-negative organisms carrying AmpC β-lactamases are resistant to most β-lactam antibiotics and can be encoded chromosomally and/or carried by plasmids. Since plasmid-mediated AmpC β-lactamases can appear in organisms lacking or having low-level expression of a chromosomal *amp*C gene, it is useful to detect and discriminate between plasmid-mediated and chromosomally expressed AmpC β-lactamases. Isolates producing AmpC β-lactamases are resistant to β-lactams, insusceptible to currently available β-lactamase inhibitors, and may develop resistance to carbapenems. Furthermore, plasmid-mediated AmpCs can spread to other organisms within a hospital or geographic region. Correct identification of an *amp*C β-lactamase gene can guide antibiotic treatment for the organism harboring the *amp*C gene.

The Philisa® *amp*C ID kit is a PCR-based molecular test that allows for multiplex identification of clinical isolates from six plasmid-mediated *amp*C gene families: MOX, DHA, ACC, EBC, FOX and CIT. An endogenous internal control is also included to reduce false negatives; it targets a conserved region common in gram-negative bacteria. Agarose gel detection is used to resolve PCR products and compare clinical samples against the external DNA controls. The Philisa *amp*C ID kit can detect both plasmid-mediated and chromosomal *amp*C genes if the genes are not from the same chromosomal origin.

The *amp*C ID kit was validated by extensive testing against clinical isolates using the Philisa® Thermal Cycler and Philisa*FAST*™ DNA polymerase. With a total PCR run time of 15 minutes, including hold times, the *amp*C ID kit can rapidly screen test samples for *amp*C-resistant genes. The purpose of this study was to demonstrate compatibility of the *amp*C ID kit with conventional thermal cycler platforms and Platinum®*Taq*, a standard *Taq* polymerase. Data generated from these experiments were compared to control *amp*C ID kit data following the Streck protocol and using the Streck Philisa Thermal Cycler and Philisa*FAST*, a high-fidelity hot-start DNA polymerase. Although Streck's rapid thermal cycler produced results twice as fast as other conventional platforms tested, amplification results were comparable between all platforms tested demonstrating that, with optimization, the Philisa *amp*C ID kit is compatible with alternative PCR platforms and DNA polymerases.

Materials and Methods

Testing was performed at Streck, Inc.

PCR Amplification: The Philisa *amp*C ID kit (Catalog No.: 250026), Philisa*FAST* DNA polymerase (Catalog No.: 250024), and associated reagents were obtained from Streck, Inc. (Omaha, NE USA). Platinum®*Taq* DNA Polymerase (Catalog No.: 10966-034) including 10X PCR Buffer, Platinum*Taq* Polymerase, and 50 mM Magnesium Chloride were provided by Life Technologies (Carlsbad, CA USA), and dNTPs (Catalog No.: N0447S) were provided by New England BioLabs (Ipswich, MA USA).

For each 25 µl reaction, reagent volumes were added as per manufacturer's instructions with the following exceptions: For Platinum*Taq* optimization experiments, 1.25 U polymerase and 2.0 mM magnesium chloride were used for each reaction. Make-up water and no template control (NTC) used MB grade nuclease-free water (Sigma, Catalog No.: W4502, St. Louis, MO USA).

PCR was carried out as per the manufacturer's instructions for the Philisa *amp*C ID kit: Hot start of 98 °C for 30 seconds, followed by 30 cycles [98 °C for 5 seconds, 58 °C for 10 seconds, and 72 °C for 7 seconds], and a final extension of 72 °C for 10 seconds. A longer conventional protocol was also tested: Hot start of 95 °C for 180 seconds, followed by 30 cycles [95 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 60 seconds], and a final extension of 72 °C for 420 seconds[1]. Rapid thermal cycling was carried out on the Philisa as well as conventional thermal cyclers: the BioRad C1000 Touch™ and the Biometra TGradient. PCR amplicons were stained with ethidium bromide and resolved on a 2.5% agarose gel. A BioRad Molecular Imager VersaDoc® was used for PCR band detection and imaging.

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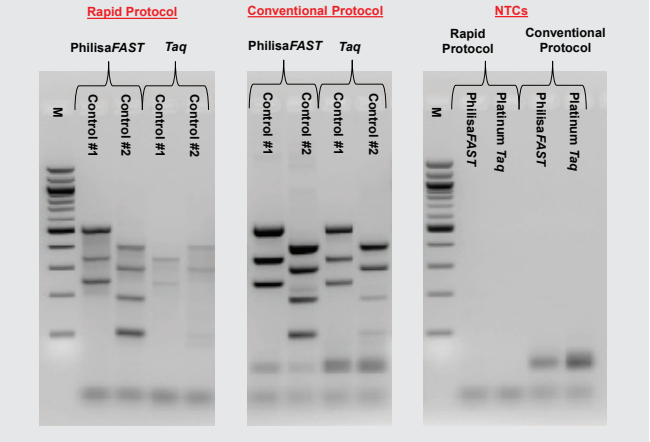
Figure 1

Agarose gel depicting Philisa *amp*C ID kit gel products following amplification of the kit DNA controls using the manufacturer's recommended protocol for rapid PCR with the Philisa Thermal Cycler and Philisa*FAST* DNA polymerase to carry out the PCR. As indicated, control #1 gel products include MOX, ACC, and FOX *amp*C gene families. Control #2 gel products include DHA, EBC, internal control, and CIT gene families.

Figure 2

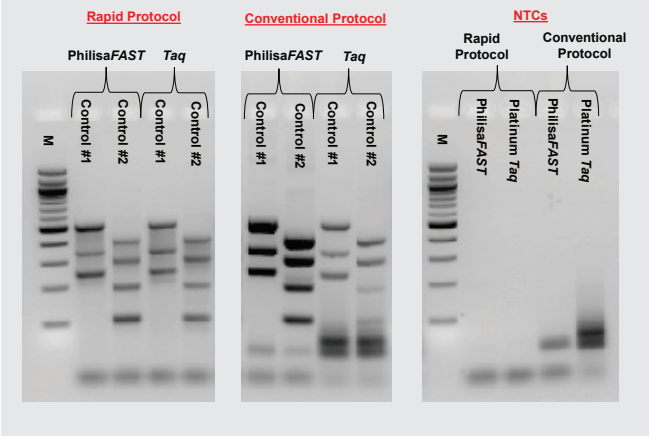


Agarose gel data, produced on the Philisa Thermal Cycler, following amplification of Philisa *amp*C ID kit controls using both rapid and conventional protocols with Philisa*FAST* and wild-type *Taq* DNA polymerase, as per each enzyme's respective IFU guidelines. NTCs are indicated as a control for identification of contamination in PCR reaction mixes.

Results and Discussion

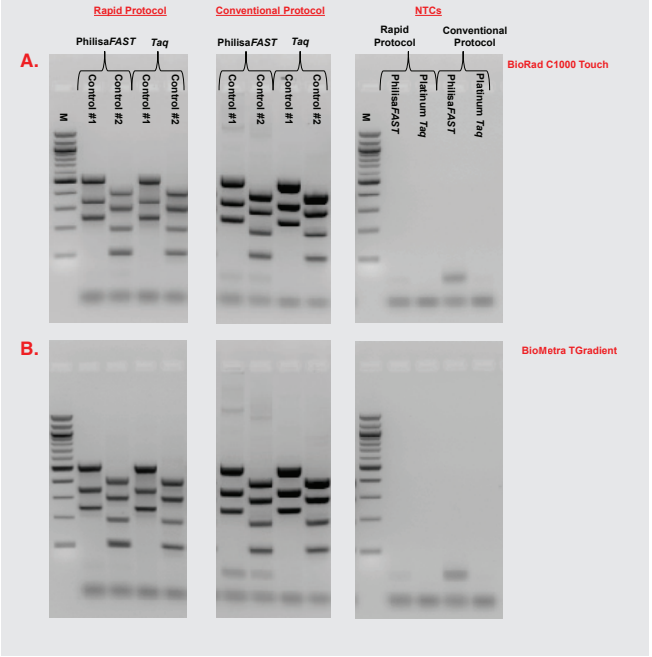
To validate the Philisa *amp*C ID kit, we tested over 300 previously characterized clinical isolates using Philisa*FAST* DNA polymerase with the Philisa Thermal Cycler. As a demonstration of the optimized kit, Figure 1 illustrates agarose gel results from typical control PCRs using the Philisa *amp*C ID kit per Streck's recommended instructions for use (IFU). The PCR results from this assay clearly indicate PCR amplification and successful resolution of all six control amplicons for the indicated *amp*C gene families and the internal control. However, the goal of this work was to demonstrate compatibility of the Philisa *amp*C ID kit with a basic *Taq* DNA polymerase and conventional thermal cycler platforms common to many research laboratories. To this end, we tested if Platinum *Taq* could amplify all multiplex targets in the Philisa *amp*C ID kit using the rapid protocol recommended in the *amp*C ID kit IFU (Figure 2). Data from these experiments indicate that Platinum *Taq* was unable to amplify all multiplex targets in the controls using the 15-minute protocol. Specifically, agarose gel data indicate complete loss of gel bands associated with MOX, FOX, internal control, and CMY amplicons (Figure 2). These data are not surprising as Streck's rapid multiplex assay uses a hot-start high-fidelity DNA polymerase with modified reaction buffer chemistry; this is primarily due to increased magnesium concentration, which supports the rapid thermal cycling. To determine if a conventional or slower protocol would counteract the reduced amplification efficiency observed with the conventional polymerase mix, we used a previously described 73-minute cycling protocol[1]. Results from this assay indicate an overall improvement in amplification efficiency for all control DNA bands when using either Philisa*FAST* or Platinum *Taq* (Figure 2). However, amplification intensity for the internal control and CMY band using Platinum *Taq* with the 73-minute protocol was still qualitatively lower than data produced with Philisa*FAST*. Most importantly, these data indicate that Platinum *Taq*, when used under the guidelines of the kit IFU, cannot be recommended for use with the Philisa *amp*C ID kit.

Figure 3



Comparison of Philisa*FAST* and *Taq* DNA Polymerase amplification of the Philisa *amp*C ID kit controls using the Philisa *amp*C ID kit with both rapid and conventional PCR protocols. Philisa*FAST* amplifications were according to the manufacturer's IFU. For amplifications with *Taq* polymerase, magnesium concentration was increased to 2 mM and DNA polymerase concentration was increased to 1.25 U per reaction. NTC samples are indicated as a control for contamination of reaction mixes.

Figure 4



Comparison of Philisa*FAST* and *Taq* Polymerase using the Philisa *amp*C ID kit with the BioRad C1000 Touch (A) and BioMetra TGradient (B) Thermal Cyclers and with both rapid and conventional PCR protocols. NTC samples are indicated as a control for contamination of reaction mixes. For experiments with *Taq* polymerase, magnesium concentration was increased to 2 mM and DNA polymerase concentration was increased to 1.25 U per reaction.

Next, we addressed if increasing both magnesium and polymerase concentration could improve amplification of the Philisa *amp*C ID kit controls using Platinum *Taq* (Figure 3) with the rapid and conventional protocols. When Platinum *Taq* concentration was increased to 1.25 U/25 µl reaction and magnesium concentration was raised from 1.5 mM to 2.0 mM, amplification of the control DNA using Platinum *Taq* was comparable to data produced with Philisa*FAST*. Interestingly, when the same reaction mixes were tested using a slow cycling protocol, Platinum *Taq* amplified the internal control and CMY targets but not as efficiently as Philisa*FAST*. If *Taq* polymerase is used with the *amp*C ID kit, these data demonstrate the reaction buffer formulation must be modified to improve amplification of all six gene families and the internal control for use with both Streck's rapid protocol and the conventional protocol (Figure 3). Furthermore, we tested the Philisa *amp*C ID kit using both protocols on alternative conventional thermal cycler platforms: the BioMetra TGradient and BioRad C1000 Touch (Figure 4). Regardless of the polymerase used or PCR protocol tested, data from these studies show the Philisa *amp*C ID kit is compatible with conventional thermal cycler platforms but requires a two-fold increase in total PCR cycling time.

As expected, the conventional protocol tested in this study contributed to qualitative increases in agarose gel band intensity for amplicons detected using the Philisa *amp*C control mixes as compared to the rapid protocol. The most obvious increase in band intensity was noted when Philisa*FAST* was paired with amplification on the Philisa Thermal Cycler using a conventional protocol (Figure 3). Furthermore, the Philisa Thermal Cycler had the shortest PCR time, producing results with the rapid protocol in ~15 minutes (Figure 3) compared to the 32-minute* BioRad C1000 Touch (Figure 4A) and 33-minute* Biometra TGradient (Figure 4B) protocol times. In conclusion, this study demonstrates the Philisa *amp*C ID kit is compatible with alternative PCR reaction chemistries and thermal cycler platforms and is useful for screening clinical isolates to detect plasmid-mediated *amp*C gene families. The Philisa *amp*C ID kit facilitates reproducible results and, when coupled with the Philisa Thermal Cycler, can reliably and rapidly screen clinical isolates, decreasing the overall time-to-results for the laboratory. It should be noted that use of the Philisa *amp*C ID kit was extensively validated with the Philisa Thermal Cycler using Philisa*FAST* DNA polymerase. While the results described here were expected for the modified protocols tested in this study, the *amp*C ID kit multiplex controls were optimized for use with the rapid protocol. Although use of a conventional protocol with increased PCR cycling times increased resolution of the multiplex controls, it can also cause non-specific amplification in PCR assays that are testing clinical samples; i.e., where the test sample may contain just one or two targets of interest. Other polymerase and/or instrument combinations are likely compatible but may require optimization and validation to amplify all control targets and prevent non-specific amplification during isolate testing. Streck technical services should be contacted for support.

* Total protocol time, including instrument preheating prior to initiation of the PCR protocol.

1. Perez-Perez, F.J., Hanson, N.D. (2002). Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *Journal of Clinical Microbiology*, 40(6):2153-2162.