Evaluation of EasyScreen™ ESBL/CPO Detection Kit Using Direct-PCR from Patient Culture and Broth Samples

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Introduction
Beta-lactam and carbapenem antibiotics are the most commonly used worldwide in the treatment of bacterial infections. The recent emergence of Extended-Spectrum Beta-Lactamases (ESBL) and carbapenemases (CPO) are a significant global concern in healthcare settings, as these enzymes may render standard treatments ineffective. Thus, accurate and rapid detection of these resistant organisms will have a significant impact on patient management.

We have developed a novel 3base™ real-time multiplex PCR assay to detect most significant and commonly encountered bacterial resistance genes TEM, CTX-M, SME, DES, IMP, NDM, OXA-23-like, OXA-48-like, OXA-51-like, MCR-1, DHA, SHV, VIM, IMI, KPC and their subtypes. The 3base™ assay is a simple and rapid molecular method that utilizes 3base™ technology to modify the 4 base wild-type DNA sequence (A, C, T, G) into a 3 base sequence (A, T, G) via a novel, patented 3base™ conversion step. The conversion process simplifies the design of multiplex PCR reactions by eliminating the large 1m differences that can be present when targeting multiple pathogens and increasing the homology between sequences (Table 1).

Methods/Materials
The sensitivity of each target was determined by using synthetic DNA constructs. In addition, validation target organisms and panels were obtained from the World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) as well as published literature. The naturally occurring TEM and SHV genes are not indicative of ESBL production, therefore the following mutations/markers are being targeted in order to differentiate the presence of ESBL from non-ESBL.

Clinical Validation
The results of 45 clinical isolates from a previous clinical trial is shown in Table 2. Two-thirds of the clinical isolates were found to have mixed infections. In summary, the EasyScreen™ ESBL/CPO Detection Kit when used with the direct-PCR method and compared against a conventional in-house method had a 97% concordance with culture and 100% agreement with Broth samples.

Table 2. 2017 QCMD validation.

<table>
<thead>
<tr>
<th>Target</th>
<th>GSL Result</th>
<th>University Hospital Galway Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>OXA-48</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>KPC</td>
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Conclusions
The EasyScreen™ ESBL/CPO Detection Kit has been developed as a useful tool in rapidly detecting resistant genes directly from cultures and broths without the need for DNA extraction purification steps (table 4). The PCR protocol can be significantly shortened to reduce the run-time given the high copy number. The turn around-time is approximately 1 hour for culture and broth samples. This test could be effective in assisting and accelerating patient management strategies.

The increased sub-type homology of the 3base™ technique expands the detection capacity of multiplex PCR for some target genes (CTX-M). Also, novel variants (such as IMP-14) or new resistant markers can be readily incorporated into existing assays given the properties of the 3base™ converted DNA sequences, thus improving the throughput of such assays. The optimized assay provides a sensitive and specific alternative for the detection of ESBL and CPO sequences and can be carried out in less than 3 hours for primary patient specimens and in approximately 1 hour for cultured isolates.

References