INTRODUCTION

Mobilized Colistin Resistance (MCR) genes represent an increased global threat for untreatable infections. These are found on plasmids; hence the gene can spread horizontally to other bacteria species including carbapenem-resistant Enterobacteriaceae (CRE). This resistance mechanism confers resistance to colistin, a last resort antibiotic for multidrug resistant pathogens. Molecular detection of this antibiotic resistance gene is essential for infection control and surveillance. The Streck ARM-D Kit, MCR (RUO) will detect the following gene families: MCR-1, MCR-2, MCR-(3-5), IC.

SUMMARY AND PRINCIPLES

MCR tests can identify specific resistance mechanisms associated with antibiotic resistance. As such, this information can be supplemented with susceptibility testing to support test results. The Streck ARM-D Kit, MCR (RUO) allows for identification of five MCR gene families: MCR-1, MCR-2, and MCR-(3-5). Additionally, an endogenous internal control (IC) that targets a conserved region common in Gram-negative bacteria is included to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. This test utilizes sequence-specific primer pairs for the PCR amplification of each family as well as fluorophore-labeled, target-specific DNA probes for detection by real-time PCR. This product has been validated with the Applied Biosystems™ (ABI) 7500 Fast Real-time PCR System.

CONTENTS

The kit includes one multiplex mix vial, containing all required primers and probes in TE buffer, pH 8.0 (10X PCR Mix), for simultaneous real-time PCR amplification of all targets. One control vial (Control Mix) containing synthetic DNA templates of the corresponding multiplex targets are also included in the kit to use as an external positive DNA control for the multiplex reaction. A premixed 2X Supermix containing buffer, dNTPs, MgCl2, and DNA polymerase are also included in each kit. The kit contents are enough for 100 reactions, including 12 reactions of the control mix.

IC * is the Internal Control Gene, 16S rRNA.

PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Use established precautions with potentially biohazardous specimens according to your laboratory guidelines.
3. Always use DNase/RNase-free plasticware/reagents and aerosol-barrier pipet tips.
4. SDS can be obtained at www.streck.com, by calling 800-843-0912, or by calling your local supplier.

STORAGE AND STABILITY

1. When stored at -20 °C, unused kit contents are stable through the expiration date.
2. Minimize the freeze-thaw cycle number for storage periods.
3. When using reagents for consecutive days, store at 4 °C. Store at -20 °C for extended storage periods.

SAMPLE EXTRACTION

The Streck ARM-D Kit, MCR (RUO) was validated with previously characterized DNA samples extracted from pure bacterial culture using the QIAGEN® DNeasy® Blood and Tissue Kit. 13ml of a 5ml overnight culture was used as per the extraction kit protocol yielding DNA concentrations that range from 10-200ng/µl, with 260/280 ratios that range from 1.4 to 2.4. Alternative growth protocols for pure bacterial cultures and nucleic acid extraction techniques/kits should also give DNA of enough yield and quality. The 30-cycle PCR assay has not been tested for use with clinical samples in which targets are present in low DNA copy numbers (e.g., direct, uncultured samples).

REACTION PREPARATION

Thaw reagents, vortex briefly to mix contents, and pulse-spin vials prior to opening. Prepare a master mix (without template DNA) according to the table below and based upon the number of samples to be processed (plus one extra reaction). Include at least one Control Mix reaction and two no-template-control (NTC) samples for each respective PCR run.

<table>
<thead>
<tr>
<th>Primer/Probe Vials</th>
<th>Control Vials</th>
<th>Cap Color</th>
<th>Target Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X PCR Mix</td>
<td>Control Mix</td>
<td>Red</td>
<td>MCR-1, MCR-2, MCR-(3-5), IC</td>
</tr>
</tbody>
</table>

PCR PROTOCOL

The following protocol has been optimized for use with the supplied Supermix 2X master mix. Some instruments may require longer extension time for signal acquisition (Detection Step). Consult your instrument manual for additional information.

**Step** | **General Protocol**
--- | ---
Hot-start | 98 °C for 30 sec
30 cycles of: | 98 °C for 5 sec 60 °C for 1 sec 72 °C for 20 sec (Detection Step)

INSTRUMENT SET-UP

Prior to each target is based on the fluorescence of the fluorophore conjugated to each target-specific DNA probe as shown in the table below. The following are general instrument set-up instructions. Parameters specific to the ABI 7500 Fast Real-time PCR System are described in the Data Acquisition and Analysis Guide which can be found on www.streck.com.

1. Familiarize yourself with the instrument's user interface.
2. Create or select a thermal profile or cycling protocol.
3. Assign control and sample wells when necessary.
4. For data interpretation, thresholds should be manually set for optimal performance (see Data Acquisition and Analysis Guide for recommended instrument-specific threshold and baseline settings).

The detection of each target is based on the optical fluorescence of the fluorophore conjugated to each target-specific DNA probe.

<table>
<thead>
<tr>
<th>Master Mix</th>
<th>Target Gene</th>
<th>Fluorophore</th>
<th>Excitation λ</th>
<th>Emission λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Mix</td>
<td>MCR-1</td>
<td>FAM</td>
<td>495nm</td>
<td>520nm</td>
</tr>
<tr>
<td></td>
<td>MCR-2</td>
<td>HEX</td>
<td>538nm</td>
<td>555nm</td>
</tr>
<tr>
<td></td>
<td>MCR-(3-5)</td>
<td>TEX615</td>
<td>596nm</td>
<td>613nm</td>
</tr>
<tr>
<td>IC</td>
<td>Cy5</td>
<td></td>
<td>645nm</td>
<td>665nm</td>
</tr>
</tbody>
</table>

DATA ACQUISITION

Observe: Each real-time PCR run must be validated with the Control Mix vials provided with the kit. If the specifications for Cq values for the DNA controls are not met, the results are considered invalid and samples must be re-evaluated. Cq values of unknown samples will vary depending on the starting DNA copy number. Visually inspect amplification curves for each unknown sample to verify results. As a general guideline, Cq values for MCR targets in unknown isolates can range from 10 to 26.

1. For Research Use Only (RUO). Not for use in diagnostic procedures.
2. The gene family targets have been tested against a considerable number of isolates with excellent sensitivity and specificity results. The PCR primers will only amplify the specified target families and will not detect other genes. Extensive testing has been done in DNA extracted from Escherichia, Klebsiella, Salmonella, and Enterobacter genera. However, given the genomic diversity of bacteria, Streck does not guarantee that all MCR genes will be detected in all Gram-negative subpecies. Results from this test should be used in combination with other laboratory tests available.

LIMITATIONS

1. The internal control (IC) primers have been designed to amplify a highly conserved gene target present in many Gram-negative bacteria. However, the IC may not successfully amplify from certain Gram-negative species or strains. Therefore, one should consider this for interpreting the absence of the IC product from a specific sample.
2. The gene family targets have been tested against a considerable number of isolates with excellent sensitivity and specificity results. The PCR primers will only amplify the specified target families and will not detect other genes. Extensive testing has been done in DNA extracted from Escherichia, Klebsiella, Salmonella, and Enterobacter genera. However, given the genomic diversity of bacteria, Streck does not guarantee that all MCR genes will be detected in all Gram-negative subpecies. Results from this test should be used in combination with other laboratory tests available.
3. Using the Streck ARM-D Kit, MCR (RUO) with alternative 4-channel real-time PCR platforms or
other enzymes not listed in this IFU is possible, but optimization may be required. Contact Streck Technical Services for assistance.

REFERENCES


ORDERING INFORMATION

Please call our Customer Service Department at 800-228-6090 for assistance. Additional information can be found online at www.streck.com.

GLOSSARY OF SYMBOLS

See the Instructions (IFU) tab under Resources on the product page at www.streck.com.

The brand and product names of the instruments are trademarks of their respective holders. See www.streck.com/patents for patents that may be applicable to this product.

Streck
7002 S. 109 Street, La Vista, NE 68128 USA