In 2012, a new pathogen emerged in the Middle East, causing severe respiratory infections with a high fatality rate among the patients. Middle East respiratory syndrome-coronavirus (MERS-CoV) is still mainly spreading in Saudi-Arabia and other countries of the Arabian Peninsula but has already reached many other countries worldwide (e.g. USA, Germany, Malaysia). Until April 2014, the number of infections remained relatively low but increased dramatically since then.

In collaboration with the group of Prof. Dr. Christian Drosten (Institute of Virology, University of Bonn Medical Center, Bonn, Germany), altona Diagnostics GmbH developed a real-time RT-PCR kit for the detection of MERS-CoV in patient samples.

The RealStar® MERS-CoV RT-PCR Kit 1.0 is the only CE-IVD marked in vitro diagnostic kit available on the market.

WHO requires two independent positive PCR results to confirm MERS-CoV cases. Therefore, the kit contains 48 reactions of an RT-PCR system targeting the Orf1a gene and another 48 reactions targeting a region upstream of the E gene (upE). Study data published in 2014 (Corman et al., 2014) shows the suitability for clinical testing (1). The kit is highly sensitive (Orf1a: 9.25 and upE: 5.35 copies/reaction) and specific and therefore facilitates reliable identification of MERS-CoV cases.

Table 1: Analytical specificity. Possible unspecific cross-reactivity of the RealStar® MERS-CoV RT-PCR Kit 1.0 was tested with patient samples previously confirmed positive for nucleic acids of the enlisted pathogens.

<table>
<thead>
<tr>
<th>Pathogen species present in sample</th>
<th>No. of samples</th>
<th>Specific signal upE / Orf1a</th>
<th>Internal Control signal upE / Orf1a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus</td>
<td>3</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>5</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Parainfluenza virus 1</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Parainfluenza virus 2</td>
<td>3</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Parainfluenza virus 4</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>5</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Human Coronavirus (hCoV) NL63</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>hCoV OC43</td>
<td>3</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>hCoV 229E</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>hCoV HKU-1</td>
<td>1</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>4</td>
<td>-/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>2</td>
<td>-/-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Figure 1: Probit analysis for upE gene and Orf1a RT-PCR assays included in the RealStar® MERS-CoV RT-PCR Kit 1.0. The limits of detection for the upE and Orf1a assay were determined using in-vitro transcribed RNA (IVT) quantified by spectrophotometry. The IVT was diluted in half-logarithmic steps (from 100 to 0.03 copies/reaction) and tested in replicates (n=13) for positive amplification and detection. The Orf1a assay detects 9.25 copies/reaction with 95% probability (95% confidence interval (CI): 7 – 14 copies/reaction); the upE-assay has got a 95% cut-off value of 5.35 copies/reaction (95% CI: 4 – 9.7 copies/reaction).