Comparison of three different real-time PCR assays for the detection of *Pneumocystis jirovecii*

Roel Nijhuis¹, Cindy van der Zee², Jorike Smink¹, Sanela Svraka¹, Peggy Godschalk¹, Erik van Hannen²

¹ Dept. of medical microbiology and immunology, Meander Medical Center Amersfoort, the Netherlands
² Dept. of medical microbiology and immunology, St. Antonius Hospital Nieuwegein, the Netherlands
³ Central laboratory for bacteriology and serology (CBLs), Tergooi Hospital, Hilversum, the Netherlands

Aim

To compare the performance of three different real-time PCR assays for the detection of PJP

Introduction

- *Pneumocystis jirovecii* is a microorganism classified as fungus that can be present in human as commensal, but also cause severe infections known as *Pneumocystis jirovecii* pneumonia (PJP)
- PJP is most often identified from immunocompromised patients
- Diagnosis of PJP is confirmed by detection of the microorganism from a bronchoalveolar lavage (BAL)
  - Molecular methods are primarily used, targeting different genes
- In our setting, a real-time PCR assay detecting the major surface glycoprotein (MSG) is used for detection of PJP
  - *In silico* analysis showed a high variety of MSG sequences (figure 1), to be divided in two major groups
- Multiple CE-IVD cleared assays are available, targeting other genes such as m1LSU or DHPS

Methods

PJP Real-time PCR assays

- Assays as stated in table 1 were used in this study
- In-house real-time PCR assay cut-off: Ct-value of 37
- CE-IVD assays were performed as stated by the manufacturer
  - PneumoGenius® identifies possible sulfa-resistance SNPs

Inclusion

- Retrospectively: 25 BAL specimens were selected, 15 PJP positive and 10 PJP negative
- Prospectively: 75 consecutively tested BAL specimens were included in the comparison

Statistics

- True positive (gold standard) was determined as 2 out of 3 positive
- Sensitivity and specificity were calculated as compared to the gold standard

Discrepancies

- Sequencing was performed to determine sequence variety as possible reason for discrepancies

Results

- Comparison of the performance of the PCRs is shown in table 2
  - Correlation of the PJP loads (copies/ml) identified by PneumoGenius® and RealStar® is shown in figure 2
  - Median load of in-house negative/CE-IVD positive assays was 236.5 (22.2-4100) and 838.5 (21.7-17500) copies/ml for PneumoGenius® and RealStar® respectively
- Retrospectively collected specimens were obtained from a total of 23 patients
  - Two positive and two negative specimens of 1 patient were included respectively. Results in this study were identical
- Prospective specimens were obtained from 65 patients
  - Of a single patient, a discrepant result was found between two specimens
    - In-house tested negative for both, PneumoGenius® tested positive for one (22.2 cop/ml), whereas RealStar® tested PJP positive in both (21.7 and 27.6 cop/ml)
- Sequencing revealed the presence of both group 1 and group 2 sequences as well as discrepant specimens
- Possible sulfa-resistance associated SNPs were found in 3 specimens. This is 11.5% (3/26) of the patients in which DHPS f43 smeltcurve analysis was successfull
  - P575 + wt in 2, T55A + P575 + wt in 1

<table>
<thead>
<tr>
<th>GS positive</th>
<th>GS negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-house PCR positive</td>
<td>23</td>
<td>0</td>
<td>69.7%</td>
</tr>
<tr>
<td>In-house PCR negative</td>
<td>10ᵃ</td>
<td>67</td>
<td>100%</td>
</tr>
<tr>
<td>PneumoGenius® positive</td>
<td>33</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>PneumoGenius® negative</td>
<td>0</td>
<td>66</td>
<td>100%</td>
</tr>
<tr>
<td>RealStar® positive</td>
<td>33</td>
<td>2ᵇ</td>
<td>100%</td>
</tr>
<tr>
<td>RealStar® negative</td>
<td>0</td>
<td>65</td>
<td>100%</td>
</tr>
</tbody>
</table>

⁰ 6 tested positive with the in-house assay with Ct >37, the remaining 4 tested negative
ᵇ Load of the RealStar® positive specimens were 21.7 and 23.3 copies/ml

Discussion

- Six out of 10 true positive PJP specimens were reported negative as a result of the cut-off value of Ct=37 used
  - No cut-off is recommended in the CE-IVD assays
- Sequencing revealed the presence of multiple MSG variations in one sample
- Discrepant results are most likely explained by better sensitivity of CE-IVD assays
  - Ct-values of concordant results show a mean difference in Ct-value of -1.5 and -3.5 for the PneumoGenius® and RealStar® respectively (data not shown)

Conclusion

- A higher positivity rate is found when using the CE-IVD assays compared to the in-house assay
- PneumoGenius® and RealStar® assays showed comparable performance