**Comparison of a new commercial real-time PCR assay (RealCycler PJIR kit, Progenie Molecular) to an in-house real-time PCR assay for the diagnosis of Pneumocystis jirovecii infections**

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### BACKGROUND

**Pneumocystis jirovecii** (P. jirovecii) is an atypical fungus that causes a life threatening pulmonary infection called Pneumocystis pneumonia (PCP) in HIV-infected patients and in non HIV-infected patients receiving immunosuppressive therapy, particularly for organ transplantation or malignancy. **P. jirovecii** infections cover a large spectrum of clinical presentations of which PCR represents only a part, and mild infections such as pulmonary colonisation may be the main part [1].

As P. jirovecii cannot be cultured, the diagnosis of P. jirovecii infections is based on the detection of the fungus in pulmonary specimens, essentially bronchoalveolar lavage (BAL) specimens using microscopic examination and polymerase chain reaction (PCR). The use of PCR assays and particularly real-time PCR assays, has improved the sensitivity of *P. jirovecii* detection, specifically in pulmonary specimens with low fungal burden.

### AIM

To compare the efficiency of the RealCycler PJIR kit (Progenie Molecular; pmPCR), to an in-house PCR assay (ipPCR) [2,3], both targets assessing the mitochondrial large subunit ribosomal RNA gene.

### Patients and specimens

- 34 patients monitored at Brest University Hospital between December 2012 and September 2015 were retrospectively enrolled in the study. Fourteen patients were initially diagnosed with PCP, 15 were considered to be pulmonary colonised by the fungus, and 5 were negative for *P. jirovecii* detection (negative control group). Patients’ characteristics are summarized in Table 1.

### METHODS

- **Comparison of pmPCR and ipPCR**
  - The 34 archival DNA specimens were examined with ipPCR and pmPCR on the same day using the same real-time PCR instrument (Applied Biosystems 7500 Real-Time PCR system).
  - Both PCR assays target the mitochondrial large subunit ribosomal RNA (mtLSU RNA) gene of *P. jirovecii* and use a TaqMan\(^\text{TM}\) probe.
  - The ipPCR assay was performed as described elsewhere by Meliani et al. and Totet al. [2,3]. The pmPCR assay was performed according to the manufacturer’s recommendations. PCR assays’ characteristics are summarized in Table 2.

### RESULTS AND DISCUSSION

#### Table 1. Characteristics of the 34 patients whose pulmonary specimens were retrospectively examined with the in-house PCR and the RealCycler PJIR kit (Progenie molecular)

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Immunocompetent</th>
<th>Non-immunocompetent</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

#### Table 2. Technical characteristics of the in-house PCR (ipPCR) and RealCycler PJIR kit (Progenie molecular; pmPCR)

<table>
<thead>
<tr>
<th>Target</th>
<th>PCR type</th>
<th>Detection of PCR products</th>
<th>Control of DNA template</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtLSU RNA</td>
<td>RealCycler PJIR kit, Progenie Molecular</td>
<td>Qualitative real-time PCR</td>
<td>Yes (Target amplification monitored by Applied Biosystems)</td>
</tr>
<tr>
<td>mtLSU RNA</td>
<td>RealCycler PJIR kit, Progenie Molecular</td>
<td>Qualitative real-time PCR</td>
<td>Yes (Target amplification monitored by Applied Biosystems)</td>
</tr>
</tbody>
</table>

#### Table 3. Qualitative results of Pneumocystis detection in 34 pulmonary patients whose specimens were examined with the in-house PCR (ipPCR) and the RealCycler PJIR kit (Progenie molecular; pmPCR)

<table>
<thead>
<tr>
<th>pmPCR</th>
<th>POS</th>
<th>NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ipPCR</td>
<td>27</td>
<td>1</td>
</tr>
</tbody>
</table>

#### Qualitative data

- **Among the 29 initially positive specimens** (from 14 PCP patients and 15 colonised patients), 27 gave positive results with both PCR assays. The 5 initially negative specimens were confirmed negative with both PCR assays (Table 3).

- **One specimen which had initially a cycle threshold (Ct) value=420 (ipPCR, B, green mark, figure 2) gave a negative result with both PCR assays. This specimen was obtained from a colonised patient harbouring a low fungal burden. This discrepant result could be explained by the low fungal burden and DNA degradation.

- **Concordant results between the two PCR assays were obtained for all DNA specimens except one (33/34).** Thus, the concordance rate between the two methods was evaluated at 97% (Cohen’s kappa, 0.905 [CI 95% : 0.72 – 1.0]). The discrepant result concerned one specimen from a colonised patient which had initially a Ct value=39.5 using the ipPCR. In the present experiment this specimen was negative with the pmPCR and confirmed to be positive with ipPCR (A, red mark, figure 1).

- **Taking the ipPCR as gold standard, the sensitivity and specificity of the pmPCR were 96.4% and 100% respectively.**

#### Quantitative data

- **Taking into account Ct values for positive samples, the correlation coefficient between the two PCR methods were estimated at 0.974 (p<0.01; R²=0.948) (Figure 1).**

- **The results of the two PCR assays for PCP patients were very close with a correlation coefficient estimated at 0.995 (p<0.01; R²=0.990) (Figure 1).**

- **A lower correlation was observed between the two PCR methods for colonised patients (higher Ct, figure 1) with a correlation coefficient estimated at 0.853 (p=0.01; R²=0.728); this result could be explained by the high Ct values (low fungal burdens) and PCR variability in specimens from colonised patients.**

- **Although the pmPCR appears to be less sensitive than the ipPCR, concordance between the two PCR assays remains excellent (Cohen’s kappa=0.8).**

- **Although the pmPCR kit does not provide an extraction control, it presents the advantage of having a ready-to-use AmpliMix (containing probe, primers, competitive heterologous internal control and buffer).**

### CONCLUSION

The RealCycler PJIR kit (Progenie Molecular; pmPCR) is a simple technique (ready-to-use AmpliMix). It shows a good concordance with a well-established in-house PCR assay (concordance rate 97%, Cohen’s-kappa, 0.905) and thus appears to be an efficient technique to detect *P. jirovecii* in PCP patients and in patients colonised by the fungus.

### ACKNOWLEDGMENTS

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