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 PCP 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 (ihPCR) [2,3], both assays targeting the mitochondrial large subunit ribosomal rRNA gene

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)

	Patients with *Pneumocystis* pneumonia	Patients with *Pneumocystis* colonisation	Negative control group
No. of patients	14	15	5
Mean age [range] (years)	58.3 [34-85]	66.3 [49-88]	62.8 [51-74]
Sex ratio M/F	10/4	9/6	2/3
Clinical underlying condition	Haematological malignancy (4), HIV infection (4), Solid tumour (3), Underlying chronic pulmonary disease[a] (2), Corticotherapy (1)	Haematological malignancy (2), Solid tumour (3), Underlying chronic pulmonary disease[a] (8), Transplantation (2)	Underlying chronic pulmonary disease[a] (4), Haematological malignancy (1)

[a]Underlying chronic pulmonary diseases : Chronic obstructive pulmonary disease, pulmonary fibrosis, chronic bronchitis, pulmonary infection or emphysema

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

	Target	PCR type	Fluorescent reporter	Detection of PCR inhibitors	Control of DNA extraction
ihPCR (Meliani *et al*, 2003; Totet *et al*, 2003)	mtLSUrRNA[a] (79bp fragment)	Qualitative or Quantitative real-time PCR	TaqMan® MGB probe (FAM)	Yes (TaqMan® exogenous internal positive control reagents, Applied Biosystems)	Yes (TaqMan® Rnase P Control reagents (VIC), Applied Biosystems)
pmPCR (RealCycler PJIR, Progenie Molecular)	mtlSUrRNA[a] (? bp fragment)	Qualitative real-time PCR	TaqMan® probe (FAM)	Yes (Competitive Heterologous Internal Control, Progenie Molecular)	No

[a]mtLSUrRNA : mitochondrial large subunit ribosomal RNA

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

		pmPCR	
		POS	NEG
ihPCR	POS	27	1
	NEG	0	6

METHODS

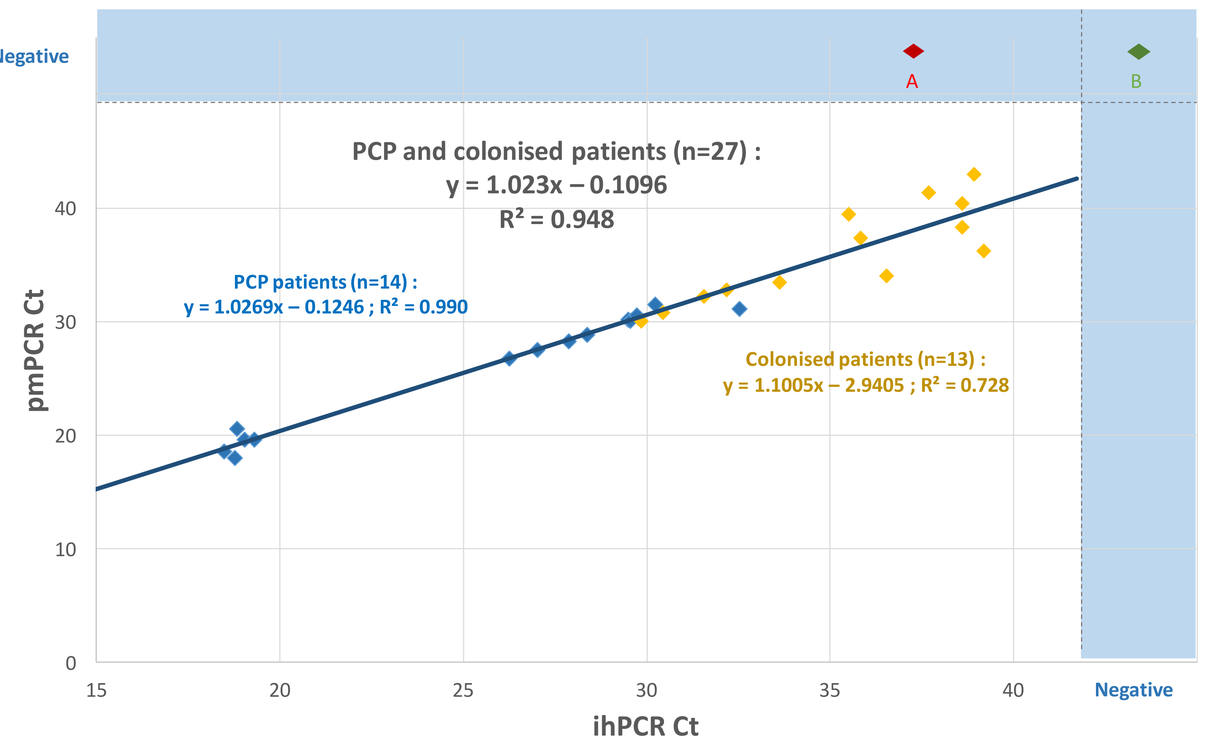
Patients and specimens

- 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.
- *P. jirovecii* detection has been initially based on microscopic examination of pulmonary specimens using May-Grünwald-Giemsa staining, indirect immunofluorescence assay (Monofluo™ Kit P. jirovecii, Biorad), and an in-house PCR assay (ihPCR) performed after DNA extraction (NucliSENS® easyMAG® sytem, bioMérieux) as reported elsewhere [2,3]. DNA extracts were stored at -80°C.

PCR assay comparison

- The 34 archival DNA specimens were examined with ihPCR 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 (mtLSUrRNA) gene of *P.jirovecii* and use a TaqMan® probe.
- The ihPCR assay was performed as described elsewhere by Meliani *et al*. and Totet *et al*. [2,3]. The pmPCR assay was performed according to the manufacturer's recommendations. PCR assays' characteristics are summarized in Table 2.
- Both PCR methods were compared using the concordance rate and Cohen's kappa coefficient for qualitative comparison and the correlation coefficient for quantitative comparison.

Figure 1. Linear correlation between cycle threshold (Ct) values obtained with the in-house PCR (ihPCR) and the RealCycler PJIR kit (Progenie molecular; pmPCR) for the 27 pulmonary specimens which were found positive for *Pneumocystis jirovecii* detection with both PCR assays.
Two specimens (A, red mark and B, green mark) which led to discrepant results were excluded from the analysis.



RESULTS AND DISCUSSION

Qualitative data

- Among the **29 initially positive specimens** (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≥40 (ihPCR, 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])**. The discrepant result concerned one specimen from a colonised patient which had initially a Ct value=39.5 using the ihPCR. In the present experiment this specimen was negative with the pmPCR and confirmed to be positive with ihPCR (A, red mark, figure 1).
- Taking the ihPCR 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** ($p<0,01$; $R^2=0.948$) (Figure 1).
- The results of the two PCR assays for PCP patients were very close with a correlation coefficient estimated at **0.995** ($p<0,01$; $R^2=0.990$) (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** ($p<0,01$; $R^2=0.728$); 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 ihPCR, 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.

REFERENCES
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