An evaluation of human β-globin gene detection to assess the quality of broncho-alveolar lavage and sputum specimens submitted for the diagnosis of Pneumocystis pneumonia (PcP)

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Objective

The use of PCR to diagnose Pneumocystis pneumonia (PcP) from clinical samples has been described for two decades and is recommended in recent guidelines for the diagnosis of PcP. Yet one component of the diagnostic pathway, specifically sampling of broncho-alveolar lavage and sputum, is poorly controlled and often the laboratory receives a portion of the sample that is not standardised in volume or portion. This study aims to evaluate the use of a human β-globin gene (HgG) PCR from respiratory samples as a method for specimen quality control, with the hypothesis that adequate levels of HgG present in a specimen is an indicator of a good quality sample.

Methods

Samples included 47 BAL and 18 sputum specimens. >3mL of BAL was centrifuged and supernatant discarded leaving 500µL - 1mL for re-suspension and transfer to a microfuge tube. 1mL of sputasol treated sputum was aliquoted to a microfuge tube. Samples were centrifuged at 8000g for 10 mins. Supernatant was discarded and the pellet ribolysed with acid washed glass beads for 45 seconds. The lysate was re-suspended in 240 µL Diasorin buffer 2 and 10µl proteinase K then incubated at 56˚ C for 10 mins. Extraction was performed as per manufacturer's instructions using the Diasorin Arrow automated extraction platform and DNA extraction cartridges. HgG and *P. jirovecii* PCR was performed using the OLM PneumID assay with 6µL template in a 20µL reaction volume. A second *P. jirovecii* PCR was performed using the FTD PCP assay using 5 µL of template in a 20 µL reaction. The PCR analyser was a RotorGeneQ. Outliers were determined using a 1.5 x IQR rule.

Results

From the total dataset of 65 specimens 47.7% (31/65) of specimens were positive for *P. jirovecii*. HgG C_T characteristics are presented in table 1 and figure 1. The mean HgG C_T value from BAL was 19.5 (IQR 21.7, 23.5) and the C_T threshold for acceptable quality specimen was determined to be C_T <26.1. By applying this threshold 10.6% (5/47) of the BAL samples had HgG C_T results indicating a poor quality specimen.

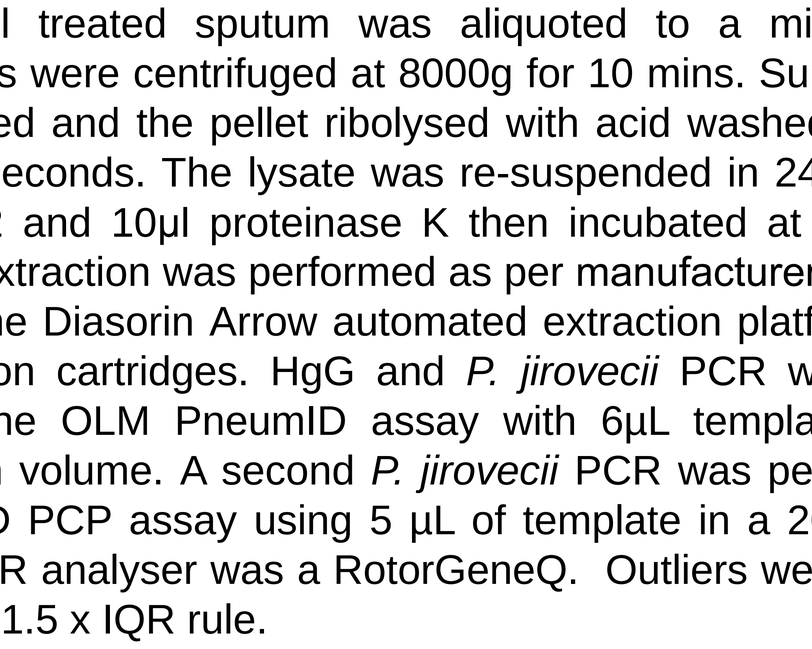

Figure 1 Scatter plot of HgG C_T values measured from 47 BAL samples

Table 1 HgG PCR C_T characteristics from DNA extracts of BAL for PcP diagnosis

n	Mean	IQR		Q threshold
47	19.5	21.7	23.5	26.1

Patient demographics for samples with a HgG below the C_T quality threshold are detailed in table 2.

Table 2 Demographics for patients with HgG PCR results from BAL measuring below the C_T 26.1 threshold

	HgG C_T	*PCP* C_T	C/mL	Underlying disease	Radiology
1	27.4	29.7	8.5 E+4	HIV low CD4	Bi-GGO
2	35.9	21.8	2.1 E+7	HIV	Bi-GGO
3	29.6	13.1	8.3 E+9	HIV	Bi-GGO
4	27.3	0	-	Adenocarcinoma	Lung Cavity
5	27.1	34.9	2.3 E+1	T2RF	RLL collapse

One patient measuring negative for *P. jirovecii* could have been reported as indeterminate, and two patients with low positive *P. jirovecii* results could have been highlighted as possibly having higher fungal burdens with a higher quality specimen.

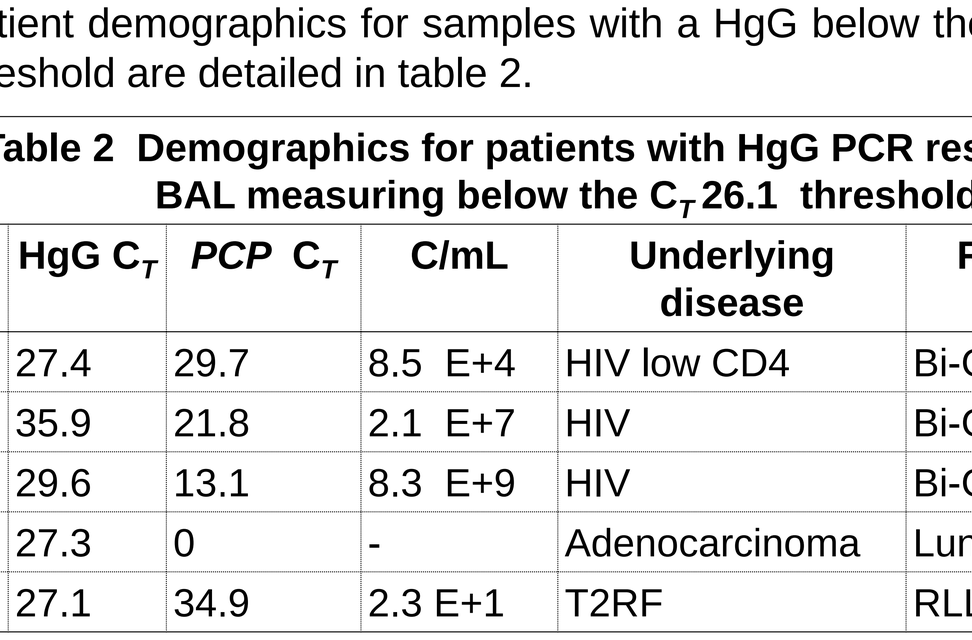

Figure 2 Scatter plot of HgG C_T values measured from 18 sputa

Table 3. HgG PCR C_T characteristics from DNA extracts of sputum for PcP diagnosis

n	Mean	IQR		Q threshold
18	18	19.2	20.3	21.9

The mean HgG C_T value and HgG threshold are presented in table 3 and figure 2. By applying this lower threshold 16.7% (3/18) of the sputum samples had HgG amplification below the low threshold. One sample was negative and two samples were positive for *P. jirovecii* with 9 E+5 and 1.9 E+6 copies per mL measured.

No significant difference (p=0.38) was observed between the measured copies per mL by the OLM PneumID assay and the FTD Pneumocystis assay indicating that the inclusion of the HgG PCR did not competitively inhibit the detection of *P. jirovecii*.

Conclusions

This study has demonstrated that human β-globin gene (HgG) can be used as a surrogate marker of respiratory specimen quality. In this study almost 90% of samples had measured HgG above the quality threshold, therefore *P. jirovecii* PCR results could be reported with confidence. When the HgG C_T was below the quality threshold, negative or low positive samples could be highlighted and interpreted with caution, as results are derived from poor quality samples. This approach is more suited to BAL samples where greater variance was observed compared with sputum.