Comparative performance of two real-time PCR for the rapid diagnosis of *Pneumocystis jirovecii* pneumonia (PJP) using non-invasive samples.

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Background.

Since the introduction of highly active anti-retriviral therapy the incidence of PJP in HIV(+) patients has been dramatically reduced. In contrast, PJP cases are increasingly diagnosed in HIV(-) immunocompromised (IC) patients (i.e.: haematological neoplasms, autoimmune diseases). In these patients, concurrence of multiple co-morbidities result in a clinical presentation of PJP difficult to distinguish fro entities such as pulmonary haemorrhage, pharmacologic toxicity or other lower respiratory tract infections. As compared to HIV(+), PJP in HIV(-) is characterised by a more acute presentation, worse outcome with higher mortality rate (approximately 35%), and lower fungal load. Lab diagnosis of PJP usually relies on the demonstration of PJ in bronchoalveolar lavage (BAL) but the severity of the presentation often prevents performing a bronchoscopy. Thus, a rapid diagnostic tool that could be applied to non-invasive samples offering a good sensitivity and specificity would be advisable.

Methods.

Between March 2016 and January 2018, paired oral washes (OW) and BAL were collected, <48 hours apart, from immunocompromised (IC) patients presenting with a clinical suspicion of PJP. PJP diagnosis was established by consensus among infectologists and microbiologists taking into consideration clinical signs and symptoms, radiological and laboratory findings. PJ detection was carried out in BAL and OW by 2 in-house rt-PCR targeting the dihydropteroate synthase (DHPS. Alvarez-Martinez et al. *Diagn Microbiol Infect Dis* 2006; 56:153) or the mitochondrial

β -D-Glucan has proven to be useful but it cannot be performed immediately on demand due to kits' design.

In this setting, real-time PCR offers a suitable diagnostic alternative to detect low fungal DNA loads in upper airway samples such as oral washes (OW). To date, several targets present in single or multiple copy in PJ genome have been described with variable diagnostic yield. Two of the most stable ones are the DHPS (single copy) and the mitochondrial RNA Small Subunit (mtSSU, multiple copy) genes. Multicopy targets may be more sensitive to detect PJ at the expenses of lowering its diagnostic specificity.

Our aim was to assess the diagnostic performance of 2 rt-PCR techniques targeting the DHPS and mtSSU genes using OW as clinical sample, and to compare their performance with results obtained in BAL samples for the non-invasive diagnosis of PJP by semi-quantification of their results.

Results.

Paired OW and LBA samples were collected from 30 consecutive adult patients presenting with an acute respiratory episode and having *P. jirovecii* infection suspicion included in the differential diagnosis. PJP was diagnosed in 16 patients, whereas 6 patients were considered to be colonized; their baseline characteristics are reflected in table 1.

Table 1	PJ INFECTION	PJ COLONISATION
Age*	60 [54.7-66.7]	66.5 [61.7-70.5]
Sex (Male/Female).	9 M/7 F	6 M/2 F
Main Baseline condition:		
-Solid neoplasm.	7	3
-Haematologic neoplasm.	3	3
-Solid organ transplant	2	0
-Stem cell transplant	1	0
-HIV+	3	0
Lymphocyte count (cells/ μ L)*.	749 (494-1115)	1105 (959-1100)

*Values expressed as Median [IQR]).

Both DHPS and mtSSU tested positive in all LBA and OW samples from the 16 PJP patients. mtSSU was able to detect colonization in 6/6 LBA and DHPS in 5/6. In contrast, all but 1 OW samples from colonised patients gave negative results by both techniques. Larger Ct values were observed for DHPS as compared to mtSSU for all combinations sample-technique (table 2; fig 1 and 2).

Table 2	PJ Infected		PJ colonised	
	OW	BAL	OW	BAL
DHPS	36.2 (34.8-36.9)	34.2 (32.8-35.7)	37.6	37.6 (37.2-37.9)
mtSSU	31.9 (29.6-34.6)	30.2 (26.8-31.6)	34.8	33.8 (33.3-33.9)

*Ct values expressed as Median [IQR]).

RNA Small Subunit (mtSSU. Valero et al. *Front. Microbiol.* 2016; 7:1413.) genes. DNA was obtained from aliquots of OW and LBA samples using a commercial kit (QiAmp mini kit, Qiagen). Assay was run in a SmartCycler apparatus (Cepheid) in a final volume of 25 μ L using Quantitect NoRox (Qiagen) as Master Mix, and RNaseP as internal control. Results were expressed as cycle threshold (Ct) values [median (interquartile range)]. Sensitivity (Se), Specificity (Sp), and AUC values were calculated.

Fig 1 and 2: Distribution of Ct values from infected and colonised patients

Ct cut-off values of 36.6 (OW-DHPS), 35.6 (OW-mtSSU), 33.5 (BAL-DHPS) and 30.9 (BAL-mtSSU) correlated with a diagnosis of PJP, with a Sp/AUC of 60/0.767 (DHPS-OW), 66.7/0.700 (mtSSU-OW), 71.4/0.893 (DHPS-BAL), and 75/0.929 (mtSSU-BAL).

Conclusions.

- ★ Both DHPS (single copy) and mtSSU (multicopy) performed similarly for the detection of PJ in OW and LBA samples. mtSSU detected 1 more colonization, what may have decreased its specificity as compared to DHPS.
- ★ Molecular techniques targeting DHPS or mtSSU seem to be equally useful for the diagnosis of PJP in non-invasive samples such as OW. Despite displaying narrow ranges of Ct that facilitate the discrimination between colonization and infection, it is advisable to interpret their results along with additional clinical and complementary tests information.