Application of real time PCR assays for the diagnosis of histoplasmosis using three molecular targets in human FFPE tissues and whole blood samples

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Introduction

Histoplasmosis is a fungal infection that causes significant morbidity and mortality in persons living with HIV/AIDS¹. Currently used diagnostic tests rely on culture and serology, lack sensitivity and often require weeks to obtain results causing significant diagnosis delays²; although a few molecular approaches have been developed, DNA-based diagnostic tools are not routinely used for diagnosis and PCR assays are not commercially available³. We recently standardized and validated a real time PCR (qPCR) assay, using three protein-coding genes as molecular targets (100-kDa, M and H antigens) in an animal model⁴. In the present study, we applied these qPCR protocols for the detection of *H. capsulatum* DNA in human samples.

Materials and methods

Formalin-fixed paraffin-embedded (FFPE) tissue samples. A total of 97 FFPE tissue samples were collected from patients diagnosed by histopathological analysis with different infectious diseases: histoplasmosis n=36, TB n=29, cryptococcosis n=6, leishmaniasis n=5, *Malassezia* infection n=2, sporotrichosis, pneumocystosis, mucormycosis, cytomegalovirus, herpes virus or *tinea* infection n=6, and from patients with non-infectious diseases: prostatic hyperplasia n=2, cholestasis n=3, appendicitis n=3, uterine prolapse, endometriosis, suprarenal hyperplasia, abdominal carcinoma or soft tissue mass n=5.

Whole blood (WB) samples. WB samples from HIV/AIDS patients with clinical suspicion of disseminated histoplasmosis were candidates for the PCR application (figure 1).

Figure 1. WB samples from patients analyzed in this study

Real time PCR amplification. Different protein-coding genes of *H. capsulatum* (100-kDa, H and M antigens) were selected and amplified in clinical samples; primers, probes and protocols were used according to López *et al*⁴ (Table 1).

Table 1. Sequences of the primers and probes for each molecular target

Primers and probes	Sequence 5'-3'	Protein-coding genes
84R22 (Forward)	5' GCAGARAATTGACCCYCAAGCC 3'	100-kDa
15F23 (Reverse)	5' GTATCCCACAGCATCMYGGAGGT 3'	
45L23	5' 6FAM-CCTTCTTGCAACTYCCYGCCT-CT-BBQ 3'	
2F (Forward)	5' CACCGAGATAAAGGTGTCGA 3'	H Antigen
2R (Reverse)	5' GGATCAGGGCTGAAACCTTC 3'	
2P	5' 6FAM-CTGCCCAACGGTCCAACCACA-BBQ 3'	
F1 (Forward)	5' CGCCRACGGGCTTTCAT 3'	M Antigen
R1 (Reverse)	5' CATCGCTTGATCCCCAACG 3'	
TM	5' 6FAM-ACCYSTTGGGTATTGCGTTGAGG-BBQ 3'	

Results

For FFPE samples, the sensitivities of 100-kDa, H and M qPCR assays were 93.9%, 91% and 57%, respectively; however, the same qPCR assays showed only 23%, 19% and 11.5% of sensitivity for 100-kDa, H y M qPCR assays when used with the WB samples. The specificity of qPCR was determined by testing samples from patients with other clinical infections and healthy controls and was 93%-100% depending on the assay and the specimen type.

Conclusions

We applied three qPCR assays for detecting *H. capsulatum* DNA in human samples, and demonstrated that the molecular protocols based on amplification of 100-kDa and H antigen can be successfully used for diagnosing this mycosis when using FFPE samples; however, we do not recommend WB for routine diagnosis of histoplasmosis by qPCR in patients with progressive disseminated histoplasmosis. Indeed, it is known that even in systemic infection caused by *Histoplasma* the fungus' presence in the blood can be transitory.

Acknowledgements: This work was supported by Colciencias (244-2013), Colombia; Corporación para Investigaciones Biológicas, Medellín-Colombia; Fondo de Investigaciones de la Universidad del Rosario (DVG154), Bogotá-Colombia; and the Mycotic Diseases Branch, Centers for Disease Control and Prevention Atlanta-USA.

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