

TITLE: Use of a low-volume sample and multiplex PCR for etiological diagnosis of infectious uveitis

Authors: Ferracioli-Oda, E.¹; Tanaka, T.¹; Gouvea, M.S.G.²; Pinho, J.R.R.²; Coelho, V.³; Yamamoto, J.H.¹

Institution: 1 - Departamento de Oftalmologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo; 2 - Laboratório de Gastroenterologia e Hepatologia Tropical (LIM-07) IMT-FMUSP; 3 - Laboratório de Imunologia - InCor - HCFMUSP

Abstract:

Uveitis, defined as any intraocular inflammation affecting mainly the uveal tract (iris, ciliary body and choroid with extension to retina and/or vitreous), is characterized as infectious, non-infectious, masquerade or idiopathic. Etiology definition relies mostly on clinical features and complementary systemic tests (i.e. serological assays) and is usually a presumed diagnosis. Several situations demand confirmatory tests for a proper differential diagnosis using mainly polymerase chain reaction (PCR) of ocular fluids (aqueous and/or vitreous humor). However, most of the tests are singleplex and require a large sample volume. In this study, we analyzed aqueous humor sample of 22 patients with active uveitis. Aqueous humor was obtained by paracentesis with a maximum volume of 200µl. A direct multiplex, real-time qualitative PCR (direct Strip PCR) has been developed by Japanese researchers for uveitis diagnosis purpose, detecting 9 pathogens associated to uveitis (herpes simplex virus 1 and 2; varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpes virus 6, human T-lymphotropic virus, *Treponema pallidum* and *Toxoplasma gondii*) using 20µl of ocular fluid. Uveitis characteristics were acute in 12 patients (9 with posterior uveitis) and chronic/recurrent in 10 patients (8 with anterior uveitis). Overall positivity was 31.8% (7/22 samples); among acute uveitis was 50% (6/12 samples). Herpes virus was detected in 4 samples: 2 for HHV6, 1 for HSV2 and 1 for EBV; *T. gondii* was detected in 2 samples and *T. pallidum* in another sample. Direct Strip PCR result was relevant for final diagnosis or treatment adjustment in 5 patients. This method seems to be very promising considering the speed (within 75 minutes), very low volume (20µl), no need for DNA extraction, testing for multiple relevant pathogens.

Keywords: infectious uveitis; aqueous humor; PCR

Supporting agency: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) - 2019/25060-2