## 2009 Research Days Abstract Form – Department of Ophthalmology – UNIFESP/EPM

2. SCIENTIFIC SECTION PREFERENCE (REQUIRED):

Review the Scientific Section Descriptions. Select and enter the two-letter Code for the one (1) Section best suited to review your abstract.

3. PRESENTATION PREFERENCE (REQUIRED) Check one:

- ☐ Paper
- ☐ Poster
- ☐ FAST Paper

4. The signature of the First (Presenting) Author (REQUIRED) acting as the authorized agent for all authors, hereby certifies that any research reported was conducted in compliance with the Declaration of Helsinki and the 'UNIFESP Ethical Committee"

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Scientific Section Descriptions (two-letter code):

(BE) OCULAR BIOENGINEERING
(CO) CORNEA AND EXTERNAL DISEASE
(CA) CATARACT
(EF) ELECTROPHYSIOLOGY
(EP) EPIDEMIOLOGY
(EX) EXPERIMENTAL SURGERY
(GL) GLAUCOMA
(LA) LABORATORY
(LS) LACRIMAL SYSTEM
(LV) LOW VISION
(NO) NEURO-OPHTHALMOLOGY
(OR) ORBIT
(PL) OCULAR PLASTIC SURGERY
(PH) PHARMACOLOGY
(RE) RETINA AND VITREOUS
(RS) REFRACTIVE SURGERY
(RX) REFRACTION-CONTACT LENSES
(ST) STRABISMUS
(TR) TRAUMA
(TU) TUMORS AND PATHOLOGY
(UV) UVEITIS
(US) OCULAR ULTRASOUND

Deadline: Oct 12, 2009

FORMAT:
Abstract should contain:
**Title**
**Author, Co-authors (maximum 6), Purpose, Methods, Results, Conclusion.**

Poster guidelines:
ARVO Abstract Book (1.10 x 1.70m)

49. FIRST (PRESENTING) AUTHOR (REQUIRED):
Must be the author listed first in abstract body.

( ) R1 ( ) R2 ( ) R3 ( ) PIBIC
( ) PG0 ( X) PG1 ( ) Fellow ( ) Technician

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Middle: Belfort

Service (Sector): Uveitis

CEP Number: 115/07

**Evaluating the presence of *Toxoplasma gondii* in peripheral blood of patients with uveitis.**

Belfort RN, Fernandes B, Miamoto C, Camargo M, Muccioli C, Belfort R Jr, Burnier MN Jr - Instituto da visão, UNIFESP, Brasil. Henry C. Witelson Ocular Pathology Laboratory, McGill University, Canada.

**Purpose**
Toxoplasmosis is the most common cause of infectious posterior uveitis worldwide. The mechanisms involved in the severity and recurrences of this particular disease are poorly understood. New diagnostic methods, such as identifying circulating parasites, are necessary to diagnose atypical cases, as well as to determine if recurrence is a local or systemic event. We have compared three methods to identify *Toxoplasma gondii* in peripheral blood of patients with uveitis.
**Methods**
RH strain of *T. gondii* was cultured on fibroblast monolayer for 5 weeks. A hemocitometer was used to determine the parasitic concentration and serial dilutions were made: $10^6$, $10^5$, $10^4$, $10^3$ and $10^2$ parasites per milliliter. Cytospins and smears were performed from samples of 3 mL of blood, spiked with different parasitic concentrations. Smears were performed from whole blood. Cytospins were done from the white blood cell layer previously separated using Ficoll. Immunohistochemistry on smears and cytospins were performed on an automated Ventana machine using antibodies for *T. gondii*. For qPCR, different concentrations of *T. gondii* were added to 1mL of blood. qPCR was performed using the DNA extracted from spiked blood and targeting a 62bp fragment of the B1 gene or the 529bp fragment. The primers used were: B1 Forward 5'- CTA GTA TCG GTG CGG CAA TGT -3'and Reverse 5'- GGC AGC GTC TCT TCC TCT TTT -3' e 529 CGC TGC AGG GAG GAA GAC GAA AGT TG- 3' and reverse 5´- CGC TGC AGA CAC AGT GCA TCT GAA TT- 3'. Twenty milliliters of blood from patients presenting with different etiology of uveitis had DNA extracted up to six hours after blood was collected, to prevent loss of DNA. The genetic material was preserved in special FTA paper plates or frozen until testes by qPCR for *T. gondii* using the methodology developed by us was performed. Tests were run in triplicate.
**Results**
The cytospins were positive for both concentrations of $10^6$ and $10^5$ toxo/ml while the smears were only positive for $10^6$ toxo/mL. The qPCR method using the 529 bp fragment as target was able to detect parasites in concentrations as low as 1 toxo/mL and the amplification product resulted in uniform melting curves. Out of 32 patients tested, only two presented positive PCR for *T. gondii* in peripheral blood.
**Conclusion**
To the best of our knowledge, this is the first side-by-side comparison of these three methods to detect *T. gondii* in peripheral blood. All three methods were able to identify *T. gondii* in peripheral blood. However, the qPCR method using the 529bp target proved to be more sensitive than qPCR using the B1 gene target, cytospins or blood smears. These results indicate that qPCR targeting the 529bp fragment is likely the most appropriate method to identify *T. gondii* in the peripheral blood of ocular Toxoplasmosis patients. Despite the high sensitivity of our method, only patients immunosuppressed presented circulating parasites in peripheral blood. Reasons for these results will be discussed in detail during the oral presentation.