EV0800
ePoster Viewing
Diagnostic parasitology

Detection of B1 gene and REP 529 in Toxoplasma gondii

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Background: Toxoplasmosis is caused by the parasite *Toxoplasma gondii*. Diagnosis of *Toxoplasma gondii* infection is normally based on serology methods, but these methods pose some difficulties for diagnosis in neonates born to infected-mothers or in immunocompromised. A positive IgG result in a neonate may reflect maternal placental transfer, while the IgM may be detected in a neonate due to placental leakage of maternal IgM. In contrast, negative anti-*Toxoplasma* antibody does not exclude Toxoplasmosis of an immunocompromised individual. Thus, direct detection of *Toxoplasma gondii* DNA using PCR, targeting the 35-fold repeated B1 gene has been commonly used for molecular diagnosis, but another sequence, the 529-bp repeat element (REP 529) was described more recently as being 200 to 300-fold repeated. Genetic studies of *Toxoplasma gondii* isolates from Europe and the United States grouped these isolates into 3 major multi-locus genotypes, types I, II and III. In Europe, type II (haplogroup 2) and type III are predominant, but in Asia, type III (haplogroup 3) is predominant. Hence, two real-time PCR, one targeting B1 gene and the other targeting REP 529, were used to detect the genome of *Toxoplasma gondii.*

Material/methods: 39 archived samples were selected. 25 were proficiency samples from QCMD (17 type II positives, 8 negatives) and 14 were clinical samples (3 unknown positives, 11 negatives). These 39 samples were extracted by EZ1 Virus mini kit v2.0 (QIAGEN) and amplified on CFX96 (Bio-Rad) using QuantiTect Multiplex No Rox Kit (QIAGEN). A quantitated, purified complete genome of *Toxoplasma gondii* DNA control, (Amplirun *Toxoplasma gondii* DNA Control, VircelI) was diluted to test the limit of detection.

Results: 76.4% (13/17) of the archived proficiency positive samples were detected by both *Toxoplasma gondii* B1 gene and REP 529 after re-extraction. The original extracts of these two samples were detected by both *Toxoplasma gondii* B1 gene and REP 529, however they were detected by either *Toxoplasma gondii* B1 gene or 529 bp repeat element after re-extraction likely due to freeze-thaw degradation. One sample was detected by only REP 529 after re-extraction, and the original extract was not available for verification. This could be due to freeze-thaw degradation. 100% (3/3) of the clinical positives were detected by both *Toxoplasma gondii* B1 gene and REP 529 after re-extraction. 100% (8/8) of the archived proficiency negative samples and 100% (11/11) of the clinical negatives were also not detected by both *Toxoplasma gondii* B1 gene and 529 bp repeat element after re-extraction. Moreover, this assay can detect at 95% confidence level, B1 gene at 0.97 copies/µL and REP 529 at 0.69 copies/µL, based on probit analysis.

Conclusions: B1 gene and REP 529 can detect *Toxoplasma gondii* in clinical and proficiency samples, but REP 529 is more sensitive than B1 gene.