Rapid Detection of Viable *Toxoplasma gondii* by Nucleic Acid Sequence-Based Amplification (NASBA), Using Primer Sets Targeting B1 rRNA Gene

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ABSTRACT

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Many diagnostic tests are currently used to diagnose chronic toxoplasmosis, yet the biggest challenge remains the rapid diagnosis of active infection, either primary or secondary. The late appearance of antibodies or their inability to emerge in case of defective immunity represents obstacles with serological testing. Molecular diagnosis relied on the detection of DNA are available, however, detecting DNA does not mean the presence of viable infectious organisms. Therefore, the switch to RNA detection technologies is necessary. In this initial study, a trial was done for early detecting genomic materials of viable circulating *Toxoplasma gondii* in the blood of 24 infected mice at variable doses, ranging from 10/ml to 10^8/ml *Toxoplasma gondii* RH strain tachyzoites, *applying* Real-time Nucleic acid sequence-based amplification technique (NASBA) and primer sets targeting B1 rRNA gene. The standard curve of real-time NASBA was created using serially diluted specific RNA samples that all generated signals for absolute genomic quantitation and crossing points ranging between 11.07 and 31.32. Fluorescence signals were created from all samples isolated from the 24 infected mice with different infection doses with quantitative genomic equivalent ranging from 1.3×10 to 9.6×10^{10} with crossing points ranging between 12.1 and 37.43, while no signals were detected from all negative control samples. In conclusion, NASBA is a relatively rapid primer-dependent molecular technique that can be performed in a single tube at a constant temperature for continuous amplification of a huge amount of nucleic acid. The technique can be used in medical diagnostics as an alternative to PCR, being quicker and sensitive to detect viable *Toxoplasma* circulating parasites.

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