Genomic and computational analysis of *Toxoplasma gondii* direct from clinical samples using selective genome whole-genome amplification (SWGA).

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Toxoplasma gondii is a protozoan parasite which infects approximately one third of the world's population. Infection usually results in mild symptoms or is asymptomatic; however, severe, life-threatening disease can occur in immunocompromised individuals and, if acquired during pregnancy may result in foetal abnormalities or death.

The Toxoplasma Reference Unit (TRU) investigates *Toxoplasma* infection in patients from England and Wales, and provides advice on patient management and risk reduction. A cost-effective method of performing whole genome sequencing on *Toxoplasma* is highly desirable. This would have a significant impact on rapid diagnosis and molecular typing, which in turn would support enhanced molecular surveillance and promote effective management of clinical infections. Whole genome sequencing (WGS) of *T. gondii* has been successful using parasites cultured in laboratory animals. For human clinical samples, however, the technology is limited because the relative levels of host genomic DNA are much higher than those of the pathogen. Selective Whole Genome Amplification (SWGA) is a new technique that has been successfully employed to specifically amplify the Malaria parasite, *Plasmodium spp.,* directly from clinical blood samples. The technique involves searching both the parasite genome and the human genome for short, e.g. 6 to 12 nucleotide, motifs that are much more common in the parasite genome than in the human genome. These motifs can be then used as oligonucleotide primer targets for the whole genome amplification reaction, thus enriching the target organism DNA concentration.

The successful outcome of this project will allow analysis of *Toxoplasma* genome sequences directly from human clinical samples, opening a valuable collection of DNA extracts and original clinical samples with accompanying clinical data for analysis. These resources would enable significantly more in-depth investigations, improving our understanding of the epidemiology, virulence and other traits of this important human pathogen, thus assisting in developing strategies for treatment, surveillance and infection prevention. The successful development of this method will also make an important contribution to the '3Rs' (Replacement, Refinement and Reduction in the use of animals in research) by precluding the need for *in vitro* isolation of *Toxoplasma* from clinical specimens prior to WGS.