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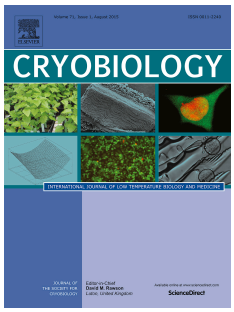

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Optimization of the cryopreservation of biological resources, *Toxoplasma gondii* tachyzoites, using flow cytometry

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ABSTRACT

The conservation of *Toxoplasma gondii* strains isolated from humans and animals is essential for conducting studies on *Toxoplasma*. Conservation is the main function of the French Biological *Toxoplasma* Resource Centre (BRC *Toxoplasma*, France, http://www.toxocrb.com/). In this study, we have determined the suitability of a standard cryopreservation methodology for different *Toxoplasma* strains using the viability of tachyzoites assayed by flow cytometry with dual fluorescent labelling (calcein acetoxymethyl ester and propidium iodide) of tachyzoites. This method provides a comparative quantitative assessment of viability after thawing. The results helped to define and refine quality criteria before tachyzoite cryopreservation and optimization of the cryopreservation parameters. The optimized cryopreservation method uses a volume of 1.0 mL containing 8 x 10^6 tachyzoites, in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% foetal calf serum (FCS). The cryoprotectant additive is 10% v/v Me_2SO without incubation. A cooling rate of ~1°C/min to -80°C followed, after 48 hours, by storage in liquid nitrogen. Thawing was performed using a 37°C water bath that produced a warming rate of ~100°C/min, and samples were then diluted 1:5 in IMDM with 5% FCS, and centrifuged and resuspended for viability assessment.

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