

# COMPARISON OF EFFICIENCY OF DIFFERENT CRYOPRESERVATION PROTOCOLS FOR *Leishmania* spp. STORAGE



Felipe do Espirito Santo Silva Pires & Elisa Cupolillo

Laboratório de Pesquisa em Leishmaniose, Coleção de *Leishmania* do Instituto Oswaldo Cruz

IOC/FIOCRUZ, Instituto Oswaldo Cruz/Fundação Oswaldo Cruz, Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ, Brasil

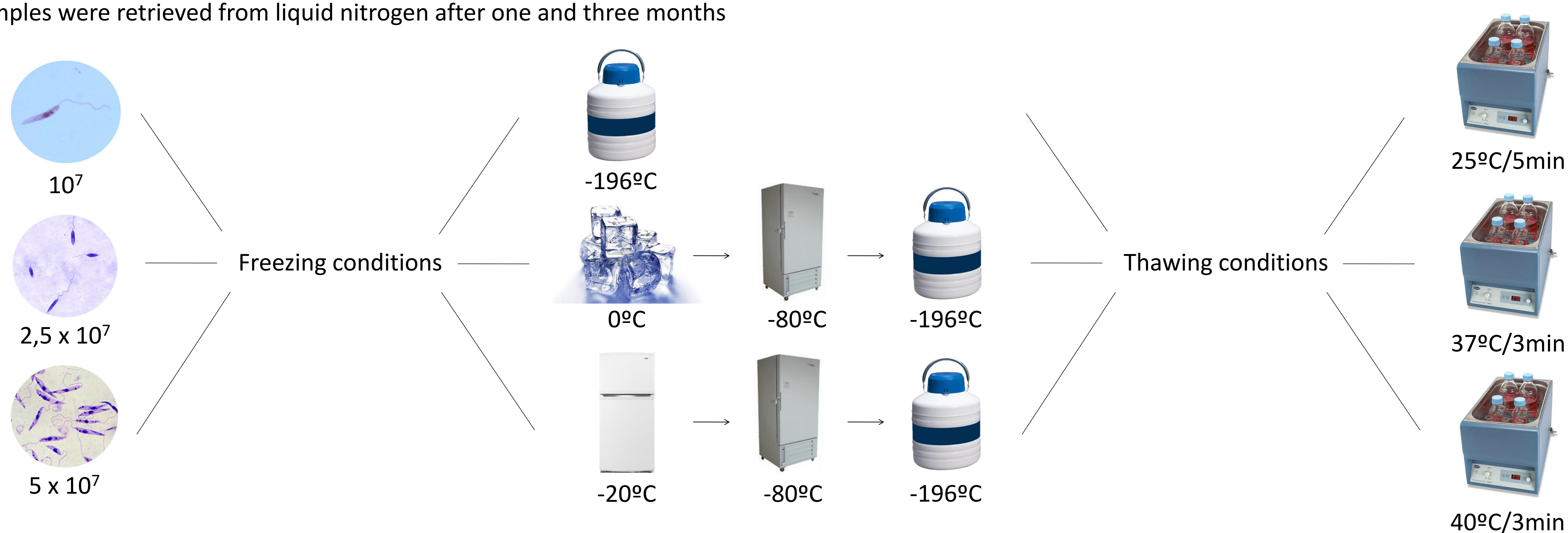
In general, the concentration and type of cryoprotectant, and the freezing-and-thawing rates are known as fact affecting the viability after cryopreservation. Despite the tremendous diversity of the genus *Leishmania*, usually the same cryopreservation protocol is employed for different species. In this study, we examined the effects of cell concentration and freezing-and-thawing conditions on the survival of *Leishmania* after cryopreservation.

## METHODOLOGY

✓ Eight species were chosen to represent the diversity of the genus: *L. (Viannia) guyanensis*, *L. (V.) lainsoni*, *L. (V.) braziliensis*, *L. (Leishmania) amazonensis*, *L. (L.) chagasi*, *L. (L.) major*, *L. colombiense* and *L. equatoriense*

✓ The cryoprotector and concentration employed were the same – 8% glycerol

✓ The samples were retrieved from liquid nitrogen after one and three months



## RESULTS

✓ It was observed that the cell densities did not affect the cell viability after freezing

✓ When frozen directly at -196° C, only *L. major* and *L. amazonensis* presented viability independent of the thawing condition

✓ An excellent viability was obtained for *L. lainsoni*, *L. major*, *L. guyanensis*, *L. equatoriense* and *L. colombiense* when the freezing rates employed were 0°C/-70°C/-196°C or -20°C/-70°C/-196°C

✓ *L. chagasi*, *L. amazonensis* and *L. braziliensis* had similar results, but the viability was better when the thawing conditions were 37°C/3min, 37°C/3min and 40°C/3min, respectively

<i>Leishmania</i> species	Freezing condition	Thawing condition	Freezing condition	Thawing condition	Freezing condition	Thawing condition
			0°C/-70°C/-196°C		-20°C/-70°C/-196°C	
<i>L. guyanensis</i>	-196		✓	i, ii, iii	✓	i, ii, iii
<i>L. lainsoni</i>	×		✓	i, ii, iii	✓	i, ii, iii
<i>L. braziliensis</i>	×		✓	iii	✓	iii
<i>L. amazonensis</i>	✓	i, ii, iii	✓	ii	✓	ii
<i>L. chagasi</i>	×		✓	ii	✓	ii
<i>L. major</i>	✓	i, ii, iii	✓	i, ii, iii	✓	i, ii, iii
<i>L. colombiense</i>	×		✓	i, ii, iii	✓	i, ii, iii
<i>L. equatoriense</i>	×		✓	i, ii, iii	✓	i, ii, iii

Thawing conditions

i 25°C/5min

ii 37°C/3min

iii 40°C/3min

Cell viability

✓ decrease of ≤ 10% in the number of viable cells

× decrease of > 10% in the number of viable cells

## CONCLUSIONS

Difference in cell surface molecules among *Leishmania* species was already reported, thus it is not surprising that different species show a distinct pattern of susceptibility to freezing-and-thawing conditions. Other protocols are being evaluated, as well as cell viability will be determined by considering the maintenance for longer periods in liquid nitrogen. At the end of this study we aim to establish a unique and standardized protocol for *Leishmania* cryopreservation.

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