实验研究

人芽囊原虫保存方法的初步研究

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摘更

目的 观察冻存剂、温度等因素对人芽囊原虫活力的影响,探索理想的人芽囊原虫保存方法。 方法 从患者阳性粪便中分离人芽囊原虫,分装到2 ml无菌冻存管内,分别加入10%二甲基亚砜

(dimethylsulfoxide, DMSO)、40%丙三醇(GL)和15%乙二醇(EG)作冻存剂,放置于不同的温度下保存,用台盼蓝染色法和原虫培养法测定细胞的活力和增殖能力。 结果 虫体在室温(18 ℃~20 ℃)下可存活3周,在4 ℃~6 ℃存活不到1周。加入冻存剂后,置-20 ℃和液氮(-196 ℃)下可存活3个月以上。用40% GL作冻存剂,于液氮低温下冻存的虫体保存半年后复苏,活力仍达41.7%,培养72 h后多见分裂相细胞。 结论 应用40% GL作为冻存剂, 在液氮中低温保存人芽囊原虫效果较佳。4 ℃不适于保存人芽囊原虫。

关键词

人芽囊原虫 保存 温度

分类号

A Preliminary Study on Preservation Methods of Blastocystis hominis

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Abstract

Objective To observe the effect of different cryoprotective agents and temperature factors on the viability of *Blastocystis hominis* so as to explore the ideal method for preservation of *B. hominis*. Methods *B. hominis* agents were obtained from a patient's fecal specimen. Having washed by normal saline and divided into tubes, the samples were cryopreserved in -20 °C refrigerator or in -196 °C liquid nitrogen with 10% DMSO, 40% glycerol and 15% ethylene glycol respectively. The thawed *B. hominis* agents were then used for culture. By trypan blue staining and microscopy, the viability and proliferation of those resuscitative cells were investigated. Results *B. hominis* survived for 3 weeks at 18 °C-20 °C while less than 1 week at 4 °C-6 °C. When stored in -20 °C refrigerator or liquid nitrogen with cryoprotective agents, they survived for more than 3 months. The cryopreservation with 40% glycerol at -196 °C for 6 months resulted in 41.7% viability of the revivified cells. Cleavage cells were easily observed after culturing for 72 hours. Conclusion Preserving *B. hominis* in liquid nitrogen with 40% glycerol is an optimal cryopreservation protocol.

Key words Blastocystis hominis Preservation Temperature

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