

实验研究

应用环介导等温扩增技术检测弓形虫

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摘要

目的 用环介导等温扩增(LAMP)技术检测弓形虫。方法 用酚-氯仿法提取弓形虫速殖子基因组DNA,设计两对扩增弓形虫B1基因的LAMP引物。以间日疟原虫、恶性疟原虫、卡氏肺孢子虫、日本血吸虫及小鼠白细胞作对照,进行LAMP反应,产物经SYBR Green I显色及电泳后观察结果,绿色判为阳性,棕色判为阴性。将弓形虫速殖子经倍比稀释为(2~3)×10⁶个/ml至(2~3)×10⁻¹个/ml等8个浓度,进行LAMP反应,验证该方法的敏感性。结果 LAMP反应结果显示,弓形虫速殖子检测管经显色后呈绿色,对照组均呈棕色。弓形虫的LAMP产物经电泳后呈LAMP特征性梯状条带,对照组均无扩增产物。LAMP技术可检测到的弓形虫速殖子最低浓度为2~3个/ml。结论 LAMP技术在弓形虫检测中显示出较好的特异性与敏感性。

关键词 [弓形虫](#) [检测](#) [环介导等温扩增技术](#)

分类号

Detection of Toxoplasma gondii DNA by Loop-Mediated Isothermal Amplification

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Abstract

Objective To detect Toxoplasma gondii DNA by loop-mediated isothermal amplification (LAMP). Methods DNA was extracted by phenol-chloroform extraction from T. gondii tachyzoites. Four primers which recognized 6 distinct regions on the B1 gene of T. gondii were designed and used for LAMP assay. To evaluate the specificity of the method, Plasmodium vivax, P. falciparum, Pneumocystis carinii, Schistosoma japonicum, and mouse leucocytes were used as controls. The parasite extract (T. gondii) was 10-fold serially diluted for evaluating the sensitivity of LAMP, and was amplified by LAMP. LAMP results were read with naked eye and analyzed by electrophoresis. Results After LAMP reaction, positive amplification was observed with T. gondii, but no positive signal was noted for the negative controls in the study. The sensitivity of LAMP assay reached up to 2-3 T. gondii tachyzoites/ml per reaction. Conclusion LAMP assay shows proper specificity and sensitivity for the detection of T. gondii.

Key words [Toxoplasma gondii](#) [Detection](#) [Loop-mediated isothermal amplification](#)

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