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微小隐孢子虫卵囊DNA提取及用于PCR检测

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摘要

目的采用3种方法提取微小隐孢子虫卵囊DNA,并用于PCR检测以进行比较。方法 微小隐孢子虫卵囊经多次冻融加热破壁后,采用螯合树脂(Chelex-100)、酚/氯仿和基因组DNA纯化系统试剂盒3种方法提取微小隐孢子虫卵囊DNA,并根据微小隐孢子虫基因序列(L16996)设计一对寡核苷酸引物,分别对3种方法制备模板进行PCR扩增分析。Chelex-100提取的DNA也用于观察PCR检测的敏感性。结果3种方法制备的微小隐孢子虫卵囊模板用于PCR检测均获得1条446 bp条带,Chelex-100提取的DNA用于PCR检测的敏感性至少达0.5个卵囊。结论3种方法提取的微小隐孢子虫卵囊DNA均可用于PCR检测,Chelex-100法是一种高效而快速的微量提取DNA方法,适用于对隐孢子虫DNA的检测。

关键词 微小隐孢子虫 卵囊 脱氧核糖核酸 分离和提纯 螯合树脂法 聚合酶链反应

分类号

Preparation of DNA from *Cryptosporidium parvum* Oocysts for PCR Detection

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Abstract

Objective To establish three methods of DNA extraction from Cryptosporidium parvum oocysts and test by PCR. Methods After three freeze-thaw cycles, three kinds of templates were extracted from the oocysts by Chelex-100, phenol/chloroform or genomic DNA purification system kit, and used for PCR detection. According to the sequence of a C.parvum gene (L16996), a pair of primers was designed and synthesized, and used for PCR. The sensitivity of the template by Chelex-100 method was also tested by PCR. Results One 446 bp PCR product was observed by agarose gel electrophoresis for all three kinds of templates. The PCR sensitivity by Chelex-100 extracted DNA reached for detection of a specimen containing only 1/2 oocyst. Conclusion The three kinds of extraction can all be served as templates for PCR detection of C.parvum oocysts, while Chelex-100 method is simpler, quicker and more reliable for DNA extraction of the parasite.

Key words *Cryptosporidium parvum* Oocysts DNA Isolation and purification Chelex-100 method PCR

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