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*Taq*Man探针实时荧光PCR方法检测粪便中微小隐孢子虫卵囊

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Detection of *Cryptosporidium parvum* in Human Stool Using *Taq*Man Real-time Polymerase Chain Reaction

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摘要 以微小隐孢子虫（*Cryptosporidium parvum*）特异性DnaJ-like蛋白基因的保守序列为模板，设计和合成特异性引物和荧光标记探针，通过检测微小隐孢子虫卵囊DNA和加标模拟样品进行敏感性分析，建立标准曲线，并对其特异性和干扰性进行评价。结果显示，该方法只对微小隐孢子虫卵囊进行特异性扩增，其他常见的肠道原虫和肠道病原菌均不能扩增；微小隐孢子虫纯卵囊基因组DNA检测的灵敏度为26个/ml；对加标粪样可检测至2 600个/ml卵囊。提示本研究建立的实时荧光PCR检测微小隐孢子虫卵囊方法具有快速、特异性和敏感性高等优点。

关键词： 微小隐孢子虫 *Taq*Man探针 实时荧光PCR 检测

Abstract： The special DnaJ-like protein gene of *Cryptosporidium parvum* was amplified through designing special primers and *Taq*Man probes within the conserved and specific regions for this gene. In this way, a rapid and stable method of real-time PCR assay for the detection of *C. parvum* was established. The specificity and sensitivity of PCR were also analyzed. By adding standard culture fluid in blank fecal sample, the sensitivity of the method was evaluated. The results showed that the detection limit of pure culture with real-time PCR assay was 26 oocysts/ml. The detection limit for *C. parvum* in artificially contaminated fecal sample was 2 600 oocysts/ml. The specificity of the method was verified with no amplification on DNA from other enteric parasites and bacteria. These results indicated that the real-time PCR method for *C. parvum* detection in fecal sample is simple, rapid, with high specificity and sensitivity.

Keywords： *Cryptosporidium parvum* *Taq*Man probe Real-time PCR Detection

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