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论著

FTA-DNA直接提取法在病原真菌分子鉴定中的应用

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摘要： 目的 建立并评价FTA-DNA直接提取法在病原真菌分子鉴定中的应用。方法 采用whatman FTA-DNA直接提取法从25个不同种属的45株培养的菌株和6例临床标本中提取病原真菌DNA,用于病原真菌的测序鉴定。配制不同浓度的孢子悬液探索该方法的检测限和安全性。结果 45株菌株扩增后均能得到1条清晰的DNA扩增片段,并成功测序。应用该方法亦成功从腹水、胸水、口腔拭子、宫颈拭子来源的临床标本中直接提取DNA并成功鉴定病原真菌。该DNA提取方法联合降落PCR能检测到1.0×10^3个cell/mL的孢子悬液,1.0×10^4个cell/mL及以下浓度的孢子悬液可以被FTA卡完全灭活。结论 FTA-DNA直接提取法可快速有效地从培养的菌株及部分临床标本中提取并保存病原真菌DNA,用于病原真菌的测序鉴定。

关键词： 真菌 分子鉴定 FTA卡 DNA提取方法

A rapid DNA extraction method for molecular identification of pathogenic fungi

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Abstract: Objective To develop and evaluate a rapid DNA extraction method using Whatman FTA cards,in molecular identification of fungi.Methods DNA was extracted from 45 isolates encompassing 25 species and 6 clinical samples by whatman FTA cards.Then PCR amplification and sequencing of the internal transcribed spacer region 1 (ITS_1) were conducted.Serial diluted suspension of conidia was prepared to determine the detection limit and security of this method.Results A clear DNA amplified fragment was obtained and successfully sequenced in all 45 isolates from clinical samples.The detection limit was approximately 10^3 cell/ mL cembined with touchdown PCR.Suspension with less than 10^4 cell/mL conidia could be thoroughly inactivated by FTA cards.Conclusions Whatman FTA technology thus represents an ultra-rapid method of fungal genomic DNA preparation for molecular identification from both cultures and clinical samples,and also potentially represents a powerful fungal DNA archiving and storage system.

Keywords: fungi molecular identification FTA cards DNA extraction methods

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