[1]赵婷婷,严磊,魏立雯,等.小鼠肝炎病毒反向斑点杂交检测方法的建立及初步应用[J].第三军医大学学报,2014,36(16):1679-1683.
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小鼠肝炎病毒反向斑点杂交检测方法的建立及初步[illegible]

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Title: Establishment and preliminary application of reverse dot blotting for detection of murine hepatitis virus

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关键词: MHV; 实验动物; 反向斑点杂交

Keywords: murine hepatitis virus; laboratory animals; reverse dot blotting

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摘要: 目的 建立一种反向斑点杂交方法（reverse dot blots, RDB）以检测小鼠肝炎病毒。 方法 用MHV四个毒株分别感染L929细胞，收获病毒提取RNA并逆转录成cDNA；根据MHV保守区基因组序列设计引物和探针，将上游引物5′端用生物素标记。进行PCR扩增，应用PCR产物进行反向斑点杂交，优化杂交条件，进行稳定性、特异性和灵敏度实验，建立反向斑点杂交法。用此法和酶联免疫吸附试验（enzyme-linked immunosorbent assay, ELISA）对41只小鼠进行检测。RDB检测结果阳性样本进行T载体克隆测序。 结果 试剂条4 ℃保质期为8个月。分别检测MHV、金黄色葡萄球菌、沙门菌和乙型肝炎病毒，仅MHV为阳性，RDB特异性为100%，且MHV PCR产物最低检测限为5 ng/μL。41只小鼠经本方法检测后，有5只MHV阳性，分布于3个群，而ELISA检测结果有5只阳性，分布于2个群，阳性样本T载体克隆测序结果与RDB检测结果一致。 结论 该方法具有简洁明了、稳定可靠、特异性好、灵敏度高等优点，可应用于实验动物小鼠肝炎病毒的检测。

Abstract: Objective To establish a method for detection of murine hepatitis virus (MHV) by reverse dot blotting (RDB). Methods L929 cells were infected with 4 MHV strains separately. MHV was harvested and RNA was extracted for reverse transcription to obtain cDNA. Primers and specific probes were designed on the

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basis of conserved sequence of MHV and forward primer was labeled with biotin. The method of RDB was developed using polymerase chain reaction (PCR) products. The stability, specificity and sensitivity of RDB were evaluated. In addition, 41 mice were tested by RDB and ELISA. The positive samples tested by RDB were detected by cloning and sequencing. Results The test strip of RDB could be kept at 4 ℃ for 8 months. The specificity of RDB was 100% for MHV (when testing with *Salmonella sp.*, *Staphylococcus aureus* and hepatitis B virus) and the limit of detection was down to 5 ng/μL MHV PCR products. In 41 mice tested by RDB, there were 5 MHV-positive mice distributed in three groups. But by ELISA, there were five MHV-positive mice distributed in 2 groups. The results by cloning and sequencing were consistent with the positive samples tested by RDB. Conclusions RDB is simple, reliable, sensitive and specific for identification of MHV of laboratory animals.

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赵婷婷, 严磊, 魏立雯, 等. 小鼠肝炎病毒反向斑点杂交检测方法的建立及初步应用[J].第三军医大学学报,2014,36(16):1679-1683.

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