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·“一带一路”专题研究·

重要虫媒病毒性传染病环介导等温扩增检测技术研究进展*

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【摘要】 登革热、基孔肯雅热、寨卡病毒热等重要虫媒病毒性传染病可引起人群发热、皮疹、关节痛和出血等主要临床症状,具有传播速度快特点,及时检测发现上述病例,采取疫情处置,对于遏制疫情蔓延具有重要意义。目前上述传染病常见的检测方法主要包括聚合酶链反应、二代测序、环介导等温扩增等分子鉴定技术,其中环介导等温扩增技术具有快速、简单、廉价、准确、灵敏度、特异性高特点,已广泛用于虫媒病毒传染病核酸分子检测。本文对上述重要虫媒病毒性传染病环介导等温扩增检测技术研究进展进行综述。

【关键词】 环介导等温扩增技术;虫媒病毒性传染病;分子检测技术;综述

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Advances in the loop-mediated isothermal amplification for detection of important arboviruses

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【Abstract】 Dengue fever, chikungunya, Zika virus disease and other important arbovirus infectious diseases could be caused by fever, rash, joint pain, hemorrhage and other main clinical symptoms, with the characteristics of the rapidity transmission, it was of great significance for curb above diseases transmission to timely detect them and take the epidemic disposal as soon as possible. At present, the common detection methods for the above infectious diseases mainly included the polymerase chain reaction, second-generation sequencing, and loop-mediated isothermal amplification and other molecular identifications, among them the loop-mediated isothermal amplification with fast, simple, cheap, accurate, sensitive and specific characteristics, has been widely used for nucleic acid molecular detection of arbovirus infectious diseases. In this paper, its advances were reviewed on the detection of important arboviruses infectious diseases.

【Key words】 The loop-mediated isothermal amplification test; arbovirus infectious diseases; molecular identification; review

***登革热、基孔肯雅热、寨卡热、黄热病、流行性乙型脑炎、西尼罗热等重要虫媒病毒性传染病可引起患者发热、皮疹、关节痛和出血等严重临床症状,对人体危害较大[1-2]。由于上述重要传染病隐性感染比例较高,临床医生较难及时诊断发现,疫情得不到及时处置,常引起疾病快速传播或蔓延,为此,及时检测发现病例,对于遏制虫媒传染病暴发或扩散具有重要的意义[3-5]。

目前常用于上述传染病的检测技术主要包括血清学检测技术和分子检测技术,其中血清学检测技术主要包括酶联免疫吸附试验(enzyme-linked immunosorbent assay, ELISA)、胶体金快速免疫层析(colloidal gold enhanced immunochronmtography assay, CGEIA)等抗原抗体检测技术,虽然血清学检测技术操作简便,所需仪器设备简单,并可同时检测大量样本,常用于疾病流行程度检测,但上述虫媒传染病IgM/IgG抗体产生一般需要5 d以上,且存在较高的抗体交叉反应、假阳性高[6-8]。分子检测技术主要包括聚合酶链式反应技术(polymerase chain reaction, PCR)、二代测序技术(Next-generation sequencing, NGS)和环介导等温扩增技术(Loop mediated isothermal amplification, LAMP)等,其中PCR、NGS检测所需时间长,如普通PCR耗时2-3 h,NGS则耗时更长,但LAMP检测技术具有快速、精确、成本低的特点,且该技术目前已发展成为逆转录等温扩增技术(reverse transcription loop-mediated isothermal amplification, RT-LAMP)、实时定量

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LAMP（quantitative real-time loop-mediated isothermal amplification）、侧向流 LAMP（Lateral flow assay Loop-mediated isothermal amplification，LFA-LAMP）、基于微流控的 LAMP、多重 LAMP（multiplex loop-mediated isothermal amplification，MLAMP）等多种 LAMP 系列技术，且已广泛应用在病毒、病原体、基因突变检测方面[9-17]。本文就 LAMP 检测技术在上述重要虫媒病毒性传染病检测研究进展综述如下。

1 RT-LAMP

该技术通过加入 SYBR Green I 等标记反应物，肉眼可直接观察颜色改变或浊度仪监测浊度变化判断检测结果[18]。Kwallah 等[19]采用 RT-LAMP 和 RT-PCR 比较检测黄热病毒（Yellow fever virus，YFV），结果发现 RT-LAMP 灵敏度为 0.29 PFU/ml，特异性为 100%，耗时仅 1 h，而 RT-PCR 灵敏度为 2.9 PFU/ml，特异性 100%，耗时 2 h；Hu 等[20]采用 RT-LAMP 和 Real-time PCR 比较检测登革病毒（dengue virus，DENV）发现，RT-LAMP 灵敏度为 10 copy/ml，特异性为 100%，耗时 20 min，而 Real-time PCR 灵敏度为 100 copy/ml，特异性为 100%，耗时 50 min；Kumar 等[21]采用 RT-LAMP 和 RT-PCR 比较检测西尼罗病毒（west Nile virus，WNV）发现，RT-LAMP 灵敏度为 0.1 PFU/ml，特异性为 100%，耗时 30 min，而 RT-PCR 灵敏度为 1PFU/ml，特异性为 95%，耗时相对较长；Benjamin 等[22]采用 RT-LAMP 和 RT-PCR 比较检测基孔肯亚病毒（Chikungunya virus，CHIKV）发现，RT-LAMP 灵敏度为 163 copy/ml，特异性为 100%，耗时 36.2 min，而 RT-PCR 灵敏度为 100%，特异度为 80%，耗时较长；Parida 等[23]采用 RT-LAMP 和 RT-PCR 比较检测流行性乙型脑炎病毒（Japanese encephalitis Virus，JEV）也发现，RT-LAMP 的灵敏度为 0.1 PFU/ml，特异性与 RT-PCR 比较为 86%，耗时 30 min，而 RT-PCR 灵敏度为 1PFU/ml，特异性为 100%，耗时较长；Lamb 等[24]建立了一种 RT-LAMP 方法检测寨卡病毒（Zika virus，ZIKV），其灵敏度为 1 copy/ml，特异性为 100%，耗时 30 min。上述结果提示，RT-LAMP 不仅灵敏度、特异性高，耗时短，且不需要昂贵的精密仪器，操作简单，尤其适用于疾病爆发时的现场检测，特别是大规模样本检测，但该技术引物设计复杂、且结果通过眼睛对浊度或颜色的改变进行判断，具有一定的主观性，且电泳时容易产生污染，会影响实验结果的准确性[25-27]。

2 实时定量 LAMP

该技术通过使用一系列不同浓度的待测样品标准溶液进行反应，并以不同标准溶液的浓度和其对应的出现阳性反应时间制作标准曲线以定量待检测样品浓度[28]。Kurosaki 等[29]采用实时定量 LAMP 检测 ZIKV 发现，其灵敏度为 10 copy/ml，特异性为 100%，耗时 15 min；Toriniwa 等[30]采用实时定量 LAMP 和实时荧光定量 PCR 比较检测 JEV 发现，实时定量 LAMP 灵敏度为 1 PFU/ml，特异性为 100%，耗时 1 h，而实时荧光定量 PCR 灵敏度为 10PFU/ml，特异性为 100%，耗时较长（3 h）；Parida 等[31]采用实时定量 LAMP 和 RT-PCR 比较检测 CHIKV 发现，实时定量 LAMP 灵敏度为 20 copy/ml，特异性为 100%，耗时 60 min，而 RT-PCR 灵敏度为 200 copy/ml，特异性为 100%，耗时 110 min；Parida 等[32]采用实时定量 LAMP 和实时荧光定量 PCR 比较检测 DENV 发现，LAMP 对 DENV-1 灵敏度为 1 PFU/ml，DENV-2，-3，-4 均为 0.1 PFU/ml，特异性均为 100%，耗时 30 min，其灵敏度是实时荧光定量的 10-100 倍，且 RT-PCR 耗时 55 min；Kumar 等[21]采用实时定量 LAMP 和实时荧光定量 PCR 比较检测 WNV 时也发现，该 LAMP 灵敏度为 0.1 PFU/ml，特异度为 100%，耗时 60 min，而 PCR 灵敏度为 1PFU/ml、特异性为 100%，耗时 3 h。上述结果提示，实时定量 LAMP 检测虫媒病毒灵敏度和特异性均较 PCR 高，在病毒感染早期，可用该技术进行定量分析，但连用实时浊度仪的 LAMP 技术在反应过程中需要频繁开盖操作，污染风险较高[33]。

3 侧向流 LAMP

该方法基于抗原抗体结合原理，阳性样品为 3 个反应步骤，首先是经过生物素化的靶向 LAMP 扩增产物与测试条上异硫氰酸（FITC）荧光素标记的 DNA 探针发生特异性杂交，随后该杂交物与胶体金标记的抗异硫氰酸荧光素抗体形成复合物，最终该复合物在检测线上与链霉亲和素反应，产生阳性信号；阴性样品中生物素化的非靶向产物不会与测试条上 FITC 标记的 DNA 探针杂交，最终不会在检测线上与链霉亲和素反应，而是直接通过检测线，停留于控制线上，产生阴性信号[34-36]。Lee 等[37]采用侧向流 LAMP 方法检测 ZIKV 发现，其灵敏度为 1 copy/ml，特异性为 100%，耗时 35 min。Deng 等[38]采用侧向流 LAMP 和 RT-PCR 比较检测 JEV，发现该 LAMP 法灵敏度为 5 pg RNA/ml，特异性为 100%，耗时 70min，而 RT-PCR 灵敏度为 5 pgRNA/ml，特异性为 100%，耗时 2 h；Cao 等[39]采用侧向流 LAMP 法检测 WNV 也发现，其灵敏度为 1.0e2copy/ml，特异性为 100%，耗时 42 min；Dauner 等[40]采用侧向流 LAMP 和实时定量 PCR 比较检测 DENV 显示，该 LAMP 灵敏度为 1 copy/ml，特异性为 100%耗时 30 min，而定量 RT-PCR 灵敏度为 10 copy/ml，特异性为 100%，耗时较长。上述结果提示，LFA-LAMP 在 LAMP 扩增之后不需要额外的样品和试剂处理即可判读结果，减少了污染和错误的可能性，同时结果判读直观，在无紫外线光源、资源有限的环境中较为适用。但该技术的测向流动分析依赖于抗原抗体结合机制以及链霉亲和素-生物素的结合机制，有可能引起与其他分子或非靶标的非特异性交叉结合，出现假阳性结果。

4 基于微流控的 LAMP

该 LAMP 技术结合微流控技术检测核酸分子的方法，将反应物通过微通道无限分离稀释后分散至单个微反应器内进行反应，反应结束后直接开盖肉眼观察计数阳性微反应器个数，以进行定性，定量[41-42]。Kaarj 等[43]采用微流控 LAMP 技术检测 ZIKV，其灵敏度为 1 copy/ml，特异性为 100%，耗时 15 min；Ganguli 等[44]采用微流控 LAMP 法检测 ZIKV，CHIKV 和 DENV，发现其检测 ZIKV 的灵敏度为 1.56e5PFU/ml，特异性为 100%，CHIKV 和 DENV 灵敏度为均 1.56e4PFU/ml，特异性为 100%，耗时 50 min。上述结果提示，微流控 LAMP 技术反应不需要开管操作，可在 15min 内观察到实验结果，样品定量简单直观，污染风险小，但该技术较为复杂，尚需要进一步完善。

5 多重 LAMP

多重 LAMP 是指在同一反应体系中加入两套或两套以上

引物，同时对多个靶基因片段进行扩增。Yaren 等[45]采用多重 LAMP 对 ZIKV、CHIKV、DNEV1 检测发现，其灵敏度 ZIKV 为 0.71 PFU/ml、DENV1 为 1.22 PFU/ml、CHIKV 为 37.8 copy/ml，特异性为 100%，耗时 30min。Li 等[46]采用多重 RT-LAMP 和多重 RT-PCR 比较检测 DENV1-4，JEV 和 WNV 发现，RT-LAMP 的准确度为 100%，其对 WNV 扩增的灵敏度为一步法 RT-PCR 的 10 倍，对 DENV1-4 和 JEV 扩增的灵敏度为一步法 RT-PCR 的 100 倍，且其特异性较高(100%)，耗时 20 min，而一步法 RT-PCR 准确度仅为 86.4%。Ball 等[47]采用多重 RT-LAMP 对 WNV 和 CHIKV 进行检测发现，其灵敏度 WNV 为 1 PFU/ml，CHIKV 为 100 PFU/ml，特异性为 100%，耗时 50-70 min。Ganguli 等[44]采用微流控 LAMP 对 ZIKV，CHIKV，和 DENV 进行检测发现，其灵敏度 ZIKV 为 1.56 e5PFU/ml，CHIKV，和 DENV 为 1.56 e4PFU/ml，特异性为 100%，耗时 50 min。Kutsuna 等[48]采用多重 LAMP 检测 DENV、CHIKV 和 ZIKV 发现，其灵敏度 DENV1 为 8 copy/ml、DENV2 为 1 copy/ml、DENV3 为 8 copy/ml、DENV4 为 2 copy/ml、CHIKV S27-African 为 20 copy/ml、CHIKV SH2830 为 8 copy/ml、ZIKV 为 8 copy/ml，特异性为 100%，耗时 45 min。Ys 等[49]采用多重 LAMP 对 ZIKV、DENV、CHIKV 检测发现，其灵敏度 ZIKV 为 5.4 e3copy/ml、DENV 为 5.7 e3copy/ml 和 CHIKV 为 4.5 e3copy/ml，特异性均为 100%，耗时 60 min。Teoh 等[50]采用多重 RT-LAMP 和多重 qRT-PCR 比较检测四种 DENV 血清型发现，多重 RT-LAMP 对四种 DENV 血清型灵敏度为 10 copy/ml，特异性为 100%，耗时 45 min，而多重 qRT-PCR 灵敏度仅为 85.2%，特异性为 100%。结果提示，多重 LAMP 在检测虫媒传染性病毒时表现出高灵敏度和特异性，且其方法简单，反应迅速，只需 20-70 min 即可得到结果，但由于多重 LAMP 引物数目过多，会加剧产物量大、分子量高或呈现梯形分布的非特异扩增产物等问题，且该实验设计复杂，对实验人员专业素质要求较高等[51-53]。

6 展望

综上 LAMP 技术均具有操作简单、灵敏度高、特异性强、设备廉价便携、耗时短等优点，但其反应所需引物设计复杂，容易产生气溶胶污染，且其产物对后续的基因测序难度较大，仍需要进一步改进完善。

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