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Review 综 述

# 基于环介导等温扩增技术的微流控芯片在病原菌检测方面的研究进展

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**摘要**：环介导等温扩增(LAMP)技术是一种新兴的核酸恒温扩增技术，与微流控芯片技术相结合，可实现对病原菌的快速检测，具有特异性强、灵敏度高、操作简单等优点。本文根据不同终产物的检测方法对目前检测病原菌的相关微流控 LAMP 芯片进行了分类与介绍，并对技术的改进和存在的问题进行了分析，以期为后续的相关研究提供参考。

**关键词**：环介导等温扩增技术，微流控芯片，病原菌

环介导等温扩增(Loop-mediated isothermal amplification，LAMP)技术是由日本荣研株式会社的 Notomi 等学者于 2000 年研究开发的一种新型体外等温扩增核酸的方法。其基本原理是针对靶基因的 6 个区域设计 4 条特异引物[一组外引物 F3、B3 和一组内引物 FIP (forward inner primer)、BIP (backward inner primer)]，同时采用具有链置换活性的 DNA 聚合酶(Bst DNA polymerase)，在 60–65 °C 恒温条件下孵育 30–60 min，从而实现对核酸 $10^9$–$10^{10}$ 倍的扩增。反应过程可分为 2 个阶段：(1) 起始阶段，在 Bst DNA polymerase 和内外引物的作用下，形成哑铃状结构，该结构是 LAMP 反应的起始结构；(2) 扩增循环阶段，在起始结构形成后，循环便只需要两条内引物参与进行，最终形成一系列由反向重复靶序列构成的茎-环状结构和多环花椰菜状结构的 DNA 混合物[1]。如需扩增 RNA，只需在反应中加入逆转录酶即可。此外，NAGAMINE 等在 2002 年通过向 LAMP 体系

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中额外添加 2 条环引物(LF/LB)，使反应时间缩短至 30 min 以内，大大提高了检测效率[2]。与普通 PCR 相比，LAMP 具有较多的优势(表 1)[3–4]，已广泛应用于病原菌的检测方面[5–8]。微流控芯片(microfluidic chip)又称芯片实验室(lab-on-a-chip)，是指在几平方厘米或者更小的芯片上构建化学或生物实验平台，因具有所需样品量少、检测效率高、成本低、集成灵活、易于便携和自动化等特点，近年来得到了迅速发展[9]。

LAMP 技术及微流控芯片技术均为新兴的快速检测技术。目前在 CNKI 中，以“环介导等温扩增”或“微流控芯片”为关键词检索 2010–2020 年间的相关文献，得到文献发表数目分别为 1418 篇和 2873 篇；在 Web of Science 中，该数量为 6491 篇和 24801 篇。具体每年发表情况如图 1 所示，可以看出这两项技术已受到了研究学者的普遍关注，但两者相结合的研究，相关文献数量报道较少，仍处于发展初期。本文首先介绍了 LAMP 和微流控芯片的结合，然后根据 LAMP 体系中产物的不同检测方法对微流控 LAMP 芯片进行了分类，综述了近几年来其在病原菌检测方面的应用及相关技术的改进。

**表 1. LAMP 相比普通 PCR 的优点**

Table 1. The advantages of LAMP compared with PCR

| Property | LAMP | PCR |
|---|---|---|
| Instrumental requirements | Thermostat water bath or thermos flask, low cost | PCR instrument, high cost |
| Speed | Quickly, 30–60 min | Slowly, 2–3 h |
| Specificity | 4–6 primers correspond to 6–8 target sites | 2 primers correspond to 2 target sites |
| Sensitivity | $10^0$ copies, lower 10–100 times than PCR | $10^1$–$10^2$ copies |
| Amplification efficiency | Highly, $10^9$ fold in 30–60 min | Lowly, $2^{30}$ fold in 2–3 h |
| Tolerance | The demand for nucleic acids quality is low, and the impurity tolerance is high, such as MEM medium, serum, plasma, urea | The demand for nucleic acids quality is high, and the impurity tolerance is low |
| Judgment of result | Easy to distinguish, such as electrophoresis, turbidity, fluorescence probe | High equipment requirements, such as electrophoresis and gel imaging system |

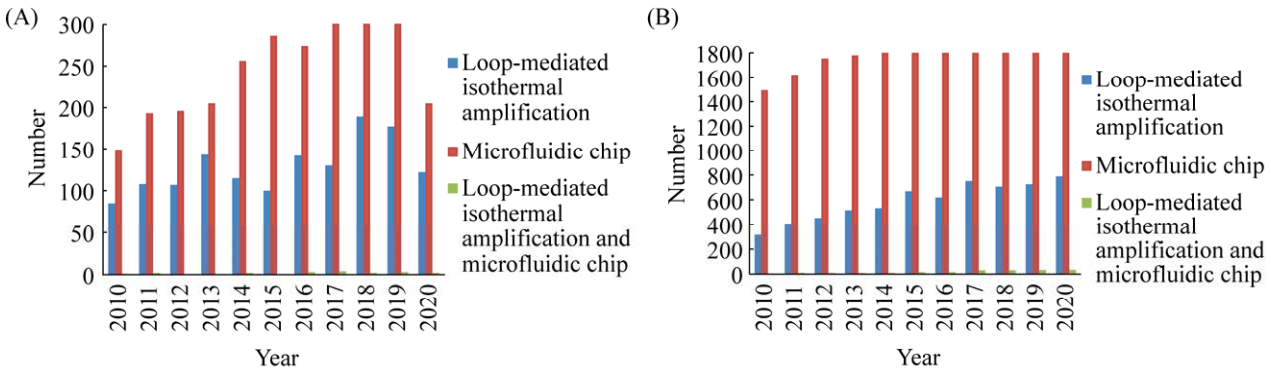


**图 1. 2010–2020 年不同数据库相关文献发表数目**

Figure 1. The number of publications related to LAMP-based technologies in different databases in 2010–2020 years. A: Number of publications with keywords of “loop-mediated isothermal amplification”, “microfluidic chip” and “loop-mediated isothermal amplification and microfluidic chip” in CNKI. B: Number of publications with keywords of “loop-mediated isothermal amplification”, “microfluidic chip” and “loop-mediated isothermal amplification and microfluidic chip” in Web of Science.

# 1 LAMP 与微流控芯片的结合

LAMP 与微流控芯片的结合顾名思义就是实现在微流控芯片上进行 LAMP 扩增反应。与传统 LAMP 相比，微流控 LAMP 芯片具有以下优势：(1) 反应体系小，可以减少试剂消耗；(2) 可实现多目标物的同时检测；(3) 不需要与其他外部连接，能够在一定程度上大大降低非特异性扩增和核酸污染的几率；(4) 预存储试剂(例如缓冲液和引物)，可减少在检测过程中的手动添加，增强检测效果；(5) 芯片具有体积小、方便携带、检测时间短等优势，可更好地满足基层实验室或现场快速检验(point-of-care testing，POCT)的需求，实现广泛的推广与应用。

# 2 微流控 LAMP 产物的检测方法

## 2.1 浊度法

LAMP 反应过程中会从 dNTP 中析出大量的焦磷酸根离子，游离的焦磷酸根离子与反应液中的 $Mg^{2+}$ 相结合，形成白色焦磷酸镁沉淀[$(DNA)_{n-1}+dNTPs=(DNA)_n+P_2O_7^{4-}$；$P_2O_7^{4-}+2Mg^{2+}=Mg_2P_2O_7$]。因此扩增结束后，用肉眼观察体系是否变浑浊或高速离心后看底部是否有白色沉淀即可判断扩增与否。但肉眼的判读具有一定的主观性，且扩增产物较少时白色沉淀不明显，容易出现误判，所以引入浊度仪可实现对终点的准确检测以及反应过程的实时监测[10]。Fang 等[11]在芯片上设计了 8 个平行反应通道，每个通道仅需加入待测样品 0.4 μL，在 1 h 内即可完成反应。单通道结合光导纤维，可实现对起始模板的定量和反应过程的监控，检测限达 10 fg/μL。Chang 等[12]将芯片与浊度仪结合，通过 RT-LAMP 反应，实现了对兰花叶中病毒 RNA 的检测。后期此研究小组对芯片进一步改造，将光纤维埋于芯片下部，加入微搅拌阀，提高了检测结果和检测效率[13]。虽然浊度法操作简单、快速，但其对反应液的透明度要求高，沉淀物不稳定，且浊度仪价格昂贵，因此该方法在微流控 LAMP 芯片中尚未得到广泛的应用。

## 2.2 染料法

基于可视化效果的染料法，因显色速度快、灵敏度较高、简单方便等特点而在微流控 LAMP 芯片中备受青睐，相关文献报道数量较多。常见的染料大致可分为两类(表 2)，一类是金属离子指

**表 2. LAMP 反应产物检测常用染料**

Table 2. Various dyes used for LAMP detection

| Dye | Mechanism | Color before and after amplification | Inhibitory effect on LAMP |
|---|---|---|---|
| HNB | Metal ion indicators, color changes from purple to blue with the reduction of $Mg^{2+}$ that can observed by naked eye | Purple/Blue | No |
| EBT | Metal ion indicators, color changes from red to blue with the reduction of $Mg^{2+}$ that can observed by naked eye | Red/Blue | No |
| Calcein | Metal ion indicators, fluorescence enhancement with the displacement of $Mn^{2+}$ from calcein to $P_2O_7^{4-}$ | Yellow/Green | No, but additional added $Mn^{2+}$ inhibit reaction |
| EB | DNA-binding dyes, intercalated with dsDNA | No color/Orange | No |
| PI | DNA-binding dyes, intercalated with dsDNA | No color/Red | Yes |
| SYBR Green I | DNA-binding dyes, intercalated with dsDNA | Dark orange/Green | Yes |
| PicoGreen | DNA-binding dyes, intercalated with dsDNA | Light orange/Green | Yes |
| EvaGreen | DNA-binding dyes, intercalated with dsDNA | Orange/Green | No |
| SYTO-81 | DNA-binding dyes, intercalated with dsDNA | Orange/Green | No |
| Gene Finder | DNA-binding dyes, intercalated with dsDNA | Orange/Green | Yes |

示剂，指示反应体系中 $Mg^{2+}$的变化情况，如羟基萘酚蓝(HNB)、铬黑 T (EBT)和钙黄绿素(Calcein)；另一类是核酸染料指示剂，与双链 DNA 分子结合后荧光增强，利用荧光信号强度指示 DNA 的数量，如溴化乙啶(EB)、碘化丙啶(PI)、SYBR Green I、PicoGreen 和 EvaGreen 等[14–15]。表 3 总结了近几年来基于染料法检测病原菌的微流控 LAMP 芯片[16]。如表 3 所示，染料试剂应用

**表 3. 基于染料法检测的微流控 LAMP 研究进展**

Table 3. LAMP-based microfluidic chips by using dyes detection

| Bacteria | Dye | Material and shape of chip | Detection time | Detection limit | Simultaneous detective numbers of bacteria | Simultaneous detective numbers of sample | Primer and DNA template are added separately | References |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | SYBR Green I | Paper | 40 min | $2\times10^{3}$ copies/μL | 6 | 6 | No | [18] |
| *Escherichia coli* | PicoGreen | Paper | 30 min | 100 CFU/mL | 1 | 1 | No | [19] |
| *Escherichia coli* | HNB | PE/Ribbon | 60 min | 30 CFU/mL | 36 | 36 | No | [20] |
| *Escherichia coli* | Calcein | PDMS/ Rectangle | 55 min | 270 copies/μL | 5 | 1 | Yes | [21] |
| *Escherichia coli* O157:H7 | EBT | PMMA/Circle | 60 min | 40 copies/μL | 24 | 1 | Yes | [22] |
| *Escherichia coli* O157:H7 | EBT | PC/Circle | 60 min | 15.2 copies/μL | 5 | 5 | Yes | [23] |
| *Escherichia coli* O157:H7 | EvaGreen | Silicon/ Rectangle | / | 3 CFU/reaction | 4 | 4 | Yes | [24] |
| *Salmonella* | HNB | CD, DVD, BD/Circle | 45 min | 65 fg/μL | 1 | 1 | No | [25] |
| *Salmonella* | Calcein | PMMA/Circle | 60 min | 30 fg/μL | 6 | 30 | Yes | [26] |
| *Salmonella* | Calcein | PMMA/ Rectangle | 70 min | 5 CFU/mL | 2 | 2 | No | [27] |
| *Salmonella* | Calcein | PDMS/ Rectangle | 70 min | 2 copies/μL | 4 | 4 | Yes | [28] |
| *Salmonella* | SYBR Green I | PMMA/Circle | 70 min | $5\times10^{-3}$ ng/μL | 4 | 16 | Yes | [29] |
| *Salmonella* | SYBR Green I | PMMA/Circle | 62 min | $2.5\times10^{-3}$ ng/μL | 16 | 16 | No | [30] |
| *Vibrio parahaemolyticus* | Calcein | PDMS/Circle | 60 min | 7.2 copies/μL | 10 | 10 | Yes | [31] |
| *Vibrio parahaemolyticus* | EvaGreen | PMMA/Circle | 30 min | 0.28 pg/μL | 24 | 1 | Yes | [32] |
| *Staphylococcus aureus* | Calcein | PDMS/Pentagon | 90 min | 24 CFU/reaction | 5 | 1 | Yes | [33] |
| *Neisseria meningitidis* | Calcein | PDMS and paper/Rectangle | 60 min | 3 copies/reaction | 4 | 1 | Yes | [34] |
| *Streptococcus pneumoniae* | Calcein | PDMS and paper/Rectangle | 60 min | 20 fg/μL | 2 | 2 | No | [35] |
| *Streptococcus agalactiae* | EvaGreen | PDMS/ Rectangle | 65 min | 20 copies/μL | 4 | 4 | Yes | [36] |

选择范围较为宽泛，大部分以 Calcein、SYBR Green I 和 EvaGreen 为主。芯片材料以有机聚合物(PDMS 或 PMMA)为主，也有部分应用硅片或纸片。芯片形状以圆形和方形为主。值得指出的是，圆形芯片近几年发展迅速，因其在一定程度上可依靠离心力，可以避免方形芯片中微阀和微泵的复杂设计。检测时间大都在 60 min 左右，检测限之间存在着一定的差距。此外，结合二极管、CCD 等成像系统，可实现对 LAMP 反应的实时监测。如 Ahmad 等[17]应用 CCD 曝光 5 s，使其信噪比提高了 8 倍，时间阈值减少到 9.8 min，提高了检测效率和灵敏度。

### 2.3 电化学法

DNA 是具有电活性的物质，基于电化学方法构建的 DNA 检测器具有简便、快速、灵敏和低成本等特点。通常是将修饰的电极固定在微流控芯片基层或通道内部，通过电极线与电化学工作站相连，实现对目标物的终点或实时监测。在检测过程中，常用的电活性物质有亚甲基蓝(MB)、Hoechst 33258、二茂铁、钴复合物和锇复合物等，检测方法包括差分脉冲伏安法(DPV)、方波伏安法(SWV)、线性扫描伏安法(LSV)和循环伏安法(CV) (表 4)。其主要机理是具有电化学活性的物质分子与 LAMP 产物结合后，通过电化学检测到的信号大小或变化值反映被测溶液中靶 DNA 片段的浓度。

相比于其他检测方法，电化学法可以大幅度提高检测的精确度和灵敏度，能够实时动态监测反应体系[37]。因此，基于 LAMP 反应的实时电化学检测方法具有很大的发展前景。

### 2.4 免疫层析试纸条法

LAMP 与免疫层析试纸条法(lateral flow dipstick，LFD)联合，可使 LAMP 结果检测变得更加简单且可视化。其原理是在反应体系中，用半抗原 A 标记一条内引物，用半抗原 B 标记一条与扩增序列碱基互补的 DNA 探针，将抗 B 抗体包被于胶体金颗粒表面，抗 A 抗体固定于检测线。当存在特异性扩增时，会形成半抗原 A 和半抗原 B 同时标记的扩增杂交物(半抗原 A 标记的扩增物-半抗原 B 标记的 DNA 探针-抗 B 抗体包被的胶体金)，该杂交物与检测线上的抗体 A 特异结合，形成肉眼可见的有色线条，表明为阳性结果；当特异性扩增物不存在时，不会显色，为阴性结果[44]。免疫层析试纸条在扩增反应后需开盖上样层析，该操作容易引起气溶胶污染，降低了其实用性，如果将其嵌入到微流控芯片的终端，可有效避免此问题。Park 等[45]采用离心式微流控 LAMP 芯片实现了对两种病原菌的同时检测，检测限为 10 CFU/μL，检测时间为 80 min。大致流程包括：首先在芯片的 LAMP 反应室内，采用两种不同的标记引物对沙门氏菌和副溶血性弧菌的目标基因进行扩增，然后在离心力的作用下，将扩增产物离心到最外侧反应室内，液体在毛细管力的作用下流经嵌入的双标记免疫层析试纸条，从而实现对目标物的检测。

**表 4.　基于电化学法检测的微流控 LAMP 研究进展**

Table 4.　LAMP-based microfluidic chips by using electrochemical detection

| Bacteria | Electroactive reagent | Electrochemical technique | Electrode | Detection time | Detection limit | Simultaneous detective numbers of bacteria | Simultaneous detective numbers of sample | Primer and DNA template are added separately | References |
|---|---|---|---|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | MB | SWV | ITO | 45 min | 16 copies/μL | 4 | 4 | No | [38] |
| *Salmonella* | MB | DPV | Gold | 60 min | 4 fg/μL | 1 | 1 | No | [39] |
| *Vibrio parahaemolyticus* | Hoechst 33258 | LSV | Carbon | 60 min | 24 CFU/ml | 1 | 1 | No | [40] |
| *Mycobacterium tuberculosis* | Hoechst 33258 | CV | Carbon | 65 min | $10^3$ copies/ reaction | 1 | 1 | No | [41] |
| *Staphylococcus aureus* | $Os[(bpy)_2DPPZ](PF_6)_2$ | SWV | Screen-printed electrode | 50 min | 200 CFU/mL | 12 | 12 | No | [42] |
| *Escherichia coli* | $Os[(bpy)_2DPPZ](PF_6)_2$ | SWV | Screen-printed electrode | 50 min | 30 CFU/mL | 12 | 12 | No | [43] |

# 3 微流控 LAMP 芯片技术的改进

## 3.1 多通路同时检测

将 LAMP 技术及微流控芯片技术相结合，最大的特色在于可以实现对多个菌或多个不用样品的同时检测，缩短检测时间，提高检测效率。如表 3 所示，其检测菌株种类或样品数量最多可达 36 个。如果在芯片中分别加入扩增引物和样品 DNA 模板成分，甚至可实现对一个样品多个菌或多个样品同一种菌的检测。

## 3.2 集成化检测

微流控芯片技术可以将样本处理、产物检测和结果输出集成于一体，从而更加适应现场检测的需求。目前，大多数微流控 LAMP 芯片的 DNA 提取与纯化过程是在芯片外进行的，不利于自动化检测。但是也有少数学者已将多个反应单元整合于芯片上，如 Wang 等[46]在磁珠表面修饰特异性结合目标物的 DNA 探针分子，实现了对耐甲氧西林金黄色葡萄球菌的分离和检测；Liu 等[47]在芯片中加入 FTA 滤膜，对 DNA 分子进行分离和浓缩；Wu 等[48]在 LAMP 反应池前加入蛇形玻璃 SPE 柱，可在 2 h 内实现对复杂基质的纯化及目标物的检测；Guo 等[49]同样加入 SPE 柱，实现了对 3 个菌的同时检测。此外，还可在芯片上加入细胞溶解池[50–51]，可直接对样品进行检测，避免了烦琐耗时的人工前处理过程。

## 3.3 数字 LAMP

数字 PCR 可实现对核酸分子的绝对定量，而由于 LAMP 的恒温特性，更适合与数字核酸扩增技术相结合。Ma 等[52]研发了自吸离散式数字化微流控 LAMP 芯片，在亲水性增强的 PDMS 芯片毛细管力作用下，进入的液滴会自发进入阵列中，无需其他微阀的控制，大大简化了芯片的构造，在 30 min 内即可对 DNA 准确定量。Manzano 等[53]对 LAMP 反应体系中指示剂染料的选择、浓度及成像最佳比率值等参数进行了优化，实现了用普通照相手机就可检测纳升内的单核酸分子。数字 LAMP 具有上样量少、绝对定量、高灵敏度、高精确度、高耐受性等优点，且流动微滴式数字 LAMP[54]还可进一步实现高通量不间断的检测。

# 4 问题和展望

综上所述，微流控 LAMP 芯片因具有特异性好、灵敏度高、检测快速、高通量、携带方便等优势，在分子诊断和病原菌检测等诸多领域具有广泛的应用价值。虽然近几年来微流控 LAMP 芯片技术得到了不断优化，但目前仍无法替代商品化的 PCR 仪。存在的主要问题如下：(1) LAMP 技术本身的缺陷，如扩增靶序列长度控制小于 800 bp、引物筛选过程烦琐、特异性扩增易出现假阳性、Bst DNA polymerase 成本高等问题；(2) 对于处理复杂样本的能力有限，仅允许从少量、简单的样品中提取核酸；(3) 许多步骤仍需手动进样，且对于芯片上的预分装或预包埋物缺乏长期有效的存储方法；(4) 对于流体的控制(如微通道、微阀、微泵等)需要高精密度和准确性，难以确保产业化的可重复性；(5) 微流控芯片中的反应体积小，对传感器的灵敏度和稳定性要求较高；(6) 芯片加工平台尚未普及，技术发展依赖性较大；(7) 自动化程度较低，应进一步开发相应的仪器和软件进行辅助。

但我们相信在未来的几年内，随着更为精细

的微机电加工、更为准确的温度和流体控制、更为灵敏的传感分析，芯片的制备会越来越精巧，LAMP 的操作会更加准确和高效；低成本、高质量的微流控 LAMP 芯片可自动化完成一系列反应，操作人员无需经过专门的培训和烦琐的样品前处理，便能够得到检测结果。基于 LAMP 和微流控芯片的突出优点，微流控 LAMP 芯片将在科研领域和商业化 POCT 平台中发挥重要的作用。

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# Advances in the detection of pathogenic bacteria by using loop-mediated isothermal amplification-based microfluidic chip

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**Abstract:** Loop-mediated isothermal amplification (LAMP) is an emerging nucleic acid amplification technique under isothermal condition, which can combined with microfluidic chip to rapidly detect the pathogenic bacteria. It is of higher specificity, higher sensitivity and simpler operation. This article classified and introduced the LAMP-based microfluidic chip for the detection of pathogenic bacteria, based on the analysis of different end-point detection methods. And also, we analyzed the improvement and existing problems of this technology, which can provide great reference value for the further studies and industry development.

**Keywords:** loop-mediated isothermal amplification, microfluidic chip, pathogenic bacteria

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