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专论与综述

重组酶聚合酶扩增技术的研究进展

沈方园，葛萧，张晓宇，张郁勃，周慧姿，李恒*

潍坊医学院医学检验学院，山东 潍坊 261053

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摘　要：重组酶聚合酶扩增(recombinase polymerase amplification, RPA)技术是一种利用重组酶(T4 UvsX)、单链 DNA 结合蛋白(T4 Gp32)和链置换 DNA 聚合酶(Bsu)等蛋白在恒温条件下扩增核酸的技术。由于其可以恒温、高效地扩增靶核酸片段，近几年在细菌、病毒、寄生虫、支原体、衣原体检测方面发挥着独特的作用，逐渐成为床旁检测(point-of-care testing, POCT)的重要技术。本文以 RPA 技术的原理为起点，对其临床应用、不足之处及改进方法进行阐述，以期为临床疾病的快速检测和诊断方面提供新思路。
关键词：重组酶聚合酶扩增技术；等温扩增技术；核酸检测

Research progress on recombinase polymerase amplification

SHEN Fangyuan, GE Xiao, ZHANG Xiaoyu, ZHANG Yubo, ZHOU Huizi, LI Heng*

School of Medical Laboratory, Weifang Medical University, Weifang 261053, Shandong, China

Abstract: Recombinase polymerase amplification (RPA) is a technology that utilizes proteins such as recombinase (T4 UvsX), single-stranded DNA binding protein (T4 Gp32), and strand replacement DNA polymerase (Bsu) to amplify target nucleic acids at constant temperatures. In recent years, RPA plays a unique role in the detection of bacteria, viruses, parasites, mycoplasma, and chlamydia for it can amplify the target nucleic acid fragment with constant temperature and high efficiency, it gradually becomes a key technology for point-of-care testing (POCT). This review takes the principle of RPA technology as a starting point, it describes the clinical application, shortcomings, and improvement of RPA, and RPA is expected to provide a new idea for the rapid detection and diagnosis of clinical diseases.
Keywords: recombinase polymerase amplification technique; isothermal amplification technique; nucleic acid testing

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*Corresponding author. E-mail: Leehome1985@163.com
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2019 年以来，新型冠状病毒感染疫情对人类生命健康造成了巨大的影响。PCR 因其灵敏度高、特异性强等优点，作为核酸扩增技术的金标准在新型冠状病毒检测中得到了广泛应用。然而，由于 PCR 反应过程中需要频繁地加热与制冷，反应全程需在 PCR 仪中进行，导致以 PCR 为基础的核酸扩增技术在快速检测和基层应用推广中受到了一定的限制。

重组酶聚合酶扩增(recombinase polymerase amplification, RPA)技术是近年来新兴的核酸扩增技术，其在恒温下就可扩增，受环境限制小[1]。RPA 技术操作简便，反应时间短，大约 20−30 min 即可获得检测结果，对大批量的样本检查和普查有实际意义。其床旁检测(point-of-care testing, POCT)尤其适合资源比较匮乏的偏远地区。除此之外，RPA 技术可与多种临床技术结合，使检测灵敏度大大增高，并且实现检测结果的可视化，观察检测结果更便捷。

本文以介绍 RPA 技术的反应原理为起点，重点介绍了 RPA 技术在感染性疾病、癌症等方面的临床应用，对其目前存在的不足之处、改进方法进行总结，并对其未来进行展望。

1 RPA 的概述

RPA 技术是在 2006 年由 Twist DX 公司提出的等温扩增技术，其反应体系主要由重组酶(T4 UvsX)、单链 DNA 结合蛋白(T4 Gp32)和链置换 DNA 聚合酶(Bsu)等多种酶组成。反应时，重组酶与引物配对形成重组酶-引物复合物，其在双链 DNA 中寻找同源序列，确定同源序列后，在重组酶的作用下，引物与之配对，DNA 双链解离为单链，此时，T4 Gp32 结合在解离的单链 DNA 上，防止 DNA 单链再次复性为双链，随后，重组酶从引物上解离，Bsu 聚合酶结合到引物的 3′末端启动新的 DNA 链合成，整个过程反复进行，实现目的 DNA 序列的指数级扩增(图 1)，反应过程中无须温度变化，在 37−42 ℃可进行，另外重组酶等温扩增所需的引物对退火温度无特殊要求，引物设计难度降低，反应无需特殊的仪器，反应时间短，对环境要求低，是具有应用前景的核酸扩增技术[2-3]。

2 其他等温扩增技术

等温扩增技术是一大类技术的总称，它们能在某一恒定的温度条件下扩增特定的 DNA 或者 RNA。该技术包含类型复杂，发展较为迅速，常见的类型包括：重组酶介导等温核酸扩增(recombinase mediated isothermal nucleic acid amplification technique, RAA)技术、环介导等温扩增(loop-mediated isothermal amplification, LAMP)技术、依赖核酸序列的扩增(nucleic acid sequence-based amplification, NASBA)技术、链置换扩增(strand displacement amplification, SDA)技术、转录介导的扩增(transcription-mediated amplification, TMA)技术、酶促重组等温扩增(enzymatic recombinase isothermal amplification, ERA)技术、解旋酶依赖性扩增(helicase-dependent amplification, HDA)和滚环核酸扩增(rolling circle nucleic acid amplification, RCA)技术[4]。下文就几种经典的等温扩增技术进行简要介绍。

重组酶介导扩增(recombinase aided amplification, RAA)技术与 RPA 有相似的原理，二者都是通过几种酶的互相作用达到等温扩增的目的，不同的是两种等温扩增技术主要酶的来源不同；RPA 技术主要依赖 T4 噬菌体重组酶 T4 UvsX、T4 Gp32 及链置换 Bsu 聚合酶发挥作用，而 RAA 技术所需主要的酶则来自于细菌或真菌，包括 UvsX、SSB 和 DNA 聚合酶 Klenow 三种[5-6]。

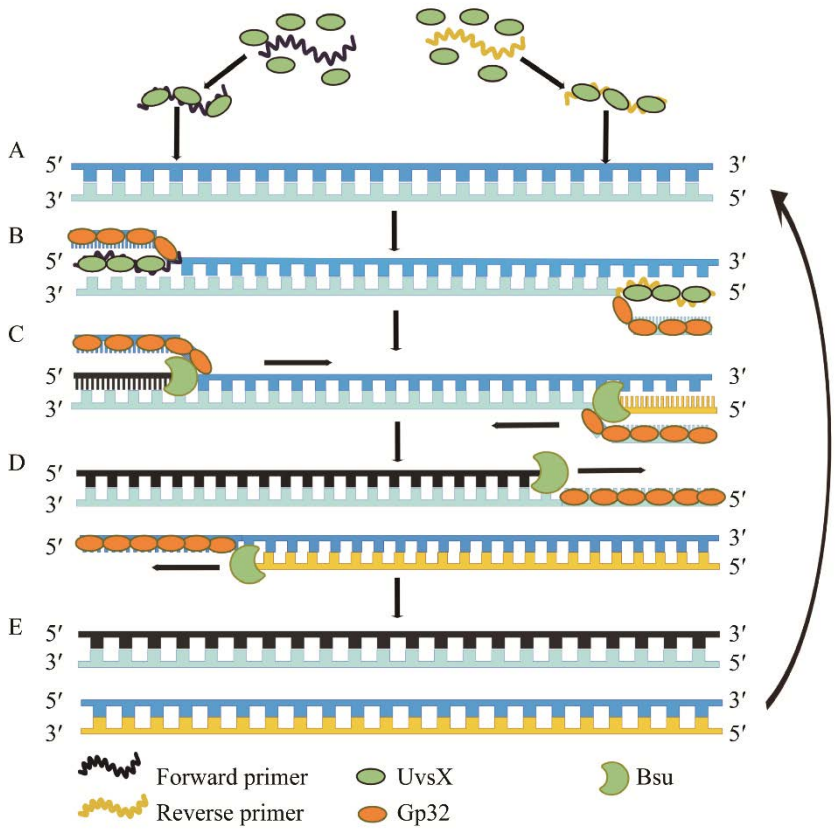

图 1 RPA 的作用原理 A：重组酶-引物复合体特异性识别 DNA 双链. B：DNA 双链解离为单链. C：单链结合蛋白绑定刚解离的 DNA 单链，防止复性为双链. D：DNA 聚合酶启动新 DNA 链的合成. E：新合成的 DNA 双链反复进行 A−E 过程，实现目的 DNA 序列扩增

Figure 1 The action principle of RPA A: Recombinase enzyme-primer complex specifically recognizes DNA double strand. B: DNA double strand dissociation to single strand. C: The single-strand binding protein binds the single strand of DNA that has just been dissociated, preventing the refolding into double strands. D: DNA polymerase initiates the synthesis of new DNA strands. E: The newly synthesized DNA double strand is repeated through the A−E process to achieve the target DNA sequence amplification.

LAMP 是 2000 年开发的等温扩增技术，其通过链置换 DNA 聚合酶如 Bst DNA polymerase 促进反应进行，最佳反应温度是 65 ℃，反应全程可分为复制起始、循环扩增、延伸 3 个阶段，与 PCR 的 3 个反应阶段不同，LAMP 反应全程不需要加热、短时间内就可合成新的 DNA[7-8]。

NASBA 于 1991 年首次提出，通过 AMV 逆转录酶、核糖核酸酶 H (RNase H)和 T7 RNA 聚合酶 3 种酶发挥作用，是由 1 对带有 T7 标签的引物引导的等温核酸扩增技术，其反应最佳温度是 41 ℃，可在 2 h 内将模板 RNA 扩增 10^9 倍，比常规 PCR 的灵敏度高约 10^3 倍[9]。

SDA 由特定的内切酶和聚合酶组成，目的 DNA 两端带有被化学修饰的限制性核酸内切酶识别序列，核酸内切酶在其识别位点将 DNA 打开缺口，DNA 聚合酶随后延伸缺口 3′端并替换 DNA 链，被替换下来的 DNA 单链可与引物结合，被 DNA 聚合酶延伸成双链[10]。

RCA 于 1998 年提出，主要由 Phi29 DNA 聚合酶构成，可以模拟自然界微生物环状 DNA 滚环复制，在具有链置换活性的 DNA 聚合酶作用下，既实现环状 DNA 的扩增，又能实现对靶核酸基因的放大，扩增产物一般是连接在固相支持物(如玻片)表面的 DNA 引物上，因此 RCA 适合在芯片上进行信号扩增[11-12]。

HDA 技术是 2004 年提出的等温扩增技术，该技术可模拟动物体内 DNA 复制，利用解旋酶解开 DNA 双链，随后单链结合蛋白与单链 DNA 结合，替代 PCR 过程中通过温度变化来进行变性-退火-延伸的过程，实现靶基因的指数级扩增[13-15]。

随着近年来临床上等温扩增技术的增多，为便于描述而进行分类，主要分为指数扩增、线性扩增和级联扩增三类进行描述(表 1)，对各种等温扩增技术优缺点进行比较(表 2)。

3 RPA 的应用

随着近年对 RPA 技术研究的不断深入，该技术的应用也在不断拓展，其在感染性疾病诊断、癌基因检测等方面的应用进一步成熟，RPA 技术已逐渐成为分子诊断领域的重要技术平台和研究工具。

3.1 RPA 在感染性疾病诊断中的应用

感染性疾病是由致病微生物通过不同方式导致人体发生感染的疾病，RPA 技术因其检测快速灵敏的特点而被广泛应用于感染性疾病的检测中。Choi 等[30]设计直接离心 RPA 微装置实时检测牛奶样品中的致病菌，装置的每个反应室中有待检测菌种的特异性探针和引物，上层注入 RPA 试剂，下层添加检测样品，试剂和待检测样品在离心力作用下等量分配到各个反应室，进行多重 RPA 反应，在 39 ℃时扩增牛奶中待检测细菌的目的基因，无须提取 DNA，可检测肠道沙门氏菌、大肠杆菌 O157:H7、副溶血弧菌等 3 种引起食物中毒的病原菌，反应室内目的基因扩增的荧光信号间隔 2 min 记录 1 次，RPA 技术扩增所需时间短，检测 30 min 可得到结果。Li 等[31]通过对沙门氏菌基因组 DNA 中的 *invA* 基因进行 RPA 等温扩增，生成 DNA 双链扩增产物，SYBR Green I 与 dsDNA 结合，通过照射发光二极管进行光敏显色和定量，该方法定量测定沙门氏杆菌的最低检测浓度为 5×10^3 CFU/mL，该方法的灵敏度和特异性分别是 90%和 95%，在 1 h 内完成检测。Wang 等[32]通过猪圆环 3 型病毒基因组的保守序列设计特异性 RPA 引物和探针，在 38 ℃扩增 20 min，RT-RPA 对猪圆环 3 型病毒具有特异性，与其他检测病原体无交叉反应，以猪圆环 3 型病毒的 DNA 为模板，用 RT-RPA 法进行扩增，RT-RPA 符合 RT-PCR 诊断标准的概率为 96.2%。

3.2 RPA 在癌基因检测中的应用

RPA 技术高效、快速、灵敏检测的特点使其应用逐渐广泛，在检测癌症基因中发挥越来越重要的作用。前列腺癌最常见的融合基因是 *TMPRSS2-ERG*，是前列腺癌的高度特异性基因，Koo 等[33]用 RT-RPA 联合辣根过氧化物催化比色反应对 *TMPRSS2-ERG* 的 mRNA 进行检测，通过肉眼可观察结果或分光光度计进行定量检测；检测提取 RNA，根据 *TMPRSS2-ERG* 设计特异性引物，继而进行 RT-RPA 扩增，扩增产物用于比色反应，扩增产物-链霉亲和素-HPR 结合物多次洗涤后再加入四甲基联苯进行显色反应，在 650 nm 处记录吸光度值，其检测极限为 10^4 copies/mL，具有单细胞水平的灵敏性，总检测时间约 75 min，不同于其他检测，RPA 扩增产物检测所需时间短，检测灵敏度高。*PIK3CA* 基因突变是结直肠癌、乳腺癌、

表 1　RPA 与其他等温扩增技术的差异

Table 1　Differences between RPA and other isothermal amplification technique

反应 Reaction	反应所需酶 Reactive enzymes	检测限 Limit of detection	特异性 Specificity	所需引物 Primers	反应最适温度 Reaction optimum temperature (℃)	反应时间 Reaction time (min)	反应模板 Template	产物检测方法 Product detection method	是否变性 Denatured or not	是否初始加热 Initial heating or not	参考文献 References
RPA	重组酶 T4 UvsX；单链 DNA 结合蛋白 T4 Gp32；重组酶辅助蛋白 T4 UvsY；链置换 DNA 聚合酶 Bsu Recombinase T4 UvsX; single-stranded DNA binding protein T4 Gp32; recombinant enzyme helper protein T4 UvsY; strand displacement DNA polymerase Bsu	10 copies/μL	High	1 pair	37	5−20	DNA	琼脂糖凝胶电泳；测流试纸条；实时荧光 Agarose gel electrophoresis; lateral flow dipstick; real-time fluorescence	No	No	[16]
NASBA	逆转录酶 AMV；逆转录酶 RNase H；T7 聚合酶 Reverse transcriptase AMV; reverse transcriptase RNase H; T7 polymerase	100 CFU/mL	High	1 pair	40−55	90−120	DNA and RNA	琼脂糖凝胶电泳；测流试纸条；酶联免疫吸附；实时荧光 Agarose gel electrophoresis; lateral flow dipstick; enzyme-linked immunosorbent; real-time fluorescence	Yes	No	[9,17]
LAMP	Bst DNA 聚合酶 Bst DNA polymerase	−	High	4−6 primers	65	30-60	DNA and RNA	浊度仪；实时荧光；琼脂糖凝胶电泳；智能手机；测流试纸条	No	Yes	[2]

(待续)

(续表 1)

反应 Reaction	反应所需酶 Reactive enzymes	检测限 Limit of detection	特异性 Specificity	所需引物 Primers	反应最适温度 Reaction optimum temperature (℃)	反应时间 Reaction time (min)	反应模板 Template	产物检测方法 Product detection method	是否变性 Denatured or not	是否初始加热 Initial heating or not	参考文献 References
								Turbidity meter; real-time fluorescence; agarose gel electrophoresis; smartphones; lateral flow dipstick			
SDA	限制性内切酶；DNA 聚合酶 Restriction endonuclease enzymes; DNA polymerase	High	High	2 pairs	37	15−20	DNA	琼脂糖凝胶电泳；实时荧光；测流试纸条；荧光偏振 Agarose gel electrophoresis; real-time fluorescence; lateral flow dipstick; fluorescence polarization	Yes	Yes	[10,17]
RCA	特殊聚合酶 Phi29 Special polymerase Phi29	High	High	1 primer or 1 pair	37	60	DNA	琼脂糖凝胶电泳；实时荧光 Agarose gel electrophoresis; real-time fluorescence	No	Yes	[11-12]
HDA	解旋酶；单链 DNA 结合蛋白 SSB；DNA 聚合酶 Helicase; single-stranded DNA binding protein SSB; DNA polymerase	High	High	1 pair	65	75−90	DNA	琼脂糖凝胶电泳；测流试纸条；酶联免疫吸附；电化学	No	No	[13-15]

(待续)

Tel: 010-64807511; E-mail: tongbao@im.ac.cn; http://journals.im.ac.cn/wswxtbcn

(续表 1)

反应 Reaction	反应所需酶 Reactive enzymes	检测限 Limit of detection	特异性 Specificity	所需引物 Primers	反应最适温度 Reaction optimum temperature (℃)	反应时间 Reaction time (min)	反应模板 Template	产物检测方法 Product detection method	是否变性 Denatured or not	是否初始加热 Initial heating or not	参考文献 References
								Agarose gel electrophoresis; lateral flow dipstick; enzyme-linked immunosorbent; electrochemistry			
TMA	RNA 聚合酶；逆转录酶 RNA polymerase; reverse transcriptase	High	High	1 pair	42	15−30	DNA and RNA	琼脂糖凝胶电泳；化学发光检测 Agarose gel electrophoresis; chemiluminescence detection	No	No	[18]
RAA	重组酶 UvsX；单链结合蛋白 SSB；重组酶辅助蛋白 UvsY；DNA 聚合酶 Klenow Recombinase UvsX; single-chain binding protein SSB; recombinant enzyme helper protein T4 UvsY; DNA polymerase Klenow	10 copies/μL	High	1 pair	37	15−20	DNA	琼脂糖凝胶电泳；测流试纸条；实时荧光 Agarose gel electrophoresis; lateral flow dipstick; real-time fluorescence	No	No	[19]
ERA	突变的重组酶、外切酶和聚合酶 Mutated recombinase enzymes, mutated exonuclease enzymes and mutated polymerases	10 copies/μL	High	1 pair	35−40	20−30	DNA and RNA	实时荧光 Real-time fluorescence	No	No	[20-21]

−：文献中未找到相关信息

−: The relevant information was not found in the literature.

表 2 各类等温技术的分类及优缺点比较

Table 2 Classification and comparison of advantages and disadvantages of various isothermal technologies

扩增类型 Amplification type	技术名称 Technical name	优点 Advantages	缺点 Disadvantages	参考文献 References
指数扩增 Exponential amplification	RPA	此方法检测无须复杂的仪器，常温就可进行检测；可定量；引物设计简单；可进行荧光终点分析；不需要热变性；快速检测；灵敏性高 This method does not require complex instruments to detect, and it can detect at room temperature; it is quantifiable; the primers' design are simple; it can be detected by fluorescence endpoint analysis; it does not need thermal denaturation; it has a rapid detection and high sensitivity	此方法琼脂糖凝胶电泳成像前产物需要纯化；核酸提取步骤较烦琐；引物之间可能会结合，出现非特异性扩增 This method's product needs to be purified before agarose gel electrophoresis imaging; the nucleic acid extraction steps are cumbersome; primers may bind to each other, resulting in nonspecific amplification	[22]
	NASBA	此方法高敏感性、高特异性；反应快，扩增效率高；保真度高 This method has high sensitivity and specificity; it has a fast response and high amplification efficiency; it has a high fidelity	此方法反应成分复杂；3 种酶反应成本高；不适合大批量样本检测；对 DNA 病毒检测无优势 This method has complex reaction components; the cost of the three enzymes reactions is high; it is not suitable for testing large sample volumes; there is no advantage to DNA virus detection	[23]
	LAMP	此方法扩增效率高；反应快；特异性强；不需要特殊设备 This method has high amplification efficiency and fast response; it has a strong specificity; it does not need special equipment	此方法对引物要求高；容易形成气溶胶，造成假阳性；产物不均一，扩增产物不能用于克隆测序 This method has high primers requirements; it is easy to form aerosols and cause false positives; and the product is not exclusive and it cannot be used for clonal sequencing	[24]
	RAA	此方法操作步骤简单；灵敏度、特异性高；反应快 This method's operation steps are simple; it has a high sensitivity and specificity; and it has a fast response	此方法对引物的要求高；前期需提取核酸，比较烦琐 This method has high requirements for primers; nucleic acid needs to be extracted in the early stage, which is more cumbersome	[17,22]
	SDA	扩增效率高、反应快；特异性强；不需要特殊设备 This method has a high amplification efficiency and fast response; strong specificity; no special equipment is required	产物不均一；循环反应中产生单、双链产物，电泳时易出现拖尾；不适合用于基因工程 This method's products are heterogeneity; single and double-stranded products are produced in the cyclic reaction, and tailing is easy to occur during electrophoresis; it is not suitable for genetic engineering	[25]
	HDA	此方法反应体系易获得；反应条件等温；操作步骤简单	此方法反应效率受解旋酶活性的影响；靶序列超过 400 bp 可显著影响扩增效率；该方法研究少，发展不成熟	[13-14]

(待续)

(续表 2)

扩增类型 Amplification type	技术名称 Technical name	优点 Advantages	缺点 Disadvantages	参考文献 References
		This method's reaction systems are readily available; reaction conditions are isothermal conditions; the steps are simple	This method's reaction efficiency is affected by helicase activity; target sequences exceeding 400 bp can significantly affect amplification efficiency; there is little research and immature development of this method	
线性扩增 Linear amplification	线性 RCA Linear RCA	此方法灵敏度高；特异性强，易操作；高通量；扩增产物磷酸化后可以进行直接测序 This method has high sensitivity and strong specificity, it is easy to operate; it has a high throughput; the amplification products can be phosphorylated and sequenced directly	此方法信号检测有背景问题，RCA 过程中未成环的锁式探针模板 DNA 或 RNA 会出现背景信号；仅限于具有环状结构的病毒、质粒及染色体的扩增，探针合成昂贵 This method's signal detection has background problems, and background signals will appear in unlooped locked probe template DNA or RNA during RCA; it is limited to the amplification of viruses, plasmids and chromosomes with circular structures, the probes are expensive to synthesize	[12]
	线性 SDA Linear SDA	此方法灵敏度高，特异性高，操作简单，反应过程不需要特殊设备 This method has high sensitivity and specificity, it has a simple operation, it does not need special equipment required in the reaction process	此方法的产物检测需要特殊仪器；靶 DNA 的含量可能会受到标本中不明抑制物的抑制 This method's product testing requires special instruments; the content of target DNA may be suppressed by unknown inhibitors in the specimen	[26-27]
级联扩增 Cascade amplification	SDA 级联放大扩增 SDA cascade amplification	此方法可检测多个靶目标，只需要单个引物；特异性高 This method can detect multiple targets; it requires only a single primer; it has high specificity	此方法操作复杂；反应时间长，产物检测方法需要特殊仪器 This method has a complex operation; it needs more reaction time; the product's detection requires special instrumentation	[28]
	RCA 级联放大扩增 RCA cascade amplification	此方法灵敏度高；特异性高；操作简单；可同时检测多个目标；可在芯片上扩增 This method has high sensitivity and specificity; it has a simple operation; it can detect multiple targets simultaneously; it can amplify on the chip	此方法芯片上扩增导致操作步骤较多；费用高且需要特殊仪器 This method's on-chip amplification has more operation steps; it is expensive and requires special instruments	[29]

胶质母细胞瘤、肺癌和皮肤癌的发病机制之一，Martorell 等[34]提出阻断式等温扩增技术检测突变的 *PIK3CA* 基因，反应溶液中含有与正常 DNA 互补的寡核苷酸(阻断剂)，目标核苷酸位于阻断剂的中心位置，此位置可达到更好的阻断效果，上游引物位于封闭寡核苷酸的附近，目的 DNA 与阻断剂的结合比其与上游引物结合更稳定，通过阻断剂与引物二者的竞争导致 RPA 扩增的正常 DNA 大大减少，优先对突变体进行扩增，通过将 RPA 扩增后产物与芯片上

的探针杂交，达到鉴定突变的目的；RPA 技术扩增快速的特点与芯片联合检测 *PIK3CA* 基因突变在一个微阵列即可完成，对突变体的检测灵敏度可达到 95%，可作为辅助手段对个体化治疗提供帮助。随着 RPA 技术灵敏、快速的特点，其在癌症检测方面的应用会逐渐广泛。

3.3 RPA 与其他技术联合应用

RPA 技术的逐渐成熟促使其与临床上其他检测技术结合，其与成簇的规则间隔短回文重复序列(clustered regularly interspaced short palindromic repeats, CRISPR)、横向流动试纸条技术(lateral flow dipstick, LFS)的联合较为常见，真正做到了高效、快速、准确检测。

3.3.1 RPA 与 CRISPR 技术的结合

CRISPR 技术是近年来飞速发展的一种分子诊断技术，RPA 与 CRISPR 技术联合可达到快速准确检测的目的。Zhang 等[35]开发了 RT-RPA 结合 CRISPR-Cas12a 比色法检测新型冠状病毒，针对新型冠状病毒基因组的 *ORF1ab* 和 *N* 设计特异性引物，使用金纳米颗粒(gold nanoparticles, AuNPs)比色读数，RT-RPA 扩增病毒基因组后得到的大量 dsDNA 识别 PAM 序列结合特异 crRNA，激活 Cas12a，在反式裂解过程中，DNA 底物会逐渐从 AuNPs 上水解，通过紫外-可见吸收光谱和肉眼观察可以对其进行监测。Zhang 等[1]统计并分析了 25 篇利用 RPA/RAA 联合 CRISPR 技术检测新型冠状病毒的研究，结果表明当等温扩增技术与 CRISPR 联用检测样本时灵敏性和特异性较高，检测灵敏度和特异性分别可以达到 98%和 99%，这表明 RPA 技术与其他检测方法联合使用能提高检测灵敏度和特异性，将来或会成为检测的新方法供人选择。Xiong 等[36]将 RPA 与 Cas12a 结合检测非洲猪瘟病毒和山羊痘病毒，将 RPA 试剂和 CRISPR/Cas12a 试剂分别加到管底和管盖，盖上反应盖进行反应，可减少气溶胶污染；RPA 与 Cas12a 联用不仅实现了 crRNA 的特异性检测，同时减少假阳性问题发生，还起到了放大检测信号的作用，使 RPA 技术检测更加精准、可视化。

3.3.2 RPA 与侧流试纸条技术的结合

RPA 与侧流试纸条联合无需特殊仪器，肉眼通过试纸条就可快速、准确观察结果。Srisrattakarn 等[37]采用 RPA 结合侧流试纸(lateral flow dipstick, LFD)方法检测耐甲氧西林金黄色葡萄球菌(methicillin-resistant *Staphylococcus aureus*, MRSA)，根据 *mecA* 基因设计特异性 RPA 引物和探针，将 RPA 扩增产物加入 SYBR 染料观察反应结果，通过与 PCR 比较检测结果，发现 RPA-LFD 法的灵敏度和特异性分别为 92.1%和 100.0%。Bian 等[38]开发 RPA-LFD 法检测志贺杆菌和肠侵袭性大肠杆菌，根据其共同的毒力基因 *ipaH* 设计 RPA 引物和检测探针；他们根据 *ipaH* 基因设计 4 种不同的引物和探针组合，筛选最适合的引物和探针，减少假阳性发生，该方法的检测极限为 1.29×10^2 copies/μL。Sun 等[39]将 RPA 与 LFD 联合使用，设计一个试纸条可同时检测 A 型流感病毒和 B 型流感病毒，根据病毒保守序列设计引物和探针，探针上带有地高辛、异硫氰酸荧光素标记，在侧流条加入抗地高辛抗体(B 型流感)、抗异硫氰酸荧光素抗体(A 型流感)与 RT-RPA 扩增后的 dsDNA 反应，若带有地高辛的检测线变红说明 A 型流感存在，带有异硫氰酸荧光素的检测线变红则说明 B 型流感存在；后期进行临床样本检测，并将检测灵敏度和特异性与 PCR 进行比较，检测一致性较高，说明 RPA 技术与试纸条联用可作为高效检测流感病毒的新技术。

3.3.3 RPA 技术与临床多种技术的结合

RPA 技术灵敏、高效的优势使其在临床应用中逐步发展，并且与其他技术联合使用也越

来越常见。Asa 等[40]将 RPA 技术联合焦磷酸盐离子探针[pyrophosphateion (PPi)-sensing probe, PK-probe]用于检测新型冠状病毒，在新型冠状病毒的全基因组研究中，PK-probe 联合 RPA 技术对新冠病毒的检测限为 1 160 copies/mL，该方法使用 RPA 扩增目的产物后再和 PPi 探针结合，根据显色反应可以快速判定检测结果。除此以外，为达到更加灵敏检测的目的，RPA 还可与絮凝测定、表面增强拉曼散射[41]、电化学、化学发光、智能手机、基于硅微环谐振器的光子等多种其他检测方法联合使用。

基于RPA技术设计的荧光型RPA试剂盒可在临床上辅助疾病的诊断，RPA 与试纸条、CRISPR 的联合使用已经成为偏远地区及 POCT 较常用的方法之一。其能够检测多种样品(表 3)，发挥着越来越重要的作用。

表 3 RPA 联合其他技术对较常见样本检测

Table 3 RPA integrated with other techniques for the more common sample detection

分类 Classification	致病菌 Pathogenic bacteria	检测方法 Detection method	检测时间 Detection time (min)	检测限 Limit of detection	特异性 Specificity	参考文献 References
Bacteria	*Staphylococcus aureus*	RPA-LF	25	10^5 CFU/mL	100%	[42]
	Vibrio cholerae	RPA-LFS	35	10 copies/μL	High	[43]
	Pseudomonas aeruginosa	RPA-LFS	40	3.05 CFU/mL	98.26%	[44]
	Salmonella	RPA-Cas13a	45	10^2 copies/μL	High	[45]
	Listeria monocytogenes	RPA-LFIA	20	9.0 CFU/mL	High	[46]
	Mycobacterium tuberculosis	AS-RPA/SYBR	20	4.0 copies/μL	98%	[47]
	Vibrio parahaemolyticus	IMS-RPA-LF	45	10^4 CFU/mL	80.3%	[48]
	Brucella	RPA-LFD	10−30	4−6 copies/mL	High	[49]
	Cryptococcus neoformans	RPA-LFS	20	1 fg/μL	High	[50]
Virus	HBV virus	RPA-LFA	−	2×10^5 IU/mL	88.2%	[51]
	HPV virus	RPA-Cas13a	35	500 copies/μL	High	[52]
	African swine fever virus	RPA-Cas12a	20	2 copies/μL	100%	[53]
	Coronavirus	RPA-CRISPR/Cas12a	−	10 copies/μL	100%	[54]
	Monkeypox virus	RPA-CRISPR/Cas12a RPA-LFS	20−30	1 copy/μL	100%	[55]
	Norovirus	RPA-CRISPR/Cas12a	40	9.65×10^2 copies/mL	High	[56]
	Lumpy dermatosis virus	RPA-CRISPR/Cas12a	15	5 copies/μL	96.3%	[57]
	H7N9	LFD-RPA	−	32 fg	100%	[58]
Parasite	Schistosoma japonicum	RPA-LFD	15−20	5 fg	100%	[59]
	Plasmodium	RPALF	12	10 parasites/μL	100%	[60]
	Trichomonas vaginalis	RPA-CRISPR-Cas12a	60	1 copy/μL	100%	[61]
	Trichomonas vaginalis	RPA-LF	25	100 fg	High	[62]
Others	Mycoplasma pneumoniae	RPA-CRISPR/Cas12a	60	2 copies/μL	100%	[63]
	Chlamydia trachomatis	RPA-LFD	20	200 copies	100%	[64]
	Mycoplasma hyopneumoniae	RPA-LFS	20	5.0×10^2 fg	100%	[65]

−：文献中未找到相关信息

−: The relevant information was not found in the literature.

4 讨论

4.1 RPA 的未来展望

RPA 技术作为一种快速、便捷、高效的核酸扩增技术，与临床上其他核酸扩增技术相比主要具有 3 个优点：(1) 检测受环境影响小。PCR 需要在反应仪器中进行，反应流程包括人工加样、设置程序、等待检测结果等多个步骤，一旦无仪器则反应无法进行；而 RPA 技术在常温下就可进行反应，反应全程无需复杂的仪器，对环境要求低，操作简单，POCT 式的检测适用于不发达地区，可减轻贫困地区因缺少仪器而检测困难的问题，拓宽 RPA 技术临床的使用范围。(2) 检测时间短。RPA 技术反应只需 20−30 min，而 PCR 反应全程需要 1−2 h，Ma 等[66]用 RPA 扩增 16、18 型 HPV 临床样本 DNA，利用 SYBR Green I 染料监测产物扩增情况，对 HPV 病毒的检测限达到 100 copies/mL，并与其他 HPV 类型无交叉，准确率达到 98.5%，20 min 内可通过肉眼观察结果。因此，RPA 能够达到快速检测的目的，对于大批量检查或疾病普查有实际意义。(3) 可与其他技术联用，提高检测灵敏度。Su 等[67]用 RPA 与 CRISPR-Cas 介导多路横向流动法可视化检测新型冠状病毒，双通道能同时检测多个不同基因，提高检测效率，检测新型冠状病毒的灵敏度达到 10 copies/μL。除此之外，RPA-LF 结合免疫磁分离(immunomagnetic separation, IMS)[68]检测金黄色葡萄球菌，可在 15 min 内得到检测结果，对金黄色葡萄球菌基因组的检出量可低至每反应 600 fg，比 PCR 的灵敏度高约 16.7 倍，对提高临床检测灵敏度具有实际意义。

同时 RPA 也存局限性和挑战，近些年来，科研人员正尝试从 4 个方面对 RPA 进行优化：(1) 避免气溶胶污染。在提取核酸和样品加样过程中，反复加样或开盖振荡反应管都易造成气溶胶污染。为减少污染，Li 等[69]将 RPA 和 Cas12 系统整合到一个试管中，即在一个试管内不开管检测，尽量减少气溶胶污染的操作步骤，在 20 min 内实现 MRSA 的可视化检测。(2) 排除抑制剂影响。核酸提取过程中试剂或培养基的选择很重要，Valasevich 等[70]发现十六烷基三甲基溴化会严重抑制 RPA 反应；Liu 等[71]发现亚硒酸胱氨酸培养基显著影响 RPA 反应，会产生大量引物二聚体，出现假阳性结果。因此，研究人员在选择培养基时应注意使用 LB 培养基，避免选用亚硒酸胱氨酸培养基 。(3) 优化引物与探针。Liu 等[72]发现同一探针、不同的引物对荧光 RPA 的效率影响较小，而同一扩增区域不同探针对荧光 RPA 的效率影响较大。Higgins 等[73]提出引物-模板复合物不匹配会影响反应，导致临床诊断易导致假阴性。Higgins 等[74]提出 Primed RPA 软件自动选择 RPA 引物和探针，通过几对引物、探针序列进行比对来确定保守序列，过滤可能与背景生物发生交叉反应的区域，旨在解决引物与探针不适配的问题。(4) 温度和搅拌。过高、过低的温度都会导致 RPA 结果不稳定，均匀的搅拌是影响 RPA 反应外在因素。Wambua 等[75]证明当搅拌 4 min 再进行 RPA 反应时，阈值荧光值会在 5−8 min 出现，无搅拌的情况下，荧光值出现的时间是 8−14 min，由此可见适当的搅拌可加快反应进程。

4.2 结语

RPA 技术作为新兴的核酸扩增技术，具有所需设备简单、反应速度快、灵敏度高等优点，克服了荧光定量 PCR 技术中仪器复杂、成本高昂等缺陷。该技术的应用价值在新型冠状病毒、非洲猪瘟病毒等病原体核酸检验中得到了较好的证明。

综合近几年国内外等温扩增技术的研究情

况，RPA 检测 POCT 化和多技术联合应用将是该技术未来几年发展的方向。POCT 的最大特点是不需要对标本进行复杂的预处理，在采样现场即可进行检测分析，快速得到检测结果。其对于近几年新型冠状病毒感染的检测有较实际的意义，POCT 式的检测可以减少因采集核酸导致的人群大量聚集，降低人群直接接触感染的概率。另外，与临床上其他检测技术结合使检测更灵敏、观察结果更直接便捷。重组酶介导的条形码/扩增诊断方法联合测序高通量方法，可一次筛选 10×10^4 个疑似新型冠状病毒感染的病人样本[76]。此外，RPA 技术还与光控 CRISPR/Cas12a、APHF 分析仪等方法联合使用，旨在提高检测灵敏度和特异性[77-78]。

综上所述，RPA 是继 PCR 后有较高实用价值的新技术。由于其还在发展阶段，需要进行不断的探索，相信随着对 RPA 技术的深入研究，以等温核酸扩增技术为基础的诊断产品会不断被开拓，RPA 将发挥自己独特的作用，其产品将更加广泛地应用到日常检测当中，为医疗事业作出贡献。

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