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侧流免疫层析技术在食品安全检测中的研究进展

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摘要:侧流免疫层析技术(lateral flow immunoassay, LFIA)不仅有效结合了层析技术的分离能力和免疫分析的高度特异性,而且操作简单、检测快速、价格低廉,为食品安全现场检测提供了一种理想的技术平台。常规的 LFIA 主要以纳米金颗粒作为信号标记材料,该方法检测灵敏度相对较低,仅能满足定性和半定量检测。为了提高 LFIA 的检测性能,近年来研究者做了大量的努力。本文总结了 LFIA 的检测原理及其在信号标记材料、信号增强方式、多元分析物同时检测、信号读出方式等方面的技术改进,并讨论了 LFIA 现有的不足以及未来的发展方向,以期为我国食品安全快速检测的发展提供技术参考。

关键词:侧流免疫层析,试纸条,食品安全,快速检测技术

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本文网刊:

Research Progress of Lateral Flow Immunoassay in Food Safety

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Abstract: lateral flow immunoassay (LFIA) not only effectively combines the separation ability of chromatography technology and the excellent specificity of immunoassay methods, but also provides an ideal platform for the on-site detection of food safety with advantages of easy operation, rapid detection and low cost. Gold nanoparticle is a commonly used signal label in the field of LFIA analysis. Unfortunately, conventional gold-based LFIA often suffers from drawbacks of low sensitivity, which can only satisfy qualitative and semi-quantitative information on analyte concentrations. To improve the detection performance of LFIA, researchers have made significant efforts. In this contribution, the basic principle of LFIA is introduced and the recent technical improvements are systematically summarized, such as developing novel signal nanoparticles, signal enhancement method, multiplex target detection and different signal readout mode, etc. Meanwhile, this study discusses the shortcomings and the trend of LFIA, which would provide technical references for the development of rapid detection methods for food safety in China.

Key words: lateral flow immunoassay; test strip; food safety; rapid detection technology

民以食为天,食以安为先,食品的安全与否直接关系到人民群众的身体健康。现代食品工业技术的不断发展以及不法商贩为了追求最大程度的经济效益,生物性污染食品事件(细菌及细菌毒素、真菌及真菌毒素等)、化学性污染食品事件(农药、兽药、重金属、激素、多环芳烃类化合物、N-亚硝基化合物等)频频发生。对相关危害成分的检测主要基于高效液

相色谱法、气相色谱法、液相色谱质谱联用法以及酶联免疫等仪器分析方法,这些方法虽然灵敏度高,但是操作繁琐,费时,需要专业的技术人员同时检测价格比较昂贵。因此开发快速、高灵敏度以及廉价的检测方法用以分析食品中的有害成分对于食品安全的监督管理尤为重要。侧流免疫层析技术(lateral flow immunoassay, LFIA)因其检测快速、所需样本

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量小、方便携带以及价格低廉等优点而广泛应用于食品安全检测中。典型的 LFIA 以球状纳米金颗粒作为信号标记材料,但是这种检测方法的结果是定性(yes/no)或半定量的,无法满足高灵敏的现场检测需求^[1]。

为了增强 LFIA 的检测性能,突破传统技术的缺陷,研究者尝试从 LFIA 的信号标记材料、信号增强方法、多元分析物同时检测以及检测信号的读出方式等方面做改进,这些方法一定程度上提高了检测的灵敏度以及检测效率。本论文主要对近年来 LFIA 的各种改进方法进行综述,以期免疫层析技术的进一步开发利用提供借鉴。

1 侧流免疫层析技术的检测原理

常规的免疫层析试纸的结构主要由样品垫、结合垫、硝酸纤维素(nitrocellulose, NC)膜、吸水垫以及 PVC 衬板 5 部分组成,其相互覆盖顺序如图 1a 所示。在硝酸纤维素膜上有用于判定检测结果的检测线(test line, T 线)和用于判定检测结果有效与否的质控线(control line, C 线)如图 1b 所示。检测原理主要基于免疫试剂抗原抗体的特异性结合, T 线上包被捕获抗原或者捕获抗体,结合垫上结合有色纳米材料标记的抗体,将待检测样品滴加到样品垫上后,层析作用的进行使得免疫试剂相互结合,最终可根

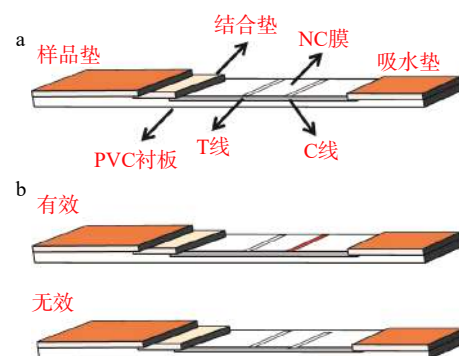


图 1 侧流免疫层析技术的示意图

Fig.1 Schematic diagram of LFIA

据 T 线区域信号颜色的有无以及信号的强度判定检测结果^[2]。

2 侧流免疫层析技术的发展方向

2.1 开发新型的信号标记材料

常规的 LFIA 通常采用 20~30 nm 的球状金纳米材料,但是该材料发光强度较弱,影响检测的灵敏度^[3]。与此同时其在制备以及存放的过程中一旦出现杂质便非常容易聚集沉淀进而影响纳米金的使用。为了改进 LFIA 的信号标记材料,近年来越来越多的纳米材料被应用于免疫层析试纸条上,表 1 列出了近年来应用于 LFIA 的纳米材料。

表 1 在侧流免疫层析技术中应用的信号标记材料

Table 1 Signal nanoparticles in LFIA

信号标记材料	检测目标物	检测限	参考文献
金纳米星	双酚A	0.073 μg/L	[4]
金纳米花	黄曲霉毒素B ₁	0.32 pg/mL	[5]
金纳米簇	克伦特罗	0.003 μg/L	[6]
	莱克多巴胺	0.023 μg/L	
聚多巴胺纳米球	呋喃唑酮的代谢物	3.5 ng/mL	[7]
多巴胺包被的纳米金颗粒	玉米赤霉烯酮	7.4 pg/mL	[8]
铂包裹的金纳米棒	空肠弯曲杆菌	75 CFU/mL	[9]
Au@Pt纳米材料	细交链孢菌酮酸	0.39 ng/mL	[10]
金二氧化硅纳米复合材料	呋喃唑酮的代谢物	0.08 ng/mL	[11]
量子点	呋喃唑酮的代谢物	0.14-0.15 μg/kg	[12]
量子点珠	戊唑醇	0.02 ng/mL	[13]
上转换发光材料	大肠杆菌O157:H7	7×10 ² CFU/mL	[14]
荧光微球	黄曲霉毒素B ₁	2.5 μg/kg	[15]
	玉米赤霉烯酮	1 μg/kg	
	脱氧血腐镰刀菌烯醇	20 μg/kg	
碳纳米材料	T-2毒素	13 μg/kg	[16]
	沙丁胺醇	10 μg/kg	
	四环素	0.0516 ng/mL	
菱形铝纳米材料	克伦特罗	1 ng/mL	[17]
间苯二酚甲醛树脂聚合物	桔青霉素	0.06 ng/mL	[18]
铂纳米颗粒	玉米赤霉烯酮	0.11 ng/mL	[19]
	鼠伤寒沙门氏菌	10 ³ CFU/mL	
聚苯乙烯包裹铂纳米材料	马铃薯病毒	0.5 ng/mL	[20]
磁性纳米材料	大肠杆菌O157:H7	2.39×10 ² CFU/mL	[21]
荧光磁纳米珠	赭曲霉毒素A	0.094 ng/mL	[22]
磁包金纳米复合材料	莱克多巴胺	0.12 ng/mL	[23]
磁性普鲁士蓝纳米颗粒	克伦特罗	0.20 ng/mL	[24]
	克伦特罗	0.20 ng/mL	

续表 1

信号标记材料	检测目标物	检测限	参考文献
乳胶微球	泰乐菌素	4 ng/mL	[26]
MoS ₂ @Au	鼠伤寒沙门氏菌	10 ² CFU/mL	[27]
氮化碳负载金纳米材料	17β-雌二醇	0.5 ng/mL	[28]
Pt-Ni(OH) ₂ 纳米片	甲氧菊酯	0.24 ng/mL	[29]
氧化石墨烯	乙草胺	0.63 ng/mL	[30]
微生物负载金纳米材料	黄曲霉毒素B ₁	0.3 ng/mL	[31]
金属聚多巴胺框架结构	赭曲霉毒素A	0.1 ng/mL	[32]
纳米金功能化花状二氧化锰纳米复合材料	克伦特罗	0.1 ng/mL	[33]
	四环素	0.045 ng/mL	[32]
	脱氧雪腐镰刀菌烯醇	0.013 ng/mL	[33]

2.2 纳米材料不标记单克隆抗体法

在传统的 LFIA 中, 显色纳米标记材料主要通过静电吸附法和共价结合法标记单克隆抗体(monoclonal antibody, mAb), 但是静电吸附法很容易受到抗体等电点、温度和 pH 等因素的影响, 而共价结合法不可避免地会阻塞 mAb 的部分抗原结合位点, 最终均导致 LFIA 的检测性能下降^[34]。为了克服纳米材料对抗体标记过程中产生的不利影响, 研究者开发出了将纳米材料不标记 mAb 的方法。研究者通过标记抗原(antigens, ag)、羊抗鼠抗体(gold nanoparticles labeled goat anti-mouse antibodies, GNPs-GAMA)等物质最大程度的暴露 mAb 的抗原结合位点, 同时开发出抗体的替代探针(噬菌体、适配体)用于特异性识别食品中的有害物质, 替代探针以其成本低、易合成、稳定性好以及保存时间长的优点使得 LFIA 的检测性能提高。表 2 总结了纳米材料标记其他物质的检测方法。

2.3 无纳米标记材料法

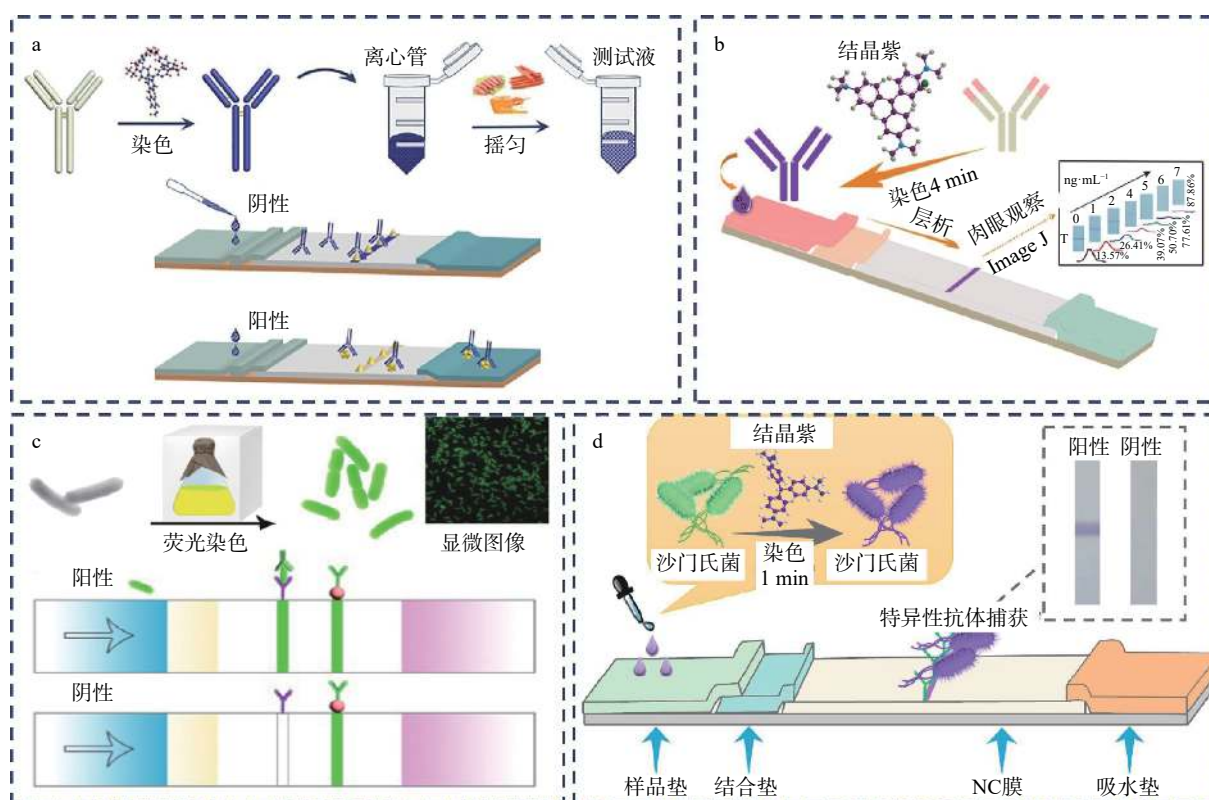
尽管纳米材料在 LFIA 中的应用非常广泛, 但是纳米材料的合成过程相对复杂且比较费时, 其和抗体之间的偶联会影响抗体的活性, 不同批次合成的纳米材料可能会存在一定的差异进而影响 LFIA 的检测性能, 同时合成的纳米探针在胶体作用下很容易发

生聚集沉淀, 需要储存在 4 ℃ 条件下。因此近年来研究者探索无纳米材料标记的 LFIA。

Xu 等^[45] 利用蛋白质染料考马斯亮蓝(coomassie brilliant blue, CBB)开发了一种新型的检测试纸条, 通过使用 CBB 对抗呋喃唑酮代谢物(3-amino-2-oxazolidinone, AOZ)的 mAb 进行染色, 染色后的 mAb 即可作为一种显色信号标签进而可代替有色纳米材料(图 2a), 该方法对 AOZ 的检测限为 2 ng/mL, 同时试纸条的制作成本比其他方法低 300 倍。Dou 等^[46] 根据结晶紫可以与蛋白质非共价结合的特性, 通过使用结晶紫对 mAb 进行染色(图 2b), 以 AOZ 为目标分析物, 对其的检测限为 1.53 ng/mL。Song 等^[47] 通过采用异硫氰酸荧光素(fluorescein isothiocyanate, FITC)染色法使得大肠杆菌和大肠杆菌的单克隆抗体均携带上黄绿色的荧光(图 2c), 这种方法对大肠杆菌的检测限可达 1 CFU/mL, 灵敏度是常规胶体金试纸的 10 倍。Bu 等^[48] 利用经典的革兰氏染色法和直接免疫法对食品中的致病菌进行检测, 通过使用结晶紫对革兰氏阴性菌沙门氏菌和革兰氏阳性菌李斯特氏菌进行染色, 染色后的病原菌即可作为显色信号(图 2d), 该方法对沙门氏菌的检测限可达 80 CFU/mL, 对李斯特氏菌的检测限为 10⁴ CFU/mL。无纳米标记材料法节省了试纸条制备过程中复杂的

表 2 纳米材料不标记单克隆抗体法
Table 2 Method by nanomaterials unlabeled mAb

所使用的纳米材料	纳米材料标记的物质	检测目标物	检测限	性能提高倍数	参考文献
三氧化二铁纳米材料	黄曲霉毒素抗原	黄曲霉毒素	0.0125 ng/mL	7倍	[35]
带正电的碳纳米材料	沙门氏菌	沙门氏菌	10 ² CFU/mL	—	[36]
四氧化三铁纳米材料	GAMA	17β-雌二醇	0.2 ng/mL	5倍	[37]
金纳米材料	GAMA	脱氧雪腐镰刀菌烯醇	500 pg/mL	10倍	[38]
		T-2毒素	50 pg/mL	10倍	
磁性纳米材料	氨苄青霉素	沙门氏菌	10 ² CFU/mL	—	[39]
普鲁士蓝纳米材料	甘露糖	大肠杆菌O157:H7	10 ² CFU/mL	—	[40]
金纳米材料	适配体	玉米赤霉烯酮	20 ng/mL	—	[41]
		汞离子	5 μg/L	—	
上转化荧光纳米材料	适配体	赭曲霉毒素A	3 ng/mL	—	[42]
		沙门氏菌	85 CFU/mL	—	
金纳米材料	噬菌体	沙门氏菌	10 ² CFU/mL	—	[43]
荧光微球	噬菌体细胞内溶素	金黄色葡萄球菌	6.6×10 ² CFU/mL	—	[44]

图2 无纳米标记材料的侧流免疫层析技术^[45-48]Fig.2 Signal nanoparticles-free LFIA^[45-48]

材料合成过程以及繁琐的标记过程, 仅仅通过染色的方法即可获得检测所需的有色信号, 节省了时间, 同时操作简单、灵敏度较高, 是一种基于染色的新型免疫层析技术。

2.4 多元分析物同时检测法

传统的 LFIA 只能用来检测一种目标分析物, 检测效率较低, 不能满足多种目标分析物的同时检测需求。为了提升试纸条的检测性能, 近年来研究者开发了能够同时检测多元目标物的试纸条。

2.4.1 单通道多条 T 线多元检测法 Han 等^[49] 利用金纳米颗粒标记八种不同待检物质的 mAb/受体, 同时在试纸条 NC 膜上建立八个检测区域以分别固定八种不同物质的捕获抗原(图 3a), 该方法实现了对喹诺酮(quinolone, QN)、黄曲霉毒素 M₁(aflatoxin M₁, AFM₁)、三聚氰胺(melamine, MEL)、四环素(tetracycline, TC)、β-内酰胺类抗生素(β-lactams, BL)、氯霉素(chloramphenicol, CAP)、磺胺类药物(sulfonamide, SA)和链霉素(streptomycin, STR)的同时检测, 检测时间少于 20 min^[49]。这种方法虽然检测效率高, 但是每条检测区域的颜色相同, 因此在检测过程中很容易混淆检测目标物。为了克服这一缺点, Zhang 等^[50] 开发出一种 T 线和 C 线颜色均不同的免疫层析试纸条用于检测鱼类中的两种海藻毒素, 该方法将红色和绿色的荧光微球分别标记抗微囊藻毒素和冈田酸的 mAb, T 线区域分别固定两种毒素的 ag, 样品的加入和层析作用的进行即可在检测线区域观察到红色和绿色的条带, C 线区域则是两

条 T 线颜色的混合色(图 3b)。检测过程在 20 min 可以完成, 对微囊藻毒素和冈田酸的检测限分别为 0.074 和 2.42 μg/kg。该方法可通过信号颜色的不同来区分不同的检测目标物, 使得结果更加容易辨别分析。

2.4.2 单通道单条 T 线多元检测法 Shu 等^[51] 采用混合杂交细胞技术制备了一种具有两个抗原识别位点的双功能抗体可用于同时识别甲基对硫磷和吡虫啉, 将此抗体固定于试纸条的一条检测线上, 结合时间分辨化学发光技术即可实现在一条 T 线上可完成对两种物质的检测(图 4a), 在 2.5 和 300 s 时即可分别采集到甲基对硫磷和吡虫啉的检测信号结果。Fabio 等^[52] 采用红色和蓝色的金纳米材料分别标记抗 AFB₁ 和伏马毒素(fumonisin B, FMB)的 mAb, 将两种分析物的抗原混合固定于一条 T 线上, 最终 T 线的颜色取决于分析物的种类、数量。检测原理基于待检物质和固定于 T 线处的相应抗原竞争结合相对应的金纳米材料标记的 mAb, 样品中存在待检物质时, 待检物质优先和 mAb 结合, 进而不与 T 线处的相应抗原结合, 即 T 线处不出现该类物质对应的标记色。而样品中不存在待检物质时, 纳米材料标记的抗体即可与 T 线处的相应抗原结合出现相应的颜色, 分析结果如图 4b 所示。该方法对黄曲霉毒素和伏马毒素的检测限分别为 1 和 50 ng/mL^[52]。该方法和单通道多条 T 线检测法相比, 在试纸条的制备过程中节约了多条 T 线的制作所需要的划线时间, 操作比较简单, 但是此法在检测目标物过多时, 会使

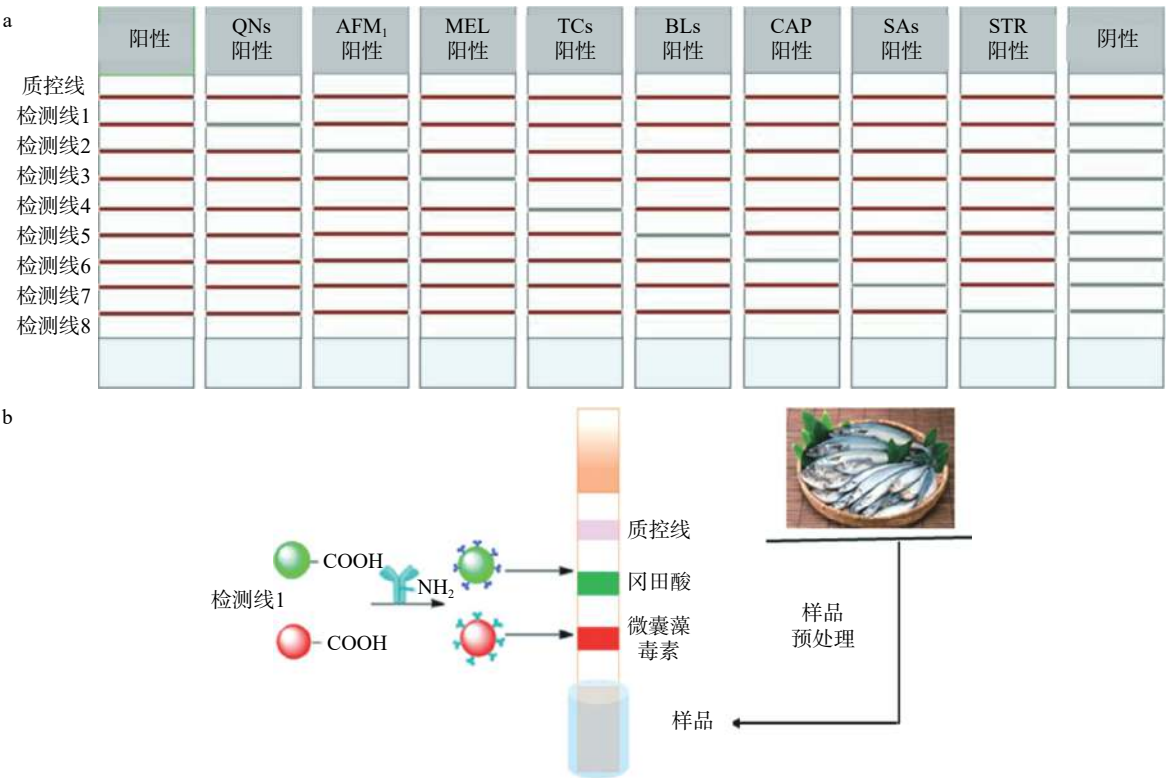


图 3 单通道多条检测线的侧流免疫层析技术^[49-50]
Fig.3 Single channel with multiple T line LFIA^[49-50]

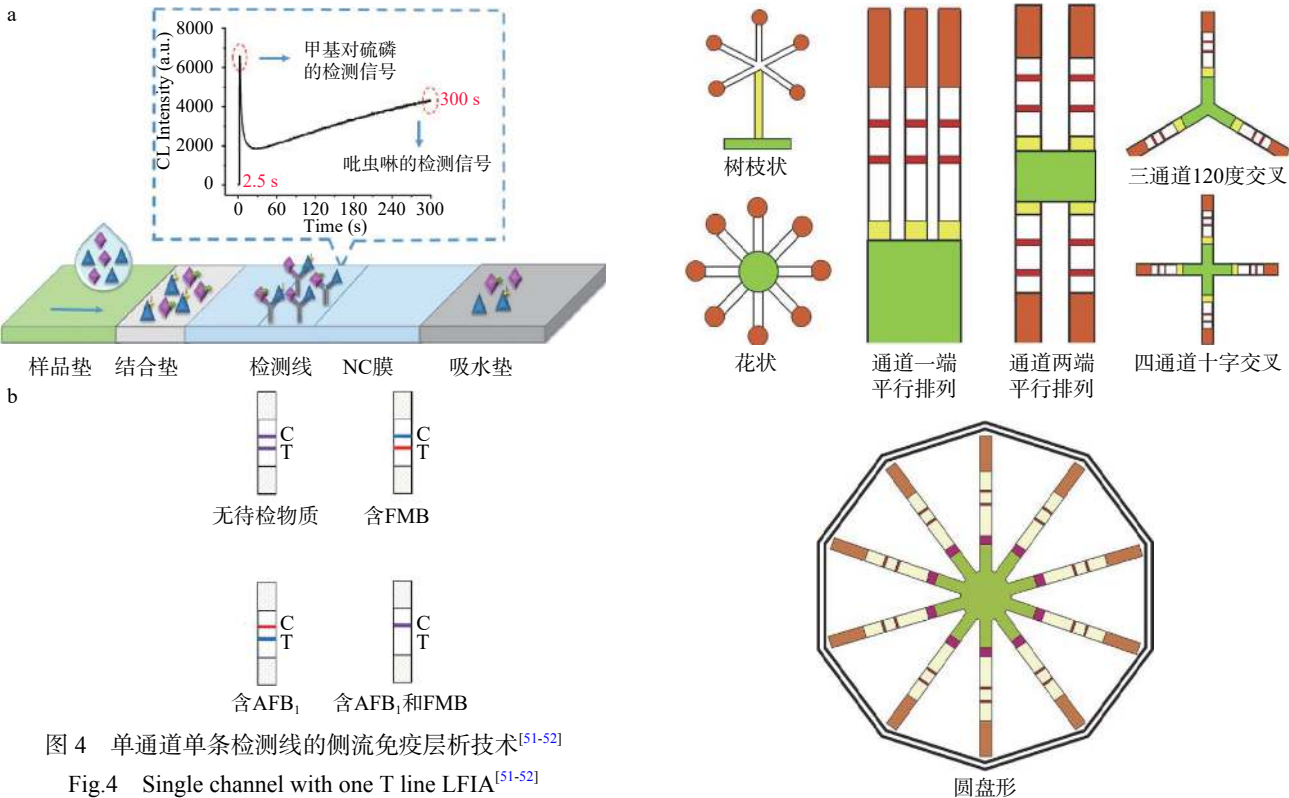


图 4 单通道单条检测线的侧流免疫层析技术^[51-52]
Fig.4 Single channel with one T line LFIA^[51-52]

结果的观察不直观。

2.4.3 多通道多元检测 近年来,为了实现 LFIA 的高通量检测,不同的试纸条结构被设计(图 5),比如三通道 120 度交叉^[53]、树枝状、花状、圆盘形、四通道垂直交叉、通道一端平行排列、通道两端平行排列等^[54]。

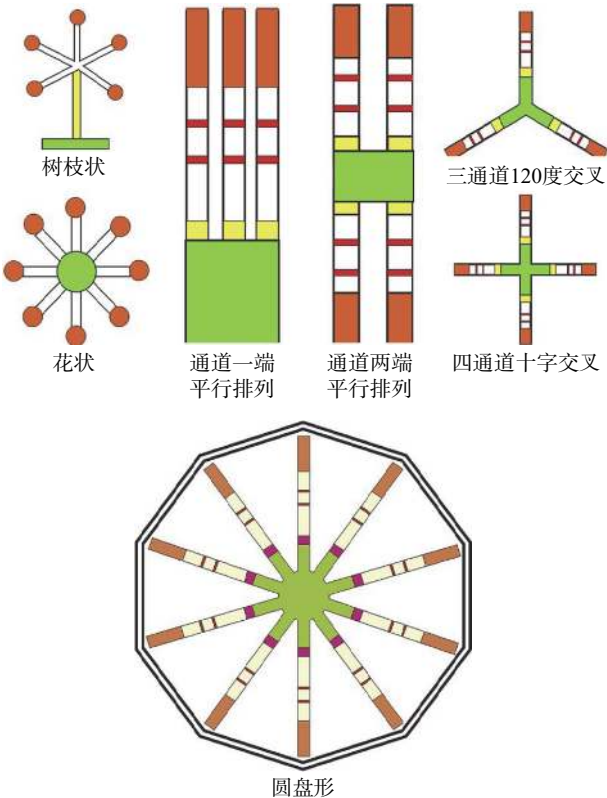


图 5 多元检测方法的检测通道结构^[53-54]
Fig.5 Channel structure of multiple detection method^[53-54]

Cheng 等^[29]开发了一种双通道的 LFIA 以同时检测两种不同的目标分析物,同时可以消除两个目标物之间的潜在交叉反应,该方法设置一个样品垫,左右两条通路分别各设置一个结合垫,一条 T 线和

C 线(图 6a), 对乙草胺、甲氧菊酯的检测限分别为 0.63 和 0.24 ng/mL。Zhao 等^[55]开发了一种具有 10 通道的检测试纸盘用以同时检测 10 种食源性致病菌(图 6b), 整个检测过程在 20 min 内可完成, 对菌体的检测限可达到 10 CFU/0.6 mg。Rong 等^[56]设计了一种可以同时检测四种目标分析物的多通道测试筒, 测试筒的顶部提供了样品进样通道, 四个流体输送通道由底盒密封, 在 420 r/min 时产生的离心力可以将装载的样品溶液以 1 mm/s 的最大流速送入四个反应室(图 6c)。整个分析工作流程只包括以下几部分: 将样品加入测试桶入口, 快速旋转以启动免疫反应的自动送样, 最后将测试桶装入读条器进行旋转和光信号扫描。该测试桶操作简单, 特别适合食品安全有害物质分析过程的现场实时检测。

2.5 多种信号的读出方式

传统的 LFIA 检测结果主要分析出现在试纸条上条带的颜色信号, 通过比色的方法对物质进行定性和定量检测。但是该方法在试纸颜色差异不明显或者样本背景颜色较深时会导致读出结果不准确, 近年来, 为了提高试纸条结果的准确性, 研究者开始探索其他的信号读出方式。

2.5.1 化学信号 酶催化反应的高效性以及出现的相关颜色反应可被仪器设备所采集, 进而使得试纸条的读数方式不仅仅限于读取条带的颜色信号, 通过采集酶催化反应出现的化学信号来提高试纸条读数的准确性。Wang 等^[40]利用普鲁士蓝纳米颗粒具有过氧化物酶活性, 将层析之后的 T 线区域剪下来放入 3,3',5,5'-四甲基联苯胺(Tetramethylbenzidine, TMB)溶液中, T 线结合的普鲁氏蓝纳米颗粒即可催化 TMB 产生蓝色的化学信号。Hui 等^[57-58]使用鲁米诺还原氯金酸制备鲁米诺还原的金纳米粒子(Luminol-reduced gold nanoparticles, LuReGNPs), 同样在免疫层析结束后将 T 线区域剪下来, 在过氧化氢和辣根过氧化物酶(Horseradish Peroxidase, HRP)存在的条件下即可收集到化学发光信号。然而上述方法均需要剪 T 线区域, 为了提升检测试纸条的使用便捷性, Deng 等^[59]开发了一个小型检测设备, 所有需要的检测试剂均预先储存于该设备中。该设备由三部分组成: 传统的试纸条、化学发光试剂垫和聚碳酸酯壳(polycarbonate holder, PCH), 试纸条的金纳米颗粒同时标记 HRP 和检测抗体, 化学发光试剂垫中储存有提前冻干的氧化剂过硼酸钠和鲁米诺。传统

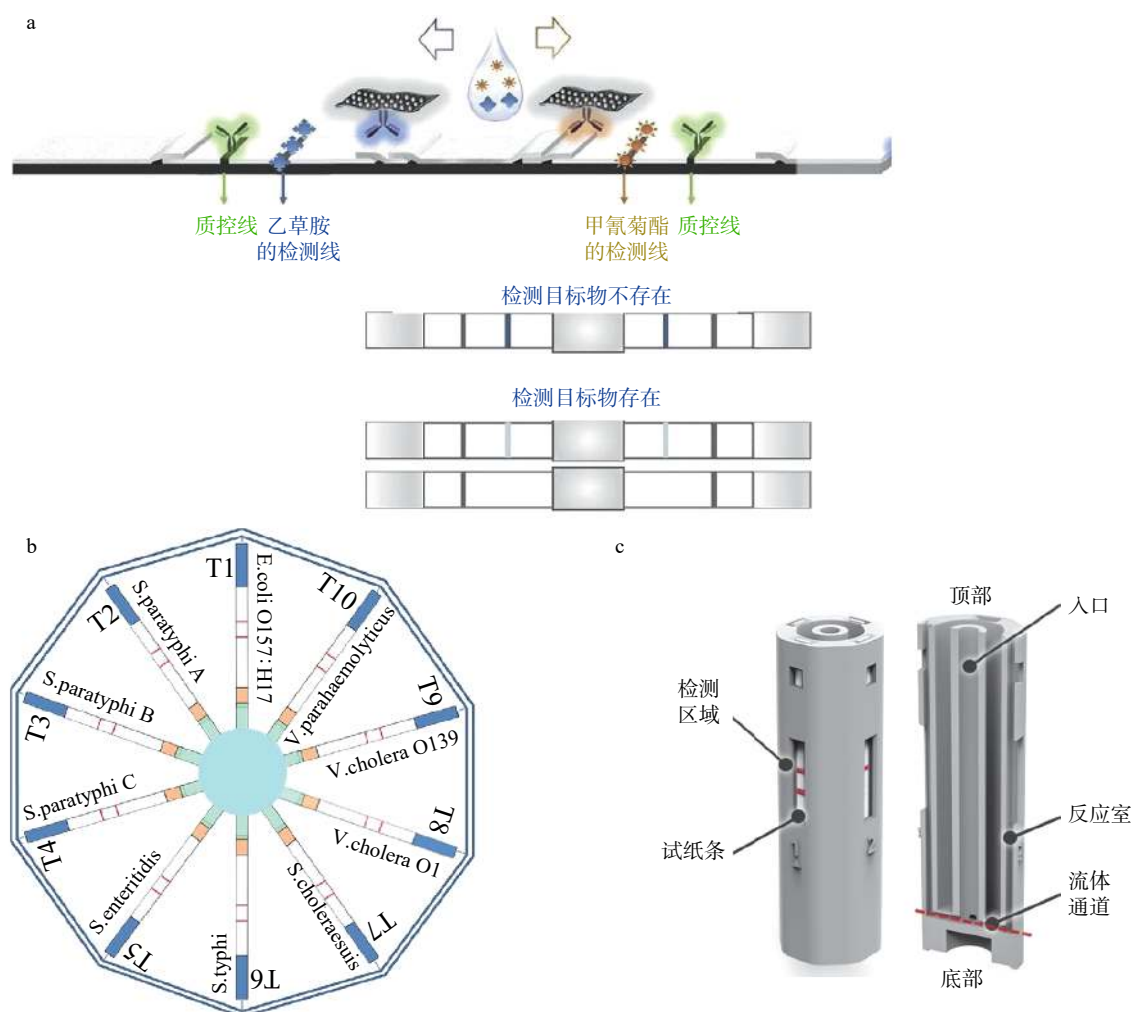


图 6 多通道多条检测线的侧流免疫层析技术^[29,55-56]

Fig.6 Multiple channel with multiplex T line LFIA^[29,55-56]

的试纸条经过反应和肉眼定性分析后,用水溶解化学发光试剂垫上的试剂,接着将试剂垫覆盖于试纸条上,试剂在 HRP 的催化下反应,生成化学发光信号用于定量检测。

2.5.2 光热信号 光热材料在近红外光的照射下可被加热,温度变化的信号可被温度计或者红外照相机采集。大多数研究表明通过读取该温度信号可提高 LFIA 的检测灵敏度^[60-62]。Zhang 等^[33]合成纳米金功能化的花状二氧化锰纳米复合材料,该纳米材料可做为光热信号的标签,在 808 nm 激光下辐照 T 线 3 min,通过使用红外照相机即可记录温度的变化。该方法对脱氧雪腐镰刀菌烯醇的检测限为 0.013 ng/mL,灵敏度是传统胶体金试纸条的 58 倍。Li 等^[63]将金纳米颗粒负载到二维黑磷纳米片上(Au nanoparticle-loaded two-dimensional black phosphorus, BP-Au),其具有较好的光热转换性能,通过结合抗体制成免疫层析试纸条的探针进而实现对恩诺沙星(enrofloxacin, ENR)的检测,该方法对 ENR 的检测限为 0.023 μg/L。Su 等^[64]也利用 MnO₂-Au 纳米复合材料的光热特性检测 AOZ,该方法对其检测限为 0.43 ng/mL。光热信号读出模式的应用提供了更为准确的结果,同时具有强大的抗干扰能力,大大提升了试纸条的检测性能。

2.5.3 拉曼信号 近年来,在 LFIA 中使用拉曼信号标记免疫探针的方法已经成为提高灵敏度的有效方法,该类信号稳定性较好,同时可以通过合理设计拉曼信号分子的结构,大大提高信号的强度进而提升检测性能。Li 等^[65]将具有拉曼信号的 5,5-二硫代双(2-硝基苯甲酸)标记金纳米颗粒,通过在 LFIA 中采集该探针的拉曼信号以实现对生鲜奶中的粘菌素的检测。该方法对粘菌素的检测限为 0.10 ng/mL,远远低于用 ELISA 方法获得的报告值和欧盟规定的最大残留限量值。Su 等^[66]开发了以金纳米颗粒为核,金纳米星为壳,DTNB 位于两种材料中间,以最后形成的夹心结构作为拉曼信号的标签,用该方法检测食品中的克伦特罗,对其检测限为 0.05 ng/mL,检测限比传统的胶体金试纸条低 200 倍。同时金纳米星形成的壳结构阻止 DTNB 被解吸,进而使得信号更加稳定。Sheng 等^[67]合成银核金壳纳米材料并将拉曼信号分子 4-硝基噻吩(4-nitrothiophenol, 4-NTP)封装在核壳材料中间,最终形成的 Ag4-NTP@Au 作为拉曼信号标签用以检测百菌清(Chlorothalonil, CHL)、吡虫啉(imidacloprid, IMI)和乙氧氟草醚(oxyfluorfen, OXY)。

2.6 增强显色信号法

2.6.1 金属染色增强法 为了提升试纸条的显色信号,研究者试图通过银增强、金增强、铜增强及铂增强方法提升信号的强度。银增强法采用对苯二酚和银盐溶液(硝酸银或乳酸银)作为增强试剂,银离子在金纳米颗粒上被还原,颗粒尺寸因还原银层的形成而

增大,同时形成的银层是黑色的,在 NC 膜的白色背景上更为明显^[68]。金增强法采用氯金酸(HAuCl₄)和盐酸羟胺(NH₂OH·HCl)通过金纳米颗粒催化 HAuCl₄和 NH₂OH·HCl 形成更大的金纳米颗粒进而使得信号颜色更强^[69-70]。铜增强法则利用抗坏血酸(ascorbic acid, AA)和硫酸铜(CuSO₄),利用还原剂抗坏血酸可以将 Cu²⁺还原为 Cu⁺,然后在金纳米颗粒存在的情况下 Cu⁺转化为铜,铜沉积于检测线上也使得信号强度增强^[71]。铂增强法先使用硝酸银、对苯二酚使得在试纸条上形成金核银壳纳米材料(Au@AgNPs),紧接着将氯铂酸(H₂PtCl₆)和 AA 加入反应体系,进而形成 Au@AgPtNPs 实现信号放大的目的。但是上述方法操作繁琐,延长了检测时间^[72]。为了解决上述方法的弊端,Yosita 等^[73]通过喷蜡打印法在试纸条上设置延迟通道和非延迟通道(图 7),免疫试剂置于非延迟通道,增强试剂(KAuCl₄和 NH₂OH·HCl)置于设置有蜡障碍的延迟通道,层析过程中增强试剂的流动速度慢于免疫试剂的流速,试纸条可通过金增强方法的原理一步实现信号增强的目的。

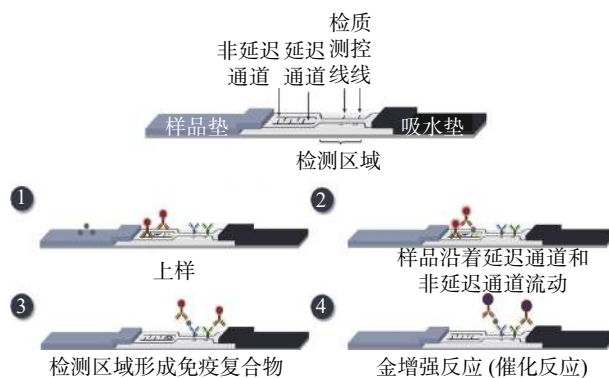


图 7 带有非延迟通道和延迟通道的侧流免疫层析技术^[73]

Fig.7 LFIA with non-delay channel and delay channel^[73]

2.6.2 酶催化染色增强法 生物酶具有高效的底物催化活性,进而会产生明显的肉眼可见的显色。利用这一特性,研究者试图在试纸条的纳米材料上同时标记单克隆抗体和辣根过氧化物,在常规读取结果完成后,把试纸条放入含有底物的溶液中,酶催化底物产生更深的显色信号,该方法使得检测灵敏度提高一个数量级^[74]。而近年来纳米模拟酶由于其较高的稳定性,较好的底物催化活性也越来越引起研究者的关注。Liu 等^[25]采用磁性普鲁士蓝纳米模拟酶作为试纸条的标记材料,同时其可催化显色底物 TMB 显色使得信号强度增强,进而提升试纸条的检测灵敏度,该方法使得检测范围提高两倍。

2.6.3 双标记增强信号法 为了提升试纸条的检测信号强度,研究者通过双标记方法使得纳米材料聚集在一起进而使得显色信号增强。Fang 等^[75]利用生物素-链霉亲和素(streptavidin, Sa)之间的高亲和性,将金纳米颗粒标记生物素化的抗体和 Sa,通过生物素-Sa 系统实现金纳米颗粒的聚集,用该方法检测吡

虫林,检测灵敏度比传统试纸条提高 160 倍。Zhong 等^[76] 通过将纳米金标记抗三聚氰胺的抗体以及抗牛血清白蛋白抗体,由于在试纸条的制备过程中会用到牛血清白蛋白封闭非特异性的结合位点,进而通过牛血清白蛋白抗原抗体系统实现纳米颗粒的聚集。该方法使得检测灵敏度提高 10~25 倍。Dou 等^[77] 将纳米金标记 mAb 和 GAMA,通过抗体和二抗结合系统实现纳米标记材料的聚集,进而起到增强检测信号的目的,该方法和传统的试纸条相比检测限提高了 5 倍。

2.6.4 增加通道障碍增强信号法 影响 LFIA 检测限和灵敏度的一个关键因素是样品、免疫试剂和预先固定在 T 线的捕获抗体/抗原之间的反应时间。近年来研究者通过降低液体在试纸条上的流动速率以增加该反应时间^[78-79]。Ioannis 等^[80] 采用激光直写技术(laser-direct write, LDW)在 NC 膜的特定区域固定液态的光聚合物,在光聚合作用下其可于 NC 膜内形成一个较窄的流体通道(图 8a),液体流动通道空间收缩使得液体流速变慢,检测区域变小,最终使得检测灵敏度提高 62 倍。Amadeo 等^[81] 在 T 线位置后的 1 mm 处设置可溶性蜡障碍,使得液体溶液短暂停留 12 min(图 8b),进而延长了免疫试剂在试纸条上的反应时间,最终使得检测灵敏度提高了 51.7 倍。

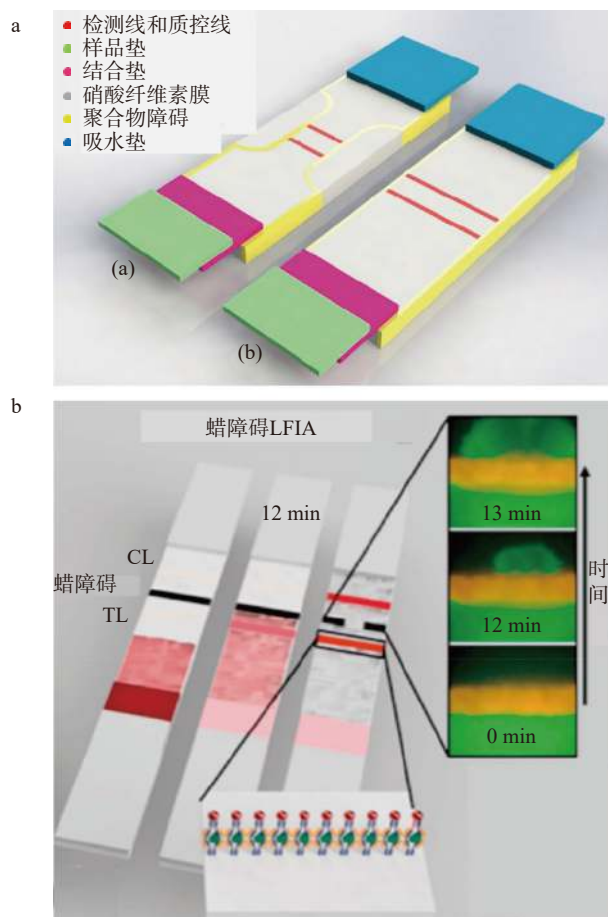


图 8 带有通道障碍的侧流免疫层析技术^[80-81]

Fig.8 LFIA with channel barrier^[80-81]

3 结语

LFIA 经过多年的不断完善和发展,为食品安全检测提供了一个方便、快捷和灵敏的技术平台。该技术已经相对比较成熟,对部分分析物的检测已实现商品化应用。目前面临的主要挑战及发展趋势简述如下:基于纳米标记材料的检测试纸存在材料合成步骤繁琐、条件较为严苛、耗时耗能、稳定性及单分散性较差的缺陷,开发性能优良的信号标记材料仍然是未来发展的一大方向;近年来大部分试纸仍然利用 mAb 来实现对目标分析物的特异性识别,但是 mAb 制备过程耗时较长,需要耗费大量的人力、物力和财力,同时不同批次间存在差异。开发可替代 mAb 的特异性识别探针诸如核酸适配体、噬菌体及其细胞内容素等对 LFIA 的发展具有重要的意义;检测灵敏度必然是考查试纸条性能优良与否的重要参数,传统的信号增强方法依赖额外的操作步骤进而使得试纸条的检测时间延长,开发便捷、省时的一步信号增强方法对提高试纸条的检测性能具有重要的意义;对多元目标物质的同时检测虽然提高了检测的效率,但是存在互相干扰的问题使得检测灵敏度相对较低,因此也依赖于高特异性识别探针技术的发展。

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