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Colloidal Gold Immunochromatography Strip Technique and Its Application in Clinical Diagnosis

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Abstract

Colloidal gold immunochromatography strip (CGIS) is a new immunoassay technique in clinic. It combines the visualization of colloidal gold technique and the specificity of immunochromatography. It has been extensively applied in clinic, laboratory and family with its excellent features at present. It has good prospects for development especially in infectious diseases and autoimmune diseases. This paper will introduce and summarize the development of CGIS technique in the disease diagnosis. Research and development of CGIS will be in the direction of more sensitive specificity and multiplexed detection in the future.

Keywords

Colloidal, Colloidal Gold Immunochromatography Strip, Disease Diagnosis

胶体金免疫层析试纸条在临床诊断中的研究进展

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摘　要

胶体金免疫层析试纸条技术(colloidal gold immunochromatography strip, CGIS)作为近年来临床上兴

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起的新的免疫检验技术，结合了胶体金检测的可视化和免疫层析检测特异性强等特点，因其操作简单迅速、成本较低等特点，适合于临床、实验室、现场及家庭的快速诊断，是目前应用广泛的检验方法，在感染性疾病、自身免疫性疾病等疾病诊断中具有良好的发展前景。本文对胶体金免疫层析试纸条检测技术在疾病诊断中的研究进展进行总结归纳，未来免疫胶体金层析试纸条将向更灵敏特异、多联检测方向进行研究和发展。

关键词

胶体金，免疫层析试纸条技术，疾病诊断

1. 引言

胶体金免疫层析试纸条检测技术基于胶体金作为示踪标志物，利用抗原抗体特异性反应，最早是由Faulk 和 Taylor 在 1971 年创立并用于免疫电镜技术[1]。胶体金颗粒具有高电子密度的特性，故在金标蛋白的抗原抗体结合处，显微镜下可见黑褐色颗粒，当这些标记物在相应的标记处大量聚集时，可在载体膜上呈现红色或粉红色斑点，从而用于抗原或抗体物质的半定量或定性。因其操作快捷简便、价格低廉、灵敏度高等优势而广泛运用于病原体检测等方面，本文将简要介绍胶体金免疫层析试纸条的制备及其在临床诊断中的应用进展，并对其存在的问题及发展前景进行分析，为该技术的深入研究及应用奠定一定的基础。

2. 胶体金免疫层析试纸条的制备及研究进展

2.1. 胶体金免疫层析试纸条的制备

胶体金(colloidal gold)也称金溶胶(goldsol)，是由氯金酸被还原成金原子后形成的金悬浮颗粒，在溶液中金颗粒呈圆形，边缘平整，界限十分清楚。金颗粒表面有大量的负电荷，由于静电的排斥力，使其在水中保持稳定状态，形成稳定的胶体，所以称为胶体金。胶体金在弱碱环境下带负电荷，可与蛋白质分子的正电荷基团形成牢固的结合，由于这种结合是静电结合，不影响蛋白质的生物特性[2] [3] [4]。制备方法多采用柠檬酸盐还原法，主要原料是氯金酸，胶体金一般通过柠檬酸三钠还原法制备，通过改变体系中氯金酸与还原剂的比例可得到所需不同直径的金颗粒。胶体金颗粒大小一般多在 1~100 nm [5]，微小的胶体金颗粒稳定均匀的呈单一分散状态悬浮在液体中，从而形成胶体金溶液。用于免疫测定时胶体金多与免疫活性物质抗原或抗体结合，这类胶体金结合物成为免疫金复合物或免疫金，胶体金标记蛋白质的原理，一般认为是由于 pH 值等于或稍偏碱于蛋白质等电点时，蛋白质呈电中性，此时蛋白质分子与胶体金颗粒相互间的静电作用较小，但蛋白质分子的表面张力却最大，处于一种微弱的水化状态，较易吸附于金颗粒的表面，由于蛋白质分子牢固地结合在金颗粒的表面，形成一个蛋白层，阻止了胶体金颗粒的相互接触，而使胶体金处于稳定状态，如果低于蛋白质的等电点时，蛋白质带正电荷，胶体金带负电荷，二者极易静电结合形成大的聚合物。如果 pH 高于蛋白质等电点时，蛋白质带负电荷，与金颗粒的负电荷相互排斥而不能互相结合。溶液 pH 值、胶体金颗粒的大小、抗体的浓度等是影响胶体金吸附的主要因素。试纸条从下到上依次由样品垫、胶体金垫、NC 膜和吸水滤纸等组成，NC 膜上标记有

检测线和指控线，对样本进行检测时，指控线显色指示试纸条有效，检测线根据实际情况呈现红色或无色，以显示阳性或阴性的结果[4] [6]。试纸条可以检测的样品为血液、尿液等，使用简便快速，便于基层和家庭使用；成本较低，不需要特殊的仪器设备及人员操作；可同时进行多项检测，节省样品；本身带有颜色结果可直接肉眼观察。

2.2. 胶体金免疫层析试纸条在临床疾病诊断中的研究进展

2.2.1. 在病原体检测中的研究进展

近年来，越来越多的免疫胶体金检测试纸条应用在传染病病原体中的检测，最早出现的是 A 群链球菌及衣原体的检测[7]，Zhang 等制备了检测血清中旋毛虫抗体的免疫胶体金试纸条[8]，Nardo 等制备的针对食物中的黄曲霉素的免疫胶体金检测试纸条，灵敏度和特异性都非常好[9]，Song 等制备出针对肺炎支原体的检测试纸条[10]，Mdluli 等制备出可以半定量检测人血液中结核分枝杆菌的试纸条，实现了结合分枝杆菌的半定量检测[11]，阪崎肠杆菌可诱发新生儿和婴儿严重的脑膜炎和败血症，死亡率很高，Blakova 等建立了检测的胶体金免疫层析试纸条，大大节省了检测时间[12]。

2.2.2. 在病毒检测中的研究进展

Reid 等制备了检测口蹄疫病毒[13]，Ma 等制备了用于检测 HIV-1 p24 抗原的免疫胶体金试纸条，为 HIV 感染的早期诊断提供了有效的方法[14]，Yang 等利用金标蛋白抗原或多肽建立了检测 O 型 FMDV 病毒抗体的胶体金试纸条检测方法[15] [16]，Cheng 等以抗原拦截的模式建立了检测 H5 和 H6 亚型禽流感抗体的胶体金试纸条[17]，Kong 等制备出可以快速检测流感嗜血杆菌的检测试纸条[18]，乙型肝炎病毒(hepatitis B virus, HBV)的免疫胶体金检测方法，与传统的酶联免疫吸附法相比操作简便迅速，Xiang 等利用双抗体夹心法建立了检测丙肝病毒(hepatitis C virus, HCV)的试纸条[19]，都可作为临床上筛查的重要手段。Sakurai 等利用荧光微球标记单抗的方法建立了检测季节性流感的试纸条[20] [21]。

2.2.3. 在激素检测中的研究进展

最早出现的抗原检测试纸条是针对人绒毛膜促性腺素(human chorionic gonadotropin, HCG)的检测[22]，目前在早早孕试纸条在医院和家庭中应用极为普遍。血液中 17α-羟孕酮浓度增高提示先天性肾上腺皮质增生症，Tripathi 等利用竞争法制备胶体金免疫层析试纸条检测血清中 17α-羟孕酮，灵敏度可以达到 2.5 ug/L [23]。

2.2.4. 在肿瘤标志物检测中的研究进展

肝癌诊断中，Yang 等制备的胶体金试纸条可以检测到 1 ng/ml 的 AFP，且标本用量少，检测时间快，10 min 即可读取结果，且假阳性率和假阴性率较低[24]。Wu 等利用双抗体夹心法制备了检测前列腺特意抗原(prostate-specific antigen, PSA)的胶体金试纸条，可作为检测前列腺癌病情变化和疗效观察的有效工具[25]，Wang 等建立了定量多联检测多种肿瘤标志物的试纸条[26]，Chen 等利用双抗体夹心法检测肿瘤标志物糖类抗原 72-4 [27]。

2.2.5. 在其它检测方面中的研究进展

人心肌肌钙蛋白 T (cTnT)是诊断急性心肌梗塞的高灵敏、高特异的血清学诊断指标，其免疫胶体金检测试纸条在国内外心血管疾病领域得到广泛应用[28]。此外还有大便隐血测试卡、血清铁质测试条、免疫球蛋白血清测试条等，在临床诊断中发挥着重要作用。

3. 展望

随着医院检验技术的迅速发展和不断更新，免疫胶体金检测技术是目前发展较快的临床诊断技术，

由于制备简便，方法敏感、特异，不需使用放射性同位素，或潜在致癌物质的酶显色底物，更能适应现代高效快速的节奏和满足疾病的诊断要求。根据胶体金的一些物理性状，如高电子密度、颗粒大小、形状及颜色反应，加上结合物的免疫和生物学特性，因而使胶体金广泛地应用于免疫学、组织学、病理学和细胞生物学等领域。免疫胶体金试纸条检测技术存在假阳性与假阴性的问题，检测灵敏度不高，检测限需要进一步发展，胶体金制备过程对无硅及清洁有较高要求等限制其广泛应用。需要寻找更多的方法使其监测范围更广一些，比如新型纳米复合材料等，将有助于改善检测限和灵敏度受限的问题，未来免疫胶体金检测试纸条技术将向更灵敏、定量、多联检测方向发展，用于疾病的诊断，相信免疫胶体金检测技术未来必定会受到越来越广泛的应用。

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