doi: 10.13241/j.cnki.pmb.2019.03.038

·技术与方法·

乙型肝炎病毒核心抗体 IgM 的蛋白芯片检测法研究 *

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摘要 目的:探索蛋白芯片技术检测乙型肝炎病毒(HBV)抗 HBc-IgM 的可行性。**方法:**应用 Nano-Plotter TM- 压电式微量喷墨点阵制备系统自制的核心抗原蛋白芯片在经 10%山羊血清封闭后加入待测血清 37℃ 孵育,PBST 清洗晾干后后再次加入检测抗体即 HRP- 抗人 IgM 抗体,芯片用伯乐成像仪检测有无信号。**结果:**24 份 HBsAg 和抗 HBc 阳性血清中经蛋白芯片检测抗 HBc-IgM 阳性率为 83.3%(20/24);24 份健康志愿者血清中经蛋白芯片检测抗 HBc-IgM 阳性率为 4.1%(1/24)。乙肝血清与健康志愿者血清化学发光信号差异明显。**结论:**蛋白芯片技术可较好地应用于定性检测 HBV 抗 HBc-IgM,为临床快速判断是否存在 HBV 感染和监测慢性肝炎 HBV 活动性提供新的辅助诊断方法。

关键词: 蛋白芯片;乙肝病毒;定性;抗 HBc-IgM

中图分类号:R446.6;R512.62 **文献标识码:**A **文章编号:**1673-6273(2019)03-560-03

Detection of Human Anti-hepatitis B Virus Core Antibody IgM by Protein Microarray*

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ABSTRACT Objective: To explore the feasibility of protein microarray for detecting human anti-hepatitis B virus core antibody IgM qualitatively. **Methods:** Protein microarray was fabricated by GeSiM Nano-PlotterTM Micropipetting System. The core antigen protein spotted on the slides with BSA spotted as control protein. All slides were blocked in a coupling buffer (10% normal goat serum with 0.1%NaN3). Then serum samples were added on the slides and incubated for 2h at 37℃ . After rinsed by PBST, the slides were added with HRP-anti-human IgM antibody and then scanned by a chemiluminescent scanner, Chemi DocTM MP System (Bio-Rad, California, USA). **Results:** The positive rate of anti-HBc IgM was 83.3% (20/24) in 24 HBsAg and anti-HBc positive serum samples detected by protein microarray, while the positive rate of anti-HBc IgM was 4.1% (1/24) in 24 healthy volunteers. The chemiluminescent signal difference was significant between hepatitis B positive and healthy serum samples. **Conclusions:** Protein microarray technology could be applied to detect anti-HBc IgM qualitatively, and provide a new auxiliary diagnostic method for clinical rapid determination of HBV infection and monitoring HBV chronic hepatitis activity.

Key words: Protein Microarray; Hepatitis B virus; Qualitative; Anti-HBc IgM

Chinese Library Classification (CLC): R446.6; R512.62 Document code: A

Article ID:1673-6273(2019)03-560-03

前言

病毒性肝炎是我国较为常见的传染病之一,以乙型肝炎病毒(HBV)感染最为常见[1,2]。病毒性肝炎是引起肝硬化及原发性肝癌最为常见的病因[3-5],对患者生命健康产生重大影响,因此如何快速、有效地检测出是否存在乙肝病毒感染是非常重要的临床问题。在乙型肝炎病毒感染早期,患者体内即可产生抗乙肝病毒核心抗原(HBc)抗体,该抗体主要包括 IgG 型和 IgM 型。IgG 型抗体分子量相对较小、维持时间较长但出现较晚,而 IgM 型抗体分子量大、维持时间较短但出现较早[6]。因此,抗 HBc 检测对近期是否存在 HBV 感染和慢性感染是否活动具有较大的诊断价值,其中又以抗 HBc-IgM 的早期诊断价值最高[7]。

* 基金项目:北京市科技委员会基金项目(Z181100001018031)

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(收稿日期:2018-05-22 接受日期:2018-06-18)

目前已有的乙肝抗 HBc-IgM 的检测方法包括胶体金斑点渗漏法、酶联免疫吸附法、电化学发光法、放射免疫法(RIA)、化学发光酶免疫分析法等[8],这些方法均存在一定程度的限制性。蛋白芯片技术是基于蛋白质与蛋白质之间相互作用,将蛋白质或抗体预先固定于固相支持物上,继而对样品中的蛋白进行高通量检测的技术[9,10],具有简单、方便、耗时少、高通量等优势。本研究旨在探索蛋白芯片法用于定量检测抗 HBc IgM 型抗体的可行性,结果报道如下。

1 材料与方法

1.1 研究对象

选取首都医科大学附属北京佑安医院北京乙型肝炎数据样本资源库中 HBsAg 与 HBc 抗体阳性血清 24 份和健康志愿者血清 24 份。

1.2 试剂和仪器

Nano-Plotter TM- 压电式微量喷墨点阵制备系统（德国 GESIM 公司），醛基芯片（上海百傲公司），伯乐成像仪(BIO-RAD CHEMIDOC MP imaging system 美国 BIO 公司)；HRP 发光底物(德国 Merck Millipore 公司),HBc 抗原(洛阳佰泰科生物技术公司),HRP 标记 - 羊抗人 IgM 抗体(洛阳佰泰科生物技术公司),10%牛血清白蛋白(美国 sigma),山羊血清(美国 sigma)。

1.3 抗 HBc-IgM 定量检测蛋白芯片的制备

用 PBS 溶液将 HBc 抗原稀释成 1 mg/mL,用 Nano-Plotter TM- 压电式微量喷墨点阵制备系统分别将 HBc 抗原和牛血清白蛋白点样至醛基玻片上,依次重复点样 3 个,点样过程如图 1 所示,点样后 4℃ 过夜,用 10%山羊血清封闭 2h,0.05%PBST 洗涤 3 次,室温干燥,4℃ 保存备用。

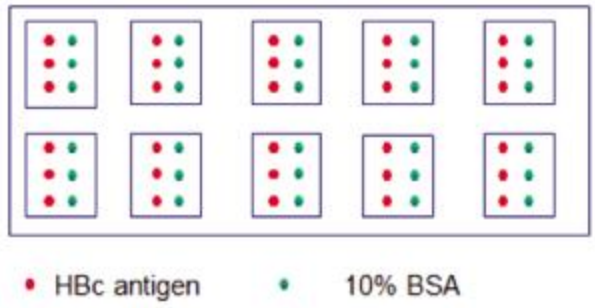

图 1 点样示意图

Fig. 1 Spotted diagram of protein microarray

1.4 检测方法

取备用的蛋白芯片至室温 5 min 平衡后加入待检测血清 15 μL/ 格,37℃ 孵育 25 min,用 0.05%PBST 清洗 3 次去除非特异性结合后加入 0.05%PBST 稀释后的 HRP- 抗人 IgM 抗体(稀释比例 1:2000)15 μL/ 格,再次 37℃ 孵育 25 min,用 0.05% PBST 清洗 3 次，晾干后加入发光底物鲁米诺 15 μL/ 格,2 min 后用伯乐成像仪检测有无信号，具体检测过程如图 2 所示,每个芯片至少设置一格空白对照组(仅不加待测血清)和一格阴性对照组(加正常血清),故每个芯片最多能检测 8 份血清。

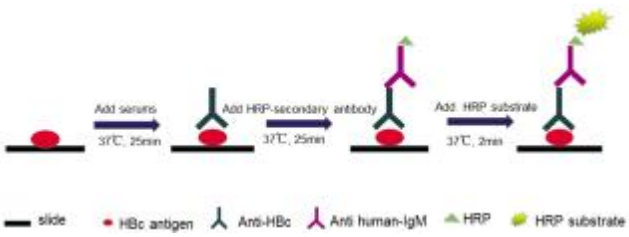

图 2 检测流程

Fig. 2 Procedure of protein microarray detecting anti-HBc IgM

2 结果

24 份乙肝 HBsAg 阳性和抗 HBc 阳性血清中，有 20 份血清经蛋白芯片检测抗 HBc-IgM 阳性,阳性率为 83.3%(20/24)；24 份健康志愿者血清中经蛋白芯片检测抗 HBc-IgM 有 3 份阳性,阳性率为 4.1%(1/24)。乙肝血清与健康志愿者血清化学发光信号差异明显,如图 3 所示,图中第 1、2、3、4 格分别代表同一份抗 HBc 阳性血清，第 6、7、8、9 格分别代表同一份健康志愿者血清,第 5、10 格代表空白对照组。不同乙肝阳性血清中化学发光信号亦存在差异,如图 4 所示,图中第 1、2、3、4、6、7、8、9 格分别代表不同抗 HBc 阳性血清，第 5、10 个分别代表空白对照和阴性对照组。

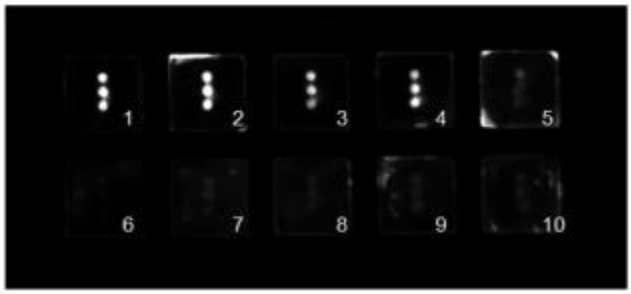

图 3 乙肝阳性血清与正常血清检测比较:1-4 抗 HBc IgM 阳性血清,6-9 正常血清,5、10 空白对照

Fig. 3 Comparison of grey intensity in hepatitis B positive serum and normal serum: 1-4wells: hepatitis B positive serums, 6-9wells: normal healthy serums, 5 and 10wells: blank control.

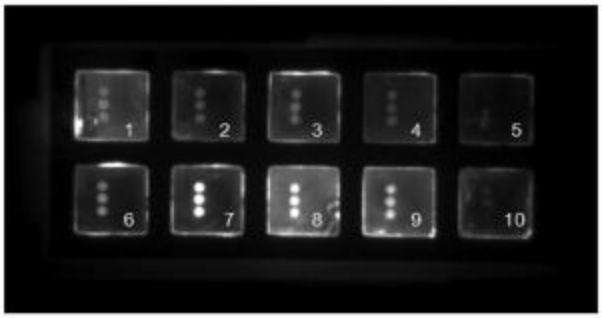

图 4 不同乙肝阳性血清检测比较:1-4 和 6-9 为乙肝阳性血清，5 和 10 为空白对照和阴性对照

Fig. 4 Comparison of detection results in different hepatitis B positive serum: 1-4wells and 6-9wells: hepatitis B positive serums, 5 and 10wells: blank control and negative control.

3 讨论

HBV 是一种嗜肝双链 DNA 病毒，呈颗粒状，基因组长约 3.2kb，整个基因组共有四个开放阅读框（ORF），分为 S、X、C 和 P 四个基因区[2,11]。S 基因区包含 S 基因和前 S 基因，编码 S 蛋白和前 S 蛋白，S 蛋白是 HBV 的包膜蛋白[12,13]，形成乙肝表面抗原 HBsAg。HBsAg 是刺激机体免疫反应形成保护抗体抗 HBs 的重要免疫原。X 基因区编码 X 蛋白，其与 HBV 的复制与致癌有关，尤其是在我国乙肝感染人群众多，慢性乙型肝炎是导致肝硬化的重要原因，继而发展原发性肝癌[14]。C 基因区编码两个蛋白，即 HBeAg 和 HBcAg，也是刺激机体免疫反应产生抗 HBe 和抗 HBc，其中抗 HBc-IgM 是机体针对 HBV 感染最早产生的抗体，与 HBV 近期感染或慢性乙肝活动期的重要标志，是乙肝病毒血清标志物的重要指标，P 基因区与 HBV DNA 多聚酶有关。

HBsAg、抗 HBs、HBeAg、抗 HBe 和抗 HBc 共称为 " 乙肝五项 " 或 " 乙肝两对半 "[15,16]，是目前乙肝病毒临床诊断和筛查的重要检测项目，在我国应用极为广泛，常用的检测方法包括电化学发光法、胶体金法、酶联免疫吸附法等[8,17,18]，检测灵敏度和特异度较好。但抗 HBc 独特性抗体即抗 HBc-IgM 尚未应用于临床大规模检测和健康筛查。鉴于抗 HBc-IgM 是 HBV 感染早期诊断的主要指标，具有重要的潜在临床价值[7]，因此如何快速与高效地检测抗 HBc-IgM 是当前面临的重要问题。

目前报道的抗 HBc-IgM 检测方法主要包括酶联免疫吸附法、免疫放射法、化学免疫分析方法、斑点渗漏法等[17-19]，但由于酶联免疫吸附法存在较多的干扰因素，如血清含有类风湿因子、加样时间差、过程复杂等诸多局限性，放射免疫法存在射线防护风险，化学免疫分析法过程复杂、耗时长、设备昂贵等缺点。蛋白芯片技术基于蛋白与蛋白之间相互作用的原理，可以大致分为抗体蛋白芯片、功能蛋白芯片、反向蛋白芯片、核酸可编程蛋白芯片、无标记蛋白芯片等[20,21]。目前蛋白芯片技术已发展成为一种成熟的蛋白质检测方法，广泛应用于细胞因子检测、抗体检测、糖蛋白检测，具有多重标记、高通量、高密度、微型化、微量样本和试剂检测等的独特优势[22-24]。在 HBV 血清学标志物检测方面，有报道称蛋白芯片技术可以有效检测 HBsAg，但尚未报道蛋白芯片技术应用于其他乙肝血清学标志物检测。

本研究结果显示，蛋白芯片技术对检测抗 HBc-IgM 效果较好，对于区分正常血清和乙肝阳性血清特异性较好，因此其可能成为一种有很好的抗 HBc-IgM 定性检测方法，为临床快速判断是否存在 HBV 感染和监测慢性肝炎 HBV 活动性提供新的辅助诊断方法。

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