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硕 士 学 位 论 文

全血 CD4+T 淋巴细胞快速定量检测
方法的建立

Establishment of Point-of-Care Counting Method for CD4+
T Lymphocytes in Whole Blood Sample

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摘要

艾滋病是 HIV 病毒引起的一种危害人生命健康的传染病。从 1981 年发现至今, 世界范围内已经有近 4000 万人感染 HIV, 每年有约 120 万人死于艾滋相关疾病。目前最为有效的艾滋治疗手段是抗逆转录治疗, 当感染者体内 CD4+ T 淋巴细胞数量在 200-350 个/mm³ 时, 是世界卫生组织推荐的艾滋病最佳治疗时机, 同时治疗过程中 CD4+T 淋巴细胞数量也是判断病程的重要指标。因此迫切需要发展快速准确的 CD4+T 细胞计数方法, 以支撑艾滋病疫情的防控。CD4+T 细胞计数金标准是流式细胞计数法, 但由于其价格昂贵、操作难度大, 一般只有省市级疾病预防控制中心或中心医院才配套有相应设施。而单就我国而言, HIV 感染比例较高的地区主要集中在云南、河南、广西、新疆等经济条件相对落后的地区, 且乡村的感染率要高于城镇。本研究主要建立现场快速的 CD4+ T 细胞检测方法, 以满足对偏远地区 HIV 感染者 CD4+T 细胞数量的检测监控。

实验通过介质捕获与荧光免疫层析技术相结合建立了全血 CD4 荧光免疫层析定量检测方法, 利用捕获柱对全血进行预处理, 排除红细胞对免疫层析检测带来的干扰, 配合荧光仪对试纸条 T 线、C 线荧光强度进行定量分析, 即可在 30 min 内完成全血 CD4+T 细胞计数。与流式细胞计数结果做对比, CD4 荧光免疫层析定量检测方法可检测全血 CD4+T 细胞个数在 50-1500 个/微升的线性范围, R² 为 0.84, 有较好的相关性。同时为整合实验流程, 省去除加样外的人工操作, 我们与北京化工大学合作开发了一台小型自动化仪器检测, 目前已完成第一台样机的制作, 未来会对仪器性能作进一步评估。但全血 CD4 荧光免疫层析定量检测方法需要对全血进行预处理, 增加了操作的复杂度, 且相应自动化仪器的开发难度也较高。因此我们又在微流控平台上进行了相关尝试, 希望可以将全血预处理和检测两部分整合, 一次性完成全血 CD4+T 细胞定量检测。

全血的微流控 CD4+T 细胞定量检测芯片平台是在微流控芯片内、配合离心机和荧光检测仪, 完成全血中 CD4+T 细胞计数。芯片采用易切割有机高分子聚合物 PMMA 和 PC 作为主体材料, 利用激光切割和胶键合方式完成芯片制造和组装。CD4 定量检测芯片是通过密度梯度差方法分离红细胞与白细胞后, 将目的细胞转移到反应腔室, 再通过免洗区域的设计完成免疫反应后洗涤, 仪器检测结果。目前该芯片已完成初步验证, 此方法可实现全血的 CD4+T 细胞计数。收

集全血样本与商品化 CD4 ELISA 试剂盒检测结果比对,当全血样本 CD4+T 细胞个数在 500-1200 个/微升时, R^2 为 0.54, 检测结果有一定趋势。之后还会对该平台做进一步优化, 并完成仪器的整合、设计。

本研究建立的两个 CD4+T 细胞定量检测平台, CD4 荧光免疫层析检测平台已基本完成, 并进入自动化仪器开发阶段; 微流控芯片平台目前完成了芯片设计验证, 下一步工作主要是优化检测体系并开发相应的自动化设备。这两个平台均是适用于乡镇等一些偏远落后地区, 操作简单、耗时短、不需昂贵仪器、可现场检测, 对 HIV 检测及抗逆转录治疗监控都具有重要意义。

关键词: CD4+T 淋巴细胞; 荧光免疫层析; 微流控芯片

Abstract

AIDS (Acquired Immune Deficiency Syndrome) is a kind of infectious disease caused by HIV (Human Immunodeficiency Virus). Since HIV was discovered in 1981, nearly 40 million people have been infected with HIV in the worldwide, 1.2 million people die each year from AIDS-related diseases. At present, the most effective means of AIDS treatment is highly active antiretroviral therapy (HAART). According to World Health Organization (WHO), while the number of CD4⁺ T lymphocytes is 200-350 per micro liter, recommended the best time for treatment of AIDS. The number of cells is also an important indicator to evaluate the therapeutic results. So developing rapid and accurate CD4⁺ T cell counting methods is urgently needed. CD4⁺ T lymphocytes counting gold standard is the flow cytometry (FCM). However because of its high cost, the operation is difficult, only developed cities and large hospital could have capability of cell counting by FCM. In China, the higher proportion of HIV infection is mainly concentrated in the areas of Yunnan, Henan, Guangxi, Xinjiang, where the economic conditions are not developed. In this study, we established a rapid CD4⁺ T cell detection method to monitor the number of CD4⁺ T cells for backward areas.

In the experiment, the whole blood CD4 fluorescent immune chromatographic test was established. The whole blood is pretreated by the capture column, and the influence caused by erythrocyte in the test is excluded. Then instrument detects results of the strip. The whole blood CD4⁺ T cells counting can be completed within 30 min. Compared with the result of flow cytometry, the fluorescent strip can detect the number of whole blood CD4⁺ T cells in the range of 50-1500 cells / μ l, R^2 is 0.84, have a good correlation. At the same time, we also cooperate with Beijing University of Chemical Technology, designing an automatic analyzer which integrates all experimental steps to finish the CD4⁺ T cells counting by instrument. Now we have completed the first prototype. However, the CD4 fluorescent immune chromatographic test method requires pre-treatment of whole blood sample,

increasing the complexity of the operation, and the development of the corresponding automation equipment is more difficult. So we try to establish another method on the microfluidic chip platform, hoping to integrate the two parts of whole blood pretreatment and result detection to complete the whole blood CD4⁺ T cell quantitative detection.

Quantitative detection chip of CD4⁺ T cells in the whole blood is on the basis of the microfluidic chip, with the centrifuge and detection equipment to complete the whole blood CD4⁺ T cells counting. The chip is made of PMMA and PC, with laser cutting and glue bonding. Chip separates erythrocytes from whole blood by density gradient centrifugation. Then the target cells transferred to the reaction chamber, and through the no-washing area to complete the immune detection. At present, the chip design has completed and the feasibility of the method has been verified. Compared with the CD4 ELISA kits, R^2 is 0.54 when the number of CD4⁺ T cells in whole blood was 500-1200 cells / μ l. We will continue to optimize the testing conditions and complete the integration of the instrument in the future.

In this study, we established two CD4⁺ T cell counting methods. One of the whole blood CD4 fluorescent immune chromatographic test platform has been completed basically and entered into the stage of designing the automatic analyzer. The other method has been verified the feasibility of testing. In the next step, we will continue to optimize the testing conditions and complete the integration of the instrument. These two platforms are all suitable for remote areas, they have the merits of simple operation, time-consuming, without expensive equipment, on-site testing. It is of great significance for HIV testing and monitoring of antiretroviral therapy.

Key words: CD4 + T lymphocyte, fluorescent immune chromatography, microfluidic chip

缩略词 (Abbreviation)

缩略词	英文全称	中文全称
AIDS	Acquired Immune Deficiency Syndrome	获得性免疫缺陷综合征
BSA	Bovine Serum Albumin	牛血清白蛋白
CB	Carbon Acid Buffer	碳酸缓冲液
CD	Cluster of Differentiation	白细胞分化抗原
CV	Coefficient of Variation	变异系数
DC	Dendritic Cell	树突状细胞
DMSO	Dimethyl Sulfoxide	二甲亚砜
DNA	Deoxyribonucleic Acid	脱氧核糖核酸
EDTA	ethylenediaminetetraacetic acid	乙二胺四乙酸
ELISA	Enzyme-Linked ImmunoSorbant Assay	酶联免疫吸附测定
FCM	Flow Cytometry	流式细胞仪分析
FITC	Fluorescein isothiocyanate isomer I	异硫氰酸荧光素
GAM	Goat Anti-Mouse	山羊抗小鼠
HA	Hemagglutinin	凝血素
HAART	Highly Active AntiretroViral Therapy	高活性的抗逆转录病毒 治疗
HIV	Human Immunodeficiency Virus	人免疫缺陷病毒
HRP	Horseradish Peroxidase	辣根过氧化物酶
IgG	Immunoglobulin G	IgG 抗体
IVD	In Vitro Diagnosis	体外诊断
LFIA	Lateral Flow Immunoassay	侧向流免疫测定
mAb	Monoclonal Antibody	单克隆抗体
mAb	Monoclonal Antibody	单克隆抗体
MES	2-(N-Morpholino)ethanesulfonic acid hydrate	2- (N-吗啉代) 乙磺酸
MHC	major histocompatibility complex	主要组织相容性复合体

缩略词

NBS	Newborn Bovine Serum	新生牛血清
NHS	N-Hydroxysuccinimide	N-羧基琥珀酰亚胺
NK	Natural Killer Cell	自然杀伤细胞
PBMC	Peripheral Blood Mononuclear Cells	外周血单核细胞
PBS	Phosphate Buffered Saline	磷酸盐缓冲液
PCR	Polymerase Chain Reaction	聚合酶链式反应
PEG	Polyethylene Glycol	聚乙二醇
PH	hydrogen ion concentration	氢离子浓度指数
POCT	Point-of-Care Testing	即时检测
PVC	polyvinylchloride	聚氯乙烯
PVP	Polyvinyl Pyrrolidone	聚乙烯吡咯烷酮
SA	Streptavidin	链霉亲和素
Tc	Cytotoxic T cell	细胞毒性 T 淋巴细胞
TCR	T Cell Receptor	T 细胞受体
Th	Helper T cell	辅助性 T 细胞
TMB	tetramethyl benzidine	四甲基联苯胺
WHO	World Health Organization	世界卫生组织

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