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Visual DNA microarrays for simultaneous detection of *human immunodeficiency virus type-1* and *Treponema pallidum* coupled with multiplex asymmetric polymerase chain reaction

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Abstract

Based on gold label silver stain and coupled with multiplex asymmetric polymerase chain reaction (PCR) analysis, we developed the visual DNA microarray for simultaneous, sensitive, and specific detection of *human immunodeficiency virus type-1 (HIV-1)* and *Treponema pallidum*. The 5′-end amino-modified oligonucleotides were immobilized on glass surface, which were used as the capturing probes to bind the complement biotinylated target DNA. The gold-conjugated streptavidins were introduced to the microarray for specific binding to biotin. The black image of microarray spots, which were the result from the precipitation of silver onto nanogold particles and bound to streptavidins, was visualized and accounted as the detection of biotinylated target DNA. Multiplex asymmetric PCR products of *HIV-1* and *T. pallidum* and *Bacillus subtilis* (used as positive control) were performed for preparing the abundant biotinylated single-stranded target DNA of which could affect detection efficiency and sensitivity of hybridization on microarray. One hundred sixty-nine clinical samples of *HIV-1* and *T. pallidum* from infected patients were tested using the homemade DNA microarrays. The results were identical to those shown in the assays of ELISA and fluorescence quantitative real-time PCR. Our results demonstrate that we have developed the visual gene detection technique, which is of high sensitivity and specificity; it may have potential in clinical applications.

Keywords: *HIV-1*; *T. pallidum*; DNA microarrays; Gold nanoparticles; Gold-conjugated streptavidins; Gold label silver stain; Multiplex asymmetric PCR

1. Introduction

Human immunodeficiency virus type-1 (HIV-1) and *Treponema pallidum* are the major causes of sexually transmitted diseases. Since 2001, the Centers for Disease Control and Prevention (2002, 2003) of the United States has reported that the syphilis had been keeping rising trend specifically among people, and the increase rate of primary and secondary syphilis has reached to 9.1%. At the same time, people with syphilis were diagnosed carrying a high risk of *HIV-1* infection. Therefore, it is necessary to develop an efficient method for the early diagnosis of *HIV-1* and *T. pallidum*.

Current detection methods for *HIV-1* include assays of immune complex dissociation (ICD assay), immunosorbent electron microscopy, and ultrasensitive enzyme immunoassay (EIA). Several assay systems have been established for the detection of *T. pallidum*-infected serum or clinical samples. Among them, *T. pallidum* hemagglutination, indirect immunofluorescence (FTA-Abs), EIA, and Western blotting technique have been used as confirmatory *Treponema* assay (Sato et al., 2004). Polymerase chain reaction (PCR), which can amplify specific DNA sequences, has also been applied to the diagnosis of *HIV-1* and *T. pallidum* (Liu et al., 2001; Zammatteo et al., 1995). However, a series of individual PCRs have to be run in parallel or sequential for the routine analysis of blood cultures that may contain 1 or multiple pathogens. The multiple individual PCRs would increase the expense and complexity for the assay.

The DNA microarray or gene chip represents the main breakthrough in molecular analysis through miniaturization

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Table 1
The primer sequences for the *HIV-1, T. pallidum*, and *B. subtilis* detection

Locus	Primer	Size (nt)	Sequence (5′→3′)
HIV-1	I-F	19	5′-AGAAGAGAAGGCTTTCAGC-3′ (868–887)
	I-R	20	5′-**T**TGGTCCTTGTCTTATGTCC-3′ (1184–1204)
T. pallidum	T-F	19	5′-TAAGCAGCATGGAGAGCCC-3′ (707–725)
	T-R	19	5′-**T**GAAATCCGCAGAGAGGAC-3′ (1252–1270)
B. subtilis[a]	B-F	20	5′-GGGAAAAACTGAAGGTGATT-3′ (713–732)
	B-R	20	5′-**A**GACTTGAGCCGTTATGGAT-3′ (868–887)

The bold letter was modified with biotin at its 5′-end. F = forward primer; R = reverse primer.
[a] Positive control.

of the assay and the capability to permit monitoring a large number of genes simultaneously (Brown and Botsein, 1999). It allows the high-throughput detection for multiple pathogens in a large number of samples. The conventional fluorescence detection method of DNA microarray relies on the expensive confocal scanner, which restricts its application in the research field and makes it unsuitable for the reading in a wide clinical and daily based practice (Bowtelld, 1999). Tremendous efforts have been devoted toward development of new labeling and detecting methodologies that can provide sensitive and low-cost detection of nucleic acids (Kwok, 2001; Lockhart and Winzeler, 2000). Taton et al. (2000) reported a novel method for DNA detection based on 2-probe sandwich hybridization/nanoparticle amplification coloring technique, which showed a new colorimetric strategy to detect genes with high sensitivity. The limitation of this method was that each applied nanogold particles must be prepared with the specific probes. Alexandre et al. (2001) used gold-conjugated streptavidins as universal detection probes to replace the nanogold-supported DNA detection probes; the result proved that the sensitivity of colorimetric detection using silver precipitation was comparable with that of detection in fluorescence using a confocal scanner. The preparation of abundant biotinylated single-stranded target DNA is the critical factor that could affect the detection efficiency and sensitivity of target DNA hybridization in microarray assay. When the 5′-end amino-modified capture probes could hybridize with biotinylated target strands denatured from conventional PCR products, the complementary strands would compete with the capture probes, which result in the decrease of the hybridization efficiency. By using the multiplex asymmetric PCR, plenty of biotinylated single-stranded target DNA could be prepared, which can greatly enhance the efficiency and sensitivity of hybridization and detection (Cao et al., 2006).

In this work, we developed a sensitive, visual, and simultaneous method to detect the virus of *HIV-1* and *T. pallidum*. It is composed of DNA microarray technology, GLSS assay, and multiplex asymmetric PCR technique.

2. Materials and methods

2.1. Design of primers and probes

To confirm the reliability of DNA amplification and gene chip detection, we chose *Bacillus subtilis* proB gene as the positive control and *Chlamydia trachomatis* trp gene as the negative control. Conserved DNA sequences of gag gene for *HIV-1*, 47k Ag for *T. pallidum*, proB for *B. subtilis*, and trp gene for *C. trachomatis* were obtained from GenBank by alignment. Primers and probes of this study were designed and selected by Primer Premier 5.0 (Premier Biosoft International, San Fransisco, CA). Except for primers for detecting *HIV-1*, they were prepared as it was described by Lin (1995). All primers and probes were synthesized and modified by Huanuo Biological Science and Technology (Shanghai, China). The reverse primers were modified with biotin at their 5′-ends and the probes with H_2N-(CH_2)6-*O*-(PO_3)-$(T)_{10}$ at their 5′-end. Ten T bases were inserted into the probes to avoid the steric hindrance affecting the immobilization on the chips and hybridization of the probes with complementary target DNA (Cao et al., 2006; Wang et al., 2005). The sequences of the primers and probes are listed in Tables 1 and 2. The lengths of DNA fragments obtained by PCR for *HIV-1, T. pallidum*, and *B. subtilis* were 392, 564, and 175 bp, respectively.

Table 2
The probe sequences for the *HIV-1, T. pallidum, B. subtilis*, and *C. trachomatis* detection

Locus	Probe	Size (nt)	Sequence (5′→3′)
HIV-1	I-P	26	5′-**G**ATACCCATGTTTTCAGCATTATCAG-3′
T. pallidum	T-P	26	5′-**C**GACCTTGTGGTAGACACGGTGGGTA-3′
B. subtili[a]	B-P	24	5′-**G**CACATATATCGGTGATAAAGAGC-3′
C. trachomatis[b]	C-P	21	5′-**C**CACCGATGAAGAGGCGTTAC-3′

The bold letter was modified with H_2N-(CH_2)6-*O*-(PO_3)-$(T)_{10}$ at its 5′-end.
[a] Positive control.
[b] Negative control.