A comparison of oligonucleotide-based microarray and real-time PCR for the detection of sexually transmitted infections

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Abstract  Many diagnostic methods for sexually transmitted infections (STIs) have been developed. Because various infection agents are associated with STIs, and because infected persons sometimes show no symptoms, the diagnosis of STIs using nucleic acid amplification tests (NAATs) has required not only simultaneous multi-targeting, but also sensitive detection. Here, we compare microarray and real-time PCR for the detection of three common STIs agents, Ureaplasma urealyticum, Mycoplasma genitalium, and Chlamydia trachomatis, using human urine samples. The detection results showed that microarray and real-time PCR technology are both effective tools for the detection of STI agents. In conclusion, real-time PCR detection offers more sensitivity and specificity than microarray, because of the quantitative method employed. But, microarray offers better performance, in terms of high-throughput and simultaneous multi-targeting.

Keywords: STIs, Real-time PCR, Microarray, DNA chip, Molecular diagnostics

Introduction

Sexually Transmitted Infections (STIs) are primarily infection by sexual contact. There are about 30 infection agents, including bacteria, viruses and parasites. STIs cause serial health problems, and increase susceptibility to other infections. Sometimes, men and women with STIs don’t even know they are infected, because they show no symptoms¹. For these reasons, sensitive diagnostic methods are applied to the control of infection. Also, because various infection agents cause infection, the diagnosis of STIs must meet simultaneous multi-targeting.

Recently, various diagnostic methods based on molecular biology, such as conventional PCR², Line probe assay³⁴, sequencing⁴, real-time PCR⁵, and microarray⁶, have been developed, and clinical validations have been accomplished. The conventional PCR method is a simple and fast diagnostic technique, and can multiplex. But the analysis of amplification must be accompanied by an electrophoresis step, and this additional experimental procedure causes contamination by aerosol of the amplicon⁷. A line probe assay can be simultaneously analyzed for various targets, but as for PCR, is vulnerable to contamination. After PCR reaction of the target gene, the sequencing of amplicon can be analyzed at a high resolution level, and allows automation of the experimental procedure. However, sequencing methods have the disadvantage of a longer turnaround time (TAT) than other methods⁸. Real-time PCR methods are the closed-tube system, which method can exclude contamination by an aerosol of amplicon, and quantitative analysis based on the Cₜ (cycle threshold) for the de-
Microarray technology can print many probes onto a substrate, such as a glass slide. High-density probes onto a microarray can simultaneously detect a large number of targets. This feature is ideal for STIs agents, because various pathogens are related to STIs, and often there is co-infection.

Here, we compared microarray and real-time PCR for detecting STIs, using human urine samples. Purified DNA samples from urines were applied to two commercial STI detection NAAT kits. Detection results showed that both microarray and real-time PCR methods were more effective tools than the established previous methods, such as conventional PCR, line probe assay, and sequencing. In conclusion, real-time PCR detection offers more sensitivity and specificity than microarray, because of the quantitative method employed. But, in terms of high-throughput and simultaneous multi-targeting, microarray offers better performance.

Results and Discussion

In order to compare the two NAATs, a total of 96 urine samples were applied, to detect for STIs. In the present study, fourteen positive samples of *U. urealyticum* were applied. One positive sample of *M. genitalium* and 2 positive samples of *C. trachomatis* were verified respectively. In the DNA extraction process, we employed an automated extraction system to minimize contamination, and then the same quantity of extracted DNA samples were applied to detect STIs using a
microarray and real-time PCR. Prior to experiment, we confirmed the performance of each diagnostic kit using the positive materials provided. In the case of microarray, *U. urealyticum*, *U. parvum*, *M. genitalium*, and *C. trachomatis* DNA materials were applied (Figure 1). The performance of the real-time PCR kit was checked by cloned DNA that included the respective target regions of STI agents. Also, internal positive controls (IPC) and partial cloned DNA of a tobacco mosaic virus were added to confirm the performance of reaction composition, and gave valid results in all reactions (Figure 4). A total of 14 urine samples represented *U. urealyticum* positive results in microarray and the real-PCR reaction (Figures 2 and 3). However, one sample (UU05) showed a close to cut-off value (of signal intensity 500), and is possibly a false negative result (Figure 3). When based on the CT value in real-time reaction, the UU5 sample was confirmed as a low positive result. In the case of *M. genitalium* and *C. trachomatis*, two NAATs were confirmed with the same positive results, and all the rest of the samples were confirmed to be negative.

During 2000, about 18.9 million people in the United States were infected by STI agents\(^5\). Various pathogens, such as bacterial, virus and parasite, were related to STIs. For early diagnosis of these infection agents,
many diagnostics, including culture\textsuperscript{11}, immunoassay\textsuperscript{12}, and NAATs\textsuperscript{13}, have been developed and applied. In this study, we compared microarray and real-time PCR techniques using urine specimens. Three common pathogens, \textit{U. urealyticum}, \textit{M. genitalium}, and \textit{C. trachomatis} were applied for comparison with two NAATs.

\textit{U. urealyticum} is part of normal flora in the genital tract, and a recognized cause of urethritis. \textit{U. urealyticum} includes two biovars and several serotypes, and because of commensal microbes, it is difficult to determine the pathogenic role of other \textit{U. urealyticum} species\textsuperscript{14}. To solve this difficulty, various target genes, 16s rRNA, 16S-23S rRNA, urease, and multiple-banded antigen (MBA), have been applied to detection\textsuperscript{15}. \textit{M. genitalium} has the smallest genome of about 580 kb, and is a recognized cause of urethritis, cervicitis, and lymphogranuloma venereum (LGV). Because of this obligate intracellular feature, an analysis method that uses culture is not a proper way to detect infection.

The microarray technique is ideal for a high throughput detection procedure, because specific probes onto substrate are physically distant from each other, and hybridization is then simultaneously accomplished in the same hybridization space\textsuperscript{17}. Also, an automated printing process can fabricate high-density microarrays, and hundreds of thousands of targets are possible. STIs often cause co-infection, and have no symptom. Therefore, an accurate and multi-targeted detection method is needed. Microarray has many advantages in this respect. First, as various pathogens are related to STIs, many probes can exactly identify one or more infectious agents. Second, in the case of co-infection, a microarray offers economic diagnosis, because only one reaction is needed for various infectious agents. Although only three STIs were accomplished in this

\begin{figure}[h]
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\caption{Hybridization plots of STIs microarray (A) \textit{U. urealyticum}, (B) \textit{M. genitalium}, and (C) \textit{C. trachomatis} positive urine samples. The Y axis represents fluorescent signal intensity.}
\end{figure}
study, an applied microarray can detect ten infectious agents, including *Nesseria gonohorreae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Haemophilus ducreyi, Candida albicans, Ureaplasma parvum, U. urealyticum, Mycoplasma hominis*, and *M. genitalium*.

The real-time PCR method offers quantitative analysis and a visual reaction process. Also, real-time PCR can be used to measure the degree of infection. Analysis based on $C_T$ in the experiment can detect simply and rapidly, using computer programs and a closed-tube system to minimize the risk of carryover contamination from the amplicon. Because the primer has been designed considering the amplification efficiency, a real-time PCR method can detect low copy infection. This advantage is ideal for the early diagnosis of STIs.

Conclusions

In this study, we compared DNA microarray and real-time PCR methods for the detection of STIs. Automated nucleic acid extraction system was used to minimize deviation and contamination, and for decision of clinical usefulness, urine samples were applied to detect STIs. All samples, including positive and negative samples, were exactly matched between the microarray and real-time PCR. The two NAATs have many advantages, which include multi-targeting and quantitative analysis, over conventional diagnostic methods, such as culture, immunoassay, PCR, and sequencing. Because of quantitative analysis, real-time PCR detection offers more sensitivity and specificity than does microarray. But, in terms of high-throughput and simultaneous multi-targeting, the microarray offers the better performance.

Materials and Methods

DNA samples

We applied urine samples to detect STIs, including *Ureaplasma urealyticum, Mycoplasma genitalium*, and *Chlamydia trachomatis*. For target gene amplification, the extraction of DNA was prepared as follows. Urine samples of one milliliter were centrifuged for 5 min at 13,000 rpm. The supernatant was discarded, and cell pellets were then mixed, using 400 μL of 1X PBS buffer until even suspension. Prepared samples were purified using an Exiprep™ bacterial genomic DNA kit and the ExiPrep™ 16 Dx automated extraction system (Bioneer, Daejeon, Korea), according to the manufacturer’s instructions. The purified DNA was quantified using a Qubit® 2.0 fluorometer (Life technology, Carlsbad, CA), and stored at 4°C.
DNA amplification
For real-time PCR tests, we used Accupower® CT, UU, MG real-time PCR kit (Bioneer), and 5 μL of purified DNA were applied for amplification. To detect the three target infection agents, samples were amplified in an ExiCycler™ 96 real-time quantitative thermal block (Bioneer), and the thermal cycle was composed as follows: 5 min denaturation at 95°C, followed by 45 cycles of 95°C for 5 seconds, and 55°C for 5 seconds. For the microarray experiment, we used a Platinum Biochip STD microarray kit (Genocheck, Ansan, Korea), and the same DNA samples were applied. The PCR condition was run with 5 min denaturation at 95°C, followed by 35 cycles of 95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, using a DNA-EN-GENE thermocycler (MJ Research, Waltham, MA).

Real-time PCR analysis
Real-time PCR analysis was accomplished by computer program based on the Cₚ value. Every PCR was run with two positive controls that synthesized de novo and no template controls. Each PCR reaction was confirmed with internal positive controls that were designed from a Tobacco Mosaic virus movement protein gene. STIs were determined by the cut-off value based on Cₚ, and all analysis processes were automatically conducted with the computer program provided.

Microarray experiment
To hybridize the microarray and amplicon, prior to microarray hybridization, PCR products were treated with one unit of Lambda exo-nuclease (ELPIS biotech, Daejeon, Korea) for 15 min at 37°C. Treated amplicon was mixed with the provided 2X hybridization solution, and applied onto the microarray, to hybridize directly for 1 hour at 53°C. Then, the microarray was washed, according to the manufacturer’s instructions. Hybridization signals were scanned by Genepix 4000B (Molecular Devices, Foster City, CA), and analyzed using Genepix 4.1 software (Molecular Devices). In this step, normalization of the hybridization results was by subtraction of the local background median value from the median value of the probe signal, and decisions on the STIs were based on the cut-off value provided.

Highlights
► Sexually Transmitted Infections (STIs) are primarily infection by sexual contact, and there are about 30 infection agents, including bacteria, viruses and parasites.
► We compared microarray with real-time PCR for detecting three common STIs agents.
► Real-time PCR detection offers more sensitivity and specificity than does microarray.
► In terms of high-throughput and simultaneous multi-targeting, microarray offers better performance than does real-time PCR.

Abbreviations
STIs, Sexually Transmitted Infections; TAT, turnaround time; Cₚ, cycle threshold; NAAT, nucleic acid amplification test

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