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Original Research

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

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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 symptoms[1]. 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 PCR[2], Line probe assay[3,4], sequencing[4], real-time PCR[5], and microarray[6], 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 amplicon[7]. 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 methods[8]. 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 C_T (cycle threshold) for the de-

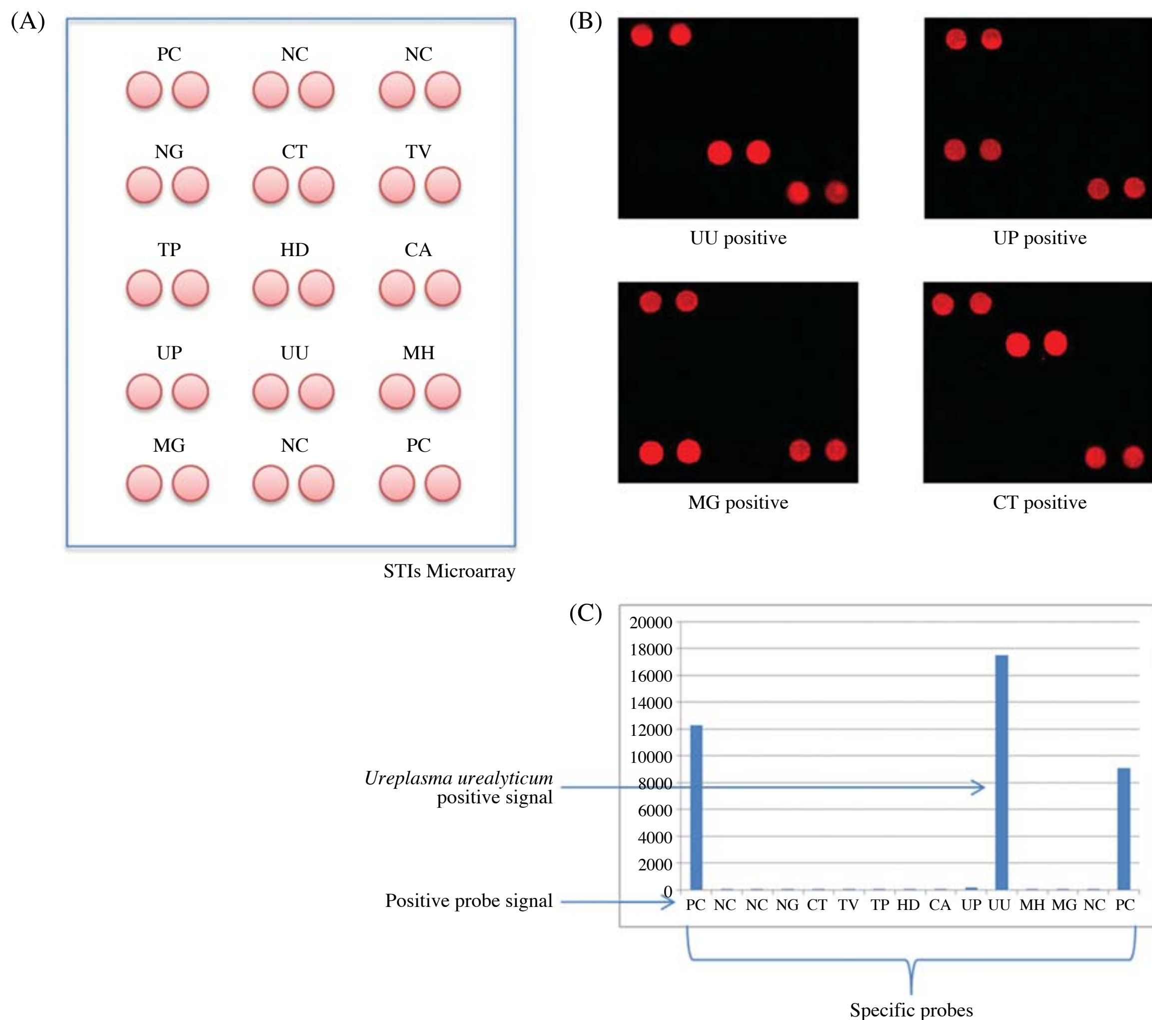

Figure 1. Microarray layout for detecting 10 STI pathogens and hybridization patterns. (A) Each probe was printed twice onto slide glass. (B) The hybridization patterns that were reacted with provided positive materials to check the performance of a microarray. (C) Signal intensity plot for the detection of *U. urealuticum* positive samples.

gree of infection[5]. 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[9]. 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

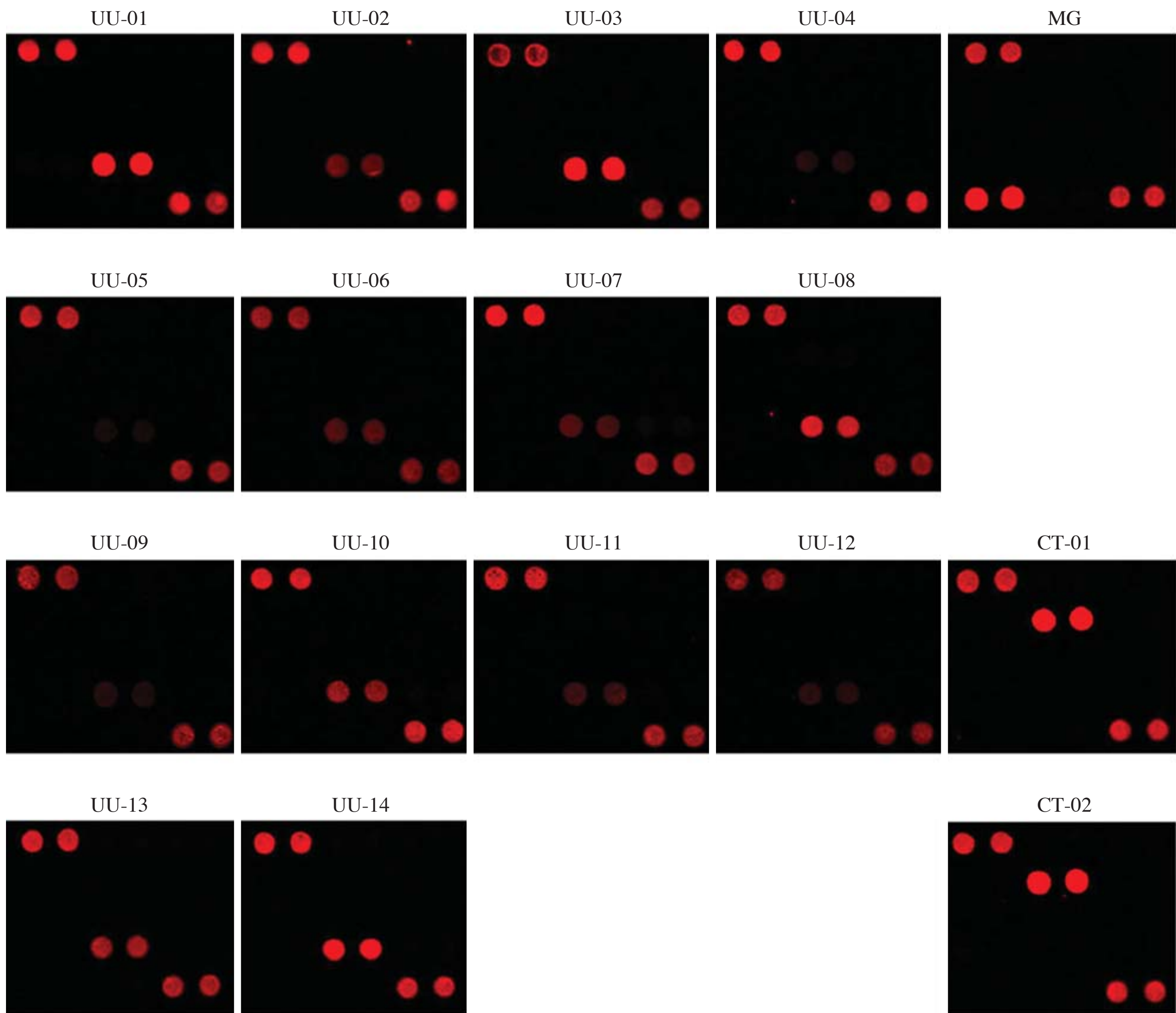

Figure 2. Hybridization patterns of *U. urealyticum* (UU), *M. genitalium* (MG), and *C. trachomatis* (CT) positive urine samples. To detect hybridization results, a 3′ end of the reverse primer for target gene amplification was labeled with CY3 fluorescent dye, and scanned using a Genepix 4000B microarray scanner.

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[10]. Various pathogens, such as bacterial, virus and parasite, were related to STIs. For early diagnosis of these infection agents,

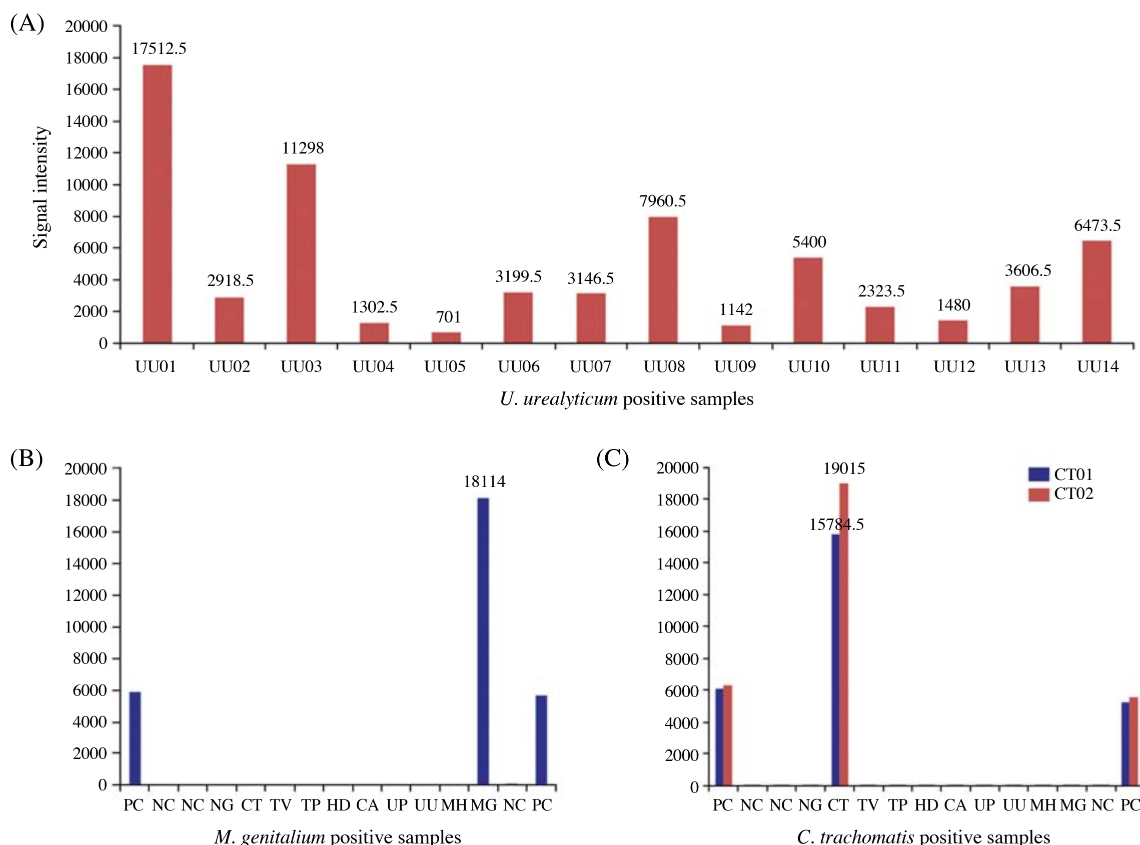

Figure 3. Hybridization plots of STIs microarray (A) *U. urealytium*, (B) *M. genitalium*, and (C) *C. trachomatis* positive urine samples. The Y axis represents fluorescent signal intensity.

many diagnostics, including culture[11], immunoassay[12], and NAATs[13], have been developed and applied. In this study, we compared microarray and real-time PCR techniques using urine specimens. Three common pathogens, *U. urealyticum*, *M. genotalium*, and *C. trachomatis* were applied for comparison with two NAATs.

U. urealyticum is part of normal flora in the genital tract, and a recognized cause of urethritis. *U. urealyticum* includes two biovars and several serotypes, and because of commensal microbes, it is difficult to determine the pathogenic role of other *U. urealyticum* species[14]. To solve this difficulty, various target genes, 16s rRNA, 16S-23S rRNA, urease, and multiple-banded antigen (MBA), have been applied to detection[15]. *M. genitalium* has the smallest genome of about 580 kb, and is a recognized cause of urethritis, cervictis and endometrictis. From previous findings, traditional detection methods, such as culture and serology, have sometimes failed[16]. Therefore, a more sensitive diagnostic procedure was developed, and its clinical usefulness verified. *C. trachomatis* is a gram-negative bacteria and obligate intracellular pathogen. *C. trachomatis* is a common bacterial STI, and about 150 million new infections occur every year. *C. trachomatis* is divided into three biovars, and each biovar has unique clinical features, such as trachoma, urethritis, conjunctivitis, 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[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

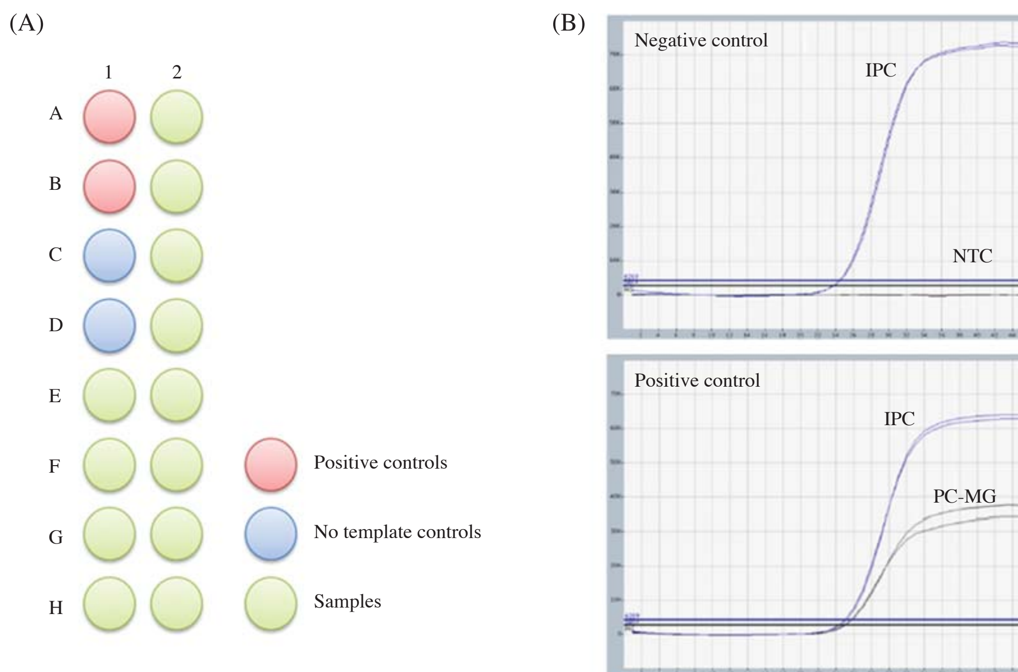

Figure 4. Real-time PCR experiment layout and amplification plots (A) Positive controls and no template controls were repeated twice, and each clinical sample was conducted one time. (B) To confirm the performance of the reaction composition, the partial movement gene of a tobacco mosaic virus was used as an internal positive control, and a cloned gene was used for positive control.

study, an applied microarray can detect ten infectious agents, including *Nesseria gonohorreae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Treponema pallidum*, *Haemophilus ducreyi*, *Candida albicans*, *Ureraplasma 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[18-20]. 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 tachomatis*. 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-ENGENE thermocycler (MJ Research, Waltham, MA).

Real-time PCR analysis

Real-time PCR analysis was accomplished by computer program based on the C_T 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 C_T, 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; C_T, cycle threshold; NAAT, nucleic acid amplification test

References

1. Takahashi, S. *et al*. Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. *J. Infect. Chemother*. **12**, 269-271 (2006).
2. Suntoke, T.R. *et al*. Evaluation of multiplex real-time PCR for detection of Haemophilus ducreyi, Treponema pallidum, herpes simplex virus type 1 and 2 in the diagnosis of genital ulcer disease in the Rakai District, Uganda. *Sex. Transm. Infect*. **85**, 97-101 (2009).
3. Safaeian, M. *et al*. Comparison of the SPF10-LiPA system to the Hybrid Capture 2 Assay for detection of carcinogenic human papillomavirus genotypes among 5,683 young women in Guanacaste, Costa Rica. *J. Clin. Microbiol*. **45**, 1447-1454 (2007).
4. Menton, J.F. *et al*. Molecular epidemiology of sexually transmitted human papillomavirus in a self referred group of women in Ireland. *Virol. J*. **6**, 112 (2009).
5. Jensen, J.S. *et al*. Use of TaqMan 5′ nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. *J. Clin. Microbiol*. **42**, 683-692 (2004).
6. Shi, G. *et al*. Fabrication and optimization of the multiplex PCR-based oligonucleotide microarray for detection of Neisseria gonorrhoeae, Chlamydia trachomatis and Ureaplasma urealyticum. *J. Microbiol. Methods* **62**, 245-256 (2005).
7. Cimino, G.D. *et al*. More false-positive problems. *Nature* **345**, 773-774 (1990).
8. De Leeneer, K. *et al*. Rapid and sensitive detection of BRCA1/2 mutations in a diagnostic setting: comparison of two high-resolution melting platforms. *Clin. Chem*. **54**, 982-989 (2008).
9. Kim, C.J. *et al*. HPV oligonucleotide microarray-based detection of HPV genotypes in cervical neoplastic lesions. *Gynecol. Oncol*. **89**, 210-217 (2003).
10. Weinstock, H., Berman S. & Cates, W.Jr. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. *Perspect. Sex. Reprod. Health* **36**, 6-10 (2004).
11. Swygard, H. *et al*. Targeted screening for Trichomonas vaginalis with culture using a two-step method in women presenting for STD evaluation. *Sex. Transm. Dis*. **31**, 659-664 (2004).

12. Almeda, J. *et al*. Evaluation of a commercial enzyme immunoassay for HIV screening in urine. *Eur. J. Clin. Microbiol. Infect. Dis*. **23**, 831-835 (2004).
13. Rogers, S.M. *et al*. NAAT-identified and self-reported gonorrhea and chlamydial infections: different at-risk population subgroups? *Sex. Transm. Dis*. **29**, 588-596 (2002).
14. Kong, F. *et al*. Comparative analysis and serovar-specific identification of multiple-banded antigen genes of Ureaplasma urealyticum biovar 1. *J. Clin. Microbiol*. **37**, 538-543 (1999).
15. Kong, F. *et al*. Phylogenetic analysis of *Ureaplasma urealyticum*-support for the establishment of a new species, *Ureaplasma parvum*. *Int. J. Syst. Bacteriol*. **49 Pt 4**, 1879-1889 (1999).
16. Jensen, J.S., Borre, M.B. & Dohn, B. Detection of Mycoplasma genitalium by PCR amplification of the 16S rRNA gene. *J. Clin. Microbiol*. **41**, 261-266 (2003).
17. Yoo, H.S. *et al*. DNA barcoding Korean birds. *Mol. Cells* **22**, 323-327 (2006).
18. Thorne, L.B. *et al*. Analytic validation of a quantitative real-time PCR assay to measure CMV viral load in whole blood. *Diagn. Mol. Pathol*. **16**, 73-80 (2007).
19. Morandi, L. *et al*. Monitoring HCV RNA viral load by locked nucleic acid molecular beacons real time PCR. *J. Virol. Methods* **140**, 148-154 (2007).
20. Keyaerts, E. *et al*. Viral load quantitation of SARS-coronavirus RNA using a one-step real-time RT-PCR. *Int. J. Infect. Dis*. **10**, 32-37 (2006).