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Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods

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SUMMARY

Objectives: The real-time PCR assay is the most sensitive test for screening and diagnosing sexually transmitted infections (STIs) and has made diagnosing these infections easier for clinicians. The aim of this study was to investigate the reliability, accuracy, and usefulness of the real-time multiplex PCR assay for the detection of seven sexually transmitted microorganisms in clinical samples.
Methods: A total of 897 specimens from 365 symptomatic patients and 532 asymptomatic volunteers were collected over a 10-month period. A total of 696 subjects provided 50 ml of first-voided urine as samples, and 201 female patients provided endocervical swab specimens. *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum*, and *Ureaplasma parvum* were tested for using five diagnostic methods: multiplex real-time PCR (Anyplex™ II), multiplex PCR (Seeplex®), strand displacement amplification (SDA, BD ProbeTec™ ET), PCR (AmpliSens®), and a commercially available Mycoplasma IST 2 Kit.
Results: Multiplex real-time PCR (Anyplex™ II) showed outstanding results in all fields, particularly sensitivity and specificity, compared with other diagnostic tools. This method yielded 100% sensitivity and high specificity for the detection of *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *M. genitalium*, and *M. hominis*. It was also useful for discriminating between *U. urealyticum* and *U. parvum*.
Conclusions: Multiplex real-time PCR was found to be an equivalent or superior modality for the diagnosis of STIs. It could be a cost-effective and rapid diagnostic tool for the simultaneous detection of multiple STI microorganisms.

1. Introduction

Sexually transmitted infections (STIs) are important individual, medical, social, and economic issues. The World Health Organization (WHO) estimates that 498.9 million new cases of syphilis, gonorrhea, chlamydia, and trichomoniasis occurred worldwide in 2008.[1] The reported prevalences of syphilis, gonorrhea, chlamydia, and trichomoniasis are 0.37%, 0.43%, 5.58%, and 2.4%, respectively, in the Republic of Korea.[2,3] It is important to increase vigilance and to recognize that STIs are often asymptomatic or cause nonspecific symptoms, that STIs can increase the infectiousness of HIV, and that regular testing for STIs is crucial.[4] Nucleic acid amplification tests (NAATs) are the most sensitive tests for STI screening and diagnosis. NAATs are more sensitive than the previously available diagnostic tests (e.g., culture, antigen detection, and nucleic acid hybridization) by approximately 20–30%.[5] NAATs provide an improvement in medical screening for STIs[6] because they can be used with noninvasively collected specimens such as first-voided urine samples and self- or clinician-collected vaginal swabs. More recently, the multiplex PCR assay has made it convenient for clinicians in many clinical fields to test for multiple causative organisms simultaneously. The multiplex PCR assay is a cost-effective diagnostic test because it allows for faster detection and a reduction in labor and reagent costs.[7–9]

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Real-time PCR comprises amplification and fluorescence detection of an amplified DNA target in the same step. Compared with conventional PCR, real-time PCR is cost-effective because it improves the detection sensitivity, decreases the amplification time, and simplifies downstream processing.[10] There are possible limitations in multiplexing. One possible limitation is PCR drift as a result of stochastic fluctuation in the interactions of PCR reagents, particularly in the early cycles, which could arise in the presence of very low template concentrations or through the assay design.[11] The reliability and accuracy of multiplex PCR for STIs have not been evaluated thoroughly. The aim of this study was to evaluate the performance and usefulness of real-time PCR assays for the detection of seven sexually transmitted microorganisms in clinical samples.

2. Patients and methods

2.1. Study population and clinical specimens

The study population consisted of two groups: symptomatic patients and asymptomatic volunteers. Three hundred sixty-five patients who were suspected of having an STI and who visited urology or gynecology hospitals in metropolitan areas were enrolled in the symptomatic patients group. Five hundred thirty-two volunteers who visited the Korean Industrial Health Association for a health examination and signed the informed consent form for this study were enrolled in the asymptomatic volunteers group. Over a period of 10 months, 897 specimens (from 365 symptomatic patients and 532 asymptomatic volunteers) were collected and tested. The specimens included 510 first-voided urine samples from men, 186 first-voided urine samples from women, and 201 endocervical swabs from women. The male participants and female volunteers were asked to provide 50 ml of first-voided urine (at least 2 h after previous urination) in a sterile 50-ml screw-cap plastic bottle. The endocervical swab specimens from female participants were collected by doctors, who followed the regular procedures for speculum examination and used manufactured collection kits. The specimens were immediately placed in a cooler and transported to the Central Diagnostic Laboratory in Seoul (Department of Laboratory Medicine, Chung-Ang University Hospital). The specimens were transported without added transport medium and were refrigerated and examined within 48 h.

2.2. Laboratory tests

Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, and *Ureaplasma parvum* were detected by four NAATs: multiplex real-time PCR (Anyplex™ II, Seegene, Seoul, Korea), multiplex PCR (Seeplex®, Seegene, Seoul, Korea), strand displacement amplification (SDA) (BD ProbeTec™ ET, Becton–Dickinson Microbiology System, Sparks, MD, USA), and PCR (AmpliSens®, PCR Kit, InterLabService Ltd, Moscow, Russia), and using a commercially available Mycoplasma IST 2 Kit (bioMérieux, Marcy l'Etoile, France) (Figure 1).

2.3. Nucleic acid amplification tests (NAATs)

2.3.1. Pretreatment of clinical specimens and DNA extraction

The swab specimens (approximately 2–3 ml) in the collection tubes were equilibrated to room temperature and mixed by

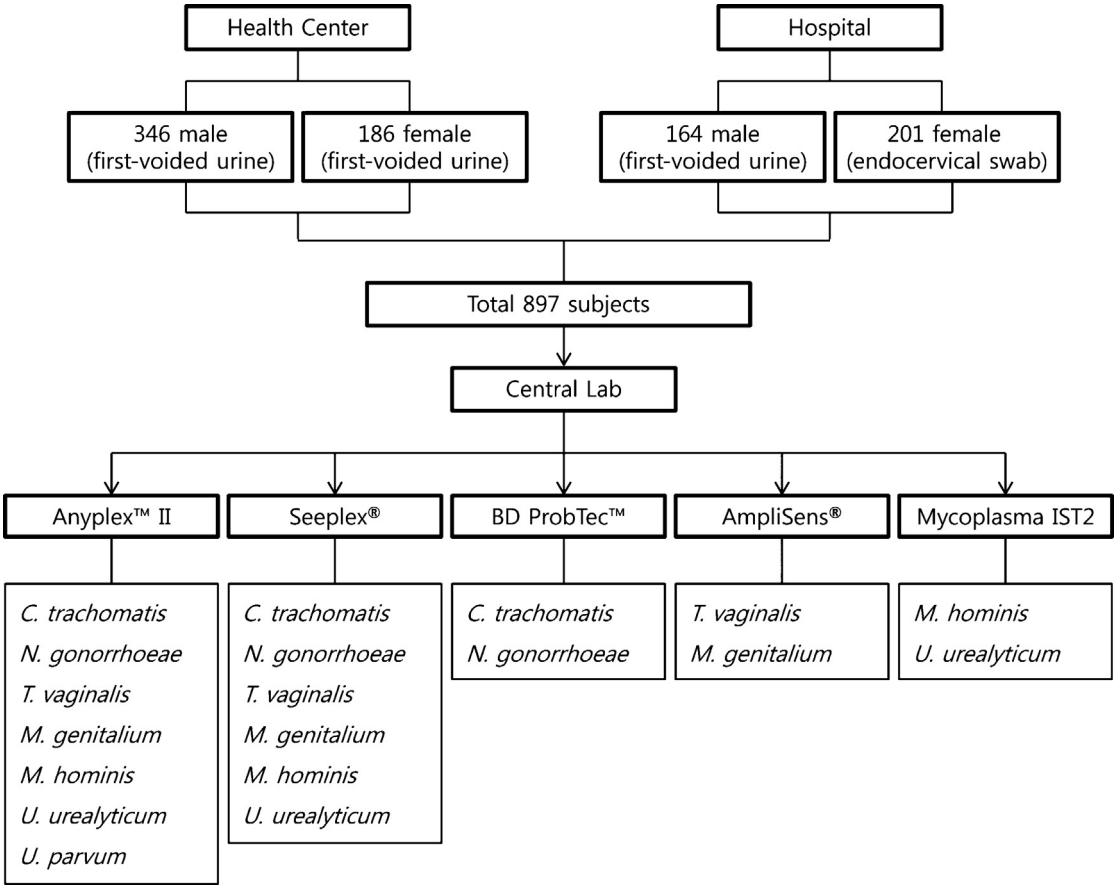

Figure 1. Flow chart showing the population and samples in the study.