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Bead-based multiplex sexually transmitted infection profiling

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Summary *Objectives:* Sexually transmitted infections are a significant cause of genital disease, infertility and hospital admissions. The economic impact is high. An accurate diagnosis is often difficult and time consuming. We report the development and validation of a novel bead-based multiplex sexually transmitted infection profiling (STIP) assay that detects 18 sexually transmitted infections using a multiplex PCR followed by Luminex bead-based hybridisation.
Methods: STIP was validated using urogenital samples pretested by commercially available quantitative PCR, microscopy or by culturing methods.
Results: STIP specifically detects *Chlamydia trachomatis*, Herpes simplex virus 1 and 2, *Treponema pallidum*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma (M.) genitalium*, *M. hominis*, *M. pneumonia*, *M. spermatophilum*, *Ureaplasma urealyticum* and *U. parvum*, and quantifies bacterial vaginosis-associated *Atopobium vaginae* and *Gardnerella vaginalis* as well as three *Candida* species and normal genital flora-associated *Lactobacillus* species. STIP reached an overall concordance of 95–100% with commercially available quantitative PCR tests. Compared to Nugent score, STIP reached a sensitivity of 95% and a specificity of 86% for bacterial vaginosis detection. *Candida* specimens, pretested by direct culturing, were identified with a sensitivity of 97% and a specificity of 99%.
Conclusions: STIP is a powerful high-throughput tool in assessing a broad spectrum of urogenital infections.
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Introduction

Sexually transmitted infections (STI) account for a variety of genital diseases (Table 1) and present a major health problem with an estimated 499 million new cases of curable cases annually worldwide.[1] According to the World Health Organisation (WHO), the incidence of gonorrhoea, trichomoniasis, syphilis and infections with *Chlamydia (C.) trachomatis* is increasing in many parts of the world.[1] In developing countries, these diseases rank among the top five entities for which adults seek health care. *Treponema pallidum (T. pallidum)*, *C. trachomatis* or *Neisseria (N.) gonorrhoeae* cause diseases in virtually all cases of infection (Table 1). Other disease-associated microorganisms, however, such as *Candida* species (vaginal candidiasis) or the bacterial vaginosis (BV)-causing *Gardnerella (G.) vaginalis* and *Atopobium (A.) vaginae* are found often in the normal genital flora.

Detection of STI by nucleic acid amplification tests, such as polymerase chain reaction (PCR) is considered to be more sensitive than conventional microscopical examination or time-consuming bacterial culturing methods. A large number of different PCR methods have been described for amplifying *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma (M.) genitalium*, *Gardnerella vaginalis*, *Atopobium vaginae* and *Candida* species in single- or multiplex formats followed by signal read-out methods, such as sequence analysis, gel electrophoresis or hybridisation with type-specific probes by different formats, such as membrane-based reverse line blot (RLB).[2–12] All of these assays have been shown to exhibit their specific advantages and disadvantages. However, the main disadvantage certainly remains that these assays usually detect only a single or a small group of STI. Thus, while being able to detect specific STI, they usually fail to assess numerous other STI simultaneously. Multiplex PCR followed by bead-based Luminex hybridisation appears to be an attractive approach to solve these limitations. Over the last years, we have developed several multiplex PCR followed by bead-based Luminex detection using specific probes to detect multiple agents simultaneously in a high-throughput fashion, e.g. for human papillomaviruses,[13–15] human polyomaviruses,[16] adeno-associated viruses,[17] cell culture contaminations[18] and single nucleotide polymorphism typing.[19] Despite of being an endpoint detection system, Luminex-based quantification of template DNA or RNA can be achieved by including a synthetic calibrator molecule into the amplification reaction.[20]

Here, we report the development and validation of a multiplex Sexually Transmitted Infection Profiling (STIP) assay that detects sensitively and specifically 18 STI and common urogenital microorganisms using a multiplex PCR followed by Luminex bead-based hybridisation. In addition, a DNA quality control was incorporated into the assay. STIP detects *C. trachomatis*, Herpes Simplex Virus (HSV) 1, HSV2, *M. genitalium*, *M. hominis*, *M. spermatophilum*, *M. pneumoniae*, *N. gonorrhoeae*, *T. pallidum*, *Trichomonas vaginalis (T. vaginalis)*, *Ureaplasma (U.) urealyticum* and *U.parvum*, and quantifies *Candida* (*C.*) *albicans*, *C. glabrata* and *C. krusei*, BV-associated *A. vaginae* and *G. vaginalis* and different *Lactobacillus* species of the normal genital flora.

Materials and methods

Clinical specimens

Liquid-based cervical cytology leftover (BD-SurePath) ($n = 20$), vaginal swabs (Multi-Collect; Abbott Molecular Inc.) ($n = 164$)and first void urine samples ($n = 28$), collected during routine gynecological health checks from women in Flanders (Belgium), were selected based on previous DNA positivity by TaqMan-based real-time quantitative PCR assays (qPCR) for *C. trachomatis*, HSV1/2, *M. hominis*, *N. gonorrhoeae*, and *T. vaginalis*, *A. vaginae*, and *U. urealyticum*.

Table 1 STIP target microorganisms, associated diseases and detection limits.

Microorganism	Disease	Detection limit (copies/PCR)
Atopobium vaginae	Bacterial vaginosis	10
Candida albicans, C. glabrata	Candidiasis	100
Chlamydia trachomatis	Pelvic inflammatory disease, infertility	10
Gardnerella vaginalis	Bacterial vaginosis	10
Herpes simplex virus 1	Herpes simplex	10
Herpes simplex virus 2	Herpes simplex	10
Lactobacillus iners	None	10
Lactobacillus crispaticus/jensenii	None	10
Mycoplasma genitalium	Pelvic inflammatory disease, infertility, cervicitis	10
Mycoplasma spermatophilum	Unclear	10
Mycoplasma pneumoniae	Unclear	10
Mycoplasma hominis	Bacterial vaginosis	10
Neisseria gonorrhoeae	Gonorrhea, pelvic inflammatory disease, infertility	10
Treponema pallidum	Congenital syphilis	10
Trichomonas vaginalis	Trichomoniasis, cervicitis, vaginitis	10
Ureaplasma urealyticum	Unclear	10
Ureaplasma parvum	Unclear	10

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