A novel rapid multiplex PCR assay for the detection of 13 bacterial and viral causes of sexually transmitted infection (STI).

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Abstract

It has been estimated that around 78-300 million new cases of genitourinary tract infections occur annually worldwide. Neisseria gonorrhoea and Chlamydia trachomatis are considered the most common causes of genital tract infections however other species such as Mycoplasma spp., Chlamydomonas vaginalis and Ureaplasma spp. are also agents of disease. Furthermore, novel markers have been identified that can improve the specificity for the detection of bacterial vaginosis (1, 2). We have developed a novel technology that has been clinically validated for the detection of HPV (3) and more recently multiplexed PCR panels for the detection of a wide range of Gastrointestinal pathogens (4). Our aims were to produce a rapid multiplex real time assay that could detect the presence of 13 causative agents of STI (see Table 1) in less than 3 hours from primary patient material. The multiplex PCR panels were designed to correspond to clinical symptom groups thus the requesting physician could select the most appropriate panel for the presenting symptoms.

Materials & Methods

Previously characterised CT/NG urine samples (1ml aliquots) and swab samples collected in COBAS sample preparation buffer (1ml) were spun at 13,000rpm for 5 minutes to pellet intact organisms and the pellets resuspended in 250µl of EasyScreen™ lysis buffer. The samples were heated at 95°C for 15 minutes and purified as below. Fifty microliters of purified DNA extracts from samples previously tested for HSV were also included in the study. The samples were heated for 15 minutes at 95°C and purified using the EZ21 (Qiagen, Hilden, Germany) or Kingfisher Flex (ThermoFisher, Waltham) according to the protocols supplied (Genetic Signatures, Sydney, Australia). Real time PCR analysis can be performed on most common hardware such as the LC480 (Roche, Pleasanton, USA). 7500 fast (Applied Biosystems, Foster City, USA), Rotorgene-Q (Qiagen, Hilden, Germany), the Smartcycler II (Cepheid, Sunnyvale, USA) and the CFX96 (Bio-Rad, Hercules, USA).

Results

The EasyScreen™ assays successfully detected all CT/NG samples from a commercially available validation panel (Zeptometrix). Using clinical samples 21/22 C. trachomatis positive samples were detected along with 22/24 N. gonorrhoea. It is expected that increasing the volume of urine tested would improve sensitivity to 100% while swab samples should be placed directly into GS sample buffer rather than into the COBAS reagent. All 20 HSV samples were detected using the assay. Interestingly HSV1/2 co-infections were not observed in 520 patients (25%). Most swab samples and a number of urine samples tested positive for the two markers of bacterial vaginitis thus further studies will be conducted to determine a suitable cut-off for reliable diagnosis of vaginitis. One CT positive sample was determined to be a LGV strain and one negative sample tested positive for the presence of HSV-1.

References