INTRODUCTION

Sexually transmitted infections (STIs) present a major public health concern worldwide with more than 1 million people acquiring an STI every day. Timely access to testing and treatment services can reduce the risk of onward transmission, however, many STIs are asymptomatic and may display similar or overlapping symptoms, thus co-infections may remain undiagnosed. As many as 35% of STI cases have been reported to have more than one STI concomitantly at the time of presentation.

The minimum number of tests recommended in the British Association for Sexual Health and HIV (BASHH) guidelines includes Chlamydia, gonorrhoea and syphilis (Treponema pallidum); symptomatic women may also be tested for Trichomonas vaginalis, which causes vaginitis and cervicitis in women and urethritis in men.

Trichomona infections are often asymptomatic and not tested for because the prevalence is assumed to be low. In addition, wet mount microscopy, the routine diagnostic method for women, is insensitive and therefore T. vaginalis infection remains under-diagnosed. New BASHH guidelines now recommend molecular approaches for T. vaginalis detection.

HSV testing is offered to both men and women in the presence of genital ulceration and tests for tropical genital ulcerative diseases such as chancre (Hemophilus ducreyi) are now advised according to patient history. Mycoplasmas and Ureaplasmas are not routinely tested for although M. hominis is now accepted as an STI, being implicated in urethritis and cervicitis, but the previous lack of recognition has lead to inappropriate treatments and a significant rise in antimicrobial resistance. U. urealyticum has also been associated with recurrent urethritis and, along with M. hominis and M. genitalium, with some cases of pelvic inflammatory disease (PID).

In this context, the need for efficient means of detecting these infections has become increasingly important. This study reports the evaluation of a multiplex array on a biochip platform for simultaneous detection of common STI pathogens from a single sample. This approach increases detection capacity, with the potential of identifying more asymptomatic infections and co-infections.

METHODOLOGY

DNA was extracted from residual urine or urogenital swab samples, obtained from an anonymous and blinded patient cohort (n=869), from the Regional Virology Laboratory (RVL, Belfast Health and Social Care Trust). Each DNA sample was simultaneously tested for the presence of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Herpes simplex 1 and 2 (HSV1, HSV2), Treponema pallidum (TP), Trichomonas vaginalis (TV), Hemophilus ducreyi (HD), Mycoplasma genitalium (MG), Mycoplasma hominis (MH) and Ureaplasma urealyticum (UU) using the STI Multiplex Array (EV3950, Randox Laboratories Limited, Crumlin, UK) on the Evidence Investigator analyser (EV3602, Randox Laboratories Limited, Crumlin, UK). The protocol involves amplification of DNA using highly sensitive primers, followed by spatial separation and detection using biochip array technology. Assay results were compared against infection detection at the RVL, using their routine procedures. Discrepant samples were re-tested.

The STI Multiplex Array detects 10 STIs in one sample

STI Multiplex Array Protocol

Sample status | Total | Infection rate (%) (relative to total samples) | Infection rate (%) (relative to positive samples)
--- | --- | --- | ---
Negative results | 575 | 66 | -
Positive results | 297 | 34 | 72
Single infections | 122 | 22 | 77
Double infections | 52 | 18 | 3
Triple infections | 10 | 7 | 3
Total infections | 62 | 7 | 21

RESULTS

Agreement with predicate assay (qPCR) was >94%. Analytical sensitivity was 100% and specificity ranged from 94% to 100% for all key targets (Table 1). Results reported for each infectious agent in Table 1 show data where both the STI Multiplex Array and at least one predicate test was performed. Discrepant results were confirmed by uniplex real-time PCR or another commercial assay.

Table 1. Sensitivity and Specificity data for the STI Multiplex Array

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>True Positive</th>
<th>False Positive</th>
<th>True Negative</th>
<th>False Negative</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Concordance (%)</th>
<th>Total tests</th>
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<td>100</td>
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<td>100</td>
<td>94</td>
<td>94</td>
<td>355</td>
</tr>
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</table>

Table 2. Infection and co-infection rates observed using the STI Multiplex Array

An example of sample report is shown (Table 3)

Table 3. Example of sample report

CONCLUSION

The data indicate that the STI Multiplex Array can accurately and simultaneously detect 10 of the most common STI pathogens from a single sample without compromising sensitivity or specificity. This has important implications in sexual health screening, particularly in asymptomatic or individualistic individuals, where identification of unexpected infection is possible. Furthermore, it facilitates the identification of co-infections, which may otherwise have been missed. This leads to increased diagnostic capabilities, which may allow tailored treatment, reducing broad spectrum antibiotic use and, in turn, the build-up of antibiotic resistance.

REFERENCES