Rapid, Sensitive and Automated Detection of 12 Bacterial and Viral Causes of Sexually Transmitted Infections with 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection Kit

Wendy Y Chen[1], Damien J Stark[2], John R Melki[1] and Douglas S Millar[1]

[1] Genetic Signatures, Level 9, Lowy Packer Building, 405 Liverpool Street, Darlinghurst 2010, [2] Microbiology Department, St. Vincent's Hospital, Darlinghurst, NSW 2010

Introduction

Sexually Transmitted Infections (STIs) have a significant impact on sexual and reproductive health, with the World Health Organisation (WHO) reporting more than 1 million STIs being acquired on a daily basis[1]. The four most commonly reported STIs are chlamydia, gonorrhoea, syphilis and trichomoniasis. Other pathogens of STI include Mycoplasma spp., Ureaplasma spp. and herpes simplex virus (HSV).

We have developed a novel 3base™ real-time PCR (RT-PCR) assay to detect the presence of 12 most significant and commonly encountered STIs in less than 4 hours from primary patient material (Table 1). The 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection kit is a simple and rapid molecular method that utilises 3base™ technology to modify the 4 usual DNA bases (A, C, T, G) into only 3 bases (A, T, G) via a novel, patented bisulphite conversion step. The conversion process simplifies the design of multiplex PCR reactions by eliminating the large Tm differences that can be present when targeting multiple pathogens (see Table 2).

Table 1. Targets included in the Genetic Signatures 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection kit.

Panel A	Panel B	Panel C	Panel D
C. trachomatis (CT)	*M. genitalium*	*Candida spp.*	*T. pallidum*
N. gonorrhoeae (NG)	*T. vaginalis*	*M. hominis*	HSV-1
LGV^	*Ureaplasma spp.*	*S. agalactiae*	HSV-2
EC*	EC*	EC*	EC*

^Lymphogranuloma venereum (LGV)
*Extraction Control

Table 2. The DNA sequence of 2 primers and probes before and after 3base™ modification

Conventional Sequence	Tm (° C)
Primer 1: GTACACACCGCCCGTCGCTCCTACC	77
Primer 2: GAAGGAGAAGTCGTAACAAG	56
Probe 1: TGAATAAAGAGGTGAAATTCTAGG	59
Probe 2: GAAGGGCCGCGAGCCCCCGCGC	87

3Base™ Sequence	Tm (° C)
Primer 1: GTATATATTGTTTGTTGTTTTATT	52
Primer 2: GAAGGAGAAGTTGTAATAAG	50
Probe 1: TGAATAAAGAGGTGAAATTTTAGG	59
Probe 2: GAAGGGTTGTGAGTTTTTGTGT	62

Methods/Materials

Nucleic acids were converted to a 3base™ form during the DNA isolation in order to yield better multiplexed PCR performance (www.geneticsignatures.com). The assay sensitivity was determined using quantified genomic DNA controls from Vircell (Granada, Spain) and assay performance assessed by using reference material from ATCC (Manassas, USA), Zeptometrix (Buffalo, USA) and QCMD (Glasgow, Scotland). The clinical performance of the assay was assessed by using over 800 clinical isolates obtained from St. Vincent's Hospital (Sydney, Australia). DNA extraction and PCR set up was performed on a GS1 automated extraction platform (Figure 1, Genetic Signatures, Sydney, Australia) resulting in a significant reduction in hands on time. PCR was performed on a CFX real-time PCR instrument (Bio-Rad, Caliofornia, USA) with integrated software calling.

Figure 1. GS1 automated extracted platform

Results

The performance of the 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection kit with the 2016 QCMD Panels is shown in Table 3. The QCMD Panels yielded 100% concordance with the expected targets.

Clinical validation

The results from testing 846 clinical STI isolates is shown in Table 4. 25.1% of the clinical isolates tested had mixed infection. An example of real-time traces generated from mixed infections are shown in Figure 2. In summary, the 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection kit had a 100% concordance with the hospital's results. Additional STI pathogens were identified.

Table 4. Results obtained using clinical isolates

Pathogen detected	*EasyScreen*™	Hospital
CT	48	42
NG	24	27
LGV	1	1[β]
M. genitalium	10	Not tested
T. vaginalis	8	4
Ureaplasma spp.	296	Not tested
Candida spp.	153	95
M. hominis	71	Not tested
S. agalactiae	98	51
T. pallidum	2	2^
HSV-1	32	25
HSV-2	19	15
Total	**762**	**259**

^ Confirmed by Reference lab

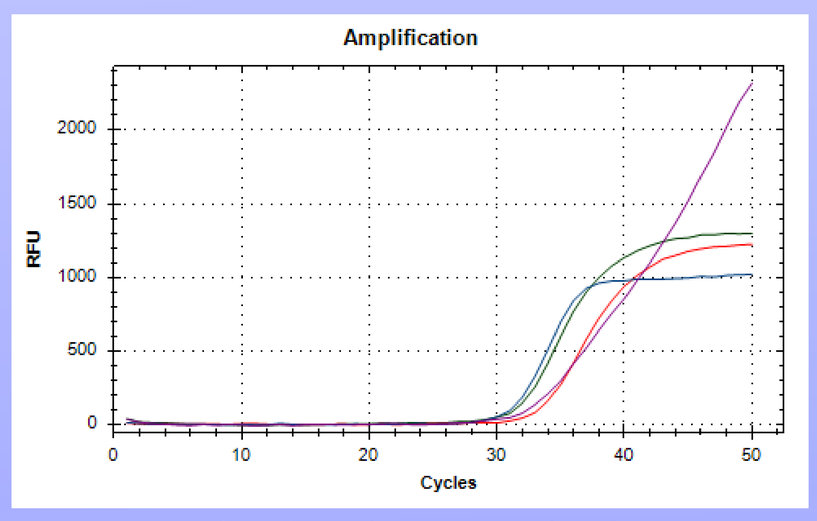

Figure 2. CT(LGV) and NG mixed infection trace signals. CT(blue), NG (red), LGV (violet) and EC (green).

Conclusions

The 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection kit provides a sensitive and specific alternative for the detection of STI pathogens. The workflow from sample processing to results requires less than 4 hours with minimal hands on time. This is particularly advantageous not just for high-throughput pathology laboratories but also in improving patient diagnosis and management.

Table 3. 3base™ *EasyScreen*™ Sexually Transmitted/Genital Detection kit performance on 2016 QCMD Panels

QCMD 2016 panel	Core samples	*EasyScreen*™ result	Educational samples	*EasyScreen*™ result
CT	4	4	1 (86.2%)[∞]	1
NG	4	4	1 (78.4%)[∞]	1
HSV-1	3	3	0	0
HSV-2	2	2	1 (91.8%)[∞]	1

[∞] % of respondents reporting the correct result

References

1. http://www.who.int/mediacentre/factsheets/fs110/en/
2. Rodriguez-Dominguez M. et al. 2015. High Prevalence of Co-Infections by Invasive and Non-Invasive Chlamydia trachomatis Genotypes during the Lymphogranuloma Venereum Outbreak in Spain. PLoS One. 10(5):e0126145

Genetic Signatures
Transforming Molecular Diagnostics