

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

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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 basis¹. 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
<i>C. trachomatis</i> (CT)	<i>M. genitalium</i>	<i>Candida</i> spp.	<i>T. pallidum</i>
<i>N. gonorrhoeae</i> (NG)	<i>T. vaginalis</i>	<i>M. hominis</i>	HSV-1
LGV [^]	<i>Ureaplasma</i> spp.	<i>S. agalactiae</i>	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: GTACACCGCCCGTGCCTACC	77
Primer 2: GAAGGAGAAGTCGTAACAAG 56	
Probe 1: TGAATAAAGAGGTGAAATCTAGG	59
Probe 2: GAAGGGCCGCGAGCCCCCGCGC	87
3Base™ Sequence	Tm (° C)
Primer 1: GTATATTTGTTTGTGTTTTATT	52
Primer 2: GAAGGAGAAGTTGTAATAAG 50	
Probe 1: TGAATAAAGAGGTGAAATTTAGG	59
Probe 2: GAAGGGTTGTGAGTTTTTGTGT	62

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

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), Zeptomatrix (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, California, USA) with integrated software calling.

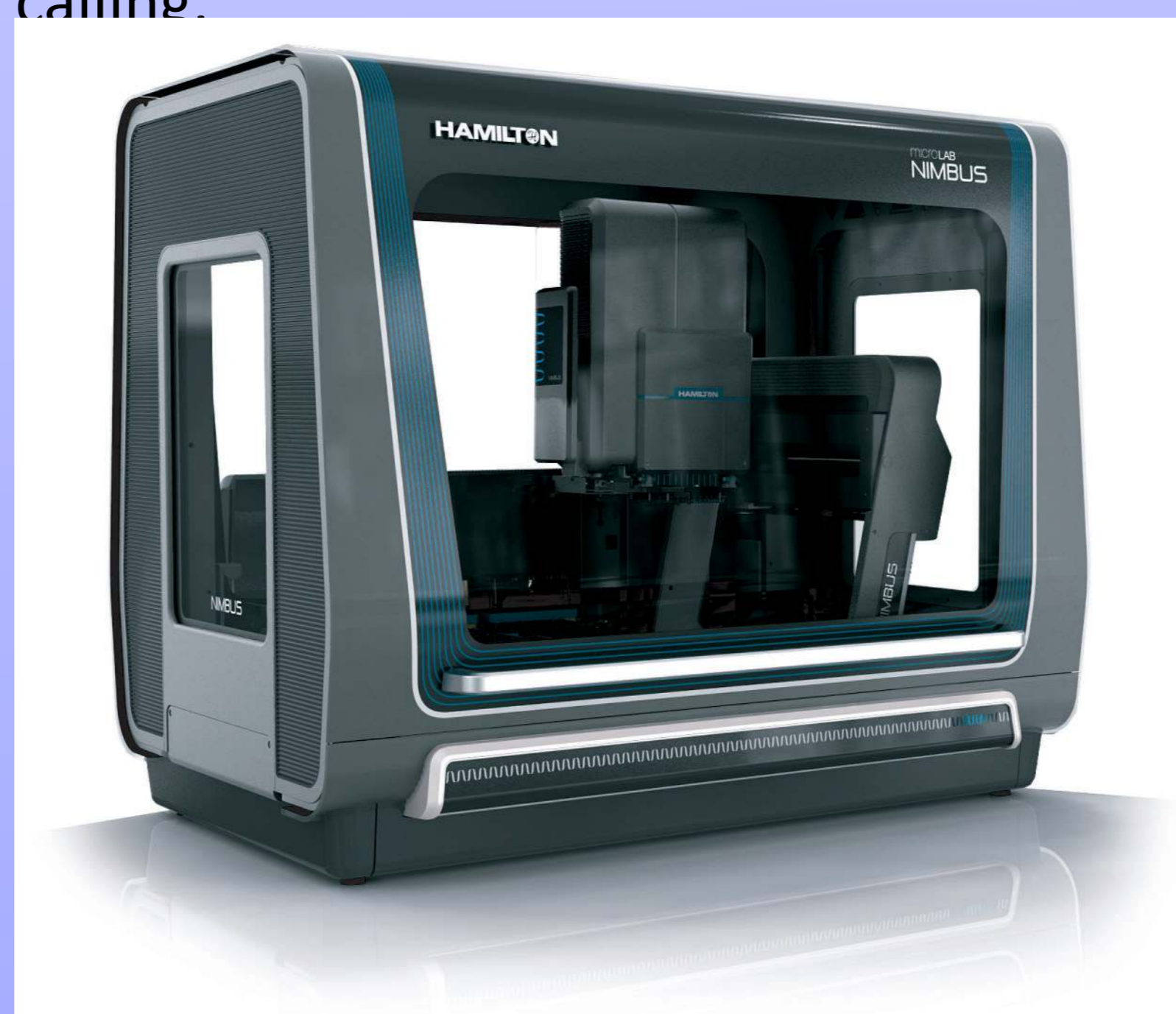


Figure 1. GS1 automated extracted platform

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
<i>M. genitalium</i>	10	Not tested
<i>T. vaginalis</i>	8	4
<i>Ureaplasma</i> spp.	296	Not tested
<i>Candida</i> spp.	153	95
<i>M. hominis</i>	71	Not tested
<i>S. agalactiae</i>	98	51
<i>T. pallidum</i>	2	2 [^]
HSV-1	32	25
HSV-2	19	15
Total	762	259

[^] Confirmed by Reference lab

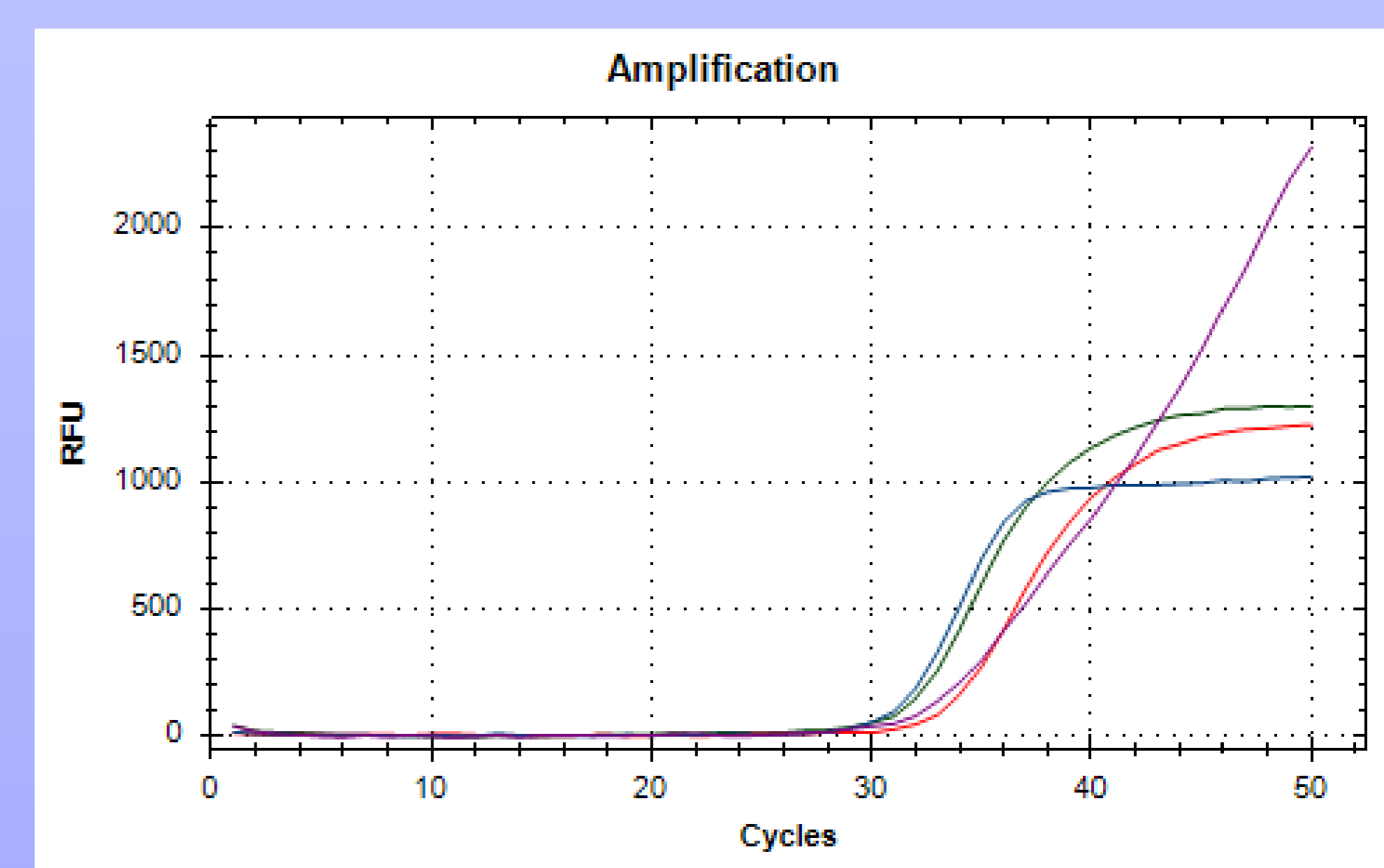


Figure 2. CT(LGV) and NG mixed infection trace signals. CT(blue), NG (red), LGV (violet) and EC (green).

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.

References

- <http://www.who.int/mediacentre/factsheets/fs110/en/>
- 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