Simultaneous detection of *Mycoplasma genitalium*, *Trichomonas vaginalis* and Lymphogranuloma venereum using *PlexPCR*™

Erskine S[1], Bromhead C[2], Dubedat S[3], Tan L[1], Walker S[1], & Mokany E[1].

[1]SpeeDx Pty Ltd, National Innovation Centre, Sydney, Australia; [2]Massey University, Wellington, New Zealand; [3]Royal Prince Alfred Hospital, Sydney, Australia.

Background

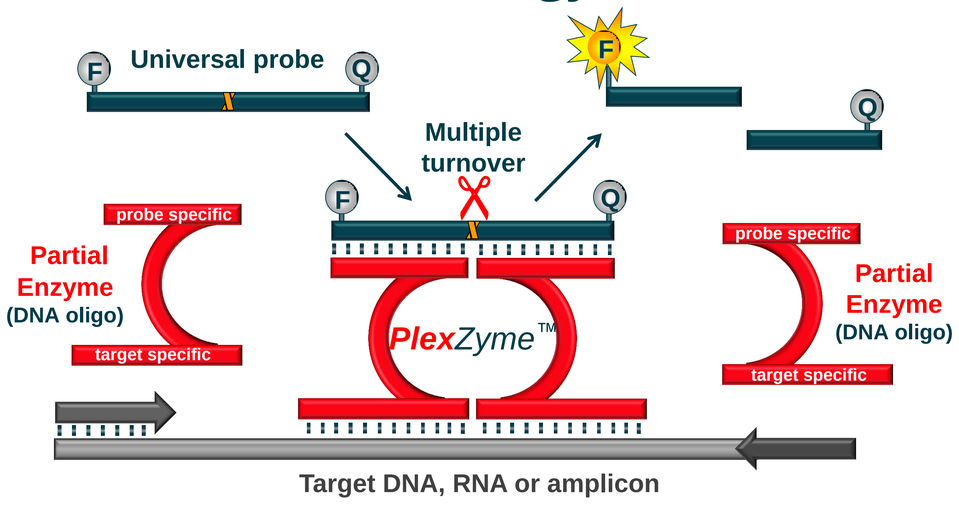
Plex*PCR*™ technology has been used to combine detection of less commonly tested causes of STIs in a single well: *Mycoplasma genitalium* , *Trichomonas vaginalis* and Lymphogranuloma venereum (LgV). The panel showed good PCR efficiency (E = 95-101%, R^2 > 0.990) and detection to 10 copies per reaction for all targets. Preliminary testing at Massey University, New Zealand, highlights the potential usefulness of the panel to detect significant co-infections in high-risk patients and from extra-genital sites.

M. genitalium is an emerging STI linked with non-gonococcal urethritis, cervicitis and pelvic inflammatory disease. In the general population, its prevalence is thought to be around 1.1-3.3%. It is the second most common cause of non-gonococcal urethritis (NGU) in men (15-35% with symptomatic NGU), and accounts for more than 1/3 of acute non-chlamydial NGU, indicating that *M. genitalium* and *Chlamydia trachomatis* act as separate causes of the condition[1].

T. vaginalis is associated with a wide range of urogenital conditions, as well as with poor birth outcomes: low birth weight, preterm delivery, pelvic inflammatory disease. Additionally, perinatal transmission of infection, causing vaginal and respiratory infections in neonates, has been reported[2].

LgV is transmitted by the rare types of chlamydia (L1-L3 biovars). Emergence of LgV has been identified in Europe, North America and Australia, predominantly in men who have sex with men[3]. Diagnosis is difficult due to the lack of testing and because the disease mimics other more common conditions. Currently, there are no commercially available NAATs that can differentiate between LgV and non-LgV chlamydial infections.

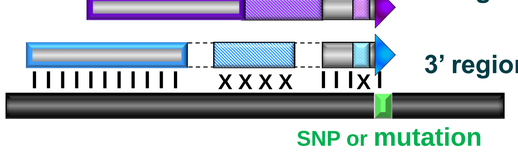
PlexPCR™ technology for NAATs



Highly specific, sensitive, universal probes and superior multiplexing capacity

PlexPrime™ design for variant detection

Multiplexed *PlexPrimers*™



Insert - unique sequence & conformation

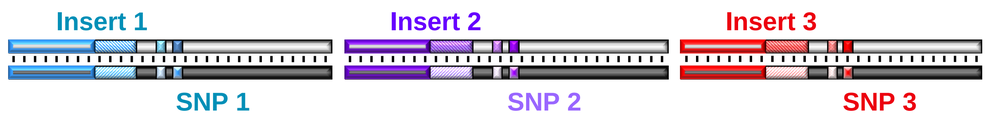
5' region - bind different target regions

3' region - match SNP or mutation

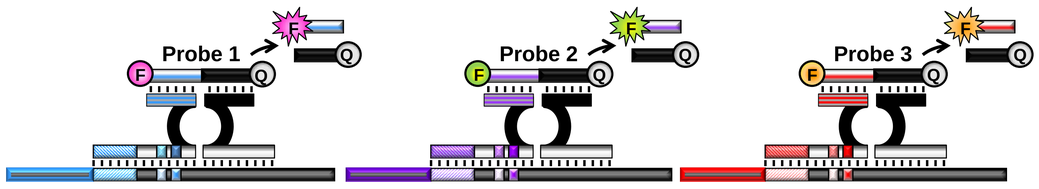
INCREASED SPECIFICITY

REDUCED COMPETITION

PlexPrime™ amplicons are distinctly different



Allele-specific *PlexZyme*™ detection



References

1. Taylor-Robinson, D, and Jensen , J, Clin. Microbiol. Rev. 2011;24:498-514
2. Kissinger, P, BMC Infectious Diseases, 2015;15(1)
3. Halse *et al*., Molecular and Cellular Probes, 2006;20(5):290-297

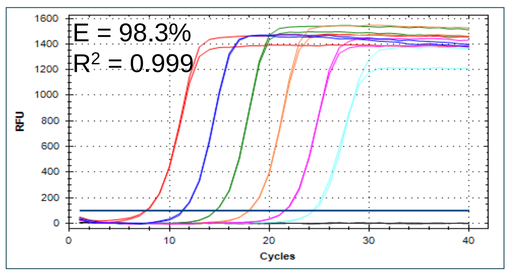
PlexPCR™ MG/TV/LgV

	Channel	Target
1 Well	1	*M. genitalium (MgPa)*
	2	*T. vaginalis* (β-tubulin)
	3	LgV (*pmpH*)
	4	Internal Control

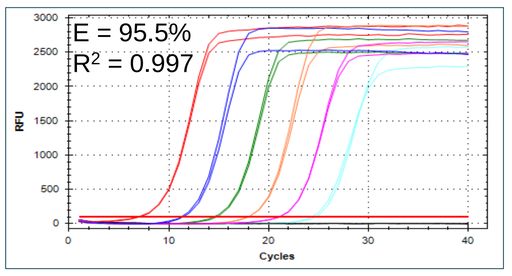
- Single well assay
- Robust and high throughput
- Rapid qPCR results (<1.5 hours)
- Multiple specimen types – urethral, rectal, throat, urine (male/female)

Analytical Performance

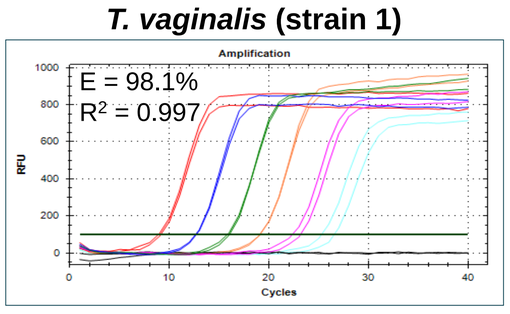
M. genitalium



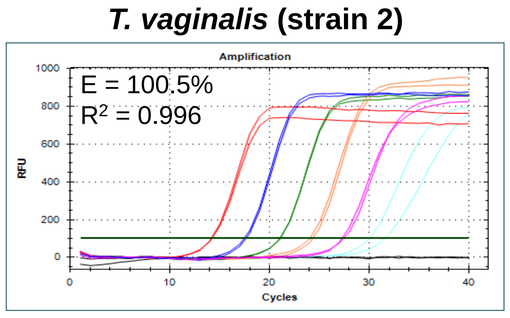
Lymphogranuloma venereum



***T. vaginalis* (strain 1)**



***T. vaginalis* (strain 2)**



Synthetic DNA Template (copies per reaction)

10^6 10^5 10^4 10^3 10^2 10

Excellent PCR efficiency. Detection at 10 copies per reaction

Clinical Evaluation

A high-risk population of patients (n = 264; 205 male, 49 female, 10 not disclosed) with Chlamydia were screened using the ***Plex****PCR*™ *M. genitalium* , *T. vaginalis* and LgV triplex to test for the presence of co-infections and LgV status. Sample sites were the rectum, throat and eye collected between 2012 and 2015.
Evaluation was performed by Dr. Collette Bromhead, Massey University, New Zealand.

264 Chlamydia-positive patients were screened

Co-infection	n	Positivity rate	Rectal	Throat	Eye	Not specified
M. genitalium	**14**	**5.30%**	**11**	**1**	**1**	**1**
T. vaginalis	**1**	**0.38%**	**1**			
LgV	**1**	**0.38%**	**1**			

Significant co-infection rates in high-risk patients
Extra-genital sites are important sites of infection

SpeeDx STI assays

TGA

Plex*PCR*™ **HSV-1&2, VZV**

CE-IVD

Resistance*Plus*™ **MG**

Plex*PCR*™ **HSV-1&2, VZV**

For Evaluation

Plex*PCR*™ **HSV-1, -2 & Syphilis**

Plex*PCR*™ **MG/TV/LgV**

Plex*PCR*™ **STI MG/TV Plus**

All assays come with an internal extraction/amplification control

Multiplexing Accelerated… visit the SpeeDx booth

SpeeDx

Plex*PCR*™ **is a flexible, rapid & cost-effective technology for multiplexed detection of targets and genetic variants**

If you are interested in multiplexing your assay and/or wanting to achieve specific single base discrimination contact **info@speedx.com.au** or for more information about ***Plex****Prime*™ & ***Plex****PCR*™ technology visit **www.speedx.com.au**