



EasyScreen Multiplex PCR for STI Infections, a Year in Review

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SEXUALLY TRANSMITTED INFECTIONS (STI's)

- The World Health Organisation (WHO)¹ report more than 1 million STI's contracted daily.
- STI's have a significant impact on sexual and reproductive health.
- Four of the most common reported STI's (WHO):
 - Chlamydia (131 million/year)
 - Gonorrhoea (78 million/year)
 - Syphilis (5.6 million/year)
 - Trichomoniasis (143 million/year)
- After a dramatic decline in STI diagnoses in the late 1980's, STI's have re-emerged as a major public health concern.

¹<http://www.who.int/mediacentre/factsheets/fs110/en/>





SEXUALLY TRANSMITTED INFECTIONS (STI's)

- HIV/AIDS pandemic in 1980's lead to behavioral changes – decrease in STI's.
- Late 1990's - increase in STI rates.
- > multiple/coinfection STI's.
- > STI's in all patient groups – especially marginalised groups.
 - MSM
 - Aboriginal and Torres Strait Islander population
 - gonorrhoea x 7, infectious syphilis x 5 and *Chlamydia* x 3

¹<http://www.who.int/mediacentre/factsheets/fs110/en/>

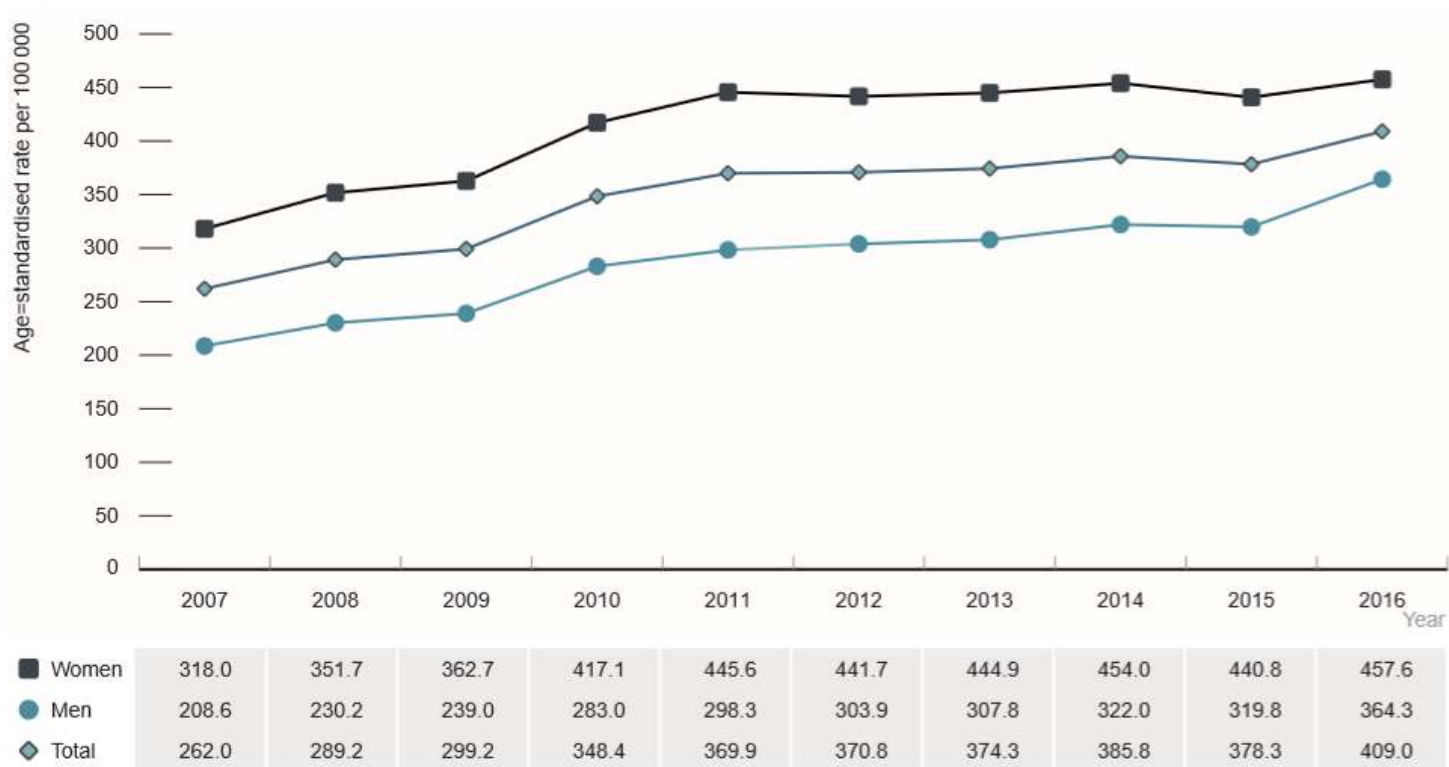


Chlamydia trachomatis

- *Chlamydia* is the most frequently diagnosed sexually transmissible infection in Australia.
- Cervicitis in women and urethritis and proctitis in both men and women.
- Chlamydial infections in women can lead to serious consequences including pelvic inflammatory disease (PID), tubal factor infertility, ectopic pregnancy, and chronic pelvic pain.
- Lymphogranuloma venereum (LGV) is caused by invasive serovars L1, L2 or L3 of *C. trachomatis*.
 - Correct diagnosis is also essential, as prolonged treatment (3 weeks) with doxycycline or a macrolide antibiotic is required for patients with LGV infections. Most commercial kits cannot distinguish LGV.

Chlamydia trachomatis

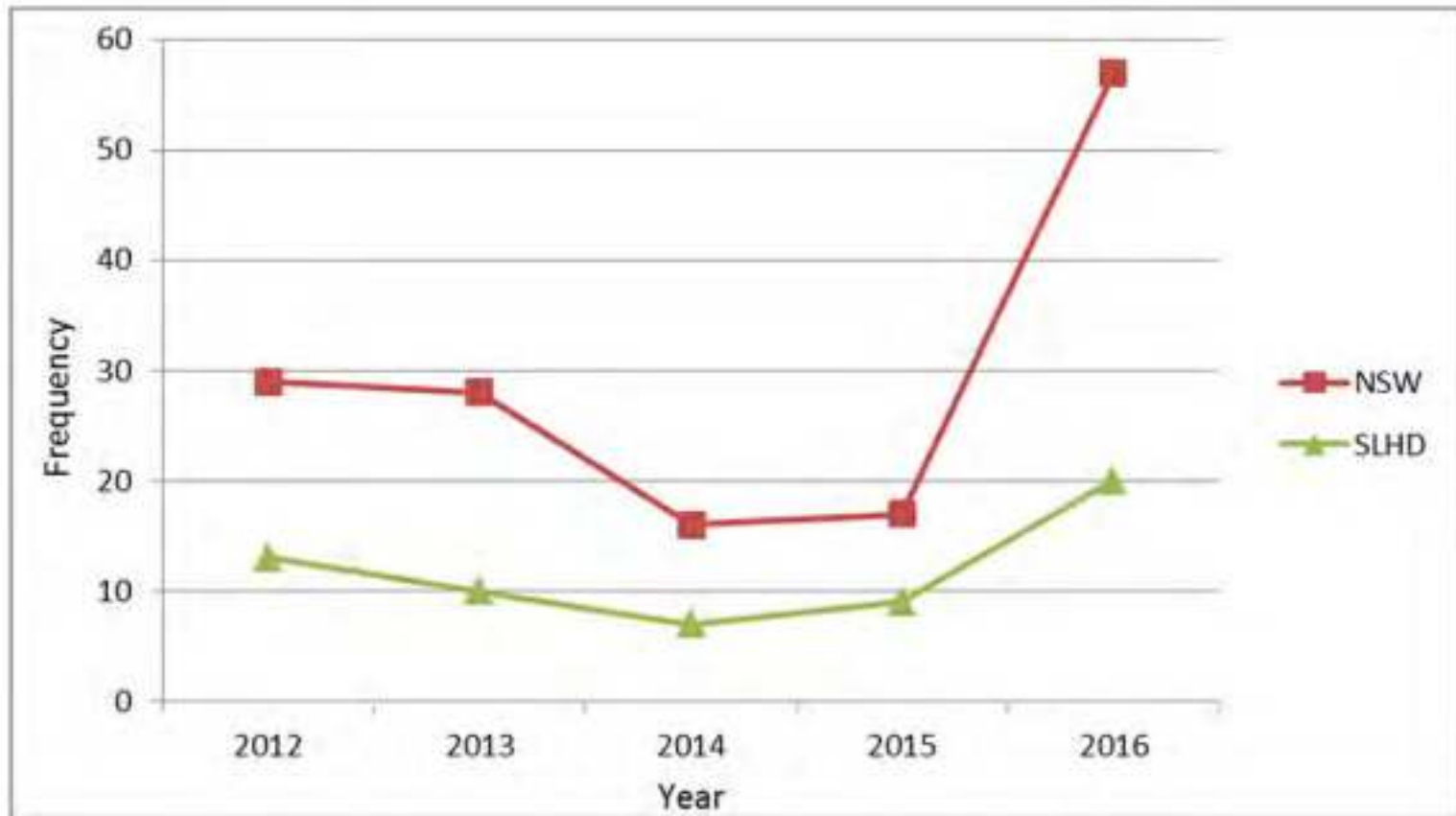
Figure 3.1.1 Chlamydia notification rate per 100 000, 2007–2016, by sex



Note: Excludes Victoria in all years, as 2015 and 2016 data were unavailable at the time of reporting.

Source: Australian National Notifiable Diseases Surveillance System

LGV NOTIFICATIONS IN NSW RESIDENTS





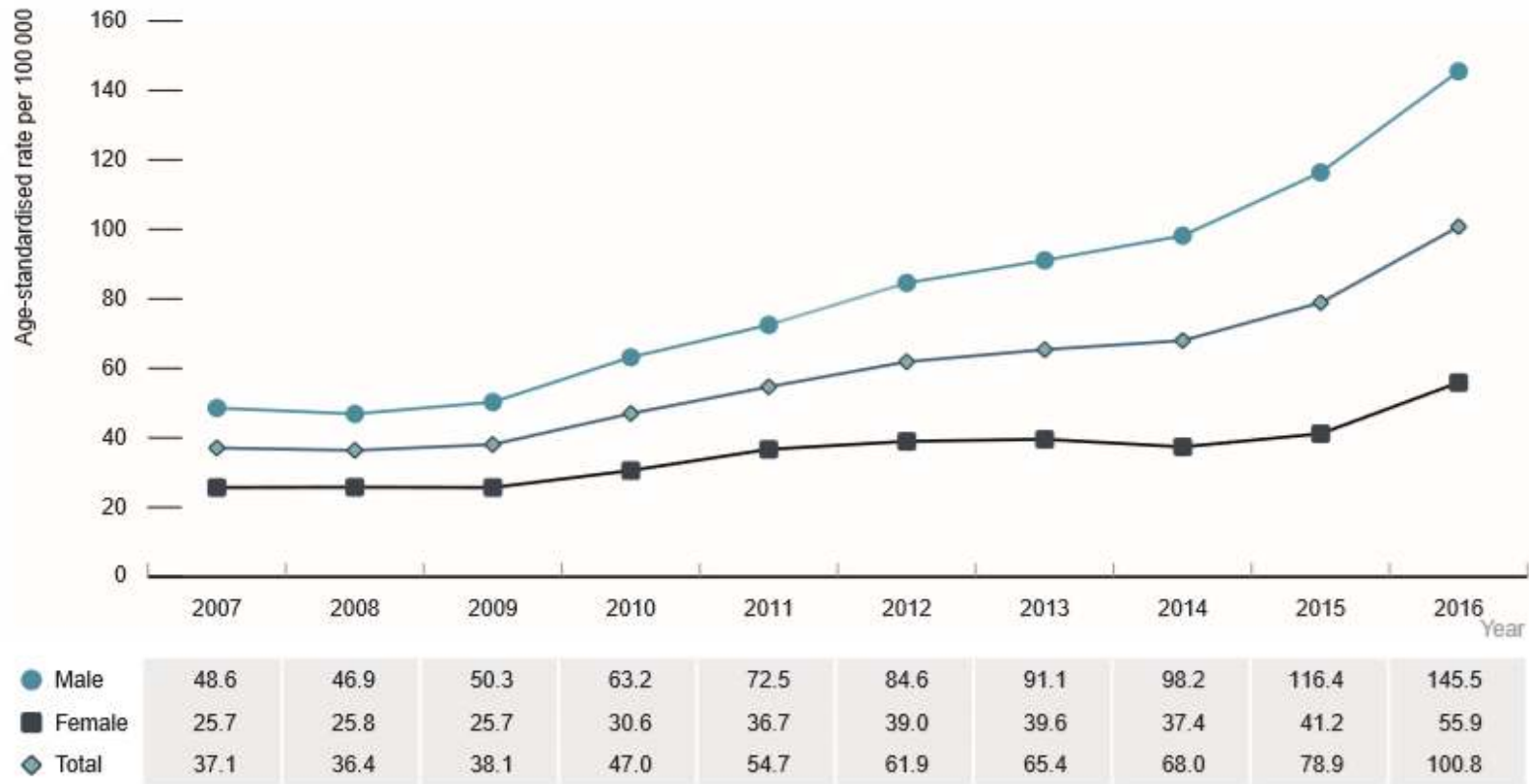
Neisseria gonorrhoeae

- Infects the mucous membranes of the reproductive tract, including the cervix, uterus, and fallopian tubes in women, and the urethra in both women and men.
- Also infects mucous membranes of the mouth, throat, eyes, and rectum.
- Between 2012 and 2016, there was a 63% increase in notification rates.
- The gonorrhoea notification rate has been higher in males than females in each of the years since 2007



Neisseria gonorrhoeae

Figure 3.2.1 Gonorrhoea notification rate per 100 000 population, 2007–2016, by sex



Source: Australian National Notifiable Diseases Surveillance System.



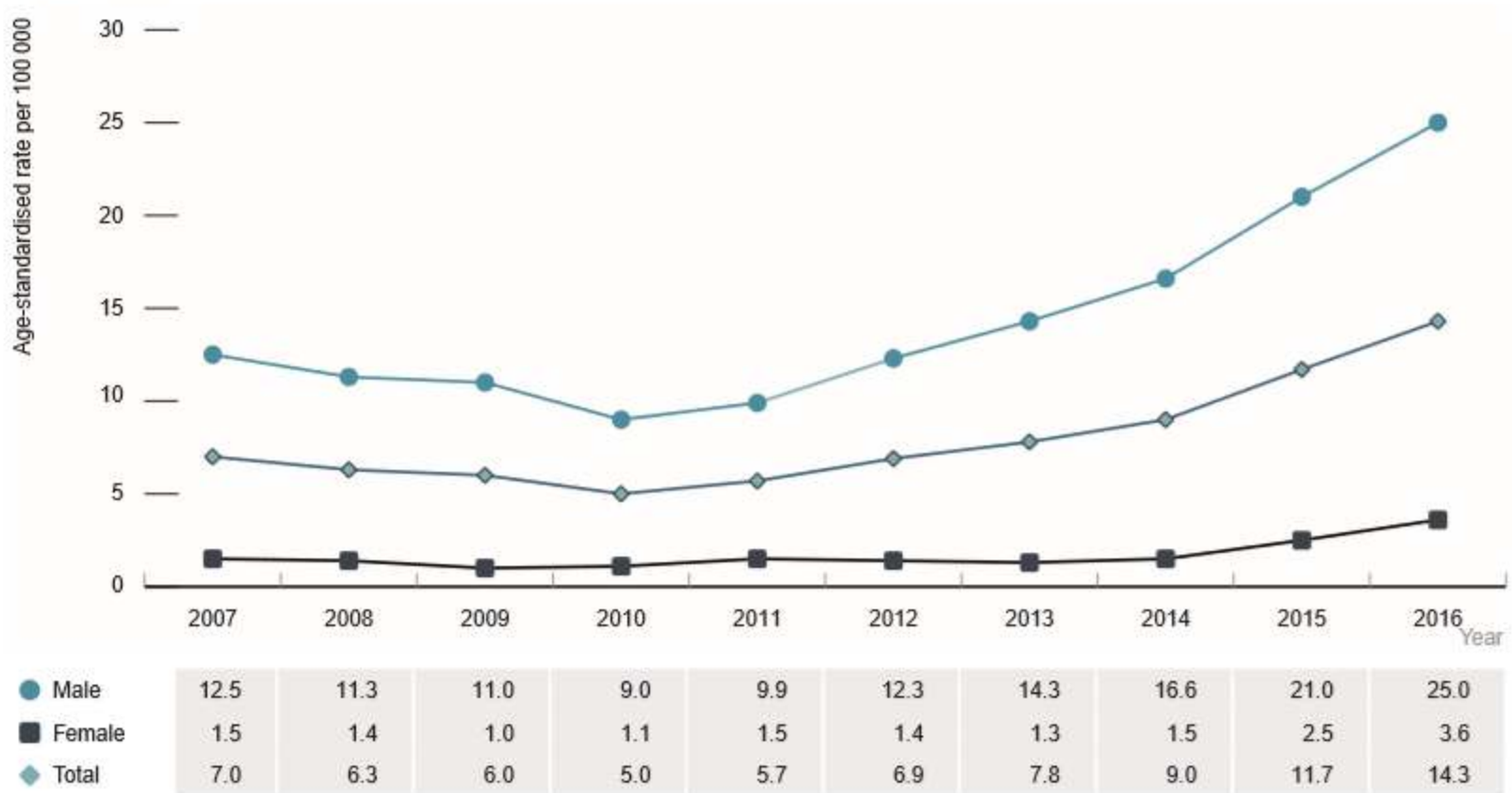
Treponema pallidum

- Syphilis is caused by the bacterium *Treponema pallidum*. Syphilis is transmitted by direct contact with a syphilitic sore, known as a chancre.
 - Primary, secondary and tertiary syphilis.
- Transmission of syphilis occurs during vaginal, anal, or oral sex.
- Congenital syphilis - from 2007–2016, 55% of the 43 congenital syphilis notifications were in the Aboriginal and Torres Strait Islander population.
- In 2016, 87% (2945) of infectious syphilis notifications were in males (majority MSM).
- From 2012–2016, the notification rate of infectious syphilis increased by **107%**.



Treponema pallidum

Figure 3.3.1 Infectious syphilis notification rate per 100 000 population, 2007–2016, by sex

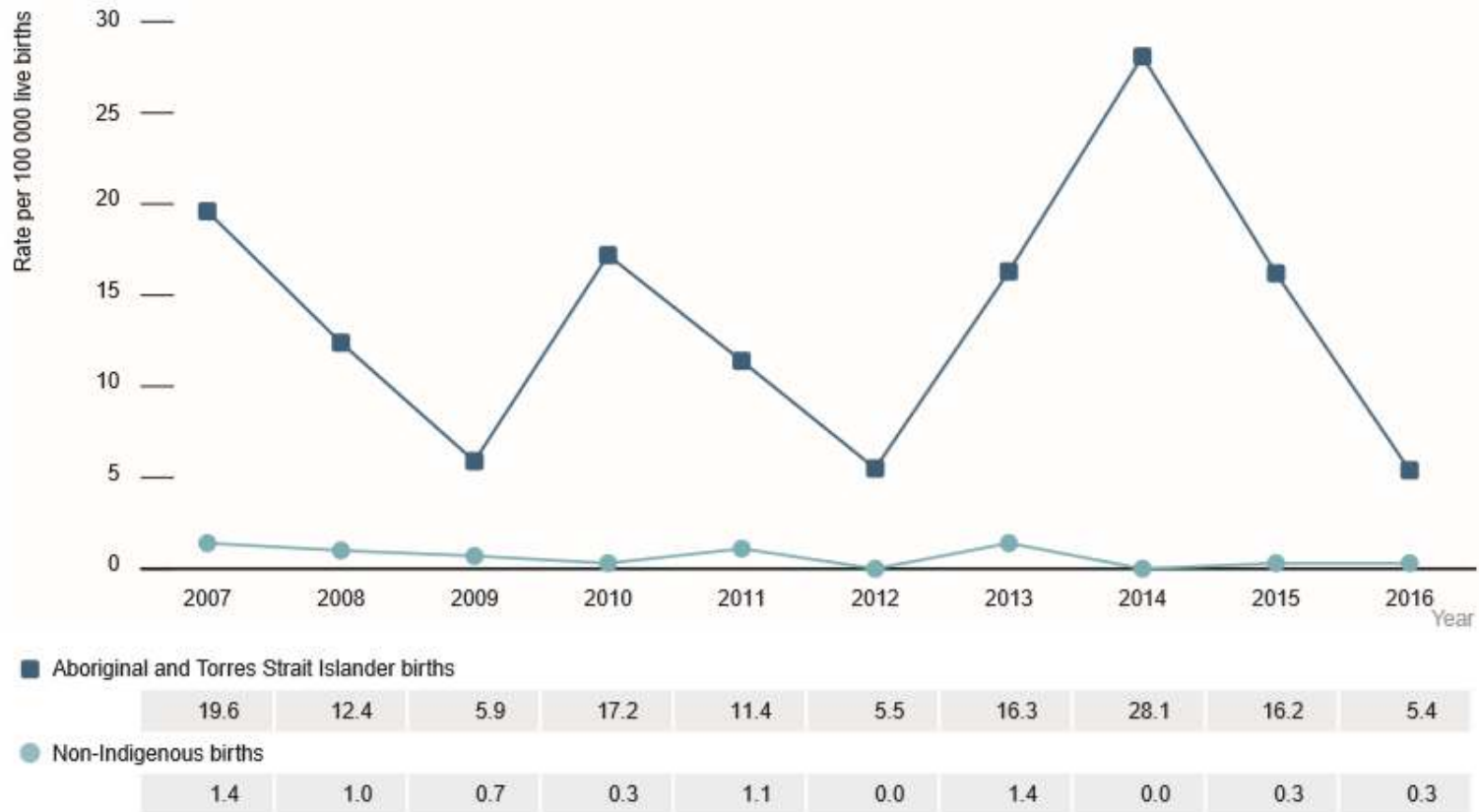


Source: Australian National Notifiable Diseases Surveillance System.



Treponema pallidum

Figure 3.3.11 Congenital syphilis rate per 100 000 live births, 2007–2016, by Aboriginal and Torres Strait Islander status



Note: 2016 rates were based on the number of live births in 2015, as 2016 births data were not available at the time of reporting.

Source: Australian National Notifiable Diseases Surveillance System.

Treponema pallidum – Tertiary and congenital syphilis

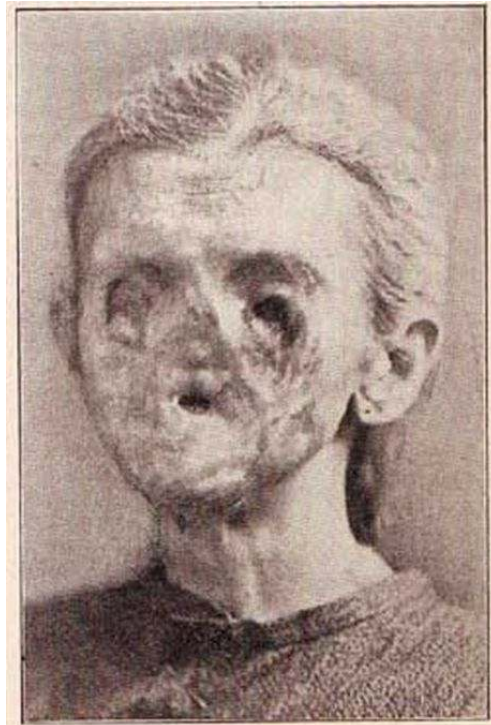


FIG. 5.—Closure of mouth from congenital syphilis.





OTHER SEXUALLY TRANSMITTED INFECTIONS

- **Trichomoniasis:** caused by a protozoan parasite *Trichomonas vaginalis*. Most women and men are asymptomatic. Mild to severe inflammation, discharge and discomfort. Infection can last months to years.
- **Herpes Simplex Virus (HSV):** HSV-1/HSV-2 are highly contagious and long lasting viral infection.
- ***Mycoplasma genitalium*:** implicated as a cause of urethritis in men and infection of the cervix in women.
- ***Ureaplasma spp.* :** Implicated bacterial vaginosis and PID in women and prostatitis or epididymitis in men. Normal flora?
- **Donovanosis:** *Klebsiella (Calymmatobacterium) granulomatis*, once a frequently diagnosed sexually transmissible infection among remote Aboriginal populations, is now close to elimination, with only two cases notified since 2011.
- **Chancroid:** *Haemophilus ducreyi*, not endemic, imported infections only. Last case reported in Australia was in 2009.

Method

- **Prospective study**
 - Laboratory and epidemiological data collected over a 12 month period from implementation of GS STI assay (16/05/17 – 16/6/18).
 - Age, sex, anatomical site, test result, clinical information, risk factors.
 - LGV typing - Jurstrand et al., (2001) targets major outer membrane protein gene (omp1 gene) as a PCR target followed by sanger sequencing.



EASYSSCREEN™ STI/GENITAL PANELS

Panel A	Panel B	Panel C	Panel D
<i>C. trachomatis</i>	<i>M. genitalium</i>	<i>Candida</i> spp.	<i>T. pallidum</i>
<i>N. gonorrhoeae</i>	<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*

*Extraction control

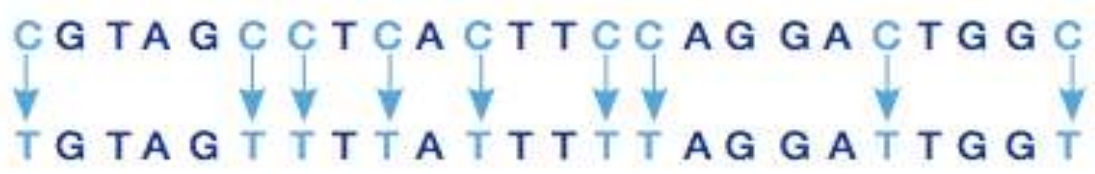


3BASE™ TECHNOLOGY- ASSISTS IN SENSITIVITY AND SPECIFICITY

Regular Cell Lysis and Preparation Steps

**Native
Sequence**

3base
conversion



**3base™
Sequence**

Standard Real-Time PCR Instrument

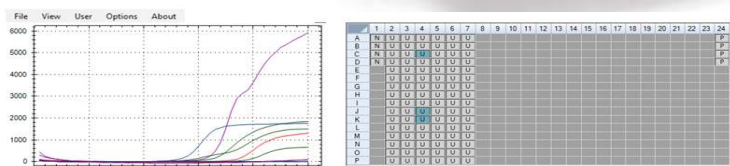
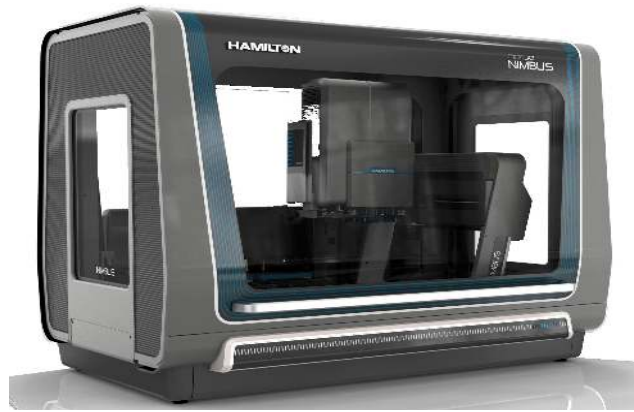
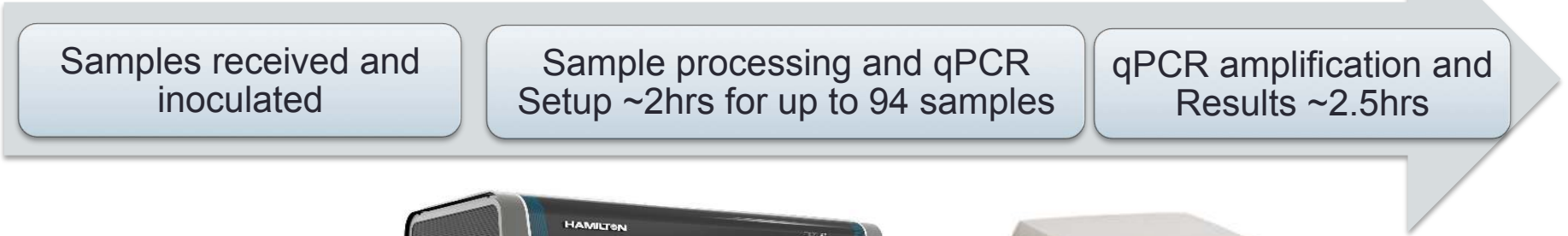
3base™ Increases Homology

	Before	After
Seq 1	G A T G G <u>C</u> G A <u>T</u> A T G G T <u>T</u> G A <u>C</u> A <u>C</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>
Seq 2	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>
Seq 3	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>
Seq 4	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>
Seq 5	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>
Seq 6	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>
Seq 7	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>
Seq 8	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>
Seq 9	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>
Seq 10	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>G</u> G A <u>T</u> A <u>T</u>	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>A</u> G A <u>T</u> A <u>T</u>

Large % of sub-species variations eliminated.

Consensus	G A T G G <u>T</u> G A <u>T</u> A T G G T <u>D</u> G A <u>T</u> A <u>T</u>
95%	75% homology over 20 bases
only 3	48 possible primer combinations

THE EASYSCREEN™ WORKFLOW



Sample ID	Well	(No pathogen targets detected)	Results	EQ
3056004	A01		N/A	N/A
3056075	A02	<i>S. agalactiae</i>	+	+
3056071	E02	<i>Ureaplasma spp.</i>	+	+
30573268	B02	<i>Ureaplasma spp.</i>	+	+
40019610	M02		-	-
30564706	A03	<i>Candida S. agalactiae</i>	+	+
40030502	B03		-	-
30564707	B03		-	-
40030504	B03		-	-
5211732	A04	<i>S. agalactiae</i>	+	+
30573264	B04		-	-
94481947	B04	<i>Ureaplasma spp. M. hominis</i>	+	+
30560721	M04		-	-
5211675	A05	<i>Ureaplasma spp. Candida</i>	+	+
40019619	E05	<i>S. agalactiae HSV 1</i>	+	+
30573273	B05	<i>Ureaplasma spp.</i>	+	+
30563662	M05		-	-
30564873	A06		-	-
40019562	E06		-	-
30573462	B06	<i>Ureaplasma spp. T. vaginalis M. hominis</i>	+	+
94481719	M06	<i>Ureaplasma spp. Candida S. agalactiae HSV 2</i>	+	+
30573564	B07	<i>Ureaplasma spp. Candida</i>	+	+
30564535	E07	<i>Ureaplasma spp. Candida</i>	+	+
30573565	B07	<i>Ureaplasma spp. Candida</i>	+	+
30573269	M07	<i>Ureaplasma spp.</i>	+	+
Positive	A24	PASS		N/A



GS1 SET-UP ON 384 WELL PCR PLATE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
A																										A
B																										B
C																										C
D																										D
E																										E
F																										F
G																										G
H																										H
I																										I
J																										J
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L																										L
M																										M
N																										N
O																										O
P																										P
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		

STI samples
12 targets
per patient

Up to 94 samples on 384 well PCR set-up



Results

- A total of 1,266 specimens from various anatomical sites were collected over a 12 month period (52% women, 48% men)
- 783 (62%) patients tested positive for 1 or more pathogens.
- A total of 519 (41%) patients returned a single positive result, with 264 (21%) testing positive for multiple pathogens (n=2-6).
- STI's were more prevalent in men (n=101) than women (n=62), with the exception of HSV which was more prevalent in women and *T. vaginalis* which was only found in women.
- Syphilis and LGV were found exclusively in MSM, with higher rates of *N. gonorrhoeae*, *C. trachomatis* and *M. genitalium* in this patient group. MSM were also more likely to have multiple STI infections.
- All 6 LGV isolates were identified as serovar L2b (99% similar to previously described strain circulating among MSM in Sydney Stark et al., 2007).

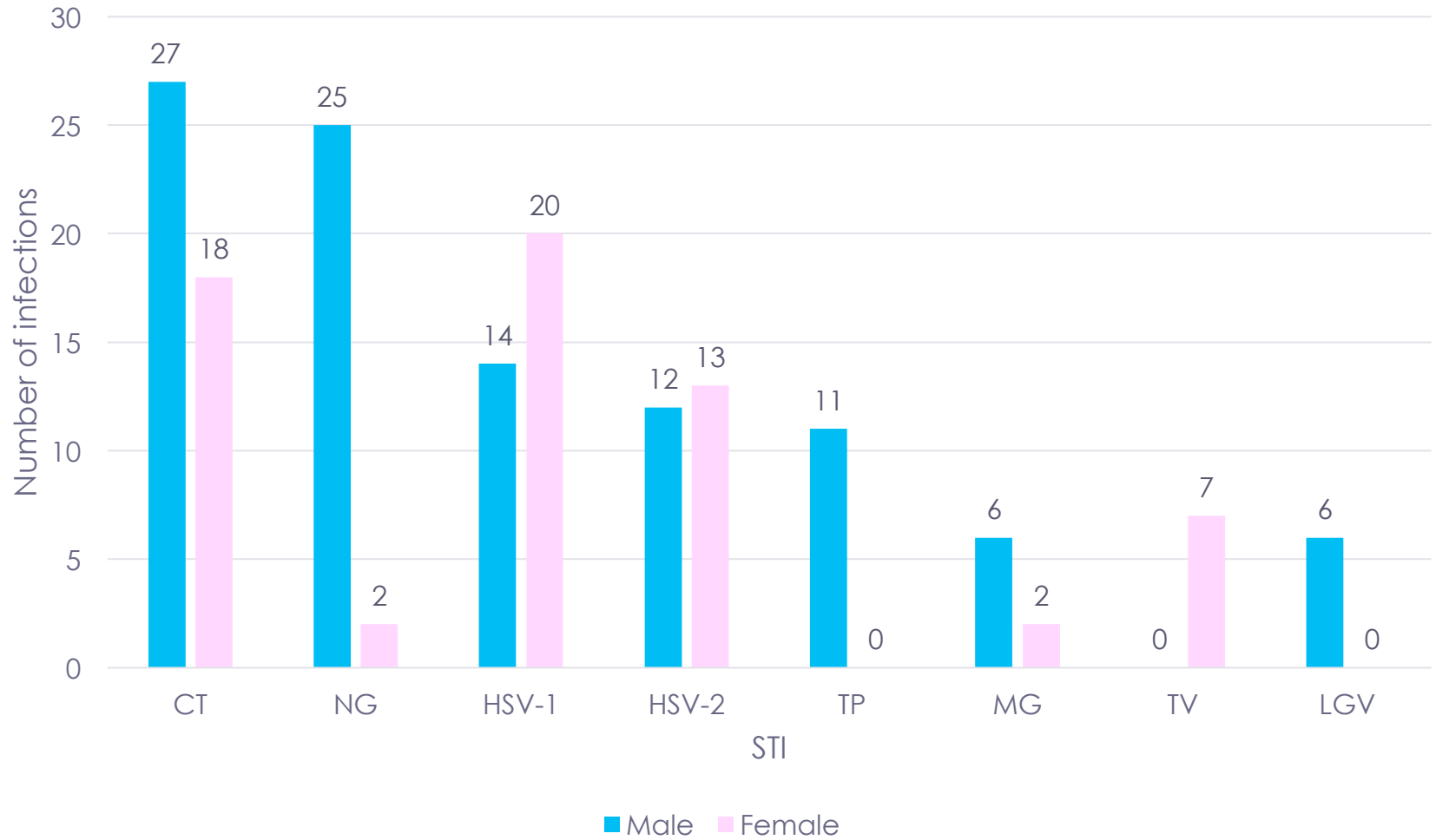


RESULTS

Pathogen detected	Number detected	% of positive samples
<i>Ureaplasma spp.</i>	557	44%
<i>Candida spp.</i>	181	14%
<i>S. agalactiae</i>	115	9%
<i>M. hominis</i>	112	8%
<i>C. trachomatis</i>	45	3.5%
HSV-1	34	2.7%
<i>N. gonorrhoeae</i>	27	2%
HSV-2	25	1.9%
<i>T. pallidum</i>	11	0.9%
<i>M. genitalium</i>	8	0.6%
<i>T. vaginalis</i>	7	0.5%
LGV	6	0.5%



STI – MALE/FEMALE DISTRIBUTION

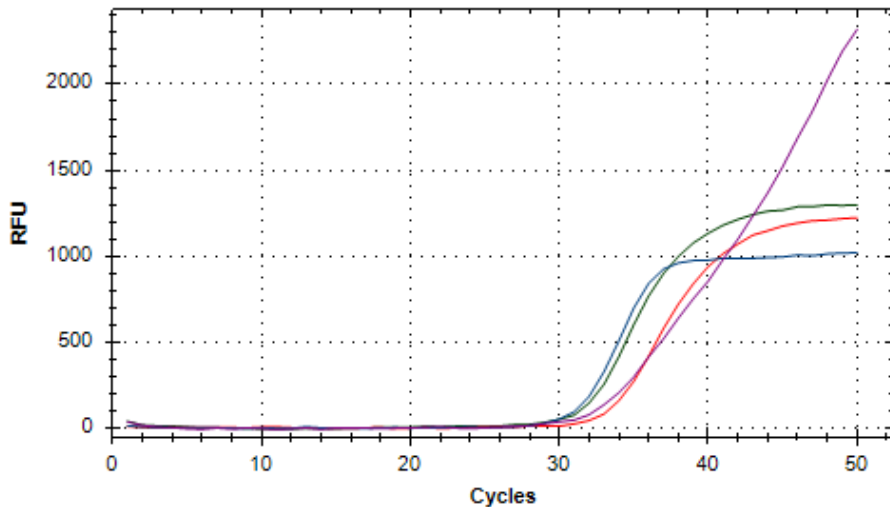


MIXED INFECTIONS

21% of the samples had mixed infection (≥ 2 pathogens detected)

1A

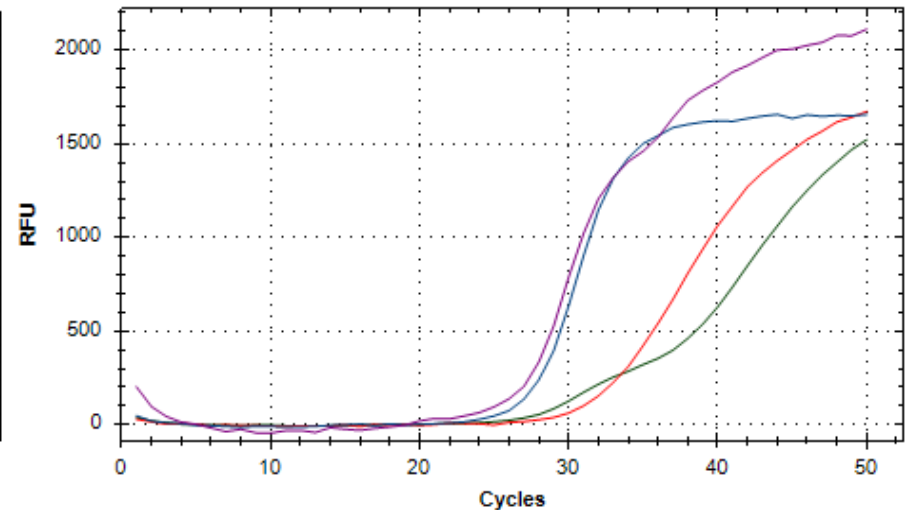
Amplification



CT (LGV) and NG mixed infection

1B

Amplification



Candida spp., *M. hominis*, *S. agalactiae*
mixed infection with EC



CLINICAL RESULTS OF *T. pallidum*

Pathogen	Sex	Age	Coinfection	Specimen type/Clinical notes	Risk factors
<i>T. pallidum</i> n=11	Male	34		Penile ulcer ?HSV	MSM
	Male	40		Penis	MSM
	Male	26		Penis ulcer, STI check	MSM
	Male	21		Penis ulcer, STI check	MSM
	Male	26	<i>Chlamydia trachomatis</i>	Rectum ?LGV	MSM
	Male	32		Penile lesion	MSM
	Male	30		Rectum lesion ? CT	MSM
	Male	33	<i>Chlamydia trachomatis</i>	Anal lesion	MSM
	Male	32		Penis gland ulcer	MSM
	Male	28		Genital lesions	MSM
	Male	50	<i>Streptococcus agalactiae</i>	Penis	MSM

All cases primary syphilitic chancres, 10/11 had yet to seroconvert at time of presentation



CLINICAL RESULTS LGV

Pathogen	Sex	Age	Coinfection	Specimen type/ Clinical notes	Risk factors	Typing
LGV n=6	Male	46		Rectal biopsy	MSM	L2b*
	Male	32	<i>Ureaplasma</i> spp.	Rectum	MSM	L2b
	Male	50	<i>Ureaplasma</i> spp., <i>Mycoplasma hominis</i>	Rectum	MSM	L2b
	Male	48	<i>Neisseria gonorrhoeae</i>	Rectum, discharge	MSM	L2b
	Male	30	<i>Mycoplasma hominis</i>	Rectum ?LGV	MSM	L2b
	Male	29		Rectum	MSM	L2b

*Typing of isolate revealed 99% sequence homology to previously characterised strain Stark et al. 2007
22% (6/27) CT isolates from male were LGV



CLINICAL RESULTS *N. gonorrhoeae*

Sex	Age	Coinfection	Specimen type/Clinical notes	Risk factors
Male	38	ND	Urethra	MSM
Male	44	ND	Urethra, mucousy discharge	MSM
Male	27	<i>Mycoplasma genitalium</i>	Urethra, urethritis	MSM
Male	51	ND	Penis ulcer, STI check	MSM
Male	26	<i>Chlamydia trachomatis</i>	Urine, STI	MSM
Male	32	ND	Urethra	MSM
Male	31	ND	Urethra	MSM
Male	32	<i>Chlamydia trachomatis</i> <i>Ureaplasma</i> , <i>Mycoplasma hominis</i>	Rectum	MSM
Male	40	<i>Chlamydia trachomatis</i> <i>Ureaplasma</i> ,	Rectum	MSM
Male	28	ND	Urethra, discharge	MSM
Male	23	ND	Urethra	MSM
Male	29	ND	Urethra, STI	MSM
Male	33	ND	Penis, urethritis	MSM



CLINICAL RESULTS *N. gonorrhoeae*

Sex	Age	Coinfection	Specimen type/Clinical notes	Risk factors
Male	27	ND	Rectum	MSM
Male	26	ND	NS	MSM
Male	25	<i>Streptococcus agalactiae</i>	Rectum	MSM
Male	48	<i>Chlamydia trachomatis</i> LGV	Rectum	MSM
Male	33	ND	Urethra	MSM
Male	33	<i>Chlamydia trachomatis</i> <i>Ureaplasma</i>	Rectum, anal sores	MSM
Male	32	ND	Urethra	MSM
Male	28	<i>Ureaplasma</i>	Rectum	MSM
Male	27	ND	Urethra	MSM
Male	32	<i>Ureaplasma</i> HSV-2	Rectum, anal lesions	MSM
Male	41	ND	Rectum	?
Male	24	ND	Urine, penile discharge 4/7	MSM



CONCLUSIONS

- STI's still remain a major public health concern in NSW with notification rates increasing.
- Screening and diagnosis are essential in addressing these increased transmission rates to allow for adequate treatment.
- The Genetic Signatures assay allows for sensitive and specific detection of multiple STI pathogens in a rapid time frame utilizing a semi-automated system that requires minimal hands on time.
- Allowed for consolidation of testing
 - multiple platform testing > single platform
 - Reduction of send away tests
 - TAT reduced



GS ASSAY ADVANTAGES TO DATE

- Additional STI pathogens were identified, even when not suspected by clinicians.
- No need to wait for seroconversion for syphilis diagnosis.
- 22% of *C. trachomatis* from men – LGV (L2b serovar).
- Multiple targets can be screened simultaneously.
- Minimal hands-on time
- Time to result 4-5 hours, runs performed daily, next day results 6 days a week.



ACKNOWLEDGEMENTS

- Jock Harkness
- Debbie Marriott
- Microbiology Department, St. Vincent's Hospital, Darlinghurst, NSW
- John R Melki
- Douglas S Millar
- Jackson Jones
- Genetic Signatures, 7 Eliza Street Newtown, 2042