



**IDENTIFICATION OF 12 STD PATHOGENS IN SEMEN USING
POLYMERASE CHAIN REACTION (PCR) AND
“FLOW-THROUGH” HYBRIDIZATION TECHNOLOGY**

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Sexually transmitted diseases (STD)

- Sexually transmitted diseases (STDs) are caused by several pathogens, including bacteria, viruses and protozoa, and can induce male infertility through multiple pathophysiological mechanisms.
- Sexually transmitted infections (STI)
- Illnesses that have a significant probability of transmission between humans by means of sexual behavior
 - vaginal intercourse
 - anal sex
 - oral sex

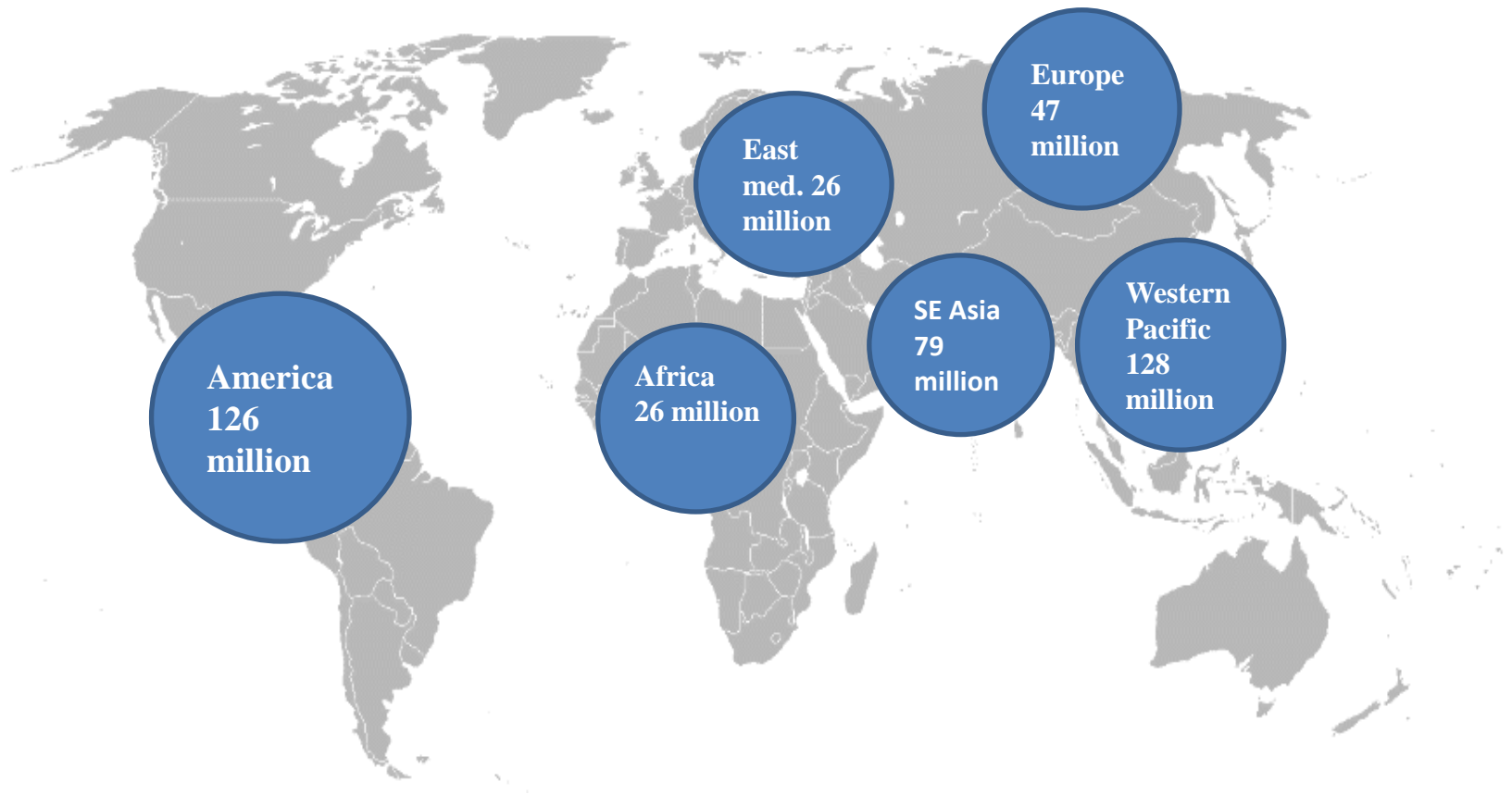
- 19 million new cases of sexually transmitted infections every year in the United State.¹
- In 2005, the World Health Organization estimated that 448 million people aged 15–49 were being infected a year with curable STIs (such as syphilis, gonorrhoea and chlamydia).²
- There were an estimated 34 million people living with human immunodeficiency virus (HIV) in 2010.³

1. Ochsendorf, F. R. Sexually transmitted infections: impact on male fertility. *Andrologia* 40, 72–75 (2008).

2. World Health Organization (WHO) Sexually transmitted infections Fact sheet N°110 August 2011 [Internet] Geneva: WHO; c2013

3. UNAIDS. UNAIDS world AIDS day report 2011 [Internet] Geneva: Joint United Nations Programme on HIV/AIDS (UNAIDS); [cited 2013 Jan 30].

High prevalence of sexual transmitted infections around the globe ¹



STDs can be bacterial, viral and parasitic

- Bacterial

Neisseria gonorrhoeae(NG)

Ureaplasma parvum(UP)

Chlamydia trachomatis (CT)

Mycoplasma genitalium(MG)

Mycoplasma hominis(MH)

Ureaplasma urealyticum(UU)

- Viral

Herpes Simples Virus 1 / 2 (HSV 1, HSV 2)

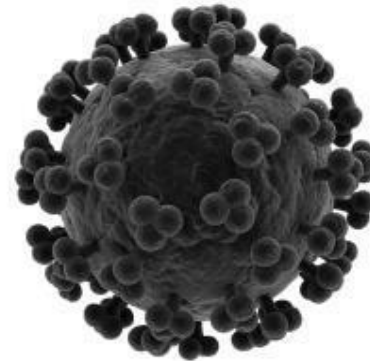
Human Papillomavirus 6 / 11 (HPV 6, HPV 11)

- Parasitic

Trichomonas vaginalis(TV)

STDs can lead to serious outcomes

- Mother to child transmission route is possible.¹
- Emerging trend of drug resistant NG.²
- STDs are associated with HIV infection³




¹Jaiyeoba, O., Amaya, M. I., Soper, D. E., & Kilby, J. M. (2012). Preventing neonatal transmission of herpes simplex virus. *Clinical Obstetrics and Gynecology*, 55(2), 510-520.

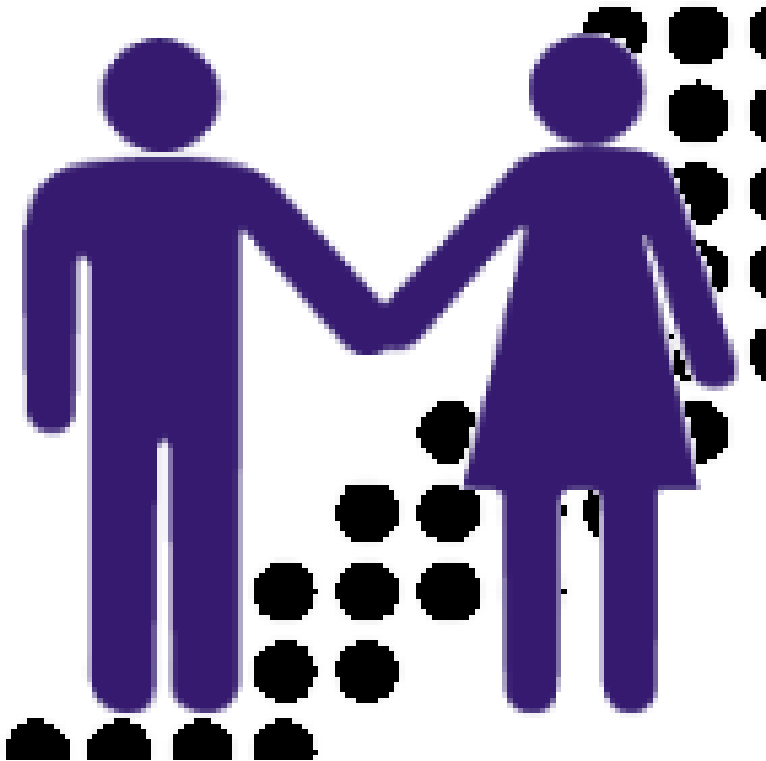
²Unemo, M., & Nicholas, R. A. (2012). Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhoea. *Future microbiology*, 7(12), 1401-1422.

³Nusbaum, M. R., Wallace, R. R., Slatt, L. M., & Kondrad, E. C. (2004). Sexually transmitted infections and increased risk of co-infection with human immunodeficiency virus. *JAOA: Journal of the American Osteopathic Association*, 104(12), 527-535

Reasons to genotype STD pathogens

- 
- Many STDs are asymptomatic
 - Different STDs have overlapping symptoms
 - Different STDs have different treatments
 - Co-infection rate is unexpectedly high

Most STD are asymptomatic



- ~85% of women have asymptomatic CT infection¹
- HPV is the most common asymptomatic STD among younger patients²
- Asymptomatic infection of NG accounts for around 10% in men and 50% in women³

¹Eng, T. R., & Butler, W. T. (Eds.). (1997). *The Hidden Epidemic:: Confronting Sexually Transmitted Diseases*. National Academies Press.

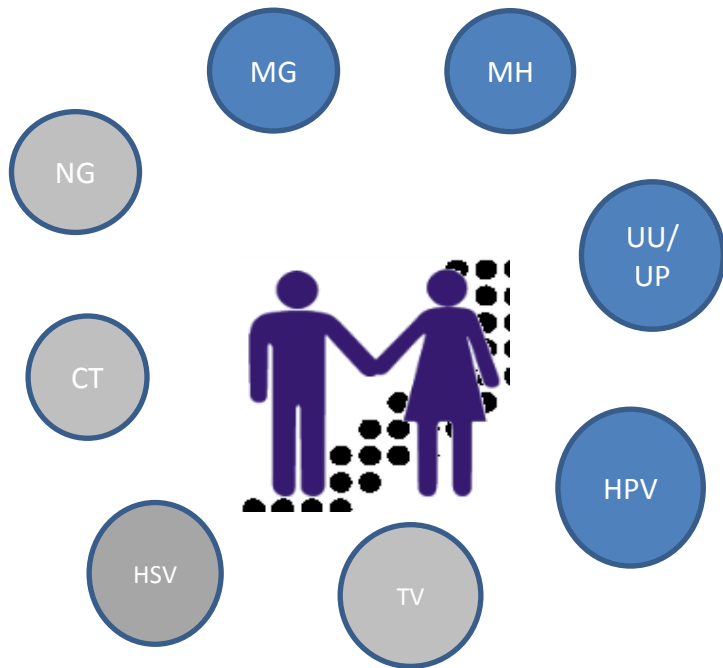
²Lehtinen, M. (2005). Preparations for implementing human papillomavirus vaccination should begin. *Euro Surveill*, 10(9), E050915.

³Shmaefsky, B. R. (2009). *Gonorrhea*. InfobasePublishing

Different STDs share common symptoms

Clinical symptoms	Causative pathogens
Urethritis	<i>N. gonorrhoeae</i> <i>C. trachomatis</i> <i>Mycoplasma genitalium</i> <i>Trichomonas vaginalis</i> HSV 1/2 <i>Ureaplasma urealyticum</i>
Cervicitis	<i>C. trachomatis</i> <i>N. gonorrhoeae</i> <i>M. genitalium</i> HSV 2 Bacterial vaginosis(BV)

STD co-infection: more than you expect



MG: 30.4% with NG¹
•MH: 3.4% with UU²
•UU/UP: High UU-MH co-infection pattern (13.91%)³
•HPV: HSV and CT are associated with HPV infections⁴

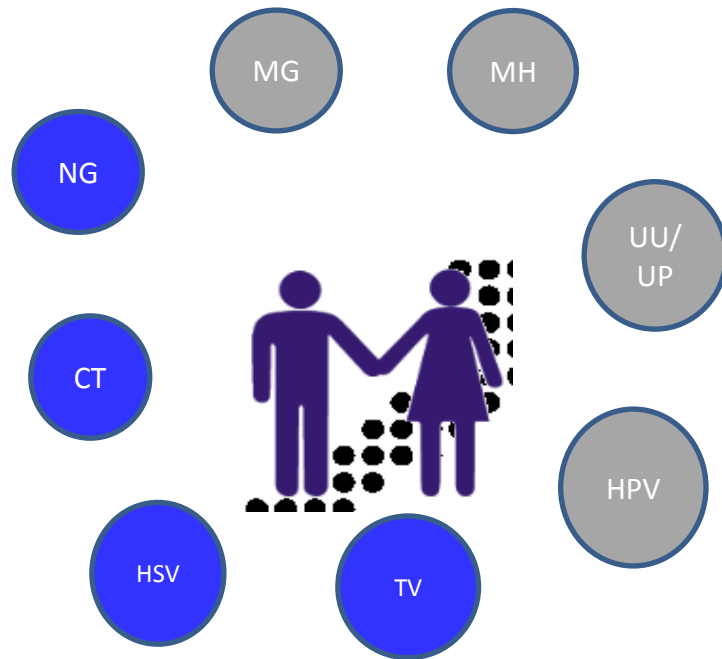
1 Moblely, V. L., Hobbs, M. M., Lau, K., Weinbaum, B. S., Getman, D. K., & Sena, A. C. (2012). Mycoplasma genitalium infection in women attending a sexually transmitted infection clinic: diagnostic specimen type, coinfections, and predictors. *Sexually transmitted diseases*, 39(9), 706-709.

2 Wang, Q.-Y., R.-H. Li, et al. (2014). "Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in female outpatients, 2009–2013." *Journal of Microbiology, Immunology and Infection*.

3 Zhu, Changtai, Liu, Jinming, Ling, Yang, Dong, Chunlei, Wu, Tingting, Yu, Xiaoyuan, . . . Cheng, Xiaowei. (2012). Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in Chinese women with genital infectious diseases. *Indian Journal of Dermatology, Venereology, and Leprology*, 78(3), 406.

4 Alberts, C. J., et al. (2013). "Association of *Chlamydia trachomatis* infection and herpes simplex virus type 2 serostatus with genital human papillomavirus infection in men: the HPV in men study." *Sex Transm Dis* 40(6): 508-515.

STD co-infection: more than you expect



- **TV:** 5-13% with MG¹
- **•HSV 1&2:** 1.4% with CT/NG²
- **•CT:** Among MG positive samples, 25% with CT co-infection³
- **•NG:** Among MG positive samples, 73.3% of female with NG co-infection⁴

1Getman, D. (2014). Prevalence of M Genitalium, T Vaginalis, C Trachomatis and N Gonorrhoeaein Women Enrolled in a Prospective Multi-Center US Clinical Study. 2014 National STD Prevention Conference, CDC.

2Vahidnia, A., et al. (2013). "A retrospective study into the prevalence of herpes simplex virus 1&2 in female patients tested for Chlamydia trachomatis and/or Neisseria gonorrhoeaeusing vaginal swabs." Clinical Microbiology and Infection 19(3): E166-E168.

3 Mobley, V. L., Hobbs, M. M., Lau, K., Weinbaum, B. S., Getman, D. K., & Sena, A. C. (2012). Mycoplasma genitaliuminfection in women attending a sexually transmitted infection clinic: diagnostic specimen type, coinfections, and predictors. Sexually transmitted diseases, 39(9), 706-709

4Gaydos, C., Maldeis, N. E., Hardick, A., Hardick, J., & Quinn, T. C. (2009). Mycoplasma genitaliumas a contributor to the multiple etiologies of cervicitis in women attending sexually transmitted disease clinics. Sexually transmitted diseases, 36(10),

HOST FACTORS

- **AGE:** Highest rate are prevalent in 20-24 yers old & followed by 25-29 & 15-19 years age groups.
- **GENDER:** Morbidity is higher in men.
- **MARITAL STATUS :** Higher among single, divorced and separated persons than among married couples

DEMOGRAPHIC FACTORS

Certain demographic factors contribute to a higher prevalence rates. They are;

- 1. Population explosion.**
- 2. Rural to Urban migration.**
- 3. Increasing educational opportunities for women.**
- 4. Delayed marriage due to the afore said.**

SOCIAL FACTORS

1. Prostitution (good time girl).
2. Broken homes.
3. Sexual disharmony.
4. Easy money.
5. Emotional immaturity.
6. Urbanization.
7. International travel.
8. Changing behavioural patterns.
10. Alcoholism.

OBJECTIVE

Identify the cause of infertility in Male.

- To access in health care facilities for diagnosis and common pathogens of STDs,
- Those causing infertility and see the cause of Chlamydia trachomatis, Neisseria gonorrhoeae, human papillomavirus(HPV) and Mycoplasma hominis transferred in female.
- Genital wart is a highly contagious sexually transmitted disease caused by some sub-types of human papillomavirus (HPV).

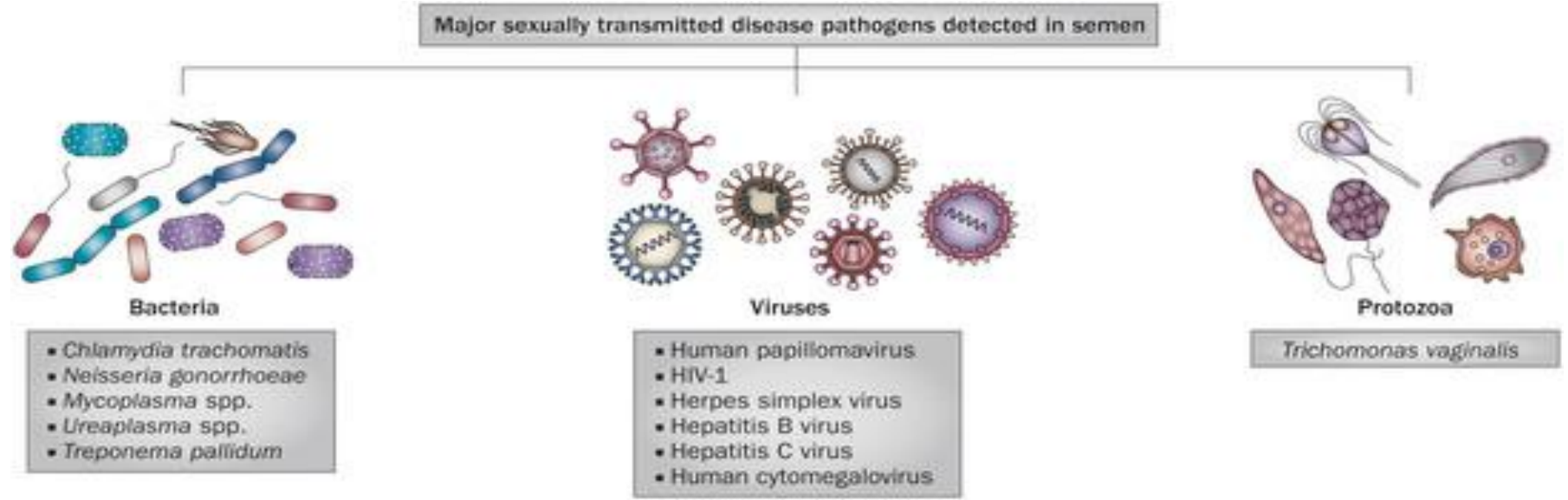
Causes of Infertility

Causes of Male Infertility

- ❖ It affects approximately 7% of all men.
- ❖ Male infertility is commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fertility
- ❖ One third of fertility problems occur solely in women.
- ❖ One third in men

The remaining third are mutual.

Sperm Abnormalities



Major sexually transmitted disease pathogens detected in semen

- Risk factors for male infertility include:
 - Aging, which can reduce sperm counts and motility and decrease the genetic quality of sperm
 - Sexually transmitted diseases, which can cause scarring in the male reproductive system or impair sperm function
 - Lifestyle factors such as smoking and substance abuse
 - Long-term or intensive exposure to certain types of chemicals, toxins, or medications

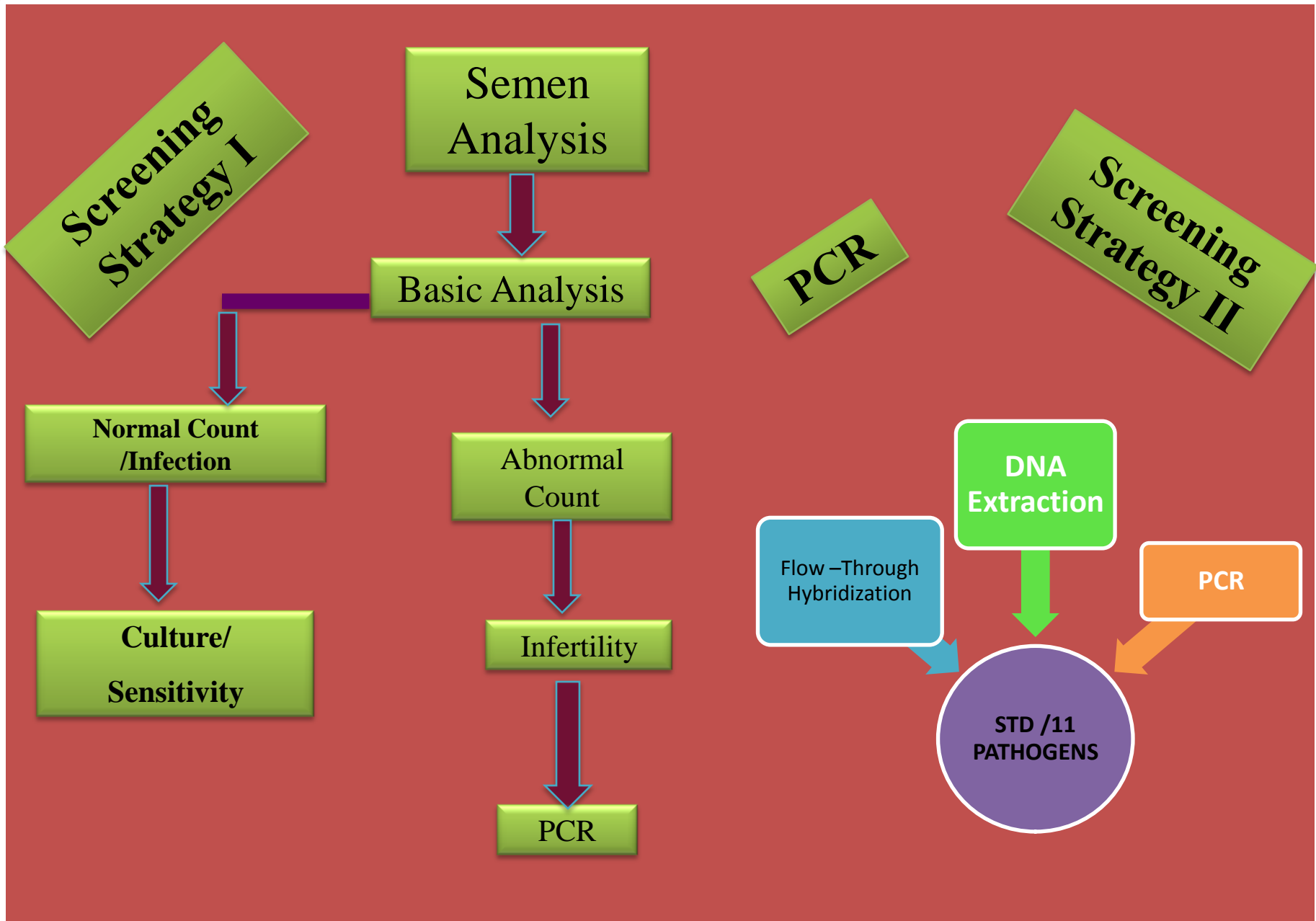
DNA damage

- Common inherited variants in genes that encode enzymes employed in DNA mismatch repair are associated with increased risk of sperm DNA damage and male infertility

Others Causes

- Environmental toxins such as chemicals
- Radiation
- Pollution
- Drugs/ Street drug use
- Stress
- Cigarette smoking
- Frequent sex/ Lifestyle habits
- Heavy metal exposure/ Poor diet
- Overuse of alcohol

Materials and Methods



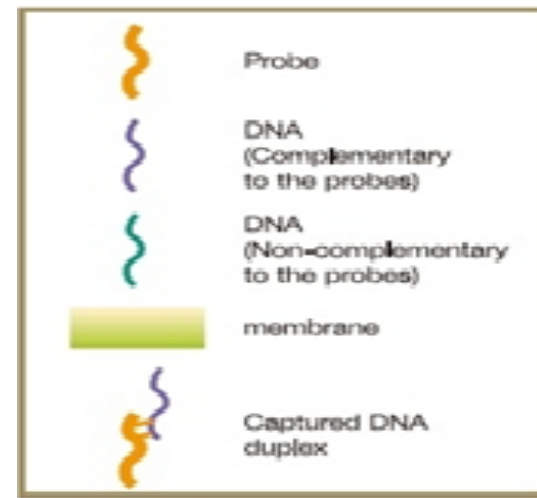
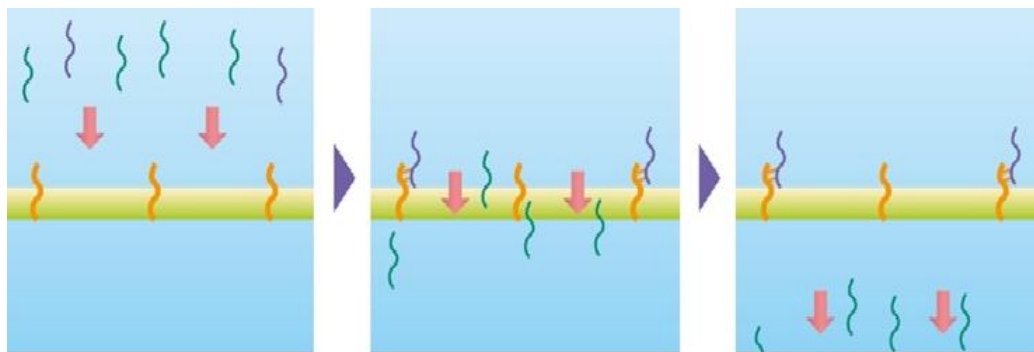
PCR-preparation

Component	Volume (μ l)
PCR premix	18.6
DNA TaqPolymerase (5U/ μ l)	0.4
25 x Primer Mix	01
DNA template	upto 5
Total	25

Thermo cycle

95 °C	10 min	
95 °C	30 sec	
61 °C	30 sec	43 cycles
72 °C	60 sec	
72 °C	7 min	
4 °C	for ever	

- The uniqueness of "Flow-through" hybridization is by directing the amplicons toward the DNA probes to form duplexes
- The "Flow-through" hybridization is changing from traditional passive to active channeling process allowing the recombination reactions to complete in seconds.
- DNA hybridization assays are known to have excellent signal-to-noise ratio.
- It enables unambiguous detection of multiple pathogens/disease genotypes in one single reaction.



Working Protocol for DiagCor STD

Hybridization-at-a-glance

Solution	Volume (μ l)	Incubation	After incubation
43 °C FT ^{pro}			
Pre-hybridization	150	2 min	Drain
Hybridization	150 + 25 PCR product	5 min	Drain
Hybridization solution wash	200 x 3	-	Drain
25 °C			
Blocking	150	5 min	Drain
Enzyme Conjugate	150	5 min	Drain
36 °C			
Wash A solution	200 x 4	-	Drain
Detection Solution	150	3 min	Drain
Wash A solution	200 x 3		Drain
Stop	150	1 min	Drain

Results

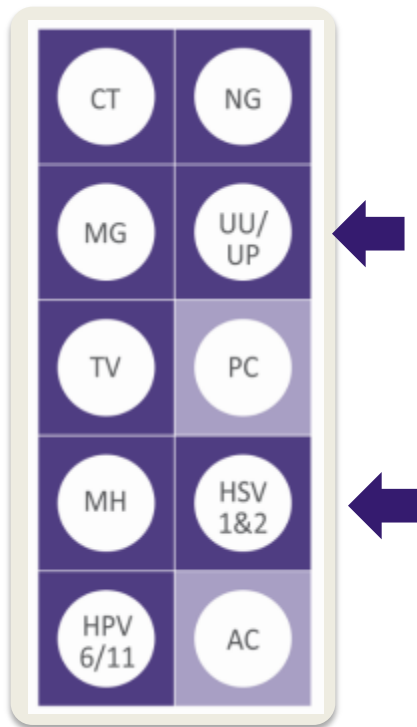
Screening Strategy I

	Rapid progressive	Slow progressive	Non progressive	Immotile	
Motility	Normal	Watery	Viscous		
Viscosity	≤ 20%	≥ 40%	≥ 40		
	Normal Form	Head Defective	Mid Piece & Neck Defective	Tail Defective	Teratozoospermia index
Sperm Morphology	≤ 20	≥30	≥20	≥10	≥2:1
	Debris	Round cell	Epithelial cells	Erythrocytes	
Culture / Sensitivity	Moderate or Heavy	≥ 10 %	≥ 4%	Present	
	Normal	Head to Head	Head to Tail	Tail to Tail	
Agglutination	≤ 10	≥ 20	≥ 10	≥ 10	

GenoFlow STD Array Test - 11 STD pathogens in 1 go

Detect 11 common STD pathogens:

1. *Chlamydia trachomatis* (CT)
2. *Neisseria gonorrhoeae* (NG)
3. *Mycoplasma genitalium* (MG)
4. *Ureaplasma urealyticum* (UU)
5. *Ureaplasma parvum* (UP)
6. *Trichomonas vaginalis* (TV)
7. *Mycoplasma hominis* (MH)
8. Human papillomavirus type 6
9. Human papillomavirus type 11
10. Herpes simplex virus type 1
11. Herpes simplex virus type 2
12. Amplification Control (AC)*
13. Positive Control (PC)*



PCR

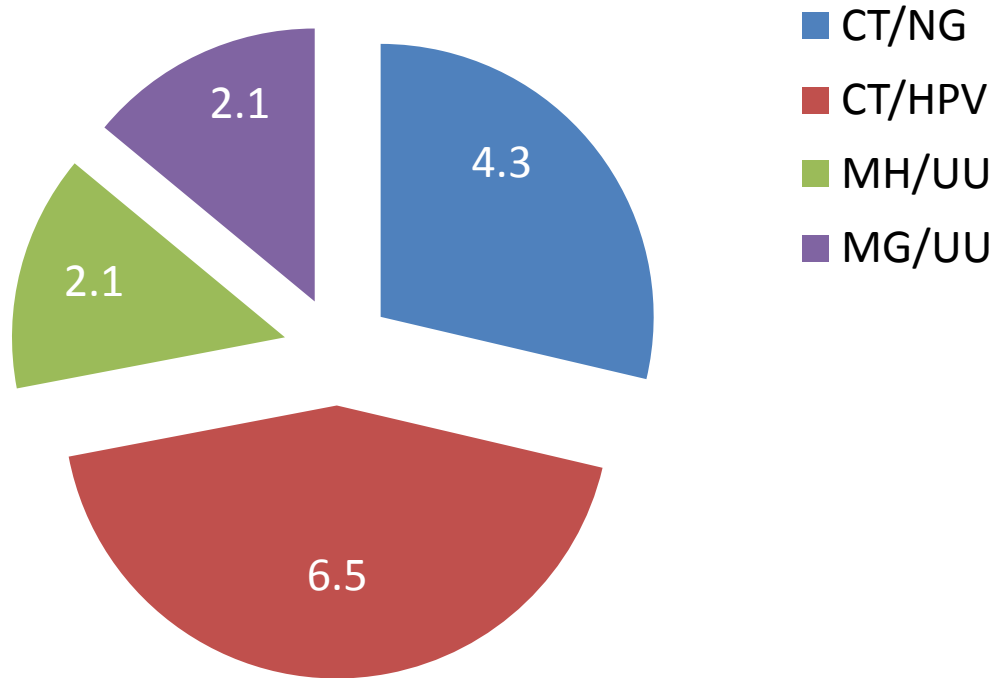


**The Target pathogens detected by Gene Flow - Through
from Semen sample**

The percentage of Target pathogens detected by Gene Flow from Semen sample to see the reason of infertility

NAME OF THE STD (N=46)	DETECTED	PERCENTAGE
Chlamydia trachomatis	02	4.3
Neisseria gonorrhoeae	05	10.8
Mycoplasma hominis	11	23.9
Treponema pallidum	01	2.1
Human Papillomavirus	01	2.1

Co -infection rate



Co- Infection	Percentage
CT/NG	4.3
CT/HPV	6.5
MH/UU	2.1
MG/UU	2.1

Higher infection rate of CT and NG in positive sample

- CT and NG infection: most important STD screening parameters
- NG infection: asymptomatic or often mistaken as vaginal or bladder infection¹. May progress to serious complications as skin pustules or petechial, septic arthritis, meningitis, or endocarditis².
- ~85% of women have asymptomatic CT infection³

¹<http://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm>

²Moran, J. S. (2007). Gonorrhoea. *BMJ Clinical Evidence*, 2007, 1604.

³Eng, T. R., & Butler, W. T. (Eds.). (1997). *The Hidden Epidemic:: Confronting Sexually Transmitted Diseases*. National Academies Press.

CT/HPV co-infection may worsen the clinical outcomes

- Co-infection may prolong both pathogen's infection, and increasing the risk of cervical cancer or infertility¹
- CT/ HPV co-infection may delay HPV lesion clearance^{2,3}
- *Monitoring and control of both pathogens are necessary*
- *The co-infection effects of other STD pathogens are also concerned*

1. Denks, K., Spaeth, E. L., Jöers, K., Randoja, R., Talpsep, T., Ustav, M., & Kurg, R. (2007). Coinfection of Chlamydia trachomatis, Ureaplasma urealyticum and human papillomavirus among patients attending STD clinics in Estonia. *Scandinavian journal of infectious diseases*, 39(8), 714-718.

2. Verteramo, R., Pierangeli, A., Mancini, E., Calzolari, E., Bucci, M., Osborn, J., ... & Degener, A. M. (2009). Human Papillomaviruses and genital co-infections in gynaecological outpatients. *BMC infectious diseases*, 9(1), 16.

3. Simonetti, A. C., de Lima Melo, J. H., de Souza, P. R. E., Brunaska, D., & de Lima Filho, J. L. (2009). Immunological's host profile for HPV and Chlamydia trachomatis, a cervical cancer cofactor. *Microbes and Infection*, 11(4), 435-442.

Conclusion

- STD screening and treatment should be a primary intervention and a standard of care in all health care settings.
- Screening for bacterial STI pathogens, *Mycoplasma hominis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are strongly recommended because these pathogens can cause serious reproductive complications such as pelvic inflammatory disease, ectopic pregnancy.
- Studies show STD and HIV co-infection increases HIV virus shedding in the patients' genital secretions.

- ***STD infection*** rates are common around the globe
- HPV/STD co-infection may ***worsen the clinical outcome***
- ***Close monitoring*** of both HPV and STD infection is necessary
- Cost effect technology should be used

STD
Multiple
infection

HPV/STD
coinfection

Worse
clinical
outcome

Close
monitoring

Cost effect
technology

THANK YOU