GG STD DNA Genotyping Chip Kit

USER'S GUIDE

Goodgene Inc.

Catalog No. GG-CI-002

GG STD DNA Genotyping Chip Kit

Manufacturer: GOODGENE INC.

Address: 11FL., MarioDigital Valley, 222-12,
Guro-Dong, Guro-Gu, Seoul,
152-050, KOREA

Product Name: GG STD DNA Genotyping Chip Kit

GG STD DNA Genotyping Chip Kit

Characteristics and Properties of GG STD DNA Genotyping Chip Kit:

Composition	Characteristics and Properties
A. Goodgene STD DNA Genotyping Chip	A white and blue paper box (29.5 × 21 × 25 ㎝)
A-1. Oligo Chip Slide	A black plastic box (9.3 × 8.0 × 3.2 cm)
① GG STD DNA Genotyping Chip	A glass slide (25 × 75 × 1 mm) to which a cover well was adhered for silicon hybridization reaction
A-2. STD Chip Reaction Solution	A silver bag (8 × 11 cm)
① Hybridization Reaction Solution (HYB I Buffer)	15 ml of transparent solution in a translucent plastic bottle
② Washing buffer S1 (Buffer S1)	25 ml of transparent solution in a translucent plastic bottle
③ Washing buffer S2 (Buffer S2)	10 ml of transparent solution in a translucent plastic bottle
A-3. Manual	Print (14.8 × 21 cm)
B. PCR Premix	A white and a blue paper box (13 × 13 × 9 cm)
B-1. STD PCR Premix	15 ㎕ of blue solution in a translucent tube
C. STD PCR Kit	A silver bag (8 × 11 cm)
C-1. Primer A1 for PCR A	Colorless solution in a translucent tube
C-2. Primer A2 for PCR A	Colorless solution in a translucent tube
C-3. Primer B1 for PCR B	Colorless solution in a translucent tube
C-4. Primer B2 for PCR B	Colorless solution in a translucent tube
C-5. Positive Control A for PCR A	20 ㎕ of transparent solution in a translucent tube
C-6. Positive Control B for PCR B	20 ㎕ of transparent solution in a translucent tube
C-7. Negative Control	20 ㎕ of transparent solution in a translucent tube
C-8 Cy5-labeled HBB Control DNA	230 ㎕ of transparent solution in a brown plastic bottle

GG STD DNA Genotyping Chip Kit

Composition of GG STD DNA Genotyping Chip Kit: 96 tests/kit

Item	Storage	Expire date
A. STD DNA CHIP	Room temperature	3 months
A-1. Oligo Chip	Dehumidity &	
- GG STD DNA CHIP	Room temperature	3 months
A-2. STD Chip Reaction Buffer		
-Hybridization Buffer (HYB I Buffer)	Room temperature	3 months
-Washing Buffer S1 (Buffer S1)	Room temperature	3 months
-Washing Buffer S2 (Buffer S2)	Room temperature	3 months
A-3. User Guide	Room temperature	1 year
B. PCR Premix	-20 ℃	6 months
C. STD PCR Kit	-20 ℃ (Dark room)	3 months
C-1. A1 Primer	-20 ℃	3 months
C-2. A2 primer	-20 ℃	3 months
C-3. B1 Primer	-20 ℃	3 months
C-4. B2 Primer	-20 ℃	3 months
C-5. Positive Control A	-20 ℃	3 months
C-6. Positive Control B	-20 ℃	3 months
C-7. Negative Control	-20 ℃	3 months
C-8. Cy5-labeled HBB Control DNA	-20 ℃ (Dark room)	3 months

GG STD DNA Genotyping Chip Kit

Intended Use: ***For In Vitro Diagnostic Use***

STD Genotyping DNA Chip Kit is an oligonucleotide chip designed to identify 9 STD (Sexually Transmitted Diseases) genotypes.

Product Description:

The Sexually Transmitted Diseases (STD) is more than 25 diseases, it is spread primarily through sexual activity, and the trends for each disease vary considerably, but together these infections comprise a significant public health problem.

Company's STD Genotyping Chip kit is a DNA chip that can analyze a total of 9 STD genotypes, such as *Neisseria gonorrhea (NG), Chlamydia trachomatis (CT), Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), Mycoplasma hominis (MH), Trichomonas vaginalis (TV), , Herpes simplex virus type 1 (HSV1), Herpes simplex virus type 2 (HSV2)*, through hybridization of each types of specific oligo probe and each specific gene region that has been enhanced with PCR that are fixed to slides.

Principle of the assay

The GG STD DNA Genotyping Chip Kit detects and identifies 9 types of STD pathogens: *N. gonorrhoeae, C. trachomatis, U. urealyticum, M. genitalium, M. hominis, Herpes simplex virus types 1* & *2, T. pallidum* and *T. vaginalis*. The GG STD Genotyping Chip Kit utilizes a oligonucleotide chip method. To detect STD genotypes, DNA is extracted from Urine, Vaginal Swab, Urethral Swab, EPS, or Semen. Next, the target DNA, which reacts to the probe, is amplified by using a labeled STD multiplex primer along with Cy5, a fluorescent marker. Labeled target DNA is then hybridized under specific conditions with Cy5, after this hybridization step, unhybridized DNA is washed from the slide, and is checked for hybridization using a fluorescent scanner (Genepix 4000B/AXON or EasyScan-1/Goodgene). The resulting spot intensity is measured. STD Genotypes are determined by comparing the spot patterns on the slide to the patterns interpretation chart.

Description of Symbols used

The following are graphical symbols used in or found on Company Corporation products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard BS EN 980:1997.

Do not reuse

Use by
Synonym for this:
Expiry Date

Catalogue Number

Temperature Limitation

Manufactured by

Contains sufficient for <n>
Tests

In vitro diagnostic
Medical device

Batch code
Synonym for this are:
Lot / Batch Number

Attention.
See Instruction for use

Upper Limit of Temperature

Date of Manufacture

GG STD DNA Genotyping Chip Kit

1. Introduction

The Biological Understanding of STD

More than 25 diseases are spread primarily through sexual activity, and the trends for each disease vary considerably. Collectively, these infections comprise a significant public health problem. While some infection rates, such as syphilis, are on the decline, others, like genital herpes, gonorrhea, and chlamydia, are on the rise and spreading at a rapid pace through the population. Because there is no single STD epidemic, but rather multiple epidemics, discussions about trends over time and populations affected must focus on each specific pathogen. Even though many STDs are difficult to track, we now know more about the frequency and trends of some sexually transmitted diseases than ever before.

What are the most serious STDs in women?

By far, women bear the greatest burden of STDs, suffering more frequent and more serious complications than men. Ten to 20 percent of women with gonorrhea and chlamydia develop one of the most serious complications, pelvic inflammatory disease (PID). PID can lead to chronic pelvic pain, infertility, and potentially fatal ectopic pregnancy. Many different organisms can cause PID, but most cases are associated with gonorrhea and chlamydia. In addition, women who are infected with an STD while pregnant can have early onset of labor, premature rupture of the membranes, or uterine infection before and after delivery. STD-related syndromes—like bacterial vaginosis—may cause harm to infants through their association with premature birth. It is estimated that 30 to 40 percent of excess preterm births and infant deaths are due to STDs and bacterial vaginosis (Goldenberg, 1996).

Urethritis, an infection often acquired via sexual contact, is categorized into gonococcal urethritis or nongonococcal urethritis (NGU) depending on the presence or absence of N. gonorrhoeae. In NGU, the infection is attributed to the pathogenic role of C. trachomatis, which has been detected in 30-40% of men with NGU (Taylor-Robinson, D. 1996. Sex. Transm. Dis. 23:86-91), or to other pathogens, including mycoplasma and ureaplasma, in chlamydia negative patients.

Neisseria gonorrhoeae

Gonorrhea is a bacterial infection of the genital tract causing a wide range of symptoms in males and females. Many carriers can remain asymptomatic allowing for the continued spread of the disease. The etiological agent, *N. gonorrhoeae*, readily infects the mucosal membranes of the lower genital tract and other sites, which can evolve into pelvic inflammatory disease affecting fertility in women, and arthritic conditions in both sexes. Culturing *N. gonorrhoeae* requires special growth conditions and transport media, and directs detection assays may not yield the required sensitivity to identify low-level infections. The polymerase chain reaction (PCR*)

assay vastly improves the ability to identify infected individuals, especially asymptomatic carriers, allowing for the commencement of antibiotic therapies to treat low level, early stage infection.

Chlamydiae trachomatis

Chlamydia is the most commonly reported infectious disease in the Korea and may be one of the most dangerous sexually transmitted diseases among women today. While the disease can be easily cured with antibiotics, millions of cases go unrecognized. If left untreated, chlamydia can have severe consequences, particularly for women. Up to 40 percent of women with untreated chlamydia will develop pelvic inflammatory disease (PID), and one in five women with PID become infertile. Chlamydia also can cause prematurity, eye disease, and pneumonia in infants. Moreover, women infected with chlamydia are three to five times more likely to become infected with HIV, if exposed.

Ureaplasma urealyticum

The *Ureaplasma urealyticum* circular genome of 0.760 Mb was sequenced in 1999 by the University of Alabama at Birmingham, Department of Microbiology. The key publication describing this sequenced genome is "The complete sequence of the mucosal pathogen *Ureaplasma urealyticum*" by J.I. Glass, E.J. Lefkowitz, J.S. Glass, C.R. Heiner, E.Y. Chen and G.H. Cassell, Nature 407: 757-762, 2000. Approximately 124 additional references pertaining to Ureaplasma species can be extracted from the Molecular Subset of MEDLINE (*U.urealyticum* References).

The *Ureaplasma urealyticum* is the smallest bacteria replicating in culture medium and do not possess peptidoglycan cell walls. This tiny microorganism can be found commensal in lower genitourinary tracts of sexually active men and women. Moreover, it cause many disorders such as NGU, postpartum fever, infertility, and pelvic inflammatory disease (McCormack, WM et al., (2000): Urethritis; Waites, KB et al., (1999): Mycoplasma & ureaplasma; Ullmann, U et al., J. Antimicrob. Chemother 43:33-36)

Mycoplasma genitalium

M. genitalium is a Gram-positive parasitic bacterium. The urogenital tract may be its primary infection site and it probably causes non-gonococcal urethritis. It adheres to host cells by means of adhesion proteins. Mycoplasmas are the smallest known organisms capable of growth and reproduction outside living host cells. Like all mycoplasmas, M. genitalium lacks a cell wall, have a very small genome (*M. genitalium* in particular has the smallest genome of known cellular organisms capable of independent replication) and a low G+C content (32%). UGA, normally a stop codon, in this organism encodes for the amino acid tryptophan.
Mycoplasma genitalium was first isolated from men with non-gonococcal urethritis (NGU) more than 20 years ago. Use of polymerase chain reaction technology has shown it to be a cause of acute NGU and probably chronic NGU, almost independently of *Chlamydia trachomatis*, but there is no substantial evidence that it causes acute or chronic prostatitis. In women, *M. genitalium* is not associated with bacterial vaginosis, but it is strongly associated with cervicitis and endometritis and serologically with salpingitis and tubal factor infertility. Further studies may show *M. genitalium* to be associated, perhaps causally, with epididymoorchitis, neonatal disease and reactive arthritis. Furthermore, its potential for enhancing HIV transmission needs to be explored. *M. genitalium* is susceptible to various broad-spectrum antibiotics, but *M. genitalium*-associated diseases are probably best treated with azithromycin
Genome length: 0.58 Mb

References:
Fraser *et al.* (1995). Science 270:397-403

Mycoplasma hominis

Current information on the role played by *Mycoplasma hominis* in placental inflammation was reviewed. *M. hominis* is associated with chorioamnionitis and funisitis, but the clinical significance of this association in not clear. Further research on the role of the organism in perinatal disease is needed. Meanwhile, in a gravely ill

infant with a history of prolonged rupture of the placental membranes, chorioamnionitis, and funisitis, *M. hominis* should probably be considered a potential pathogen, cultures for this organism should be performed, and therapy with an antibiotic regimen effective against *M. hominis* should be instituted.

Treponema pallidum

Development of DNA-based technologies for syphilis diagnosis will help in a couple of ways: detection of the etiologic agent in genital ulcers, through use of multiplex panels, and detection of *T. pallidum* m tissues especially cerebrospinal fluid (CSF), m order to help make more accurate diagnosis of neurosyphilis.
The DNA sequence of *T. pallidurn* was completed very recently. One hopes that analysis of the genomic sequence will lead to insights about the physiology of the organism, and therefore to solutions of the very old problem of in vitro cultivation outside of animals. Availability of the DNA sequence will predictably enable the development of molecular strain typing tools, which has been entirely lacking in syphilis research until now.

Herpes Simplex Virus

Genital herpes can be caused by either *herpes simplex virus type 1* (HSV-1) or type 2 (HSV-2). The pathogenesis of HSV genital infection is complex and involves primary, latent and recurrent infections (Stanberry, 1996). The primary infection begins with the sexual transmission of HSV to anogenital sites. The virus replicates at these mucocutaneous sites but also spreads via sensory nerve fibers beyond the portal of entry to sacral dorsal root ganglia. Once in the ganglia, HSV actively replicates in some neurons while establishing a lifelong latent infection in other neurons. After recovery from the primary infection, latent infection can reactivate and spread back along sensory nerve fibers to mucocutaneous sites to cause recurrent anogenital HSV infections. Genital HSV infections, both primary and recurrent, can produce recognizable signs and symptoms or can be asymptomatic. During the infection, whether symptomatic or not, virus is

present in the genital tract and thus there is the potential for the spread of virus to susceptible sexual partners or, in the case of the pregnant woman, to her fetus or newborn infant.

The incubation period for primary genital herpes ranges from 2 to 20 days, with an average of 6 days (Wald and Corey, 1997). For symptomatic primary infection the clinical signs and symptoms may range from very mild to extremely severe. Individuals may experience itching, tingling, burning and pain in the anogenital area. Some develop classic vesicular ulcerative lesions. On dry skin the lesions may begin as small clear vesicles with an erythematous base.

These may persist for several days. When the thin skin covering the vesicle is lost the lesions become shallow yellow-gray ulcers, which eventually crust over; healing occurs with loss of the crust. On chronically moist tissues vesicles are very short-lived and rapidly progress to the ulcer stage. Crusts rarely develop: rather, healing occurs as the ulcer re-epithelializes. It takes 7-10 days for progression from vesicle to complete healing, but during the primary infection individuals typically develop new crops of vesicles for the first 1-2 weeks of the illness. Consequently, the average duration of lesions, from first vesicle to complete healing, are 16 days for males and 20 days for females in subjects experiencing classic genital herpes (Wald and Corey, *1996;* Brown *et al.*, 1979).

Other local symptoms may include urethritis, dysuria and lymphadenopathy. It should be noted that there is great variability in the course of primary genital herpes. In some the infection may be so mild as to not be recognized as genital herpes, whereas in others the classic lesions may last for more than 35 days (Wald and Corey, 1996; Benedetti *et al., 1994).*

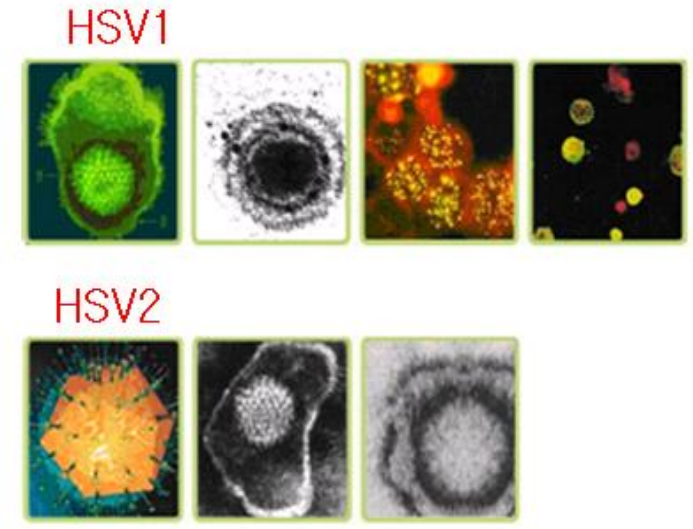
During classic primary infection approximately 40% of males and 70% of females also experience fever, headache and myalgias. This flu-like illness typically lasts 2-7 days. About 10% of males and 40% of females will also mild or non-classic as to be unrecognized as genital herpes. Recurrent genital herpes can be mistaken for a variety of cutaneous and urogenital disorders.

The number of symptomatic recurrences experienced by an individual varies greatly from person to person. Three factors- gender, virus type and severity of the primary infection - somewhat predict the frequency of recurrent infections (Benedetti *et al.*, 1994; Reeves *et al.* 1981a). Males tend to have recognizable recurrences more frequently than do females. Individuals who have primary genital herpes caused by HSV-1 are less likely to experience recurrent infections and have fewer recurrences than do those with genital HSV- 2 infection. People who have very severe primary infection lasting longer than 35 days also tend to have more recurrences (Benedetti *et al.*, 1994).

The risk of asymptomatic shedding was greater in subjects infected with HSV-2 than HSV-1, and in those experiencing frequent symptomatic recurrences. Also, subjects with newly acquired infections were more likely to shed HSV than those who had been infected for more than 1 year. The high frequency with which HSV-2 is shed from the genital tract supports the opinion that asymptomatic or unrecognized shedding is responsible for most perinatal and sexual transmission of HSV-2 (Corey, 1998; Mertz *et al.*, 1985). This observation has important implications for the development of vaccines to control genital herpes.

Herpes simplex viruses types 1 and 2 are members of the Herpesviridae family of viruses. The HSV virion has a dense core surrounded by three layers: an icosahedral protein capsid, an amorphous protein layer called the

tegument, and an outer envelope studded with glycoproteins. The core contains a linear double-stranded DNA genome of about 150 kilobase pairs which encodes at least 81 genes (Whitley and Roizman, 1997). Approximately 50% of the HSV-1 and HSV-2 DNA sequences are homologous, and the two viruses are closely related both genetically and biologically (Stanberry *et al.*, 1997).



2. The Basic Principle of the DNA Chip

2.1. The Basic Understanding of the DNA Chip

The principle of the DNA chip is based on reverse hybridization. In 'Southern Blot Hybridization' that has been traditionally used, the probe, made on the basis of known genetic information, is made to react to the DNA of the specimen, separated by gel electrophoresis, is fixed to another membrane, to found out the sequence of the complementary DNA in the specimen.

On the other hand, in reverse hybridization, the oligonucleotide DNA made on the basis of known genetic information or the probe made by using cDNA is fixed to a hard plate like a glass plate or a semiconductor, and fluorescent material or a radio isotope is fixed to the target DNA (the DNA amplified by mRNA or PCR) extracted from the specimen. On the basis of the principle that hybridization reaction is caused only in the probe that shows the sequence of complementary DNA, qualitative and quantitative analyses are performed.

2.2. The Basic Principle of the Product

Common causative organisms of sexually transmitted diseases (STD) include *Neisseria gonorrhea (NG), Chlamydia trachomatis (CT), Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), Mycoplasma hominis (MH), Trichomonas vaginalis (TV), Treponema pallidum (TP), Herpes simplex virus type 1 (HSV1) and Herpes simplex virus type 2 (HSV2),* all of which are difficult to detect by conventional culture or immunologic assay. STD commonly show mixed infection. Therefore, we need a new molecular test which can reliably detect all the major causative organisms of STD. We herein have developed a prototype oligonucleotide microarray to detect genotype of 9 types and evaluated its diagnostic value.

GG STD Genotyping Chip® is the first DNA chip for double typing 9 STD types. Genomic DNAs of NG, CT, UU, MG, MH, TV, TP, HSV type1 and HSV type2 were isolated from samples of voided urine or cervical swab from adults (about 37,853) attending STD clinics in Seoul, Korea and their genotypic information were analyzed by PCR, cloning and sequencing. In addition, plasmid DNA library of genomic DNA of each organism was established.

Multiple oligonucleotide probes were designed for gene of 16S rRNA, antigen gene, repeated target gene, 16-23S intergenic region, cryptic plasmid, and human beta-globin gene, and were spotted onto microscopic glass slide to produce DNA chip of STD.

The grid of this chip was designed to be able to analyze eight different samples on a single chip.

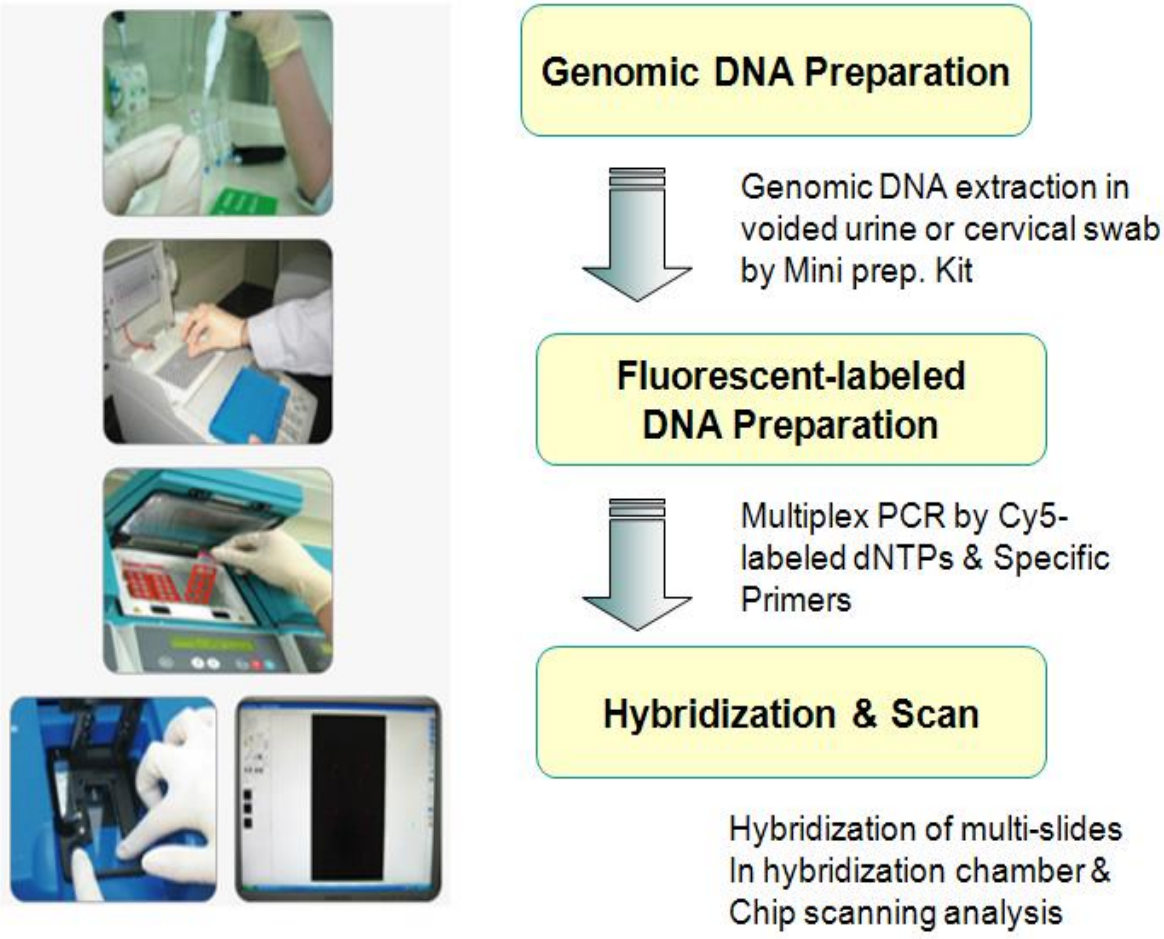
GG STD DNA Genotyping Chip Kit

PCR products from plasmid clones and clinical samples containing DNA of NG, CT, UU, MG, MH, TV, TP, and HSV, and urine samples from adult men without evidence of STD were applied onto the DNA chip and hybridization reaction were carried out, which were then analyzed by fluorescence scanner.

The working principle of the GG STD DNA genotyping Chip® is the hybridization of a fluorescent-labeled STD type specified target to the GG STD DNA genotyping Chip®, carrying STD specific DNA-probes. Before hybridization, STD specific targets are amplified in a multiplex PCR, using STD type specific primer pairs.

The final step comprises scanning and evaluation of the GG STD DNA genotyping Chip®. The GG STD DNA genotyping Chip® is spotted on a coated glass-slide (25mm x 75mm x 1mm), which fits into most commercial scanner. The GG STD DNA genotyping Chip® innovative design allows the control of all working steps to avoid false positive or false negative results. The GG STD DNA Genotyping Chip® kit is ready to use. Each kit includes 8 tests on one chip slides and all necessary solutions.

3. Summary of Usage



3.1. Genome DNA should be extracted from clinical specimens by using DNA Extraction Mini Kit (not supplied)

3.2. On the basis of the genome DNA extracted by using primer A1 & A2 (PCR A), the primer B1 & B2 (PCR B), PCR premix and target DNA should be amplified through chain reaction (PCR).

3.3. Hybridization reaction should be performed under proper conditions by using GG STD DNA Genotyping Chip.

3.4. It should be scanned by using GenePix 4000B (Axon, USA).

4. Protocol and Procedure

4.1. Extracting Genome DNA from Specimens – Intron Kit (not supplied)

Extracting Genome DNA from the Specimens (the Urine, Vaginal Cervix Swab, Urethral Swab, EPS, and Semen).

- **Test Preparation (Intron Kit)**

1) Set the water bath (or heat block) to 65℃.
2) This protocol is for isolation of genomic DNA from 1 to 1.5 milliliters of specimen solution ($5\times10_6$ cells).
3) Add 280ml of 100% ethanol to buffer UB (concentrated) and stor at room temperature. After it was opened, the lid should be closed so that ethanol cannot be vaporized.
4) Add 320ml of 100% ethanol to buffer UWA (concentrated) and store at room temperature. After it was opened, the lid should be closed so that ethanol cannot be vaporized.
5) The above reagents can be stored for a year on the premise that those are stored in a darkroom at room temperature. The storage period is not influenced by adding additives.
6) Store Proteinase K at -15 ~ -20℃ and can be stored for a year
7) Necessary reagents and equipments are not provided:
   - 1×PBS, 100% ethanol, centrifuge, vortex, and water bath.

- **Test Method**

➢ **If, Urine:**

1) Centrifuge the 15~50 ml of urine at 3,000 rpm for 15 min.
2) Discard the supernatant and re-suspend the pellet in 400㎕ of PBS.
3) Transfer solution into a sterile 1.5 ml micro-centrifuge tube and centrifuge at full speed for 2 min.
4) Continue to protocol 3.

➢ **If, Other Samples (Vaginal Swab, Urethral Swab, EPS, Semen and so on):**

1) Pipet 1.5 ml of specimen solution into the 1.5ml microcentrifuge tube (not provided) , centrifuge at 12,000 rpm for 2 minutes.

2) Remove supernatant and resuspend the pellet in 100 ㎕ of 1xPBS by pulse-vortexing.

3) Add 200 μℓ of Buffer UG.5. Add 10 μℓ of Proteinase K and 5 μℓ of RNase A solution and mix well vortex.
4) Incubate at 65℃ for 10 minutes and briefly spin down.
5) Note: For complete lysis, mix 5-6 times during incubation by inverting tube.
6) After lysis completely, add 250 μℓ Buffer UB to the lysate, and mix well by pipetting or gently inverting 5 to 6 times. DO NOT vortex.
7) Briefly centrifuge at 6,000xg for 10 seconds to remove drop from inside the lid.
8) Add 250 μℓ of 80% ethanol to the lysate and mix well pipetting or gently inverting 5 to 6 times. DO NOT vortex.
9) Briefly centrifuge at 6,000xg for 10 seconds to remove drop from inside the lid.
10) **Note**: It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA.
11) Carefully transfer lysate (800 μℓ) from step 10 to a spin column (in collection tube) without wetting the rim and centrifuge at 13,000 rpm for 1 minute on RT. Place the column in a clean collection tube, and discard the collection tube containing the flow-through.
12) Add 700 μℓ of Buffer UWA into the spin column and centrifuge at 13,000 rpm for 1 minute. Place the column in a clean collection tube, and discard the collection tube containing the flow-through.
13) Add 700 μℓ of Buffer UWB into the spin column and centrifuge at 13,000 rpm for 1 minute.
14) Discard the filtrate and centrifuge again for 1 minute to dry the membrane. Discard the filtrate and collection tube altogether.
15) **Note**: It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from collection tube without contacting with the filtrate, since this will result in carryover of ethanol.
16) Place spin column in a new 1.5 ml tube (not supplied) and add 50 μℓ of Buffer UE or ultra pure water to the center of column and incubate at RT for 1 minute.
17) Centrifuge at 13,000 rpm for 1 minute to elute.
18) Genome DNA can be used to PCR immediately after extraction, and can be stored at -20℃ for a long time.

4.2. Extracting Genome DNA from Specimens- LaboPass Kit (not supplied)

- **Test Preparation**

1) Set the water bath (or heat block) to 65℃.
2) This protocol is for isolation of genomic DNA from 1 to 1.5 milliliters of specimen solution ($5\times10_6$ cells).
3) Add 93ml 100% ethanol to buffer BW (concentrated) and store at room temperature.
4) Add 92ml 100% ethanol to buffer NW (concentrated) and store at room temperature.
5) If Buffer TB contains precipitates, dissolve by heating to 60℃ with gentle agitation.
6) The above reagents can be stored for a year on the premise that those are stored in a darkroom at room temperature. The storage period is not influenced by adding additives.
7) Store Proteinase K at -15 ~ -20℃ and can be stored for a year
8) Necessary reagents and equipments are not provided:
   - 1×PBS, 100% ethanol, centrifuge, vortex, and water bath.

- **Test Method**

➢ If, Urine:

1) Centrifuge the 15~50 ml of urine at 3,000 rpm for 15 min.
2) Discard the supernatant and re-suspend the pellet in 400㎕ of PBS.
3) Transfer solution into a sterile 1.5 ml micro-centrifuge tube and centrifuge at full speed for 2 min.
4) Continue to protocol 3.

➢ If, Other Samples (Vaginal Swab, Urethral Swab, EPS, Semen and so on):

1) Pipet 1.5 ml of specimen solution into the 1.5ml microcentrifuge tube (not provided), centrifuge at 12,000 rpm for 2 minutes. Remove supernatant and resuspend the pellet in 100 ㎕ of 1xPBS by pulse-vortexing.
2) Centrifuge at 13,000±100 rpm for 2 minutes, and remove supernatant.
3) Add 200 microliters buffer TL and briefly spin down. (if uric acid or semen sample, adds 300 microliters buffer TL).
4) Add 20 microliters proteinase K and mix by vortex.
5) Incubate at 65℃ for 10 - 30 minutes.
6) Briefly centrifuge at 13,000±100 rpm for 10 seconds to remove drop from inside the lid.

7) Add 400 microliters buffer TB and mix well. (if uric acid or semen sample and blood sample, adds 600 microliters buffer TB and add 200 microliters ethanol, respectively)
8) Centrifuge at 13,000±100 rpm for 10 seconds to remove drop from inside the lid.
9) Carefully transfer lysate from step 9 to a spin column (in collection tube) without wetting the rim.
10) Centrifuge at 13,000±100 rpm for 1 minute.
11) Place the column in a clean collection tube, and discard the collection tube containing the flow-through.
12) Add 700 microliters buffer BW and centrifuge at 13,000±100 rpm for 1 minute.
13) Place the column in a clean collection tube, and discard the collection tube containing the flow-through.
14) Add 500 microliters buffer NW and centrifuge at 13,000±100 rpm for 3 minutes.
15) Place the column in a clean collection tube (not provided)
16) Add 60 microliters autoclaved ultra pure water to the center of the membrane and incubate at Room Temperature (15 ~25℃)for 2 minutes.
17) Centrifuge at 13,000±100 rpm for 3 minutes.
18) Genome DNA can be use for PCR immediately after extraction or can be store at -15 ~ -20℃ for 3 months.

Note: Genome DNA can be observed under UV by electrophoresing at 3% agarose gel immediately after extraction. The ratio of the 260 nm and 280 nm absorbance reading should be greater than 1.5 (approximately ≤ 50ng/μℓ) and electrophoresing at 3% agarose gel immediately after extraction.

4.3. PCR for STD Chip Reaction

- Test Preparation

1) The expiration date of the premix is valid for a year under the condition of -20℃, but the wall of the tube may be frosted during storage. In that case, it should be centrifuged at 6,000xg for 30 seconds and the premixed reagent should be get together to the bottom of the tube so that it can be used.
2) Necessary reagents and equipments are not provided:
   - Autoclave ultra pure water, 1mM Cy5-dCTP and PCR instrument (T3, Biometra, Germany or GeneAmp2720, Appliedbiosystem, USA)

➢ Mixture PCR A (5 Plex)

Prepare the PCR solution using the table below.

Additive Reagents	Dosage (per reaction)
Premix	15 ㎕
Ultra Pure Water	6 ㎕
Primer A1	1 ㎕
Primer A2	1 ㎕
0.05nM Cy5-dCTP	2 ㎕
Template Genome DNA	5 ㎕

Note: In case of positive control, use 1 ㎕ of positive control DNA (supplied).

1) Prepare premix on ice.
2) Add autoclaved ultra pure water to the 1.5-ml master mix tubes as much as those need.
3) Add primer sets (A1 and A2) and 0.05nM Cy5-dCTP to master mix tube as many as those need, and mix well.
4) The mixtures, prepared in the above process, add 10 microliters to the premix tube
5) Add 5 microliters of templates to premix tube and mix well.
6) Centrifuged at 13000 rpm for 10 seconds to remove drop from inside the lid and reacted in the PCR instrument as in the following.

Process	Temperature	Time	Cycle
Pre-denaturation	95℃	10 min	1
Denaturation	95℃	30 sec	40
Annealing	58℃	30 sec	
Extension	72℃	30 sec	
Final - Extension	72℃	7 min	1

➢ Mixture PCR B (4 Plex)

Prepare the PCR solution using the table below.

.

Additive Reagents	Dosage (per reaction)
Premix	15 ㎕
Ultra Pure Water	6 ㎕
Primer B1	1 ㎕
Primer B2	1 ㎕

0.05n Cy5-dCTP	2 ㎕
Template Genome DNA	5 ㎕

Note: In case of positive control, use 1 ㎕ of positive control DNA (supplied).

1) Prepare premix on ice.
2) Add autoclaved ultra pure water to the 1.5-ml master mix tubes as much as those need.
3) Add primer sets (A1 and A2) and 0.05nM Cy5-dCTP to master mix tube as many as those need, and mix well.
4) The mixtures, prepared in the above process, add 10 microliters to the premix tube
5) Add 5 microliters of templates to premix tube and mix well.
6) Centrifuged at 13000 rpm for 10 seconds to remove drop from inside the lid and reacted in the PCR instrument as in the following.

Process	Temperature	Time	Cycle
Pre-denaturation	95℃	10 min	1
Denaturation	95℃	30 sec	40
Annealing	58℃	30 sec	
Extension	72℃	30 sec	
Final - Extension	72℃	7 min	1

Optional- STD amplified DNA

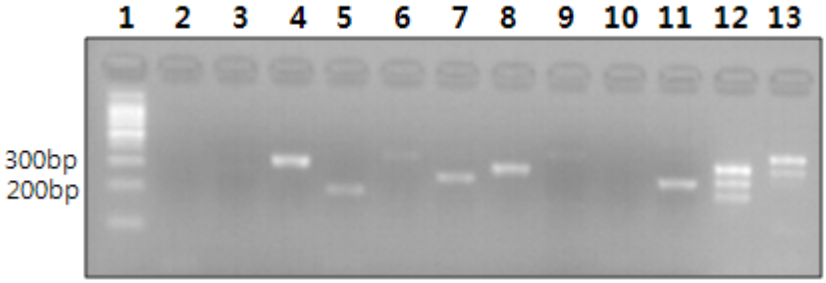
Amplified DNA can be observed by electrophoresing 5 microliters of 3% agarose gel. In case electrophoresis is performed for 30 minutes at 110 volt current, the products, of which lengths are

A type: NG(284bp, MH(333bp), UU(373bp), MG(207bp), TV(262bp),

B type: CT(321bp), HSV1(384bp), HSV2(400bp), TP(260bp), respectively.

The 100-bp ladder should be used for the standard electrophoresis.

[A Result of STD-Amplified DNA]



Lane 1: 100bp ladder marker
Lane 2: Negative control
Lane 3: NG
Lane 4: MH
Lane 5: MG
Lane 6: UU
Lane 7: TV
Lane 8: CT
Lane 9: HSV1
Lane 10: HSV2
Lane 11: T P
Lane 12: Positive control A
Lane 13: Positive control B

4.3. STD DNA Chip Reaction

A. Test Preparation

1) Prepare of heat block at 95℃ for denatuaration of PCR product
2) Prepare of Hybridization Chamber (Incubation oven or water bath) at 52℃ for chip reaction and put wet tissue (or plastic water box) at the edge of the bath for keep moisture.
3) Prepare washing solution 1 and 2 by using buffer S1 and S2 as in the following.
   a) Washing Solution 1
      - Add 5ml of buffer S1 and 2ml of buffer S2 to 993ml of distilled water and mix well.
   b) Washing Solution 2
      - Add 3ml of buffer S1 to 997ml of distilled water and mix well.
   c) The solution, prepared through the above process, can be stored for 3 months under the condition of room temperature.
   d) Necessary reagents and equipments are not provided:

   A square container (glass jar) or a coated lock & lock box, Hybridization chamber (or Incubation Oven), and Horizontal shaker.

B. Hybridization Reaction

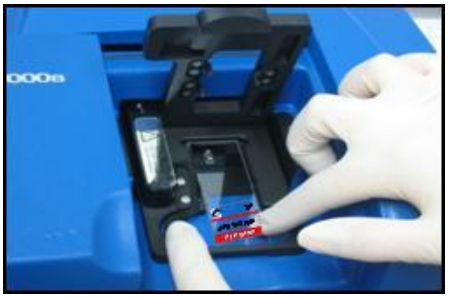
1) This entire procedure must be performed with gloves on.
2) Add 44 microliters ultra pure water into 1.5 milliliters or 200 microliters tube.
3) Add 10 microliters PCR A products, 10 microliters PCR B products, 1 microliters of Cy5-HBB oligo and mix well.
4) Incubate at 95℃ for 3 minutes.
5) Place ice for 5 minutes.
6) Briefly centrifuge at 13000rpm for 30seconds to remove drop from inside the lid.
7) Add 65 microliters HYB I solution and mix well by using the pipette.
8) The reaction solution, prepared in advance, should be poured into the inlet on the cover slip on the surface of the chip. In that case, the chip and the cover well should be checked whether bubbles are being between them and whether those are well adhered. In case bubbles are observed, those should be removed by wiping off with glove on.
9) Hybridization reaction should be performed at the bath (hybridization chamber or incubator) at 52℃ for 30 minutes.

C. Washing after Hybridization Reaction

1) After hybridization reaction, the cover well should be taken off from the chip into Washing Solution I.
2) Washing Solution 1, prepared in advance, should be poured into the washing container so that the reaction chip can be sunken. The reacted chip should be washed at 230 rpm, room temperature for 2 minutes by using the reciprocating shaker.
3) Washing solution 1 should be taken off after use and then repeat step 2 & 3 once again.
4) Washing solution 2 should be newly poured there and it should be washed at 230 rpm for 2 minutes.
5) Washing solution 2 should be taken off after use.
6) In case buffer remains on the chip after washing, it should be removed by using Kim Wipe. However, the reacted region should not be touched by hand.

D. Scanning

1) Turn on the power of the scanner.
2) Click the icon “GenePix Pro 5.0”  on the screen.
3) Activate the window “Hard Ware Diagnostic”  and set to wavelength 635, PMT 800, Pix size 10 ㎛, Lines to average 1 and Focus Position 0 ㎛.
4) Carefully open the cover of the scanner, and the chip should be set in so that the label is down and the reacted region is up.



5) Click the icon “Preview Scan”  so that the region to be scanned can be controlled.
6) Click the icon “Data Scan”  to check the color signal.
7) Click the icon “Grid”  so that only the reacted region on the scanned image can be set to column x Row 6x8, column & Row spacing 375 ㎛ and Feature diameter 220 ㎛. In case the grid needs to be moved, click the icon “  ” so that the grid can be accurately put to the spot.
8) Click the icon “Align Features in All Blocks”  .

9) Click the icon "Analyze"  and "Results" should be saved in the form of "gpr" file by clicking the icon "  ".
10) The saved file "gpr" should be called out from Excel Program, and SBR value (Signal-to-Background Gray Level Ratio) should be calculated by the formula "F635 Median ÷ B635 Median".
11) HBB is used as an internal control, so 6 spot signals must be detected at (≥2.5) greater than or equal to 2.5 SBR.
12) Patient results should be judged with SBR values of 32 kinds of spots (=F635 Median/B635 Median).

4.4. Result Analysis

A. Positive

SBR values of 6 spots of each HBB are ≥ 2.5 and SBR value of blank are ≤ 1.5, and SBR values of 2 spots of STD genotypes are ≥ 2.5, the relevant STD genotype should be judged to be suitable or **positive**.

B. Negative

SBR values of all 6 spots of each HBB are ≥ 2.5 and SBR value of blank are ≤ 1.5 but SBR value of 2 spots of 9 kinds of STD genotypes are <2.5 and, the relevant STD genotypes should be judged to be **negative**.

C. Retest

If SBR value of one spot of STD genotypes ≤ 2.5 and one ≥ 2.5, retest complete run from PCR amplification. If retest remains unsuccessful, retest f recollection of specimens.

[Criterion of SBR Results]

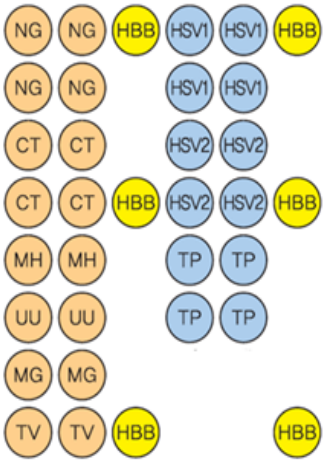
STD probe	**Result**
SBR 2.5 and Upward	Positive
SBR 2.5 and Downward	Negative

D. STD Gene Genotypes

The subtypes of STD genes should be selected with reference to the following table.

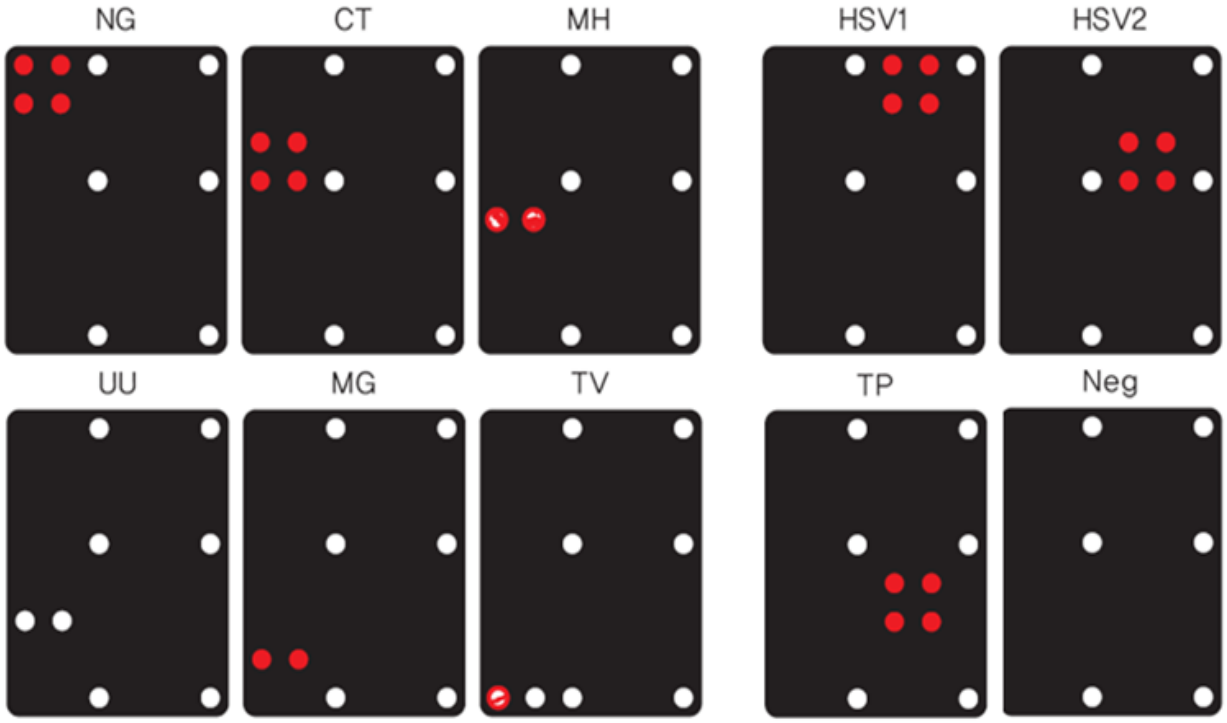
1) As shown in the following figure, in the case of NG, CT, MH, UU, MG, TV, HSV-1 &2, TP, spots should be arranged to genotypes in two.
2) HBB spots should be arranged as shown in the following figure, so as to corner marker.

GG STD DNA Genotyping Chip Kit



NG	*Neisseria gonorrhoeae*
CT	*Chlamydia trachomatis*
MH	*Mycoplasma hominis*
UU	*Ureaplasma urealyticum*
MG	*Mycoplasma genitalium*
TV	*Trichomonas vaginalis*
HSV1	*Herpes simplex virus type 1*
HSV2	*Herpes simplex virus type 2*
TP	*Treponema pallidum*

E. Examples of Positive



Directions for Use

A. This product should be used for in vitro diagnosis, and should not be used for other purposes.
B. This product should be used in accordance with the manual.
C. Specimens should be carefully treated so that those cannot be touched with the human body, because those may be latent pathogenic bacteria, virus and fungi. Those should be treated with protective on (poly glove, latex glove, etc.).
D. Specimens should not be touched with the human body, and the one who treated them should wash hands after test.
E. Other contents should be stored at the place and temperature fixed in the manual. In particular, Primer A1, A2, B1 and B2 should be stored at -20℃ and HBB corner marker should be wrapped by a silver pack and should be stored in the dark room at -20℃ because their performances become weak when those are exposed to light. Likewise, in the case of primers, only necessary quantity should be defrosted before use but should not be again frosted because their performances become weak if frosting is alternated with defrosting. The box of PCR primers, the silver pack of STD PCR kit and RNase A should be stored at -20℃ but the compositions in the box of GOODGENE STD DNA Genotyping Chips should be stored at room temperature.
F. Directions for the Use of DNA Chips

1) Chips should be handled with the powder-free glove on.
2) Only the label of the chip should be handled with the powder-free glove on.
3) The opened product should be stored after sealing up by using the box or the silver pack, in which dehumidifying agents, or the desiccators that can remove moisture.
4) Chips should be used within 3 months after the product was opened. In case 4 months elapses after it was opened, SBR value indicated 80 and upward. Although the value does not affect test, the fluorescent signal of the probe may be weakened 10 to 20%. Thus, it should be used within 3 months.
5) After reaction, chips should be scanned and should be stored in a darkroom.
6) Storage conditions: Although the GG HPV DNA Genotyping Chip kit, except PCR kit, is shipped at Room Temperature (15~25℃) and protected from light until the expiration date printed on the label.

***Important Note**:* PCR kit must be stored at -15~ -20°C. Liquid components should be mixed well before use! Precipitation can occur in the Hybridization Buffer. To dissolve the precipitate, incubate the buffer at room temperature.

7) Do not reuse the kit components.
8) Chip guard caution and Waste: Use caution when handling chip guard material. Edges and corners are sharp. Failure to take caution could result in personnel injury if not handled properly. In case of used chip, please discard into properly.
9) Equipment & Consumable to be supplied by user: When working on reagents, always wear a suitable lab coat, disposable gloves and protective goggles.
   - Fluorescent Scanner (Cy5, 635nm)
   - Hybridization Chamber or Hybridization Oven
   - PCR machine (Thermal Cycler)
   - Microcentrifuge
   - Pipette, single channel P100
   - Pipette, single channel P200
   - Pipette, single channel P1000
   - Pipette, single channel P20
   - As needed Pipette tips for pipettes listed above; full racks
   - 0.2 ml and 1.5ml or 2ml microcentrifuge tubes
   - Vortexer
   - Timer
   - Reciprocating Shaker
   - Ethanol (96 ~ 100%)
   - Pipet tips
   - Disposable gloves (powder free)

List of Acronyms

STD – Sexually Transmitted Disease
DNA – Deoxyribonucleic acid
NG - *Neisseria gonorrhea*
C T - *Chlamydia trachomatis*
UU - *Ureaplasma urealyticum*
MG - *Mycoplasma genitalium*
MH - *Mycoplasma hominis*
TV- *Trichomonas vaginalis*
TP - *Treponema pallidum*
HSV1 - *Herpes simplex virus type 1*
HSV2 - *Herpes simplex virus type 2*
HPV- *Human papillomavirus*
HIV- *Human Immunodeficiency virus*
PCR – Polymerase chain reaction

References

1. The History of Nongonococcal Urethritis: Thomas Parran Award Lecture. Taylor-Robison, D. 1996. Sex. Transm. Dis. 23:86-91
2. The complete sequence of the mucosal pathogen *Ureaplasma urealyticum.* J.I. Glass, E.J. Lefkowitz, J.S. Glass, C.R. Heiner, E.Y. Chen and G.H. Cassell, Nature 407: 757-762, 2000
3. Principles and practice of infectious diseases, Churchill Livingstone, Philadelphia. W.M. McCormack and M.F. Rein, In: G.L. Mandell, J.E. Bennett and R. Dolin, Urethritis 1208–1218, 2000.
4. Waites, KB et al., (1999): Mycoplasma & ureaplasma;
5. Comparative in-vitro activity of levofloxacin, other fluoroquinolones, doxycycline and erythromycin against *Ureaplasma urealyticum* and *Mycoplasma hominis*. Ullmann U, Schubert S, Krausse R. Journal of Antimicrobial Chemotherapy 1999;43: 33-36.
6. The minimal gene complement of Mycoplasma genitalium. Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., Fritchman, J.L., Weidman, J.F., Small, K.V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T.R., Saudek, D.M., Phillips, C.A., Merrick, J.M., Tomb, J.-F., Dougherty, B.A., Bott, K.F., Hu, P.-C., Lucier, T.S., Peterson, S.N., Smith, H.O., Hutchison III, C.A., and Venter, C. 1995. Science 270: 397-403.
7. Frequent genital herpes simplex virus 2 shedding in immunocompetent women. Effect of acyclovir treatment.Wald A, Corey L, Cone R, et al. J Clin Invest. 1997;99:1092-1097.
8. Detection by complementation of defective or uninducible (herpes simplex virus type 1) virus genomes latent in human ganglia. BROWN, S. M., SUBAK-SHARPE, J. H., WARREN, K. G., WROBLEWSKA, Z. & KOPROWSKI, H. (1979) *Proceedings of the National Academy of Sciences,* U.S.A. 76, 2364 2368.
9. Risk of recurrence after first episodes of genital herpes. Relation to HSV type and antibody response. *WC Reeves, L Corey, HG Adams, LA Vontver, and KK Holmes NEJM 305:315-319, 1981*
10. Recurrence rates in genital herpes after symptomatic first-episode infection. Benedetti, J., L. Corey, and R. Ashley. 1994. Ann. Intern. Med. 121:847-854
11. Raising the consciousness for identifying and controlling viral STDs: fears and frustrations. Corey, L. (1998). *Sexually Transmitted Diseases* 25, 58-69
12. Frequency of acquisition of first episode genital infection with herpes simplex virus from symptomatic and asymptomatic source contacts. Mertz, G.L., Schmidt, O. & Jourden, J.L. *Sex. Tramm. Dis.* 12, 33−39 (1985).
13. Herpes simplex viruses. Whitley RJ, Roizman B: *Clinical virology,* New York, 1997, Churchill Livingstone.
14. The epidemiology of herpes simplex virus infections in adolescents. Stanberry LR, Rosenthal SL. Herpes 1999; 6:12–5.