

Prevalence of nonviral reproductive tract infections/sexually transmitted infections in female patients with cervicovaginal discharge: Excerpts from a regional reference center in North India

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Abstract

Background: To study the prevalence of common nonviral reproductive tract infections/sexually transmitted infections (RTI/STI) prevailing among females who presented to our regional STI reference center and to ascertain the association of various symptoms with different RTI/STIs. **Materials and Methods:** A retrospective analysis of female patients presenting to our STI Regional center located in the Department of Medical Microbiology in PGIMER, Chandigarh, was done between April 2018 and December 2019 for patients presenting with cervico-vaginal discharge. Two to three swabs were collected from each patient. The first swab was subjected to wet mount, gram stain, Potassium hydroxide (KOH) test, and culture on blood agar, the colonies obtained were identified by matrix-assisted laser desorption time of flight mass spectrometer (MALDI TOF-MS). Second swab was used for DNA extraction and detection of *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), and *Chlamydia trachomatis* (CT) by polymerase chain reaction. The third swab, when available, was inoculated onto pleuropneumonia-like organisms (PPLo) broth. **Results:** One thousand and thirteenth of 1472 (69%) female patients were symptomatic and the most common presenting symptoms were vaginal discharge (707/1013 [69.8%]), infertility (266/1013 [26.2%]), genital itching (60/1013 [5.9%]), lower abdomen pain (47/1013 [4.6%]) and burning micturition (16/1013 [1.6%]). The most prevalent RTI/STI was bacterial vaginosis (BV) 18.2% (269/1472), followed by vulvovaginal candidiasis (VVC) 6.8% (100/1472) and trichomoniasis (TV) 1.9% (28/1472). Five cases each of *Mycoplasma genitalium* and *Ureaplasma urealyticum*, three of NG and one of CT were also identified. Coinfections were seen in 40 (2.7%) cases. The most common causative agent responsible for VVC in our study was *Candida albicans* (65%). **Conclusion:** RTI/STIs were common among women and 69% were symptomatic. BV was the most common STI present in 18.2%, followed by VVC (6.8%) and trichomoniasis (1.9%).

Key words: Bacterial vaginosis, chlamydia trachomatis, polymerase chain reaction, trichomoniasis

Introduction

Sexually transmitted infections (STIs) are one of the most prevalent infections, being caused by more than 30 pathogens (including bacteria, parasites, and viruses). Yet, they remain one of the most neglected human infections. The World Health Organization 2018 report on global STI surveillance documented that more than 1 million STIs are newly acquired every day.^[1] Further, it was estimated that 376 million new infections with one of the four STIs:

Chlamydia, gonorrhoea, syphilis, and trichomoniasis occur annually worldwide.^[1]

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The commensal *Lactobacilli* play an important role in maintaining the vaginal pH in the range of 3.5–4.5, thus keeping a check on the invading microbes. However, alteration in the number of *Lactobacilli*, due to douching, hormonal changes, sexual exposure, menstrual periods, etc., make the cervico-vaginal epithelium vulnerable by providing a favorable milieu for colonization by potentially pathogenic microbes leading to STI.^[2-4] Among the different STIs, bacterial vaginosis (BV) is the most common form of vaginal infection in women of reproductive ages. It can be a chronic recurrent infection, which is typically characterized by a reduction of *Lactobacillus* with subsequent overgrowth of *Gardnerella vaginalis* and other anaerobic bacteria, such as *Atopobium vaginae*, *Bacteroides* spp., *Mobiluncus* spp. and *Prevotella* spp.^[2,5,6] Vulvo-vaginal candidiasis (VVC), another common STI, affects up to 75% of women of reproductive age group at least once in their lifetime. Though the majority of the infections are caused by *Candida albicans*, the incidence of non-*albicans* *Candida* infection is increasingly reported.^[7] Trichomoniasis, caused by the parasite *Trichomonas vaginalis* (TV), is the most common curable STI worldwide. Trichomoniasis is associated with a two-fold increased risk of transmission of other STIs, including human immunodeficiency virus (HIV).^[8,9]

Reproductive tract infections (RTI)/STIs present as a syndrome with an overlap of not only several symptoms but, in many cases, several pathogens also. A proper understanding of the pattern of STDs prevailing in different geographic regions of a country is necessary for proper planning and implementation of STD control strategies. There is a paucity of literature regarding prevalence and pattern of various RTI/STIs in North India. Hence, as a part of the Regional STI Reference center, we studied the prevalence of common nonviral RTI/STIs from cervico-vaginal specimens of females representing different sections of society in our setup and their association with the different symptoms.

Materials and Methods

Study design

A retrospective analysis of data from female patients presenting to the Regional STI Reference, Research and Training Laboratory in the Department of Medical Microbiology at PGIMER, Chandigarh over a period of 21 months (April 2018–December 2019) was analyzed.

The following inclusion criteria were used in the study:

1. Specimen type - only swab specimens (cervical swab/vaginal swab/cervicovaginal swab and ulcer swab) and urine specimens were included; serum specimen received for serological investigations were excluded
2. Patient type - female patients presenting with symptoms of RTI/STI at our tertiary care hospital [as elaborated in Table 1], high-risk groups like female sex workers and transgenders who were approached with the help of nongovernmental organizations (NGOs) and slum population visiting the dispensary attached to our institute. Only those patients were included wherein the age of the patient was between 18 and 45 years and they were not currently undergoing any treatment for RTI/STI
3. Exclusion criteria - Pregnant females, HIV/HBsAg/hepatitis C virus positive females and females from whom only blood specimen was received were excluded from the study.

Specimen collection and processing

According to laboratory policy, a sterile speculum was gently inserted after explaining the procedure to the patient. The genital area was visually inspected for the presence of vaginitis or cervicitis or both. A cotton swab (Hi-Media Laboratories, Mumbai, India), predipped in sterile normal saline, was used to collect the cervical swab specimen and the vaginal specimen was collected from the lateral vaginal wall. Two to three swabs were collected from each patient.

The first (vaginal or cervicovaginal) swab was used to prepare a wet mount, gram stain and inoculated onto blood agar. Briefly, the swab was smeared onto a clean glass slide and inoculated onto blood agar followed by placing the swab in a screw-capped tube (Hi-Media Laboratories, Mumbai, India) containing 0.5 mL of 0.9% saline to carry out wet mount microscopy for detection of TV. The swab was vigorously rotated in the saline and pressed against the side of the tube to express as much fluid as possible. One drop of the expressed fluid was placed on a glass slide with a coverslip and examined at a magnification of $\times 40$ within 4 h of collection of the specimen. The smear on the glass slide was heat-fixed and stained with Gram's stain; reporting was done for the presence of BV (a Nugent score of 0–3 was interpreted as negative for BV and a score of 4–6 as intermediate while a score of 7–10 was interpreted as consistent with BV) and yeast. The second swab (cervical or cervicovaginal) was used to inoculate onto chocolate agar and it was then squeezed thoroughly into a falcon tube containing molecular grade water for DNA extraction. The DNA was extracted using Chelex® (Sigma Aldrich, MO, USA) and amplified for detecting the presence of TV, *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) using polymerase chain reactions (PCRs), as described earlier^[10,11] [Supplementary File]. The chocolate agar and blood agar plates were incubated in a CO₂ incubator (Binder, GmbH Germany) at 37°C for at least 72 h, the plates were examined for the presence of any visible growth. The colonies so obtained were spotted onto the MALDI-TOF MS target plates and identified using MALDI Biotyper (Bruker Daltoniks, GmbH, Germany).

The third swab, whenever available, was used to inoculate PPLO broth for the isolation of *Mycoplasma* spp. and *Ureaplasma urealyticum*. The urine specimens obtained were subjected only to PCR, as described earlier^[10,11] [Supplementary File].

Data collection and analysis

Clinical history was collected from all patients with regard to symptoms of STI. The confidentiality of all patients was maintained. High-risk behavior (as informed by the NGOs) was kept anonymous and not included in analysis. The age, specific symptoms, and results of the three swabs were maintained and analyzed in Excel (Microsoft Inc., USA).

Statistical analysis

The analysis was performed using GraphPad (GraphPad Software Inc., USA). Discrete variables were presented as percentages and Chi-square with Fisher's exact test was applied for the analysis of categorical variables. A $P < 0.05$ was considered to be statistically significant.

Results

Out of 3217 specimens received in our regional STI laboratory during the study, 2179 (67.7%) were from female patients and the remaining 1028 (31.9%) were from male

Table 1: Symptom-matrix of the clinical presentation of female patients with sexually transmitted infections

| Symptom | Total | Discharge | Itching | Primary infertility | Secondary infertility | Burning micturition | Lower abdominal pain |
|------------------------|-------|-----------|---------|---------------------|-----------------------|---------------------|----------------------|
| Discharge (n=767) | | | | | | | |
| Discharge | 707 | 642 | 3 | 45 | 17 | - | - |
| Genital itching | 60 | 4 | 32 | 1 | 1 | 6 | 16 |
| Infertility (n=266) | | | | | | | |
| Primary infertility | 201 | 45 | 1 | 155 | - | - | - |
| Secondary infertility | 65 | 17 | 1 | - | 47 | - | - |
| Symptoms of UTI (n=62) | | | | | | | |
| Burning micturition | 16 | - | 6 | - | - | 7 | 3 |
| Lower abdominal pain | 46 | - | 17 | - | - | 2 | 27 |

n=1095; A total of 1013 patients were symptomatic with 1095 symptoms. UTI=Urinary tract infection

patients. Out of 2179 female specimens, 707 were serum specimens sent for rapid plasma reagin/Venereal disease research laboratory and were hence excluded from the study. Of the remaining 1472 specimens, 1433 were swab specimens (97.4%) (1372 [93%] were cervicovaginal swabs, 56 vaginal swabs [3.8%], 3 cervical swabs [0.2%] and 2 ulcer swabs [0.1%]) and remaining 39 (2.6%) were urine specimens. The mean age of study patients was 31.71 years and all were married.

Results of clinical profile of patients

On examination, the inflammation of the cervicovaginal canal was seen in 383 patients, of whom 236 (51.6%) had vaginitis, 126 (51.6%) had cervicitis, and 21 (47%) had vaginitis and cervicitis both [Figure 1]. The maximum occurrence of inflammation was observed in the age group of 25–35 years (51.6%) followed by 18–25 years (23.7%) for vaginitis and 35–45 years (23.8%) for cervicitis. Females aged >45 years had inflammation in <10% of cases.

Out of 1472 female patients, 459 (31%) were asymptomatic and 1013 (69%) had one or more symptoms of RTI/STIs [Tables 1 and 2]. The most common presenting symptom was vaginal discharge in 707 (69.8%), followed by infertility in 266 (26.2%), genital itching in 60 (5.9%) patients, lower abdomen pain in 47 (4.6%), and burning micturition in 16 (1.6%) of female patients. Among 266 patients having infertility, primary infertility was seen in 201 (75.6%) and secondary infertility was seen in 65 (24.4%) and two patients (0.1%) had a genital ulcer.

Results of microbiological investigations

Wet mount examination, gram stain, and culture were performed for all 1433 swab specimens. TV was observed on wet mount examination in 26 cases (1.8%). Gram stain showed BV (score 7–10) in 269 (18.7%) patients; BV intermediate (score 4–6) in 323 (31.8%) patients; Gram-positive budding yeast cells in 100 (9.8%) patients and Gram-negative diplococci suggestive of NG in 2 (0.2%) patients. The culture showed growth of small transparent colonies in 2 specimens and creamy white colonies in 100 specimens. MALDI-TOF MS identified the transparent colonies as NG and creamy white colonies as *Candida* species. Six different species of candida were identified, the most common being *C. albicans* (65%) followed by *Candida glabrata* (24%). *Mycoplasma* spp. and UU were observed in PPLO broth in five cases each. Swabs from two patients of genital ulcer did not reveal any organisms targeted in the present study.

PCR for CT, NG, and TV was performed in 1314 of the 1472 specimens. PCR was found to be positive in 15 cases (1.1%); 11 were TV, 3 were NG and 1 was CT.

Prevalence of different sexually transmitted infections among females

The most common STIs prevalent in our study were BV 18.2% (269/1472) followed by VVC in 6.8% (100/1472) and TV in 1.9% (28/1472). *Mycoplasma hominis* and *Ureaplasma urealyticum* were detected in 5 (0.33%) cases each; NG in 4 (0.27%) and CT in 1 (0.06%). There were 40 (2.7%) cases of coinfection [Table 3], with two patients having a triple infection and one patient having a quadruple infection. The most common causative agent responsible for VVC in our study was *C. albicans* (65%), followed by *C. glabrata* (24%).

Results of the association of different symptoms with different STIs are shown in Table 2.

Among the various symptoms, vaginal discharge was found to have significant association with BV ($P = 0.0033$, odds ratio [OR] = 1.74, 95% confidence interval [CI] 1.2, 2.51) and TV ($P = 0.001$, OR = 11.92, 95% CI 1.61–88.26). Among the characteristics of discharge, watery discharge was associated with TV ($P = 0.0001$, OR = 0.01, 95% CI 0.002). Greenish discharge was associated with TV ($P = 0.0001$, OR = 1518.75, 95% CI 180.32, 12791.62) and white discharge was associated with VVC ($P = 0.0001$, OR = 37.04, 95% CI 7.29, 188.11).

Infertility, as a whole, was found to be associated with VVC ($P = 0.045$, OR = 0.54, 95% CI 0.29, 0.99) and TV ($P = 0.025$, OR = 0.22, 95% CI 0.05–0.93), however no association was found when primary and secondary infertility were evaluated separately. No association was seen among symptoms of urinary tract infection like burning micturition and lower abdominal pain with either BV or VVC or TV [Table 2].

Discussion

In the present study, the mean age of female patients with RTI/STI was 31.9 years. This is lower than the mean age of 36 years as reported by Sarkar *et al.* but falls within the range of 25–35 years, as reported by Rajalakshmi and Kalaivani.^[12,13] In our study also more than half of the patients (51%) with vaginitis, cervicitis, and combination of both were aged between 25 and 35 years [Figure 1]. This is higher than that reported in the study by Garba, wherein 33% of symptomatic Nigerian women belonged to this age group.^[14]

Out of the 1013 symptomatic females, the most common symptom observed in our study was vaginal discharge seen in 707 (69.8%) patients followed by infertility in 266 (26.2%), genital itching in 60 (5.9%) patients, lower abdomen pain in 47 (4.6%) and burning micturition in 16 (1.6%). These findings are in tune with the study by Narayankhedkar *et al.* wherein the most common presenting

symptoms were vaginal discharge followed by vulval itching/irritation, malodor, pain abdomen, and dysuria.^[15] The incidence of vaginal discharge among all female patients of our study (32.4% [707/2179]) was higher than that reported by Sarkar *et al.* where it was found to be 25% for nontribal females and 14% for tribal females.^[12]

BV was found to be the most common STI in our study, with a prevalence of 18.2%. Our findings are in concordance with a community study by Narayankhedkar *et al.* wherein BV was identified in 17.3% of women of the reproductive age group in Mumbai.^[15] Other studies have reported a large variation in the prevalence of BV, ranging from 5 to 50%, mostly arising due to the selection of the specific patient population, economic status and educational background of the patients, and microbiological tools used for the detection of BV.^[16] Rajalakshmi and Kalaivani., among their asymptomatic patients, noted a higher prevalence of 59%, while Bitew *et al.* reported a prevalence of 48.6% among women with vaginitis attending a hospital in Kochi.^[13,17] In the east of Yemen, BV was reported among 39.2% of pregnant women.^[18] We found a significant association between BV and discharge ($P = 0.005$; i.e., women with BV were 1.69 times more likely to have vaginal discharge than those without BV. No significant association of BV was seen with infertility and urinary tract infection (UTI). This is in accordance with Garba, who also found that the symptoms of vaginal discharge were significantly associated with BV.^[14]

The second most prevalent STI in our study was VVC contributing to an overall prevalence of 6.8%. This is slightly lower than 7.8% reported by Rajalakshmi and Kalaivani. and 7.5% reported by Pawanarkar *et al.*^[13,19] These differences could be attributed to variations in patient populations and detection methods selected in these studies. A statistically significant association between VVC infection and the presence of white discharge ($P = 0.0001$, OR = 72.4, 95% CI 15.61, 335.81) was seen, which corroborates with findings of Narayankhedkar *et al.*^[15]

A weak association of candidiasis with infertility was seen with a $P = 0.045$ (OR = 0.54, 95% CI 0.29, 0.99); the association was lost when primary and secondary infertility were analyzed separately. Although studies have shown that *C. albicans* have sperm immobilizing activity, how much of this actually contributes to infertility is not yet determined.^[20,21] A previous study done by Vander *et al.* in mice showed that when sperm immobilizing strains of

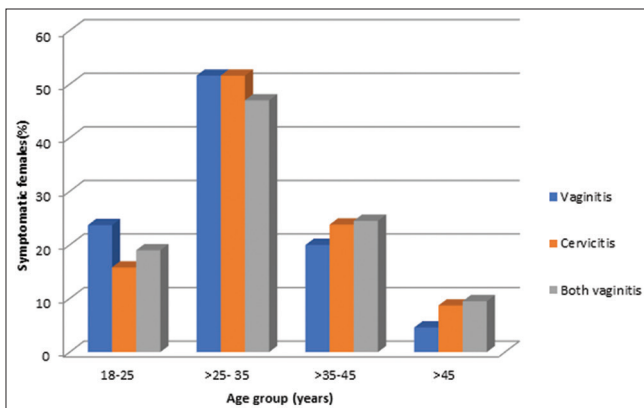


Figure 1: Bar-graph depicting the vaginitis, cervicitis and both in different age groups

Table 2: Association of bacterial vaginosis, vulvo-vaginal candidiasis and Trichomoniasis with different symptoms of sexually transmitted infections

| Presenting sign/symptom | Total n=1472, n (%) | Bacterial vaginosis | | | Candidiasis | | | Trichomonas vaginalis | | |
|-------------------------|---------------------|---------------------|-------------------|----------|-------------|---------------------|----------|-----------------------|---------------------------|-------|
| | | Score (7-10), n (%) | OR (95% CI) | n (%) | P | OR (95% CI) | n (%) | P | OR (95% CI) | n (%) |
| Symptomatic | 1013 (69) | 196 | | 78 (5) | | 27 (1.83) | | | | |
| Discharge | 707 (69.8) | 153 (15) | 1.69 (1.17-2.44) | 60 (6) | 0.16 | 1.48 (0.86-2.56) | 26 (2.6) | 0.001 | 11.64 (1.57-86.2) | |
| Watery | 675 | 149 | 1.63 (0.5-4.78) | 57 | 0.49 | 0.74 (0.22-2.53) | 8 | 0.0001 | 0.01 (0-0.02) | |
| Green | 19 | 1 | 0.092 (0.03-1.47) | 0 | - | - | 18 | 0.0001 | 1518.75 (180.32-12791.62) | |
| White | 13 | 2 | 0.744 (0.14-2.98) | 11 | 0.0001 | 72.4 (15.61-335.81) | 0 | - | - | |
| Infertility | 266 (26) | 47 (4.7) | 0.86 (0.6-1.24) | 13 (1.3) | 0.045 | 0.54 (0.29-0.99) | 2 (0.2) | 0.025 | 0.22 (0.05-0.93) | |
| Primary infertility | 201 | 37 | 0.76 (0.62-1.38) | 10 | 0.138 | 0.57 (0.29-1.13) | 1 | 0.028 | 0.15 (0.02-1.12) | |
| Secondary infertility | 65 | 10 | 0.52 (0.37-1.49) | 3 | 0.63 | 0 | 1 | 0.028 | 0.55 (0.07-4.15) | |
| Symptoms of UTI | 62 (6) | 9 (0.9) | 0.69 (0.34-1.43) | 5 (0.5) | 0.81 | 1.06 (0.41-2.71) | 0 | - | - | |
| Asymptomatic | 459 (31) | 73 (5) | - | 23 (1.6) | - | - | 7 | - | - | |

OR=Odds ratio; CI=Confidence interval; UTI=urinary tract infection

Table 3: The incidence of mixed infections seen in females patients

| Coinfection | n=1472, n (%) |
|-------------------|---------------|
| BV + VVC | 14 (0.95) |
| TV + VVC | 2 (0.14) |
| BV + TV | 14 (0.95) |
| BV + MH + UU + TV | 1 (0.07) |
| TV + MH + UU | 2 (0.14) |
| MH + TV | 2 (0.14) |
| BV + UU + TV | 1 (0.07) |
| BV + UU | 2 (0.14) |
| BV + NG | 2 (0.14) |

BV=Bacterial vaginosis; VVC=Vulvovaginal candidiasis; NG=*Neisseria gonorrhoea*; UU=*Ureaplasma urealyticum*; MH=*Mycoplasma hominis*

C. albicans were intravaginally injected in female mice, it showed a decrease in fertility in all groups as compared to control mice.^[20] However, they did not cause clinical and histopathological changes in the genital tract of mice, thus showing that their colonization in the genital tract might impair sperm motility. Mathur *et al.* showed that in patients with chronic vaginal candidiasis of 5 years duration, a high titer of auto-antibodies to ovary was present, which might be due to the presence of one or more cross-reactive antigens on ovarian follicle and *Candida* spp.^[22] Thus chronic vaginal candidiasis might be associated with decreased ovarian function, thereby affecting the fertility outcome.

Trichomoniasis, the third most prevalent STI, was detected in 1.9% of patients in our study. Narayankhedkar *et al.* also reported a similar prevalence of 1.8% for TV.^[15] Other studies have reported differences in prevalence ranging from <1% in the USA to 4.8% in Senegal.^[23,24] A greenish discoloration was found to be significantly associated with TV discharge ($P = 0.0001$). This is in concordance with the typical characteristics of TV-associated vaginal discharge reviewed by Petrin *et al.*^[25]

The other less common STIs, prevalent among the female patients, were NG detected in 4 (0.27%) patients and CT in one patient (0.06%). MH and UU were detected in 5 patients each. Maraki *et al.* have reported a prevalence of 0.6% for MH and 1.7% for UU.^[26]

Mixed microbial etiologies were observed in 40 (2.7%) patients in our study with maximum cases of coinfection seen in association with BV (BV + TV IN 0.95% and BV + VVC in 0.95%) [Table 3]. Narayankhedkar *et al.* reported a mixed infection in 6.4%^[15] and Khan *et al.* reported BV + VVC as the most common coinfection in 3.8% of cases.^[27] It might be because women with BV tend to alter the vaginal pH thus predisposing to the acquisition of other coinfections such as TV and *Candida*.^[28]

Conclusion

STIs were not uncommon among females in North India. The most common STI was BV diagnosed in 18.2% cases followed by VVC and trichomoniasis, coinfections were also encountered. BV was associated with vaginal discharge, VVC with white creamy discharge, and trichomoniasis with watery and greenish discharge. Cervicovaginal swab specimens served as a useful tool for the detection of common agents of STIs.

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Conflicts of interest

There are no conflicts of interest.

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Upcoming Conferences - 2023

- **Dermacon International 2023**
2nd International Dermacon and 51st National Conference of IADVL
Dates: 23rd - 26th February 2023
Venue: Jio World Convention Centre, Mumbai
<https://www.dermaconinternational2023mumbai.com>

- **25th World Congress of Dermatology Singapore, 2023**
World Congress at your doorstep
Dates: 3rd - 8th July 2023
Venue: Suntec Singapore Convention & Exhibition Centre
<https://www.wcd2023singapore.org>

- **21st ACSICON Nagpur 2023**
National Conference of Association of Cutaneous Surgeons of India
Dates: 5th-7th October 2023
Venue: Nagpur
<https://www.acsicon2023.com>

- **ASTICON Kolkata 2023**
47th National Conference of IASTD & AIDS.
Venue: Kolkata

Supplementary File

Samples: Vaginal/cervical/cervicovaginal swab and first-catch urine

PCR: Neisseria gonorrhoeae

Cycling parameters

| Opa | Temperature and cycles | Por | Temperature and cycles |
|--------------|------------------------|--------------|------------------------|
| Denaturation | 95° - 1 min | Denaturation | 95° - 30 s |
| Annealing | 65° - 45 s 35 cycles | Annealing | 55° - 30 s 35 cycles |
| Elongation | 72° - 1 min | Elongation | 72° - 1 min |
| Elongation | 72° - 7 min | Elongation | 72° - 7 min |
| Storage | 4° - infinite | Storage | 4° - infinite |

PCR: Chlamydia trachomatis

Cycling parameters

| Cryptic Plasmid | Temperature and cycles | Hjcp | Temperature and cycles |
|-----------------|------------------------|--------------|------------------------|
| Denaturation | 95° - 1 min | Denaturation | 95° - 30 s |
| Annealing | 55° - 45 s 35 cycles | Annealing | 60° - 30 s 35 cycles |
| Elongation | 72° - 1 min | Elongation | 72° - 1 min |
| Elongation | 72° - 7 min | Elongation | 72° - 7 min |
| Storage | 4° - infinite | Storage | 4° - infinite |

PCR: Trichomonas vaginalis

Cycling parameters

| Tvk | Temperature and cycles |
|--------------|------------------------|
| Denaturation | 94° - 45 s |
| Annealing | 60° - 30 s 40 cycles |
| Elongation | 72° - 45 s |
| Elongation | 72° - 7 min |
| Storage | 4° - infinite |