

Evaluation of DNA Based Techniques for the Diagnosis of Human Vaginal

Trichomoniasis in North Indian Population

Running title: Diagnostic methods for human vaginal trichomoniasis.

ABSTRACT:

Introduction: Human trichomoniasis due to *Trichomonas vaginalis* is a curable sexually transmitted infection. It may lead to symptomatic vaginitis or asymptomatic carrier state. The symptoms and signs mimic other pathologies and conventional techniques of diagnosis have its own limitations. Therefore, the present study aimed to evaluate a newly established in-house PCR based on *pfoB* gene and compared it with conventional wet smear examination method and 18S rRNA gene based PCR technique for the diagnosis of *T. vaginalis* in symptomatic and asymptomatic subjects.

Material and Methods: Four hundred women in age group 20-57 years attending the Obstetrics and Gynecology out Patients Department (OPD) of Nehru Hospital attached to Post Graduate Institute of Medical education and Research (PGIMER) Chandigarh, India were included in the study. Based on the symptoms and signs, 344 (86%) women were categorized as symptomatic and 56 (14%) as asymptomatic. Vaginal swabs collected from all the women were processed by three techniques including wet smear, 18S rRNA and the *pfoB* gene based PCR techniques for the detection of *T. vaginalis*.

Results: The main presenting symptom in majority of symptomatic patients was vaginal discharge. The highest numbers of *T. vaginalis* positive patients were found in the sexually active age group of 20 to 40 years. The amplifications of *pfoB* and 18S rRNA gene by PCR

revealed significantly higher positive cases (20.7% and 18.6%, respectively) than the wet smear (6.6%) method. The diagnostic efficacy and kappa value estimated by the three techniques were 86.8-100% and 35.5-66%, respectively.

Conclusions: The combined application of any two of the three techniques used in the present study may be useful for the diagnosis of *T. vaginalis* infection in symptomatic and asymptomatic subjects. This information may help clinicians to make a timely and accurate diagnosis.

KEYWORDS: *T. vaginalis*, Trichomoniasis, diagnosis, *pfoB* PCR, 18S rRNA PCR, wet smear examination.

1. INTRODUCTION

Human vaginal trichomoniasis is the most common non-viral sexually transmitted infection (STI) (1) with adverse but significant effects on public health. The prevalence of *Trichomonas* infection in developing countries is increasing year by year, up to the level of approximately 12-30%, and is prevalent mainly in women of the sexually active age (1, 2). Many of the *Trichomonas* infected people are asymptomatic, but a greater tendency was reported of the *Trichomonas* infected women to become symptomatic (3). A varied clinical presentation of trichomonas involves severe inflammatory manifestation such as urethritis, vulvo-vaginitis, and cervicitis leading to vaginal discharge, itching, dysuria, foul smell and dyspareunia in females. Women with known chronic infection are at greater risks of atypical pelvic inflammatory diseases, HIV infection and cervical cancer (4, 5). An early detection of *T. vaginalis* would be a crucial factor for treatment, reduction and prevention of negative health outcomes in women (6, 7).

A primitive conventional technique for visualization of trichomonas trophozoites is by wet smear examination. This method is quick and cheap but has a limited sensitivity (60-

70%). Although, culture technique yields relatively higher sensitivity (98.5%) and specificity and is considered as a 'gold standard' for the diagnosis of Trichomoniasis (8), it requires well established facilities and expertise, which may not be available in all the diagnostic centres.

Various sero-diagnostic techniques have been applied to demonstrate the presence of specific antibodies in serum and vaginal secretions (VS) with variable sensitivity (10) like detection of antibody response to cysteine proteinase 30 (CP30) (11). *Trichomonas* infection was reported in 36.5% and 96.7% of women investigated by wet smear and ELISA, respectively (12). Significantly higher levels of anti-trichomonad IgA antibodies were found in *T. vaginalis* patients as compared to controls (13). Monoclonal antibodies against *T. vaginalis* proteins were also used for detection of Trichomoniasis (14). Though less specific, the rapid XenoStrip-Tv test in vaginal swab samples from women attending STI clinics was found to be more sensitive than the wet smear examination (15). The XenoStrip-Tv test was used, but could not prove to be applicable for point-of-care diagnostic assay. Another relatively simple test, the OSOM (One Step One Minute) trichomonas rapid test is an immune-chromatographic capillary flow (dipstick) assay used for VS and showed a better sensitivity and specificity as compared to the wet smear examination (16). The only limitations of OSOM test was that it was performed on frozen samples in batches in a research setting. Therefore antibody detection assays demand well-established facilities and expertise, which may not be available in all the diagnostic centers.

In order to improve the sensitivity and specificity of the diagnostic techniques, recombinant DNA techniques with high sensitivity have increasingly been used for the diagnosis of microbial infections. Correspondingly, the development of PCR techniques for trichomonas detection have been described, including the use of oligonucleotide probe test with a sensitivity and specificity of 80-90% and 95%, respectively (17-19). Another study reported that the affirm-VPIII test in VS of both symptomatic and asymptomatic patients was

77 better for the detection of vaginitis as compared to wet smear (20). The Affirm assay was
78 more likely to identify *Candida* and *Gardnerella* than *Trichomonas*. Affirm-VPIII is more
79 expensive, and is also classified as a moderately complex test, which is based on the
80 principles of nucleic acid hybridization (20). The newly established pyruvate:ferredoxin
81 oxidoreductase proprotein gene (*pfoB*) has also been exploited for performing *T. vaginalis*
82 specific diagnostic assays (21, 35). In our earlier report, *pfoB* gene PCR did not show any
83 cross reactivity with the genome of other microorganisms or human DNA (21, 35). Despite
84 all these efforts, PCR based diagnostic methods are still in experimental stages in the
85 developing countries. Moreover, lack of facilities and relevant expertise in the conventional
86 diagnostic laboratories has impeded the routine use of DNA based techniques.

87 The *pfoB* gene contains highly conserved DNA sequences that are useful for
88 performing diagnosis assays (22-24). Given the variable sensitivity and specificity of the
89 applied techniques for the diagnosis, including the DNA based methods (17), the present
90 study was performed to evaluate the possibility of applying *pfoB* gene based PCR for the
91 diagnosis of *T. vaginalis* in comparison to the earlier techniques (18S rRNA PCR and wet
92 smear examination). Furthermore, the utility of combined use of these techniques was
93 revealed for the diagnosis of symptomatic and asymptomatic *Trichomonas* infection.

94 **2. PATIENTS AND METHODS**

95 **2.1 Patients**

96 For this study, 400 women with ages of 20-57 years who visited the Obstetrics and
97 Gynecology out Patients Department (OPD), Nehru Hospital attached to the Postgraduate
98 Institute of Medical Education and Research (PGIMER), Chandigarh (India) were enrolled.
99 The patients with complaint of vaginal discharge, itching, foul smell, pruritus, dysuria,
100 dyspareunia and abdominal pain were categorized as suggestive of trichomoniasis and
101 labelled as symptomatic. Subjects attending the OPD for routine ante/postnatal follow-up,

family planning advice and infertility without any symptoms and signs suggestive of trichomoniasis were labelled as asymptomatic. The per-vaginal (P/V) and per-speculum (P/S) examination of all the women was conducted after obtaining an informed consent and the clinical findings and relevant history was recorded on a pre-planned proforma.

2.2 Sample collection and processing

During P/S examination, two vaginal swabs were collected from posterior vaginal fornix from each subject using commercially available sterile wet cotton swabs (HiMedia, India). All the vaginal swabs were transported to the department of Medical Parasitology, PGIMER within half an hour of collection. The first swab was subjected to wet smear examination and the second swab was stored at -20°C for the subsequent DNA extraction.

2.3 Wet smear examination

The wet smear examination was performed to detect the presence of motile Trichomonads. For this, a drop of normal saline (0.90% NaCl) was put on a glass slide and the swab was gently rolled in the saline to make a thin smear. A clear coverslip was placed over the smear and the presence of *T. vaginalis* was examined under a light microscope as described previously (8).

2.4 Genomic DNA isolation and Polymerase Chain Reaction

The total genomic DNA was isolated from each of the samples as described earlier (21, 25, 35). In brief, phosphate buffered saline (PBS, 1mL) was added to the vial containing dry swabs and incubated for 10 minute at 4°C, mixed by vortexing thoroughly and squeezed. An aliquot of the sample (400 µl) was centrifuged at $11,000 \times g$ at 4°C for 10 minutes. The supernatant was discarded and the cell pellet was suspended in 40 µl of PBS followed by centrifugation at

11,000 × g at 4°C for 5 minutes. Then the total genomic DNA was isolated from the pellet as described earlier (21, 25, 35). The acquired nucleic acids were either stored at 4°C for further use or used directly as template DNA (5 µl) for PCR assay. The PCR was performed separately using the oligonucleotides specific to 18S rRNA gene (GenBank accession number U17510) and *pfoB* gene (GenBank accession number U16823) of *T. vaginalis*, that generate DNA fragments of 312 bp and 338 bp respectively. The PCR procedure and the specific oligonucleotides for 18S rRNA gene were the same as those in previous report (17). For *pfoB* gene, the amplification was carried out in a 25 µl volume reaction mixture containing 1×Taq DNA Polymerase Buffer, 200 µM of the deoxyribonucleoside triphosphates (Bangalore Genei, India), 5 pmoles of forward (pfoBF 5'-CAAAGTCAACATGGCTATGAT-3') and reverse primer (pfoBR 5'-GAAGACCTGTGTGGATGGATGT-3'), 5 µl of genomic DNA sample, 1.0 U of Taq DNA Polymerase (Bangalore Genei, India). PCR included a negative control (sterile water) and a positive control (1 ng of purified genomic DNA of *T. vaginalis*). After an initial denaturation of 96°C for 5 minute, PCR was achieved in a thermal cycler (I-cycler, BioRad, USA) for 35 cycles using the following conditions: cycling at 96°C for 20 seconds, 56°C for 30 seconds and 72°C for 25 seconds. A final extension was performed at 72°C for 7 minutes. The amplicons (20 µl) were analyzed by electrophoresis in a TAE buffer using 2% (w/v) agarose gel containing 0.5 µg/ml of ethidium bromide. After electrophoresis, the DNA bands were visualized on a UV transilluminator (Alpgen, USA) (Figure 1).

2.5 Statistics and calculations

A Chi-square test of homogeneity and Kruskal-Wallis test were used for the statistical analysis. PCR data were analyzed by using InStat programme. Standard formulae were used to make a comparison of the sensitivity, specificity and positive/negative predictive values of the diagnostic methods (26, 27). Due to lack of an efficient Gold Standard Criteria for

diagnosis of *T. vaginalis* and lower sensitivity of wet smear examination, we evaluated the diagnostic efficacy and kappa value of *T. vaginalis* by multiple techniques in various combinations. The diagnostic efficacy and kappa value of the techniques was calculated after defining the best possible measure of ‘true positives’ as criteria, ‘a’; Positive by all the three techniques.

For this, the following four discrete measures were defined.

- a). Positive by all the three techniques: wet smear, 18S rRNA gene and *pfoB* amplifications.
- b). Positive by wet smear and 18S rRNA gene PCR.
- c). Positive by wet smear and *pfoB* PCR.
- d). Positive by 18S rRNA and *pfoB* amplifications.

2.6 Ethical Approval

The institutional ethical committee of Dr. B.R Ambedkar Centre for Biomedical Research, University of Delhi, Delhi, India (EC. No: F-50-2/Eth.Com/ACBR/11/2110) and Postgraduate Institute of Medical Education and Research, Chandigarh, India (EC. No: PGI/IEC/2012/1549-50) approved this project. Written consent was obtained from all the adult subjects included in this study.

3. RESULTS

3.1 Subjects and Symptomatology

Among all the patients, 344 were symptomatic subjects and 56 were asymptomatic. The main presenting symptom was vaginal discharge in 305 (76.25%), followed by vaginal discharge and itching in 159 (39.75%), vaginal discharge, itching and pruritus in 70 (17.5%), vaginal discharge, itching, pruritus, and dyspareunia in 44 (11%), vaginal discharge, itching, pruritus,

dyspareunia and abdominal pain in 36 (9%) patients. All the 56 asymptomatic subjects had a normal appearing vagina and cervix. Assessment of the symptoms in the symptomatic group of patients was made using Chi-square test of homogeneity. The distribution of symptomatic patients with reference to their symptoms was found to be homogenous (Chi-square=621.50) and significant ($p=0.000001$).

3.2 Clinical findings of the symptomatic subjects

Patients were categorized into four age groups (20-30, 31-40, 41-50 and >50 years) in order to find out which sexually active age group is most vulnerable to *T. vaginalis* infection. Clinical details of the symptomatic patients are summarized in Table 1. Subjects belonging to the age group of 20-30 ($n=122$) and 31-40 years ($n=119$) showed more symptoms and signs compared to the age group 41-50 ($n=82$) and >50 ($n=21$) suggestive of trichomoniasis. Vaginal discharge was the main presenting complaint followed by abdominal pain, itching and foul smell among all the age groups. P/V examination of symptomatic patients showed inflammation/erosion in 16 (2.6%) followed by uterus bulky 10 (3.2%). Similarly, a P/S examination of symptomatic patients suggested cervix (Cx) erosion as a clear clinical indication in 114 (33.7%), followed by Cx bleeding in 31 (9%), strawberry Cx in 10 (2.9%), multiparous Cx in 2 (0.6%) and normal Cx in 187 (53.8%) (Table 1).

3.3 Distribution of symptoms of the symptomatic patients in various age groups

Significantly, higher number of women in the sexually active age groups of 20-30 and 31-40 years presented the clinical complaints (Table 1). Therefore, for further statistical analysis these age groups were combined and labeled as 20-40 years age group. Among the symptomatic patients, the frequency of presenting symptoms in age group 20-40 years were significantly higher than in the age groups 41-50 and >50 years ($p<0.01$; Figure 2A). P/S

examination of the cervix (Cx) of symptomatic patients showed that Cx erosion was the main signs in the age group 20-40 years while the frequency of Cx bleeding and strawberry were significantly higher in the age groups >50 years ($p<0.01$; Figure 2B). P/V examination of the uterus (Ut) of symptomatic patients showed no significant signs in the age group 20-40 as compared to the other two age groups (Figure 2C).

3.4 Detection of *T. vaginalis* by wet smear examination, 18S rRNA gene and *pfoB* PCR

Out of the 400 patients, *T. vaginalis* was detected in 12 (3%), 28 (7%) and 30 (7.5%) women by wet smear, 18S rRNA gene PCR and *pfoB* PCR, respectively. The percentage of positivity in the asymptomatic group was higher than that in the symptomatic group ($p=0.037$) detected by *pfoB* method and no significant difference ($p=0.08$) was detected by 18S rRNA PCR method (Figure 2A).

By wet smear examination, 10 (2.91%) out of the 344 symptomatic and 2 (3.57%) out of the 56 asymptomatic women were found positive for *T. vaginalis*, suggesting a similar proportion of positive patients in both the groups ($p=0.27$; NS). However, 18S rRNA gene PCR showed a higher efficiency, by which 21 (6.1%) symptomatic and 7 (12.5%) asymptomatic women were positive, suggesting the proportion of positive patients in the latter group was higher but not significant ($p=0.08$; NS). Interestingly, the *pfoB* PCR showed 22 (6.4%) symptomatic and 8 (14.3%) asymptomatic women positive, suggesting the proportion of positive patients in the latter group was significantly higher ($p=0.03$) (Figure 3A).

In order to decipher the status of *T. vaginalis* infection among different age groups, the frequency of *T. vaginalis* detection by the various methods was calculated. It was

found that the highest numbers of *T. vaginalis* positive patients were in the sexually active age group of 20-40 years (Figure 3B).

3.5 Sensitivity, specificity, positive and negative predictive value and diagnostic efficacy of the techniques

Based on the best possible measure of ‘true positive’ as criteria ‘a’; ‘Positive by all the three techniques (wet smear, 18S rRNA, *pfoB*)’, the sensitivity of the wet smear, 18S rRNA and *pfoB* was 100% each in samples from symptomatic and asymptomatic subjects respectively. Specificity of the wet smear was 98.4% and 100% in symptomatic and asymptomatic subjects respectively. Specificity of 18S rRNA and *pfoB* based DNA detection methods was 95.1% and 94.8 in symptomatic and 90.2% and 88.5% in asymptomatic subjects, respectively (Table 2). The data suggests that the kappa value and diagnostic efficacy of criteria ‘a’; can be considered as the best combination for the diagnosis.

4. DISCUSSION

The status of presenting symptoms based on the age groups of *T. vaginalis* patients detected in the present study has not been delineated well before. This aspect of population study throws light on ‘which age group of patients is more prone to *Trichomonas* infection’ in order to target a specific population age group for diagnosis. Significantly higher number of women in the sexually active age group of 20-40 years presented with the clinical complaints suggestive of trichomoniasis, compared to other age groups. This is understandable since trichomoniasis is a STI (1).

A rapid and efficacious diagnostic test can be critical for the control of STIs like trichomoniasis. Wet smear and culture methods are the most frequently used methods of

diagnosis for *T. vaginalis* detection. However, time limit for wet smear examination, following sample collection and lower sensitivity limits its use as an effective diagnostic technique. Therefore, developing countries have adopted a diagnosis system based on symptoms and signs of women as the primary strategy for management of trichomoniasis. However, up to 50% of *T. vaginalis* infections may be asymptomatic leading to considerable under-treatment (28).

In the study conducted in India, the prevalence of *T. vaginalis* infection in Mysore (South India) was 8.5% (29), in Chandigarh (North India) was 3.6% in symptomatic and 2.7% in asymptomatic cases positive by wet smear examination (30). In the present study when both the groups were taken together, the percentages of positivity revealed by *pfoB* and 18S rRNA gene PCR (20.7% and 18.6%, respectively) were significantly higher as compared to that detected by wet smear examination. Though the PCR for detection of *T. vaginalis* in VS showed moderately high sensitivity (80-95%) as compared to the wet smear examination (31-32), the only diagnostic assay for detection of *T. vaginalis* cleared by the FDA is NAAT aptima *T. vaginalis* assay (33). The Aptima (TMA) *T. vaginalis* assay is a nucleic acid amplification test that utilizes target capture, transcription-mediated amplification, chemiluminescent probe hybridization to detect *T. vaginalis* 18s rRNA gene and a high sensitivity 96.7% and specificity 97.5% has been reported (34). The sensitivities of OSOM rapid antigen test varied from 82-92.5% and specificity was 100% (34). Although DNA based diagnostic tests have improved the sensitivity of trichomonas diagnosis (19), yet these are still in limited use in the developing countries. Therefore, the evaluation of newer DNA based diagnostic tests for screening of human trichomoniasis in symptomatic and asymptomatic subjects is of significance from public health importance.

It is suggested that the PCR based diagnostic methods are important tools in aiding epidemiologic evaluations based on prevalence and morbidity of the disease (32). This

study evaluated a newly established *pfoB* gene-based PCR method in combination with the known conventional techniques (wet smear) in symptomatic and asymptomatic subjects. Gene *pfoA* is *T. vaginalis* specific which translate into a cell-binding protein and is involved in adherence of *T. vaginalis* to the host's vaginal epithelial cells (23, 24). The *pfoB* has a highly conserved and unique DNA sequences that was exploited for performing *T. vaginalis* specific diagnostic assays. Previously we found that *pfoB* gene is specific for *T. vaginalis* and does not show any cross reactivity with the genome of other microorganisms and human DNA samples (21).

In the present study, an efficiency of the techniques was calculated and only 3.2% were found positive by wet smear method, thus, in both symptomatic and asymptomatic groups, large numbers of positive patients detected by PCR were found negative by wet smear. Intriguingly, we also observed that a percentage of *T. vaginalis* infection detected by DNA based diagnostic methods among the symptomatic patients was less (6.4%) as compared to the asymptomatic women (14.3%). One probable reason for a less percentage of positivity among the symptomatic group could be prior treatment of symptomatic patients with metronidazole, as described earlier (36). Overall, the *pfoB* gene followed by 18S rRNA gene PCR showed the highest positivity among the *T. vaginalis* infected patients as compared to the wet smear examination method. Positive by any two techniques out of wet smear, 18S rRNA gene and *pfoB* detection by PCR was found to be from 46% to 100% in both symptomatic and asymptomatic subjects. Thereby our study suggests that the diagnostic efficacy and kappa value of criteria 'a' can be termed as the best combination as a diagnostic tool. Recently we evaluated the possibility to use *pfoB* gene PCR for the diagnosis of *T. vaginalis* in the patients. *pfoB* gene PCR diagnosis showed similar specificity and sensitivity even with the dry swab samples that were transported from clinic at an ambient

temperature in confirmation to our earlier observation (35). This knowledge may further add to the prevalence and the comparative diagnosis as a predictor of the *Trichomonas* infection, which could be helpful in the management of trichomoniasis.

5. CONCLUSION

The vaginal discharge is the main presenting symptom in majority of symptomatic patients. The highest number of *T. vaginalis* positive patients was found in the sexually active age group of 20 to 40 years. The *pfoB* and 18S rRNA gene based PCR techniques are more efficient as compared to traditional wet smear examination. *T. vaginalis* infected asymptomatic subjects are known to spread infection to their sexually active partners, therefore the proposed set of methods for diagnosis may prove to be a robust method for identification and early detection of *T. vaginalis* positivity among the asymptomatic subjects as well. The results showed that the PCR techniques (both for 18S rRNA gene and *pfoB*) are better for detection of *T. vaginalis* as compared to the conventional wet smear examination. As shown in this study and in other recent study, the *pfoB* gene based in-house PCR assay is useful in a clinical setting of the developing countries offering convenience and affordability for the diagnosis of *T. vaginalis*. It would be worthwhile to encourage further studies to explore the feasibility and cost-effectiveness of these diagnostic tests.

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Table 1: Clinical details of the symptomatic patients with respect the different age groups.

Age groups (years)		20-30	31-40	41-50	> 50	
		N=344 (%)	N=122 (%)	N=119 (%)	N=82 (%)	N=21 (%)
Clinical profiles						
<i>Complaints</i>						
	Vaginal discharge	305 (88.7)	116 (95.1)	108 (90.7)	71 (86.6)	10 (47.6)
	Itching	141 (41.0)	45 (36.9)	56 (47.1)	33 (40.2)	7 (33.3)
	Pruritus-vulva	95 (27.6)	29 (23.8)	35 (29.4)	28 (34.1)	3 (14.3)
	Dysuria	91 (26.4)	29 (23.8)	33 (27.7)	25 (30.5)	4 (19.0)
	Dyspareunia	62 (18.0)	26 (21.3)	23 (19.3)	11 (13.4)	2 (9.5)
	Abdominal pains	195 (56.7)	76 (62.3)	73 (61.3)	39 (47.6)	7 (33.3)
	Foul smell discharge	131 (38.1)	52 (42.6)	49 (41.1)	24 (29.3)	6 (28.6)
Physical Examination						
<i>P/S</i>						
	Cx normal	187 (53.8)	66 (54.1)	70 (58.8)	41 (50)	10 (47.6)
	Cx erosion	114 (33.7)	40 (32.8)	35(29.4)	32 (39)	7 (33.3)
	Cx bleeding	31 (9.0)	7 (5.7)	9 (7.6)	12 (14.6)	3 (14.3)
	Cx strawberry	10 (2.9)	2 (1.6)	1 (0.8)	3 (3.6)	4 (19)
	Cx multiparous	2 (0.6)	0	1 (0.8)	1 (1.2)	0
<i>P/V</i>						
	Ut normal	306 (91.9)	103 (84.4)	102 (85.7)	80 (97.5)	21 (100)
	Ut mobile	8 (1.7)	3 (2.5)	4 (3.4)	1 (1.2)	0
	Ut bulky	10 (3.2)	2 (1.6)	5 (4.2)	2 (2.4)	1 (4.8)
	Ut enlarged	4 (0.6)	2 (1.6)	1 (0.8)	1 (1.2)	0
	Ut inflamed/erosion	16 (2.6)	6 (4.9)	5 (4.2)	4 (4.9)	1 (4.8)

Abbreviations: P/S, Per-Speculum examination; P/V, Per-Vaginal examination; Cx, cervix; Ut, uterus.

Table 2: Comparative analysis of wet smear examination, 18S rRNA and *pfoB* gene based diagnostic methods for *T. vaginalis* detection in symptomatic (N= 53) and asymptomatic (N =17) cases based on four (a to d) criteria of ‘true positives’.

Method	Samples	No. of patient (%)	Sensitivity (%)		Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Diagnostic efficacy (%)	Agreement with ‘gold standard’ (kappa value)
Wet smear	Symptomatic	10 (2.9%)	a	100	98.4	50	100	98.5	66
			b	100	98.4	50	100	98.5	66
			c	100	99.4	80.6	100	99.4	86.6
			d	35.7	98.4	97.2	50	95.8	39.5
	Asymptomatic	2 (3.57%)	a	100	100	100	100	100	54.6
			b	100	100	100	100	100	100
			c	100	88.5	25	100	88.9	100
			d	40	100	100	93.9	90.1	54.6
18S rRNA gene	Symptomatic	21 (6.10%)	a	100	95.1	23.8	100	95.2	36.9
			b	100	95.1	23.8	100	95.2	36.9
			c	62.5	95.1	23.8	99.0	94.4	32.2
			d	100	97.8	66.7	100	97.9	78.9
	Asymptomatic	7 (12.5%)	a	100	90.2	28.6	100	86.8	41
			b	100	90.2	28.6	100	88.8	41
			c	100	90.2	28.6	100	86.8	41
			d	100	90.2	28.6	100	86.8	81.3
<i>pfoB</i>	Symptomatic	22 (6.39%)	a	100	94.8	22.7	100	94.9	35.5
			b	100	94.8	22.7	100	94.9	35.5
			c	100	95.7	36.4	100	95.8	51.6
			d	100	97.5	63.6	100	97.6	76.6
	Asymptomatic	8 (14.28%)	a	100	88.5	25	100	88.9	36.2
			b	100	88.5	25	100	88.5	36.2
			c	40	100	100	93.9	90.2	36.2
			d	100	93.9	62.5	100	94.4	74

(a) Positive by all the three techniques i.e wet mount, 18S rRNA gene, *pfoB* detection by PCR.

(b) Positive by only wet mount and 18S rRNA gene

(c) Positive by only wet mount and *pfoB*

(d) Positive by only 18S rRNA gene and *pfoB*

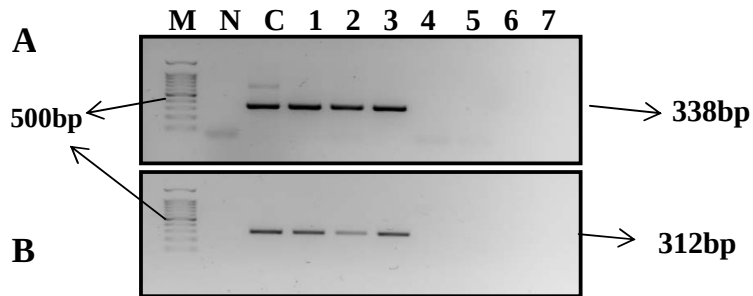
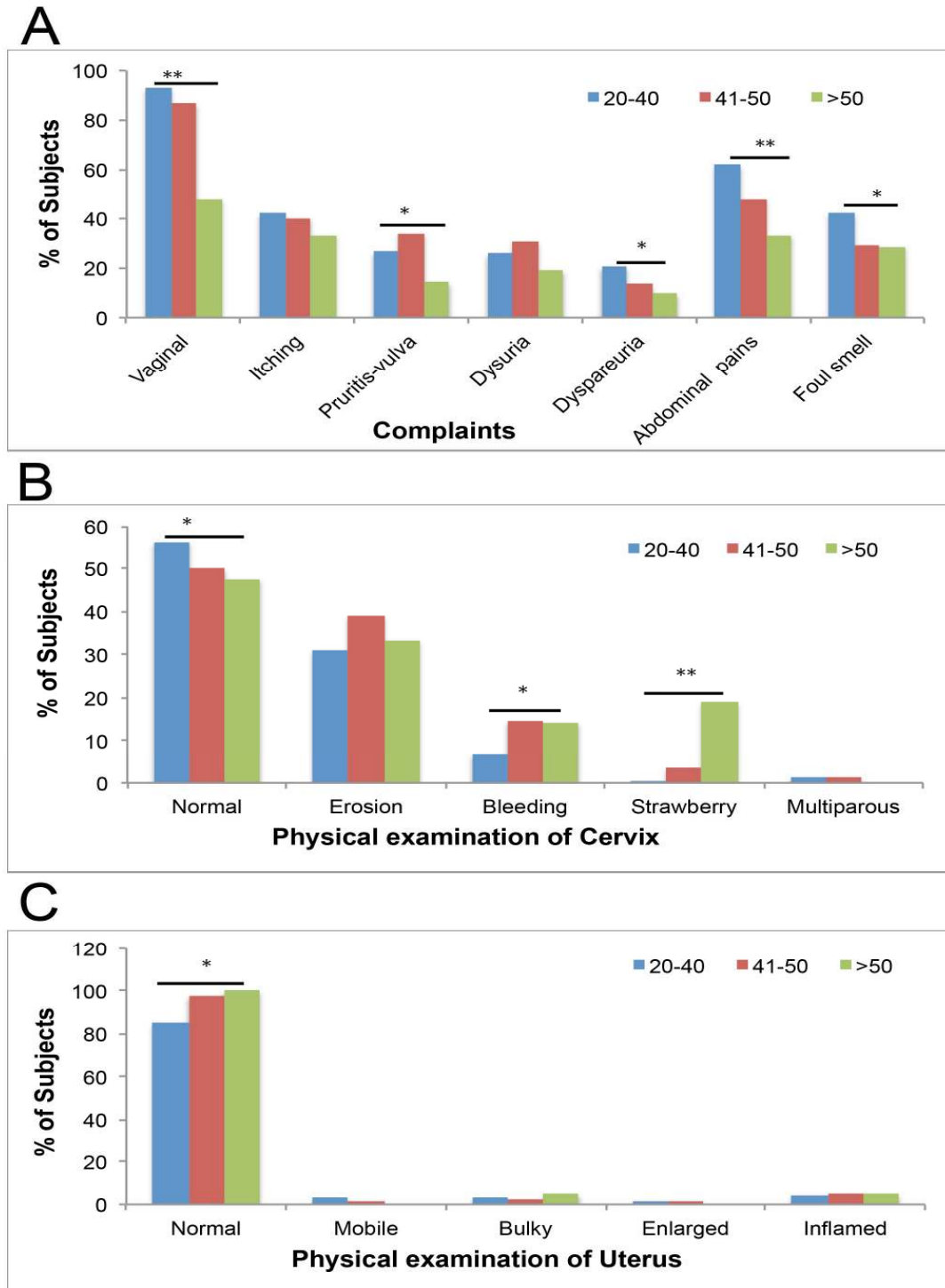


Figure 1. Agarose gel showing amplification of *pfoB* (A) and *18S rRNA* (B) gene of *T. vaginalis* from randomly selected clinical samples. Lane M: 100bp DNA marker, Lane N: Negative control, Lane C: Positive control, Lanes 1-7: Samples from *T. vaginalis* infected patients. In Panel A; Lane 1-3 are TV (+) and Lane 4-7 are TV (-) with *pfoB* while in Panel B; Lane 1-3 are TV (+) and Lane 4-7 are TV (-) with *18S rRNA* gene.



469

470 **Figure 2.** Distribution of symptoms of the symptomatic patients in the age groups 20-40, 41-
 471 50 and >51years. Distribution of the percentage of patients was assessed with respect to the
 472 type of complaints (A) received, physical examination of the cervix (B), and uterus (C). P
 473 values are * $p<0.05$, ** $p<0.01$.

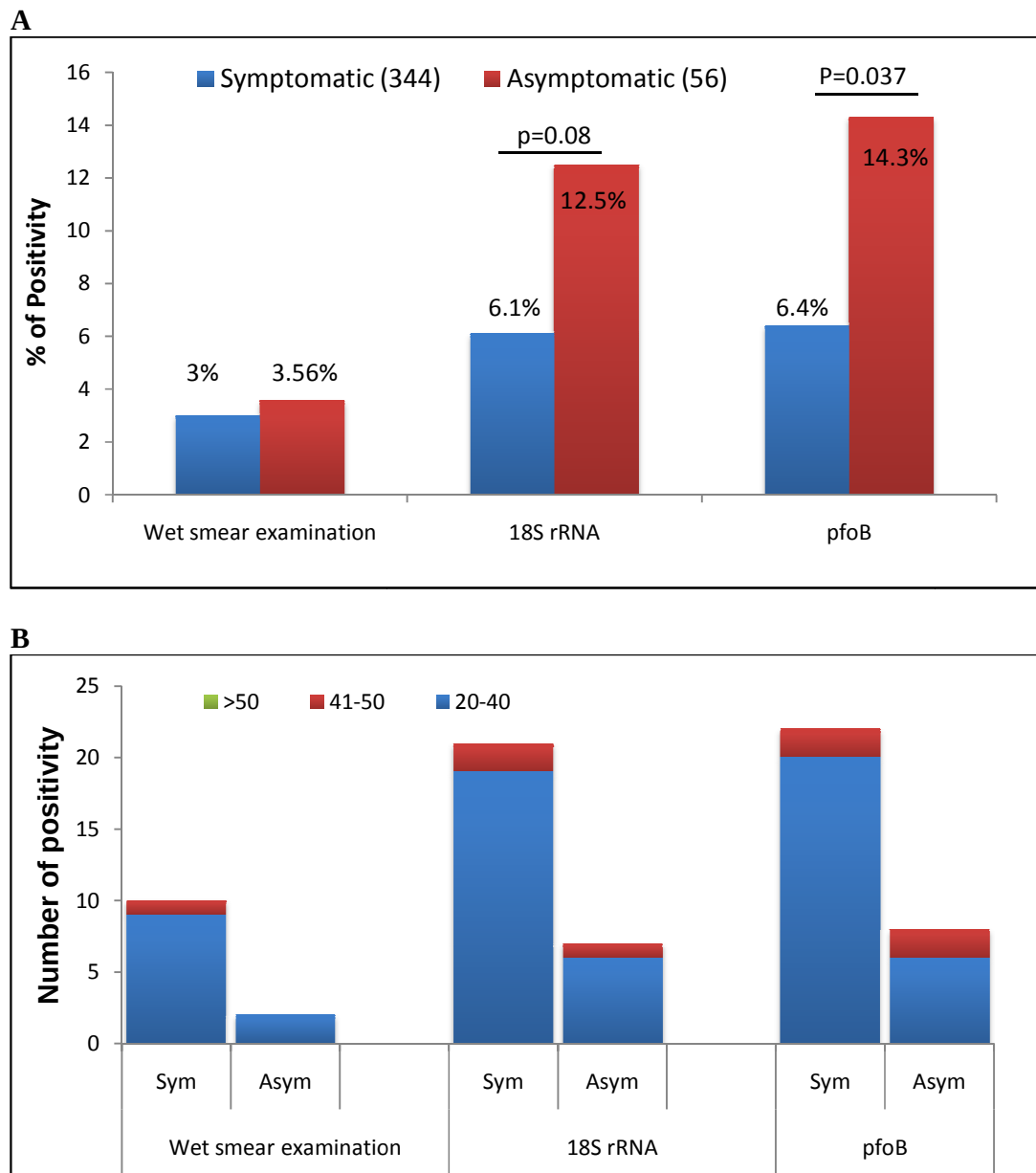


Figure 3. Detection of *T. vaginalis* by various methods (N=400). (A) Percentage of positivity in both symptomatic and asymptomatic women. (B) Number of positive patients is graphically represented with respect to age-groups in both symptomatic and asymptomatic women.