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Identification study for suspected women with *Trichomonas vaginalis* by PCR technique in AL-Najaf AL-Ashraf province

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Abstract

The study was conducted on 450 out patients and 30 healthy women, whom have visited the department of infertility at Al-Sadder medical city and Al-Zahra Hospital in Najaf Province during the period from January till August, 2012. The infection with *T.vaginalis* in clinical suspected women determine by using PCR technique, the infection women numbers and percent by PCR test gave 58 and 12.88% respectively. DNA of *T.vaginalis* parasite was determined by PCR was using five specific primers (BTUB 9/2, BGL 1/2, TVA 5/6, TV 1/2 and OP 1/2) whereas the number, percent and sensitivity of infected women of these primers were 58 (12.88%) (100%), 56 (12.44%) (96.55%), 53 (11.77%) (91.37%) and 52 (11.55%) (89.65%) respectively, from comparing between these primers to evaluate the efficiency in diagnostic of *T.vaginalis* .Indicated that BTUB 9/2, BGL 1/2 the highest primers and OP 1/2 the lowest primers in diagnostic efficiency.

Introduction

T.vaginaalis is an extra cellular pathogen covered by a dense glycocalyx thought to play role in host-parasitic interactions. The main component of the glycocalyx is lipophosphoglycan (LPG, apolysaccharide anchored in the plasma membrane by inositol phosphoceramide (Felix *et.al*, 2005).It is lacks mitochondria, some necessary enzymes cytochromes, instead uses the hydrogenosome to accomplish fermentative carbohydrate metabolism, the hydrogenosome appears to have common ancestry with mitochondria based on similarities in protein import. Nutrient is taken up by transport through the cell membrane and by phagocytosis. The organism is able to maintain energy requirements by the use of a small amount of enzymes to provide energy via glycolysis of glucose to glycerol and succinate in the cytoplasm (Singh *et al*., 2010).

It is produces soluble factors that have been reported to have the ability to damage target cells *in vitro*, and it has been hypothesized that these factors may play a role in the pathogenesis of human Trichomoniasis, one of these factors is lytic factor (LF) which have been an important virulence factor of *T.vaginalis* mediating the destruction of host cells and contributing to tissue damage and inflammation in Trichomoniasis (Lubick & Burg-ness , 2004).

The traditional clinical diagnosis of vaginal infection is based on information from patient, clinical finding observed during the vaginal specimen. This last one provides the most objective information. A microscope wet amount examination of the specimen permits, detection of the motile protozoa *T. vaginalis* (Daron *et al*, 2009).

Nucleic acid amplification techniques have been developed an applied for the detection of sexually transmitted pathogens in clinical specimens, nucleic acid amplificatication test have advantages with respect to sensitivity, specificity and rapidity for detection of sexually transmitted pathogens compared with culture technique (Johnson *et al*., 2000 and Stellrecht *et al*., 2004). PCR techniques using specific primer sets for the detection of *T.vaginalis* have been validated on clinical specimens (Crucitti *et al*., 2003). The current study aimed to estimate the prevalence of *T.vaginalis* in AL-Najaf AL-Ashraf province by used PCR techniques.

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Materials and Methods

The study was conducted on 450 women with pelvic inflammatory disease and 30 of healthy women as control groups, when all these cases were examined and defined as suspected with *T.vaginalis* by obstetrician when attended to AL-Zahra, maternity and paediatric, AL-Sadder teaching hospital in AL-Najaf province from January to August 2012. A careful history was taken from each patient according to the following Questionnaire sheet:

Case No:	Date:
Name :	Age :
Address:	
Economic status:	Type of contraception:
Education Level:	Vaginal discharge:
Colour: Oder:	
Cervical ulcer :	
History of vaginal infection:	
*Treatment use:	
*Any disease :	

***Any women using drug or undergo disease removal from current study.**

Sample collection

From suspected women vaginal discharge was carefully collected from the posterior vaginal fornix after putting the patient at a lithotomic position and taking swab after opening the vagina by a sterile speculum, the swab are immersed in a tube with ml of a sterile normal saline (Shaio & Lin, 1997). The swab was examined by PCR technique.

DNA Extraction

1-The cell pellets were resuspended in 500 µl of 0.5% Tween 20 (sigma).

2- Boiled for 20 min in water bath at 100Cº.

3- Pelleted by centrifugation at 12,000 rpm for 5min.

4- The supernatant was discarded, and the pellet was emulsified by vigorous flicking in 25 µl of chloroform phase.

5- The aqueous phase containing water-soluble components including nucleic acids was harvested by centrifugation at 12,000 rpm for 2min.

6- The tube which contains DNA was stored at−20Cº till used for amplification. 7- Five microliters were used in a 20 µl PCR mixture. rapid boiling method (Men-Fang *et al.*, 1997). Procedure of

Primers:

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Five specific of primers were used in PCR , (BTUB 9/2,BGL1/2,TVA5/6,TV1/2 and OP1/2) for all strain (Paces *et al*.,1992), the sequences of primer seen in table (1) , and Cycling parameters of genes amplification are seen in table (2):

Table (1): Primers used for *T.vaginalis* identification (Paces *et al*., 1992).

Primers	DNA sequences (5 `---3`)
BTUB9 (reverse)	5'-(CATTGA TAACGA AGC TCTTTA CGAT3').
BTUB2(forward)	5'(GCATGTTGT GCC GGA CAT AAC CAT3').
TVA5(reverse)	5'-(GATCATGTT CTA TCTTTT CA3').
TVA6 (forward)	5¹-(GAT CAC CACCTT AGT TTA CA3¹).
TV1(reverse)	5'-(TAA TGG CAG AAT CTT TGG AG3').
TV2(forward)	5'-(GAA CTT TAA CCG AAG GAC TTC3').
BGL1(reverse)	5'-(CTT CAT CCA CGT TCA CC3').
BGL2(forward)	5'-(GAA GAG CCA AGG ACA GGT AC3').
OP1(reverse)	5'-(GTGAAA TCTC ATT GGGGTATTAACTT-3')
OP2(forward)	5'-(GTTTTATTTATCACTGGAAAATAACGCTT3').

Identification of *T.vaginalis* by PCR:

Infection of *T.vaginalis* initially confirmed by PCR amplification, as following: 1- 5µl of Deionized Distilled water was added to PCR premix tubes.

2- 5µl of primer (2.5 ml from each R-primer and F-primer) was added to the mixture 3- 5µl of sample was added to mixture, as seen in table (2). 4- PCR-mix tubes were closed and transferred them into the thermocycler only when temperature reaches 95C° and start the following programs according to the type of primers as seen in table (1):

Table (1): Cycling parameters of genes amplification

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Primers	steps	Initial denaturation	Denaturation	Annealing	Extension	Final extension
BTUB9/2	Temp.(C°)	95	95	57	72	72
	Time	5min.	45sec.	45sec.	1min.	7min.
	Cycle	45				
BUA 5/6	Temp.(C°)	95	94	47	72	72
	Time	5min.	1min.	1min.	1min.	7min.
	Cycle	35				
TV ½	Temp.(C°)	95	94	58	72	72
	Time	5min.	10sec.	45sec.	15sec.	7min.
	Cycle	40				
BGl ½	Temp.(C°)	95	95	57	72	78
	Time	5min.	1min.	1min.	1min.	5min.
	Cycle	40				
OP ½	Temp.(C°)	94	94	47	72	72
	Time	2min.	45sec.	45sec.	1min.	10min.
	Cycle	30				

Table (2): PCR reaction mixture component

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Component	Reaction size (20µl reaction)
DNA polymerase	1Unit
Each dNTP (dATP,dCTP,dGTP,dTTP)	250 µM
KCl	30 mM
$MgCl_2$	1.5 mM
Stabilizer and tracking dye	5 µM
Tris-HCl(pH 9)	10 mM

Analysis of PCR Products:

Polymerase chain reaction products were analysed by 2% agarose gel electrophoresis which prepared according to (Men-Fang *et al*.,1996) as the following:

1- 100 ml of TBE (pH 8.3) was added in beaker contain 2 gm. of agarose.

2- The solution was heated to boiling (using water bath), the allowed to cool at 50-60 Cº and (0.5mg/ml) ethidium bromide was added.

3- The agarose gel was assembled to casting tray and comb was positional at one of the tray after both edges were sealed and agarose was allowed to gel at room temperature.

4- The comb was carefully removed and the gel was replaced in electrophoresis chamber, and the chamber was filed with TBE buffer until, the buffer reached (0.5-1 mm) over the surface of the gel. 5- DNA was mixed with 6× loading dye in case of PCR products without loading buffer, and was loaded in the wells of the agarose gel.

6- The cathode was connected to the well side of the unit and the anode to the other side.
7- The gel electrophoresis was run at 70 V. for 1h.

8- The DNA band was observed by under U.V transilluminater.

Interpretation of Results:

The sample was considered to be positive for *T.vaginalis* DNA if the band observed on agarose gel in target place according to the type of primer which used as seen in table (3):

Table (3): Type of primers used in present study and place of band.

Primer type	Place of band in positive sample
BTUB 9/2	**112 bp**
TVA 5/6	**102 bp**
TV 1/2	**312 bp**
BGL 1/2	**102 bp**
OP 1/2	**290 bp**

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Results

A total of 450 specimens were tested with different primer set. The number and percentage of *Trichomonas* infection, as defined by PCR technique were 58 and 12.88% respectively .Fifty eight specimens were positive by five primer sets, seen in table (4), number and percentage of vaginal swab were detected by used two primers BTUB 9/2 and BGL 1/2 were 58 and 12.88% respectively, by used TVA 5/6 primer were 56 and 12.44% respectively, by used TV 1/2 primer were 53 and 11.77%respectively,and by used OP 1/2 primer were 52 and 11.55% respectively. The result revealed that the tow primers sets BTUB 9/2 and BGL 1/2 gave the highest sensitivity (100%), but the OP 1/2 has less one of sensitivity (89.65%),and the other primer (TVA 5/6 and TV 1/2) had sensitivity between them (96.55% and 91.37%) respectively, these results are shown in table (4). The current study shown the effected of five primer (BTUB 9/2, BGL1/2, TVA5/6, TV1/2 and OP1/2) on DNA genomic of *T.vaginalis* are shown on agarose gel electrophoresis, as seen in figure (1),(2),(3),(4) and (5) respectively.

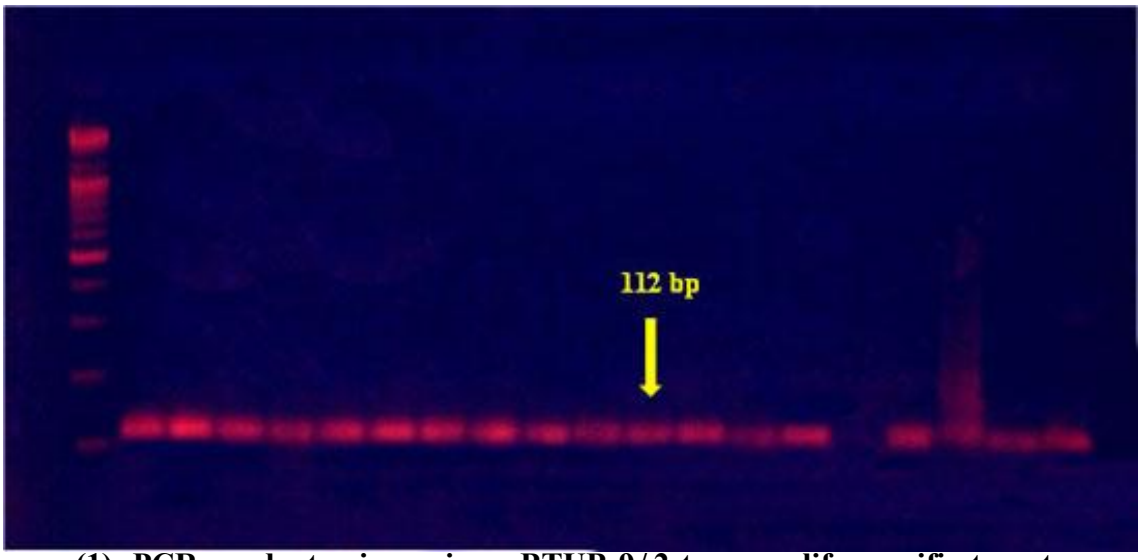

Figure: (1). PCR product using primer BTUB 9/2 two amplify specific target sequence from genomic DNA of *T.vaginalis* .M-DNA ladder 100bp.C-negative control. Other lane represents the PCR product (112 bp).

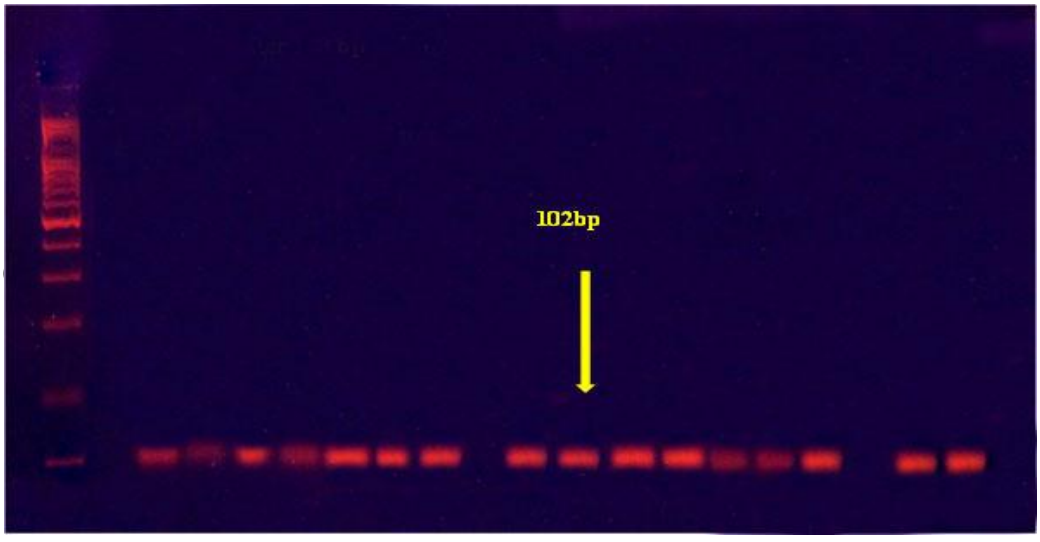

Figure: (2). PCR product using primer BGL1/2 two amplify specific target sequence from genomic DNA of *T.vaginalis* .M-DNA ladder 100bp.C-negative control. Other lane represents the PCR product (102 bp).

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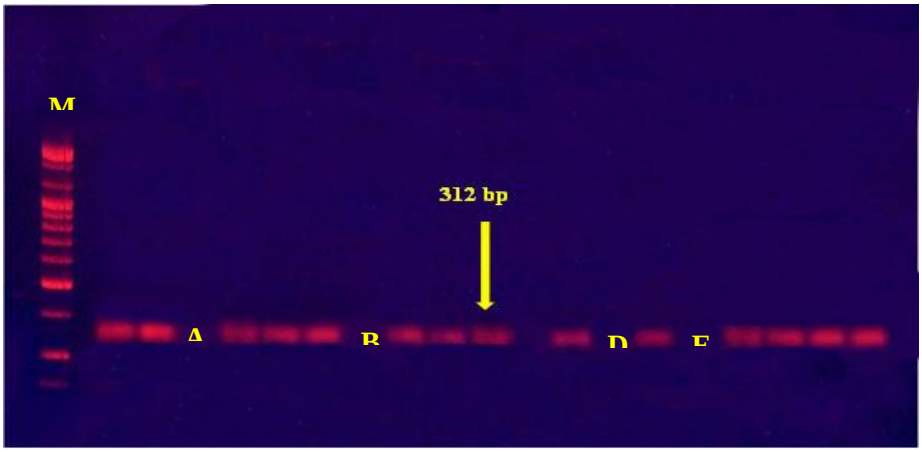

Figure: (3). PCR product using primer TVA5 / 6 two amplify specific target sequence from genomic DNA of *T.vaginalis* .M-DNA ladder 100bp.C-negative control. Other lane represents the PCR product (102bp) A, B and C represent negative sample.

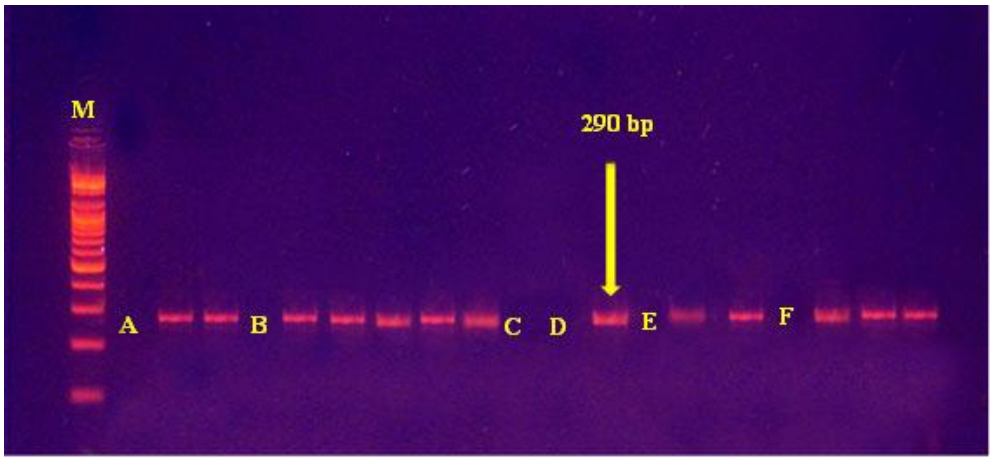

Figure: (4). PCR product using primer TVA1/ 2 two amplify specific target sequence from genomic DNA of *T.vaginalis* .M-DNA ladder 100bp.E-negative control. Other lane represents the PCR product (312 bp) A, B, C and D represent negative sample.

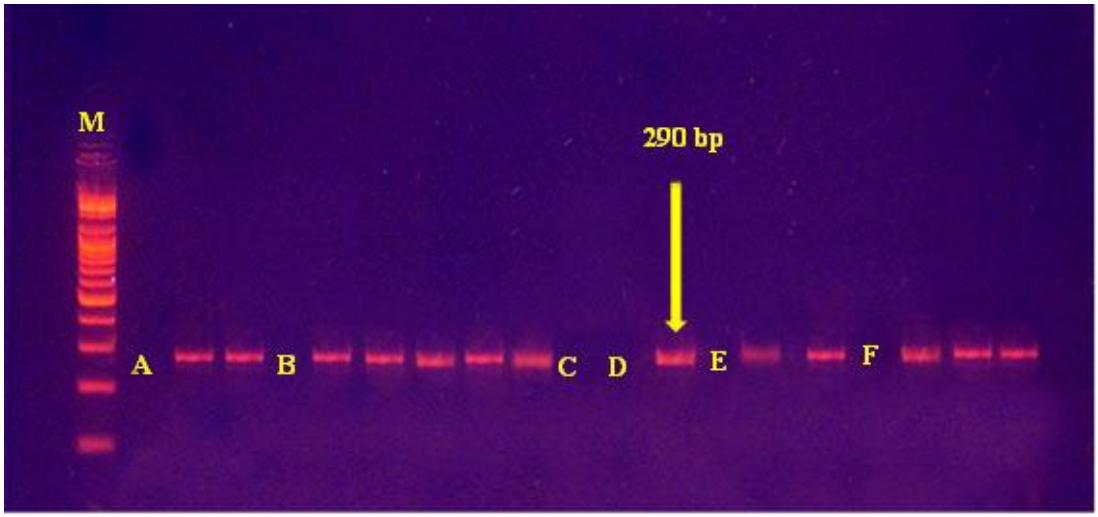

Figure: (5). PCR product using primer OP1/2 two amplify specific target sequence from genomic DNA of *T.vaginalis* .M-DNA ladder 100bp.E-negative control. Other lane represents the PCR product (290 bp) A, B, C, D and F represent negative sample.

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Table (4): Comparison between the sensitivity of primers sets used in detection of *T. vaginalis*

Primer	Positive case		Sensitivity %
	No.	%	
BTUB 9/2	58	12.88	100%*
BGL 1/2	58	12.88	100%*
TVA 5/6	56	12.44	96.55%
TV 1/2	53	11.77	91.37%
OP 1/2	52	11.55	89.65%
Total of sample examine	450	0.00	0.00

***The highest sensitivity primers used in detection of *T. vaginalis*.**

Discussion

The purpose of this study was the detection of *T. vaginalis* by used specific primer sets by PCR technique and also identification the sensitivity and specificity between the PCR technique and other detection method which used in this study (wet amount smear, culture, *T. vaginalis* rapid test), also to compare between the sensitivity of primer set which used in this study.

The resulting of study revealed that the highest positive cases which the number and percent were 58 (12.88%) respectively from the 450 suspected cases examination by the PCR method .these result agreement with study of (Crucitti, 2003),who proved that PCR technique more sensitivity than the other method which used for diagnosis of *T.vaginalis* the number and percent of women infected with *T.vaginalis* parasite was 40 and 9.61% respectively, and by culturing method was 29 and 6.97% respectively, another study agreement with the resulting of present study was the study of Amany *et al.*,(2008),who recorded that the sensitivity of wet amount smear was (33.30%) compared to sensitivity of PCR method (57.14%).The highest sensitivity of (BTUB 9/2 and BGL 1/2) primers sensitivity 100% may be due to that the two primers were specific to strain of *T.vaginalis* which found in Iraq, but the other primers (TVA 5/6, TV 1/2 and OP 1/2) were less specificity than the primers recorded above (Mayata *et al.*, 2000).A DNA don't suspect degradation owing to the characteristic endogenous nuclease activity of *T. vaginalis*, which would have resulted in the cleavage of DNA fragments and thus primarily affect the PCR assay with larger target sequences (Riley *et al*., 1992). The target of the primer set BGL1/2 and TVA5/6 is the shortest one, 102bp, comparable to the target size of the BTUB 9/2 primer set, which is 112bp; the size of the TV1/2 target is 312 bp, comparable to the target size of OP1/2 primer set which is 290 bp.

The presenting result found that the performance of different primer set was different from has been described above. There were also important differences between the primer sets. The explanations for these differences can be multiple strain variability of *T. vaginalis*, suitability the primer set and probe, and choice of gold standard.

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References

Amany, A. A.; El-Aal, F. A. and Maha, H.E. (2008): Assessing feasibility of using samples for diagnosis of *T.vaginilis* by both wet mount and nested PCR. International Journal of Medicine, 1(4): 154-156.

Crucitti ,T.; Van, D. E. and Tehe, A., (2003): Comparison of Culture and different PCR assay for detection of *Trichomonas vaginalis* in self-collected vaginal swab specimens. Sex Transm infect; 79: 393 – 398.

Daron, C.E. (2009): Office laboratory diagnosis of vaginitis: clinician- performed tests compared with a rapid nucleic acid hybridization test available on line at (http:/ nd articles. Com/P/articles/mi – m – m 0689/is – n6– v4lai -179136341), 02. 05.

Felix, D.B.; Okumura, C.Y.; Colocoussi, A. and Johnson, P.J. (2005). *Trichomonas vaginalis* Lipophosphoglycan mutants have Reduced Adherence and cytotoxicity to human Ectocervical cells. American society for Microbiology, 4(11): 1951 – 1958.

Johnson, R.E.; Green, T.A.; schachter, J.; Jones, R.B.; Hook, E.W.; Black, C.M.; Martin, D.H.; and Louis, M.E. (2000): Evaluation of nucleic acid amplification tests as reference test men. J Clin Microbial 38: 4382 – 4386.

Lubick, K.J. and Burg-ness, D.E. (2004): Purification and analysis of a phospholipase A-like Lytic factor of *Trichomonas vaginalis*. 72(3): 1284 – 1290.

Men-fang, S.; Pey-Ru, L. and Jah-Yao, L.I., (1997): Colorimetric One-Tube Nested PCR for Detection of Trichomonas *vaginalis* in vaginal Discharge. J. of Clinical. Microbiology. 35 (1): 132-138.

Shaio, M.F.; Lin, P.R. and Liu, J.Y. (1997): Colorimetric one - tube Nested PCR for detection of *T. vaginalis* in vaginal discharge, J. of clinical Microbiology, 35(1): 132-138.

Singh, S.; Singh, G.; Gautam, B.; Varadwaj, P. and Farmer, R. (2010): *Trichomonas vaginalis* genome analysis using bioinformatics approaches 3(1): 976 – 944.

Riley, D.E.; Roberis, M.C. and Takayama, T.(1992): Development of Polymerase Chain reaction-based diagnosis of *Trichomonas vaginalis*. J. Clin Microbiol, 30:465- 72.

Johnson, R.E.; Green, T.A.; schachter, J.; Jones, R.B.; Hook, E.W. ; Black, C.M.; Martin, D.H.; Louis, M.E. (2000). Evalution of nucleic acid amplification tests as reference test men. J Clin Microbiol 38: 4382 – 4386.

Stellrecht, K.A.; Woron, A.M. ; Mishrik, N.G. and venezia, R.A. (2004): comparison of multiplex PCR assay with culture for detection of genital mycoplasmas .J. Clin. Microbiol 42, 1528 – 1533.