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# PREDICTION OF TRICHOMONIASIS IN WOMEN COMPLAINING VAGINAL DISCHARGE BY DIFFERENT METHODS AND DETERMINE SOME IMMUNOLOGICAL MARKERS.

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## Abstract

Trichomoniasis is the most prevalent non-viral sexually transmitted infection (STD) in the world caused by the vaginotropic extracellular protozoan parasite *Trichomonas vaginalis*.

The present study was conducted in Babylon Province during the period from October, 2017 to June, 2018 in AL-Hilla Teaching Hospital and AL-Zahraa Hospital, to diagnosis *Trichomonas vaginalis* using two methods direct microbial examination and molecular method by polymerase chain reaction using specific primer pairs of *T. vaginalis* B-Tubulin 9/2 and study levels of some immunoglobulins and cytokines among infected women using immunological methods. A total of (125) blood and fluid vagina samples (110) from infected women and (15) from control women were taken from women aged between (15-45) years. Two vaginal swabs (one for direct microscopical examination and the other for molecular test) and a blood sample were collected from each woman. These women suffer from acute secretions, itching, yellowish green discharge and other symptoms.

The results showed that the total frequency of infection by *T. vaginalis* was (20.9%) in microscope test, while in PCR technique was (13.6 %).

The highest infected number and percentage of infected women was 24 (21.81%) at the age (26-35) years, while the lowest infected at the age (36-45) years was 5 (4.54%) respectively. Also the results revealed the frothy yellow to green discharge 31 (81.57%) while the malodor discharges gave 29 (23.6 8%).

The results revealed a significant increase ($P<0.01$) in serum concentration of proinflammatory cytokines interleukin (8 and 12) and IgG and IgM in *T. vaginalis* infection patients in comparison with healthy control group.

***Key words:*** *Genes; Immunoglobulin's; Cytokines; Infected women*.

## Introduction

Trichomoniasis, simply, is a sexually transmitted disease typically asymptomatic in men and resulting in vaginitis with a copious, frothy discharge and itching in women, caused by *Trichomonas vaginalis* (Arab-Mazar and Niyyati, 2015).

*Trichomonas vaginalis* is an anaerobic, flagellated protozoan parasite which is the causative agent of trichomoniasis. Unlike many protozoan parasites, it possesses trophozoite form and lacks the cyst stage. The organism is most commonly isolated from vaginal secretions in women and urethral secretions in men (Kadir *et al*., 2014).

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May have different the symptoms of infected Women with Trichomoniasis, are a frothy yellow-green vaginal discharge and vulvar irritation. While men with Trichomoniasis may have nongonococcal urethritis. The inflammation of the vaginal epithelium led to redness, swelling and leukocyte infiltration (Scott *et al*., 2005).

Globally, a sexually transmitted infection is considered a major public health problem. Infections with trichomoniasis estimated 143 million new infections annually (Francis *et al*., 2008; WHO, 2016).

There are a plethora of published studies revealed that at least 80% of *T. vaginalis* infections are asymptomatic. However, even asymptomatic infections

are a public health concern. Trichomoniasis is linked to various inflammatory diseases such as prostatitis, pelvic inflammatory disease (PID) as well as to increase in the risk of Human Immunodeficiency Virus (HIV) acquisition. It is also associated with infertility in men and women as well as co-infections with different STIs (Poole and McClelland, 2013).

Diagnosis of trichomoniasis by microscopic examination is considered the most tradition method, Wet mount preparations are useful for giving clear images of fresh specimens under the microscope (Van Der Pol, 2015).

The different samples can be used for the laboratory investigation of trichomoniasis. Vaginal sample (vaginal swab and vaginal secretions), urine and dried blood spot (DBS) are the sample of choices. Vaginal samples have better sensitivity than urine sample to examine the trophozoites to detect anti-trichomonal antibody by enzyme immunoassay (EIA) (Mason *et al*., 2005).

Enzyme immunoassay (EIA) detects exposure to trichomonal infection with >90% sensitivity in men and women (Mason *et al*., 2001) amplification, with increased diagnostic performance have been developed (Hobbs and Seña, 2013).

These tests are validated for the detection of *T. vaginalis* in women and men as well as PCR is the only method currently available for rapid laboratory diagnosis (Samra *et al*., 2011).

## Materials and Methods

### Sample collection

After insertion, endocervical swab was obtained by a specialized gynecologist. The swab was inserted (1-2) cm into the endocervical canal followed by a few rotations (Siegel *et al*., 2007). The swab was stored in (-20°C) until using in PCR technique. By using syringe, 3 ml of venous blood was taken from each woman for the serological test. After that 3ml of the blood sample was placed in a gel tube and left standing to clot, then the tube was centrifuged at 3000 rpm for 10 minutes to collect the serum. All serum was stored in the refrigerator at -20°C until using in ELISA.

### Examination of sample (wet mount examination)

Immediately, one drop from each tube was applied to a glass slide, covered with a cover slip and examined under the microscope by using the high power objective (X40) for the presence of *T. vaginalis*. The wet mounts were examined for at least ten minutes (Philip *et al*., 1987). Positive results were defined as the presence of one or more Trichomonads with characteristic motility (jerky movement) and morphology. The Trichomonads may be inactive and non-motile as in chronic or asymptomatic condition. The wet mount is also used to demonstrate the presence of clue cells in vaginal secretions; these cells were epithelial cells covered by masses of bacteria of varying morphology (Rockett *et al*., 2004).

### DNA extraction:

- **DNA extraction from vaginal swab**

Before DNA extraction, swabs were taken from the freezer and left at room temperature till thawing. The vaginal cotton swab was transferred into Eppendorf tube. Extraction was performed according to the manufacturer company (FAVORGEN Genomic DNA Mini Kit Cultured Cell/USA)

### PCR technique

Pcr protocol was used to investigate B-tubline gene (BTUB) using primers produced by primer company-UAS. Table 1, shows sequences of primers and PCR product sizes of BTUB and TLR4 genes.

**Table 1:** Primer sequences and characteristics.

| Primer | Primer structure (5' – 3') | Segment |
|---|---|---|
| BTUB9 | 5' CATTGATAACGAAGCTCTTTACGAT3' | 112bp |
| BTUB2 | 5' GCATGTTGTGCCGGACATAACCAT3' | |

For B-tubline gene amplification, each pcr tube has contained 12.5 µl master mix with standard buffer, 0.5µl from each R-primer and F-primer, 5 µl of template DNA and 6.5µl of free nuclease water (total volume :25 µl). PCR-mix tubes were closed and transferred then into the thermocycler. The amplification was performed in the PCR tubes and the procedure is as follows in table 2.

### Agarose Gel Electrophoresis

Polymerase chain reaction products were analysed by 2% agarose gel electrophoresis (w/v) using TBE 0.5 X.

### Immuno Tests

- Enzyme immunoassay (EIA) Detection:
- Interleukin – 8 (IL-8):

The Assay Max Human Interleukin -8 (IL- 8) ELISA kit was achieved according to the manufacturing company

**Table 2:** Cycling parameters of genes amplification.

| Primers | Steps | Initial denaturation | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|
| BTUB9/2 | Temp. (C°) | 94 | 94 | 60 | 68 | 68 |
| | Time | 30 sec. | 30 sec. | 45 sec. | 45 sec. | 5 min.. |
| | Cycle | | 45 | | | |

(Elabscience/USA).

**Interleukin – 12 (IL-12)**

The Assay Max Human Interleukin -12 (IL- 12) ELISA kit was achieved according to the manufacturing company (Elabscience/USA).

**Measurement of IgG and IgM Concentration in women Infected with Trichomoniasis by Immune Diffusion Technique**

The Mancini (1965) method has adopted in this test, in brief, the plate was opened and left to stand for about 5 minutes at room temperature so that any condensed water in the wells evaporated, then wells were filled with 5 ìl of undiluted patient samples.

The plate was closed with the lid, the samples was left for about 20 min for diffusing into the gel, then left to stand, overturned into the envelope, at room temperature for 48 hours, then the spreading of the antibody was observed in a round shape as the diameter of the circle increased in the sample. The diameter of the ring formed in the agar plate was then measured with an ocular lens inserted from 1-20 mm and the measurements were compared with antibody concentration in the table attached with the test kit.

**Statistical analysis**

Data processing and the statistical analysis were performed using Statistical Package for the Social Sciences (SPSS; version 18.0). The results were given as mean ± standard deviation (Mean ± S.D). Statistical analysis for the significance of differences of the quantitative data was conducted by using ANOVA test for single factor means. Unpaired, Unequal Variances, Student's t test used for the determination of significant differences between means of different immunoglobulin's and cytokines used in this study and TLR4. The probability levels were indicated as follows (*p < 0.05; **p < 0.01; ***p < 0.001 and ****p < 0.0001 and similarly for other symbols).

## Results

**Total percentage of *T. vaginalis* infection by two methods of diagnosis (Microscopic Examination and PCR).**

In the present study the patient's women were married, non-pregnant aged between 15 and 45 years. Out of (110) high vaginal swab specimens collected from women suspected of having trichomoniasis and (15) healthy women as a control group were examined by microscope and polymerase chain reaction technique, the results showed that the positive samples which was diagnosed by microscopic constitute 23 (20.9%), while those by PCR technique was 15 (13.6 %). In otherwise control after examination all individuals were negative (0% positive). These results are shown in table 3.

**Table 3:** Total percentage of *Trichomonas vaginalis* infection detected by two methods of diagnosis (Microscopic examination and PCR.

| Methods of diagnosis | Total samples | No. P+ (%) | No. P- (%) |
|---|---|---|---|
| Microscopic examination | 110 | 23 (20.9) | 87 (79.09) |
| PCR | 110 | 15 (13.6) | 95 (86.36) |
| Control | 15 | 0 (0) | 15 (100) |

**Molecular techniques**

**• Detection of *Trichomonas vaginalis* using PCR diagnosis:**

The *T. vaginalis* specific primers, PCR amplified a fragment size of 112 bp in positive test samples. No amplification was detected in the negative control samples. The results showed that the extracted DNA of swabs contain parasite DNA as shown in fig. 1, Which revealed the present of single band of amplified DNA with product size of 112 bp.

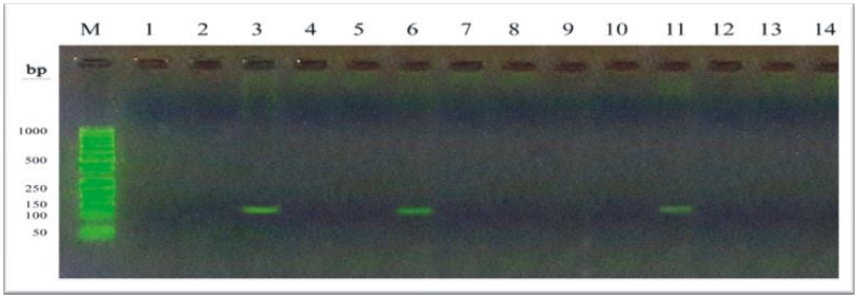


**Fig. 1:** Representative agarose gel image of the amplified PCR product from *T. vaginalis* DNA from collected high vaginal swabs, using red safe dye. M is the 50 bp molecular weight marker; Lane 1 is negative control; Lanes 2 - 14 are the field test samples; Lane 3, 6 and 11 were samples positive for *T. vaginalis*; Lane 2, 4, 5, 7, 8, 9, 10, 12, 13 and 14 were samples negative for *T. vaginalis.*

**The infection percentage of *T. vaginalis* according to age groups**

Table 4, demonstrates that the percentage of infection with trichomoniasis in women according to the age group. The highest incidence of *T. vaginalis* infection occurred in age group of (26-35) years with the percentage of (47.06%), followed by the age group of (15-25) years with the percentage of (32.14%) and followed by the age group of (36-45) years with the percentage of (16.13%).

**Table 4:** The infection percentage of *Trichomonas vaginalis* according to age.

| Age group | Total No. Examined women | No. of infected women | Percentage |
|---|---|---|---|
| 15-25 | 28 | 9 | 32.14 |
| 26-35* | 51 | 24 | 47.06 * |
| 36-45 | 31 | 5 | 16.13 |
| **Total** | **110** | **38** | **34.55** |

*The highest infection with *T.vaginalis.*

### The infection percentage of *T. vaginalis* according to infection symptoms

**• Vaginal discharge color:**

As table 5, indicates that, the percentage of infection with trichomoniasis in women according to vaginal discharge color, the highest infection found with Yellow to green discharge which the number and percentage of infection was 31 (81.57%), followed by the women with bloody discharge 5 (13.15%), followed by the women with clear (white) discharge 2 (5.26%).

**Table 5:** The vaginal discharge color and infection with *T. vaginalis.*

| Symptoms Abnormal vaginal discharge | | No. Positive | Percentage % |
|---|---|---|---|
| Color | White | 2 | 5.26 |
| | Yellow to green | 31* | 81.57 |
| | Bloody | 5 | 13.15 |
| Total | | 38 | 100 |

*The highest infection with *T.vaginalis.*

**• Vaginal discharge odor:**

The results reveal that the number and ratio of the women with malodor vaginal discharge 29 (76.31%), while the women with odorless vaginal discharge had the number and percentage of 9 (23.68%), these results as shown in table 6.

**Table 6:** The vaginal discharge odor and infection with *Trichomonas vaginalis.*

| Symptoms Abnormal vaginal discharge | | No. Positive | Percentage % |
|---|---|---|---|
| Odor | Malodor | 29* | 76.31 |
| | Odorless | 9 | 23.68 |
| **Total** | | **38** | **100** |

*The highest infection with *T.vaginalis.*

## Discussion

The findings suggest that the difference between two methods is due to the results caused by different specificity and sensitivity, wet-mount microscopy, which is commonly used in routine tests, a rapid, inexpensive screening technique, but having low sensitivity shows a sensitivity of (38%) depend on the time, experience, the immediate examination of the specimen, the trophozoites loose movements after the protozoan has been removed from body temperature and may be the use of dry swabs or delayed transportation of the specimen to the laboratory these factors that contribute to low test sensitivity with wet-mount microscopy (Figueroa-Angulo *et al*., 2012; Nathan *et al*., 2015).

The high sensitivity and specificity of PCR reported in this study would offer a useful rapid screening tool. This could reduce spread and transmission of the infection, in particular from asymptomatic patients but its availability and cost effectiveness limit its use in routine diagnostic laboratories according to (Smooker *et al*., 2010).

The finding of the present study suggests that the age groups of (26-35) have higher incidence with this infection than the age group of (15-25) years. This result can be explained by the fact that the ability of the parasite to alternate the vaginal environment for its survival (Huppert *et al*., 2005). The result also shows that the lowest rate of infection in the age group of (36-45) years, these results are identical with the study of (Dahab *et al*., 2012) who proved the prevalence rates tend to decrease in elderly women (40-45) years, may probably be related to the development of acquired immunity to infection with increase of age.

The reason of the malodor may be because of the metabolic by products of anaerobic *T. vaginalis* and other anaerobes that increased the concentration during Trichomoniasis which may contribute to the malodor discharge. The fishy discharge odor may be caused by volatilization of amine, most commonly putrescence and cadaverine, which are produced by bacterial metabolism (Coexistence between *Gardenrella vaginalis* - with *T. vaginalis*), the amine odor often increases with menses and after intercourse when the amine is volatilized by alkalization (Al Gazali *et al*., 2017).

As a mucosal pathogen, *T. vaginalis* must adhere to epithelial cell monolayer and once in contact with host cells, the parasite undergoes a drastic morphological shift that leads to tight association to the target cells (Figueroa-Angulo *et al*., 2012).

"Through the recognition of pathogens or their products, TLRs can induce the production of cytokines such as IL-12 and IL-18 in APCs. These cytokines function as instructive cytokines and drive naïve T cells to differentiate into $T_H1$ cells. Pathogens are also captured in multiple ways, including phagocytosis, endocytosis or via TLRs themselves" (Akira *et al*., 2001)

The finding of the present study also revealed a highly

significant increase in the concentration of (IL-8) cytokines in serum of patient infected with *T. vaginalis* compared to the healthy control group. This result agrees with study of (Nam *et al.*, 2012) proved that the human neutrophils and macrophages stimulated by *T. vaginalis* which produced the (IL-8) and pro-inflammatory cytokines tumor necrosis factor and IL-1β.

(Nguyen *et al*., 2005) revealed that the IL-1β, IL-2, IL-6 and IL-8 present in higher level which correlated with infection agent example *T. vaginalis*.

The result of (Fichorova *et al*., 2006) showed that LPG stimulates a significantly increased IL-8 production in the absence of cell toxicity and at low baseline levels of endogenous IL-1 and TNF-α.

These findings suggest that the production of IL-8 upon *T.vaginalis* infection may be secondary to cytopathic effects and the release of the early response proinflammatory cytokine TNF-α and IL-1β by damage epithelial cells (Al-Qadhi, 2014).

Data from the present study showed highly significant increase in the concentration of proinflammatory IFN-γ and IL-12.

In serum of patients infected with *T. vaginalis* in comparison with control group, suggesting that cytokines and chemokines provide a mechanism for initiation, amplification or containment of inflammation during disease status. This result agrees with study of (Li *et al*., 2018) which proved that *T. vaginalis* induced proinflammatory cytokines production in macrophages through the activation of MAPK *via* TLR2.

The most striking result to emerge from the data is that a significant increase in the concentration of, IgA, IgE and IgG and IgM in serum of infected with *T. vaginalis* patients compared to control group. This agrees with the experimental trichomoniasis conducted by paintlia *et al*., (2002) on mice infected with symptomatic and asymptomatic isolates of *T.vaginalis* alone. This increase in the concentration of IgA, IgE, IgG and IgM cooperates with increase in the B-lymphocyte which generate IgA, IgE, IgG and IgM responses (Vojdani, 2009; Finkelman *et al*., 1990).

Another study demonstrated that the concentration of IgG and IgM significantly increase in serum of infected with *T.vaginalis* patient in comparison with control group. This demonstrates an incitement of the humoral immune response during the infection with *T. vaginalis* (Gould *et al*., 2003; Kaur *et al*., 2008).

## Conclusions

This study showed a low prevalence of *T. vaginalis* infection in the study population.

PCR appears to be the most sensitive and specific method for detection of genital infections with *T. vaginalis*.

Also, there is a significant increase in the cytokines (IL-8 and IL-12) and immunoglobulin's (IgG and IgM) in the serum of women infected with *T. vaginalis*.

This indicates a stimulation of the cellular and humoral immune response during the infection with *T. vaginalis*.

## References

Akira, S., K. Takeda and T. Kaisho (2001). Toll-Like Receptors: Critical Proteins Linking Innate and Acquired Immunity. *Nature Immunology.*, **2:** 675.

Al-Qadhi, B.N. (2014). Research Article Prediction Of Acute Trichomoniasis In Women Complaining Vaginal Discharge By Different Immunological Methods.

Al-Gazali, B.S., M.K. Al-Hadraawy and H.A. Al-Fatlway (2017). Study Level Of Interleukin-1β And Interleukin-2 In Women Infected With *Trichomonas vaginalis* Parasite. *Al-Qadisiyah Medical Journal.*, **10:** 138-143.

Arab-Mazar, Z. and M. Niyyati (2015). *Trichomonas vaginalis* Pathogenesis: A Narrative Review. *Novelty In Biomedicine.*, **3:** 148-154.

Dahab, M., W. Koko, E. Osman and A. Hilali (2012). Prevalence and Transmission Of *Trichomonas vaginalis* Infection Among Women In Khartoum State, Sudan. *Journal Of Public Health and Epidemiology.*, **4:** 34-38.

Fichorova, R.N., R.T. Trifonova, R.O. Gilbert, C.E. Costello, G.R. Hayes, J.J. Lucas and B.N. Singh (2006). *Trichomonas vaginalis* Lipophosphoglycan Triggers A Selective Upregulation Of Cytokines By Human Female Reproductive Tract Epithelial Cells. *Infection and Immunity.*, **74:** 5773-5779.

Figueroa-Angulo, E.E., F.J. Rendón-Gandarilla, J. Puente-Rivera, J.S. Calla-Choque, R.E. Cárdenas-Guerra, J. Ortega-López, L.I. Quintas-Granados, M.E. Alvarez-Sánchez and R. Arroyo (2012). The Effects Of Environmental Factors On The Virulence Of *Trichomonas vaginalis*. *Microbes and Infection.*, **14:** 1411-1427.

Finkelman, F.D., J. Holmes, I.M. Katona, J.F. Urban Jr, M.P. Beckmann, L.S. Park, K.A. Schooley, R.L. Coffman, T. R. Mosmann and W.E. Paul (1990). Lymphokine Control of *In Vivo* Immunoglobulin Isotype Selection. *Annual Review Of Immunology.*, **8:** 303-333.

Francis, S.C., C.K. Kent, J.D. Klausner, L. Rauch, R. Kohn, A. Hardick and C.A. Gaydos (2008). Prevalence Of Rectal *Trichomonas vaginalis* And Mycoplasma Genitalium In Male Patients At The San Francisco Std Clinic, 2005-2006. *Sexually Transmitted Diseases.*, **35:** 797.

Gould, H.J., B.J. Sutton, A.J. Beavil, R.L. Beavil, N. Mccloskey, H.A. Coker, D. Fear and L. Smurthwaite (2003). The Biology

of ige and The Basis of Allergic Disease. *Annual Review Of Immunology.,* **21:** 579-628.

Hobbs, M.M. and A.C. Seña (2013). Modern Diagnosis of *Trichomonas vaginalis* Infection. *Sex Transm Infect.,* **89:** 434-438.

Huppert, J.S., B.E. Batteiger, P. Braslins, J.A. Feldman, M.M. Hobbs, H.Z. Sankey, A.C. Sena and K.A. Wendel (2005). Use Of An Immunochromatographic Assay For Rapid Detection of *Trichomonas vaginalis* In Vaginal Specimens. *Journal Of Clinical Microbiology.,* **43:** 684-687.

Kadir, M.A., M.A. Sulyman, I.S. Dawood and S. Shams-Eldin (2014). *Trichomonas vaginalis* And Associated Microorganisms In Women with Vaginal Discharge In Kerkuk-Iraq. *Ankara Medical Journal.,* **14**.

Kaur, S., S. Khurana, R. Bagga, A. Wanchu and N. Malla (2008). Antitrichomonas IgG, IgM, IgA and IgG Subclass Responses In Human Intravaginal Trichomoniasis. *Parasitology Research.,* **103:** 305.

Li, L., X. Li, P. Gong, X. Zhang, Z. Yang, J. Yang and J. Li (2018). Trichomonas vaginalis Induces Production of Proinflammatory Cytokines In Mouse Macrophages Through Activation Of Mapk And Nf-Kb Pathways Partially Mediated By Tlr2. *Frontiers In Microbiology.,* **9:** 712.

Mason, P.R., P.L. Fiori, P. Cappuccinelli, P. Rappelli and S. Gregson (2005). Seroepidemiology of *Trichomonas vaginalis* In Rural Women In Zimbabwe And Patterns of Association with Hiv Infection. *Epidemiology & Infection.,* **133:** 315-323.

Mason, P.R., S. Gregson, L. Gwanzura, P. Cappuccinelli, P. Rapelli and P.L. Fiori (2001). Enzyme Immunoassay For Urogenital Trichomoniasis As A Marker Of Unsafe Sexual Behaviour. *Epidemiology & Infection.,* **126:** 103-109.

Nam, Y.H., A. Min, S.H. Kim, Y.A. Lee, K.A. Kim, K.J. Song and M.H. Shin (2012). Leukotriene B4 Receptors Blt1 And Blt2 Are Involved In Interleukin-8 Production In Human Neutrophils Induced By *Trichomonas vaginalis*-Derived Secretory Products. *Inflammation Research.,* **61:** 97-102.

Nathan, B., J. Appiah, P. Saunders, D. Heron, T. Nichols, R. Brum, S. Alexander, P. Baraitser and C. Ison (2015). Microscopy Outperformed In A Comparison Of Five Methods For Detecting *Trichomonas vaginalis* In Symptomatic Women. *International Journal of Std & Aids.,* **26:** 251-256.

Nguyen, H.H., T.R. Broker, L.T. Chow, R.D. Alvarez, H.L. Vu, J. Andrasi, L.R. Brewer, G. Jin and J. Mestecky (2005). Immune Responses To Human Papilloma virus In Genital Tract Of Women With Cervical Cancer. *Gynecologic Oncology.,* **96:** 452-461.

Paintlia, M., S. Kaur, I. Gupta, N. Ganguly, R. Mahajan and N. Malla (2002). Specific Iga Response, T-Cell Subtype and Cytokine Profile In Experimental Intravaginal Trichomoniasis. *Parasitology Research.,* **88:** 338-343.

Philip, A., P. Carter-Scott and C. Rogers (1987). An Agar Culture Technique To Quantitate *Trichomonas vaginalis* From Women. *Journal Of Infectious Diseases.,* **155:** 304-308.

Poole, D.N. and R.S. Mcclelland (2013). Global Epidemiology Of *Trichomonas vaginalis*. *Sex Transm Infect.,* **89:** 418-422.

Rockett, J.C., G.M. Buck, C.D. Lynch and S.D. Perreault (2004). The Value Of Home-Based Collection Of Biospecimens In Reproductive Epidemiology. *Environmental Health Perspectives.,* **112:** 94.

Samra, Z., S. Rosenberg and L. Madar-Shapiro (2011). Direct Simultaneous Detection Of 6 Sexually Transmitted Pathogens From Clinical Specimens By Multiplex Polymerase Chain Reaction And Auto-Capillary Electrophoresis. *Diagnostic Microbiology And Infectious Disease.,* **70:** 17-21.

Scott, K., M. Manunta, C. Germain, P. Smith, M. Jones, P. Mitchell, D. Dessi, K. Branigan Bamford, R.I. Lechler and P.L. Fiori (2005). Qualitatively Distinct Patterns of Cytokines Are Released By Human Dendritic Cells In Response To Different Pathogens. *Immunology.,* **116:** 245-254.

Siegel, J.D., E. Rhinehart, M. Jackson, L. Chiarello and H.C.I.C. P.A. Committee (2007). 2007 Guideline For Isolation Precautions: Preventing Transmission of Infectious Agents In Health Care Settings. *American Journal of Infection Control.,* **35:** S65-S164.

Smooker, P.M., R. Jayaraj, R.N. Pike and T.W. Spithill (2010). Cathepsin B Proteases Of Flukes: The Key To Facilitating Parasite Control? *Trends In Parasitology.,* **26:** 506-514.

Van Der Pol, B. (2015). Clinical And Laboratory Testing For Trichomonas vaginalis Infection. *Journal Of Clinical Microbiology.,* Jcm. 02025-15.

Vojdani, A. (2009). Detection Of IgE, IgG, IgA and IgM Antibodies Against Raw And Processed Food Antigens. *Nutrition & Metabolism.,* **6:** 22.

WHO (2016). *Sexually Transmitted Infections (Stis).* Http://Www.Who.Int/Mediacentre/Factsheets/Fs110/En.