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Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Egyptian Women Suffering from Infertility

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Abstract

Chlamydial and gonococcal infections are recognized as two of the major causes of sexually transmissible human bacterial infection which may lead to infertility. In this cross sectional study, we aimed to determine the prevalence of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* among Egyptian women using different microbiological methods. One hundred and fifty cervical swabs were collected, of which 100 were from infertile women. Culture and ELISA technique were used for screening of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* individually. In addition, PCR was used for all examined samples. For *C. trachomatis*, 3 cases were positive for antigen detection by ELISA. Moreover, in obtained results of PCR, DNA was detected in 4 samples, and three of them from infertile group. So based on PCR results, the sensitivity and specificity of ELISA were 75% and 100% respectively. Furthermore, 3 samples were positive for gonococcal infections by PCR, and two of them were taken from infertile women. Positive results of two samples were verified by culture. The estimated sensitivity and specificity of culture method were 66.7% and 100% respectively. Results of this study indicate that PCR is a valuable method for detection of gonococcal and chlamydial infection and it is suitable for the confirmation of ELISA results for *C. trachomatis* diagnosis. Culture method is less sensitive than PCR for detection of *N. gonorrhoeae*. The prevalence of such infections is higher among infertile women.

Keywords

***Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Female Infertility, Culture, Polymerase Chain Reaction, Enzyme-Linked Immunosorbant Assay**

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1. Introduction

Chlamydial and gonococcal infections are recognized as two of the major causes of sexually transmissible human bacterial infection throughout the world. Infertility due to obstruction of fallopian tubes is one of the main severe and lasting consequences of infection with gonorrhea and *Chlamydia* [1]. Women often suffer silently from these infections; as many as 70% to 80% of women who are infected do not experience any symptoms [2]. The majority of infected women cannot be distinguished from uninfected women by clinical examination.

It is estimated that up to 40% of women with untreated *Chlamydia trachomatis* infection will develop (pelvic inflammatory disease) PID which is defined as any combination of endometritis, salpingitis, tubo-ovarian abscess, or pelvic peritonitis [3]. Of those with PID due to chlamydial or gonococcal source, 20% will become infertile, 18% will experience debilitating chronic pelvic pain and 9% will have a life-threatening ectopic pregnancy. Prevalence levels among general populations of African women are highly variable, initially it is attributed to gonococcal infection but now more often (up to 70%) associated with chlamydial disease [4].

In the area of reproductive health, most Egyptian women subjected to one or more potentially septic procedures such as female circumcision, traditionally induced abortion and delivery by traditional midwives. Many husbands also get some of sexual experiences before marriage [5].

Marcia C. Inhorn has studied infertility and the new reproductive technologies (NRTs) in Egypt for many years. He said that the most salient and clear-cut need is for the prevention of the many preventable causes of infertility in Egypt as in other parts of the world. Studies of the prevalence of these infections in Egypt have been hindered by low participation rates, and little is known about the rates among the youngest married women. For all these reasons, improved means for prevention and control of early diagnosed cases are urgently needed [5].

N. gonorrhoeae and *C. trachomatis* were the main cause of male urethritis in study made in Ain Shams University, Egypt [6].

As *C. trachomatis* is an obligate intracellular pathogen, the cell culture remains the reference method and it has 100% specificity but it is not recommended for routine use because of its technical complexity, the long turn-around time and it is unsuitable in developing countries. There is no clinical or microbiological reference standard for diagnosis of *C. trachomatis* infection [7].

Culture was earlier considered the gold standard, however PCR studies suggested that the sensitivity of the culture even in expert laboratories was as low as 75% to 85% and is no longer considered a reference method of new diagnosis assay [8]. In a study in diagnosing women with suspected genital *C. trachomatis* infection using PCR and direct antigen detection methods, the sensitivity of PCR compared to culture was 81.25% and specificity was 90.74%. They found that the ELISA sensitivity was (56.25%) and specificity was (64.81%) and they said that the reevaluation of ELISA depending upon multiple tests as gold standard might increase its sensitivity and specificity [8]. Another study found that the ELISA method has a specificity of 94.8% and verification of positive results can further improve the specificity of this test [9]. PCR procedure also could be suitable for the confirmation of ELISA results, as it has a high concordance rate compared to the rapid test [10].

From all previous studies, we conclude that the ELISA role in screening for *C. trachomatis* needs further studies to evaluate the accuracy of this method.

The current preferred laboratory method for the diagnosis of *N. gonorrhoeae* infections is the isolation and confirmation of culture results by Gm stain and chemical tests. However, if the specimens require long transportation times or have been exposed to extreme temperatures, culture is less sensitive than the nucleic acid methods. The processing time for PCR method is shorter than for cultural methods [11]. Manal M. Amin also found that culture sensitivity and specificity were 58.2% and 100%, respectively, in comparison to the standard PCR test [6]. In a study used culture and three nucleic acid amplification tests, the sensitivities of *N. gonorrhoea* culture and PCR were 65 and 95.8%, respectively and specificity were 100% and 99.4% respectively [12]. In another study, the sensitivity and specificity of PCR testing as compared to true-positives (infected patients) were 96.3% and 98.2% for gonorrhea, respectively [13].

In this work, we tried to evaluate the diagnostic efficacy of detection methods which were culture for *N. gonorrhoeae* and ELISA for *C. trachomatis* in terms of sensitivity, specificity, positive and negative predictive values compared to polymerase chain reaction (PCR) method which was accurate enough to act as the gold standard to establish the most reliable and easy technique for diagnosis of both organisms.

We also aim to find the prevalence of *Chlamydia*/gonorrheal infection among Egyptian women and finding out the correlation between infection presence and infertility by comparison between infection prevalence in infertile and fertile women.

2. Materials and Methods

Inclusion criteria: This project was approved by the Scientific Research Ethic Committee of Faculty of Pharmacy, Suez Canal University. Samples were collected after obtaining written informed consent from 150 women with age ranging from 20 - 45 years old, who attended Adam's Infertility center and Gynecology Outpatient Department of Hai Elsalam Medical center and KILO 11 medical center.

Specimen type: The main way to obtain specimens for Gonorrhea and Chlamydia testing is the endo-cervical swab. Cervical swabs were collected from the 150 women accompanied by the medical history for each one as shown in **Table 1** and **Table 2**.

Table 1. Medical condition of the infertile group.

Total	**100**
Previous miscarriage	4
Tubal blockage	3
PID	12
Pain with intercourse + discharge	34
Asymptomatic	47

Table 2. Medical condition of the fertile (control) group.

Total	50
Discharge + pelvic pain	26
asymptomatic	24

Sample Collection and Transportation: 3 endo-cervical swabs were taken from each patient. Dacron swabs were used for collecting cervical discharges from the endo-cervix. One swab was collected and inoculated directly on Modified Thayer Martin (MTM) agar after getting at room temperature for *N. gonorrhoeae* culture. The inoculated specimen was placed in a container containing a humid atmosphere of 5% - 10% CO_2 and transported to the laboratory accompanied by the filled in appointment card for testing of each patient within 2-5 hours.

Additional 2 swabs were obtained and placed in transport medium 2-sucrose phosphate saline (2SP) and preserved at 4˚C - 8˚C one of them is for ELISA and the other one is for PCR.

2SP medium contained: (0.0146 M K2HPO4.3H20, 0.054 M KH2PO4, 0.2 M sucrose, 2.5 mg/l amphotericin B, 100 - 120 mg/l gentamicin, 10 g/l bovine serum albumin and 0.0025% phenol red solution). 2SP medium is stored at room temperature for 6 hours, at 2˚C - 8˚C for 1 week and at minus 20˚C for 1 month [14].

2.1. Culture Method

Plates were incubated at 37˚C in 5% - 10% carbon dioxide for 36 hours and more than 48 hr because most old cultures would not survive storage condition [11]. Supplemental CO_2 were supplied by CO_2-generating bags. After incubation, the plates were examined for the presence of characteristic morphology of *N. gonorrhoeae* colonies. After 18 - 24 hours of incubation, typical gonococcal colonies started to appear as Grey to white in color, transparent to opaque, convex to flat, and having a diameter of approximately 0.5 - 1.0 mm [15].

A frozen culture is prepared, cells are suspended in both brain heart infusion broth with 20% glycerol and trypticase-soyabean broth with 20% glycerol and preserved in −20˚C [16].

Colonies were then further identified by Gm stain. Gram stained film was prepared and examined for pus cells and Gram negative intracellular and extra cellular gonococci. They are mainly identified in pairs (diplococci) with the coffee-bean-shaped bacterial cells arranged with the concave sides opposing each other. The color of Gram-stained gonococci is pink-red [15] (**Figure 1**).

Figure 1. Typical colonies of *Neisseria gonorrhea* on modified Thayer martin media (MTM).

Finally we confirmed culture results by applying **Rapid positive oxidase test**.

Identification of cytochrome c oxidase is frequently performed using commercial discs or strips impregnated with, e.g., dimethyl-p-phenylenediamine hydrochloride are also available. A single colony was applied on a disc or strip, eventually pre-saturated with distilled water, using a sterile loop. A deep-purple or blue coloration appearing in 10 - 20 seconds denotes a positive oxidase reaction. Absence of color change indicates a negative test result.

Detection of oxidase-positive Gram-negative dipl-ococci is considered sufficient for their presumptive identification as *N. gonorrhoeae* in routine diagnostics [11] (**Figure 2**).

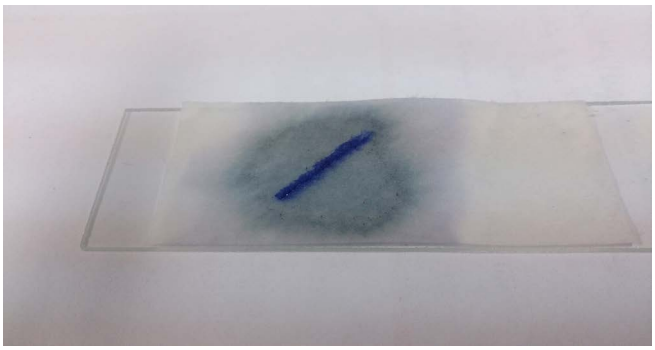

Figure 2. Positive result of oxidase test.

2.2. Enzyme-Linked Immunosorbant Assay (ELISA) Method

Chlamydia trachomatis IgG were detected using a double-antibody sandwich micro assay technique to assay the level of human *Chlamydia trachomatis* (CT) in samples (Human *Chlamydia trachomatis* (CT) ELISA Kit-Bio medical assay company—China).

The Human CT polyclonal antibodies were pre-coated onto well plate. Standard and samples were pipetted into the wells and Human CT present in a sample was bound to the wells by the immobilized antibody. The biotinylated detection antibodies were added to the wells and then followed by washing with PBS or TBS buffer.

After washing away unbound biotinylated antibody, Avidin-Biotin-Peroxidase Complex is *Chlamydia trachomatis* (CT) in swabs pipetted to the wells. The wells were washed again, a TMB substrate solution was added to the wells and the color changed after adding acidic stop solution.

The intensity is proportional to the amount of Human CT bound and measured at 450 nm ± 10 nm.

2.3. Polymerase Chain Reaction (PCR) Method

PCR kit used in the study is an in vitro nucleic acid amplification test for qualitative detection of *Chlamydia trachomatis* and *Neisseria gonorrhea* DNA individually, in the clinical material which is endo-cervical swabs by using electrophoretic detection of the amplified products.

It is based on the amplification of pathogen DNA specific region using specific primers. After PCR, the amplified product is detected in agarose gel. PCR kit also contains the Internal Control (IC) which is used to confirm the final results of clinical samples amplification.

2.3.1. DNA Extraction

Total DNA was extracted from the pellet by using a (QIAmp DNA Mini Kit) with a bacterial DNA protocol.

The pellet was resuspended in 180 ml of buffer ATL (QIAGEN) with 20 ml of proteinase and then incubated at 56˚C with occasional vortexing until the pellet was completely lysed, which took 30 min. After lysis of the sample, 200 ml of buffer AL was added to the sample and the mixture was incubated for 10 min at 70˚C. The mixture was then combined with 200 ml of absolute ethanol and mixed by pulse vortexing for 15 s. The mixture was applied to a spin column, which holds a silica gel membrane, and spun for 1 min at 6000 ×g. The spin column was washed with 500 ml of buffer AW2 by centrifugation at 20,000 ×g for 3 min. The DNA bound on a membrane was eluted by centrifugation with 50 ml of buffer AE after incubation for 5 min at room temperature. The resulting DNA extracts were stored at −20˚C until PCR assessment.

2.3.2. Amplification and Detection of DNA Products

1) For *Chlamydia trachomatis*

Chlamydia trachomatis DNA is detected using the amplification kit (Ref. K014-Chlamydia trachomatis; Genekam Biotechnology AG, Germany). Steps of amplification were done as written in manual.

The micro tubes were marked with a sample number and with control +ve and control −ve. 8 µl of tube A was added to each tube, 10 µl of tube B was added to each tube, 2 µl of extracting DNA template was added, 2 µl of solution "positive control" was added to control +ve tube, and 2 µl of solution "negative control" was added to control −ve tube.

The tubes were put in the Eppeddorf thermo-cycler and run the program shown in **Table 3** which will be 60 cycles program.

The gel Agarose 1% was prepared in TAE (1×) buffer, the gel was getting dried then TAE (1×) buffer was added in gel chamber.

After PCR step was finished test tubes were moved from thermo-cycler, 8µl from each amplicon specimen or control (+ve, −ve) to new empty test tube, 2 µl of dye was added to each test tube, mixed and added the content of each tube to the lane carry the same name of the test tube. 10 µl of marker was added to the first lane of electrophoresis. The gel was run for 60 min. at 120 Volt. The gel was viewed under UV trans-illuminator.

315 bp band of amplicon appeared in control +ve and +ve samples for *Chlamydia trachomatis*, no band in control negative or negative samples (**Figure 3**).

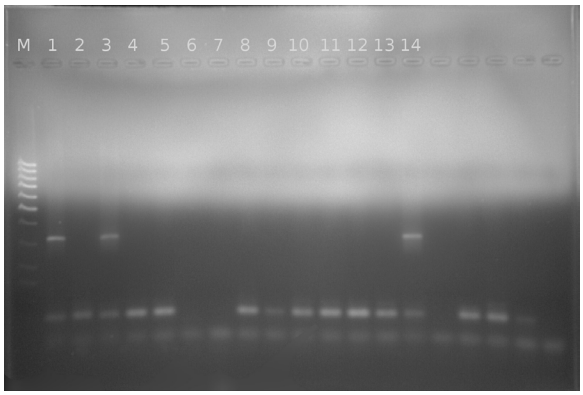

Figure 3. Interpretation of PCR results for *C. trachomatis*. Lane M: molecular weight ladder: 100 bp (max 1000 bp), lane 1: *C. trachomatis* positive control, lane 2: negative control, lanes 3, 14: positive specimen for *C. trachomatis* (315 bp).

Table 3. PCR program for *Chlamydia trachomatis*.

1. 75 seconds at 95˚C	
2. a—45 seconds at 94˚C b—45 seconds at 62˚C c—60 seconds at 72˚C	repeat step 2. for 4 cycles
3. a—45 seconds at 94˚C b—45 seconds at 61˚C c—60 seconds at 72˚C	repeat step 3. for 4 cycles
4. a—45 seconds at 94˚C b—45 seconds at 60˚C c—60 seconds at 72˚C	repeat step 4. for 4 cycles
5. a—45 seconds at 94˚C b—45 seconds at 59˚C c—60 seconds at 72˚C	repeat step 5. for 4 cycles
6. a—45 seconds at 94˚C b—45 seconds at 58˚C c—60 seconds at 72˚C	repeat step 6. for 4 cycles
7. a—45 seconds at 94˚C b—45 seconds at 57˚C c—60 seconds at 72˚C	repeat step 7. for 4 cycles
8. a—45 seconds at 94˚C b—45 seconds at 56˚C c—60 seconds at 72˚C	repeat step 8. for 4 cycles
9. a—45 seconds at 94˚C b—45 seconds at 55˚C c—60 seconds at 72˚C	repeat step 9. for 4 cycles
10. a—45 seconds at 94˚C b—45 seconds at 54˚C c—60 seconds at 72˚C	repeat step 10. for 4 cycles
11. a—45 seconds at 94˚C b—45 seconds at 53˚C c—60 seconds at 72˚C	repeat step 11. for 4 cycles
12. a—45 seconds at 94˚C b—45 seconds at 52˚C c—60 seconds at 72˚C	repeat step 12. for 20 cycles

Table 4. PCR program for *Neisseria gonorrhea*.

1. 120 seconds at 94˚C	
2. a—60 seconds at 94˚C b—60 seconds at 55˚C c—60 seconds at 74˚C	repeat step 2. for 35 cycles

2) **For *Neisseria gonorrhoeae***

Neisseria gonorrhoeae DNA is detected using the amplification kit (Ref. K406-Neisseria gonorrhoeae; Genekam Biotechnology AG, Germany).

The micro tubes were marked with a sample number and with control +ve and control –ve. 8 µl of tube A was added to each tube, 10 µl of tube B was added to each tube, 2 µl of extracting DNA template was added, 2 µl of solution "positive control" was added to control +ve tube and 2 µl of solution "negative control" was added to control –ve tube then placed in the thermal cycler, thermo-cycler programed as in **Table 4**.

The amplification products then electrophoresed using gel Agarose 1% in TAE (1×) buffer containing ethidium bromide and view under ultraviolet light.

The bands appear at 390 bp in +ve controls *Neisseria gonorrhoeae* and positive samples and no bands in –ve control or negative samples (**Figure 4**).

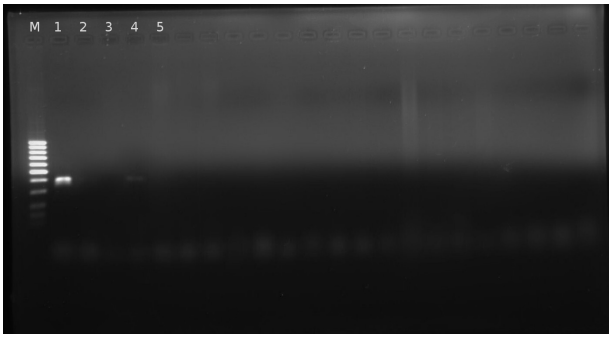

Figure 4. Interpretation of PCR results for *N. gonorrhoeae*, Lane M: molecular weight ladder: 100 bp (max 1000 bp), lane 1: *N. gonorrhoeae* positive control, lane 2: negative control, lane 4: positive specimen *N. gonorrhoeae* (390 bp).

3. Results

This study is one of few studies in Egypt that has focused on the detection of *C. trachomatis* in and *N. gonorrhoeae* using endocervical swab specimens. The mean age of the 150 women enrolled in this study was ranging from 20 - 45 yrs.

The infertile group composed of 100 women, 47 of them were asymptomatic, 34 had pain with intercourse and discharge, 12 had PID symptoms, 4 had previous miscarriage and 3 were suffering from tubal blockage. 26 women of the control group had discharge with pelvic pain, while 24 women were free of all signs and symptoms. Symptomatic cases from both groups were 79 cases.

Chlamydia trachomatis

Table 5 shows the results of different microbiological techniques used Identification methods of *Chlamydia trachomatis* in this study.

C. Trachomatis DNA was detected by PCR in the endocervix of 4 out of 150 cases (2.67%), 2 of them belong to infertile women with tubal blockage, 1 specimen belongs to asymptomatic, infertile women and 1 specimen is from control group from a woman with discharge and pelvic pain, which means that infertility group showed the higher percentage of active *C. Trachomatis* infection compared to the control group, also tubal blockage is the common symptom in chlamydial infection in this group.

Out of 79 suspected cases from both groups (pain with intercourse, discharge, PID symptoms, previous miscarriage and tubal blockage) we found 3 +ve chlamydial infection with a percentage (3.8%).

ELISA method detected the infection in 3 out of 4 cases detected by PCR method.

PCR was considered as gold standard technique.

Disease prevalence: 2.67%.

Among the 4 chlamydial cases identified by PCR, 3 cases were true +ve by ELISA as shown in **Table 6**.

ELISA has 75% sensitivity, 100% specificity;

Positive Predictive Value: 100;

Negative Predictive Value: 99.3.

Neisseria gonorrhoeae

Table 7 shows the results of different microbiological techniques used Identification methods of *Neisseria gonorrhoeaea* in this study.

From 150 samples, 3 gonococcal cases were identified by PCR (2%), 2 of them belonged to the infertile group, both cases suffered from vaginal discharge and pain with intercourse. The third case was from control group and she was suffering from vaginal discharge and pelvic pain.

Out of 79 suspected cases from both groups gonorrheal infection was found in 3 cases (3.8%).

Culture method detected N. gonorrhea in 2 cases out of 3 cases were detected by PCR, which means that infertility group showed the higher prevalence of active *N. gonorrhoeae* infection compared to asymptomatic group. Vaginal discharge and pelvic pain were the common symptoms in infected cases.

PCR was considered as gold standard technique.

Disease prevalence: 2%.

The 2 samples detected by culture showed typical colonies of *Neisseria gonorrhoeae* and confirmed by Gm stain and rabid oxidase test.

Table 5. Identification methods of *Chlamydia trachomatis* (150 cases).

Test	+ve cases			
	Infertile	Fertile	Total	%
ELISA	3	0	3	2
PCR	3	1	4	2.67

Table 6. ELISA test results comparing to the golden standard test PCR in diagnosis of chlamydial infection.

Test		PCR					
		+ve		−ve		Total	
		NO.	%	NO.	%	NO.	%
ELISA	+ve	3	75	0	0	3	2
	−ve	1	25	146	100	147	98
	Total	4	100	146	100	150	100

Table 7. Identification methods of *Neisseria gonorrhea* (150 cases).

Test	+ve cases			
	Infertile	Fertile	Total	%
Culture	1	1	2	1.3
PCR	2	1	3	2

Culture method has 66.7% sensitivity, 100% specificity;
Positive Predictive Value: 100;
Negative Predictive Value: 99.3 (**Table 8**).

Table 8. Culture test results comparing to the golden standard test PCR in diagnosis of gonococcal infection.

Test		PCR					
		+ve		−ve		Total	
		NO.	%	NO.	%	NO.	%
Culture	+ve	2	66.7	0	0	2	1.3
	−ve	1	33.3	147	100	148	98.7
	Total	3	100	147	100	150	100

In general, we found that *Chlamydia* and gonorrheal infections are found low prevalence among Egyptian females.

4. Discussion

The World Health Organization (WHO) estimates that the main curable STIs (gonorrhea, chlamydial infection) are commonest in the 15 - 44 age groups [17]. They are the most common cause of cervicitis and urethritis, and their sequelae (pelvic inflammatory disease, chronic pelvic pain, tubal factor infertility, and reactive arthritis) [18].

Chlamydial infections are primarily an issue of women's health care since the manifestations and conse-

quences are more damaging to the reproductive health in women than in men [19]. Most urogenital *C. trachomatis* and gonococal infections are initially asymptomatic but may subsequently cause considerable long-term morbidity. Consequently, accurate diagnosis of both infections requires the use of specific laboratory techniques. The important progress in laboratory diagnosis of chlamydial infection includes the development of non-viability-dependent test [20].

This study was performed to determine the prevalence of *C. trachomatis* and *N. gonorrhoeae* infection among women attending gynecology and infertility centers in Egypt. 150 cases were examined for both infections by different techniques.

As PCR method is the most sensitive and accurate method for infection detection we use it as golden standard and compare other diagnosing methods with it.

Value of direct antigen detection by ELISA in diagnosing *C. trachomatis* compared to PCR was illustrated in **Table 6**. It was positive in 4 (2.67%) cases. 4 chlamydial cases were identified by PCR, 3 (2%) cases were true positive by ELISA. ELISA had 75 % sensitivity100% specificity, NPV was (99.3%) and PPV was (100%).

This is in agreement with Chernesky who reported that Sensitivity of ELISA varies from 65 to 75% [21]. On the other hand, some authors reported that sensitivity of ELISA was 50 and 58%, respectively and the specificity was 100% by both studies [22] [23]. A study made in Mansoura University Hospital, Egypt used ELISA and PCR techniques to detect *C. trachomatis* in Egyptian women attending Gynecology Clinic; they were consulting for symptoms suggestive of genital infection. Antigen detection by ELISA was positive in 28 (40%) of symptomatic cases. The sensitivity was (56.25%), specificity (64.81%) in this study [24]. Similar results were reported by some authors who found that antigen detection by ELISA had a low sensitivity of (48%) while specificity was (92.9%) [25]. The results obtained by a study made in Gaza, Palestine found that prevalence rate of *C. trachomatis* was 20.2% from women attending gynecology and infertility clinics in Gaza. The sensitivity was 73% for the ELISA versus 100% PCR based method; the specificity was 94% for the ELISA and 98% for the PCR. PCR proved to be superior and more efficient in the diagnosis of *C. trachomatis* than ELISA [26]. Bébéar and de Barbeyra concluded that the diagnosis of *C. trachomatis* is best made by using nucleic acid amplification tests, because they perform well and do not require invasive procedures for specimen collection [27]. The CDC also recommended molecular biological technique for confirmation of positive results [28].

Of 150 specimens examined for gonorrhea, 2 (1.3%) were reported as positive by cervical culture, results are shown in **Table 7**. Culture method has 66.7% sensitivity, 100% specificity comparing to PCR results. Positive Predictive Value: 100, Negative predictive Value: 99.3 (**Table 8**).

A similar result was found by Charlotte A. Gaydos in a study made on Female Soldiers using culture and PCR techniques, PCR detected more N. gonorrhoeae (3.3%) than routine cervical culture (2.1%). The sensitivity of culture method in this study (63.0%) is very close to our results [13]. With same value, in a study made by E. van Dyck, culture method shows (67.8%) sensitivity [12]. Also, Gilson and Mindel, reported that amplification assays have a sensitivity of at least 90% compared with 60% - 70% for culture [29]. An Egyptian study found that the sensitivity and specificity of culture were 58.2% and 100%, respectively, in comparison to the standard PCR test [6]. In all previous studies, specificity of culture method was (100%) like in our study.

Out of 4 *C. trachomatis* infected cases, 2 of them belong to infertile women and 1 case were from a symptomatic woman with discharge and pelvic pain from the control group, which means that infertility group showed the higher prevalence of *C. trachomatis* compared to asymptomatic group, also tubal blockage is the common symptom in chlamydial infection in this group. Out of 79 suspected cases from both groups we found 3 +ve chlamydial infection with a percentage (3.8%).

Also out of 3 gonococcal cases, 2 cases were belonged to the infertile group, both cases suffered from vaginal discharge and pain with intercourse. The third case was from control group and she was suffering from vaginal discharge and pelvic pain. Out of 79 suspected cases from both groups gonorrheal infection was found in 3 cases (3.8%).

5. Conclusion and Recommendations

In summary, we concluded that the PCR procedure is suitable for the confirmation of ELISA results for *C. trachomatis* diagnosis. Culture method for detection of *N. gonorrhea* is highly specific but it is less sensitive than PCR method which is less difficult than cultural method and allows high throughput processing of clinical specimens. The processing time for PCR method is shorter than for cultural method.

In addition, different females in different localities in Egypt should be evaluated to assess combination of tests to find the simplest, rapid and accurate technique that facilitates the diagnosis and epidemiological studies for prevalence rates of *C. trachomatis* and *N. gonorrhoeae* infection in Egyptian females.

Our study also suggests that all infertile or symptomatic women should be screened for *C. trachomatis* and *N. gonorrhoeae*. The prevalence of infection in infertile women is higher than fertile, and is higher in symptomatic women than asymptomatic.

Further studies are needed on a bigger number of cases using molecular techniques for diagnosis STDs in Egypt.

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References

[1] Garnett, G.P. (2008) How Much Infertility Does Chlamydia Cause. *Sexually Transmitted Infections*, **84**, 157. http://dx.doi.org/10.1136/sti.2008.031989

[2] Stamm, W.E. and Cole, B. (1986) Asymptomatic Chlamydia Trachomatis Urethritis in Men. *Sexually Transmitted Infections*, **13**, 163-165. http://dx.doi.org/10.1097/00007435-198607000-00010

[3] Paavonen, J. (1996) Chlamydial Pelvic Inflammatory Disease. *Human Reproduction Update*, **2**, 519-529.

[4] Johnson, R.E., Newhall, W.J., Papp, J.R., Knapp, J.S., Black, C.M. and Gift, T.L. (2002) Screening Tests to Detect Chlamydiatrachomatis and *Neisseria gonorrhoeae* Infections. *Morbidity and Mortality Weekly Report*, **51**, No. RR-15.

[5] Inhorn, M.C. (2003) Global Infertility and the Globalization of New Reproductive Technologies: Illustrations from Egypt. *Social Science & Medicine*, **56**, 1837-1851. http://dx.doi.org/10.1016/S0277-9536(02)00208-3

[6] Amin, M.M., Emara, A., El-Din, H.B. and Elghandour, T.M.A. (2007) Detection of *Neisseria* Gonorrhea, Chlamydia Trachomatis and *Mycoplasma* Genitalium in Acute Male Urethritis Using Multiplex PCR. *Egyptian Journal of Medical Laboratory Sciences*, **16**, 65-76.

[7] Ostergaard, L. (1999) Diagnosis of Urogenital *Chlamydia* Trachomatis Infection by Use of DNA Amplification. *APMIS*, **89**, 5-36. http://dx.doi.org/10.1111/j.1600-0463.1999.tb05672.x

[8] Hagdu, A. (1996) The Discrepancy in Discrepant Analysis. *The Lancet*, **348**, 592-593. http://dx.doi.org/10.1016/S0140-6736(96)05122-7

[9] Malenie, R., Joshi, P.J. and Mathur, M.D. (2006) Chlamydia Trachomatis Antigen Detection in Pregnancy and Ts Verification by Antibody Blocking Assay. *Indian Journal of Medical Microbiology*, **24**, 97-100. http://dx.doi.org/10.4103/0255-0857.25179

[10] Mohamed, N.H. and Shara, T.M. (2001) Evaluation of Different Techniques in Diagnosing *Chlamydial* Endocervical Infection among Egyptian Females. *The Egyptian Journal of Hospital Medicine*, **2**, 138-147.

[11] Ng, L.-K. and Martin, I.E. (2005) The Laboratory Diagnosis of *Neisseria gonorrhoeae*. *Canadian Journal of Infectious Diseases & Medical Microbiology*, **16**, 15-25.

[12] Van Dyck, E., Ieven, M., Pattyn, S., Van Damme, L. and Laga, M. (2001) Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Enzyme Immunoassay, Culture, and Three Nucleic Acid Amplification Tests. *Journal of Clinical Microbiology*, **39**, 1751-1756. http://dx.doi.org/10.1128/JCM.39.5.1751-1756.2001

[13] Gaydos, C.A., Crotchfelt, K.A., Shah, N., Tennant, M., Quinn, T.C., Gaydos, J.C., McKee Jr., K.T. and Rompalo, A.M. (2002) Evaluation of Dry and Wet Transported Intravaginal Swabs in Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in Female Soldiers by PCR. *Journal of Clinical Microbiology*, **40**, 758-761. http://dx.doi.org/10.1128/JCM.40.3.758-761.2002

[14] Dubuis, O., Gorgievski-Hrisoho, M., Germann, D. and Matte, L. (1997) Evaluation of 2-SP Transport Medium for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Two Automated Amplification Systems and Culture for Chlamydia. *Journal of Clinical Pathology*, **50**, 947-950. http://dx.doi.org/10.1136/jcp.50.11.947

[15] Savicheva, A., Sokolovsky, E., Frigo, N., Priputnevich, T., Brilene, T., Deák, J., Ballard, R., Ison, C., Hallén, A., Domeika, M. and Unemo, M. (2007) Guidelines for Laboratory Diagnosis of *Neisseria gonorrhoeae* in East-European Countries. *Acta Medica Lituanica*, **14**, 65-74.

[16] Harbec, P.S. and Turcotte, P. (1996) Preservation of *Neisseria gonorrhoeae* at −20˚C. *Journal of Clinical Microbiology*, **34**, 1143-1146.

[17] World Health Organization (WHO) (2008) Global Incidence and Prevalence of Selected Curable Sexually Transmitted Infections.

[18] Millman, K., Black, C.M., Johnson, R.E., Stamm, W.E., Jones, R.B., Hook, E.W., *et al*. (2004) Population-Based Genetic and Evolutionary Analysis of *Chlamydia trachomatis* Urogenital Strain Variation in the United States. *Journal of Bacteriology*, **186**, 57-65. http://dx.doi.org/10.1128/jb.186.8.2457-2465.2004

[19] Peipert, J.F. (2003) Genital Chlamydial Infections. *New England Journal of Medicine*, **349**, 24-30. http://dx.doi.org/10.1056/NEJMcp030542

[20] Boyadzhyan, B., Yashina, T., Yatabe, J.H., Patnaik, M. and Hill, C.S. (2004) Comparison of the APTIMA CT and GC Assays with the APTIMA Combo 2 Assay, the Abbott LCx Assay, and Direct Fluorescent-Antibody and Culture Assays for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Journal of Clinical Microbiology*, **42**, 3089-3093. http://dx.doi.org/10.1128/JCM.42.7.3089-3093.2004

[21] Chernesky, M.A. (2005) The Laboratory Diagnosis of *Chlamydia trachomatis* Infections. *Canadian Journal of Infectious Diseases & Medical Microbiology*, **16**, 39-44.

[22] Malik, A., Jain, S., Hakim, S., Shukla, I. and Rizvi, M. (2006) *Chlamydia trachomatis* Infection & Female Infertility. *Indian Journal of Medical Research*, **123**, 770-775.

[23] Mania-Pramanik, J., Potdar, S. and Kerkar, S. (2006) Diagnosis of *Chlamydia trachomatis* Infection. *Journal of Clinical Laboratory Analysis*, **20**, 8-14. http://dx.doi.org/10.1002/jcla.20092

[24] El-Fatah Agha, S.A., El-Mashad, N., Rakha, S.A. and El Metwally, A.E.G. (2011) Value of Direct Antigen Detection Methods in Diagnosing Women with Suspected Genital *Chlamydia trachomatis* Infection. *African Journal of Microbiology Research*, **5**, 1215-1219.

[25] Mahilum-Tapay, L., Laitila, V., Wawrzyniak, J.J., Lee, H.H., Alexander, S., Ison, C., Swain, A., Barber, P., Ushiro-Lumb, I. and Goh, B.T. (2007) New Point of Care *Chlamydia* Rapid Test—Bridging the Gap between Diagnosis and Treatment: Performance Evaluation Study. *British Medical Journal*, **335**, 1190-1194. http://dx.doi.org/10.1136/bmj.39402.463854.AE

[26] El Qouqa, I.A., Shubair, M.E., Al Jarousha, A.M. and Sharif, F.A. (2007) Prevalence of *Chlamydia trachomatis* among Women Attending Gynecology and Infertility Clinics in Gaza, Palestine. *International Journal of Infectious Diseases*, **13**, 334-341. http://dx.doi.org/10.1016/j.ijid.2008.07.013

[27] Tong, C.Y.W., Donnelly, C. and Hood, N. (1997) Lowering the Cut off Value of an Automated Chlamydia Enzyme Imunoassay and Confirmation by PCR and Direct Immunofluorescent Antibody Test. *Journal of Clinical Pathology*, **50**, 681-685. http://dx.doi.org/10.1136/jcp.50.8.681

[28] Gilson, R.J. and Mindel, A. (2001) Recent Advances: Sexually Transmitted Infections. *British Medical Journal* (*Clinical Research Ed.*), **322**, 1160-1164. http://dx.doi.org/10.1136/bmj.322.7295.1160

[29] Bébéar, C. and Barbeyrac, B. (2009) Genital *Chlamydia trachomatis* Infections. *Clinical Microbiology and Infection*, **15**, 4-10. http://dx.doi.org/10.1111/j.1469-0691.2008.02647.x