A Comparative Study of Laboratory Diagnosis ofGenital Chlamydial Infections InWomen By Immunofluorescence And By Conventional Staining Method.

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Abstracts: **Objective:** The major objective of this study was to carry out concurrent testing of cervical swab samples with both conventional and fluorescent staining method using Giemsa stain & direct fluorescent antibody stain (DFA) respectively. The study would enable us to establish an appropriate, effective and sensitive method of diagnosis of genital chlamydial infections within the present set-up.**Material & methods:** The study "A Comparative Study of laboratory Diagnosis of Genital Chlamydial Infections in Women by Immunofluorescence and Conventional Staining Method" was conducted on 50 patients attending the OPD of a tertiary care hospital of Ahmedabad. Results: Out of the total 50 cases tested, four samples were found positive containing dark-purple inclusion bodies of Chlamydia trachomatis surrounding the nuclei of the host cells in Giemsa and the same were positive for elementary bodies of C. trachomatis by the DFA staining. Based on these samples, the prevalence of is 8% only.**Conclusion:** The present comparative study of Giemsa and DFA staining for Chlamydia trachomatis infections in females showed that both methods are equally sensitive for the detection of the microorganism.[Jain M NJIRM 2014; 5(4) :1-3]

Key Words: Chlamydia trachomatis, Genital Chlamydia infections

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Introduction: Genital chlamydial infection has become most common sexually transmitted infection worldwide.[2] The World Health Organization (WHO) estimates that approximately two million new cases of Chlamydia occur annually.[3] in females; the disease is manifested in the form of urethritis, mucopurulent cervicitis, endometritis, salpingitis and pelvic inflammatory disorder (PID). C.trachomatis may also cause infertility, ectopic pregnancy, premature deliveries and perinatal morbidity. Genital chlamydiasis is also associated with cervical metaplasia and atypia. It is also considered a HPV co-factor that increases the incidence of cervical carcinoma[3]. More than13.5% women below the age of 25 years have lower genital tract infections reducing to less than 4.9% in women over the age of 25 years.[4]

In India, 20-30% of women with mucopurulent cervicitis & 30-60% of women with salpingitis& pelvic inflammatory diseases have chlamydial infections.[2]

Conventional diagnostic methods i.e. staining of smear though inexpensive but the infections may be undiagnosed in some patients while immunofluorescent technique which is more specific & higher sensitive, is carried out to detect direct antigen in smear.

Materials & methods: Selection criteria of patients: Female patients aged between 15-55 years with complaints of burning micturition, itching in genital areas and cervical discharges were taken for the study. These represent a set of sexually active females. Patients with complaints of vaginal discharge were not included in the study.
Collection of samples: Two Cervical swabs were collected by per speculum examination. Sterile cotton swabs were inserted in the cervical canal for 1 cm. and rotated for 15-20 seconds.

Swabs were kept in sterile test tubes and two smears were prepared on glass slides for each sample. One of these slides was stained with Giemsa stain & the other was stained with fluorescent stain according to the instructions in the kit manual.

An accurate laboratory diagnosis of chlamydial infection is very valuable for the physician to prescribe the right treatment and for epidemiological control.

Kit Source: The Giemsa stain was used from the standard stock available with the Department. The Direct Fluorescence Assay (DFA) test kit used in the study is the Chlamydia T procured from Bioscientifica S.A., Iturri 232/4 (C1427ADD), Buenos Aires, Argentina.

Method of Giemsa staining: The 6 to 8 mm smear was prepared on a clean, sterile glass slide. Each slide was fixed with methyl alcohol for 2 - 3 minutes and stained with Giemsa stain. After that each slide was examined under oil immersion for the epithelial cells, pus cells and inclusion bodies of Chlamydia trachomatis and for the presence of any other cocci or bacilli.

Inclusion bodies appear as blue-mauve to dark-purple bodies surrounding the nucleus. The nuclei of the host cells are dark-purple and the cytoplasm is light-blue.

Method of Direct Fluorescent Antibody (DFA) staining:A clean slide was taken and a circle was traced. A drop of distilled water was put into the circle and the swab was rolled back and forth on the slide to cover the area inside the circle. The smear was air-dried and fixed by 0.5ml of dehydrated acetone then stained with DFA. Each smear was scanned in a fluorescent microscope at 40x for screening and then finally at 100x.

The smear was observed for the presence of extra cellular elemental bodies. If present, they appear against a reddish background as bright apple-green bodies of round shape and smooth borders (300nm approximately).

Positive diagnosis is made when at least 10 elemental *Chlamydia trachomatis* corpuscles[5] are found associated to cells in a clinical sample. Inclusion bodies were compared with those in the positive control slide provided in the kit.

Negative result: A sample with visible cells but without C. trachomatis inclusion corpuscles isinterpreted as negative.

Results & Discussion: For the total 50 cases tested, four samples were found positive containing dark-purple inclusion bodies of *Chlamydia trachomatis*surrounding the nuclei of the host cells in Giemsa and the same were positive for elementary bodies of *C. trachomatis* by the DFA staining. More than 10 apple-green coloured elementary bodies were seen against a red background in DFA staining. This criterion was chosen on the basis of recommendations for *C. trachomatis* detection in DFA testing by CDC, Atlanta.[5]In the remaining samples, no inclusion bodies could be observed by Giemsa staining and no elementary bodies could be observed by the DFA staining method. Based on these samples, the prevalence of *Chlamydia trachomatis* is 8% only. The low figure obtained in the present study could be because of the small size of the study. In a similar study, the prevalence was reported as 10% and in some it reached up to 40% also.[5]

In a majority of the samples, more than five epithelial cells were seen in the smears however in 3 cases out of the 50, epithelial cells were not found. In these cases possibility of missing Chlamydia inclusion bodies is high.

Table 1: Comparison of Patient's Data according To Age of Patients

Age group (in years)	Total patients	Positive
16-25	23	1
26-35	23	2
36-45	4	1
>45	0	0

Table 2: Comparison of Patient's Data according To Occupation

Occupation	Total patients	Positive
House wife	41	4
Workers	9	0

According to table no.1& 2 genital infection of Chlamydia trachomatis can occur in any age group but in the study it is more in 26-35 years of age groupspecially in housewife patients.

Table3: Comparison Of Microscopy Results

Microscopy results	Total patients	Positive
Pus cells &> 5 epithelial cells are seen	39	4
Pus cells are not seen	11	0

Conclusion: The present study of"A Comparative Study of laboratory Diagnosis of Genital Chlamydial Infections in Women by Immunofluorescence and Conventional Staining Method"Giemsa and DFA staining for *Chlamydia trachomatis* infections in females shows that both methods are equally sensitive for the detection of the microorganism. The literature reports that DFA is the more sensitive method of detection for C. trachomatis in genital specimens.

The limitation in the present study was that the number of cases investigated was small so the result we obtained shows similar results for both the methods.

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