

## A COMPARATIVE STUDY FOR DETECTION OF MYCOBACTERIA BY DIRECT AFB SMEAR EXAMINATION, CULTURE BY LOWENSTEIN JENSEN MEDIA, FLUORESCENT SENSOR TECHNOLOGY BASED BACTEC™ MICRO MGIT™ SYSTEM & PCR

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### ABSTRACT

**Background:** Female Genital Tuberculosis is an important cause of infertility in developing countries about 5-16% of cases among women in India. Though the actual incidence may be underreported due to asymptomatic presentation of genital tuberculosis. This study was conducted for early detection of *Mycobacteria* by polymerase chain reaction and compare the results of direct AFB smear examination, conventional method Lowenstein Jensen media, Bactec Micro MGIT & Polymerase Chain Reaction. **Materials & Methods:** A total of 160 genital samples were received from suspected cases of female genital tuberculosis during the year 2013. These samples were processed by modified petroff's method for detection of *Mycobacteria* was done by direct AFB smear examination, conventional method Lowenstein Jensen media, BACTEC Micro MGIT & Polymerase chain reaction. **Results:** Out of 160 genital samples, a total of 9.3% (15/160) were positive, 8/15 (53.3%) were detected by PCR, 5/15 (33.3%) isolates by BACTEC, 5/15 (33.3%) was isolated by LJ and 1/15(6.66%) only by Direct AFB smear examination. Mean time to detection of growth has seen earlier in BD BACTEC Micro MGIT for the detection of *Mycobacteria*. **Conclusion:** In our study PCR came out to be most sensitive method among all followed by MGIT & LJ both, and then direct AFB smear examination.

**Keywords:** BACTEC Micro MGIT, Direct AFB smear, Genital tuberculosis, Lowenstein Jensen Media (LJ), Polymerase Chain Reaction (PCR), Tuberculosis.

### INTRODUCTION:

Female Genital tuberculosis is a major cause of tubal factor infertility in developing countries (1). It is the root cause in about 5-16% of cases of infertility among Indian women, though the actual incidence may be underreported due to asymptomatic presentation of Genital Tuberculosis and paucity of investigations (2, 3). Genital tract TB is a chronic disease that often presents with low grade symptomatology and very few specific complaints. Presenting symptoms are generally varied, infertility being

the most frequent clinical presentation (43-74%). Other clinical presentations include oligomenorrhoea (54%), amenorrhoea (14%), menorrhagia (19%), abdominal pain (42.5%), dyspareunia (5-12%) and dysmenorrhoea (12-30%). The fallopian tube is the initial site of involvement, affected in almost all cases followed by endometrium in 50-90 % of cases. Symptoms are usually mild and local which include pelvic pain, menstrual disorders, vaginal bleeding and/or poor general health (4).

Though various options are available for confirming the diagnosis based on the collective evidences from imaging techniques, direct visualization by endoscopy, serology, and histopathology of material from genital tract, culture and polymerase chain reaction (PCR) test. Laparoscopy generally detects macroscopic changes, such as peritubal adhesion, tubercles on the tubes and small tube ovarian masses that commonly are seen in chronic cases. Female genital TB, being non-infectious, has been neglected by healthcare providers, but is an important cause of both significant morbidity and short and long term sequelae for the affected women(5).

Early diagnosis and treatment of genital tuberculosis with in reproductive age can improve conception rate significantly. Early diagnosis of tuberculosis and initiating optimal treatment would not only enable a cure of an individual patient but will also prevent the transmission of infection and disease to others in the community by allowing the appropriate treatment and giving better prognosis. So it will affect epidemiological control by preventing the spread of disease by rapid diagnosis of genital tuberculosis.

Conventional solid media such as Lowenstein Jensen Media, is time consuming taking up to 6-8 weeks for detection of growth of *Mycobacteria*, it is less sensitive as well and can detect TB only when the sample contains at least 100 organisms/ml. Although microscopy for acid fast bacteria is a rapid procedure but it is also less sensitive and can detect TB only when the sample contains at least 10,000 organisms/ml. BD BACTEC Micro MGIT (Mycobacteria Growth Indicator tube) system is a rapid and sensitive method, amongst other culture methods for early diagnosis of pulmonary and extra pulmonary TB. Molecular methods like

Polymerase Chain Reaction (PCR) are highly specific, sensitive and very fast as compared to the gold standard culture method(6).

Thus rapid and reliable diagnosis of tuberculosis is essential to initiate timely and appropriate treatment. So, it is here that the clinical Microbiology laboratory can play a critical role resulting in curbing the spread of this potentially life threatening disease. Early diagnosis of genital tuberculosis allowing the appropriate treatment and better prognosis will affect epidemiological control by preventing the spread of disease by rapid diagnosis of genital tuberculosis.

In our study we have compared microscopy, BD BACTEC™ Micro MGIT™, Lowenstein Jensen media and PCR technique in the diagnosis of genital Tuberculosis in infertile female patients.

#### **MATERIALS AND METHODS:**

The present study was conducted on suspected cases of genital tuberculosis, whose collected samples were received in the department of Microbiology & Immunology, Mahatma Gandhi Medical College & Hospital from Jaipur Fertility Centre, Jaipur during the year 2013. The PCR samples got tested at Path Corp Pvt. Lab at New Delhi.

**Samples:** 160 genital samples were collected from suspected cases of genital tuberculosis which include endometrial tissue in normal saline, Hydrosalpinx fluid, Product of conception, Pouch of douglas, Cystic fluid, Menstrual blood, Uterine aspirate fluid etc in a sterile container.

**Inclusion criteria:** The study subjects included females having genital tuberculosis and presenting with complaints like primary infertility, secondary infertility, pain abdomen,

abortion, ectopic pregnancies and menstrual irregularities etc, from age group of 20-45 yrs mostly affected the age group 30-40 yrs.

**Sample Processing:** Endometrial tissue were obtained in a tube containing normal saline were crushed by electric tissue homogeniser for 1 minute and then digested and decontaminated by using 4% NaOH-NALC modified petroffs method (7).

**Conventional Culture Method:** Smears were prepared by deposit of the processed sample and then two drops of this digested and decontaminated sample was inoculated to LJ media bottle slant and incubated at 37 degree Celsius for a maximum period of 6-8 weeks.

### **BACTEC™ Micro MGIT™**

For culture on liquid media, deposit was inoculated on BACTEC Micro MGIT Media. Prior to sample inoculation in MGIT tubes, 800 micro litre of PANTA which had been reconstituted with growth supplement, was added and finally 500 micro litre of the concentrated sample was inoculated into the media and the contents of the tube were mix well. LJ media were checked twice weekly for first two weeks & then every week for growth, manually for maximum period of 8 weeks. BACTEC Micro MGIT tube were checked every alternate day in 1<sup>st</sup> week and then bi-weekly in a 2<sup>nd</sup> week and then once in a week proceeded up to till 6 weeks. For reading the tubes, a positive control and negative control are important for correctly interpreting the results. Using the Micro MGIT calibration tube, place the tubes in the BACTEC Micro MGIT fluorescence reader. If reading above 14 mark-up to 20 are considered as positive where as tubes of mark 1-13 are considered as negative. Smears were made from BACTEC Micro MGIT tubes to confirm the

presence or absence of *Mycobacteria*. The identification of Mycobacterial species in positive cultures done by Biochemical test (Niacin test & Nitrate test) and MPT64 Ag card test (BD MGIT™ TB<sup>C</sup> Identification Test & SD Boline standard diagnostics Inc.).

### **Molecular identification (Viable Prokaryotic mRNA)**

PCR samples got tested at Path Corp Diagnostics Pvt Ltd. at New Delhi (Naraina Industrial Area). As per the details obtained from Path Corp Diagnostic Pvt Ltd, Real time PCR detected viable prokaryotic bacteria mRNA by reverse transcriptase for *Mycobacterium tuberculosis* (viable prokaryotic mRNA) & the primers used in PCR is specific for M.Tb. A positive signal would indicate the presence of recently viable organisms. If it's less than 70 copies /ml considered as negative and if greater than 70 copies /ml considered as positive.

### **RESULTS:**

Out of 160 samples received, a total of 15(9.3%) were positive by combination of all the method used. Overall positivity by PCR alone was 8/160(5%), by BACTEC Micro MGIT 5/160(3.1%), by Lowenstein Jensen Media 5/160 (3.1%) and by direct AFB smears 1/160(.62%). Out of total 15 genital positive samples, 13 were endometrial tissue, 8/13(61.5%) were detected by PCR, 4/13(30.7%) isolated by BACTEC, 4/13(30.7%) isolated by LJ media and one sample was isolated by direct AFB smear (7.6%). Out of 2 Hydrosalpinx fluid samples, 1 came out to be positive by BACTEC and other one by LJ as shown in table no 1.

12 samples were positive either by PCR or BACTEC or combination of both, 1/12 (8.3%) isolated by both PCR and BACTEC, 7 samples

were isolated by PCR alone and 4/12 (33.33%) isolated by BACTEC alone (Table no 2).

Out of 12 positive samples, 1/12 (8.3%) isolated by both PCR and LJ, 7/12(58.33%) were isolated by PCR alone and 4/12(33.33%) isolated by LJ alone but these were diagnosed as NTMs (Table no 3).

Out of 8 positive isolates by BACTEC and LJ, 2/8 (25%) positive by both culture, 3/8 (37.5%) was isolated by BACTEC alone and 3/8 (37.5%) were isolated by LJ (Table no 4).

Mean time to detection of growth (TTD) by BACTEC was 12.4 days and 20.2 days by L.J. The sensitivity of MGIT & LJ is equal except the average time to detection of growth which was lesser in MGIT (avg 12.4 days) than LJ (20.2 days).

The Contamination rate of BACTEC Micro MGIT was found to be less 1.25% (2/160) than Lowenstein Jensen media 1.8% (3/160).

Identification of Mycobacterium Species by the Four Different Culture Methods is shown in Table no 5. For Non tuberculosis *mycobacteria* (NTM) PCR detection rate was none because the primers used in PCR is specific for M.Tb.

## DISCUSSION:

The present study demonstrated that PCR provided better isolation rate of *Mycobacterium tuberculosis* amongst all, followed by MGIT & LJ both and then direct AFB smear examination. Several studies have evaluated clinical utility of PCR in mycobacterium infection. In our study PCR showed a positivity rate of 5%, which is slightly varied from Tanu Rana *et al* (8) who reported the positivity rate of 2.8%. The low positivity rate of our study due to mRNA detection as mRNA present only in viable Mycobacteria. Some of the earlier studies have higher reported which is varied from 23.78% to 56% (9-13).

In the present study, BACTEC Micro MGIT showed a positivity rate of 3.1% which is equal to LJ except on average time to detection of growth which was seen earlier in BACTEC (12.4 days) in contrast to L.J. method (20.2 days). This has great implication on the treatment failure and emerging of multi drug resistance tuberculosis in patients. Contamination rate of BACTEC Micro MGIT was found to be less 1.25% (2/160) than Lowenstein Jensen media 1.8% (3/160) which may be attributed due to longer transportation time of the specimen from the fertility centre to the laboratory, leading to overgrowth of contaminants.

In the present study, LJ showed a positivity rate of 3.1% which is also confirmed by other findings study who have reported 3.2%, 5.4%, 5.5%, 5.6% respectively(13-15). Among BACTEC and LJ, BACTEC Micro MGIT was found to be most efficient method to isolate *Mycobacterium* as it detects earlier than LJ but for maximum recovery of *Mycobacteria*, important to use both type of media.

Smear microscopy is simple and easy to perform thus making it more chosen technique but it has poor sensitivity. In our study it showed a positivity rate of 0.62% which is in consistence with the findings of Bhanu *et al* (13) who reported a positivity rate 1.6% respectively. Srivastva *et al* (14) reported the lowest positivity rate of .45% as compared to our study. The low AFB smear and culture positivity in endometrial tissue is due to the paucibacillary nature of the endometrial sample (13).

In our study PCR was found to be most sensitive and fastest method for the detection of *Mycobacteria* among all the methods used. Seven samples were negative by PCR those positive by culture identified as NTM, the primers used by path corp. laboratory are specific only for *Mycobacterium tuberculosis* and detect

only mRNA which is present only in viable *Mycobacterium* so it helpful in diagnose active genital tuberculosis but now with rising incidence of infections due to NTM it is important to also include primers which are genus specific. False positive result may be come due to cross contamination as it is performed in closed tube so risk of contamination is minimized and false negative result may be due to PCR inhibitors so care should be taken to prevent the false results. So early diagnosis of tuberculosis by molecular method will helpful to initiate appropriate treatment leading to successful pregnancies and change in psychology of the patients and prevent the transmission of infection and disease in community and gives better prognosis.

### CONCLUSION:

Real time PCR is very rapid and sensitive test for diagnosis of genital tuberculosis and has great utility in providing diagnosis in clinically relevant time. However, further improvement can be done in the test by doing multiplex PCR with genus and MTB specific primers to detect all the Mycobacteria.

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**Table1: Specimen-wise Percentage of Positivity by the Four Different Methods**

Type of specimen	Total No. of positive	Positive by PCR	Positive by BACTEC	Positive by LJ	Positive by AFB smear
Endometrial Tissue	13	8/13 (61.5%)	4/13 (30.7%)	4/13 (30.7%)	1/13 (7.6%)
Hydrosalpinx fluid	2	0 (0%)	1/2 (50%)	1/2 (50%)	0 (0%)

**Table 2: Distribution of Isolates Recovered in Combination of PCR (Polymerase Chain Reaction) and Liquid Culture Media and in Each System Alone**

Total no. of positive samples by PCR & BACTEC	Positive by both PCR and BACTEC	Positive by PCR alone	Positive by BACTEC alone
12	1/12 (8.3%)	7/12 (58.33%)	4/12 (33.33%)

**Table 3: Distribution of Isolates Recovered in Combination of PCR (Polymerase Chain Reaction) and Solid Culture Media (Lowenstein Jensen Media) and in Each System Alone**

Total no. of positive samples by PCR & LJ	Positive by both PCR and LJ	Positive by PCR alone	Positive by LJ alone
12	1/12 (8.3%)	7/12 (58.33%)	4/12 (33.33%)

**Table 4: Distribution of Isolates Recovered in Combination of BD BACTEC Micro MGIT and Lowenstein Jensen Media and in Each Culture Alone**

Total No. of positive samples by BACTEC & LJ	Positive by both BACTEC and LJ	Positive by BACTEC alone	Positive by LJ alone
8	2/8 (25%)	3/8 (37.5%)	3/8 (37.5%)

**Table 5: Identification of Mycobacterium Species by the Four Different Culture Methods**

Mycobacterium species	Total no. positive	Positive by PCR	Positive by BACTEC	Positive by LJ	Positive by AFB Smear
<i>Mycobacterium tuberculosis</i>	8	8/8 (100%)	1/8 (12.5%)	1/8 (12.5%)	1/8 (12.5%)
Non tuberculous <i>mycobacteria</i>	7	0 (0%)	4/7 (57.1%)	4/7 (57.1%)	0 (0%)
<b>Total</b>	<b>15</b>	<b>8</b>	<b>5</b>	<b>5</b>	<b>1</b>