Comparative evaluation of Ziehl-Neelsen staining, Fluorescent staining and Culture with Polymerase Chain Reaction in the diagnosis of Genital Tuberculosis in infertile women - One year cross sectional study at Tertiary Care Hospital,Belagavi.

By

REG.NO.BI0114001

Dissertation

Submitted to the

KLE University, Belagavi, Karnataka

In partial fulfillment

of the requirements for the degree of

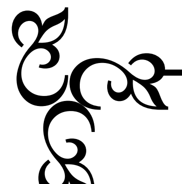
DOCTOR OF MEDICICNE
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,

JAWAHARLAL NEHRU MEDICAL COLLEGE,

KLE UNIVERSITY, BELAGAVI,KARNATAKA.

APRIL 2016



KLE UNIVERSITY, BELAGAVI, KARNATAKA

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **" Comparative evaluation of Ziehl-Neelsen staining, Fluorescent staining and Culture with Polymerase Chain Reaction in the diagnosis of Genital Tuberculosis in infertile women - One year cross sectional study at Tertiary Care Hospital, Belagavi "** is a bonafide research work done by **REG. NO. BI0114001**

Dr.Sumati Hogade MD.

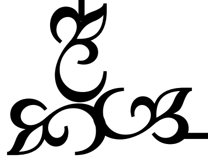
Professor & Head,
Department of Microbiology,
Belagavi – 590010

Date:
Place: **BELAGAVI**

Dr.N.S.Mahantashetti MD.(paed)

Principal,
J.N.Medical College,
Belagavi – 590010

Date:
Place: **BELAGAVI**



LIST OF ABBREVIATIONS USED

AFB	-	Acid Fast Bacilli
ADC	-	albumin-dextrose-catalase
DNA	-	Deoxyribose Nucleic Acid
EPTB	-	Extrapulmonary tuberculosis
FGTB	-	Female genital tuberculosis
HIV	-	Human Immunodeficiency Virus.
IGRA	-	Interferon-gamma release assays
LAM	-	Lipoarabinomannan
L-J	-	Lowensten Jensen
MAbs	-	monoclonal antibodies
OADC	-	oleic acid, albumin, dextrose and catalase
PIM	-	Phosphatidyl-myo-inositol mannosides
PB	-	Proskauer and Beck (medium)
μl	-	Microlitre.
PCR	-	Polymerase chain reaction
TB	-	Tuberulosis
WHO	-	World Health Organization
ZN	-	Ziehl-Neelsen

ABSTRACT

Comparative evaluation of Ziehl-Neelsen staining, Fluorescent staining and Culture with Polymerase chain reaction in the diagnosis of Genital Tuberculosis in infertile women-One year cross sectional study at Tertiary Care Hospital, Belagavi.

Background:

Tuberculosis is a chronic infectious disease caused by *Mycobacterium Tuberculosis* (*Mtb*).Female Genital tuberculosis (FGTB) is associated with infertility. Still, the true epidemiology of this disease remains unknown due to lack of highly sensitive and specific tests. Genital tuberculosis not only causes tubal obstruction and dysfunction but also impairs implantation due to endometrial involvement and ovulatory failure from ovarian involvement. Clinical diagnosis of FGTB is challenging due to varied clinical presentation. Microscopy and Culture to detect *Mycobacteria* though specific have low detection rate due to paucibacillary nature of FGTB.Molecular detection of *Mtb* DNA by Polymerase chain reaction (PCR) is rapid and sensitive being immensely employed to diagnose FGTB.

Objective:

To compare the diagnostic ability of Ziehl-Neelsen staining, Fluorescent staining and Culture with Polymerase Chain Reaction in the diagnosis of Genital Tuberculosis in infertile women.

Methodology:

The Cross-sectional study for one year duration (Jan-Dec 2015) was conducted on eighty cases of female infertililty at KLE'S Dr. Prabhakar Kore Hospital and Research Center,Belagavi who met the inclusion and exclusion criteria, after obtaining their informed consent. Institutional Ethical Clearance (MDC/DOME/318) was sought.Endometrial tissue sample was collected in a sterile universal container containing normal saline.The sample was homogenised and subjected for Ziehl-Neelsen staining, Culture(Lowenstein-Jensen media) and DNA TB-PCR .The samples were processed in Biosafety cabinet II , adhering to Universal safety precautions in the department of Microbiology, JNMC, Belagavi.

Results and Discussion:

Out of 80 Samples, 39 cases were clinically suspected to have Genital TB and the remainder had no clinical suspicion. Out of 80 samples tested , Acid-fast bacilli was not detected in the microscopy.No organisms were grown in culture.DNA TB-PCR was positive in 4 of the 39 clinically suspected cases of FGTB while no *Mtb* DNA was detected in other 51 infertile cases who did not have clinical suspicion.

Conclusion:

FGTB being paucibacillary, diagnosis by Conventional methods (Microscopy and Culture) have limitations and low detection rate.Culture (8 weeks) is gold standard test. Molecular test like PCR which is rapid and more sensitive may help in early diagnosis of FGTB and can be a chief tool to confirm diagnosis in clinically suspected cases. False negative PCR was a limitation in this study.

Keywords

Female Genital Tuberculosis, Smear Microscopy, LJ Media, DNA TB-PCR, IS*6110*.

CONTENTS

SL. NO.	TOPIC	PAGE NO.
1	INTRODUCTION	1-3
2	OBJECTIVES	4
3	REVIEW OF LITERATURE	5-59
4	METHODOLOGY	60-70
5	PHOTOGRAPHS	71-74
6	RESULTS	75-84
7	DISCUSSION	85-89
8	CONCLUSION	90
9	SUMMARY	91
10	BIBLIOGRAPHY	92-101
	ANNEXURES	
11	ANNEXURES I – ETHICAL CLEARANCE LETTER	102
	ANNEXURE II – CONSENT FORM	103-116
	ANNEXURE III – DATA COLLECTION INSTRUMENT	117-123
	ANNEXURE IV– H37 Rv LETTER	124

	ANNEXURE V – STAIN AND MEDIA PREPARATION	**126-130**
	ANNEXURE VI – MTB GROWTH CHART AND REPORT FORMATS	**131-133**
	ANNEXURE VII - MASTER CHART.	**134-137**

LIST OF TABLES

SL. NO.	DESCRIPTION	PAGE NO.
1	Controls and media used for the biochemical identification of mycobacteria	43
2	Distinctive properties of commonly cultivable Mycobacteria encountered in Clinical specimens	44
3	Key biochemical reactions to help distinguish Mycobacteria belonging to the same Mycobacterial group.	45
4	Age distribution of patients studied	75
5	Diagnosis distribution of patients studied	76
6	Clinical Suspicion of FGTB distribution of patients studied	77
7	ZN Stain of patients studied in relation to Clinical suspicion of FGTB	78
8	Fluorescent Stain of patients studied in relation to Clinical suspicion of FGTB	79
9	Culture of patients studied in relation to Clinical suspicion of FGTB	80

10	**PCR of patients studied in relation to Clinical suspicion of FGTB**	**81**
11	**Age distribution of patients studied in relation to PCR findings**	**82**
12	**Diagnosis of patients studied in relation to PCR findings**	**83**

LIST OF FIGURES AND GRAPHS

SL. NO.	DESCRIPTION	PAGE NO.
1	**Anatomy of Female Genital tract showing Tuberculosis in order of frequency**	**07**
2	**The structure of the *Mycobacterium tuberculosis* cell wall**	**26**
3	**A flow chart for specimen processing for isolation of Mycobacteria**	**33**
4	**Steps in PCR –Denaturation,Annealing and Extension**	**47**
5	**Bar graph showing age distribution of patients studied**	**75**
6	**Bar graph showing diagnosis distribution of patients studied**	**76**
7	**Pie graph showing clinical Suspicion of FGTB distribution of patients studied**	**77**

8	**Bar graph showing ZN Stain of patients studied in relation to Clinical suspicion of FGTB**	**78**
9	**Bar graph showing Fluorescent Stain of patients studied in relation to Clinical suspicion of FGTB**	**79**
10	**Bar graph showing Culture of patients studied in relation to Clinical suspicion of FGTB**	**80**
11	**Bar graph showing PCR of patients studied in relation to Clinical suspicion of FGTB**	**81**
12	**Bar graph showing Age distribution of patients studied in relation to PCR findings**	**82**
13	**Bar graph showing diagnosis of patients studied in relation to PCR findings**	**83**

LIST OF PHOTOGRAPHS

SL. NO.	DESCRIPTION	PAGE NO.
1	**Biosafety cabinet -2**	71
2	**ZN staining of *Mtb* H37Rv strain**	71
3	**Fluorescent staining of *Mtb* H37Rv Strain**	72
4	**LJ media**	72
5	**LJ medium with *Mtb* growth**	72
6	**Photograph of gel documentation of *Mtb* DNA**	73
7	**Photograph of eppendorf mastercycler gradient**	74
8	**Photograph of gel documentation system.**	74

INTRODUCTION

A dread disease in which the struggle between soul and body is so gradual, quiet and solemn, and the result so sure that day by day, and grain by grain, the mortal part wastes and withers away. A disease … which sometimes moves in giant strides and sometimes at a tardy sluggish pace, but, slow or quick, is ever sure and certain.[1]

—Charles Dickens: *Nicholas Nickleby*

Tuberculosis (TB) is a chronic infection caused by *Mycobacterium tuberculosis(Mtb).* It is a major public health problem in many developing countries.[2] An estimated 30 million persons have active TB and 7–10 million people die each year because of this infection globally.[3] TB can affect any organ in the body and presents as pulmonary (PTB) and extrapulmonary (EPTB) tuberculosis . Female Genital TB (FGTB) is one such type of EPTB responsible for a significant proportion of women presenting with infertility.[2]The incidence of FGTB is high in India and accounts for 1–19% of cases of total infertile cases diagnosed each year. [4]

Female Genital TB (FGTB) poses a great challenge for the treating clinicians in terms of diagnosis and treatment in a woman in her reproductive age, when she has the most important role in the family. It causes significant morbidity, short and long term sequelae, especially infertility in the affected women in reproductive age in India.The age of presentation of FGTB is lower in developing countries than in developed countries possibly due to younger age at marriage and child-bearing. FGTB is caused by *Mycobacterium tuberculosis* in most cases and mostly occurs secondary to a focus elsewhere in the body which is usually healed or quiescent and becomes active after a long latent period. Chance of FGTB increases if the primary infection occurs near time of menarche. Primary focus is usually pulmonary (in 50% cases) or

extra-pulmonary TB like lymph nodes (40%), bones and joints (5%), urinary or gastro-intestinal tract(5%). It is also estimated that 8-15% women with pulmonary TB may have co-existent genital TB which is usually silent. However, primary genital TB can rarely occur in women whose male partners have active genitourinary TB (e.g. tuberculosis epididymitis) by transmission through infected semen.The spread of TB from lungs and other sites is usually by haematogenous or lymphatic route, less commonly by direct contiguous spread from nearby abdominal organs like intestines or abdominal lymph nodes can cause genital TB.[(5)] It not only causes tubal obstruction and dysfunction but also impairs implantation due to endometrial involvement and ovulatory failure from ovarian involvement. [(6)]

Unlike pulmonary tuberculosis, the clinical diagnosis of FGTB is difficult because in majority of cases the disease is either asymptomatic or has varied clinical presentation. [(7)] The diagnosis requires high index of suspicion. [(8,9)] The diagnosis of FGTB is challenging due to its paucibacillary nature as well as the lack of any concrete laboratory investigation technique. Laparoscopy being the clinical gold standard may miss the early changes of tubercular pathology whereas histopathological examination (HPE) can be confirmatory only when it demonstrates acid fast bacilli (AFB) in the tissue which is very scarce in the case of the endometrial biopsy specimen. Certain tests such as Mantoux test, erythrocyte sedimentation rate (ESR) and serum adenosine deaminase levels have also shown poor sensitivity in this regard. Similarly, microbiological techniques including Ziehl–Neelsen (ZN) stain, Lowenstein–Jensen (LJ) culture and BACTEC Mycobacterial Growth Indicator Tube (MGIT) culture are associated with the disadvantages of low sensitivity and long turn around time which prevents the early diagnosis of the disease when irreparable damage to the fallopian tubes and subsequent infertility can be avoided.[(4)]

A combination of improved traditional techniques and application of molecular diagnostic methods are being used these days as a tool for rapid detection and identification of mycobacteria in clinical specimens and positive cultures.[10] Recently, Nucleic Acid Amplification techniques (NAAT) such as Polymerase Chain Reaction (PCR) targeting various genes such as IS*6110*, *mpt64* and ESAT-6 have been reported to be more sensitive, specific and less time consuming.[4,7,11,12]

OBJECTIVES

To compare the diagnostic ability of Ziehl-Neelsen staining, Fluorescent staining and Culture techniques with Polymerase Chain Reaction in the diagnosis of Genital Tuberculosis in infertile women.

REVIEW OF LITERATURE

FEMALE GENITAL TUBERCULOSIS (FGTB)

Genital tuberculosis in women is an infection of female genital tract with tubercle bacilli. The infection mostly being secondary to a nidus elsewhere in the body. Thus by the time the genital lesion is found which can be at any age, the primary has often healed and is inscospicuous. In about 50% affected women a past history of an extragenital infection can be elicited and a further number of patients can recall if questioned closely, contact with the disease in childhood or adolescence.

The tubercle bacilli reach the female genital tract by one of the following modes,

- **Hematogenous:**

This mode of transmission accounts for at least 90% of cases, the primary focus being quite often situated in the lungs,lymph nodes, urinary tract, bones and joints---in that order.

- **Descending:**

In this type, infection reaches the pelvic organs by direct or by lymphatic spread from infected adjacent organs such as the peritoneum, bowel and mesenteric nodes.

- **Ascending:**

Female having coitus with a male suffering from urogenital tuberculosis can be one of the modes of contracting infection.[13]

Etiological agent:

In most of the cases it is generally the human bacillus (*Mycobacterium tuberculosis*) which is found as the etiological intracellular microbe.In countries where cattle or milk is free from tubercle bacilli, the human bacillus is found in 95% of cases of genital tract infection.

When the pelvic disease is secondary to tuberculous peritonitis, or when the primary focus is in the lymph nodes or bowel, the bovine bacillus(*Mycobacterium bovis*) is likely to be involved .[14]

Pathology: The various genital organs affected by TB in order of frequency are

- Fallopian tubes (90-100% cases),
- Endometrium (50-80% cases),
- Ovaries (20-30% cases),
- Cervix (5-15%) and
- Vagina and vulva (1% cases).[15]

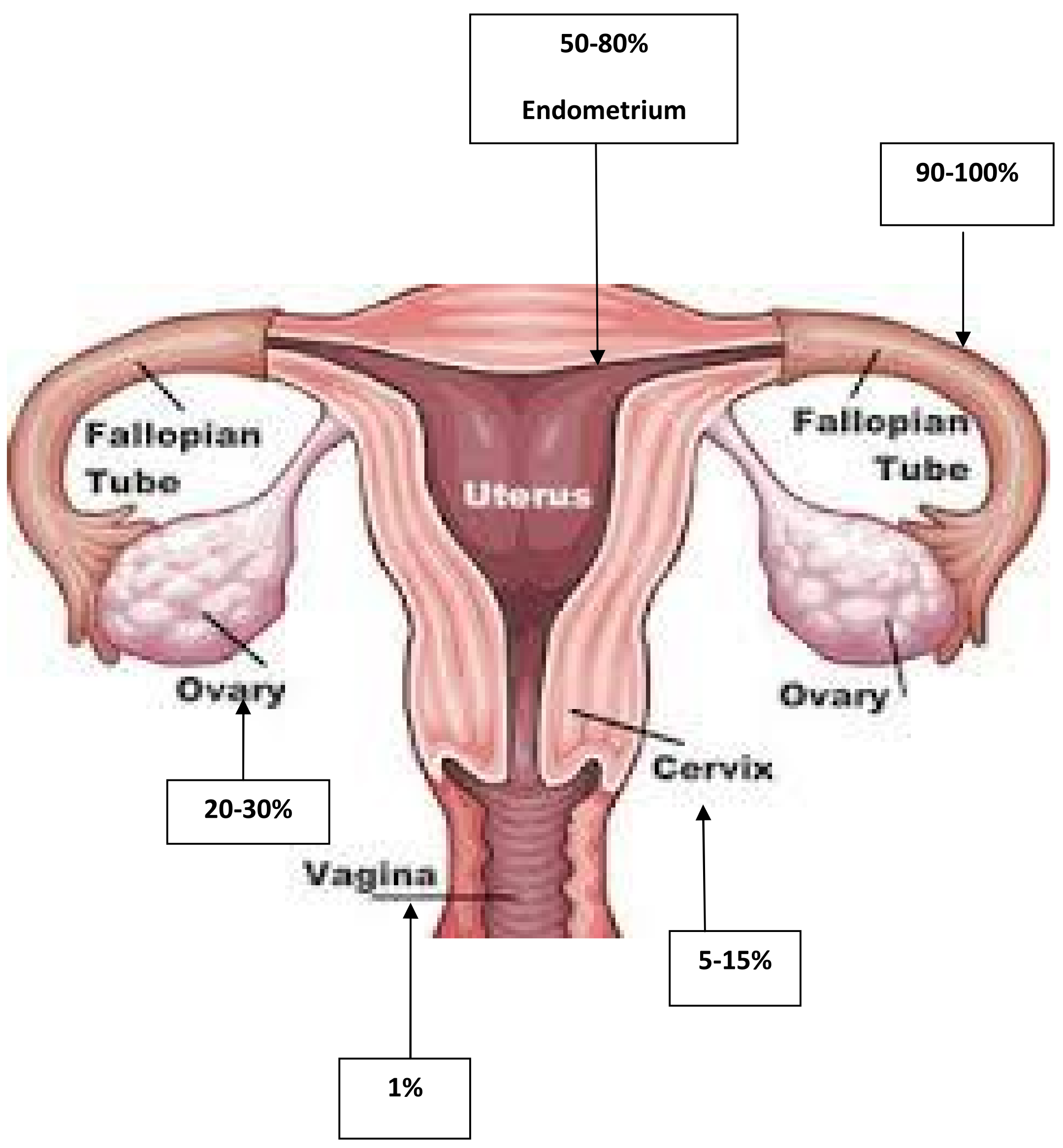


Fig.1-Anatomy of Female Genital tract showing infection of *Mtb* in order of frequency.

www.shutterstock.com 8763040

- **Vulva and vagina :**

Tuberculosis of the vulva and vagina usually takes the form of shallow,superficial,indolent ulcers with undermined edges.The ulceration tends to spread slowly, healing in some areas with the formation of scar tissue.A vulvar hypertrophic lesion is less common and mostly represents inflammatory induration and odema resulting from fibrosis and lymphatic obstruction.

- **Cervix:**

Clinically recognisable Tuberculosis of the cervix can be ulcerative but more often appears as a bright red papillary erosion which bleeds easily. It can be confused with carcinoma. As a histological finding , Cervical Tuberculosis is not uncommon in cases of endometrial tuberculosis.

- **Uterus:**

The uterus usually looks normal to the naked eye although a typical tuberculous ulcer may rarely be seen in the endometrium. Extensive involvement can result in collections of caseous material to form a type of pyometra, or to cause abscesses in the myometrium. Adhesions and partial obliteration of the cavity are also described.

However tuberculous endomeritis is only recognised by histological and bacteriological examination of the tissues.The finding of endometrial tuberculosis almost always means that the tubes are infected, but tuberculous salphingitis can exist without associated endometritis. It begins in the submucosa at the outer ends of the tubes and gradually progresses inwards, bombarding the endometrium with bacilli.

- **Fallopian tubes:**

The appearance of tuberculous tubes varies widely and depends to some extent on whether the infection is blood borne or spreads directly from the peritoneum or bowel.In the one case the disease is primarily endosalphingitis, in the other an exosalphingitis with the possibilities of tubercles on the surface and of dense surrounding adhesions.

Sometimes the tubes look completely normal more often they appear red,oedematous and swollen when the infection is active and fibrosed when it is chronic.They do not have tubercles on their surface.Tubercles when seen on the tubes and the peritoneum at laparoscopy or laparotomy are not always caused by tuberculosis.They may be an end-result of any chronic inflammation,whether infectious or non-infectious e.g.talc or starch granulomas,or peritoneal deciduous.The tubal lumina are closed in only 50% of cases in which there is bacteriological and histological evidence of endometrial disease.In this respect, however it seems likely that many examples of tubal obstruction of uncertain etiology represent completely inactivated and unprovable old tuberculous lesions.In tuberculosis the obstructions are typically multiple and the tube wall is thickened and shotty.Sometimes a localised closure at the outer end results in the formation of a hydrosalpinx or a pyosalpinx with thick fibrous walls which can become calcified or even ossified .A tuberculous pyosalphinx is often remarkably free from adhesions and may be so large that the patient's complaint is the presence of an abdominal tumour.

A hypertrophic form of endosalphingitis has the macroscopic and sometimes microscopic appearances of adenocarcinoma and can be mistaken for such.

Secondary infecton is often present and may itself account for symptoms or for exacerbations of the disease.

- **Ovaries:**

The ovaries are infected in atleast 30% of cases of tuberculous salphingitis but ovarian Tuberculosis without tubal involvement is rare.The disease can manifest by way of surface tubercles, adhesions and thickening of the capsule, retention cysts and sometimes by caseating abscess cavities in the substance of the ovary.Often,however the ovaries have a normal macroscopic appearance and the diagnosis is only revealed by laboratory studies of excised tissue.

Clinical features

Tuberculosis of uterus and adnexa:

This is often a silent disease.It may be present 20 or more years without producing any symptoms,the woman remaining in apparently excellent health.The presence of pelvic tuberculosis is most often revealed by the investigation of infertility and discovered in women 20-40 years.

It should be routine practice to look for bacteriological and histological evidence of endometrial tuberculosis in all cases of infertility, amenorrhoea and also when there are grounds for clinical suspicion.Symptoms are as follow,

1) Infertility: Primary Tuberculosis is the commonest symptom.This is a feature of 70% of cases and occurs even when the tubes are open reflecting abnormal tubal and endometrial function.

2)Ectopic pregnancy: Every woman who has or has had a tubal pregnancy should also be suspected of having tubal tuberculosis.

3)Menstrual disturbances:In approximately 50 % of cases the menstrual function is normal.In the others it is generally held that the change is mostly in the direction of menorrhagia and polymenorrhoea.Amenorrhoea or oligomenorrhoea is also seen.

4)Intermenstrual discharge:blood stained discharge noted in case of ulceration of of cervix or endometrium.

5)Pain:An intermittent chronic ache in the lower abdomen often of long standing is noted in 20-30% of cases.

6)General disturbances: Conditions such as malaise, loss of weight, night sweats and pyrexia are only seen during an unusually active phase of the disease.

DIAGNOSIS:

I.CLINICAL:

Some of the guidelines are,

- TB should be suspected and excluded in every woman whose infertility or amenorrhoea is not explained by other causes.
- Any virgin having symptoms and signs of chronic pelvic infection should be assumed to have TB until it is proved to the contrary.
- Any pelvic infection which is slow to respond to the ordinary methods of treatment is suspect and so is one which shows an exacerbation after curretage or tubal patency tests or one which is not accompanied by polymorphonuclear leucocytosis.
- Sometimes a previous history of peritonitis,appendicectomy with slow healing, pleurisy and a prolonged illness in childhood or a history of TB affecting other members of the family and childhood contacts provides the lead.
- The finding of an active or healed extragenital lesion should always raise suspicion,as should the radiological demonstration of calcification in the tube or ovary or the classical appearance of isthmica nodosa on a hysterosalphingogram performed before the diagnosis is suspected or made.

An advantage is taken of the fact that the endometrium is nearly always involved and can be obtained for examination by endometrial biopsy or aspiration.Specimens should be preferrably be taken from the cornual regions.The most preferred time to collect sample is during the week preceding menstruation.This

is because the tubercles and bacteria are mostly found in the surface layers which are shed during menstruation.[14]

II.LABORATORY DIAGNOSIS:

1) Complete blood count, ESR, Mantoux test, HPR, Abdominal scan, Laparoscopy.

2) **Microbiology diagnosis:**

A) Microscopy

B) Cultivation

C) Nucleic Acid Amplification test-PCR

D) Guinea pig inoculation test.[13]

A) Microscopy: **(i) Ziehl-Neelsen stain**:

Mycobacteria possess cell walls that contain mycolic acids, which are long-chain, multiple cross-linked fatty acids. These long-chain mycolic acids probably serve to complex basic dyes, contributing to the characteristic of acid-fastness that distinguishes mycobacteria from other bacteria.

The mycolic acids and lipids in the mycobacterial cell wall probably account for the unusual resistance of these organisms to the effects of drying and harsh decontaminating agents, as well as for acidfastness.

Three types of staining procedures are used in the laboratory for rapid detection and confirmation of acid-fast bacilli: **Ziehl-Neelsen, Kinyoun and**

fluorochrome Acid-fast stained smears of clinical specimens require at least 10^4 acid-fast bacilli per milliliter for detection from concentrated specimens.

In extra-pulmonary lesions the bacilli are sparse in number. The smear positivity is low as stated in few previous studies conducted similarly [15-22] There are studies showing comparatively higher positivity [23-25] by smear microscopy.

Although mycobacterial demonstration in examination of sputum is the mainstay of diagnosis of pulmonary TB where there is a high bacterial load,the very low sensitivity for diagnosis of paucibacillary disease limits its practical utility in EPTB[26]

(ii) Fluorescent Microscopy:

Fluorescent staining is more sensitive than the conventional carbolfuchsin stains, because

- the fluorescent bacilli stand out brightly against the background
- the smear can be initially examined at lower magnifications (250× to 400×), and therefore more fields can be visualized in a short period.
- In addition, a positive fluorescent smear may be restained by the conventional Ziehl-Neelsen or Kinyoun procedure, thereby saving the time needed to make a fresh smear.

Screening of specimens with rhodamine or rhodamine auramine will result in a higher yield of positive smears and will substantially reduce the amount of time needed for examining smears.

One drawback associated with the fluorochrome stains is that many rapid-growers may not appear fluorescent with these reagents. It is recommended that all positive fluorescent smears be confirmed with a Ziehl-Neelsen stain or examination by another technologist.[15]

B) Culture on LJ media:

Accurate identification of *M. tuberculosis* through culture is presently the yardstick for diagnosis and remains the gold standard. However, in spite of inoculation into multiple media,the rate of MTB isolation is low .

Similar detection rates of *M. tuberculosis* by culture have been reported earlier[5,7, 26 ,27]

The possible reasons for the low incidence of culture positivity in endometrial tissue could be

- due to paucibacillary nature and a substantial number of TB lesions of the genital tract are bacteriologically mute[7,19].
- The low rate of positivity in culture may also be due to the presence of a bacteriostatic substance which inhibits the growth of the bacilli. [7,28]

In a few of the studies carried out earlier, varied range of culture positivity from 2% to 7.8% was noted.[19,20,22-23,29,30]. No growth of MTB was noted in one of the previous studies. [31]

C) PCR

The studies done by Bhanu et al,Thangappah et al,Rana et.al showed high sensitivity and specificity by PCR technique in diagnosing FGTB compared to conventional methods. However PCR has few drawbacks**,**

False positive PCR results with negative clinical findings could be

- due to the early disease with low number of bacilli or
- with latent infection which are picked by PCR when women are still asymptomatic and before the structural damage to the tube has taken place.
- Contamination dead bacilli or previous infection or asymptomatic TB at another site.

The possible explanation for the false negative results of PCR could be,

- due to paucibacillary nature of the specimen, and
- the portion of the specimen taken for PCR would not have had any *M. tuberculosis*.
- The analyzed specimen may also contain inhibitors of PCR. [(7)]

The most widely used primers to detect *M. tuberculosis* in clinical specimens by PCR are from the insertion element IS *6110*. It has already been reported that 40 per cent of the strains of *M. tuberculosis* isolated from patients in Chennai had only a single copy of IS*6110* and 4 per cent did not carry even a single copy of IS*6110*. [(32)]

Based on the results of PCR, various studies have shown that a positive PCR result should be given due importance. Therefore, in clinically suspected cases, in the presence of positive PCR results, an infertile woman should be considered as having GTB and should be treated.

A negative PCR may result in missing the diagnosis in a few cases. Therefore, when GTB is suspected clinically, but the PCR results are negative, it indicates the need for further evaluation using other diagnostic tests and repeat testing to confirm/exclude diagnosis.[7]

IS*6110*:

IS6110 has been most commonly used for detection of *Mycobacterium tuberculosis (Mtb)* but has certain limitations as it is reported that *Mtb* isolates from certain parts of India may have no copy or very low copy numbers of IS6110 element and this may lead to false negative results. [19,33]

A significant proportion (40%) of south Indian isolates have been reported with single copy or lacked this sequence and thus, precluded its use in the context of Indian isolates of *M. tuberculosis*

Based on the copy number, *M. tuberculosis* isolates were classified into four groups,

(i) lacking IS*6110* element;

(ii) low copy number (1-2);

(iii) intermediate copy number (3-5); and

(iv) high copy number (6-19).

Copy number higher than 19 however was not observed in any of the isolates studied. At the national level, 56 per cent of the isolates showed high copy number of IS*6110*, 13 per cent showed intermediate copy number, 20 per cent showed low copy number, whereas11 per cent isolates lacked IS*6110* element. At the regional level, there was not much difference in the RFLP profiles of isolates (IS*6110* copy numbers/patterns) from different parts of the country.[34]

The IS*6110* sequence is present in most clinical isolates of *M. tuberculosis*; however, 4/378 (1%) isolates and 19/80 (24%) isolates in two studies from southern India showed an absence of even a single copy of IS*6110*. [35-37]

Treatment:

- General
- Chemotherapy
- Surgery

Chemotherapy:

- Multiple drug therapy in adequate doses and for sufficient duration is the main stay in the treatment of TB including FGTB.
- Genital TB is classified under category 1 being seriously ill extra-pulmonary disease treatment as per guidelines for national programmes by WHO.
- To ensure quality-assured drugs in adequate doses, a full six-month course pack box is booked for an individual patient in the DOTS centre with fixed drug combipack (FDC) of isoniazid, rifampicin, pyrazinamide and ethambutol thrice a

week for the first two months (intensive phase) under direct observation followed by combination blister pack of isoniazid and rifampicin thrice a week for the next four months (continuation phase).

- Rarely, FGTB, cases can have relapse or failure categorizing them into category II which includes two months intramuscular injections of streptomycin thrice weekly along with other four drugs (RHZE) of category I under direct supervision of DOTS centre health worker for the first two months followed by four drugs (RHZE) thrice a week for another month (Intensive phase), followed by continuation phase with three drugs isoniazid (H), rifampicin (R) and ethambutol (E) thrice a week for another five months.[36-38] Patients not opting for DOTS treatment must take daily therapy of RHZE for two months (intensive phase) followed by RH for four months (continuation phase).[36]
- Treatment of multi drug resistant (MDR) FGTB is the same as for pulmonary MDR-TB with second line drugs and is needed for long duration (18-24 months).[36-38]

Surgery:

- The modern short course chemotherapy consisting of rifampicin and other drugs is highly effective for the treatment of FGTB with rare need of surgery.
- However, limited surgery like drainage from residual large pelvis or tubo ovarian abscesses, or pyosalpinx can be performed followed by ATT for better results.

Outcome for fertility in FGTB is only good when ATT is started in early disease.

- However, cases of advanced TB with extensive adhesions in pelvis and uterus are usually untreatable with very poor prognosis for fertility.
- Tuboplasty performed after ATT does not help much with chances of flare up of the disease and risk of ectopic pregnancy, should women conceive.
- Surgical therapy usually consists of total abdominal hysterectomy and salpingoophorectomy in cases of persistence of pelvic masses after complete medical treatment.[13]

MYCOBACTERIA

❖ Definition:

The genus Mycobacteria is currently the only genus in the family mycobacteriaceae, order actinomycetales and related to other mycolic acid – containing genera.The minimal standards for including a species in this genus are:

- Acid –alcohol fastness(i.e. resist decolourisation by acidified alcohol after being stained with basic fuchsin dye);
- The presence of mycolic acid containing 60 -90 carbon atoms which are cleaved to C_{22}-C_{26} fatty acid methyl esters by pyrolysis; and
- A G + C content of the DNA of 61-71mol% with the only expression being *M.leprae* (more than 51%).

Mycobacteria are non-motile, non-spore forming, weakly Gram –positive aerobic or microaerophilic, straight or slightly curved rod shaped bacteria (0.2-0.6x1.0-10 um) [39-41]

❖ History

TB was recognized as a clinical entity as far back as 1000 BC. In 1744, Morgagni described the first case of genital TB. The word tuberculosis was first used in 1834, although Koch did not discover the tubercle bacilli until 1882. [42] In 1874, Armauer Hansen identified a rod shaped (*Bacillus leprae*) in a tissue biopsy from a patient and suggested that it was the etiological agent of leprosy(Hansen 1880). Eight years later, Robert Koch identified another rod shaped bacillus (*Bacterium tuberculosis*) as the causative agent of tuberculosis and formulated Koch's postulates for establishing a causal relationship between a suspected pathogen and a given

disease (Koch 1882). These two species were subsequently renamed *Mycobacetrium Leprae* and *Mycobacterium Tuberculosis* , respectively and placed in the genus *Mycobacterium* ('fungus bacterium', named to reflect a mould- like pellicle formed by MTB on liquid media).[15]

❖ **Classification:**

Currently there are about 100 species in th genus Mycobacteria. [39] Mycobacteria are divided in two major groups based on fundamental differences in epidemiology and association with disease i.e those belonging to *Mycobacterium tuberculosis* complex and those referred to as *nontuberculous mycobacteria* (NTM).

I.MYCOBACTERIUM TUBERCULOSIS COMPLEX

1.*M. tuberculosis.*

2.*M. bovis .*

3.*M. bovis BCG .*

4.*M. africanum.*

5.*M.canettii.*

6.*M. microti.*

II.NONTUBERCULOUS MYCOBACTERIA

A.Slow-Growing .

a)Nonphotochromogens	b)Photochromogens	c)Scotochromogens'
M. avium complex	*M.Kansasi*	*M. szulgai*
M. celatum	*M.asiaticum*	*M. scrofulaceum*
M. ulcerans	*M.marinum*	*M. interjectum*
M. gastri	*M.intermedium*	*M. gordonae*
M. genavense		*M. cookii*
M. haemophilum		*M. hiberniae*
M. malmoense		*M. lentiflavum*
M. shimoidei		*M. conspicuum*
M. simiae complex		*M.heckeshornense*
M. xenopi		*M. tusciae*
M. terrae complex		*M. kubicae*
M. heidelbergense		*M. ulcerans*
M. branderi		*M. bohemicum*
M. triplex		
M. conspicuum		

B. Rapid-Growing

a) Potentially Pathogenic	b) Rarely Pathogenic	
M. fortuitum	*M. agri*	*M.murale*
M.chelonae	*M.aichiense*	*M.neoaurum*
M. abscessus	*M.austroafricanum*	*M. obuense*
M. smegmatis	*M.aurum*	*M. parafortuitum*
M. peregrinum	*M.brumae*	*M. phlei*
M. Mucogenicum	*M.chitae*	*M. pulveris*
M. fortuitum third biovariant sorbitol-positive	*M.chubuense*	*M. rhodesiae*
M. fortuitum third biovariant sorbitol-negative†	*M.duvali*	*M. senegalense*
M. Wolinskyi	*M.duvali*	*M. sphagni*
M. goodiii	*M.fallax*	*M. thermoresistible*
M. septicum	*M.flavescens*	*M. tokaiense*
M. mageritense	*M.gadium*	*M. vaccae*
M. canariasense	*M.gilvum*	
M. alvei	*M.hassiacum*	
M. novocastrense	*M.komossense*	
M.immunogenum	*M.moriokaense*	

C.Noncultivatable

1.*M. leprae*

*This box is not inclusive, but rather lists only the prominent mycobacteria isolated from humans.† Currently consists of genomospecies with new species names proposed of *M. bonikei, M. houstonense, M. neworleansense,* and *M. brisbanense.*[15]

In 1959 Runyon classified NTM into 4 groups based on phenotypic characters ie. Growth rate and pigmentation as mentione below.[15]

Runyon Group Number	Group Name	Description
I	Photochromogens	Colonies of NTM that develop pigment following exposure to light after being grown in the dark and take more than 7 days to appear on solid media
II	Scotochromogens	Colonies of NTM that develop pigment following exposure to light after being grown in the dark and take more than 7 days to appear on solid media
III	Nonphotochromogens	Colonies of NTM that are nonpigmented regardless of whether they are grown in the dark or light and take more than 7 days to appear on solid media
IV	Rapid-growers	Colonies of NTM that appear on solid media in less than 7 days.

- **Nutritional requirements of Mycobacteria**

The basic nutritional requirements of mycobacteria growing in axenic media are similar to those of other bacteria which include **carbon, nitrogen, oxygen, phosphorous, sulfur, iron, sodium, potassium, magnesium and trace elements (zinc and manganese).** *M genavense, M paratuberculosis and M.haemophilum* being fastiduous are the exceptions. [39,43]

❖ **Cellwall structure and components**

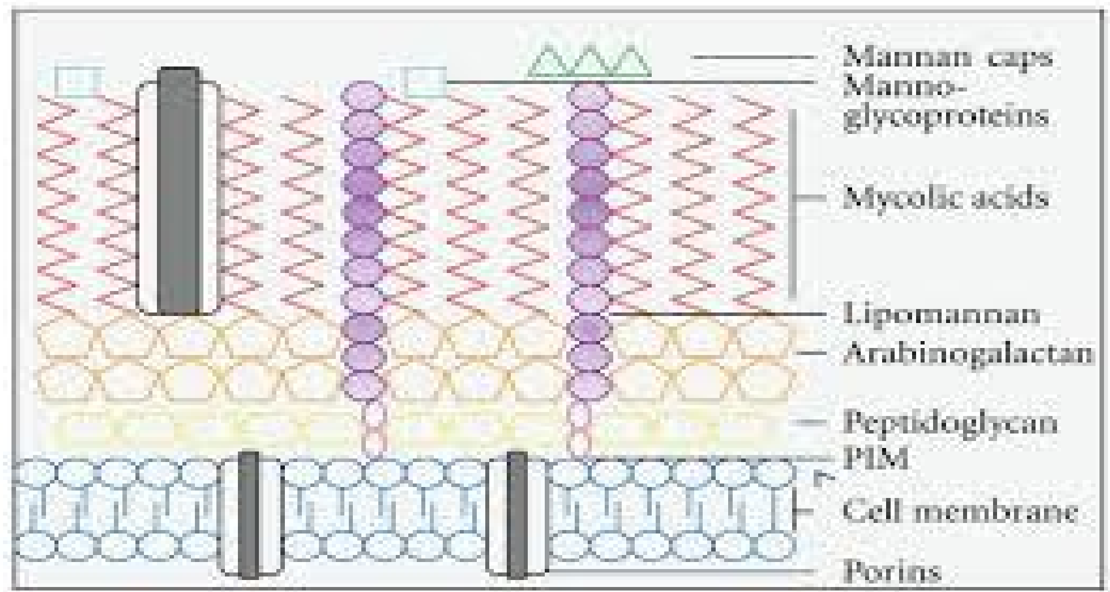


Fig.2 <u>The structure of the *Mycobacterium tuberculosis* cell wall</u>. This figure shows a schematic representation of the major components of the cell wall and their distributions. The inner layer is composed of peptidoglycan which is covalently linked to arabinogalactan layer.The outer membrane contains mycolic acids, glycolipids like (mannose-capped) lipomannan, and mannoglycoproteins. <u>www.hindawi.com/journals/jir/2011/405310/fig1/</u> [accessed on 22.10.2016]

Mycobacteria have a complex outer envelope composed of several distinct layers.[45,46] The plasma membrane (innermost asymmetric bilayer structure) layer contains proteins, phosphatidylinositol mannosides and lipoarabinomannan inserted in it.Next is the peptidoglycan layer which determines the shape of the cell. It contains repeating disaccharide units of N-acetylglucosamine-(beta1-4)-N-glycolylmuramic acid cross-linked via L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanine tetrapeptides. About 10%of the N-glycolylmuramic acid residues are covalently attached to a branched-chain polysaccharide, arabinogalactan via phosphodiester bonds.Distinct arabinose residues of the arabinogalactan molecules are esterified to high-molecular-weight mycolic acids.Finally the outer

surface of the MTB is found by the intercalation of medium –chain(eg mycocerosates) and short chain (eg acylglycerols) lipids,glycolipids and peptidoglycolipids into the uneven hydrophic layer of mycolic acids.Proteins (eg porins,transport proteins) are found throught the various layers.

1.Lipids:

High content of lipid in the outer surface of mycobacteria contributes to several biological features namely hydrophobicity of mycobacteria, their tendency to form clumps and cords,their resistance to common lysis procedures and their ability to survive for a long period of time. [(47)]

2.Lipoarabinomannan:

LAM is anchored in the plasma membrane and extends through the cellwall to the surface of the mycobacterium.Biological activities of LAM include strong seroreactivity, inhibition of interferon-gamma-mediated activation of macrophages, induction of cytokine production and relaese by macrophages, scavenging reactive oxygen intermediates,and suppression of T-cell proliferation [(48)]

3.Mycolic acids:

The mycolic acids are alpha-branched,beta-hydroxy fatty acids with 60-90carbon atoms in the primary chain.The alkyl branches are attached to the alpha position and typically contain 22-26 carbon atoms.

Each species of Mycobacterium appears to synthesize a unique set of mycolic acids, and this has been exploited for identifying mycobacteria [(49)] The resulting pattern is compared with a library of reference patterns to identify the species.

4.Acetylated trehalose:

It is a cell surface component contributing to virulence of *Mtb*.

5.Mycosides:

The outer surface of *Mtb* contains heterogenous group of biologically and immunologically active medium and short chain lipids.They are,

1)peptidoglycans,which contain mycoserosic acid,sugars and amino acids and

2)phenol-phthiocerol glycosides.

6.Antigens:

Tuberculins, Soluble antigens being cytoplasmic and secreted proteins and soluble carbohydrates, Insoluble antigens being cellwall components, membrane –bound proteins,high molecular weight carbohydrates,lipids,mycolic acids ,phenolic acids.

Secreted antigens have been suggested to be key targets of a protective immune response to *Mtb*. [50]

- **Mycobacterial genomes**[51]

The complete genome sequence of the best-characterized strain of Mycobacterium tuberculosis, H37Rv, has been determined andanalysed in order to improve our understanding of the biologyof this slow-growing pathogen and to help the conception of new prophylactic and therapeutic interventions. The genome comprises 4,411,529 base pairs, contains around 4,000 genes,and hasavery high guanine+cytosine content that is reflected in the biased amino-acid content of the

proteins. M. tuberculosis differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation.

❖ Innate Immunity and Host Defense

After the inhalation of infected aerosols into the lungs of the host, the first encounter of mycobacteria is with alveolar resident macrophages. Mycobacteria that escape the initial intracellular destruction can multiply and disrupt the macrophage, after which chemokines are released, attracting monocytes and other inflammatory cells to the lung. Inflammatory monocytes will differentiate into macrophages, which readily ingest but do not destroy the mycobacteria. In this stage of the infection, the mycobacteria grow logarithmically and blood-derived macrophages accumulate, but little tissue damage occurs. Two-to-three weeks after infection, T-cell immunity develops and antigen-specific T lymphocytes arrive, proliferate within the early lesions or tubercles, and release proinflammatory cytokines such as interferon- (IFN) that will activate macrophages to kill the intracellular mycobacteria. Subsequently, the early logarithmic bacillary growth stops, and central solid necrosis in these primary lesions or granuloma inhibits extracellular growth of mycobacteria. Several scenarios may follow, with infection becoming stationary or dormant in some individuals, or progressive in the lung, or with hematogenous dissemination in a minority of patients. In addition, reactivation can occur months or years afterwards, under conditions of failing immune surveillance. Granuloma often contains central caseous necrotic tissue, which gives rise to cavities and aerogenic spread of mycobacteria.

The macrophage is a pivotal cell in these events, as it is involved in phagocytosis and killing of mycobacteria as well as in the initiation of adaptive T-cell immunity. Phagocytosis of *Mtb* involves different receptors such as the scavenger receptors, the mannose receptor (MR), and complement receptors. Phagocytosis can involve both uptake of the bacilli after opsonization with complement factors, or it can be initiated as a nonopsonic event.

In vitro experiments have shown that complement receptor 3 (CR3) mediates approximately 80% of complement-opsonized *Mtb* phagocytosis Nonopsonic phagocytosis is an important process in the primary infection of the lung, because complement factors are largely absent in the alveolar space.

Macrophages can eliminate mycobacteria through different mechanisms, such as production of reactive oxygen and nitrogen species, acidification of the phagosome, and phagosome fusion with the lysosomes .The fate of intracellular mycobacteria is also influenced by autophagy, a cellular process though which cytoplasmic components, including organelles and intracellular pathogens, are sequestered in a double-membrane-bound autophagosome and delivered to the lysosome for degradation .Activation of autophagy leads to phagosome maturation, an increased acidification in the phagosome, and killing of mycobacteria in macrophages .However, once inside the cell, *Mtb* often evades destruction by the innate microbial machinery ,one of the main mechanisms being the inhibition of phagosome-lysosome fusion .[44]

❖ LAB DIAGNOSIS:

Following are the tests employed to diagnose tuberculosis,

1. Demonstrating the bacillus by microscopy.
2. Isolating it in culture
3. Transmitting the infection to experimental animals
4. Demonstrating hypersensitivity to tuberculoprotein
5. Using molecular diagnostic methods.[52]

TB is a contagious airborne infection.The infective dose of *Mtb* for humans is low i.e 50% infective dose fewer than ten bacilli.Hence specimens suspected of TB or known TB must be considered potentially infectious and handled with appropriate precautions. [53]

Precautions:

Biosafety Level 2 practices, containment equipment, and facilities for preparing acid fast smears and culture are strongly recommended. Of great significance, all aerosol-generating procedures must be performed in a class II A or B or III biological safety cabinet (BSC). If *M. tuberculosis* is grown and then propagated and manipulated, Biosafety Level 3 practices are recommended.[15]

Specimen collection and transport:

The different specimens subjected for the diagnosis of TB are,

- Pulmonary
- Gastric lavage
- Feces

- Urine
- Tissue and body fluids
- Blood
- Wound, skin lesions and aspirates.

Contaminated specimens like sputum,urine should be subjected to digestion and decontamination procedures before testing. The most frequently used method is decontamination with 4% sodium hydroxide. Commonly used digestion-decontamination methods are as follows,

- Sodium hydroxide (NaOH) method,
- Zephiran-trisodium phosphate method, and
- *N*-acetyl-L-cysteine (NALC)-NaOH method.

Tissues or body fluids collected aseptically usually do not require the digestion and decontamination methods used with contaminated specimens. [15]

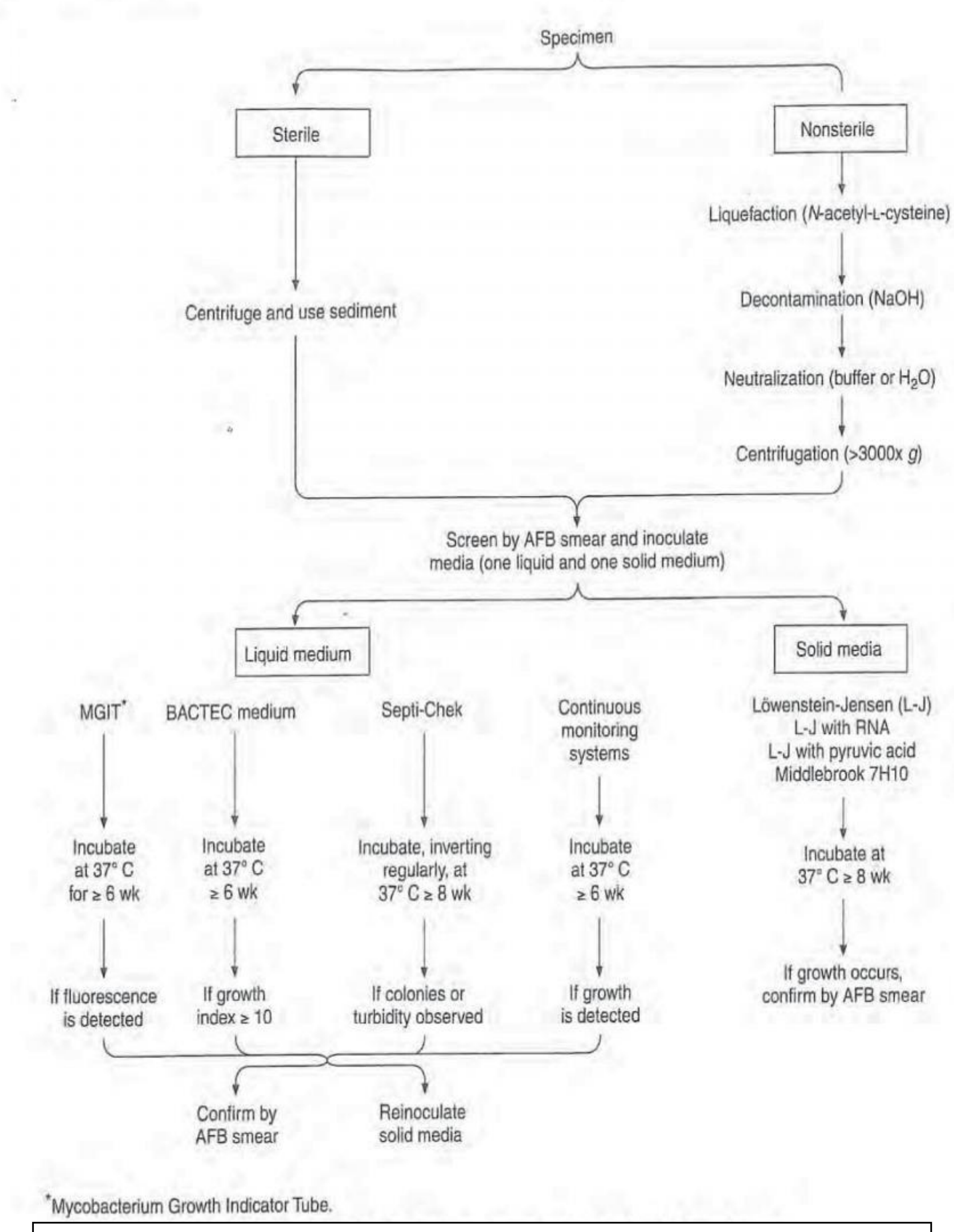


Fig:3- A flow chart for specimen processing for isolation of Mycobacteria.(15)

- **Microscopy:**

Acid-Fast Stain

- The **acid-fast stain** is an important differential stain because it stains cells of the genus *Mycobacteria* .Cells of these bacteria have large amounts of waxy lipid in their cell walls, so they do not readily stain with the Gram stain. Modern microbiological laboratories commonly use a variation of the acid-fast stain developed by Franz Ziehl (1857–1926) and Friedrich Neelsen (1854–1894) in 1883. Other method being used isKinyoun staining.
- **Fluorescent stain:**

  Auramine – stained smears are examined with a fluorescence microscope at a lower power (250x). If fluorescent particles are seen,they must be observed with higher magnification to confirm the typical morphology of AFB. Ideally, all fluorochrome-positive smears should be confirmed by carbol-fuchsin staining method.[10]

- **Cultivation of Mycobacteria**

Media for the cultivation of mycobacteria have mainly been developed for the isolation *Mtb* from clinical specimens such as sputum, body fluids and tissue. Specimens from clinical sites are frequently contaminated with normal body flora and require exposure to strong acids or alkali in order to recover mycobacteria. However, decontamination with sodium hydroxide (4%), which is one of the most widely used methods for sputum decontamination.

The first successful isolation of *Mtb* was by Robert Koch in 1882 using coagulated bovine serum; however, on such a nutritionally poor medium, the bacterial

colonies are minute. Since this time, there have been many culture media described.They are

- Egg-based,
- Agar based,
- Liquid media

Both liquid and solid media may be made selective using either antibiotics or chemical agents.

The basic components of most mycobacterial-culture media originate from the work of Proskauer and Beck in 1894. The medium they developed is still used by some reference collections to maintain mycobacterial species.

- **Egg-Based Culture Media**

The use of egg-based media was introduced by Dorset m 1903 , and they are the most commonly used media for isolation of mycobacteria from clinical specimens. Most egg-based media are composed of whole eggs, organic and inorganic salts, asparagine and glycerol as a carbon source. In order to observe the buff-colored colonies of *M tuberculosis* against the similar

color of the egg medium, it is usual practice to add a colored dye to the medium before coagulation. Although several dyes have been recommended, the most widely-used is malachite green, which has the additional advantage of being slightly inhibitory to small numbers of contaminating organisms.

The three most commonly-used egg-based media are

i.Lowenstein-Jensen (LJ),

ii.American Trudeau Society (ATS) and

iii.Ogawa (22)

- **Agar-Based Culture Media**

The most widely used agar-based media are those developed by Middlebrook and co-workers. These are the '7H' media, and have been developed through a series of changing formulae with increasing numbers. Each slight alteration to the constituents has led to a change in medium number.

Agar has been shown to be inhibitory for mycobacterial species, and most agar-based media contain substances which reduce the toxicity. These substances vary from activated charcoal to bovine albumin or blood.

Middlebrook 7H10 and 7H11 are the most useful of agar-based media and both have similar constituents. Medium 7H11differs from 7H10 agar by the addition of casein hydrolysate, which improves the growth of some fastidious strains of *M tuberculosis*. It is important to note details of the preparation and storage of Middlebrook media because they are affected adversely by exposure to daylight and to high temperature.

- **Liquid Culture Media**

The most useful liquid media are those of

- Proskauer and Beck ,
- Sauton,

- Kirchner ,
- Dubos and
- Middlebrook .

Proskauer and Beck (PB) and Sauton's are defined media whereas the others contain animal protein. PB is also useful for cultivation of large amounts of *Mtb* and some other species in the form of surface pellicles.

Sauton's medium has been used to produce large-scale growth of *Mtb* and some other species for producing monoclonal antibodies (MAbs).

As neither PB nor Sauton's medium are freely available commercially they must be made m-house, and batch-to-batch variation frequently occurs. In addition, surface growth may take several weeks to reach maximum yield. It is important when growing mycobacteria as surface pelicles that the inoculum is floated onto the surface of the medium to obtain maximum aerobic growth.

Kirchner's medium is most useful for isolation of *Mtb* from clmical specimens other than sputum. The medium may be made selective and greatly increases the isolation rate of tubercle bacilli from specimens such as tissue and body fluids, which usually have very small bacterial populations. As with other selective-culture media, Kirchner may be used to recover *Mtb* from contaminated cultures.

Dubos-liquid medium has been widely used in experimental situations and is avaiable commercially. As it contains Tween-80, it yields dispersed growth of species such as *Mtb*, which otherwise produce large clumps of bacilli. It has also been used at double-strength to cultrvate *M microti* .

Middlebrook 7H9 medium is probably the most useful of liquid media for research investigations. The medium is available commercially, is easily soluble and autoclavable. However, it requires the addition of an albumin-dextrose-catalase (ADC) supplement before use and this exposes the medium to potential contamination.It is important with Middlebrook media to note that 7H10 and 7H11 agar are supplemented with oleic acid, albumin, dextrose and catalase (OADC). 7H10 agar contains MgSO, sodium glutamate, malachite green and agar, otherwise 7H10 agar and 7H9 liquid medium are the same.Liquid 7H9 has only ADC added, because it is usually used with the addition of Tween-80 and therefore sufficient oleate is released during growth.

` It is important to recognize the value of the Middlebrook media for both research and diagnostic work. They constitute a series of defined liquid and solid media. Liquid 7H9, with the addition of Tween-80, is most useful when viable counts are required as cells grow in very small colony-forming units.Agar-based media such as 7HlO and 7H11 have the advantage of not being affected by proteolytic contaminants as are egg-based media. The agar is relatively clear, allowing colonial morphology to be examined and variant or contaminating colonies to be distinguished. Agar-based media have less protein content than egg-based media and therefore cause less nonspecific protein binding of antibiotics and drug susceptibilityty testing.

Selective Culture Media

There have been numerous attempts to produce culture media that are selective for mycobacterta; however, most of these have been specifically for the isolation of M tuberculosis from clinical specimens. They are,

Gruft described a selective egg medium that contained penicillin and nalidixic acid in addition to malachite green. This medium is usually used for culture of *Mtb* following some form of decontamination. Petran and Vera used Lowenstein-Jensen medium supplemented with cyclohexamide and nalidixic acid for selection. By varying the concentration, these agents could also be used in Middlebrook 7H10and 7H11 agar.

One of the most widely-used selective media was described by Mitchelson et al. Four antimicrobial agents, polymyxin B, amphotericin B, carbemcillin and trimethoprrm lactate (PACT) were added to Middlebrook 7H 10 agar. This selective medium can be used for direct culture of *Mtb* from sputum or other clinical specimens and to recover *Mtb* from contaminated cultures . Mitchlson et al. used this combination of selective agents in liquid Kirchner medium in place of guinea pig inoculation for the isolation of *Mtb* from clinical specimens. In subsequent work, it was found to increase greatly the isolation of *Mtb* from tissue specimens when compared to solid media .[54]

Commonly Used Liquid Media Systems to Culture and Detect the Growth of Mycobacteria [15]

1) BACTEC 460 TB (Becton Dickinson Diagnostic Systems, Cockeysville, Md)

2) Septi-Chek AFB System (Becton Dickinson)

2) Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson)

Continuous Growth Monitoring Systems

1) ESP Culture System II (TREK Diagnostic Systems, Cleveland, Ohio)

2) BacT/Alert System (bioMérieux, Inc., Durham, NC)

3) BACTEC MGIT 960 (Becton Dickinson)

- **Storage and Maintenance of Cultures**[15]

A variety of methods are available for the storage and maintenance of mycobacterial cultures and the method selected will depend on the frequency of use.

1.Short- Term Storage: If strains are in frequent use, it may be necessary to maintain a working culture by frequent subculture in either liquid or solid media.

2.**Long-Term Storage:**There are several ways of storing mycobacteria long term and the method of choice will depend on the intended laboratory application. The methods most frequently used are,

- lyophilization by freeze-drying = Lyophilization is a process by which water is removed by evaporation of a frozen bacterial suspension or

- freezing at very low temperatures in a suitable support medium= Preservation by freezing is probably the most widely used method for both mycobacteria and most other bacterial species. However, cells may be damaged both during freezing and subsequent thawing. Cells may be protected during freezing by the addition of various protecting agents. The most frequently used are glycerol and dimethyl sulfoxide (DMSO). Storage temperatures

used are usually -20, -70, or -80°C in commercially available freezers, -140°C in liquid nitrogen vapor phase and -196°C in liquid nitrogen liquid phase.

In laboratories that do not have very low temperature storage, it is possible to store fully grown LJ cultures at -20°C for several years . However, this is not the most satisfactory method as the entire slope has to be used after thawing and may lead to selection of cells.

Conventional Phenotypic Tests

Growth Characteristics. The preliminary identification of mycobacterial isolates depends on their rate of growth, colonial morphology, colonial texture, pigmentation, and, in some instances, the permissive incubation temperatures of mycobacteria. Despite the limitations of phenotypic tests, the growth characteristics of mycobacteria is helpful to determine a preliminary identification [15]

. The traditional methods are well established, standardised, reproducible and relatively inexpensive. Though the biochemical tests help for further characterisation but do not always allow an accurate characterisation at the species level. The current identification scheme of mycobacteria mainly relies on molecular methods along with some key phenotypic tests.[39]

The most useful biochemical tests for characterising species within MTB complex are,[55]

- Niacin accumalation tests
- Catalase test: Room temperature

  At 68^0 C.

- Nitrate reductase test
- Pyrazinamidase test
- Inhibition by thiophene-2-carboxylic acid hydrazide.

  Few other tests useful for other mycobacteria include,

- Arylsulfatase
- Iron uptake
- Sodium chloride tolerance
- Tellurite reduction
- Tween 80 hydrolysis
- Urease

	Control Organisms		Result				
Biochemical Test	Positive	Negative	Positive	Negative	Medium Used and Amount	Duration	Incubation Conditions
Niacin	*M. tuberculosis*	*M. intracellulare*	Yellow	No color change	0.5 mL DH_2O	15-30 min	Room temperature
Nitrate	*M. tuberculosis*	*M. intracellulare*	Pink or red	No color change	0.3 mL DH_2O	2 hours	37° C bath
Urease	*M. fortuitum*	*M. avium*	Pink or red	No color change	Urea broth for AFB	1, 3, and 5 days	37° C incubator without CO_2
68° C Catalase	*M. fortuitum* or *M. gordonae*	*M. tuberculosis*	Bubbles	No bubbles	0.5 mL phosphate buffer (pH 7)	20 min	68° C bath
SQ Catalase	*M. kansasii* or *M. gordonae*	*M. avium*	>45 mm	<45 mm	Commercial medium	14 days	37° C incubator (with CO_2)
Tween 80	*M. kansasii*	*M. intracellulare*	Pink or red	No color change	1 mL DH_2O	5 or 10 days	37° C incubator (in the dark, without CO_2)
Tellurite	*M. avium*	*M. tuberculosis*	Smooth, fine, black precipitate (smokelike action)	Gray clumps (no smokelike action)	Middlebrook, 7H9 broth	7, then 3 additional days	37° C incubator (with CO_2)
Arylsulfatase	*M. fortuitum*	*M. intracellulare*	Pink or red	No color change	Wayne's arylsulfatase medium	3 days	37° C incubator (without CO_2)
5% NaCl	*M. fortuitum*	*M. gordonae*	Substantial growth	Little or no growth	Commercial slant with and without 5% NaCl	28 days	37° C incubator (with CO_2)
TCH	*M. bovis*	*M. tuberculosis*	No growth (i.e., susceptible)	Growth (i.e., resistant or ≥1% of colonies are resistant)	TCH slant	3 weeks	37° C incubator (with CO_2)

Table.1- Controls and media used for the biochemical identification of mycobacteria [15]

Mycolic acid analysis

Mycolic acid composition is constant for all strains of a given species and varies from species to species. An HPLC method for analysis of mycolic acid esters has been standardised and demonstrated as to be as rapid and reliable method for identifying many MTB species

A fully integrated automated FL-HPLC System, SMIS (Sherlock Mycobacteria Identification System, MIDI, Inc,Newark,DE) was developed with a library containing entries for 26 mycobacterial species or groups.[39]

Group/Complex	Species	Optimal Temp (° C)	Usual Colonial Morphology[a]	Niacin	Growth On TCH (10 μg/mL)[b]	Nitrate Reduction	Semiquantitative Catalase (>45 mm)	68° C Catalase	Tween Hydrolysis, 5 Days	Tellurite Reduction	Tolerance to 5% Nacl	Iron Uptake	Arylsulfatase, 3 Days	Growth on Macconkey Agar	Urease	Pyrazinamidase, 4 Days
TB	*M. tuberculosis*	37	R	+	+	+	−	−	−[c]	∓	−	−	−	−	±	+
	M. bovis	37	Rt	−	−	−	−	−	−	∓	−	−	−	−	±	−
	M. africanum	37	R	V	V	V	−	−	−	−	−	−	−	−	+	−
Photochromogens	*M. marinum*	30	S/SR	∓	+	−	−	−	+	∓	−	−	∓[d]	−	−/+	+
	M. kansasii	35	SR/S	−	+	+	+	+	+	∓	−	−	−	−	+	−
	M. simiae	37	S	±	+	−	+	+	−	+	−	−	−		±	+
	M. asiaticum	37	S	−	+	−	+	+	+	−	−	−	−		−	−
Scotochromogens	*M. scrofulaceum*	37	S	−	+	−	+	+	−	∓	−	−	V	−	V	±
	M. szulgai	37	S or R	−	+	+	+	+	∓[c]	±	−	−	V	−	+	+
	M. gordonae	37	S	−	+	−	+	+	+	−	−	−	V	−	V	±
Nonphotochromogens	*M. avium* complex	35-37	St/R	−	+	−	−	±	−	+	−	−	−	∓	−	+
	M. genavense[e]	37	St	−	+	−	+	+	+			−	−		+	+
	M. gastri	35	S/SR/R	−	+	−	−	−	+	∓	−	−	−	−	∓	−
	M. malmoense	30	S	−	+	−	−	±	+	+	−	−	−		−	+
	M. haemophilum[f]	30	R	−	+	−	−	−	−	−	−	−	−	−	−	+
	M. shimoidei	37	R	−	+	−	−	−	+		−	−	−	−	−	+
	M. ulcerans	30	R	−	+	−	−	+	−		−		−		−	−
	M. flavescens[g]	37	S	−	+	+	+	+	+	∓	+	−	−	−	+	+
	M. xenopi[h]	42	Sf	−	+	−	−	+	−	∓	−	−	±	−	−	V
	M. terrae complex (*M. terrae*, *M. triviale*,[i] *M. nonchromogenicum*)	35	SR	−	−	+	+	+	+	−	−	−	−	V	−	V
Rapidly growing	*M. fortuitum* group	28-30	Sf/Rf	−	+	+	+	+	V	+	+	+	+	+	+	+
	M. chelonae	28-30	S/R	−/+	+	−	+	V	V	+	−	−	+	+	+	+
	M. abscessus	28-30	S/R	−		−	+	V	V	+	+	−	+	+	+	
	M. smegmatis	28-30	R/S	−	+	+	+	+	+	+	+	+	−	−		

Plus and minus signs indicate the presence or absence, respectively, of the feature; blank spaces indicate either that the information is not currently available or that the property is unimportant. *V*, Variable; ±, usually present; ∓, usually absent.

See *Clinical microbiology procedures handbook*, vol 2, The American Society for Microbiology, Washington, DC, 2004, and Vincent V, Brown-Elliott BA, Jost KC, Wallace RJ: In Murray PR, Baron EJ, Pfaller MA, et al: *Manual of clinical microbiology*, ed 8, The American Society for Microbiology, Washington, DC, 2003, for other mycobacterial species' biochemical reactions and additional biochemical reactions on the mycobacteria included in this table.

[a] *R*, Rough; *S*, smooth; *SR*, intermediate in roughness; *t*, thin or transparent; *f*, filamentous extensions.

[b] *TCH*, Thiophene-2-carboxylic acid hydrazide.

[c] Tween hydrolysis may be positive at 10 days.

[d] Arylsulfatase, 14 days, is positive.

[e] Requires mycobactin for growth on solid media.

[f] Requires hemin as a growth factor.

[g] Young cultures may be nonchromogenic or possess only pale pigment that may intensify with age.

[h] Strains of *M. xenopi* can be nonphotochromogenic or scotochromogenic.

[i] *M. triviale* is tolerant to 5% NaCl, and a rare isolate may grow on MacConkey agar.

Table.2-Distinctive properties of commonly cultivable Mycobacteria encountered in Clinical specimens.[15]

Mycobacterial Group	Key Biochemical Tests
M. tuberculosis complex	Niacin, nitrate reduction, susceptibility to TCH if *M. bovis* is suspected
Photochromogens	Tween 80 hydrolysis, nitrate reduction, pyrazinamidase, 14-day arylsulfatase, urease, niacin
Scotochromogens	Permissive growth temperature, Tween 80 hydrolysis, nitrate reduction, semiquantitative catalase, urease, 14-day arylsulfatase
Nonphotochromogens	Heat-resistant and semiquantitative catalase activity, nitrate reduction, Tween 80 hydrolysis, urease, 14-day arylsulfatase, tellurite reduction, acid phosphatase activity
Rapidly growing	Growth on MacConkey agar, nitrate reduction, Tween 80 hydrolysis, 3-day arylsulfatase, iron uptake

Table.3-Key biochemical reactions to help distinguish Mycobacteria belonging to the same Mycobacterial group'.[15]

- **Gene amplification methods for direct detection of M.tuberculosis sequences from clinical specimens:**

Gene amplification techniques have made a major impact on the diagnosis of mycobacterial diseases. Gene amplification techniques are highly sensitive and under optimum conditions may detect 1-10 organisms. If systems are adequately standardized, evaluated and precautions for avoiding the contamination are taken, these assays can play a very useful role in early confirmation of diagnosis in paucibacillary extra-pulmonary forms of tuberculosis. These methods may be classified as those based on Polymerase Chain Reaction (PCR) and others based on Isothermal Amplification Reactions.

A.PCR Amplification:

A variety of PCR methods have been developed for detection of specific sequences of M. Tuberculosis and other mycobacteria .These PCR assays may target

either DNA or rRNA/and these could be based on conventional DNA based PCR, nested PCR, multiplex PCR and RT-PCR.

Targets include insertion and repetitive elements, various protein encoding genes, ribosomal rRNA etc. Developments in this area have been very rapid and a large number of PCR assays targeting different gene stretches of *M. tuberculosis* have been described.[56]

B.Non –PCR Amplification:

- Ligase chain reaction
- Nucleic- acid sequence-based amplification
- Transcription mediated amplification
- Strand displacement amplification [10]

POLYMERASE CHAIN REACTION (PCR)

PCR is a technique developed by Kary Mullis in the 1980s. PCR is based on using the ability of DNA Polymerase to synthesize new strand of DNA complementary to the given template strand. Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group, it needs a primer to which it can add the first nucleotide. This requirement makes it possible to delineate a specific region of template sequence that the researcher wants to amplify. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons).

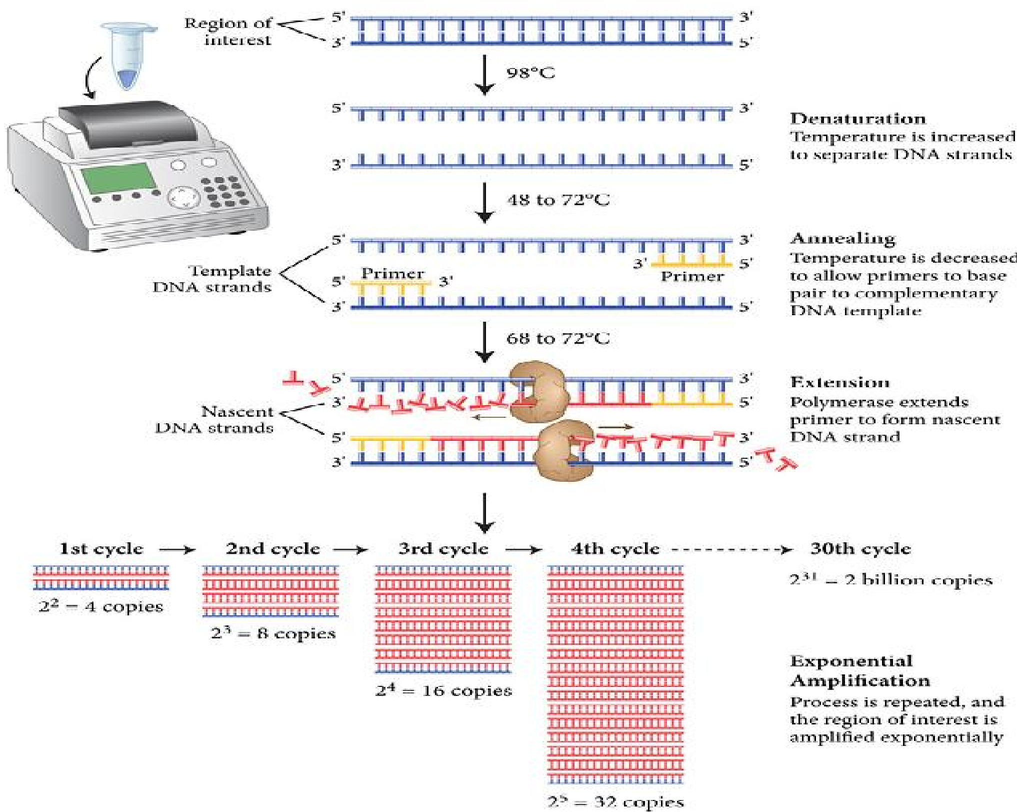


Fig.4 - Steps in PCR –Denaturation, Annealing and Extension. https://www.neb.com/~/media/NebUs/Page%20Images/Applications/DNA%20Amplification%20and%20PCR/pcr.jpg

The PCR assay involves three steps,

A) Sample preparation for Extraction of DNA

B) Amplification of target

C) Analysis of amplified product.

A) Sample preparation for Extraction of DNA comprises of

- release of nucleic acid from microorganisms
- Stabilisation of nucleic acid against degradation
- Removal of amplification inhibitors

- Concentration of the target into a small volume.[10]

Many different methods are used for release of nucleic acid from mycobacteria.

The commonly used method for extraction of DNA are,

- Boiling[59,60]
- Freezing and thawing[58,60]
- Sonication[57,59]
- Enzymatic lysis[59]
- Use of detergent and alkali[60] etc

Following nucleic acid extraction from sample, the DNA is kept at -20deg C until further use.

B) Amplification:

The amplication reaction consists of three steps .They are,

- Denaturation to single strands
- Annealing(primers binding)
- Primer extension by action of DNA polymerase.

The components of the PCR reaction mixture/master mix are mentioned below,

1. DNA template

It is the sample DNA that contains the target sequence. At the beginning of the reaction, high temperature is applied to the original double-stranded DNA molecule to separate the strands from each other.

2. DNA polymerase

It is a type of enzyme that synthesizes new strands of DNA complementary to the target sequence. The first and most commonly used of these enzymes is *Taq* DNA polymerase (from *Thermis aquaticus*), whereas *Pfu* DNA polymerase (from *Pyrococcus furiosus*) is used widely because of its higher fidelity when copying DNA. Although these enzymes are subtly different, they both have two capabilities that make them suitable for PCR: 1) they can generate new strands of DNA using a DNA template and primers, and 2) they are heat resistant.

3. Primers

Primers are short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer. The primers can be classified,depending upon the target they amplify into genus specific and spcies specific.

- The list of *Mtb* complex specific primers include target from IS*6110*,38KDa protein coding gene,MPB64 protein coding gene,mtp40 protein coding gene.

- Genus specific primers are those amplifying Targets from 65Kda protein coding gene, 32Kda protein coding genes,16S rRNA coding gene and dnaJ gene.

IS*6110*:

The insertion sequence IS*6110* is a member of insertion sequence family IS3. It is present in multiple copies in most strains of MTB. Many workers have developed a pcr assay using various base pair fragments of this insertion sequence as target.Of these, the 123bp target and 245 bp target are found to be specific for *Mtb* complex

4. Nucleotides (dNTPs or deoxynucleotide triphosphates)

They are single units of the bases A, T, G, and C, which are essentially "building blocks" for new DNA strands.

5. PCR buffer

6. Sterile double distilled water.

Except primers for any PCR reaction,the contents of “master mix” is almost the sample. Amplification is performed in a thermocycler. Following are the steps in amplification process,

- DENATURATION

The DNA obtained from the clinical sample is in the double standard form. It is melted at 92-95 deg C for 30-69 seconds to obtain two different strands without causing any damage to the DNA molecule. This step is called Denaturation.

- ANNEALING

The reaction mixture is then brought down to 50-55 deg C. At this lower temperature the primers from thereaction mixture flank on target DNA strand at defined, specific locations. This flanking is called Annealing.

The annealing temperature is different for different primers and it depends on the length and GC content of the primer. The annealing temperature can be calculated using the formula. Annealing temperature is usually 5^0 C below Tm.

Tm = 2(A+T) +4 (G+C)

Where, Tm = melting temperature

A = Adenine

T = Thymine

C = Cytozine

G= Guanine

- EXTENSION

In the third step the annealed primers are extended. The DNA extends in 5'-3' direction. The step cycle program of the thermal cycler is set to required denaturation, annealing and extension temperatures for a total of 30 cycles. At the end of each cycle

number of target DNA molecule is theoretically doubled. This program causes the instrument to heat and cool to the required temperature as quickly as possible.

C) Analysis of PCR

One of the most important steps after amplification is the authentication of the resultant Product. So PCR product analysis is mainly done by

1) Size estimation of the product by electrophoresis.
2) Hybridization
3) Restriction enzyme mapping
4) Sequencing
5) Microarray analysis.

Identification of the expected PCR product by estimating it's size is the simplest method. The amplified prodcut is subjected to electrophoresis in 2% agarose gel. The size of the product is estimated by running DNA markers of known size.[(10)]

- **Genotypic identification of mycobacterial species**

New PCR-based genotyping techniques include

a) Spacer oligonucleotide typing (spoligotyping),
b) IS6110-based restriction fragment length polymorphism (RFLP) and
c) Mycobacterial interspersed repetitive unit (MIRU) typing.

Genotyping is useful in analyzing suspected outbreaks of tuberculosis in institutions such as hospitals, schools and prisons When used together for strain

typing, spoligotyping and MIRU can discriminate strains which are not part of the chain of transmission. In certain cases however, these tests can also return false-positive results.This is circumvented with the advent of DNA fingerprinting.

a) Spoligotyping

Spoligotyping can be used for both detection and typing of *M tuberculosis*, through PCR amplification of a highly polymorphic direct repeat locus in the genome of *M tuberculosis*.

b) DNA fingerprinting

This type of test, using *IS*6110-based restriction fragment length polymorphism, has proven useful in phylogenetic studies of tuberculosis bacilli particularly since IS6110 is unique for the *M tuberculosis* complex.

RFLP DNA fingerprinting is the gold standard for strain typing in mycobacteriology and this method of genotyping has been standardized in order to increase the inter- and intra- laboratory comparability, so that it could be used for subspeciation of *M tuberculosis*. Roughly one week is needed before the results can be interpreted. The disadvantages of RFLP genotyping are that a large cell mass is required and that comparison is difficult since the results are band patterns, hard to convert into digital formats.

c) Mycobacterial interspersed repetitive unit typing

Mycobacterial interspersed repetitive unit typing is a technique based on variable numbers of tandem repeat at 12 loci in the genome of *M tuberculosis*.

Molecular typing of other mycobacterial species

Pulsed –field gel electrophoresis, RFLP and PCR-method targeting insertion sequences are methods employed for mycobacteria other than MTB complex.

- **Immunodiagnostic tests:**

Cytokine detection assays:Interferon-gamma release assays (IGRA s)

Cytokine detection assays measure the cell-mediated immune response elicited against a MTB.IGRAs measure the interferon (IFN)-gamma released by sensitized White blood cells.

a) QuantiFERON-TB Gold

QuantiFERON-TB Gold (Cellestis Limited, Carnegie, Victoria, Australia) is an ELISA test which detects the release of IFN-gamma in fresh heparinized whole blood from sensitized persons upon incubation with synthetic peptides simulating ESAT-6 and culture filtrate protein-10 (CFP-10).

b) QuantiFERON-TB Gold In-Tube

The QuantiFERON-TB Gold In-Tube (Cellestis Limited, Carnegie, Victoria, Australia) was developed to overcome the limitation of QuantiFERON-TB Gold, which could only be used in facilities where blood testing could begin within a few

hours of its collection. This test uses a mixture of 14 peptides representing ESAT-6, CFP-10 and a part of TB7.7.

c) T-SPOT.TB

T-SPOT.TB (Oxford Immunotec Limited, Abingdon, United Kingdom, 2008) incubates peripheral blood mononuclear cells with mixtures of peptides (ESAT-6, CFP-10) and uses an enzyme-linked immunospot assay (ELISpot) to detect increase in the number of cells that secrete IFN-gamma (spots in each test well).

d) Monokine-amplified IFN-gamma release assays (MIGRAs)

Given that IFN release leads to subsequent release of IFN-responsive chemokines such as MIG and IP-10, recent studies have investigated whether measurement of these chemokines might provide a sensitive tool for the detection of mycobacterial infection and antigen-specific T-cell responses.

- **Other diagnostic approaches**

a) Detection of anti-mycobacterial superoxide dismutase antibodies.

Superoxide dismutase (SOD) has been evaluated for its role in establishing mycobacterial phylogeny based on the immunological relatedness among mycobacteria.

b) MPB 64 patch test

MPB 64 is a mycobacterial antigen specific for *M tuberculosis* complex. This test is particularly useful since it can discriminate between latent infection and active disease.

c) FAST Plaque TB

FAST Plaque TB (BIOTEC Laboratories Ltd, FIND – Foundation for Innovative New Diagnostics) is a test that uses mycobacterio-phages to detect *M tuberculosis* directly from sputum samples. The principle of this assay, as described by the manufacturing companies, is that a specific mycobacteriophage infects cells of the *M tuberculosis* complex present in the specimen.

It only detects live bacilli, thus reducing the possibility of false positives.

d) FAST Plaque-Response

This assay is an extension of the technique used for FAST Plaque TB, which allows the early detection of rifampicin resistance through the use of mycobacteriophages. [61]

❖ **Anti-tubercular sensitivity tests.** [10]

1.Conventional methods to determine susceptibility of MTB isolates to Antimycobacterial agents.

- Absolute concentration method
- Resistance ratio method
- Proportion method

Automated systems like BACTEC 460TB,BACTEC MGIT 960,ESPII Culture System and MB/BacT Alert 3D are commonly used as the turnaround time is short compared to conventional methods[52]

2.Molecular methods to determine susceptibility of MTB isolates to Antimycobacterial agents.

- Line probe Assay
- GeneXPERT
- Luciferase-reporter Mycobacteriophage assay
- FAST PLAQUE TB-RIF

TUBERCULOSIS IN WOMEN (62)

'uberculosis (TB) is a contagious airborne disease. ;lobally, it is the greatest cause of death of people ving with HIV, and ranks alongside HIV as a top ıfectious disease killer. While more men than women re diagnosed with TB and die from it, TB can have ›articularly severe consequences for women, ›specially during their reproductive years.

3URDEN OF TUBERCULOSIS IN WOMEN

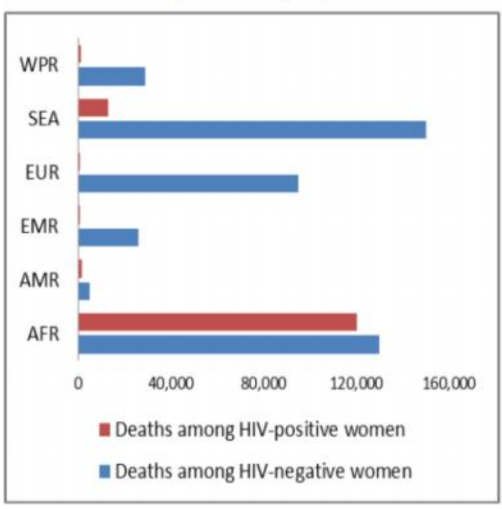
In 2014, an estimated 3.2 million women fell ill with TB.

TB is one of the **top five killers of women** among adult women aged 20–59 years. 480 000 women died from TB in 2014, including 140 000 deaths among women who were HIV-positive.

Of the 330,000 HIV-related TB deaths among adults (age ≥15) globally in 2014, just over 40% were among women, accounting for about a third of all AIDS-related deaths among female adults.

Almost 90% of these HIV-associated TB deaths among women were in Africa.

Estimated number of TB deaths among women, disaggregated by WHO region and HIV status, 2014



IMPACT OF TB ON MATERNAL HEALTH

- TB among mothers is associated with a six-fold increase in perinatal deaths and a two-fold risk of premature birth and low birth-weight.
- Genital TB, which is challenging to diagnose, has been identified as an important cause of infertility in high TB-incidence settings.
- TB in pregnant women living with HIV increases the risk of maternal and infant mortality by almost 300%.
- In Africa, TB rates are up to 10 times higher in pregnant women living with HIV than in pregnant women without HIV infection.
- Facility-based studies in a number of high HIV-burden settings found TB accounted for 15-34% of indirect causes of obstetric mortality.
- Evidence from India has found that TB among mothers living with HIV, is associated with more than double the risk of vertical transmission of HIV to the unborn child.

The **WHO Global TB Programme** together with WHO regional and country offices: develops policies, strategies and standards; supports the efforts of WHO Member States; measures progress towards TB targets and assesses national programme performance, financing and impact; promotes research; and facilitates partnerships, advocacy and communication.

SOCIO ECONOMIC FACTORS

- TB is a disease of poverty affecting vulnerable groups. The vast majority of TB deaths are in the developing world where gender inequities are all too common.
- Malnutrition and food insecurity can exacerbate the risk of TB disease; other threats such as rising tobacco use and diabetes among women also result in increased TB burden.
- Globally, more men than women fall ill with TB annually. However in some settings, such as Afghanistan, parts of Pakistan bordering Afghanistan and Iran, more women than men are detected with TB.
- Stigma and discrimination in some settings can mean women ill with TB are ostracized by their families and communities.
- Cultural and financial barriers can act as major obstacles for women seeking care resulting in delayed presentation and more severe illness.
- TB mainly affects women when they are economically and reproductively active, the impact of the disease is also strongly felt by their children and families.

ENDING TB BY 2030

The WHO End TB Strategy, serves as a blueprint for countries to reduce TB incidence by 80% and TB deaths by 90%, and to eliminate catastrophic costs for TB-affected households by 2030. Ending the TB epidemic is also a Sustainable Development Goal target.

Protecting and promoting human rights, ethics, and equity is a key principle of the End TB Strategy.

WHAT CAN BE DONE?

- **COMMITMENT:** Mobilize support at global and national levels to remove underlying risk factors and assure gender-equitable access, including women-friendly services for TB prevention, diagnosis, treatment, care and support.
- **COLLABORATION:** Foster strategic partnerships and synergies across the health system. TB, HIV, maternal, neonatal and child health programmes and primary care services should collaborate to maximize the entry points to TB care for women at all levels.
- **INTEGRATION:** Integrate TB screening and investigation into reproductive health services, including family planning, antenatal and postnatal care. Emphasis should be given to girls and women living with HIV in high HIV and TB prevalent settings.
- **DATA COLLECTION:** Improve the recording and reporting of TB data disaggregated by sex and age, including for TB treatment initiation and outcomes.
- **MONITORING SYSTEMS:** Promote the implementation of integrated patient monitoring systems for HIV, PMTCT and TB care to capture data and ensure successful follow-up of the patient in HIV and TB prevalent settings.
- **DIAGNOSTIC SCALE-UP:** Xpert MTB/RIF should be used as the initial test for TB diagnosis in people living with HIV or who are suspected of multidrug- resistant TB. The uptake of Xpert MTB/RIF needs to be scaled up. Xpert MTB/RIF is more effective at detecting TB than sputum microscopy with no significant difference in performance by sex or HIV status.
- **RESEARCH AND DEVELOPMENT:** Advocate for increased research for the development of new diagnostics and new drugs which also take into account the specific needs of women living with HIV as well as pregnant and lactating women, as well as relevant operational and social science research.

For more information please access www.who.int/tb

© World Health Organization November 2015

METHODOLOGY

All women clinically diagnosed as cases of infertility attending Obstetrics and Gynaecology Out-Patient and In-Patient Department of KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi with their informed consent formed the study group.Ethical clearance was sought from Institutional Ethical Committee.

Study design: Cross-sectional study.

Study period: January 2015 to December 2015

Sample size: 80 cases

Sample calculation:

$$n = \frac{Z^2 . \text{Sensitivity (100-sensitivity)}}{d^2 . PR}$$

Where,

Z =95% confidence interval=1.96

Sensitivity=57%

PR=Prevalance =4-9%

d =error =8

Hence, n=36.7(40) suspected FGTB cases

Plus 40 infertile cases =80 cases.

- A woman was said to be suspected of having Genital tuberculosis if she has had findings suggestive of tuberculosis at laparoscopy with one or more of the following findings: A definite past history of tuberculosis, in the presence of

active extra-genital tuberculosis, characteristic features on HSG, elevated ESR, positive Mantoux test, evidence of calcification/ complex adnexal mass by scan.

Sampling procedure: Convenience sampling

Sample collection:

1) Endometrial tissue
2) Fluid from Pouch of Doughlas, where ever possible.

Endometrial tissue is obtained by dilatation and curettage, aspiration biopsy or by hysterolaparoscopically.

Samples are subjected for Ziehl –Neelsen staining, Fluorescent staining, Culture on Lowenstein-Jensen medium and *Mtb* DNA Polymerase chain reaction targetting IS*6110*

Inclusion Criteria: All cases of primary and secondary infertility.

Exclusion Criteria:Patients who are not willing to give their consent.

Statistical Analysis: to compute the Sensitivity and Specificity of Microscopy, Culture and PCR in the diagnosis of Female Genital Tuberculosis.

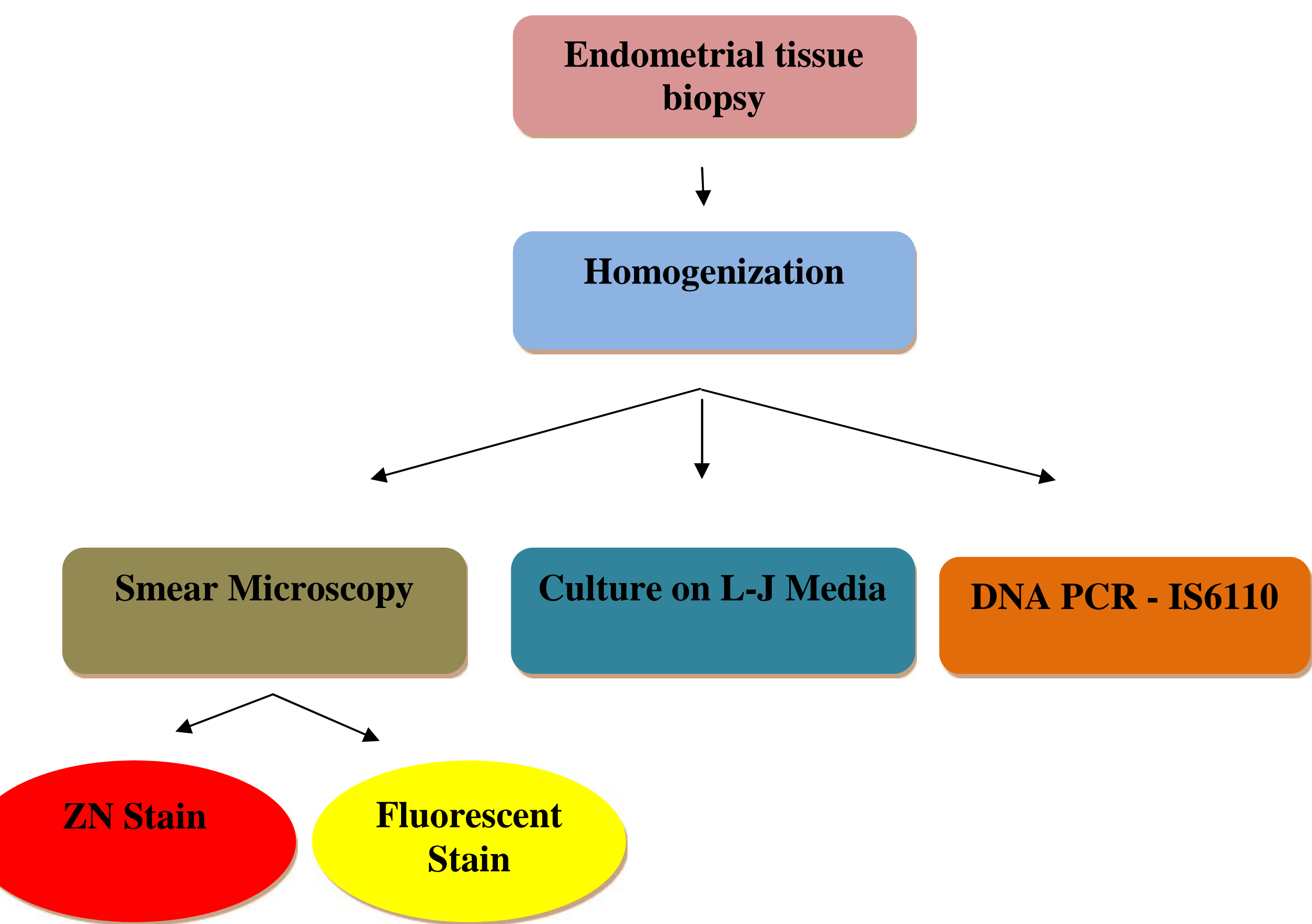
Sample transport and processing:

Endometrial tissue biopsy sample collected from the study group was transported in a sterile container with normal saline to the microbiology laboratory.

The sample was processed in Biosafety cabinet II, adhering to Universal safety precautions in the Department of Microbiology, JNMC, KLE University Belagavi.

Preparation of PCR reagents, addition of template DNA and analysis of amplified products were done in three different rooms to avoid carry over contamination. Reagents were aliquoted and each aliquot was used only once.

Overview of Sample processing for *Mtb* ISOLATION



A. Ziehl-Neelsen Staining: [10]

Principle: The capability of certain bacteria of binding to Carbol Fuchsin dye due to the presence of long chain fatty (Mycolic) acid so that it is not destained by Acid alcohol.Once stained the cellwall holds the dye 'Fast' giving the characteristic red colour.

Specimen: A smear measuring 2x1cm is prepared from homogenised tissue sample,air dried and heat fixed.Sample handling is done in BSC -2 after adhering to universal safety precautions.

Materials:1)Equipment:3”x1” glass slides

2) Reagents:i)CarbolFuchsin

ii) 20% Sulphuric acid

iii) Methylene blue

Quality control: i) Unstained positive control smears of *Mtb*(H37Rv) have been prepared.

ii) Control smears reviewed before the patients smears are read.

iii) If control slide is unacceptable,review procedures and reagent preparations.

Unacceptable control slides include the following,

a) Bacilli in positive control not stained.

b) Background is not properly decolourised.

Procedure:

1) Place the slide on the staining rack.

2) Flood the slide with CarbolFuchsin.

3) Heat the slide slowly and intermittently until steaming with the help of bunsen burner, but short of boiling.

4) Stain for 5min.If the stain dries,add more stain without heating.

5) Rinse the slide thoroughly under slow running stream of water.

6) Immerse the slide in Coplik jar containing 20% Sulphuric acid for 1 min.Repeat this step several times until film is very faintly pink.

7) Rinse the slide thoroughly under slow running stream of water.

8) Flood the slide with Methylene blue –counter stain for 1 min.Do not blot but air dry.

9) Examine the smear with 100x oil immersion objective.

Observation:

Acid fast bacilli appear as red coloured bacilli against blue background.

Limitation of procedure:

1) Acid- fastness do not stand alone and final interpretation must always be correlated with culture results.

2) Organisms other than *Mtb* may demonsatrate various degrees of acid fastness. Rhodococcus spp.,Nocardiaspp., Legionella micdadei and the cysts of Cryptosporidium spp.Use differences in smear morphology and correlation and culture appearance and other biochemical tests.

3) Rapidly growing *Mtb* may stain poorly or not at all.

B.Fluorescent staining: [10]

Principle: AFB emit a bright yellow fluorescence when acctivated with a short -wave ultra violet light. The potassium permanganate counterstain causes the non specific background debris to fluoresce a pale yellow in contrast with the bright yellow appearance of AFB.

Specimen : A smear measuring 2x1cm is prepared from homogenised tissue sample,air dried and heat fixed.Sample handling is done in BSC -2 after adhering to universal safety precautions.

Materials: 1) Equipment:3”x1”glass slide.

2) Reagents: i) Auramine O

ii) 0.5% Acid alcohol

iii) 0.1% Potassium permanganate

Quality control: Positive control: Mycobacterium spp.bacilli with yellow fluorescence

Procedure:

1) Place the slide on staining rack.

2) Flood surface of the smear with Auramin O stain. Let stand at room temperature for 15 min.

3) Rinse with tap water and drain.

4) Decolorize with 0.5% acid alcohol for 2min.

5) Rinse with tap water and drain.

6) Air dry,do not blot.

7) Observe the smear as soon as possible using the fluorescence microscope.If there is a delay –refrigerate the smears at 4^{0}C in the dark.

8) Examine the dry film by fluorescence microscopy with 4mm objective.

Observation:

Tubercle bacilli fluoresce as bright yellow rods against a dark bacground.

Procedure notes:Fluorochrome stained smears may be directly resatined with any of the carbolfuchsin staining procedures.This is done to confirm positive slides and to study the morphology of the organisms present.

Limitations of procedures:

1) Few organisms other than mycobacteria may demonstrate varying degree of acid fastness.

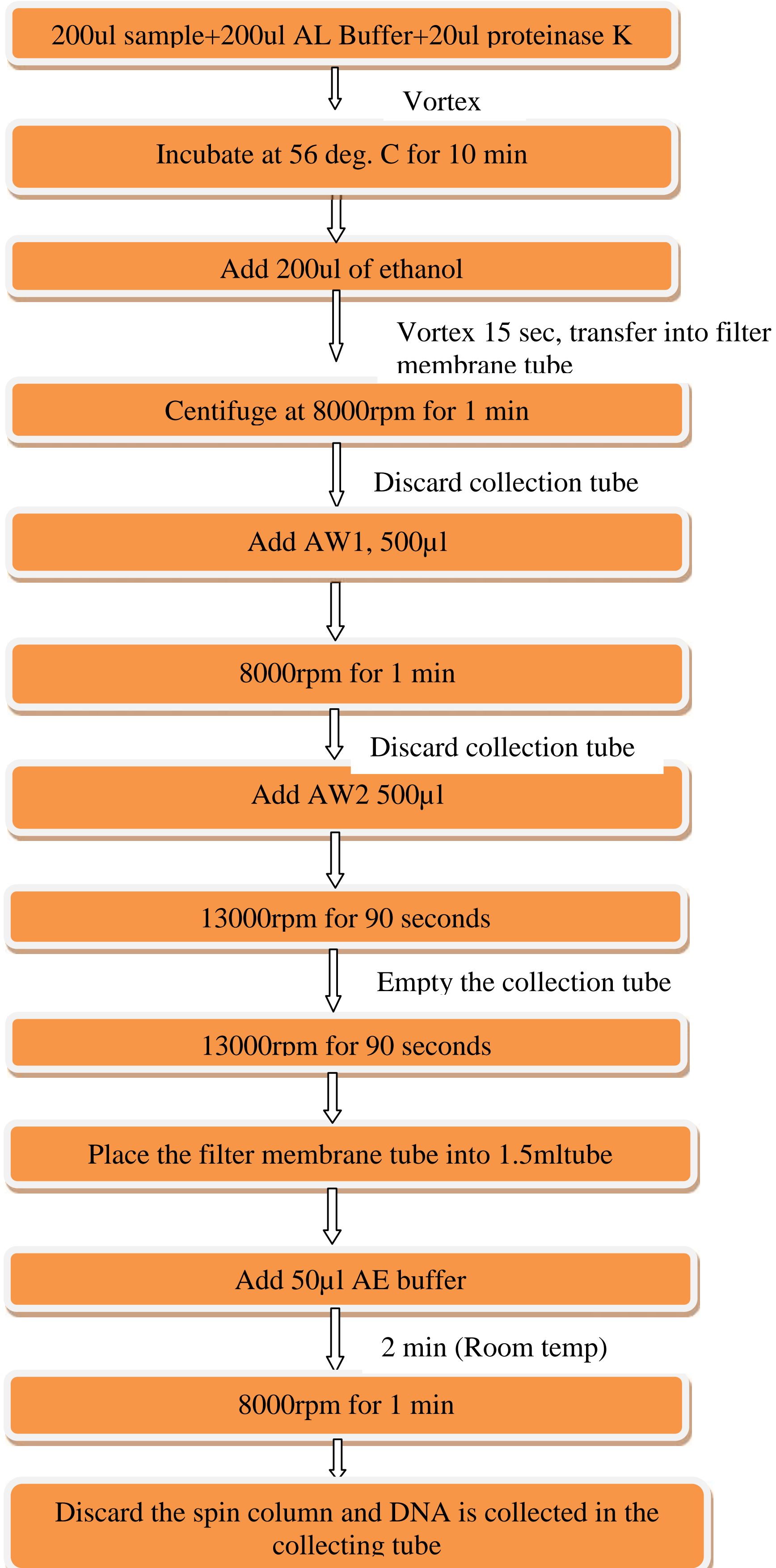
2) Dead acid fast bacilli may still stain positive in fluorescent stains, a feature that must be considered when using smears to assess efficacy of treatment.

3) Most species of rapidly growing *Mycobacteria* will not fluoresce in fluorochrome-stained smears and Carbol Fuchsin procedure is recommended.

C.<u>Inoculation on LJ Media</u> :[52]

- Bring LJ slants from refrigerator to room temperature.
- Tissue homgenenate is inoculated with sterile loop.
- Incubate at 37deg for 8 weeks.
- Observe for growth.
- Record the growth in the Growth Recording Chart mentione in ANNEXURE VI a.

4.i. DNA extraction by Qiagen method



The DNA thus collected in the collecting tube (eppendorf tube) was stored at -20^0C untill amplification was carried out.

ii) Amplification of mycobacterial DNA

PCR was performed using Eppendorf Mastercycler Gradient with standard 25 µl working volume .Precautions were taken to avoid false positivity. Preparation of PCR reagents, addition of template DNA and analysis of amplified products were done in three different rooms to avoid carryover contamination. Reagents were aliquoted and each aliquot was used only once.

Single sample calculations:

DNA template	2µl
Forward Primer	1µl
Reverse Primer	1µl
PCR Master Mix	12.5 µl
H_2O	8.5 µl
Total Volume	25µl

PCR SET UP: Cycling parameters for Single cycle(Total=35 cycles)

Initial denaturation	94^0 C	2min
Denaturation	94^0 C	5min
Annealing	60^0 C	20s
Extension	72^0 C	10s
Final Extension	72^0 C	2min

iii) <u>Post amplification analysis:</u>

Gel documentation: Detection of amplified products was done by agarose gel electrophoresis (2%) at 80 volts for 45 min. Gel was stained with ethidium bromide and viewed under UV transilluminator .The *IS*6110 primers amplify a fragment with a length of 123bp.

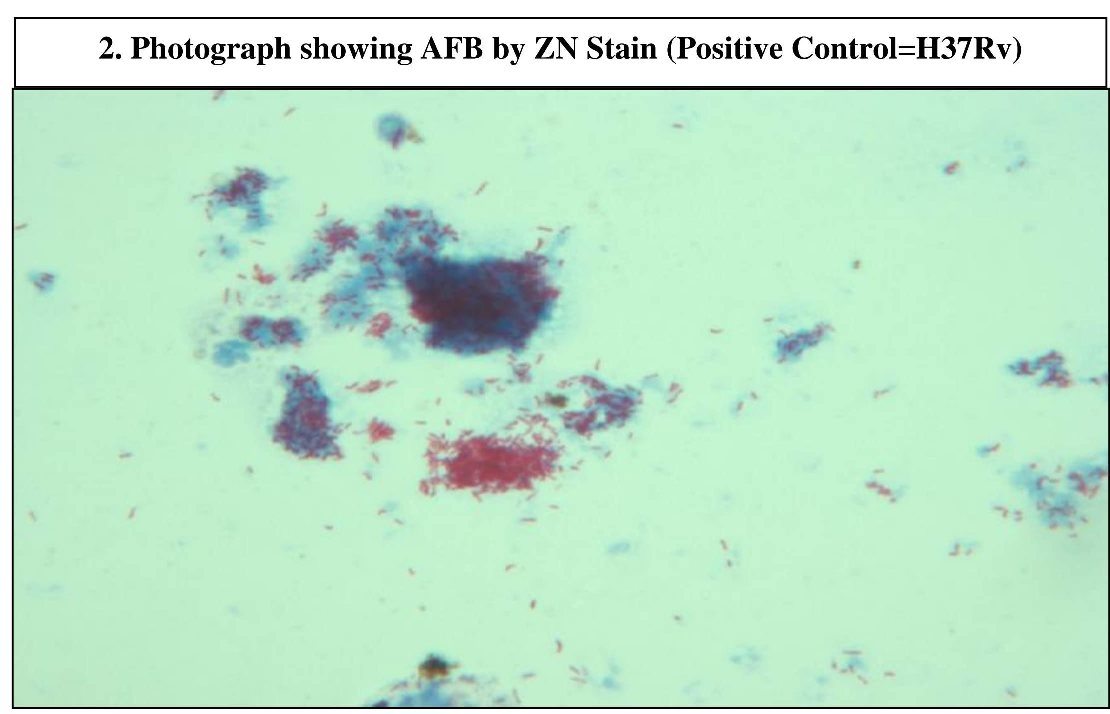
DNA extraction Kit and PCR chemicals were obtained from QIAGEN and the assay was performed as per the protocol mentioned.[74]

Disinfection and discard:5% Lysol is used to discard specimens.

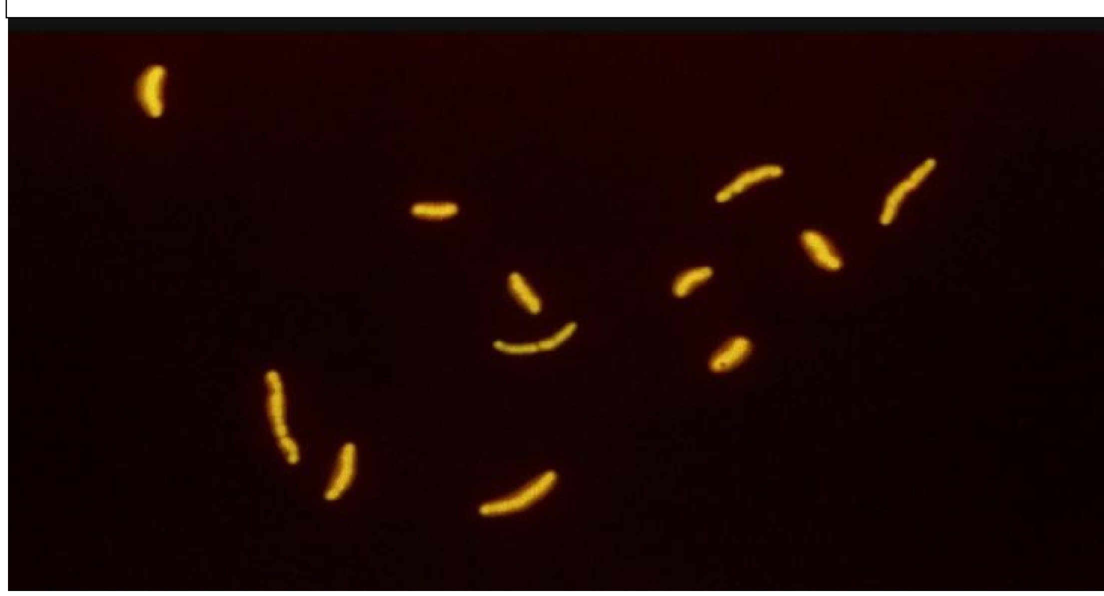
ANNEXURE - II – PHOTOGRAPH

1. Biosafety Cabinet -2

2. Photograph showing AFB by ZN Stain (Positive Control=H37Rv)

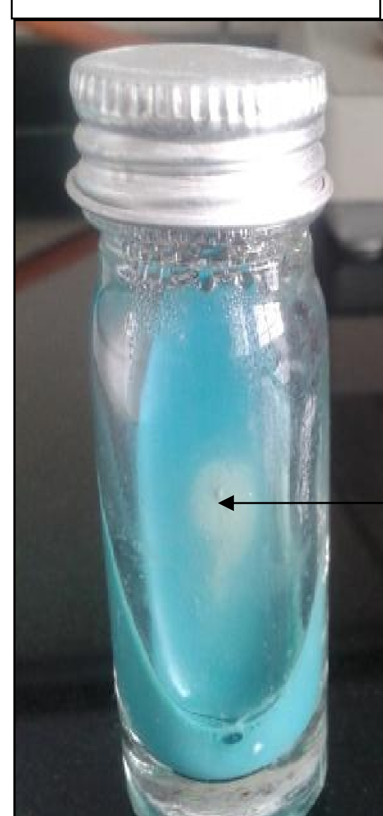


3. Photograph showing AFB by Fluorescent Stain (Positive Control=H37Rv)

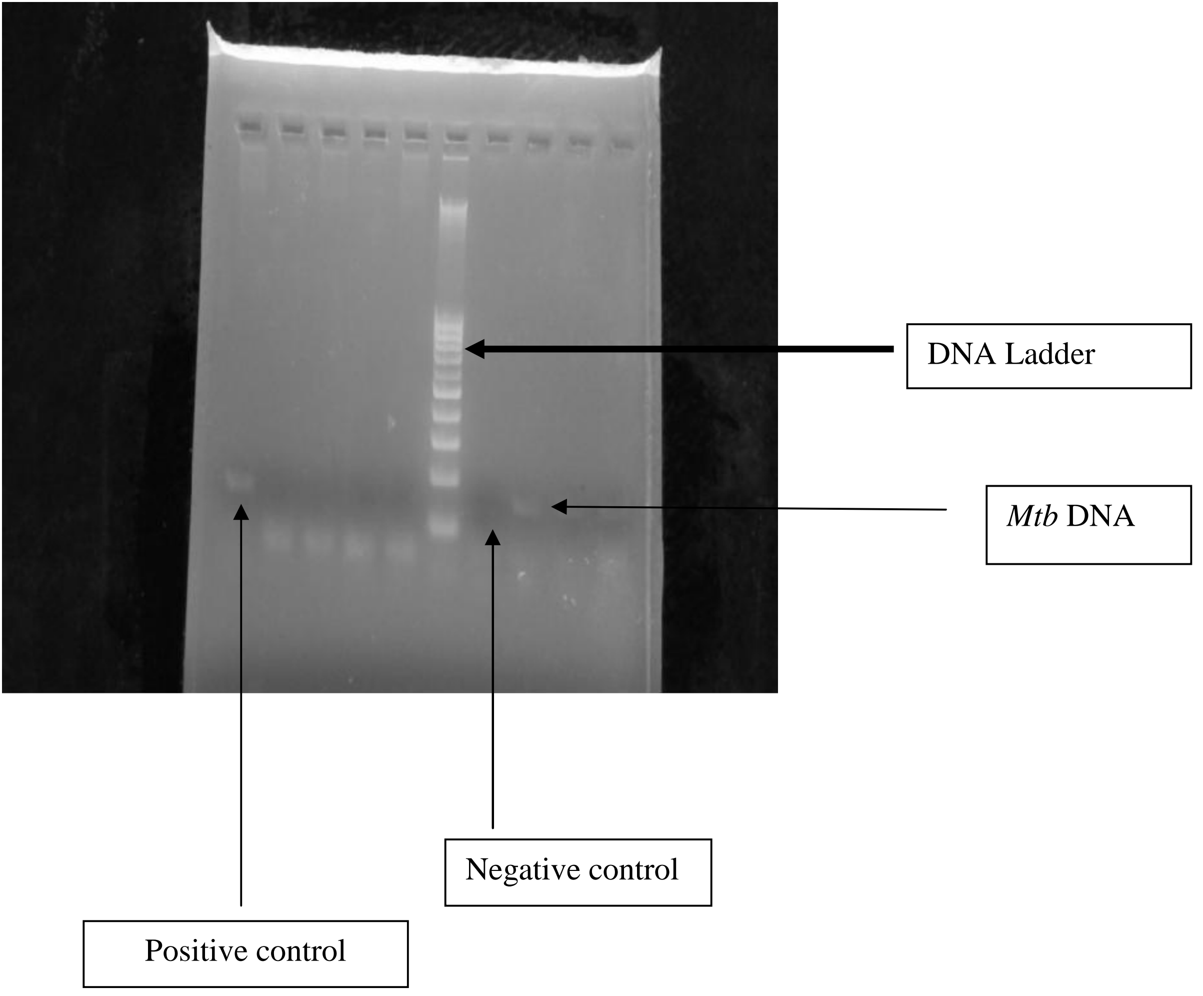


4. L-J Medium

5. L J Medium with *Mtb* Growth (H37Rv)

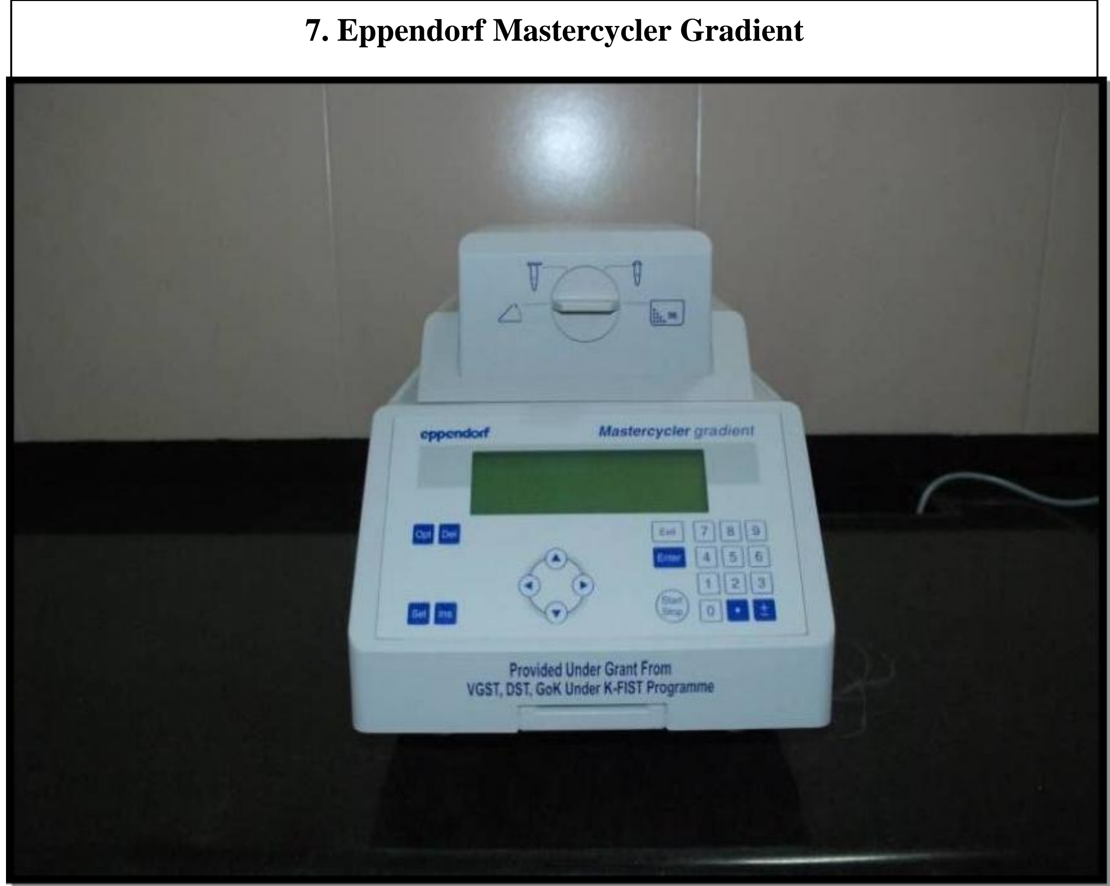


Mtb Growth



6. Photograph showing Gel Documentation of *Mtb* –DNA (123bp), Positive Control (H37Rv), Negative Control, DNA Ladder 100bp, Housekeeping gene (143bp).

7. Eppendorf Mastercycler Gradient



8. Gel Documentation System (SynGene, U.K)

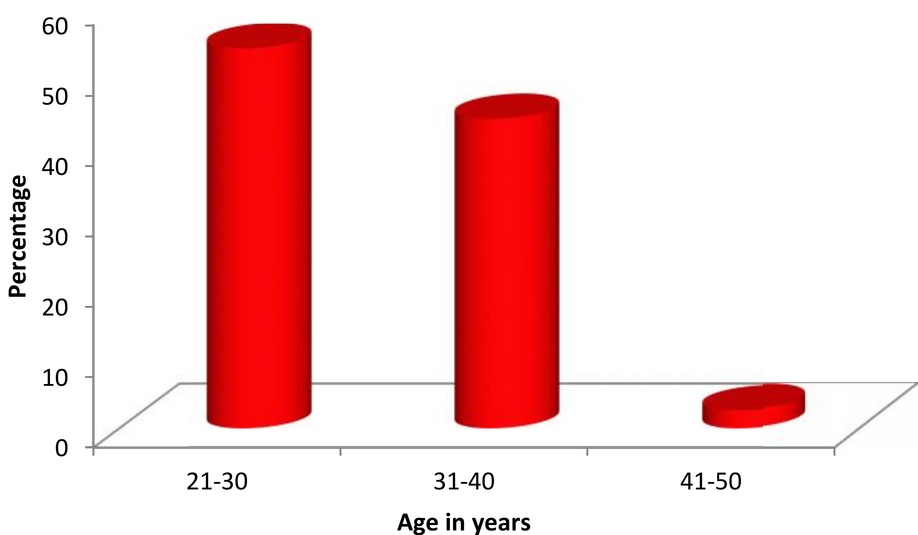
RESULTS

Study design: A Cross-sectional study

Table 4: Age distribution of patients studied

Age in years	No. of patients	%
21-30	43	53.8
31-40	35	43.8
41-50	2	2.5
Total	80	100.0

Fig 5: Bar graph showing age distribution of patients studied

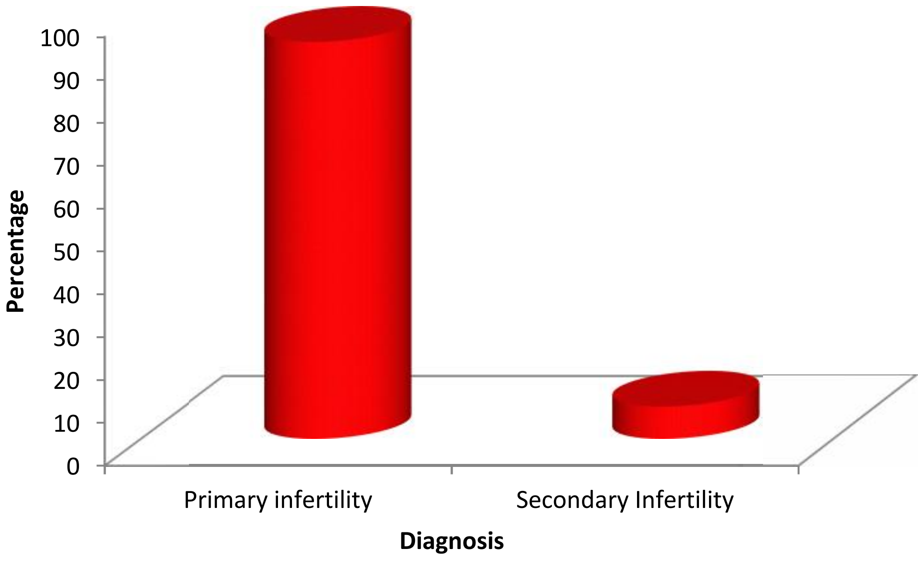


Out of 80 patients studied, 53.8% (43/80) were in the age group between 21-30 years,43.8 % (35/80) were in the age group between 31-40years and 2.5%(2/80) patients belonged to the age group of 41-50 years.

Table 5: Diagnosis distribution of patients studied.

Diagnosis	No. of patients	%
Primary Infertility	74	92.5
Secondary Infertility	6	7.5
Total	80	100.0

Fig. 6: Bar graph showing diagnosis distribution of patients studied.

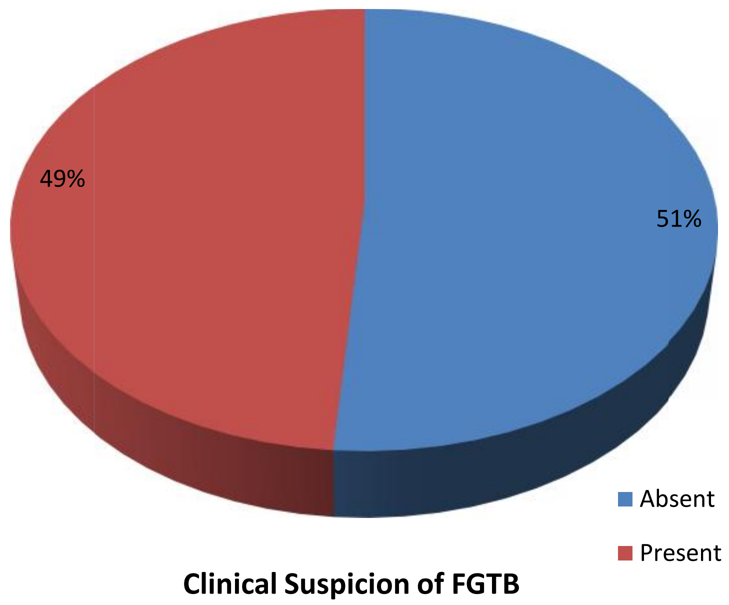


Of the 80 patients evaluated, 92.5 % (74/80) were diagnosed to have primary infertility and 7.5% (6/80) were diagnosed as cases of secondary infertility.

Table 6: Clinical Suspicion of FGTB distribution of patients studied.

Clinical Suspicion of FGTB	No. of patients	%
Absent	41	51.3
Present	39	48.8
Total	80	100.0

Fig. 7:Pie- chart showing Clinical Suspicion of FGTB distribution of patients studied.



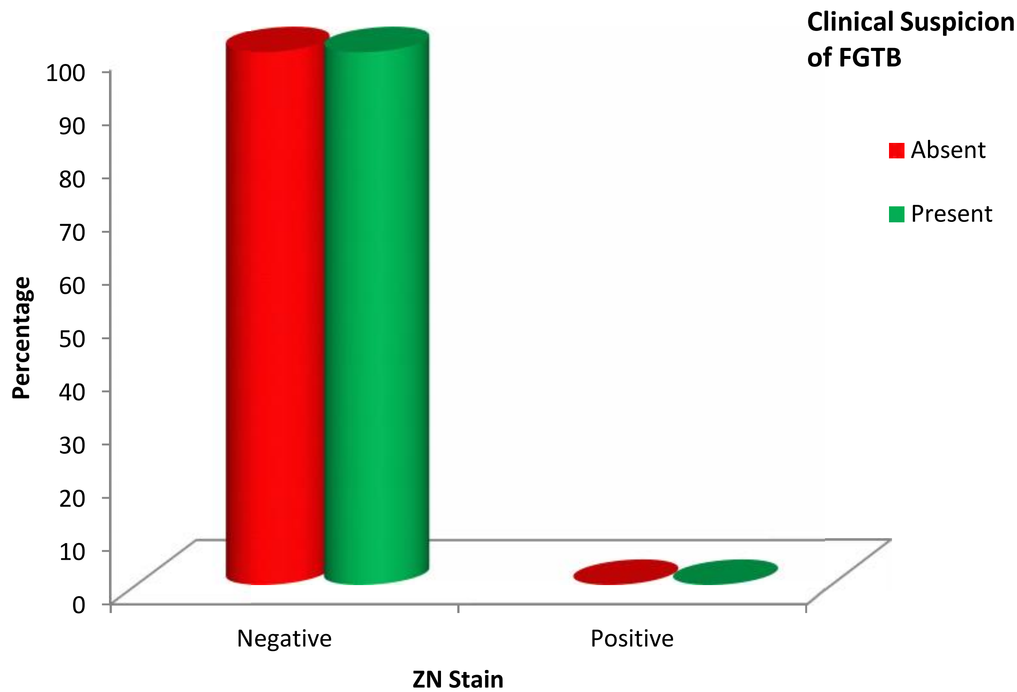
Of the 80 infertility cases evaluated, 48.8% (39/80) had clinical suspicion of FGTB while 51.3% (41/80) had no clinical suspicion of FGTB.

Table 7: ZN Stain of patients studied in relation to Clinical suspicion of FGTB

ZN Stain	Clinical Suspicion of FGTB		Total
	Absent	Present	
Negative	41(100%)	39(100%)	80(100%)
Positive	0(0%)	0(0%)	0(0%)
Total	41(100%)	39(100%)	80(100%)

p=1.000, Not significant, Fisher Exact test

Fig 8: Bar graph showing ZN Stain of patients studied in relation to Clinical suspicion of FGTB.



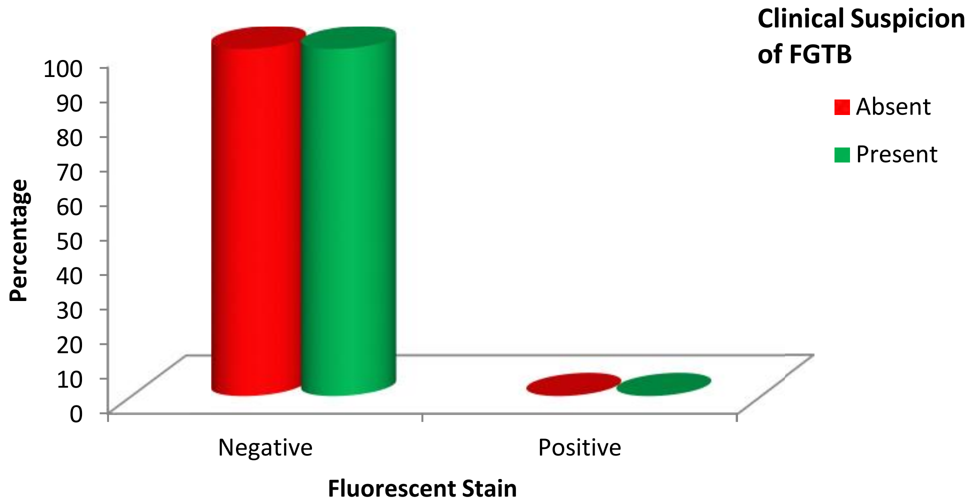
No Acid –fast bacilli was detected in the 80 samples thus examined.*p*-value is not significant by Fisher Exact test.

Table 8: Fluorescent Stain of patients studied in relation to Clinical suspicion of FGTB

Fluoroscent Stain	Clinical Suspicion of FGTB		Total
	Absent	Present	
Negative	41(100%)	39(100%)	80(100%)
Positive	0(0%)	0(0%)	0(0%)
Total	41(100%)	39(100%)	80(100%)

p=1.000, Not significant, Fisher Exact test

Fig 9: Bar graph showing Fluorescent Stain of patients studied in relation to Clinical suspicion of FGTB



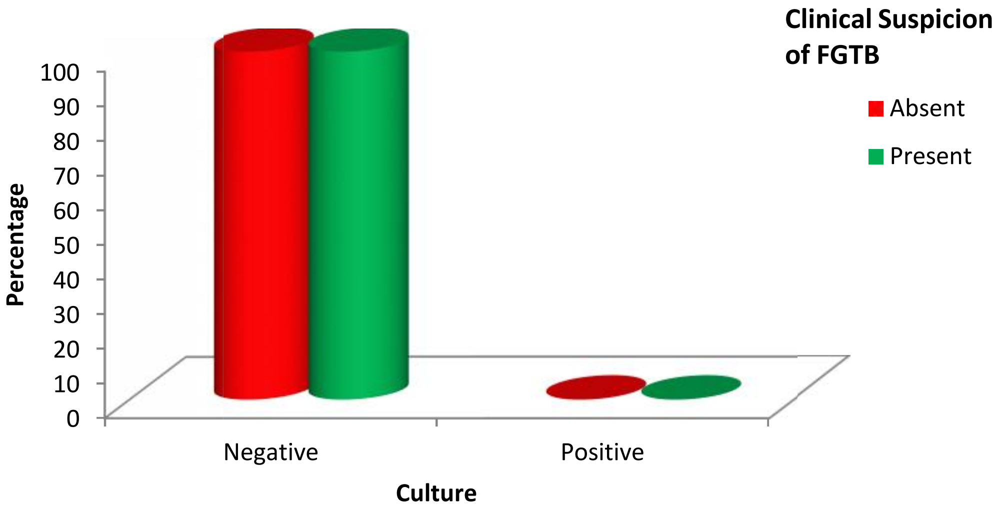
No Acid –fast bacilli was detected by Fluorescent microscopy from the samples thus tested. p-value is not significant by Fisher Exact test.

Table 9: Culture of patients studied in relation to Clinical suspicion of FGTB

Culture	Clinical Suspicion of FGTB		Total
	Absent	Present	
Negative	41(100%)	39(100%)	80(100%)
Positive	0(0%)	0(0%)	0(0%)
Total	41(100%)	39(100%)	80(100%)

p=1.000, Not significant, Fisher Exact test

Fig. 10: Bar chart showing culture of patients studied in relation to Clinical suspicion of FGTB



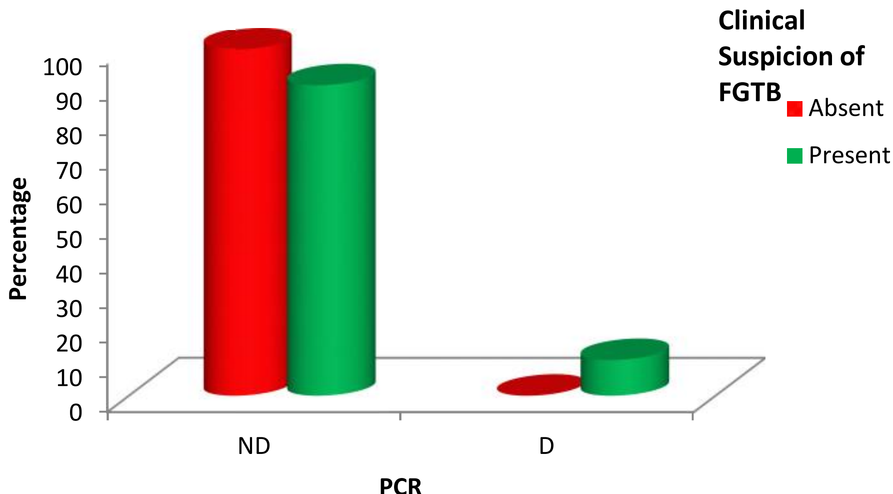
No growth resembling *MTB* on LJ medium was noted at the end of 8 weeks of incubation. *p*-value is not significant by Fisher Exact test.

Table 10: PCR of patients studied in relation to Clinical suspicion of FGTB

PCR	Clinical Suspicion of FGTB		Total
	Absent	Present	
ND	41(100%)	35(89.7%)	76(95%)
D	0(0%)	4(10.3%)	4(5%)
Total	41(100%)	39(100%)	80(100%)

p=0.05, significant, Fisher Exact test

Fig. 11: Bar chart showing PCR of patients studied in relation to Clinical suspicion of FGTB



MTB DNA was detected in 10.3 %(4/39 suspected cases) and was not detected in the remaining 41 cases who had no clinical suspicion of FGTB .*p*-value is just significant.

Table 11: Age distribution of patients studied in relation to PCR findings

Age in years	PCR		Total
	D	ND	
21-30	2(50%)	41(53.9%)	43(53.8%)
31-40	2(50%)	33(43.4%)	35(43.8%)
41-50	0(0%)	2(2.6%)	2(2.5%)
Total	4(100%)	76(100%)	80(100%)

p=1.000, Not significant, Fisher Exact test

Fig 12: Bar chart showing age distribution of patients studied in relation to PCR findings.

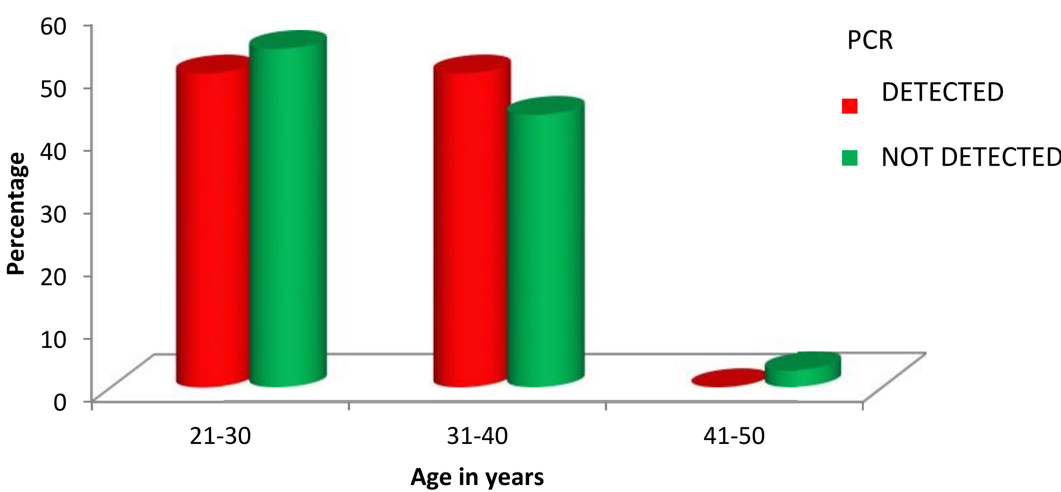


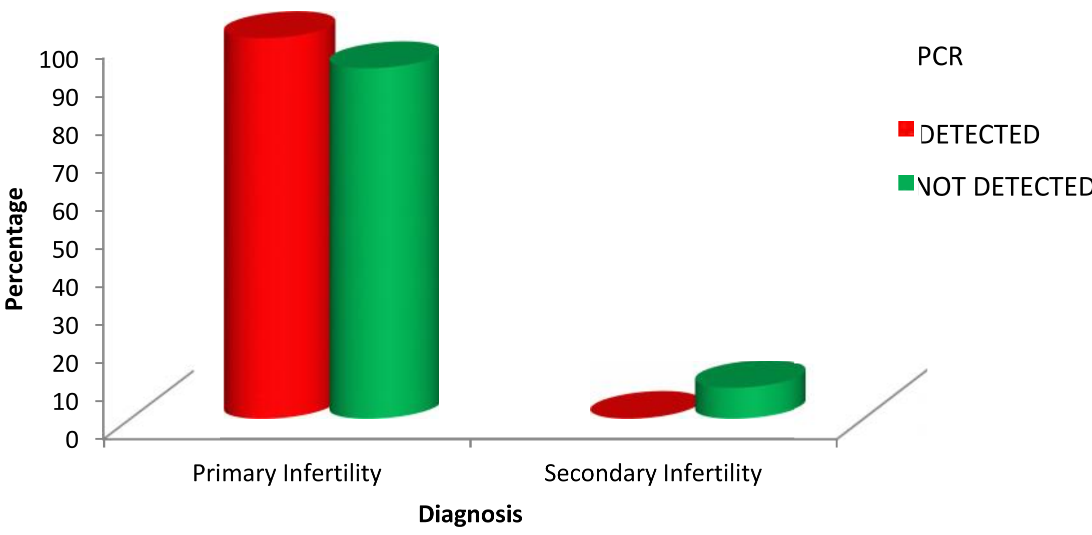
Table 12: Diagnosisof patients studied in relation to PCR findings

Diagnosis	PCR		Total
	D	**ND**	
Primary Infertility	4(100%)	70(92.1%)	74(92.5%)
Secondary Infertility	0(0%)	6(7.9%)	6(7.5%)
Total	4(100%)	76(100%)	80(100%)

p=1.000, Not significant, Fisher Exact test

Sensitivity=10.25%
Specificity=100.0%
PPV=100.00%
NPV=53.95%
Accuracy=56.25%

Fig .13: Bar graph showing diagnosisof patients studied in relation to PCR findings



Statistical Methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, Cases of the samples should be independent

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.Sensitivity, Specificity, PPV, NPV, Accuracy are computed to find the diagnostic relationship

Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value: $0.01<P \leq 0.05$)

** Strongly significant (P value: $P\leq 0.01$)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

DISCUSSION

TB is a one of the known causes of infertility in women .There is a global increase in the spread of TB due to HIV co- infection and drug resistance (MDR and XDR TB). Hence when investigating cause of infertility in women, she should be also evaluated for FGTB. Actual frequency of female genital tuberculosis is unknown despite different published data from various countries as it is often discovered incidentally or remains 'undetected' in symptomless patients. Genital tuberculosis is usually an indolent infection and takes years to manifest clinically after initial infection.[64] Effective chemotherapy had reduced all forms of TB including FGTB until the HIV co-infection reversed the trend over the last decade.[65]

The present study was undertaken to compare the diagnostic ability of conventional techniques namely Ziehl-Neelsen staining, Fluorescent staining and Culture on L-J medium with molecular method namely Polymerase Chain Reaction in diagnosing Genital Tuberculosis in women presenting to Gynaecology department with Infertility as their diagnosis.

Infertility (clinical definition) is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.[66]In the present study, 80 endometrial biopsy specimens were tested from 80 women diagnosed to have infertility. Out of 80 cases of infertility, 39 cases had a clinical suspicion of FGTB based on the clinical profile and laparoscopic evaluation. In the present study, 92.5 %(74/80) cases had primary infertility and 7.5 % (6/80) had secondary infertility. Genital TB generally affects women of reproductive age group, most of which are asymptomatic. The majority of the

patients in this study were in theage group of 21–30 years. Epidemiological profile of infertility patients in previous studies has also shown maximum cases of infertility in the age group of 25-35 years.[4,25,67,68]

In the present study, Mantoux test was negative in these patients. All the patients were from urban area with no history of stay in overcrowded areas. All the patients were non-reactive to HIV-I and II test.

The gold standard method for diagnosis of TB is Culture in this study. No acid fast bacilli was detected in all the 80 specimens tested microscopically, using ZN and Fluorescent staining techniques which could be attributed to sparse and uneven distribution of bacilli in sample tissue.However, AFB staining has very low sensitivity as it requires presence of atleast 10^4 organisms/ml in the sample.[4] The results of the present study matched with one of the previous studies conducted i.e no AFB was detected by smear microscopy from EPTB.[69]

Fluoroscent microscopy also has low senstivity in EPTB because of paucibacillary nature of the disease .No acid fast bacilli was detected in all the 80 specimens tested microcopically using this technique which is lower than one of the studies thus conducted previously .[64]

No *Mtb* growth was noted on LJ medium at the end of 8 weeks of incubation. Such comparable results were also evident in few of the similar studies conducted in the past.[31,72] However the detection rates by culture from the present study was quite low when compared to few of the studies conducted in the past. [7, 23,70,71] Culture requires as little as 10^2 organisms/ml. In addition to the non-availability of culture facilities in many laboratories, *M. tuberculosis may* take long incubation time (up to 8 weeks) to grow in LJ medium. Besides technical drawbacks in demonstrating *M.*

tuberculosis in the laboratory, a substantial number of TB lesions of the genital tract are bacteriologically mute. [64]

In the present study, Conventional PCR detected *Mtb* DNA in 10.3% (4/39 suspected cases) and no *Mtb* DNA was detected in the other cases. These findings were comparable with the results of the previous studies showing low positivity. [67,72].However Compared to few of the earlier studies thus conducted, the positive results were lower in the present study. [7,8,19,71,73]

There are several possible reasons for false negativity viz.,

- the paucibacillary nature of the disease, or
- Presence of PCR inhibitors like RBCs,or
- absence or low number of copies of IS*6110* in *Mtb* DNA.

IS*6110* is specific for M. tuberculosis complex and generally occurs in 1-20 copies per cell, which are dispersed in the *M. tuberculosis* genome and it is an ideal target for amplification, one locus, the direct repeat region, has an high frequency of carriage of IS*6110* and has been proposed as a “hot spot” for integration of this element, although most of the copies are located at a single site .These insertion elements are present in multiple copies on the genome of *M. tuberculosis*, with 16 copies of IS*6110*, 6 copies of IS*1081* and 2 copies each of IS*1547*. The variable copy number of IS*6110* among different strains of the tubercle bacilli has led to its extensive use as a genetic marker to investigate the epidemiology of tuberculosis. [51]

A significant proportion (40%) of south Indian isolates have been reported with single copy or lacked this sequence [33] The high false negative result is an important limitation in this study. A negative PCR may result in missing the diagnosis in a few cases. Therefore, when FGTB is suspected clinically, but the PCR results are

negative, it indicates the need for further evaluation using other diagnostic tests and repeat testing to confirm/exclude diagnosis.[7]

The common problem raised during the PCR assays is the high risk of false positive results due to common laboratory contamination or presence of killed or dormant bacilli in the patient specimens. It was taken care by proper control checks and good laboratory practice so that chances of false positive results be kept to a minimal.In this regard,DNA extraction,amplification and post amplification was done in different rooms to avoid carry-over contamination.

High false negative results, inability to perform multiple sampling were limitations of the current study.

From this study we deduce that the advantages of IS*6110* PCR are that it is very rapid, easy to perform method and result can be issued for early treatment and to prevent further transmission of tuberculosis infection. Further, IS*6110* PCR test proved to be more sensitive even when both smear examination and culture results were considered in conjunction. PCR techniques are highly sensitive and under optimum conditions may detect 1-10 organisms. The main advantage of PCR is that it is a rapid and specific molecular technique which allows detection of mycobacteria within 4–5 h compared to culture on LJ which has a poor detection rate and requires a 8 weeks to get the result. [75]

CONCLUSION

FGTB poses a great diagnostic challenge inspite of great advances in diagnostics. Culture and ZN staining are the tests employed for definitive diagnosis of this disease. The diseasse being paucibacillary, the bacteriologic confirmation by the conventional techniques is often difficult in terms of sensitivity and long duration. Due to poor yield of conventional diagnostic methods there is considerable delay in the treatment. The molecular method based on In-Vitro amplification of *Mtb*DNA using PCR has immensely met with great success in early diagnosis of this disease.

Based on the results of our study, we conclude that PCR tests for DNA specific to *M. tuberculosis* may be of hope for a rapid and accurate diagnostic test for FGTB and it helps where conventional diagnosis fails and provisional diagnosis of tuberculosis is made on the basis of clinical presentation and laparoscopic examination without evidence of AFB. PCR based methods may have a great potential for rapid diagnosis, treatment and prevention of FGTB. Due to the short turn-around time of PCR, it has utility in providing the results in clinically relevant time and thus helps to initiate the treatment timely.

SUMMARY

- A total of 80 female patients with the diagnosis of infertility formed the study group. Out of which 72 cases were diagnosed as having primary infertiliy and 6 of them had secondary infertility.
- Out of 80 cases, 39 cases had a clinical suspicion of FGTB and the remainder had no clinical suspicion. Based on the clinical profile and laparoscopic evaluation of patients, a diagnostic criteria were derived to suspect tuberculosis. A woman was said to be suspected of having genital tuberculosis if she has had findings suggestive of tuberculosis at laparoscopy with one or more of the following findings: A definite past history of tuberculosis, in the presence of active extra-genital tuberculosis, characteristic features on HSG, elevated ESR, positive Mantoux test, evidence of calcification/ complex adnexal mass by scan.
- Out of 80 patients studied ,53.8% (43/80) were in the age group between 21-30 years , 43.8%(35/80) were in the age group between 31-40years and 2.5%(2/80) patients belonged to the age group of 41-50 years.Majority were in the age group of 21 -30 years.
- Smear microscopy by ZN staining and Fluorescent staining did not detect any AFB in the study.
- No *Mtb* growth was seen on L-J media at the end of 8 weeks of incubation.
  Culture is the gold standard in the study.
- *Mtb* DNA was detected in 10.3% (4/39 suspected cases) and was not detected in the remaining 41 cases who had no clinical suspicion of GTB. *p*-value is just significant. Sensitivity=10.25%; Specificity=100%;PPV=100.00%; NPV=53.95%; Accuracy=56.25% are the values of *Mtb* DNA PCR deduced from the present study.

BIBLIOGRAPHY

1. Ryan KJ, Ray CG, Sherris JC. *Sherris Medical Microbiology*, 6th edn.Mc Graw Hill;2010.p. 489-506.

2. Muir DG, Belsey MA: Pelvic inflammatory disease and its consequences in the developing world. Am J Obstet Gynecol. 1980; 138:913.

3. Raviglione MC,O'Brien RJ.Tuberculosis.In:Fauci AS,Braunwal E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL et.al(eds).*Harrissons' principles of internal medicine*.New York:McGraw-Hill,2001:1024-35.

4. Sethi S, Dhaliwal L, Dey P, Kaur H, Yadav R, Sethi S. Loop-mediated isothermal amplification assay for detection of Mycobacterium tuberculosis complex in infertile women. Indian J Med Microbiol [Internet]. 2016;34(3):322. Available from:http://www.ijmm.org/text.asp?2016/34/3/322/188323. [accessed on 13.10.2016].

5. Arora R, Sharma JB. Female Genital Tuberculosis - a diagnostic and therapeutic challange. Indian J Tuberc.2014; 61(2): 98-102

6. Roy H, Roy S, Roy S. Use of polymerase chain reaction for diagnosis of endometrial tuberculosis in high risk sub-fertile women in an endemic zone. J Obstet Gynecol India 2003; 53:260-3.

7. Thangappah RBP, Paramsivan CN, Narayan S. Evaluating PCR, culture and histopathology in the diagnosis of Female Genital Tuberculosis. I J Med Res 2011;134:40-6.

8. Baxi A, Neema H, Kaushal M, Sahu P, Baxi D. Genital tuberculosis in infertile women: Assessment of endometrial TB PCR results with laparoscopic and hysteroscopic features. J Obstet Gynecol India. 2011;61(3):301–6.

9. Patil A, Patange RP. Assessment of Conception Rate in Infertile Couples with Genital Tuberculosis Diagnosed with Multiplex Polymerase Chain Reaction. IJRTST .2013;9(1):136–41.

10. Washington W Jr, Stephen A, William J, Elmer K, Gary P, Paul S et al.*Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th ed.China Lippincott Williams & Wilkins;2006.p.1067-8

11. Saraswat P, Swarankar ML, Bhandari A, Soni RR. Detection of active Female Genital Tuberculosis by Molecular method. Int J Pharma. Bio Sci 2010;1:338-42.

12. Bose M. Female genital tract tuberculosis: How long will it elude diagnosis? Indian J Med Res 2011;134:13-4.

13. Dutta DC.Genital Tuberculosis.In:Konar H(ed.). D.C.Dutta Text book of Gynaecology,6th edn.Calcutta;New central book agency(p) ltd;2013:132-8.

14. Kumar P, Malhotra N.*Jeffcoates Principles of Gynaecology, 7th* edn. Jaypee publications;2008 .

15. Forbes BA,Sahm DF,Weisfeld AS.*Bailey and Scott's Diagnostic Microbiology*. 12th edn.St.Louis, Missouri: Mosby; 2007.p.478-509

16. Agarwal J, Gupta, JK.Female genital tuberculosis – a retrospective clinicopathological study of 501 cases.Indian J Pathol Microbiol .1993,36(4): 389–97.

17. Srivastava I, Bhatambare GS, Deshmukh AB, Bajpai P, Bajpai T, Singh T. Original Research Article Genital Tuberculosis : Evaluating Microscopy , Culture Histopathology and PCR for Diagnosis All Play Their Role. 2014;3(4):439–45.

18. Misra R, Sharma SP, Jina R, Pant N, Srivastava D K. Female genital tract tuberculosis with special reference to sterility in Eastern UP. J Obstet Gynaecol India. 1996; 104-9

19. Bhanu NV, Singh UB, Chakraborty M, Suresh N, Arora J, Rana T, et al. Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. J Med Microbiol. 2005;54(10):927–31.

20. Kulshrestha V, Kriplani A, Agarwal N, Singh UB, Rana T.Genital tuberculosis among infertile women and fertility outcome after antitubercular therapy. Int J Gynaecol Obstet 2011;113:229-34

21. Rana T, Singh UB, Kulshrestha V, Kaushik A, Porwal C, Agarwal N, et al. Utility of reverse transcriptase PCR and DNA-PCR in the diagnosis of female genital tuberculosis. J Med Microbiol. 2011;60(4):486–91.

22. Malhotra B, Sinha S, Hooja S, Vyas L. Rapid diagnosis of Genital tuberculosis by Real Time PCR: JSAFOG;4(1): 39-42.

23. Kumar P, Shah NP, Singhal A, Chauhan DS, Katoch VM, Mittal S, et al. Association of tuberculous endometritis with infertility and other gynecological complaints of women in India. J Clin Microbiol 2008;46:4068-70.

24. Rozati R, Roopa S, Rajeshwari CN. Evaluation of women with infertility and genital tuberculosis : 2006;56(5):423–6.

25. Namavar Jahromi B, Parsanezhad ME, Ghane-Shirazi R. Female genital tuberculosis and infertility. Int J Gynaecol Obstet 2001;75:269-72.

26. Roy A, Mukherjee S, Bhattacharya S, Adhya S, Chakraborty P. Tuberculous endometritis in hills of Darjeeling- a clinicopathological and bacteriological study. Indian J Pathol Microbiol 1993; 36 : 361-9.

27. Srivastava N, Manaktala U, Baveja CP. Role of ELISA (enzyme-linked immunosorbent assay) in genital tuberculosis. Int J Gynaecol Obstet 1997; 57 : 205-6.

28. Soltys MA. An anti-tuberculous substance in tuberculous organs. J Comp Pathol 1953; 63 : 147-52

29. Manjari M, Khanna S, Arora U, Kahlon SK, Gulati VL, Pushpa, et al. Tuberculous endometritis in sterile females: a Clinicopathological study. Indian J Tuberc 1995; 42 : 227-8.

30. Deepjyoti VG, Kiran M, Neera A, Sunil G. Isolation of mycobacteria from cases of infertility in women. J Obstet Gynaecol India 1990; 40 : 803-5

31. Baum SE,Dooley DP,Wright J,Kosh ER,Storey DF.Diagnosis of culture negative Female genital tract tuberculosis with involvement of Polymerase chain reaction.J.Reprod Med 2001;46:929-32.

32. Narayanan S, Parandaman V, Narayanan PR, Venkatesan P, Girish C, Mahadevan S, et al. Evaluation of PCR using TRC(4), and IS 6110 primers in detection of tuberculous meningitis. J Clin Microbiol 2001; 39 : 2006-8.

33. Chauhan DS , Sharma VD, Parashar D, Chauhan A, Singh D, Singh HB et al. Molecular typing of Mycobacterium tuberculosis isolates from different parts of India based on IS6110 element polymorphism using RFLP analysis. Indian J Med Res., 2007; 125: 577-81

34.Narayanan, S., S. Das, R. Garg, L. Hari, V. B. Rao, T. R. Frieden, and P. R.Narayanan.2002. Molecular epidemiology of tuberculosis in a rural area of high prevalence in south India: implications for disease control and preven-tion. J. Clin. Microbiol.40:4785–4788.

35.Radhakrishnan, I., Y. K. Manju, R. A. Kumar, and S. Mundayoor.2001.Implications of low frequency of IS6110 in fingerprinting field isolates of Mycobacterium tuberculosis from Kerala, India. J. Clin. Microbiol.39:1683.

36. Sharma JB: Tuberculosis and obstetric and gynecological practice. *In*: Studd J, Tan SL, Chervenak FA, (editors), Progress in Obstetric and Gynaecology Philadephia: *Elsevier* 2008; 18: 395-427.

37. Arora R, Rajaram P, Oumachigui A, Arora VK. Prospective analysis of short course chemotherapy in female genital tuberculosis. *Int J Gynaecol Obstet* 1992; 38(4): 311-4.

38. World Health Organization. Global Tuberculosis Control: A short update to the 2009 report. WHO/HTM/TB 2009, 426.Geneva:WHO;2009.

39. Good RC and Shinnick TM.Mycobacterium.In:Collier L ,Balows A and Sussan M (eds).*Topley and Wilson's Microbiology and Microbial Infections,Systemic bacteriology,* Vol.2.9th edn.London:Edward Arnold; 1998 .p.549-76.

40. Levy-Frebault V and Portaels F. Proposed minimal standards for the genus and for description of new slowly growing Mycobacterium species.Int J Syst Bacteriol. 1992;42:315-23.DOI:10.1099/00207713-42-2-315[Accessed on 20.09.16]

41.Wayne LG and Kubica GP.The mycobacteria.In:Sneath PHA ,Mair NS,et al.(eds).*Bergey' s manual of systematic bacteriology*,8th

edn.Baltimore,MD:Williams and wilkins;1986.p.1436-57. DOI: 10.1099/ijs.0.01963-0 [accessed on 20.09.16]

42. Morgagni JB: De Sedibus et Causis meorboram. Epistola. 1744;38-34.
43. Wheeler PR and Ratledge C.*Metabolism of mycobacterium, protection and control*.Washington DC: American society for microbiology Press; 1994 .p.353-85.
44. Kleinnijenhuis J, Oosting M, Joosten LAB, Netea MG, Van Crevel R. Innate Immune Recognition of *Mycobacterium tuberculosis*. Clin Dev Immunol [Internet]. 2011;1–12. Available from: http://www.hindawi.com/journals/jir/2011/405310/ [accessed on 21.10.16]
45. Besra GS and Chatterjee.D.Lipids and carbohydrates of *Mycobacterium tuberculosis*.In:Bloom BR(ed).*Tuberculosis:Pathogenesis,Protection and Control.*Washington,DC:American Society for Micrbiology Press; 1994.p.285-306.
46. Brennan PJand Draper P. Ultrastructure of Mycobacterium Tuberculosis.In:Bloom BR(ed.).*Tuberculosis:Pathogenesis,Protection and Control*. Washington,DC:American Society for Micrbiology Press. 1994.p.271-84.
47. Brennan PJ, Hunter SW.Reappraisal of the chemistry of mycobacterial cellwalls,with a view to understanding the roles of individual entities in disease processes.In:Ayoub EM, Cassell,GH(eds).*Microbial determinants of virulence and host response*.Washington ,DC: American Society for Micrbiology Press, 1990.p.55-75
48. Kaplan G,Gandhi RR .Mycobacterium leprae antigen-induced suppression of T-cell proliferation in vitro.J Immunol, 1987;138:3028-34.

49. Minnikin DE,Hutchinson IG..Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. J Chromatogr.1980;188:221-33.

50. Horwitz MA, Lee BWE. Protective immunity against tuberculosis induced by vaccination with major extracellular proteins of Mycobacterium tuberculosis.Proc Natl Acad Sci USA, 1995;92:1530-34.

51. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature. 1998;393(6685):537–44.

52. Ananthanarayan R,Paniker CKJ.Mycobacterium-I-M.tuberculosis.In:Kapil A.(ed.)Textbook of Microbiology.9th edn.India:Universities Press (India)Private Limited;2013.p.345-58

53. CDC. *Biosafety in Microbiological and Biomedical Laboratories*.4th edn,HHS Publication(CDC) 017-040-00547-4.US Government Printing Office,Washington,DC.1999. Available at www.cdc.gov/od/ohs/biosfty/bmb14. [accessed on 13.06.16]

54. Bryan WA. Mycobacteria-General Culture Methodology and Safety Considerations.In:Parish T,Stoker NG.*Methods in molecular biology,vol.101,Mycobacteria protocols*. Humana Press Inc,Totowa,NJ.p.15-30

55. Vincent V, Jost JKC. Mycobacterium :Phenotypic and genotypic identification .In:Murray PR, Baron EJ(eds).*Manual of clinical microbiology*,8th edn.Washington DC: American Society for Micrbiology Press .2003.p.560-84.

56. Joshi M, Deshpande JD. Polymerase Chain Reaction : Methods , Principles and Applications. Int J Biomed Res [Internet]. 2010;1(5):81–97. Available from: www.ssjournals.com [accessed on 01.10.16]

57. Mullis KB.The unusual origin of the Polymerase chain reaction.Scientific Americans.1970;262:56-65

58. Buck GE,O'Hara LC,Summersgill JT.Rapid simple method for treating clinical specimens containing M.Tuberculosis to remove DNA for Polymerae Chain Reaction.J.Clin Microbiol 1992;50(5):1331-34.

59. Cousins DV,Witon SD,Francis BR,Gow BL.Use of polymerase chain reaction for rapid diagnosis of tuberculosis. J.Clin Microbiol 1992;30(1):255-58.

60. Baek CH,Kim S,Ko YH and Chu KC.Polymerase Chain Reaction deyection of Mycobacterium tuberculosis from fine needle aspirate for the diagnosis of cervical tuberculous Lymphadenitis.Laryngoscope 2000;110(1):30-4.

61. Anochie PI, Onyeneke EC, Ogu AC, Onyeozirila AC, Aluru S, Onyejepu N, et al. Recent advances in the diagnosis of mycobacterium tuberculosis. Germs. 2012; 2(3): 110–20.

62. Palenzona C. Tuberculosis in women. Praxis (Bern 1994). 1959;48:983–7.

63. Duguid FP. Staining methods.In:Collee JG,Fraser AG,Marmion BP,Simmons A(eds.). *Mackie and McCartney Practical Medical Microbiology,* 14th edn.India;Elsevier.2012.p.800-03.

64. Kashyap B, Srivastava N, I RK, Jhamb R, D KS. Diagnostic dilemma in female genital tuberculosis- staining techniques revisited. Iran J Reprod Med [Internet].

2013;11(7):545–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/246397891) 14.10.2016

65. Roya R,Sreenivasagari R,Cheruvu Naga R.Evaluation of women with infertility and genital tuberculosis.J obstet Gynecol India .2006:56(5):423-26

66. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009*. Fertil Steril [Internet]. 2009;92(5):1520–4. Available from: http://dx.doi.org/10.1016/j.fertnstert.2009.09.009 [accessed on 16.10.2016]

67.Prasad S, Singhal M, Negi SS, Gupta S, Singh S, Rawat DS, et al. Targeted detection of 65 kDa heat shock protein gene in endometrial biopsies for reliable diagnosis of genital tuberculosis. Eur J Obstet Gynecol Reprod Biol 2012;160:215-8.

68. Sankar MM, Kumar P, Munawwar A, Kumar M, Singh J, Singh A, et al. Usefulness of multiplex PCR in the diagnosis of genital tuberculosis in females with infertility. Eur J Clin Microbiol Infect Dis 2013;32:399-405.

69. Biswas D,Agrawal M,Ali A,Bhargava R.Anti ES-31/41 antibodies:A potential tool in the sero diagnosis of extra-pulmonary Tuberculosis.Indian J Tuberc.2003;50:203-7

70. Mani R, Nayak S, Kagal A, Deshpande S, Dandge N, Bharadwaj R. Tubercular endometritis in infertility: A bacteriological and hist opathological study. Indian J Tuberc 2003;50:161.

71. Khanna A, Agrawal A. Markers of genital tuberculosis in infertility. Singapore Med J 2011;52:864-7.

72). Kohli MD, Nambam B, Trivedi SS, Sherwal BL, Arora S, Jain A. PCR based evaluation of tubercular endometritis in infertile women of North India. J Reprod Infertil 2011;12:9-14.

73. Gupta N, Sharma JB, Mittal S, Singh N, Misra R, Kukreja M. Genital tuberculosis in Indian infertility patients. Int J Gynaecol Obstet 2007;97:135-8

74.Dna F, Buffy PS, Cultured L, Dried TS. QIAamp ® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook For DNA purification from Whole blood Buffy coat Body fluids Lymphocytes Cultured cells Tissue Swabs Dried blood spots February 2003. Blood. 2003. p.37-8

75.Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Microbiol. 2012;66(1):20–36.

ANNEXURE I – ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2471350
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 318

Date: 27/05/2015

To,
Dr.
PG student in Microbiology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"COMPARATIVE EVALUATION OF ZEIHL –NEELSEN STAINING, FLUORESCENT STAINING AND CULTURE WITH POLYMERASE CHAIN REACTION IN THE DIAGNOSIS OF GENITAL TUBERCULOSIS IN INFERTILE WOMEN – ONE YEAR CROSS – SECTIONAL STUDY AT TERTIARY CARE HOSPITAL, BELAGAVI"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr.Hema Dhumale)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr.Ganga Pilli)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE II - CONSENT FORM

CONSENT FORM FOR PARTICIPANTS IN RESEARCH

TITLE: Comparative evaluation of Ziehl-Neelsen staining, Fluorescent staining and Culture with Polymerase Chain Reaction in the diagnosis of Genital Tuberculosis in infertile women One year cross-sectional study at Tertiary Care Hospital, Belagavi.

Study Investigator: Dr. ____________________,
Post Graduate Student,
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum – 590 010.

Guide: Dr. ____________________ ,
Professor, Dept of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum – 590010.

Background/Introduction

A research study called "Comparative evaluation of Ziehl-Neelsen staining, Fluorescent staining, Culture with Polymerase Chain Reaction in the diagnosis of Genital Tuberculosis in infertile women One year cross-sectional study at Tertiary Care Hospital, Belagavi, is being conducted. It involves women between ages of 15 and 45 years diagnosed as cases of infertility. You are invited to participate in this study.

Female Genital tuberculosis (FGTB) is an infectious disease that causes considerable morbidity and mortality. It is a major socio-economic burden in India mostly affecting the reproductive age group (15-45 years).It is one of the major causes for severe tubal disease leading to infertility. FGTB is a prime suspect in about 5-16% of cases of infertility among Indian women though the actual incidence may be under reported due to asymptomatic presentation of the disease and paucity of investigation. Hence this study may help to diagnose the FGTB at the earliest by combination of conventional and modern molecular techniques.

Eligibility

All clinically diagnosed cases of infertility in women, between 15 and 45 years of visiting Obstetrics and Gynaecology Out Patient Department and In Patient Department of KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum with their informed consent.

What will happen if I participate in the study?

During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.Endometrial tissue is obtained by aspiration biopsy or by dilatation and curettage or directly at hysteroscopy from cases clinically diagnosed as infertile.

Risks

There are no major risks of being in the project.

Voluntary participation

Your participation is voluntary and the care provided to you will not be affected whether you choose to participate or not. You may withdraw from

participation at any time without any reason. All data collected about you during the study will be kept in our files for research purposes only.

Payment

You will not receive money or gifts for your participation. You will not be reimbursed for expenses.

Benefits

Your participation may help to evaluate the efficacy of conventional and molecular techniques in the diagnoses of FGTB.

Confidentiality

All the collected information will be given a number instead of using your name to protect your privacy. The identifiable information about you will be accessed only by the research team. The information provided by you will be used for scientific/educational purposes without your identification.

Who do I contact if I have questions about the study during my participation?

If you have any project-related enquiry, you may contact Dr _____________ at +_____________. In case you have any questions about your rights as a participant, you may contact Dr _________, Chairman, K.L.E University's JNMC Institutional Ethics Committee for Human Subjects Research, Belgaum at +_____________ or extension no.___ .

Authorization to publish results

When the results of research are published or discussed, in a conference no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

CONSENT STATEMENT

I, the undersigned ____ have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

________________________ _______________ ___________________

Signature/Left thumb print of Person-Providing consent

Name

Date: DD/ MM/ YYYY

________________________ _______________ ___________________

Signature/Left thumb print of Witness Name Date: DD/ MM/ YYYY

________________________ _______________ ___________________

Signature of Person Obtaining consent Name Date: DD/ MM/ YYYY

// ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸುವ ಸಹಮತಿ ಪತ್ರ.//

ಶಿರ್ಷಿಕೆ:

ಸಂತಾನವಂಚಿತ/ಸಂತಾನಹೀನ ಬಂಜೆ ಮಹಿಳೆಯರಲಲ್ಲಿ ಜನನೇಂದ್ರಿಯದ ಕ್ಷಯರೋಗದ ಅಂಶವನ್ನು ಝೀಲ-ನೀಲಸನ್ ಸ್ಟೇನಿಂಗ, ಪ್ಲೋರೋಸೆಂಟ ಸ್ಟೇನಿಂಗ ಮತ್ತು ಕಲ್ಚರದೊಳಗೆ ಪಾಲಿಮರಸ್ ಚೈನ್ ರೆಯಾಕ್ಷನ (ಪಿ.ಸಿ.ಆರ್) ಬಳಸಿ ಕಂಡುಹಿಡಿಯುವ ಬೆಳಗಾವಿಯ ಧರಸೆರಿ ಕೇರ್ ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ನಡೆಯುವ ಒಂದು ವರ್ಷದ ತುಲನಾತ್ಮಕ ಕ್ರಾಸ ಸೆಕ್ಸನಲ್ ಅಧ್ಯಯನ.

ಅಧ್ಯಯನ ನಡೆಸುವವರು: ಡಾ||.

ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿ,
ಮಿನಿ ಜೀವ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜಿ. ಎನ್. ಎಮ್. ಸಿ,
ಕೆ.ಎಲ್.ಇ, ವಿಶ್ವ ವಿದ್ಯಾಲಯ,
ಬೆಳಗಾವಿ – ೫೯೦೦೧೦.

ಮಾರ್ಗದರ್ಶಕರು: ಡಾ||.

ಪ್ರಾದ್ಯಾಪಕರು,
ಮಿನಿ ಜೀವ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜಿ. ಎನ್. ಎಮ್. ಸಿ,
ಕೆ.ಎಲ್.ಇ, ವಿಶ್ವ ವಿದ್ಯಾಲಯ,
ಬೆಳಗಾವಿ – ೫೯೦೦೧೦.

ಪೂರ್ವ ಪರಿಚಯ & ಹಿನ್ನಲೆ:

ಇದು ಒಂದು ಸಂಶೋಧನಾ ಅಧ್ಯಯನವೆಂದು ಕರೆಯಲ್ಪಡುವ ಸಂತಾನವಂಚಿತ/ಸಂತಾನಹೀನ ಬಂಜೆ ಮಹಿಳೆಯರಲಲ್ಲಿ ಜನನೇಂದ್ರಿಯದ ಕ್ಷಯರೋಗದ ಅಂಶವನ್ನು ಝೀಲ-ನೀಲಸನ್ ಸ್ಟೇನಿಂಗ, ಪ್ಲೋರೋಸೆಂಟ ಸ್ಟೇನಿಂಗ ಮತ್ತು ಕಲ್ಚರದೊಳಗೆ ಪಾಲಿಮರಸ್ ಚೈನ್ ರೆಯಾಕ್ಸನ (ಪಿ.ಸಿ.ಆರ್) ಬಳಸಿ ಕಂಡುಹಿಡಿಯುವ ಧರಸೆರಿ ಕೇರ್ ಆಸ್ಪತ್ರೆ, ಬೆಳಗಾವಿ ಯಲ್ಲಿ ನಡೆಯುವ ಒಂದು ವರ್ಷದ ತುಲನಾತ್ಮಕ ಕ್ರಾಸ ಸೆಕ್ಸನಲ್ ಅಧ್ಯಯನ. ಈ ಅಧ್ಯಯನವು ೧೫ ರಿಂದ ೪೫ರ ವಯೋಮಿತಿಯ ಸಂತಾನವಂಚಿತ/ಸಂತಾನಹೀನ ಬಂಜೆ ಮಹಿಳೆಯರನ್ನು ಒಳಗೊಂಡಿರುತ್ತದೆ. ಇದರಲ್ಲಿ ಸಂಬಂದಪಟ್ಟ ಮಹಿಳೆಯರು ತಮ್ಮ ಸ್ವ ಇಚ್ಛಯಿಂದ ಭಾಗವಹಿಸಬಹುದು.

ಅಧ್ಯಯನದ ಉದ್ದೇಶ:

ಸಾಕಷ್ಟು ಮಹಿಳೆಯರಲ್ಲಿ ಜನನೇಂದ್ರಿಯ ಕ್ಷಯರೋಗ ಒಂದು ಮಾರಕ ರೋಗವಾಗಿ ಮಾರ್ಪಟ್ಟು ಇದು ಮಹಿಳೆಯರಲ್ಲಿ ಅಂಗಹೀನತೆ ಮತ್ತು ಮರಣಾವಸ್ಥೆಯನ್ನು ತರುವ ರೋಗವಾಗಿದೆ. ಇದು ಭಾರತ ದೇಶದಲ್ಲಿ ಸಾಮಾನ್ಯವಾಗಿ ಮಹಿಳೆಯರಲ್ಲಿ ಗರ್ಭ ಧರಿಸುವ ೧೫ ರಿಂದ ೪೦ ವರ್ಷ

ವಯಸ್ಸಿನ ಮಹಿಳೆಯರಿಗೆ ಸಾಮಾಜಿಕ ಮತ್ತು ಆರ್ಥಿಕವಾಗಿ ಹೊರೆಯಾಗಿ ಪರಿಣಮಿಸಿದೆ. ಈ ರೋಗದಿಂದ ಸಾಕಷ್ಟು ಮಹಿಳೆಯರು ಸಂತಾನರಹಿತರಾಗಿ ಬಂಜೆಯರಾಗುತ್ತಿದ್ದಾರೆ. ಈ ಮಾರಕ ರೋಗದ ಬಗ್ಗೆ ಸೂಕ್ತ ಮಾಹಿತಿ ಮತ್ತು ನಿರ್ದಿಷ್ಟ ಸಂಶೋಧನೆಯ ಅಭಾವದಿಂದ ಶೇ. ೫ ರಿಂದ ೧೩ ಪ್ರತಿಶತ ಮಹಿಳೆಯರಲ್ಲಿ ಈ ರೋಗವು ಕಂಡು ಬರುತ್ತಿದೆ.

ಆದುದದರಿಂದ ಈ ಜಟಿಲ ಸಮಸ್ಯೆಯನ್ನು ಗಮನಿಸಿ ಮೇಲೆ ತಿಳಿಸಿದ ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯದ ಪರಿಣಿತ ತಂಡ ಮಹಿಳೆಯರಲ್ಲಾಗುವ (ಎಫ್.ಟಿ.ಟಿ.ಪಿ) ರೋಗವನ್ನು ಈ ಆಧುನಿಕ ವಿಧಾನದಿಂದ ಪತ್ತೆ ಹಚ್ಚುವಲ್ಲಿ ಕಾರ್ಯೋನ್ಮುಖವಾಗಿದೆ.

<u>ಅರ್ಹತೆ:</u>

ಈ ಸೂಕ್ತ ಚಿಕಿತ್ಸಗಾಗಿ ೧೫ ರಿಂದ ೪೫ ವಯಸ್ಸಿನ ಸಂತಾನವಂಚಿತ ಎಲ್ಲ ಮಹಿಳೆಯರಿಗೆ ಚಾರಿಟೇಬಲ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಬೆಳಗಾವಿಯ ಸ್ತ್ರೀರೋಗ ವಿಭಾಗದಲ್ಲಿ ಹೊರ ರೋಗಿಯಾಗಿ ಸಂದರ್ಶಿಸಬಹುದು. ಈ ವಿಭಾಗದಲ್ಲಿ ಸಂಭಂದಪಟ್ಟ ಮಹಿಳೆಯರಿಗೆ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ.

ಪರೀಕ್ಷಾರ್ಥಿಗೆ ಲಾಭ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವ ಮಹಿಳೆಗೆ ಈ ರೋಗಕ್ಕೆ ಸಂಬಂಡಪಟ್ಟಂತೆ ಅವರ ಭಾಷೆಯಲ್ಲಿ ತಿಳಿಯುವ ಹಾಗೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲಾಗುತ್ತದೆ. ಭಾಗವಹಿಸುವ ಮಹಿಳೆಯು ತಮಗೆ ತಿಳಿದದ್ದನ್ನು ಯಾವುದೇ ಆತಂತವಿಲ್ಲದೇ ಸೂಕ್ತ ವಿವರಣೆಯೊಂದಿಗೆ ಉತ್ತರ ನೀಡಬೇಕಾಗುತ್ತದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ/ ಅಧ್ಯಯನದಲ್ಲಿ ಅವಸ್ಯಕತಾನುಸಾರ ಆಧುನಿಕ ತಂತ್ರಜ್ಞಾನದ (ಹಿಸ್ಟರೊಸ್ಕೋಪಿ) ಬಳಕೆಯ ಬಗ್ಗೆ ಸಂಪೂರ್ಣ ವಿವರಣೆಯೊಂದಿಗೆ ತಿಳಿಸಲಾಗುತ್ತದೆ.

<u>ಪರೀಕ್ಷಾರ್ಥಿಯಾಗಿ ಭಾಗವಹಿಸುವಿಕೆ:</u>

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಮಹಿಳೆಯು ಪರೀಕ್ಷಾರ್ಥಿಯಾಗಿ ಭಾಗವಹಿಸುವದು ಕೇವಲ ತಾತ್ಕಾಲಿಕವಾಗಿದ್ದು ಇದರಲ್ಲಿ ಭಾಗವಹಿಸುವ ಪ್ರತಿ ಮಹಿಳೆಯು ಸ್ವ-ಇಚ್ಛೆಯಿಂದ ಭಾಗವಹಿಸಬಹುದು. ಇದರಲ್ಲಿ ಯಾವುದೇ ಕಾನೂನು ಕಟ್ಟಳೆಗಳಿರುವದಿಲ್ಲ. ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಸಂಶೋಧನೆಯಿಂದ ಹೊರ ಹೋಗುವ ಸಂಪೂರ್ಣ ಅಧಿಕಾರವಿರುತ್ತದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸುವ ಪ್ರತಿ ಮಹಿಳೆಯು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಮುಂದುವರೆಯುವ ನಿರ್ಗಮಿಸಲು ಸಂಪೂರ್ಣ ಸ್ವತಂತ್ರಳು. ಇದರಲ್ಲಿ ಯಾವುದೇ ಅಡಚಣಿಯಾಗದಂತೆ ಸಂಶೋಧಕರು ಪರೀಕ್ಷರ್ಥಿಗೆ ಸಂಪೂರ್ಣ ಭರವಸಿಯನ್ನು ನೀಡುತ್ತಾರೆ.

ಸಂಭಾವಣೆ:

ಇದೊಂದು ಸಂಶೋಧನಾ ಅಧ್ಯಯನವಾಗುರುವದರಿಂದ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಸಂಶೋಧನಾರ್ಥಿಯಾಗಿ ಬರುವ ಪರೀಕ್ಷಾರ್ಥಿಗೆ ಯಾವುದೇ ತರಹದ ವಾಹನ ಭತ್ಯೆ ಇನ್ನಿತರ ಯಾವುದೇ ಬತ್ಯೆಯನ್ನು ನೀಡಲಾಗುವದಿಲ್ಲವೆಂದು ನನಗೆ ಮನವರಿಕೆ ಮಾಡಿಕೊಡಲಾಗಿದೆ.

ಉಪಯೋಗ:

ಇದು ಒಂದು ಸಂಶೋಧನಾ ಅಧ್ಯಯನಾಗಿದ್ದು ಸಂತಾನವಂಚಿತ/ಸಂತಾನಹೀನ ಬಂಜೆ ಮಹಿಳೆಯರಲಲ್ಲಿ ಜನನೇಂದ್ರಿಯದ ಕ್ಷಯರೋಗದ ಬಗ್ಗೆ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ. ಇದರಿಂದ ಸಾಮಾನ್ಯ ಮಹಿಳೆಯರಿಗೆ ಬಹಳ ಉಪಯುಕ್ತವಾಗಿದೆ.

ಗೌಪ್ಯತೆ:

ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಪಟ್ಟಂತೆ ಪರೀಕ್ಷಾರ್ಥಿಯಾಗಿ ಭಾವವಹಿಸುವ ಪ್ರತಿ ಮಹಿಳೆಯ ದಾಖಲಾತಿಗಳನ್ನು ಮತ್ತು ವಿವರಗಳನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ. ಭಾಗವಹಿಸುವ ಪ್ರತಿ ಮಹಿಳೆಯ ಗುತುತಿಗಾಗಿ ಅವರಿಗೆ ಸಂಬಂಧಪಟ್ಟಂತೆ ನಿಗದಿತ ಪತ್ರ ವ್ಯವಹಾರಕ್ಕಾಗಿ ಒಂದು ಸಂಖ್ಯೆಯನ್ನು (ಕೋಡ ನೆಂ.) ನೀಡಲಾಗುತ್ತದೆ. ಇದರ ಮುಖಾಂತರವೇ ಅವರನ್ನು ಗುರುತಿಸಲಾಗುತ್ತದೆ. ಈ ಪತ್ರ ವ್ಯವಹಾರವು ಕೇವಲ ಸಂಶೋಧನೆ ವಿಷಯವಾಗಿಯೇ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಯಾವುದೇ ಸಂಧರ್ಭದಲ್ಲಿ ಪರೀಕ್ಷಾರ್ಥೀಯ ಹೆಸರು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಬಳಸಲಾಗುವದಿಲ್ಲ. ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಭಂದಪಟ್ಟಂತೆ ಅದ್ಯಯನದ ಸಂಪೂರ್ಣ ಮಾಹಿತಿಯನ್ನು ವೈದ್ಯಕೀಯ ವಿಷಯವಾಗಿ ವೈದ್ಯಕೀಯ ಪತ್ರಿಕೆಯಲ್ಲಿ ಪ್ರಕಟಿಸಲಾಗುವದೆಂದು ಪರಿಕ್ಷಾರ್ಥಿಗೆ ಸಂಪೂರ್ಣ ವಿವರಣೆಯೊಂದಿಗೆ ಅವರಿಗೆ ತಿಳಿಯುವ ಭಾಷೆಯಲ್ಲಿ ತಿಳಿಸಲಾಗುತ್ತದೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವ ಪ್ರತಿ ಮಹಿಳೆಯ ಎಲ್ಲ ಸಂಶೋಧನೆಗೆ ಸಂಬಂದಪಟ್ಟಂತೆ ಎಲ್ಲ ವಿಷಯಗಳನ್ನು ಗೌಪ್ಯವಾಗಿಡುವ ಜವಾಬ್ದಾರಿ ಸಂಶೋದನಾದಿಕಾರಿಯದಾಗಿರುತ್ತದೆ. ಅದರ ಸಂಪೂರ್ಣ ಭರವಸೆ ಸಂಶೋಧನಾಧಿಕಾರಿ ನೀಡುತ್ತಾರೆ.

<u>ಸಮ್ಮತಿ ಪತ್ರ</u>

ಈ ಕೆಳಗೆ ಸಹಿ ಮಾಡಿರುವ ನಾನು ಶ್ರೀಮತಿ: ________________ __ ನನ್ನ ಸ್ವ ಇಚ್ಛೆಯಿಂದ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುತ್ತಿದ್ದೇನೆ. ಈ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಪ್ರಧಾನ ಸಂಶೋಧಕರು ಹಾಗು ಅಧ್ಯಯನದ ಪ್ರತಿನಿಧಿ ನನಗೆ ತಿಳಿಯುವ ಭಾಷೆಯಲ್ಲಿ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡಿದ್ದಾರೆ. ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂದಪಟ್ಟಂತೆ ತಪಾಸಣೆಗಳ ಉಪಯೋಗ ಮತ್ತು ಅಡಚಣೆ ಕುರಿತು ನನ್ನ ಆಡು ಭಾಷೆಯಲ್ಲಿ ತಿಳಿಸಿದ್ದಾರೆ ಹಾಗು ತಿಳಿದುಕೊಂಡಿದ್ದೇನೆ. ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಯಾವುದೇ ಸಕಾರಣ ನೀಡದೇ ಈ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವತಂತ್ರಳು ಮತ್ತು ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂದಪಟ್ಟಂತೆ ನನಗೆ ಸಾಕಷ್ಟು ಸಮಯ ನೀಡಿ ನನ್ನ ಎಲ್ಲ ಸಂಶಯಗಳನ್ನು ಕುಲಂಕುಶವಾಗಿ ಚರ್ಚಿಸಿ ಸೂಕ್ತ ಪರಿಹಾರ ಕಂಡುಕೊಂಡಿದ್ದೇನೆ.

ಭಾಗವಹಿಸುವ ಪರೀಕ್ಷಾರ್ಥಿಯ ಸಹಿ & ಹೆಸರು:

ಸಾಕ್ಷಿದಾರರ ಸಹಿ ಮತ್ತು ಹೆಸರು.

೧. ಮಹಿಳೆಯ ಸಹಿ / ಎಡಗೈ ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು   ಹೆಸರು & ವಿಳಾಸ: ದಿನಾಂಕ:

೨. ಪಾಲಕರ/ಸಂಬಂಧಿಕರ ಎಡಗೈ ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು   ಹೆಸರು & ವಿಳಾಸ: ದಿನಾಂಕ:

೩. ವೈದ್ಯಾಧಿಕಾರಿಗಳ ಹೆಸರು:   ಸಹಿ:   ದಿನಾಂಕ:

संशोधन प्रक्रिय में भाग लेनेका सहमती पत्र

शिर्षीके :

संतानवंचित संतानहिन बंज महीलावोमें होनेवाले जननेंद्रीय क्षयरोग संबधीत जेल निलसन स्टेनींग, प्लोरोसेंट स्टेनिंग और कल्चरमें पालीमरस चौन रियाक्षन स्टेनींग (पीसीआर) हे विधानमें चलानीवाली थरशरी केर हॉस्पीटल में एकवर्षीय तुलनात्मक अद्ययन.

Study Investigator :

Post Graduate Student,
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum - 590 010.

Guide :

Dr.
Professor
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum - 590 010.

पुर्व पीठीका :

हे एक संशोधन अध्यन बोलनेवाले संतानहिन संतानवंचित बंज महीलांओमें होनेवाले जननेद्रीय क्षयरोग अंश जील - नीलसन स्टेनिंग प्लोरोसेंट स्टेनींग और कल्चरमें पालीमरस चौन रियाक्षन स्टेनींग (पीसीआर) हे विधानमें चलानीवाली थरशरी केर हॉस्पीटल में एकवर्षीय तुलनात्मक अद्ययन. हे अध्यन 15 ते 40 वर्ष वयोमान का संतानहिन बंज महीलायोको अध्यन में स्व इच्छेने सहभाग लेणेके लिए सम्मती पत्र.

आपको इस अध्यन में स्वईच्छेने भाग लेनेके लिए मुक्त स्वागत है.

उद्येश :

बहुतसे बहुत महिला में जननेद्रीयाचा क्षयरोग प्रमुख रोग से पिडीत (एफजीटीबी) हो रहे है. इस रोगसे बहुत महीला अंगहिन अकाल मृत्यु होरहे है. हे रोग पुरा भारत देश में सामान्य महीलाहोमें सामाजी और आर्थीक संकष्ट में पढरहे. हे रोग 15 ते 45 वर्षीय महीलोमें मारक रोग बनके प्रतीशत 5 -16 % महीला अधुनिक तंत्रझान और सुक्त संशोधना सुक्त चिकीत्सा अभावके कारण हे रोग मारक बनके जा रहे है.

इस दिश्यामें महीलाहोमें होनीवाले ए रोग सुक्त समय सुक्त संशोधन और अधुनिक तंत्रझान का आवश्यकता जरुरी आहे. इस अध्यनमें भाग लेनेवाले महीलाओको सहकारी है.

जानकारी:

15 ते 45 वर्षीय संतानहिन बंज महीला थरशरी केर हॉस्पीटल व संशोधना केंद्र बेळगांव, स्त्रिरोग विभागमें बाह्य रोगी विभाग में संपुर्ण माहीती दिया जायेगा.

.....2

.....2....

<u>लाभ :</u>

हे अध्यन में भाग लेनेवाले प्रती अभ्थींयों ए रोग संबधीत कुच प्रश्न पुचता जायेगा. आप इस संबधीत सुक्त प्रश्न आपना भाषामें पुचताच करेंगे. आप आपनी भाषामें उत्तर देणा होगा. इस अध्यन में हिस्ट्रोस्कोपी विनुतन तंत्रज्ञान उपयोगके संबधीत जानकारी दिया जायेगा.

<u>अपाय / दोका :</u>

इस अध्यन में भाग व तपासने में कोईबी शारीक और मानसीक संबधी कोई त्रासदायक नही होनेका बारे में समजाया और समजलिया है.

<u>भाग लेने का स्वेच्छिक स्वरुप</u>

आप इस अध्यन में आपने स्व इच्छेसे भाग ले रहे है. इस अध्यनसे भाग लेने से इंकार करणे के लिए पूरी तरहसे आप स्वतंत्र है. इस अध्यनसे सहमत होते अथवा ने होणेसे कोंही कारवाई / जुरमाना नाही होगा. इस भारे में मेरे भाषा में समजाया और समजलिया है.

<u>उपयोग :</u>

इस अध्ययन / तपासन में भाग लेने केवल तातपुर्तीक है. इस संबधीत कोही प्रकार के नियम वगैरे नाही. कोणत्याही परीस्थीत कोणत्याही कारण न पुचता इस अध्यन और तपासने में बाहेर हाटाने में मै संपुर्ण स्वतंत्र है. मेरे को समजाया है. इस तपासने संबधीत कोई विचार सर्व विचार गुप्त रका जायेगा. और इस तपासने संबधीत सर्व विचार अध्यन संबधीत वापरथा जायेगा , इस के बारे में मेरे को समजाया है.

<u>वेतन :</u> इस अध्ययन संबधीत अध्ययन में भाग लेनेवाले प्रती अभ्यर्थी को कोई तरहका आर्थीक सहायता नाही दिया जायेगा . इस जानकारी मै समजलिया हु.

<u>गौपनीयता</u>

इस अध्ययन में आपके भाग लिए जाने के रिकाई गुप्त रखें जाएमें। परन्तु गुप्त कामजात जो आपकी पहचान आपके नाम द्धारा करते है अध्ययन अधिकारी को, जोंच के समय कौरपोरेट गुणवता जॉचकर्ती को तथा इन्सटीट्यूशनल रिवयु बोर्ड (आई आर बी) को और अन्य नियत्रंण संस्थाओ को जरुरत पडने पर उपलब्ध होगें। अगर यह जानकारी किसी भी प्रकार से प्रकाशित होती है तो उसमें आपका नाम नही होगा।

.....3....

सम्मती पत्र

मै ए सहमती देती हूं श्रीमती ....
वय .... इस अध्यन में भाग लेना मेरी आपनी इच्छानुसार है. यह अध्ययन एक अनुसंधान प्ररियोजना है तथा इस अध्ययन से मुझे कोई चिकित्सा लेना होगा. अध्ययन अवधि के दौरान उठने वाले प्रश्नो का उत्तर लेना मेरा अधिकार है. मुझे यह बता दिया गया है कि यदि अध्ययनके दौरान किसी नई महत्नवपूर्ण जानकारी का ज्ञान होता है. तो मुझे उससे अवगत कराया जाएगा. में किसी भी समय अध्ययन से अपना नाम वापस ले सकता हू , भविष्य में मिलने वाली चिकित्सा सुविधा व भावी अध्ययनो के लिए होने वाले चयन का इससे कोई सरोकार नाही है. मुझे अपने स्वास्थ्य की रक्षा अथवा अध्ययन के करार उल्लघंन करने पर किसी भी समय अध्ययन से हटाया जा सकता है. मैने सुचित सहमति पत्र को पढ और समज लिया है तथा अध्ययन आदिलेख का पालन करने मे मुझे कोई परेशानी नही है. मैने सहमती पत्र को अध्ययनपुर्वक पढ लिया है तथा अध्ययन , संभावित दुष्प्रभाव अथवा सहमति पत्र के बारे मे सभी प्रश्नो का मुझे संतोषप्रद उत्तर मिल गया है . मुझे इस बात की जानकारी है की अध्ययन प्रक्रियाओं की अवश्कताओं के अनुसार मेरे जैविक नमुनो को नाम रहित किया जायेगा अथवा नष्ट किया जायेगा .

1. महीला का सही /धावेबोटका निशान | नाम और पत्ता | ता :

2साक्षीदार का सही /धावेबोटका निशान | नाम और पत्ता | ता :

3. वैद्याधीकारी नाम | सही | ता.

संशोधन प्रक्रिय मध्ये भाग धंनेचा सम्मती पत्र

मूल न झालेला संतानहिन बांज महिलांचे जननेंद्रीयातील क्षयरोग ग्रस्त अंश संबधीत जील - नीलसन प्लोरोसेंट स्टेनींग व पॉलीमरस चैन रियाक्सन हा विधान वापरुन चलनारे थरशरी केर हॉस्पीटल बेळगांवी मध्ये एक वर्षाचा क्रॉस विभागाचा तुलनात्मक अध्ययन .

Study Investigator : Dr.
Post Graduate Student,
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum - 590 010.

Guide : Dr.
Professor
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belgaum - 590 010.

हे एक संशोधन अध्यन म्हणुन बोलनारे संतानहिन मुल न झालेले महीलांचा जननेंद्रीयातील क्षयरोगाचा अंशाचा जील - नीलसन आणी प्लुरोसेंट स्टेनींग पद्दत वापरुन चलनारे तुलनात्म अध्यन थरशरी केर हॉस्पीटल बेळगांव येथे चालनारे एक वर्षाचा क्रॉस अध्यन. हे अध्यन 15 ते 40 वर्ष वयाचा संतानहिन बांज महीलासाठी अध्यन मध्ये स्व इच्छेने भाग घेण्याचा सम्मती पत्र.

फारचा महीला मध्ये जननेद्रीयाचा क्षयरोग प्रमुख रोग होऊ लागले. हे महिलाना अंगहिन आणी अल्पवहीन मृत्यु अणणारे रोग आहे. हे पुरा भारत देशामध्ये सामान्य गर्भवती होत असलेल्या 15 ते 45 वयाचा महीलाना सामाजीक व आर्थीक खुब लोड होऊ लागले. ह्या रोग संबंध खुब महीला संतानहीन व बांज होऊ लागले आहेत. हे मारक रोगचा सुक्त माहीती व सुक्त संशाधनाचा अभावामुळे प्रतीशत 5% ते 16% महीला मध्ये हा रोग दीसुन येण्यात आलेले आहे. हा रोगाचा प्रभावाने महीला मध्ये संतानही व बांज होण्याचे शकत आहे.

यांची सुक्त पारंपरीक पद्दत आणी आधुनीक तंत्रझान वापरुन करणारे हे अध्यन महीला मध्ये (FBTB) रोगाचा बद्दल पत्ता लावण्याचा अत्यंत सहकारी होतो.

पात्र :

सगळे सुक्त चिकीत्सा घेतलेले 15 ते 45 वयाचा संतानहिन बांज महीलाना थरशरी केर हॉस्पीटल बेळगांवी येथे संशोधना केंद्राचा स्त्रिरोग विभागाचा बाह्य रोगी विभाग मध्ये संपुर्ण माहीती देता येईल.

...2

.....2....

लाभ :

हे अध्यन मध्ये भाग घेतलेल्या अभर्थीला (मी) ह्या रोग संबधीत कांही प्रश्न विचारीता येईल. आपला भाषा मध्ये आपल्याला संमजोसारखे उत्तर द्यावा लागेल. ह्या अध्यन मध्ये हिस्ट्रोस्कोपी म्हणुन विनुतन तंत्रज्ञान वापर करायची मला संपुर्ण माहीती आहे.

अपाय व दोका :

ह्या अध्यन मध्ये भाग व तपासनेमुळे मला कांही त्रास व दोका नाही म्हणुन मला समजले आहे.

परिक्षार्थी म्हणून भाग घेणे :

ह्या अभ्यासात / तपासनेमध्ये भाग घेणे फक्त तातपुर्तीक आहे. या संबधीत कांही प्रकारचे नियम वगैरे नाही. कोणत्याही परीस्थीत कोणत्याही कारण न सांगता हे अध्यन व तपासने मधुन बाहेर येयाला मी संपुर्ण स्वतंत्र आहे. म्हणुन मला समजुन आलेले आहे. ह्या तपासने संबधीत कांही विचार सगळे विचार गुप्त ठेवणात येईल. आणी या ह्या तपासने संबधीत सगळे विचार अध्यनीचा संबधीत वापरला येईल म्हणुन मला समजला आहे.

वेतन : ह्या तपासने संबधीत मला कोणत्याही वेतन बत्त पैसा न दिला जाईल म्हणुन मला समजला आहे.

गौप्यता :

ह्या अध्यन व तपासने संबधीत कांही सगळे विषय व विचार संशोधन करणारे व्यवक्ती /अधीकारी गुप्त ठेवला जाईल. आणी या संबधीत एक कोड नंबर दिला जाईल. या कोड नंबर वर सगळ विचार विनीमय माहीती वळकला जाईल. या संबधीत दाखला विशयक आणी वैज्ञानीक विषयासाठी प्रकटनाला शक्यता असतो. या प्रकटनामध्ये अभ्यर्थीचा नांव प्रकटन न करायची संपुर्ण जबाबदारी अधिकारी वर्ग घेईल.

....3....

सम्मती पत्र

या खालील सही केलेले मी ....
वय .... मी माझे स्व इच्छेने या अध्यन व तपासने मध्ये भाग होत आहे. या तपासने संबधीत मला माझा भाषा मध्ये संपुर्ण विवरण करुन माहीती दिले आहे. या अभ्यास व तपासने संबधीत उपयोग व अडचणी मधुन कोणत्याही परीस्थीत बाहेर येयायचा व अध्यन सोडायचे संपुर्ण स्वतंत्र आहे. तीतुन मला संपुर्ण बरोसा आहे. या अध्यन व तपासने संबधीत मला विचार व संशय बदल अतिशय वेळ देऊन माझा भाषा मध्ये मुक्त चर्चा करुन मी परीहार घेतली आहे.

1. महीळाचा सही /द्यावेबोटाचा निशान नांव व पत्ता ता :

2 साक्षीदार सही /द्यावेबोटाचा निशान नांव व पत्ता ता :

3. वैद्याधीकारी नाव सही ता.

DATA COLLECTION INSTRUMENT

Title: Comparative evaluation of Ziehl-Neelsen staining, Fluorescent staining and Culture with Polymerase Chain Reaction in the diagnonsis of Genital Tuberculosis in infertile women- One year cross sectional study at Tertiary Care Hospital,Belagavi.

Screening ID No:

ID No:

OPD No:

IPD No:

Unit:

Date:

D	D	M	M	Y	Y

Patient's Name :
F: ______ M: ______ S: ______

Age: ______ Years

Address H.No : ______ Street: ______ Place: ______

Taluka: ______ District: ______

Tel No: ______ Mob No: ______

I) SCREENING:

1) Is the patient clinically diagnosed as infertile

a) Yes b) No ☐

2) If yes to Q1, tick the following

a) Primary Infertility ☐

b) Secondary Infertility ☐

3) Is the patient suffering from pulmonary tuberculosis

a) Yes b) No ☐

4) If yes to Q3, then is she on treatment

a) Yes b) No ☐

5) Is the patient having past h/o pulmonary tuberculosis

a) Yes b) No ☐

6) If yes to Q5, has she taken treatment before

a) Yes b) No ☐

7) Any family member suffering from pulmonary tuberculosis

a) Yes b) No ☐

8) If yes to Q7, then treatment taken

a) Yes b) No ☐

II) Informed consent

9) Is the woman willing to participate in the study

a) Yes   b) No   ☐

10) Is the woman 18 years of age

a) Yes   b) No   ☐

11) If yes to Q10, has she signed the informed consent form

a) Yes   b) No   ☐

12) If the woman < 18 years of age has her parent / guardian signed the informed consent form

a) Yes   b) No   ☐

III) Socio - demographic information
Fill in the box approriate option

13) Education   ☐

a) Illiterat
b) Read
c) Write
d) Primary
e)Secondary
f) Graduate
g) Post Graduate

14) Occupation   ☐

a) Housewife
b) Labourer

c) Professional

15) What type of socio economic card she has ? ☐
a) White
b) Green
c) Yellow
d) PAN (Income Tax)

16) Place of residence ☐
a) Urban
b) rural

17) Area ☐
a) Over crowded
b) Not crowded

IV) OBS history

18) Since how long the woman is married (Married Life) ☐ Years

19) Are her menstrual cycles regular ☐
a) Yes
b) No
c) Don't know

20) In case of secondary infertility any h/o pregnancy ☐
a) Yes
b) No

21) If yes to Q20, what was the outcome ☐
a) Live birth
b) Still birth
c) Abortion

V) Examination

22)
Height Cms

23)
Weight Kgs

24) BMI

25) Is CVS normal
a) Yes
b) No

26) Is RS normal
a) Yes
b) No

VI) Investigation

27) HB % gm/dl

28) TC cell /ml

29) DC cell /ml

30) ESR mm at 1st hours

31) HIV I and II Screening

a)
Positive b) Negativ

32) Chest x-ray findings-suggestive of pul. TB
Cavitatory Lesion present
a) Yes b) No

33) USG findings suggestive of GTB
a)Present b)Absent

34) If ans to Q33, present then specify
a) Ascites
b) Thickened peritoneum
c) Endometrial involvement
d) Adnexal mass
e)Adhesions
f) Loculated fluid

35) HSG findings suggestive of GTB
a) Present b)Absent

36) If ans to Q35, present then specify
a) Tubal occlusion
b) Beaded appearance of tubes
c) Rigid pipe appearance
d) Tubal dilatation
e) Peritubal adhesions
f) Endometrial TB
g) Others

37) Laparoscopy findings suggestive of GTB
a) Present b)Absent

38) If ans to Q37, present then specify
a) Tubal block with hydrosalphinx

b) Tubo ovarian mass
c) TB salphingitis
d) Beaded tubes
e) Tubal adhesion
f) Tubercles on uteres
g) Others

39) After examination is she eligible

a) Yes b) No ☐

40) If no to Q39, specify ____________________

ANNEXURE - IV– H37 Rv LETTER

GOVERNMENT OF KARNATAKA

To,
Dr. Jyoti M. Nagamoti,
Professor and Head of the Department,
Department of Microbiology,
J.N.M.C,
KLE University,
Belagavi- 590001.

Bangalore
16th Sep 2015

(Through proper channel)

Sub: Issue of MTb H37Rv strain as per the letter No. 2154, dated 31/08/2015

Madam,

As per the letter No. 2154, dated 31/08/2015, the above mentioned MTb H37Rv strain is issued to the Department of Microbiology, J.N.M.C, Belagavi for academic purpose.

Dr. Anil S,
Director,
State TB and Demonstration Centre,
Intermediate Reference Laboratory,
Bengaluru-29.

STDC Director
State TB Training and Demonstration Centre
SDS TB & RGICD Campus
Hosur Road, Bangalore-560 029

ANNEXURE V – STAIN AND MEDIA

1.ZN stain preparation [63]

Safety:Some of the reagents are highly corrosive.In preparing them and in carrying out the staining procedures it is advisable to wear spectacles or goggles,disposable gloves and a waterproof apron.

A)Carbol Fuchsin:

i)Basic fuchsin(powder)=5g

ii)Phenol (crystalline)=25 g

iii)Alcohol(95% or 100%Ethanol)=50ml

iv)D/w=500ml

- Dissolve the Fuchsin in the phenol by placing them in one litre flask over a boiling water-bath for about 5min.
- Shake the contents from time to time
- When solution is complete,add the alcohol and mix thoroughly
- Add d/w
- Filter the mixture through a Whatman No.1 paper into the inuse bottle.

B) Sulphuric acid(20%) decoloriser

i)Concentrated Sulphuric acid (98%,1.835g/ml)=100ml

ii)D/W =400ml

- Pour he water into a large flask
- Place the flask in 5-8cm of cold water in the sink
- Add the acid in 500ml lots over 10 min,pouring slowly down the side of the flask into the water.
- Mix gently
- The mixture will become hot.
- Allow it to cool and decant into a labelled bottle for use.

C)Loeffler' s Methylene blue:

i)Saturated solution of methylene blue in alcohol=150ml

ii) KOH,0.01% in water=500ml

- Dissolve the solution of methylene blue in KOH,0.01% in water and filter it through Whatman no.1 filter paper.

ANNEXURE

2.Fluorochrome stain preparation[63]

A)Primary stain

i. Auramine O =1.5g

ii. Ethanol 95%=10ml

iii.Phenol crystals=3g

iv.d/w=87ml

- Solution 1:Dissolve 1.5g of Auramine O in 10ml of 95% Ethanol.
- Solution 2:Dissolve 3g of phenol crystals in 87 ml of d/w

- Working solution:combine solution 1 and 2 .label it.
- Shelf life=3 months.Filter and store in dark,stoppered bottle.

B) Acid alcohol (0.5%)

i. Concentrated hydrochloric acid=0.5ml

ii.70%ethanol= 95.5ml

- Add concentrated hydrochloric acid to ethanol.
- Shelf life=3 months at room temperature.

C) Potassium permanganate (0.5%)

- Dissolve 0.5g of potassium permanganate in 100ml of distilled water.

ANNEXURE

3.LJ medium preparation [63]

1.LJ base from Hi-media:Dissolve 37.24 gm of LJ base in 600ml of DW.Sterilize it by autoclaving.

2. Egg homogenate= 12 eggs.

A) Day-1 preparation:

i.Conical flask

- Take one 500ml conical flask.
- Put at least 15-20 non drilled glass beads of about 4mm diameter.
- Plug it with cotton.cover it with paper.
- Sterilise in hot air oven.

ii.Measuring cylinder

- Take 1L measuring cylinder.
- Cover its mouth with atleast 4-5 layers of gauze cloth
- Tie it loosely about 1cms from the mouth.
- Cover it with paper and sterilise it in hot air oven.

iii.McCartney bottles

- Take McCartney bottles 20ml dried, sterilize them in hot air oven.

(McCartney bottles autoclavable from Hi Media)

B) Day-2 preparation:

Preparation of medium.

- Take cotton and spirit.
- Wipe the surfaces of all eggs with spirit swab.wipe the knife or spatula with spirit.
- Light the spirit lamp.
- Take the conical flask with glass beads.
- Open the paper.
- Flame the mouth.
- Gently break egg one by one with spatula into the conical flask.
- After breaking each egg rotate the conical flask horizontally on the surface so that you get a good homogenate.
- Once all the eggs are broken and homogenate is ready.
- Now open the paper cover of measuring cylinder.
- Slowly pour the homogenate into the gauze tied over the mouth of the measuring cylinder.gauze acts likeseive.all well formed homogenate is filtered into measuring cylinder.(do not tie too many layers or too tightly)
- Now once all homogenate is added,measure the volume of homogenate in measuring cylinder.
- For 1000ml of egg homogenate add 600ml of autoclaved LJ base into the same measuring cylinder.
- Check the colour.Many a times we need to add little about 5-6 ml of malachite green as green colour now has to be something like TCBS.The colour fades after inspissation (always mix gently as because of egg you tend to get bubbles)

- Now fill the McCartney bottles upto 8-10ml.take care that you donot create lot of bubbles.
- Put the bottles in inspissator where water is already added into water tank.
- Arrange them at about 30 degree angle to get good slant.
- You can keep them in water bath also,but take care that water does not enter into the bottle.
- 82 degree C for one hour.switch off.let the bottles in inspissator until temp comes down to room temp,check for the slants.
- Keep one slant in incubator for sterility checking for 24 hours.

MTB GROWTH RECORDING CHART (L-J MEDIUM)

Sl. No.	Sample code	3rd day date	I week date	II Week date	III Week date	IV Week date	V Week date	VI Week date	VII Week date	VIII Week date	Pigmentation	Morphology atypical/typical	Remarks
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

KLE University's

Jawaharlal Nehru Medical College, Belgaum-10

Department of Microbiology

Lab No.: Name : OPD/IPD No. :

Age/Sex: Ward:

Ref. By:

Microbiology Report

1.Ziehl-Neelsen staining : The studied smear is .... for Acid –Fast bacilli.

2.Fluorescent staining : The studied smear is .... for Acid –Fast bacilli.

3.Culture on L-J media : .... seen at the end of 8 weeks of incubation.

Date: Section (I/C) Bacteriologist

KLE University's

Jawaharlal Nehru Medical College, Belgaum-10

Department of Microbiology

Lab No.: Name : OPD/IPD No. :

Age/Sex: Ward:

Ref. By:

MTB/NTM DNA Qualitative PCR

Specimen : Endometrial Tissue

Methodology : Conventional PCR

Primer used : Inbuilt primer from Qiagen PCR kit targeting IS*6110* gene.

Report :

Interpretation

DETECTED: MTB/NTM DNA is present in the sample tested.

NOT DETECTED: Absence of MTB/NTM DNA in the sample tested

Date: **Molecular Biology section** **Microbiologist**

(I /C)

KEY TO MASTER CHART

NOGC = No organism grown in culture

D = Detected

ND = Not Detected

ZN Stain = Ziehl-Neelsen Stain

PCR = Polymerase chain reaction

B = Endometrial biopsy

Serial no.	Age(yrs)	Diagnosis	Sample	ZN Stain	Fluorescent Stain	Culture	PCR
1	22	primary infertility	EB	Negative	Negative	NOGC	ND
2	35	primary infertility	EB	Negative	Negative	NOGC	ND
3	30	primary infertility	EB	Negative	Negative	NOGC	ND
4	34	primary infertility	EB	Negative	Negative	NOGC	ND
5	36	primary infertility	EB	Negative	Negative	NOGC	ND
6	33	secondary infertility	EB	Negative	Negative	NOGC	ND
7	28	primary infertility	EB	Negative	Negative	NOGC	ND
8	29	primary infertility	EB	Negative	Negative	NOGC	ND
9	28	primary infertility	EB	Negative	Negative	NOGC	ND
10	23	primary infertility	EB	Negative	Negative	NOGC	ND
11	24	primary infertility	EB	Negative	Negative	NOGC	ND
12	32	primary infertility	EB	Negative	Negative	NOGC	ND
13	25	primary infertility	EB	Negative	Negative	NOGC	ND
14	33	primary infertility	EB	Negative	Negative	NOGC	ND
15	30	primary infertility	EB	Negative	Negative	NOGC	ND
16	36	primary infertility	EB	Negative	Negative	NOGC	D
17	28	primary infertility	EB	Negative	Negative	NOGC	ND
18	26	primary infertility	EB	Negative	Negative	NOGC	ND
19	22	primary infertility	EB	Negative	Negative	NOGC	ND
20	31	primary infertility	EB	Negative	Negative	NOGC	ND
21	22	primary infertility	EB	Negative	Negative	NOGC	ND
22	28	primary infertility	EB	Negative	Negative	NOGC	ND
23	28	primary infertility	EB	Negative	Negative	NOGC	D
24	29	secondary infertility	EB	Negative	Negative	NOGC	ND
25	31	secondary infertility	EB	Negative	Negative	NOGC	ND
26	26	secondary infertility	EB	Negative	Negative	NOGC	ND
27	41	secondary infertility	EB	Negative	Negative	NOGC	ND
28	35	primary infertility	EB	Negative	Negative	NOGC	ND

29	26	primary infertility	EB	Negative	Negative	NOGC	ND
30	38	primary infertility	EB	Negative	Negative	NOGC	ND
31	28	primary infertility	EB	Negative	Negative	NOGC	D
32	34	primary infertility	EB	Negative	Negative	NOGC	ND
33	29	primary infertility	EB	Negative	Negative	NOGC	ND
34	26	primary infertility	EB	Negative	Negative	NOGC	ND
35	30	primary infertility	EB	Negative	Negative	NOGC	ND
36	35	primary infertility	EB	Negative	Negative	NOGC	ND
37	28	primary infertility	EB	Negative	Negative	NOGC	ND
38	36	primary infertility	EB	Negative	Negative	NOGC	D
39	34	primary infertility	EB	Negative	Negative	NOGC	ND
40	30	primary infertility	EB	Negative	Negative	NOGC	ND
41	32	primary infertility	EB	Negative	Negative	NOGC	ND
42	28	primary infertility	EB	Negative	Negative	NOGC	ND
43	28	primary infertility	EB	Negative	Negative	NOGC	ND
44	31	primary infertility	EB	Negative	Negative	NOGC	ND
45	33	primary infertility	EB	Negative	Negative	NOGC	ND
46	32	primary infertility	EB	Negative	Negative	NOGC	ND
47	36	primary infertility	EB	Negative	Negative	NOGC	ND
48	32	primary infertility	EB	Negative	Negative	NOGC	ND
49	32	primary infertility	EB	Negative	Negative	NOGC	ND
50	31	primary infertility	EB	Negative	Negative	NOGC	ND
51	27	primary infertility	EB	Negative	Negative	NOGC	ND
52	26	primary infertility	EB	Negative	Negative	NOGC	ND
53	33	primary infertility	EB	Negative	Negative	NOGC	ND
54	32	primary infertility	EB	Negative	Negative	NOGC	ND
55	29	primary infertility	EB	Negative	Negative	NOGC	ND
56	35	primary infertility	EB	Negative	Negative	NOGC	ND
57	38	primary infertility	EB	Negative	Negative	NOGC	ND
58	24	primary infertility	EB	Negative	Negative	NOGC	ND

59	25	primary infertility	EB	Negative	Negative	NOGC	ND
60	26	primary infertility	EB	Negative	Negative	NOGC	ND
61	28	primary infertility	EB	Negative	Negative	NOGC	ND
62	37	primary infertility	EB	Negative	Negative	NOGC	ND
63	36	primary infertility	EB	Negative	Negative	NOGC	ND
64	31	primary infertility	EB	Negative	Negative	NOGC	ND
65	22	primary infertility	EB	Negative	Negative	NOGC	ND
66	30	primary infertility	EB	Negative	Negative	NOGC	ND
67	22	primary infertility	EB	Negative	Negative	NOGC	ND
68	28	primary infertility	EB	Negative	Negative	NOGC	ND
69	35	primary infertility	EB	Negative	Negative	NOGC	ND
70	29	primary infertility	EB	Negative	Negative	NOGC	ND
71	26	secondary infertility	EB	Negative	Negative	NOGC	ND
72	24	primary infertility	EB	Negative	Negative	NOGC	ND
73	37	primary infertility	EB	Negative	Negative	NOGC	ND
74	29	primary infertility	EB	Negative	Negative	NOGC	ND
75	29	primary infertility	EB	Negative	Negative	NOGC	ND
76	41	primary infertility	EB	Negative	Negative	NOGC	ND
77	35	primary infertility	EB	Negative	Negative	NOGC	ND
78	38	primary infertility	EB	Negative	Negative	NOGC	ND
79	28	primary infertility	EB	Negative	Negative	NOGC	ND
80	34	primary infertility	EB	Negative	Negative	NOGC	ND