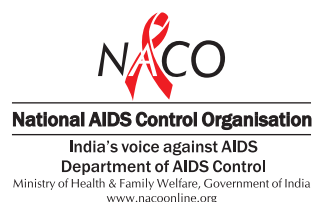


# Training of Laboratory Technicians to Deliver STI/RTI Services

## Participant's Handout





TRAINING OF  
LABORATORY TECHNICIANS  
TO DELIVER STI/RTI SERVICES

**PARTICIPANT'S  
HANDOUT**



MAY 2011





सत्यमेव जयते

**Sayan Chatterjee**

*Secretary & Director General*



*Department of AIDS Control, NACO, Ministry of Health and Family Welfare, Government of India*

## MESSAGE

The prevention, control and management of STI/RTI is a well recognized cost effective strategy for controlling the spread of HIV/AIDS in the country as well as to reduce reproductive morbidity among sexually active population. Individuals with STI/RTI have a significantly higher chance of acquiring and transmitting HIV. Moreover STI/RTI are also known to cause use infertility and reproductive morbidity. Controlling STI/RTI helps decrease HIV infection rates and provides a window of opportunity for counselling about HIV prevention and reproductive health.

An operational framework for convergence between National AIDS Control Programme Phase III and Reproductive and Child health Programme Phase II under National Rural Health Mission has been developed. This will bring about uniformity in implementation of STI/RTI prevention and control through the public health care delivery system. Through this, the availability and reach of standardized STI/RTI care at all levels of health facilities will be ensured.

The NACP III Strategy and Implementation Plan (2007-2012) makes a strong reference to expanding access to a package of STI management services both in the general population as well as for high risk behavior groups.

For nation-wide training of health functionaries on STI/RTI management standardized training modules and training aids/job-aids for various functionaries involved in provision of STI/RTI care have been developed to train doctors ANMs/Nurses, and to technicians on Syndromic Case Management of STI/RTI.

I am sure that these comprehensive operational guidelines will help towards ensuring the provision of quality STI/RTI services across the country.

(Sayan Chatterjee)

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अपनी एचआईवी अवस्था जानें, निकटतम सरकारी अस्पताल में मुफ्त सलाह व जाँच पाएँ  
Know Your HIV status, go to the nearest Government Hospital for free Voluntary Counselling and Testing





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## PREFACE

Sexually transmitted infections and reproductive tract infections (STIs/RTIs) are important public health problems in India. Studies suggest that 6% of the adult population in India is infected with one or more STIs/RTIs. Individuals with STIs/RTIs have a significantly higher chance of acquiring and transmitting HIV. Moreover, STIs/RTIs are also known to cause infertility and reproductive morbidity. Controlling STI/RTIs helps decrease HIV infection rates and provides a window of opportunity for counseling about HIV prevention and reproductive health.

The implementation framework of National Rural Health Mission (NRHM) provided the directions for synergizing the strategies for prevention, control and management for STI/RTI services under Phase II of Reproductive and Child Health Programme (RCH II) and Phase III of National AIDS Control Programme (NACP III). While the RCH programme advocates a strong reference "to include STI/RTI and HIV/AIDS preventions, screening and management in maternal and child health services", the NACP includes services for management of STIs as a major programme strategy for prevention of HIV.

These modules are intended as a resource document for the programme managers and service providers in RCH II and NACP III and would enable the RCH service providers and NACO service provider in organizing effective case management services for STI/RTI through the public health care system.

(P.K. Pradhan)







सत्यमेव जयते

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## FOREWORD

Community based surveys have shown that about 6% of adult Indian population suffers from sexually transmitted infections and reproductive tract infections. The prevalence of these infections is considerably higher among high risk groups ranging from 20-30%. Considering that the HIV epidemic in India is still largely concentrated in the core groups, prevention and control of sexually transmitted infections can be an effective intervention to reverse the HIV epidemic progress.

Syndromic Case Management (SCM) is the cornerstone of STI/RTI management, being a comprehensive approach for STI/RTI control endorsed by the World Health Organization (WHO). This approach classifies STI/RTI into syndromes, which are easily identifiable group of symptoms and signs and provides treatment for the most common organisms causing the syndrome. Treatment has been standardized through the use of pre-packaged colour coded STI/RTI drug kits. SCM achieves high cure rates because it provides immediate treatment on the first visit at little or no laboratory cost. However, it goes hand in hand with other important components like counseling, partner treatment, condom promotion and referral for HIV testing.

As per the convergence framework of NACO-NRHM for STI/RTI service delivery, uniform service delivery protocols, operational guidelines, training packages & resources, jointly developed by NRHM & NACO are to be followed for provision of STI/RTI services at all public health facilities including CHC and PHC. As per joint implementation plan, NACO/SACS would provide training, quality supervision and monitoring of STI/RTI services at all health facilities, thus overseeing the implementation. For tracking access, quality, progress and bottlenecks in STI/RTI program implementation, common information and monitoring system jointly developed by NACO and NRHM would be followed.

As a step to take convergence forward, it is envisaged that a resource pool of trainers is created at state and district level so as to enable roll out trainings for service providers in the public health care delivery system using the jointly developed training material and through the cascade models of trainings. The ultimate aim is to ensure high quality STI/RTI service delivery at all facilities with best utilization of resources available with both NACP III and RCH II/NRHM.

(Aradhana Johri)

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**अपनी एचआईवी अवस्था जानें, निकटतम सरकारी अस्पताल में मुफ्त सलाह व जाँच पाएँ**  
**Know Your HIV status, go to the nearest Government Hospital for free Voluntary Counselling and Testing**





सत्यमेव जयते



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## ACKNOWLEDGMENT

Reproductive tract infections (RTIs) including sexually transmitted infections (STIs) present a huge burden of disease and adversely impacts the reproductive health of people. The emergence of HIV and identification of STIs as a co-factor have further lent a sense of urgency for formulating a programmatic response to address this important public health problem.

The comprehensive training modules on the Prevention and Management of STI/RTI have come through with the coordinated and concerted efforts of various organizations, individuals and professional bodies, who have put in months of devoted inputs towards it.

The vision and constant encouragement of Ms K Sujatha Rao, IAS, Secretary Health and Family welfare, Shri K Chandramouli, IAS, Secretary and Director General NACO, Ms Aradhana Johri, IAS, Additional Secretary NACO and Shri Amit Mohan Prasad, IAS, Joint Secretary RCH, Ministry of Health and Family Welfare is sincerely acknowledged, under whose able leadership these modules have been developed.

The technical content has been jointly developed by STI division, Department of AIDS Control (National AIDS Control Organization) and Maternal Health Division of MoHFW. The National Institute for Research in Reproductive Health (NIRRH), Mumbai under ICMR initiated and lead the process of reviewing the existing training material and developing updated training modules through the organization of a number of meetings and workshops. The preparation and design of material also involved the technical assistance, funding support and other related support provided by WHO, UNFPA, FHI and many other experts in the field.

Thanks are due to Dr. Anjana Saxena, Deputy Commissioner, Maternal Health Division, Dr. Himanshu Bhushan, Dr. Manisha Malhotra, and Dr. Dinesh Baswal, Assistant Commissioners Maternal Health Division for their constant technical inputs, unstinted support and guidance throughout the process of developing these guidelines. The hard work and contributions of Dr. Ajay Khara, then Assistant Director-General, and NACO STI team comprising of Dr. Shobini Rajan, Deputy Director, Dr. Bhriгу Kapuria, Technical Officer, Dr. TLN Prasad, and Dr. Aman Kumar Singh, Technical Experts and Dr. Naveen Chharang, Assistant Director at NACO have been invaluable in shaping the document.

Sincere appreciation is due to Dr. Sanjay Chauhan, Deputy Director, NIRRH who coordinated the whole process along with his team comprising Dr. Ragini Kulkarni, Research Officer and Dr. Beena Joshi, Senior Research Officer at NIRRH. Special mention is made of contribution of Dr. Deoki Nandan, Director, NIHFW, Delhi and for all those who coordinated the piloting of the module through State Health Directorates and State AIDS Control Societies of Uttar Pradesh, Madhya Pradesh, Assam, Kerala, West Bengal and Gujarat. I also thank to Public Health Foundation of India (PHFI) for providing assistance to print these modules.

(Dr. Sunil D. Khaparde)



## LIST OF ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ANC	Ante Natal Care
ART	Anti Retroviral Therapy
ANMs	Auxiliary Nurse Midwives
BV	Bacterial Vaginosis
CA	Candidiasis, yeast infection
CHCs	Community Health Centres
CMV	Cyto MegaloVirus
CDC	Centre for Disease Control
DNA	Deoxy Ribonucleic Acid
EC	Emergency Contraception
ESR	Erythrocyte Sedimentation Rate
EIA	Enzyme Immuno Assay
ELISA	Enzyme Linked Immuno Sorbent Assay
Endo	Endogenous
FP	Family Planning
FHI	Family Health International
FTA-Abs	Fluorescent Treponema Antibody Absorption Test
GUD	Genital Ulcer Disease
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSV	Herpes Simplex Virus
Iatro	Iatrogenic
IPHS	Indian Public Health Standards
ICTC	Integrated Counselling and Testing Centre
IDUs	Intravenous Drug Users
IM	Intramuscular
IU	International Units
IUD	Intra Uterine Device
IV	Intravenous
KOH	Potassium Hydroxide
LCR	Ligase Chain Reaction
LGV	Lympho Granuloma Venereum

LHV	Lady Health Visitor
MOHFW	Ministry of Health and Family Welfare
MSMs	Men having Sex with Men
MCH	Maternal and Child Health
MHA-TP	Micro Haemagglutination Assay for antibodies to Treponema Pallidum
MTCT	Mother-To-Child Transmission
MVA	Manual Vacuum Aspiration
NACP	National AIDS Control Program
NRHM	National Rural Health Mission
NPCP-III	National AIDS Control Program-Phase III
NIRRH	National Institute for Research in Reproductive Health
NACO	National AIDS Control Organization
NGO	Non Governmental Organization
NGU	Non Gonococcal Urethritis
PHC	Primary Health Centre
PLHAs	Persons Living with HIV/AIDS
PAP Test	Papanicolaou Test
PPTCT	Prevention of Parent-To-Child Transmission of HIV
PSI	Population Services International
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PID	Pelvic Inflammatory Disease
ROM	Rupture of Membrane
RPR	Rapid Plasma Reagin
RTI	Reproductive Tract Infection(s)
RCH	Reproductive and Child Health Program
RCH-II	Reproductive and Child Health Program-Phase II
STI	Sexually Transmitted Infection
STD	Sexually Transmitted Disease
SACS	State AIDS Control Society
TPHA	Treponema Pallidum Haemagglutination Test
TI	Targetted Intervention
TV	Trichomonas Vaginalis
UTI	Urinary Tract Infection
UNFPA	United Nations Population Funds
VCT	Voluntary Counseling and Testing
VDRL	Venereal Disease Research Laboratory
WBC	White Blood Cells
WHO	World Health Organization

# Contents

<b>Handout No.</b>	<b>Topic</b>	<b>Page No.</b>
1	Programme Objectives and Schedule	1
2	Understanding Common STI/RTI	5
3	Laboratory Tests for STI/RTI	17
4	Care of Microscope	31
5	Bio-medical Waste Disposal	35
6	Disinfection and Standard Precautions	41
7	<b>Annexures</b>	
	I References and Source	51
	II Core Group Members	52
	III List of Contributors	53





## PROGRAMME OBJECTIVES AND SCHEDULE

Sr. No.	Topic	Page No.
1	Objectives of the training programme	2
2	Schedule of the 2 day workshop	3

## 1. Objectives of the Training Programme

**By the end of this programme, laboratory technicians will be:**

- More knowledgeable on STI/RTI, their causative agents and complications;
- Able to understand the seriousness of complications of common STI/RTI, if left untreated and its long term implications on reproductive health;
- Able to assist doctors and other health care providers in collection of various body fluid samples such as blood; vaginal; cervical; urethral; urine; rectal samples;
- Able to perform minimal laboratory tests for STI/RTI for diagnosis of common STI/RTI based on the infrastructure and facilities available at PHC;
- More knowledgeable on standard work precautions for prevention of STI/RTI.

## 2. Schedule of the Two Day Workshop for Laboratory Technicians

Days/ Timings	Module: Topic and Duration	Contents
<b>Day 1 (Morning)</b>		
<b>09 00 hrs</b>	<b>Module 1:</b> Introductory Module (1 hr. 30 min)	<ul style="list-style-type: none"> <li>Getting to know each other</li> <li>Program objectives and schedule</li> <li>Pre-test</li> </ul>
<b>10 30 hrs</b>	<b>Module 2:</b> Understanding Common STI/RTI (1 hr)	<ul style="list-style-type: none"> <li>Basic information on common STI/RTI</li> <li>Different types of STI/RTI and its causative organisms</li> <li>Complications of STI/RTI</li> </ul>
<b>11 30 hrs</b>	<b>Module 3:</b> Laboratory test for STI/RTI (1 hr)	<ul style="list-style-type: none"> <li>Role of laboratory test in control of STI/RTI</li> <li>Tests for STI/RTI</li> </ul>
<b>12 30 hrs</b>	<b>Module 4:</b> Disinfection and Universal Work Precautions (1 hr)	<ul style="list-style-type: none"> <li>Standard work precautions</li> <li>Use of personal protective equipment</li> <li>Use of disinfectants</li> </ul>
<b>13 30 hrs</b>	<b>LUNCH BREAK</b>	
<b>Day 1 (Afternoon) LAB PRACTICAL</b>		
<b>14 30 hrs</b>	Laboratory Diagnosis for STI/RTI (3 hrs)	<ul style="list-style-type: none"> <li>Practical demonstration by facilitator on minimal laboratory tests for STI/RTI including sample collection</li> </ul>
<b>Day 2 (Morning) LAB PRACTICAL</b>		
<b>9 00 hrs</b>	Laboratory Diagnosis for STI/RTI (5 hrs)	<ul style="list-style-type: none"> <li>Performance of various laboratory tests for STI/RTI by participants</li> </ul>
<b>13 00 hrs</b>	<b>LUNCH BREAK</b>	
<b>Day 2 (Afternoon) LAB PRACTICAL</b>		
<b>15 00 hrs</b>	Disinfection and standard work precautions (1 hr 30 min)	<ul style="list-style-type: none"> <li>Demonstration by facilitator and performance by participants on standard precautions and use of disinfectants</li> </ul>
<b>16 30 hrs</b>	Post test (30 min)	



## UNDERSTANDING COMMON STI/RTI

Sr. No.	Topic	Page No.
1	What are STI/RTI?	6
2	Epidemiological determinants of STI/RTI	7
3	Body sites where STI/RTI can occur in men and women	8
4	Common STI/RTI and their causative organisms	11
5	Complications of STI/RTI	12

## 1. What are STI/RTI?

It is critical to understand or get familiarized with the key terms generally used while providing reproductive and child health services, and communicating with clients having STI/RTI. So before beginning the discussion on diagnosis and treatment of STI/RTI, we will first ensure to understand some of the key terms and basic concepts in this area.

### What are RTI?

Reproductive tract infection is a broad term that includes sexually transmitted infections as well as other infections of the reproductive tract that are not transmitted through sexual intercourse. In women, RTI includes infections of the outer genitals, vagina, cervix, uterus, tubes, or ovaries. In men, RTI involves the penis, testes, scrotum, or prostate. RTI are caused by bacteria, viruses, or protozoa that person gets either through sexual contact or by non-sexual route.

### What are STDs?

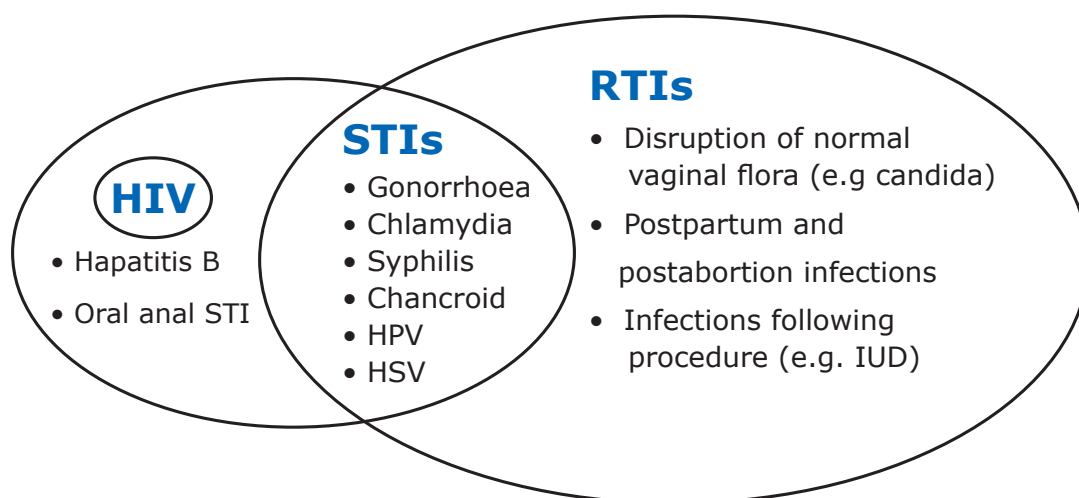
STDs means sexually transmitted diseases caused by microbes that are passed from one person to another through sexual contact. The terminology is used to describe the diseases that are acquired through sexual contact. Sexually transmitted organisms may also be sometimes transmitted by nonsexual modes of transmission.

### What are STI?

The term "Sexually Transmitted Infections" (STI) is newer term used to indicate that infections caused by microbes may not manifest as symptoms and do not always result in a disease.

### STI versus STD

Historically, the terminology used to describe infections and diseases acquired through sexual contact has demonstrated the social stigma attached to these infections. As these terms became laden with moral judgments and as medical and public health professionals began to see the need for a more accurate, technical description, the term 'STI' was approved by WHO and hence became the standardized term.



**Figure 1:** Reproductive Tract Infections (RTI); Sexually Transmitted Infections (STI); and HIV Infection

## 2. Epidemiological determinants of STI/RTI

RTI include both sexually transmitted infections (STI) and non-sexually transmitted infections. STI caused by bacteria, virus, or protozoa that are passed from one person to another through sexual contact. RTI, which are not sexually transmitted, can be caused by disturbances of the normal endogenous flora and by medical interventions that may provoke iatrogenic infection. Some infections can be easily cured by using antibiotics or other drugs, while few others are incurable. HIV, which causes AIDS, is a viral STI. An understanding of these differences is essential in order to provide effective care and to give good advice to patients with reproductive tract complaints.

### Factors that are contributing to high prevalence of STI/RTI

Though the STI are infectious diseases, however, more than with other infectious diseases, STI transmission depends mainly on human behavior. A person with many sexual Partners is much more likely to acquire a STI than a person with one Partner. A person with many partners also has more opportunity to infect others.

In fact, most STI transmission occurs within a small part of the population that has multiple sex Partners. This does not mean, that the rest of the community is not at risk for STI infection. A woman who has sex only with her husband can still get a STI if her husband has other partners. For these reasons, control of STI in any community requires effective strategies that reach those with the greatest number of sex partners.

Clinical services can contribute to STI control, but they are not enough. There are many reasons for high prevalence of STI/RTI, which include lack of access to health care and medicines, lack of awareness of STI, and in-out migration.

- STI such as syphilis, gonorrhoea and chancroid spread more rapidly in places where communities are disrupted, migrant labour is common and commercial sex networks are active.
- Iatrogenic infections are more commonly seen where the STI/RTI in high prevalence, and where health care providers do not have the training or supplies to perform procedures safely. Postpartum and post abortion infections are more common where medical services and follow-up care are not provided safely.
- Endogenous infections, such as yeast infection and bacterial vaginosis, are common worldwide and are influenced by environmental, hygienic, hormonal and other factors like co existent diabetes and immune compromised states like AIDS

### 3. Body sites where STI/RTI can occur in men and women

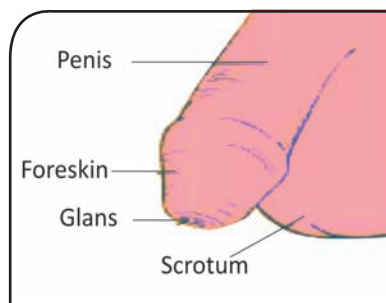
For understanding different body sites where STI/RTI could occur in men and women, you must have knowledge about sexual and reproductive anatomy.

#### Male sexual and reproductive organs

##### External male genitals

The external male genitals consist of the penis and the scrotum. The **penis** is a cylindrical structure with the capacity to be flaccid or erect. The penis provides passage for both urine and semen.

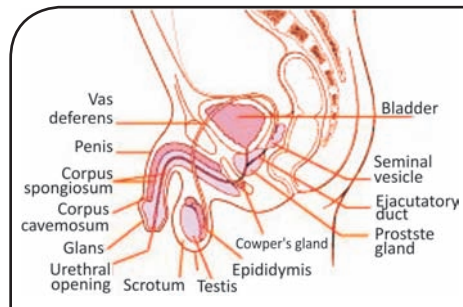
The tip of the penis, is known as the **glans (glans penis)**, is the part of the penis that is most sensitive and has the most nerve endings. The glans is covered by the foreskin, or **prepuce**, in men who are not circumcised. The **scrotum** is a pouch of skin hanging directly under the penis that contains the testes. The scrotum protects the testes and maintains the temperature necessary for the production of sperm.





## Internal male genitals

The internal male genitals are: the testes, the epididymis, the vasa deferentia, the seminal vesicles, the prostate gland, and the Cowper's glands. The **testes**, the paired, oval-shaped organs that produce sperm and male sex hormones, are located in the scrotum. They are highly innervated and sensitive to touch



and pressure. The testes produce **testosterone**, which is responsible for the development of male sexual characteristics and sex drive (**libido**).

The **epididymis** are the two highly coiled tubes against the backside of the testes where sperm mature and are stored until they are released during ejaculation. **The vas deferens** is the paired tubes that carry the mature sperm from the epididymis to the urethra.

The **seminal vesicles** are a pair of glandular sacs that secrete about 60% of the fluid that makes up the semen in which sperm are transported. Seminal fluid provides nourishment for sperm.

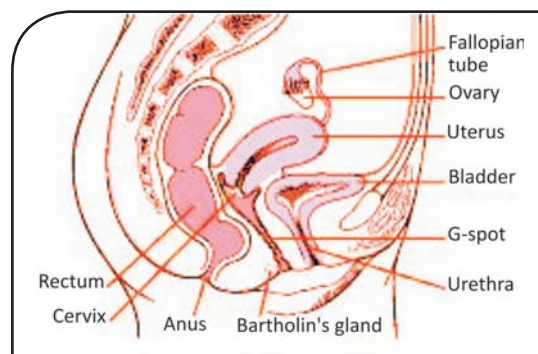
The **prostate gland** is a walnut-sized, glandular structure that secretes about 30% of the fluid that makes up semen.

The **Cowper's glands** are two pea-sized glands under the prostate gland. These glands produce pre-ejaculatory fluid in the urethra that acts as a lubricant for the sperm and the urethra as semen flows out of the penis.

## Female sexual and reproductive organs

### External female genitals (Vulva)

The external female genitals are: the mons pubis, the clitoris, the labia majora, and the labia minora. Together, along with the opening of the vagina, they are known as the **vulva**.



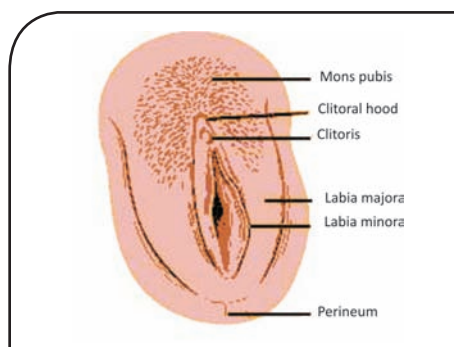
## Internal female genitals

The internal female genitals are: the vagina, the cervix, the uterus, the fallopian tubes, and the ovaries.

The **fallopian tubes (oviducts)** are a pair of tubes that extend from the upper uterus, extending out toward the ovaries (but not touching them), through which ova (eggs)

travel from the ovaries toward the uterus and in which fertilization of the ovum takes place.

The **ovaries** are two organs located at the end of each fallopian tube that produce ova (releasing one per month from puberty to menopause). The ovaries produce **estrogen** and **progesterone**, the hormones responsible for the development of sex characteristics.



## Where STI/RTI occur?

### STI/RTI in females

In women, RTI involve the outer genitals, vagina, cervix, and are referred to as lower reproductive tract infections. Infections in the uterus, fallopian tubes, and ovaries are considered upper reproductive tract infections.

It also leads to

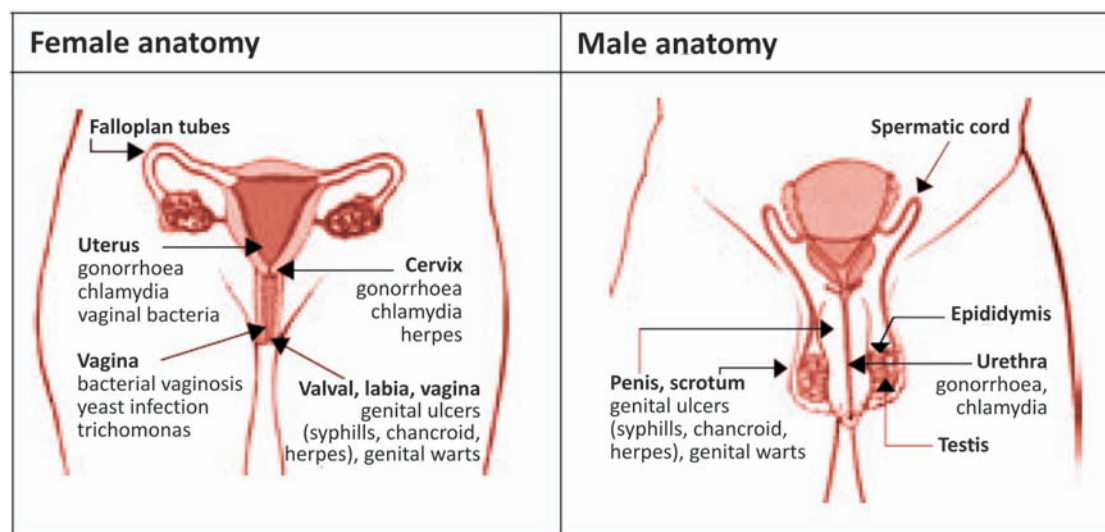
- Disruption of normal vaginal flora (candida and bacterial vaginosis)
- Postpartum and postabortion infections
- Infections following procedures (e.g. IUD insertion)

### STI/RTI in males

RTI generally begin in the lower reproductive tract (the urethra). If untreated, they may ascend through the vas deferens (sperm tube) to the upper reproductive tract (which includes the epididymis and testes). It also leads to prostatitis and epididymitis

**Note:** In general, RTI in men are easier to identify and treat, as they are more likely to be symptomatic.

### Sites of STI/RTI in females and males



**Source:** Adopted from "Integrating STI/RTI care for reproductive health, sexually transmitted and other reproductive tract infections, A guide to essential practice-2005 WHO".

## 4. Common STI/RTI and causative organisms

Any individual can become infected with a sexually transmitted infection (STI) or reproductive tract infection (RTI), regardless of age, background, or socioeconomic class.

RTI that are not sexually transmitted are even more common. They are:

- Bacterial vaginosis
- Vaginal yeast infection

### There are over 20 STI. But 11 most common are:

1. Syphilis
2. Gonorrhoea
3. Chlamydia
4. Trichomoniasis
5. Chancroid
6. Herpes simplex virus (HSV)
7. Genital and cervical warts or human papilloma virus (HPV)
8. Hepatitis B (HBV)
9. Human immunodeficiency virus (HIV)
10. Scabies
11. Pubic lice

**The common STI/RTI and the causative organisms are as follows:**

<b>Diseases or syndromes</b>	<b>Infectious agent/s</b>
Syphilis	Treponema pallidum
Molluscum contagiosum	Poxvirus
Chancroid	Haemophilus ducreyi
Chlamydial infection	Chlamydia trachomatis
Gonorrhoea	Neisseria gonorrhoea
Trichomonas infection	Trichomonas vaginalis
Yeast infection	Candida albicans
Bacterial vaginosis (BV)	Mixed infection by Gardnerella vaginalis, Mycoplasma hominis, vaginal anaerobes
Pelvic inflammatory disease (PID)	Mixed infection by Neisseria gonorrhoea, Chlamydia trachomatis and/or vaginal anaerobic bacteria
Hepatitis B, Hepatocellular carcinoma	Hepatitis B Virus
AIDS	Human immunodeficiency virus (HIV)
Genital and anal warts	Human papilloma virus (HPV)
Scabies	Sarcoptes scabiei
Pubic lice	Phthirus pubis

## 5. Complications of STI/RTI

STI/RTI if left untreated can cause serious complications in males, females and neonates. Millions of men, women, and children all over the world are affected by the long-term complications of RTI and STI. These infections can lead to numerous serious, long-term, and sometimes deadly complications, particularly in women. Some STI/RTI can also cause pregnancy-related complications or congenital infections. Unfortunately, many a times most of the STI/RTI are asymptomatic or the symptoms and signs of many infections may not appear until it is too late to prevent serious consequences and damage to the reproductive organs.

In addition, the complications of RTI and STI affect even more than an individual's health. The morbidity associated with them has a profoundly adverse effect on the quality of life and economic productivity of many women and men, their families, and consequently, entire communities.

## Complications of STI/RTI in males

### Infertility

Infection of the upper reproductive tract can occasionally result in partial or complete blockage of the sperm ducts, and disorders in sperm production. This can cause low sperm counts in semen or abnormal sperm, which contribute to male infertility.

### Carcinoma of the penis

Infection with Human papilloma virus (HPV) is associated with the development of penile cancer.

## Complications of STI/RTI in females

### Pelvic inflammatory disease

Some of the most serious consequences of RTI in women occur when an infection of the lower genital tract (cervix or vagina) or outside organisms reach the upper genital tract (uterus, fallopian tubes, ovaries and surrounding structures). Infection may become generalized and life threatening, and resulting tissue damage and scarring may cause infertility, chronic pelvic pain and increased risk of ectopic pregnancy.

### Adverse outcomes of pregnancy

RTI such as chlamydia, gonorrhoea, syphilis, genital herpes etc. are responsible for the adverse outcomes of pregnancy. In addition to ectopic pregnancy, other poor pregnancy outcomes that are linked to RTI include -

- Fetal wastage - spontaneous abortion or stillbirth.
- Low birth weight due to premature delivery or intra-uterine growth retardation.
- Congenital or perinatal infections - eye infections causing blindness, infant pneumonias and mental retardation.

### Infertility

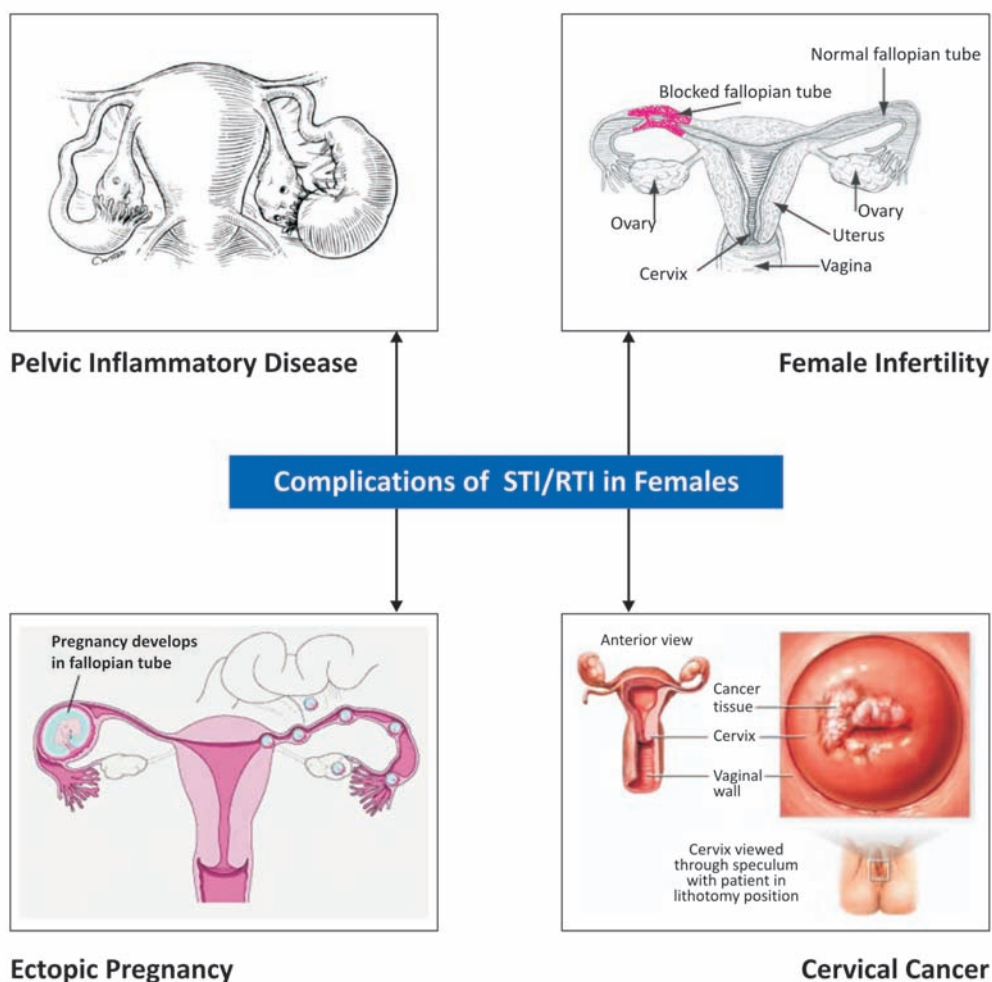
Infertility often follows after untreated pelvic inflammatory disease in women, and epididymitis and urethral scarring in men. In fact, complications of RTI are the most important preventable causes of infertility in regions where childlessness is most common. Repeated spontaneous abortion and stillbirth often due to STI such as syphilis are other important reasons why couples are unable to have children.

## Ectopic pregnancy

The tubal scarring and blockage that often follows PID may be total or partial. Fertilization can still occur with partial tubal blockage but risk of implantation in the fallopian tubes or other site outside the uterus (ectopic pregnancy) is high. Ruptured ectopic pregnancy, along with complications of abortion and postpartum infection, is a common preventable cause of maternal death in places with high prevalence of STI/RTI and PID.

## Cervical cancer

Infection with Human papilloma virus (HPV) appears to be strongly associated with the development of cervical cancer. It is the most common genital cancer among women in India. Cervical cytological screening (Papanicolaou smears) facilities are still not available in the primary health care facilities and therefore majority of diagnosed cases are detected in advanced stages when treatment has lower successful outcome.



## Complications of STI/RTI in new born babies

### Congenital syphilis

Congenital syphilis results from the transmission of *Treponema pallidum* infection from an infected pregnant woman to her fetus. Maximum transmission (up to 100%) occurs if the mother herself is in the primary or secondary stages of the disease and this transmission rate drops to 10% to 30% if the mother is in the late latent stage.

### Gonorrhoea

An untreated *Neisseria gonorrhoea* infection in pregnant woman results in its transmission to her neonate. The neonate may present with only conjunctivitis, which usually appears within the first four days of life and may progress to panophthalmitis unless treated.

### Chlamydia

*Chlamydia trachomatis* can be vertically transmitted from an infected pregnant woman to her neonate and may cause only conjunctivitis or have systemic infection like pneumonitis.

### Human immunodeficiency virus (HIV)

Most of the HIV transmission takes place during delivery but it must be remembered that HIV is also transmitted through breast milk (14%).

### Herpes simplex viruses 1 & 2 (HSV1 & HSV2)

The herpes simplex virus has a very high intrapartum transmission rate (75% to 90%) and can lead to localized or central nervous system or disseminated disease in the affected neonates with a very high rate of long-term residual sequelae.

### Hepatitis B virus

Hepatitis B virus infection in the mother can be transmitted to the neonate. Neonatal infections result in higher carrier rates with more chances of long term sequelae.

There are a number of other infections like Cytomegalovirus, Candida, Trichomonas and other organisms that are transmitted from the mother to the neonate and can cause serious morbidity.

## **Prematurity**

STI/RTI in pregnancy especially bacterial vaginosis and trichomoniasis may result in preterm delivery, which can lead to prematurity and associated complications in the neonate.

## **Low birth weight**

Low birth weight can be a result of prematurity or intrauterine growth retardation caused due to associated STI/RTI in pregnancy.



## LABORATORY TESTS FOR STI/RTI

Sr. No.	Topic	Page No.
1	Definitions of Common Terms	18
2	Role of the Laboratory in STI/RTI control	19
3	Laboratory Tests for RTI and STI	19

## 1. Definitions of common terms

**Antigen:** A molecule, which is recognized by the immune system and induce an immune reaction (the organism itself)

**Antibody:** A class of serum proteins, which are induced in response to the immune reaction following contact with antigen (an infectious organism)

**False positives:** Uninfected people diagnosed as positive

**False negatives:** Infected people diagnosed as negative (missed infections)

### Sensitivity:

- How good a test is at identifying people who are infected?
- Higher the sensitivity, the lower the rate of false negatives (missed infections)
- Example: if sensitivity of a test is 95% and 100 infected people are tested, 95 will have positive test results and 5 will have negative test results (even though they are infected)
- The minimum number of organisms needed in a sample for a test to be positive varies from one type of test to another. The lower the number of organisms that can be detected, the greater the sensitivity of the test. The new amplified DNA techniques [e.g., polymerase chain reaction (PCR), ligase chain reaction (LCR)] are extremely sensitive and can detect between 1 and 50 organisms in the sample tested.

### Specificity:

- How good a test is at identifying people who are not infected?
- Higher the specificity, the lower the rate of false positives
- Example: if specificity of a test is 95% and 100 people who are not infected are tested, 95 will have negative test results and 5 will have positive test results (even though they are not infected) Sensitivity & specificity are used to give an indication of how good a diagnostic test is. Ideally one would like a test that has 100% sensitivity (i.e. everyone who is infected tests positive) and 100% specificity (i.e. everyone who is not infected tests negative).

## 2. Role of the laboratory in STI/RTI control

1. Screening and detection of disease in those without symptoms who seek health care for other reasons. eg. Antenatal women.
2. Screening groups of people who may be at risk for a STI/RTI but have no symptoms.
3. Testing a sample of the population to see what percentage is infected (prevalence) and how many new infections are occurring in a certain time period (incidence).
4. Conducting simple studies to check on the accuracy of syndromic management (validation).
5. Testing for antimicrobial resistance.
6. Sentinel surveillance of STI.
7. Making an etiologic diagnosis for patients who present with STI/RTI symptoms.
8. Simple laboratory tests improve the diagnostic sensitivity and specificity of syndromic approach to symptomatic STI/RTI, particularly in women.

## 3. Laboratory Tests for RTI and STI

- A. Microscopic examination:** Directly visualizing the organism on vaginal/cervical smear under the microscope. Examples: Wet mount for *Trichomonas vaginalis*, *Candida* (budding cells), Bacterial vaginosis (BV), Gram staining for gonococcus, BV causing organism, *Candida* and Dark field microscopy for *Treponema pallidum*.
- B. Detection of antigen** (a specific molecule from the infecting organism itself): Example: Enzyme immunoassay (EIA) for chlamydia, gonorrhoea and other infections.
- C. Antibody tests:** To measure the body's response of producing antibodies to the infecting organism. Examples: EIA used for *Treponema pallidum* or HIV antibodies.
- D. Culture of different organisms** (Growing the organism in the laboratory). Examples: *Culturing Trichomonas vaginalis*, *Candida albicans* and other species, *Chlamydia trachomatis* and *N.gonorrhoea*.
- E. Detection of DNA of the organism using non-amplified techniques:** Example: Nucleic acid hybridization used for herpes.

- F. Detection of DNA of the organism using amplified techniques:**  
Examples: PCR (polymerase chain reaction), LCR (ligase chain reaction) and TMA (transcription mediated amplification) used for chlamydial infection and HIV infection.
- G. Other:** Use of Vaginal pH for BV.

## What are the laboratory tests for detecting common STI/RTI and how they are performed?

### 1. Vaginal pH

The pH of vaginal fluid should be measured using pH paper of appropriate range (3.8 to 6.0). The vaginal fluid sample is collected with a swab from the lateral and posterior fornices of the vagina and the swab is then touched directly on to the paper strip. Alternatively, the pH paper can be touched to the tip of the speculum after it has been withdrawn from the vagina. Care must be taken not to use any jelly (eg K.Y jelly) or disinfectant (eg. savlon) before doing pH test. Contact with cervical mucus must be avoided since it has a higher pH. The normal vaginal pH is 4.0. In bacterial vaginosis (BV), the pH is generally elevated to more than 4.5.

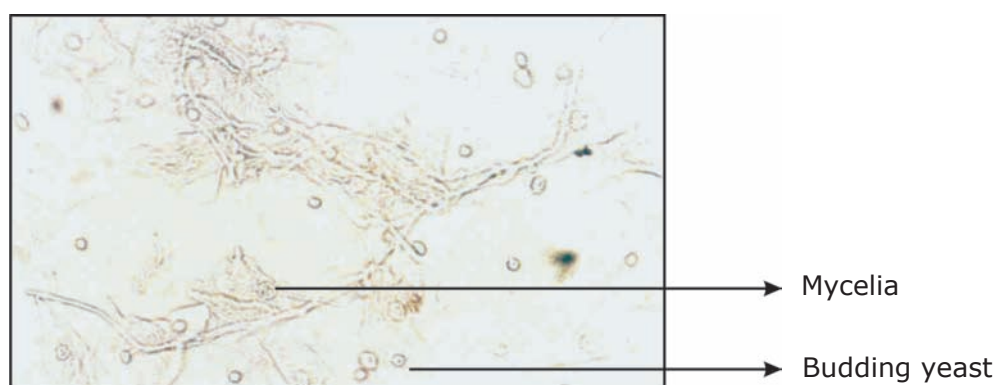
The vaginal pH test has the highest sensitivity (less false negativity) of the four characteristics used for identification of BV, but the lowest specificity (more false positivity); an elevated pH is also observed if the vaginal fluid is contaminated with menstrual blood, cervical mucus or semen, and in women with a *T. vaginalis* infection. In simple words it means that if pH test is negative the result can be taken as it is but if it is positive one has to rule out the other factors contaminating the sample such as menstrual blood, cervical mucus or semen or presence of *T. vaginalis* infection.

### 2. Wet mount microscopy

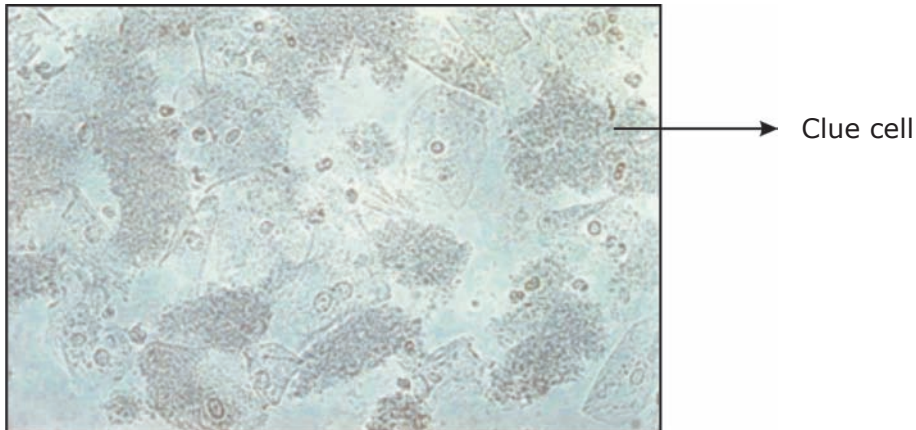
Wet mount microscopy is the direct microscopic examination of vaginal discharge for the diagnosis of trichomoniasis, candidiasis and bacterial vaginosis (Box 1).

**Box 1: Wet mount microscopy examination of vaginal discharge**

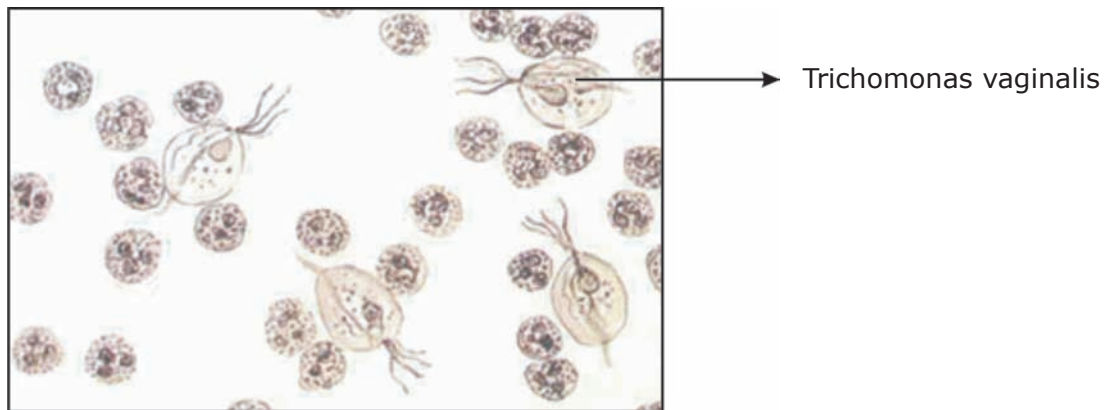
<b>Collect specimen</b>	Take a specimen of discharge with a spatula from the sidewalls or deep in the vagina where discharge accumulates.
<b>Prepare slide</b>	Mix specimen with 1 or 2 drops of saline on a glass slide and cover with a cover slip.
<b>What to look for</b>	<ul style="list-style-type: none"> <li>• Examine at 100X magnification and look for typical jerky movement of motile trichomonads (ovoid, globular, pear-shaped flagellated protozoan).</li> <li>• Examine at 400X magnification to look for yeast cells (round to ovoid cells with typical budding) and trichomonads.</li> <li>• To make identification of yeast cells easier in wet mount slides, mix the vaginal swab in another drop of saline and add a drop of 10% potassium hydroxide to dissolve other cells. Note any fishy odour to suggest BV.</li> <li>• Presence of clue cells (squamous epithelial cells covered with many small coccobacillary organisms). Wet mount shows stippled granular cells without clearly defined edges because of the large numbers of adherent bacteria present and an apparent disintegration of the cells. The adhering bacteria are predominantly <i>G. vaginalis</i>, sometimes mixed with anaerobes).</li> </ul>
<b>Important</b>	Look for evidence of other vaginal or cervical infections as multiple infections are common.



**Figure 1:** Potassium hydroxide preparation of vaginal fluid showing budding yeast and mycelia



**Figure 2:** "Clue cells" in vaginal wet mount (x 400)



**Figure 3:** Trichomonas vaginalis in a wet mount of vaginal discharge (x 400)

**Box 2: Clinical criteria for Bacterial Vaginosis (BV)**

BV can be diagnosed using simple clinical criteria with or without the aid of a microscope	
<b>Collect specimen</b>	Take a specimen of discharge from the sidewalls or deep in the vagina where discharge pools (or use discharge remaining on speculum). Note color and consistency of discharge. Touch pH paper to discharge on swab or speculum and note pH.
<b>Prepare slide</b>	<ul style="list-style-type: none"> <li>Place specimen on a glass slide. Add a drop of 10% potassium hydroxide (KOH) and note for any fishy smell.</li> <li>Make a wet smear with 0.9% normal saline, cover with cover slip and see under microscope for clue cells.</li> </ul>

Contd...

<b>What to look for</b>	<p>The diagnosis of BV is based on the presence of at least 3 of the 4 following characteristics</p> <ul style="list-style-type: none"> <li>• Homogeneous white-grey discharge that sticks to the vaginal walls</li> <li>• Vaginal fluid pH &gt;4.5</li> <li>• Release of fishy amine odour from the vaginal fluid when mixed with 10% potassium hydroxide (positive Whiff test)</li> <li>• "Clue cells" visible on microscopy on wet preparation</li> </ul>
<b>Important</b>	<p>Look for evidence of other vaginal or cervical infections as multiple infections are common.</p>

### 3. Whiff test

Women with BV often complain of a foul vaginal smell. This odour is due to the release of amines, produced by decarboxylation of the amino acids (lysine and arginine) by anaerobic bacteria. When potassium hydroxide is added to the vaginal fluid, these amines immediately become volatile, producing the typical fishy odour.

Place a drop of vaginal fluid on a glass slide and add a drop of 10% potassium hydroxide. Hold the slide close to nose to detect the amine odour. After a positive reaction, upon standing the specimen will quickly become odourless because the amines will be rapidly and completely volatilized.

### 4. Gram stain microscopy

A gram stain of a vaginal smear has a higher specificity (i.e lesser false positivity) for the diagnosis of bacterial vaginosis (BV) than a wet mount preparation. Moreover, a Gram stain allows good evaluation of the vaginal bacterial flora. Normal vaginal fluid contains predominantly lactobacillus species and exceedingly low numbers of streptococci and coryneform bacteria. In BV, lactobacilli are replaced by a mixed flora of anaerobic bacterial morphotypes and *G. vaginalis*. However, gram stain microscopy has a very low sensitivity for detecting *N.gonorrhoea* among women; culture remains the method of choice.

**Box 3: Gram stain microscopy of vaginal smears**

<b>Collect specimen</b>	A Gram stain slide can be prepared at the same time as the wet mount by rolling the spatula/swab on a separate slide.
<b>Prepare slide</b>	<ol style="list-style-type: none"> <li>1. Heat fix.</li> <li>2. Stain with crystal violet (60 seconds) and rinse.</li> <li>3. Stain with iodine (60 seconds) and rinse.</li> <li>4. Decolorize with acetone-ethanol for few seconds (until the liquid runs clear).</li> <li>5. Stain with safranin (30 seconds) and rinse.</li> <li>6. Gently blot dry and examine under oil immersion (1000X) and count each type of organisms.</li> </ol>
<b>What to look for</b>	<ol style="list-style-type: none"> <li>1. Lactobacilli (large Gram positive bacilli) only: Normal</li> <li>2. Mixed flora, mainly lactobacilli with a few short rods (cocci/bacilli): Considered normal</li> <li>3. Presence of clue cells; mixed flora, mainly Gardnerella and anaerobic bacteria with a few lactobacilli diagnose as BV</li> <li>4. Presence of clue cells, mixed flora of Gram-positive, Gram-negative and Gram-variable rods; no lactobacilli diagnose as BV</li> </ol>
<b>Important</b>	Look for evidence of other vaginal or cervical infections as multiple infections are common.

**\*Nugent score**

**Scoring system (0 to 12) from Gram-stained vaginal smears**

<b>Total score</b>	<b>Lactobacillus morphotypes (large Gram positive bacilli)</b>	<b>Gardnerella and Bacteriodes spp. morphotypes (small Gram negative/Gram variable bacilli)</b>	<b>Mobilincus curved Gram-negative/variable bacilli</b>
0	0 + (>30/oif)	0 (0/oif)	0 (0/oif)
3	1 + (6-30/oif)	1 + (<1/oif)	1+ (</oif)
6	2 + (1-5/oif)	2 + (1-5/oif)	2+ (2-5/oif)
9	3 + (<1/oif)	3 + (6-30/oif)	3+ (6-30/oif)
12	4 (0/oif)	4 + (>30/oif)	4+ (.30/oif)



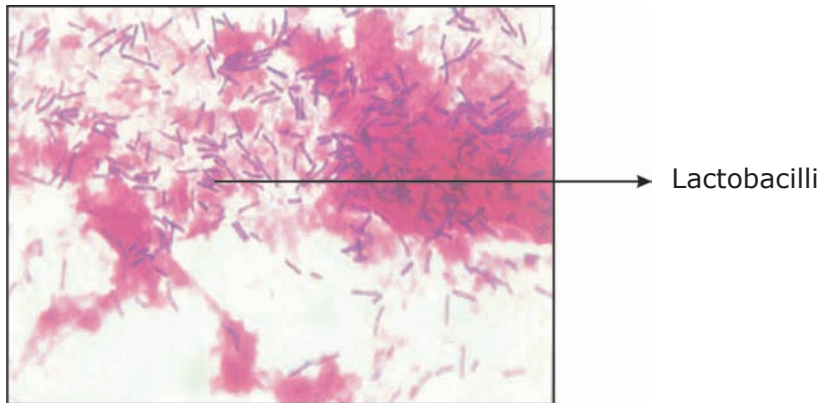
Morphotypes are scored as the average number seen per oil immersion field (oif). Note that less weight is given to curved Gram negative/variable rods. Total score = lactobacilli + *G. vaginalis* and *Bacteriodes* spp. + curved rods.

### Interpretation of Nugent score

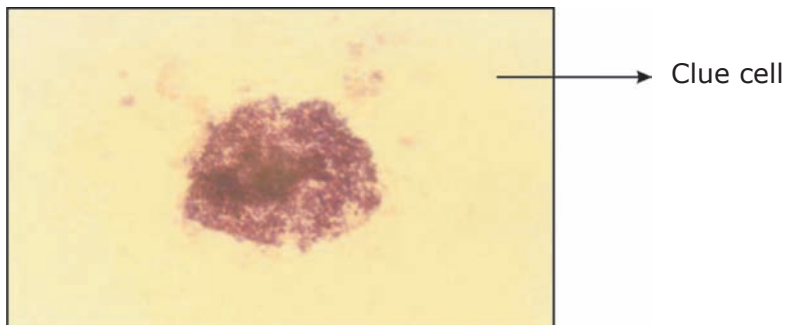
0-3 = normal, never treat

4-6 = intermediate, decide on symptoms for treatment

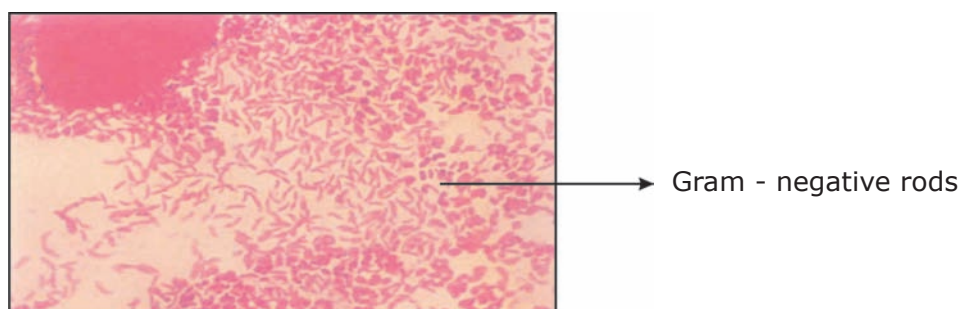
7-or more = BV infection, Treat



**Figure 4:** Gram stained vaginal smear showing a normal flora of lactobacilli (x 1000)



**Figure 5:** Gram stained vaginal smear with typical "clue cell" (x 1000)



**Figure 6:** Gram stained vaginal smear showing large Gram- negative rods (*Mobilincus mulieris*) (x 1000)

### Use of gram stain for diagnosis of cervical infection

1. The Gram stain method in female does not provide conclusive evidence of the presence of gonococcal infection. Presence of intracellular gram negative diplococci indicates infection but their absence does not rule out infection.
2. The costs associated with the method, including the cost of maintaining microscopes, outweigh the benefits in terms of improved quality of care.

### Use of gram stain for diagnosis of Urethral/Ano-rectal infection

1. For men, gram stain microscopy of urethral discharge smear will show pus cells and gram-negative intracellular diplococci as well as extra cellular diplococci in case of gonorrhoea.
2. In case of non-gonococcal urethritis more than 5 neutrophils per oil immersion field (1000X) in the urethral smear or more than 10 neutrophils per high power field (400X) in the sediment of the first void urine, in the absence of N. gonorrhoea, is observed.
3. The Gram stain method in male provide conclusive evidence of the presence of gonococcal infection.

## 5. Rapid plasma reagin (RPR) test for syphilis

The current non-treponemal tests for syphilis are Venereal Disease Research Laboratory Test (VDRL Test) and Rapid Plasma Reagin (RPR) test. RPR test is most suitable for the primary health care set-up.

### Procedure of RPR test

- Seek consent
- Use a sterile needle and syringe. Draw 5 ml of blood from a vein. Put in a plain test tube
- Let the test tube stand for 20 minutes to allow serum to separate (or centrifuge 3–5 minutes at 2000–3000 rpm). In the separated sample, serum will be on top.
- Use sampling pipette to transfer the serum. Take care not to include any red blood cells from the lower part of the separated sample.
- Hold the pipette vertically over a test card circle. Squeeze teat to allow one drop (50 µl) of serum to fall onto a circle. Spread the drop to fill the circle using a toothpick or other clean spreader.

**Important:** Several samples may be done on one test card. Be careful not to contaminate the remaining test circles. Use new tip and spreader for each sample. Carefully label each sample with a patient name or number

- Attach dispensing needle to a syringe. Shake antigen.\* Draw up enough antigen for the number of tests done (one drop per test).
- Holding the syringe vertically, allow exactly one drop of antigen to fall onto each test sample. Do not stir.
- Rotate the test card smoothly on the palm of the hand for 8 minutes (or rotate on a mechanical rotator.)

### Interpreting results

After 8 minutes rotation, inspect the card in good light. Turn or tilt the card to see whether there is clumping (reactive result). Test cards include negative and positive control circles for comparison.

#### Interpretation of test results

1. Non-reactive (no clumping or only slight roughness): Non reactive for syphilis
2. Reactive (highly visible clumping): Reactive for syphilis
3. Weakly reactive (minimal clumping): Reactive for syphilis

**Note:** Weakly reactive can also be more finely granulated and difficult to see than this illustration

\* Make sure antigen was refrigerated (not frozen) and has not expired.

### Correlation and confirmation of test results

- Tests for syphilis detect antibodies, which are evidence of current or past infection.
- Non-treponemal tests (such as RPR test and VDRL test ) are the preferred tests for screening. These tests detect almost all cases of early syphilis, but false positives are possible. RPR test can be performed without a microscope.
- Quantitative RPR test titres can help evaluate the response to treatment.

- Treponemal tests, such as Treponema pallidum haemagglutination test (TPHA), fluorescent Treponema antibody absorption test (FTA-Abs), microhaemagglutination assay for antibodies to Treponema pallidum (MHA-TP), if available, can be used to confirm non-treponemal test results.

Quantitative RPR test titres can help evaluate the response to treatment.

## RPR Quantitative Slide test

Additional Equipment and Reagent

- 1) Micropipette (1000ul) with blue plastic tips.
- 2) Normal Saline (0.9%)
- 3) Test tubes or Cuvettes-6 to 8 per reactive serum.
- 4) Rubber teats

### Procedure:

#### A) Preparing sera in dilutions -

- 1 Take 6 test tubes (cuvette), label them from 1-6 and keep them in a rack.
- 2 Pipette 0.5 ml normal saline in each tube.
- 3 Pipette 0.5 ml of test serum in tube 1 and mix well. (Serum dilution = 1:2).
- 4 Take 0.5 ml of diluted serum from test tube 1 and add to tube 2. Mix well and transfer 0.5 ml to test tube 3, mix well and go on adding 0.5 ml of diluted serum to next tube till tube 6 is reached. The dilution obtained in these 6 tubes are 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 respectively.
- 5 Dilutions can be done upto 1:1024 levels by following above described procedure.

#### B) Performing quantitative testing of diluted sera -

- 6 Take a RPR card and add 0.5 ml of serum from the sixth tube on one circle as shown in figure 9.
- 7 Similarly add 0.05 ml of serum from the tube no 5, 4, 3, 2 and 1 in the remaining circles respectively.
- 8 In circle 1 take 0.05 ml of neat, undiluted serum as for the qualitative test.

- 9 Positive and negative controls for each qualitative test should be incorporated.
- 10 Add 1 drop (1/60 ml) of RPR antigen to each circle with a 18 gauge needle and syringe.
- 11 Rotate the card on a RPR/VDRL rotator for 8 min or as per manufacturers instruction making a diameter of 3/4 inch and rotating at a speed of 180 RPM.
- 12 Observe the card immediately under 10 x magnification of light microscope.
- 13 Report the titer as the highest dilution of serum that shows a reactive result.

**Reporting:**

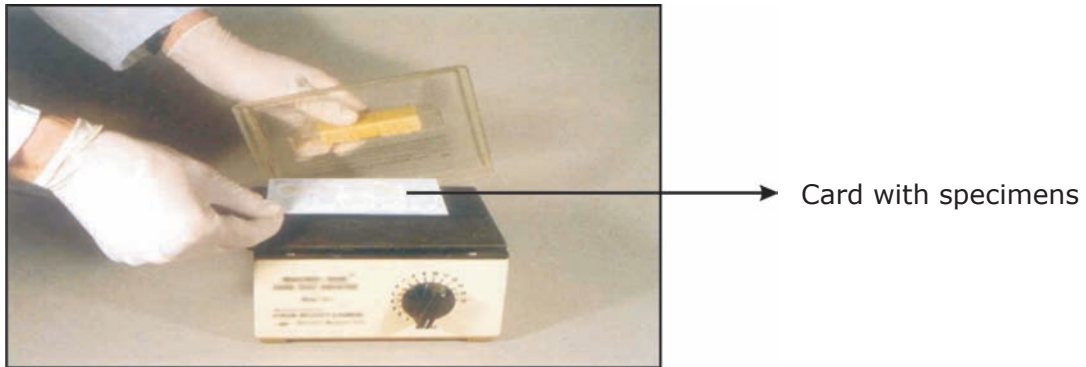
Undiluted Serum	Serum Dilution*					Report
	1:2	1:4	1:8	1:16	1:32	
(1:1)*						
R	W	N	N	N	N	Reactive undiluted only or 1 dil +
R	R	W	N		N	Reactive, 1:2 dilution, or 2 dils
R	R	R	W	N	N	Reactive, 1:4dilution, or 4 dils
W	W	R	R	W	N	Reactive, 1:8 dilution, or 8 dils
N (ROUGH)	W	R	R	R	N	Reactive 1:16 dilution, or 16 dils
W	N	N	N	N	N	Weakly reactive, undiluted only or 0 dil
*R = Reactive                      W = Weakly Reactive                      N = Non-reactive						
+ A titre of 1:1 means that the serum was reactive in a dilution of 1 to 1. This may also be termed as "1 dil"						

The following table can be used to interpret syphilis test results.

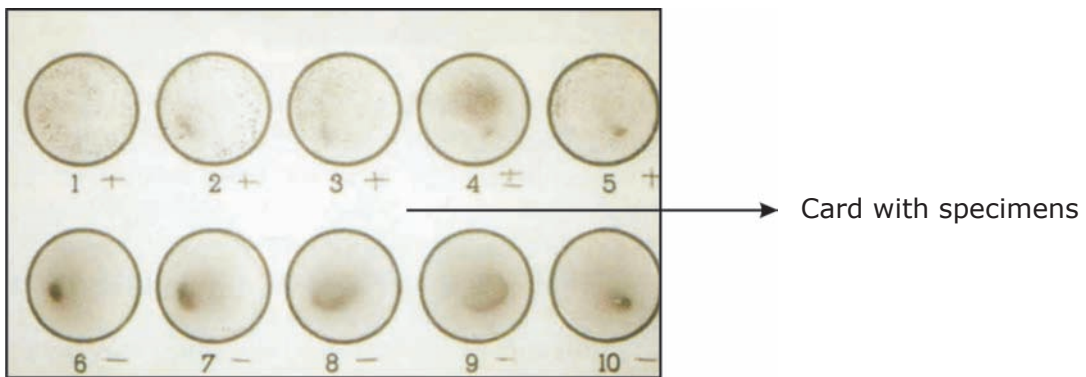
**Note:** where additional tests are not available, all patients with reactive RPR or VDRL should be treated.

**Interpreting serological test results**

	RPR	RPR titre	TPHA
Active infection	+	>1:8	+
Latent syphilis	+	Often <1:4	+
False positive	+	Usually <1:4	-
Successful treatment	+ or -	2 titres decrease (e.g. from 1:16 to 1:4)	+



**Figure 8:** Test serum is mixed with antigen and the card is placed on appropriate rotator



**Figure 9:** Reading RPR test results for 10 undiluted sera showing reactive (1,2,3,5, 4: Borderline) and non-reactive samples (6 – 10). The presence of small to large flocculated clumps indicates reactivity, whereas no clumping or a very slight roughness indicates non-reactivity

## CARE OF MICROSCOPE

Sr. No.	Topic	Page No.
1	How to Take Care of Microscope	32
2	Common Difficulties in Microscopy	33

## 1. How to take care of Microscope

The microscope is an instrument of precision, and care must be taken to preserve its accuracy. Proper handling and maintenance of the microscope is very important.

1. The instrument should be kept at uniform temperature, vibration free environment and not exposed to sunlight or any source of heat.
2. When not in use it must be protected from dust under a transparent plastic cover or in its box or at least with a clean clothe.
3. Put plenty of dry blue silica gel into a shallow plate and place it in a box when the microscope is kept in it. Silica gel is blue in colour when it is dry but when it becomes wet it turns pinkish. As soon as the silica gel becomes pink, change or heat it until it turns blue again and then use it.
4. Avoid exposing the microscope to moisture. Humidity may allow fungus to grow on the lens and cause rusting of the metal parts.
5. If the microscope has to be moved, it should be lifted by the upright limb and not held by the body-tube. Carefully place the microscope on the table. Before shifting the instrument to another place make sure you turn the eyepiece in the opposite direction by using the screw provided.
6. Switch off the source of light when not in use but is sure that you have reduced the intensity with the help of the rolling switch.
7. Clean the source of light on a regular basis.
8. With binocular microscopes dust may collect on the surfaces of the prism. This may be removed by passing a soft camel hair brush down the eyepiece tubes after removing the eye-pieces. (Do not open or remove the prisms, as this will alter the optical alignment.)
9. The oil immersion objective must be cleaned each day before and after use by wiping the front lens with a cotton handkerchief or fine tissue paper (lens paper).
10. Lens paper is used to clean eyepiece and condenser.
11. When cleaning the objective, do not use spirit or alcohol as these can damage them. Benzol or xylol must be used to remove dried oil, and if the oil is hard, repeated applications on a soft cloth are necessary.



12. Never let the oil immersion lens touches the smear & do not use excessive oil.
13. Use the fine focusing knob only while using the oil immersion lens. Do not remove the slide without lowering the stage.
14. Clean the remaining exposed non-lens parts of the microscope with a fresh piece of lint cloth.
15. On no account should the component parts of an objective be unscrewed.
16. Never rub the surface of a lens with a dry cloth because any hard particles on its surface may scratch the glass.

## 2. Common difficulties in Microscopy

Those beginning microscopy may encounter a number of troubles and the following hints are given to help overcome them:

### i. Inability to obtain a sharp image with the oil-immersion objective:

- Check that there is no dirt or dried oil adherent to the front lens of the objective.
- Check that the microscope slide carrying the object has not been put in upside down.
- Check that the immersion oil being used is not so viscous that the slide adheres firmly to the objectives and travels upwards with movements of the coarse adjustment.
- Check whether the specimen slide and cover slip has a film of dried immersion oil and dirt on it by a previous viewer.

If none of these steps improves matters, exchange the objective.

### ii. A dark shadow passes into the field with loss of definition of image.

The movement of an air bubble in the immersion oil usually causes this. Raising the objective so that the contact between the oil and the objective is broken and then refocus may cure the trouble.

**iii. Poor illuminations or the field of view in semidarkness:**

- Check that the flat and NOT the concave surface of the mirror is being used and adjust the mirror so that the light beam fills the field of view.
- Check that the condenser has been racked up to its full height. Occasionally it slips downwards in its mounting ring and must be pressed up as far as possible before it can be racked up close to the microscope slide.
- Check that the substage iris diaphragm is fully open.

**General instructions:**

- Wear gloves while handling blood or serum
- Do not perform mouth pipetting
- Use sodium hypochlorite to clean any spillage of blood or serum
- Report any accidents-however minor
- Use clean, dry test tubes for all tests
- Haemolysed, chylous, contaminated and highly icteric sera are unsatisfactory for testing
- Avoid bubble formation while diluting
- Sodium hypochlorite is used in discarding jars
- Store positive sera for controls
- Samples received from outside hospitals are tested only if found sterile after subculture on to blood agar

All samples are considered potentially infectious-especially for HIV & hepatitis B & C. Therefore adequate infection control precautions should be taken while handling blood and serum.

## BIO-MEDICAL WASTE DISPOSAL

Sr. No.	Topic	Page No.
1	Waste Disposal	36
2	Health Hazards Associated with Poor Hospital Waste Management	36

## 1. Waste disposal

Every health care facility generates waste. It is estimated that in most of the health care settings, about 85% of the waste generated is non-hazardous, about 10% is infectious wastes and 5% non-infectious wastes but hazardous waste. All these facilities generate waste, which require safe disposal.

**Medical waste:** The term medical waste is used to describe any waste, which is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining there to, or in the production or testing of biological specimens.

**Clinical waste:** It is defines as any waste coming out of medical care provided in hospitals or other medical care establishments. This definition does not include medical waste resulting from medical care in the home.

**Pathological waste:** This category includes human tissues, organs, body parts removed during surgery or autopsy or other medical procedures, and specimens of body fluids and their containers.

**Infectious waste:** Include all kind of wastes, which may transmit viral, bacterial, or parasitic diseases. In addition to infectious medical wastes, it includes infectious animal wastes from laboratories, and veterinary practices.

**Hospital waste can be categorized into:**

Non-hazardous	Hazardous
Biodegradable	Infectious
Non-biodegradable	Toxic

## 2. Health hazards associated with poor hospital waste management

- Injuries from sharps to all categories of hospital personnel;
- Nosocomial infection in patient from poor infection control and poor hospital waste management;
- Risk of infections outside hospitals for waste handlers and the general public;
- Risk associated with hazardous chemicals, drugs being handled by persons handling wastes at all levels, and
- Disposable, recycled, repacked and solid without being even washed.

The rising trends of HBV & HIV infection in the community and among health care providers has lead to an increasing awareness about the risk associated with this lackadaisical practice and the need to evolve and implement strategies for safe and sustainable methods of disposal of waste material generated at different sites in health care delivery system.

### Route of transmission of infection from infectious waste

- Through non-infected skin or by cuts and puncture of intact skin.
- Through mucous membranes such as splashing into eyes.
- Inhalation of dust particles containing germs.
- By ingestion through contaminated hands, contaminated water or foodstuffs.

### Categories of persons exposed to risk of infection

- Other patients attending the health care facilities.
- Medical and paramedical person providing health care.
- Persons involved in collecting and disposing the waste material.
- Those involved in clearing the instruments, floor, surfaces and washing of glassware and linen.
- If potential waste gets mixed with solid waste from other activities, the entire chain of workers/persons involved in solid waste disposal.
- If some of the disposable items are introduced in the market as sterile without being sterilized, the patients who receive treatment are at risk.

The Ministry of Environment and Forests has a classification, which is notified in the Bio-medical handling and management rules. These have been appended below:

Category	Type of waste	Treatment & disposal option
1	Human anatomical	Incineration/deep burial
2	Animal waste	Incineration/deep burial
3	Microbiology & biotechnology	Local/A/MW/incineration
4	Waste sharps	Chemical disinfection/A/MW & mutilation/shredding
5	Discarded medicine & cytotoxic drugs & disposal in landfills	Incineration/destruction

Category	Type of waste	Treatment & disposal option
6	Soiled wastes (linen)	Incineration/A/MW
7	Solid wastes (non sharp disposables)	Chemical disinfection/A/MW& mutilation/shredding
8	Incineration ash	Municipal landfills
9	Chemical waste	Chemical Disinfection & drain/landfills

**A**= Autoclave **MW** = Microwave

## Segregation and storage

Segregation at the point of generation of waste. It helps to reduce total treatment cost, reduce the impacts of this waste on the community and reduce the chances of infecting health care workers. Storage in different containers for different types of waste at the site of generation. Ministry of Forest and Environment has notified the types of containers and their colour codes for storage of different categories of hospital wastes as appended below:

### Colour coding & containers for bio-medical waste disposal

Colour code	Container Type	Category	Treatment options
Yellow	Plastic bags	1, 2, 3 & 6	Incineration/deep burial
Red	Disinfectant container/Plastic bag	3, 6 & 7	A/MW/Chemical disinfection
Blue/white transparent	Plastic bag/puncture proof container destruction & shredding	4 & 7	A/MW/Chemical disinfection, destruction & shredding
Black	Plastic bag	5, 8 & 9	Disposal in landfills

**A**= Autoclave **MW** = Microwave

## Handling and treating

The term refers to process that modify the waste in some ways before it is taken to its final resting place. Treatment is required to disinfect or decontaminate by chemical disinfection of infectious waste right at source. The following should be kept in mind while dealing with infectious waste:

- Infectious waste should be separated at the point of generation itself.

- Bins with lids lined with polythene bags, or with inner chamber for bucket should be used.
- A bins and bags should be labeled with biohazard symbol and if required, for the types of waste they have to be used for.
- If the hospital does not have a facility to treat the waste, it has to be sent to common facility.
- Personnel involved in infectious waste handling should be provided with protective wear and should be properly trained.
- Polythene bags placed in the bins have to be changed with each shift or when they are 3/4th full.
- Polythene bags carrying waste have to be sealed/tied at the top during transportation.

### Biohazard symbol

Chemical Bags containing infectious waste should have biohazard symbol.



### Chemical disinfection

It has a wide application in small health care facilities. A good disinfectant is bleach. The conc. prescribed by WHO is 10gm of bleach in 1 lit. water. The bleach solution should be prepared at the beginning of the shift.

### Disposal of needle sharps

Needles are either cut by a needle cutter & put in 1% Sodium hypochlorite or autoclaved or/and shredded or destroyed by needle destroyer.

### Disposal of other sharps

Scalpels, blades, ampoules, broken pieces of glass containers etc. should be put in a puncture proof container chemical disinfection/autoclaving/micro waving mutilation/shredding.

### Disposal of liquid waste

Hospital generates liquid waste, which is either infectious or chemical in nature. To avoid the exposure to general public, it is necessary that the waste be properly treated. The liquid pathological waste should be treated with a chemical disinfectant. The solution then be treated with a reagent to neutralize it. This can be flushed in sewer system.

### **Non-radioactive general waste**

Office waste-can sent for recycling. Kitchen waste can be composted. Non-biodegradable waste can be disposed off in municipal bins. Disposable biodegradable waste-biodegradation can be achieved by bio-digestion (using bacteria or earth worms or pit composting).



## DISINFECTION AND STANDARD PRECAUTIONS

Sr. No.	Topic	Page No.
1	Standard precautions for prevention of STI/RTI	42
2	Disinfection of instruments	47

## 1. Standard precautions for prevention of STI/RTI

The terms “standard precautions” and additional (transmission-based) precautions have replaced previous terms such as universal blood and body fluid precautions, universal precautions and barrier nursing.

Standard precautions require that health care workers assume that the blood and body substances of all patients are potential sources of infection, regardless of the diagnosis or presumed infectious status.

Additional (transmission-based) precautions are needed for diseases transmitted by air, droplets and contact.

A number of RTI can be spread from patient to health care provider or to other patients if basic precautions are not followed. Hepatitis B and C viruses and HIV are incurable infections that are easily transmitted by reuse of contaminated sharps. Because RTI are often asymptomatic, it is not possible to know which patients have an infection. For this reason, all the health care workers should follow standard precautions.

Standard precautions include the following-

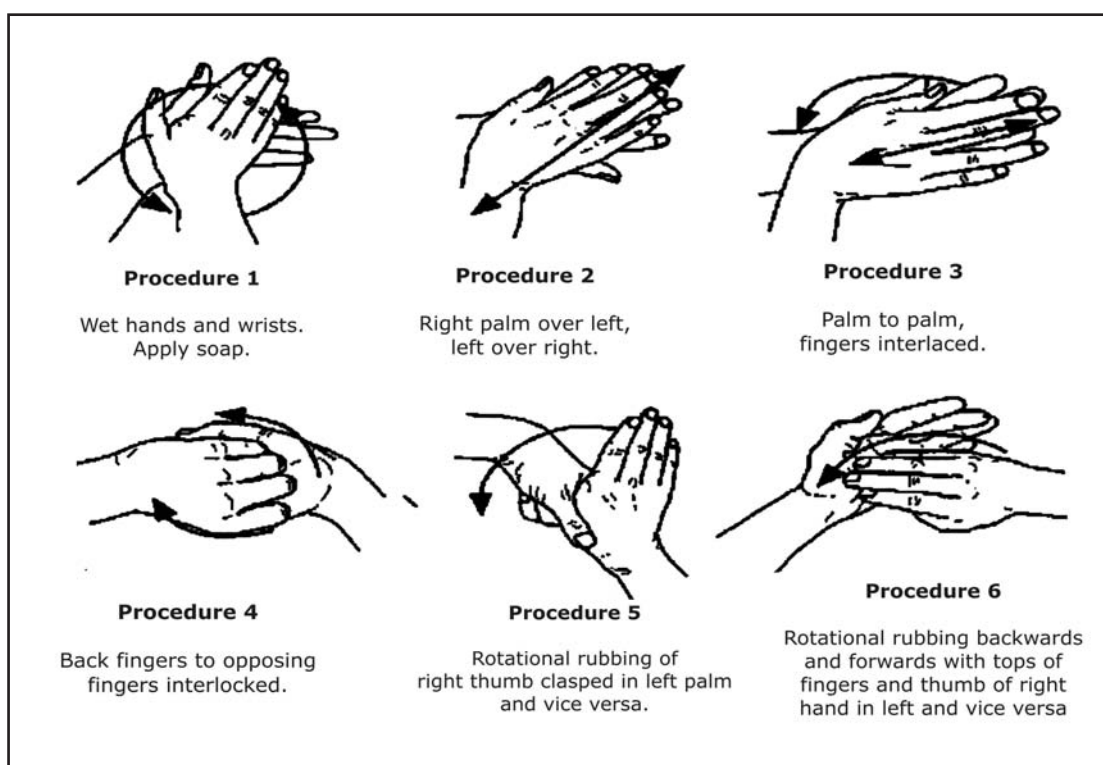
1. Hand washing and antisepsis (hand hygiene)
2. Use of personal protective equipment when handling blood, body substances, excretions and secretions
3. Appropriate handling of patient equipment and soiled linen
4. Prevention of needle-stick/sharp injuries
5. Management of health care waste

### i. Hand washing and antisepsis (hand hygiene)

Hand washing breaks the chain of infection transmission and reduces person—to-person transmission. It is the most important way to kill germs on the skin. You need to wash your hands even more thoroughly and for a longer time in the following situations:

- before and after helping someone give birth;
- before and after touching a wound or broken skin;
- before and after giving an injection, or cutting or piercing a body part;
- after touching blood, urine, stool, mucus, or fluid from the vagina;
- after removing gloves; and
- between contact with different patients

The hands must be washed for a minimum of 10-15 seconds, count to 30 as you scrub your hands all over with the soapy lather. Use soap or other disinfectant to remove dirt and germs. Use a brush or soft stick to clean under your nails, then rinse, using running water. Do not reuse the same water. Immersion of hands in bowls of antiseptics is not recommended. Common towels must not be used as they facilitate transmission of infection. If there is no clean dry towel, it is best to air-dry hands.



**Figure:** Hand washing procedures

**Source :** World Health Organization, Regional Office for Western for Pacific. Interim guidelines for national SARS Preparedness. Manila: WHO,2003, Page 45.

- Hand washing is the simplest and most cost-effective way of preventing the transmission of infection
- The hands must be washed for a minimum of 10-15 seconds with soap or other disinfectant
- Common towels must not be used as they facilitate transmission of infection

## ii. Use of personal protective equipment when handling blood, body substances, excretions and secretions

Using personal protective equipment offers protection by helping to prevent micro-organisms from:

- Contamination of hands, eyes, clothing, hair
- Being transmitted to other patients and staff

### Personal protective equipment includes:

- Gloves
- Masks
- Aprons
- Gowns
- Caps/hair covers

### Gloves:

- Use of gloves (clean, non-sterile) or a piece of plastic for handling dirty bandages, cloths, blood, vomit or stool.
- Disposable gloves should not be reused
- Gloves must be changed not only between contacts with different patients but between tasks/procedures on the same patient to prevent cross-contamination between different body sites.

Personal protective equipment must be used effectively, correctly and at all times where there is contact with patient's blood, body fluids, excretions and secretions may occur

## iii. Appropriate handling of patient equipment and soiled linen

Ensure that all reusable equipment is cleaned and reprocessed appropriately before being used on another patient. Keep bedding and clothing clean. This helps in keeping sick people comfortable and helps in preventing skin problems. Handle clothing and/or sheets carefully, which are stained with blood, urine, stool or other body fluids. Separate from other laundry for washing. Dry laundry thoroughly in the sun if possible or iron after drying.

#### **iv. Prevention of needle-stick/sharp injuries**

All the used disposable syringes and needles, scalpel blades and other sharp items should be placed in a puncture resistant container having a proper lid. These containers must be located close to the area. Never recap or bend needles.

#### **v. Management of health-care waste**

Daily collection of waste must be encouraged and uncollected, long stored waste or waste within the premises must be avoided. The bio-medical waste should be segregated into containers/bags at the point of its generation into colour-coded containers/bags. Following table gives the colour, coding, type of containers used and multiple treatment options for disposal of the bio-medical waste.

**Table: Management of health care waste**

Colour coding	Type of container	Waste category	Treatment and disposal
Yellow/Red	Plastic bag	Human anatomical waste (Human tissues, organs, body parts)	Incineration/deep burial*
Blue/white translucent	Puncture proof container	Waste sharps (Needles, syringes, scalpels, blades, glass etc. that may cause puncture and cuts)	Chemical treatment# / autoclaving/ shredding##
Black	Plastic bag	Discarded medicines (Wastes comprising of outdated, contaminated and discarded medicines)	Incineration, destruction and drug disposal in secured landfills
Yellow/Red	Disinfected container/ plastic bag	Solid waste (Items contaminated with blood and body fluids including cotton, dressings, linen, beddings or other material contaminated with blood)	Incineration/ autoclaving
-	-	Liquid waste (waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities)	Disinfection with chemical treatment# and discharge into drains

\* Deep burial should be done in a secure area. Burial should be 2 to 3 meters deep and at least 1.5 meters above the groundwater table.

# Chemical treatment using at least 1% hypochlorite solution or any other equipment chemical re-agent. It must be ensured that chemical treatment ensures disinfection

## Shredding must be such so as to prevent unauthorized use of sharp waste.

Standard precautions require that health care workers assume that the blood and body substances of all patients are potential sources of infection, regardless of the diagnosis or presumed infectious status. Additional (transmission-based) precautions are needed for diseases transmitted by air, droplets and contact. A number of RTI can be spread from patient to health care provider or to other patients if basic precautions are not followed. Hepatitis B and C viruses and HIV are incurable infections that are easily transmitted by reuse of contaminated sharps. Because RTI are often asymptomatic, it is not possible to know which patients have an infection. For this reason, all the health care workers should follow standard precautions.

## 2. Disinfection of instruments

Disinfect or sterilize equipment and instruments. Instruments must first be washed and then disinfected if they are to be used to:

- Cut or pierce skin;
- Give an injection;
- Cut the cord during childbirth;
- Examine the vagina, especially during or after childbirth, a miscarriage, or an induced abortion;
- Perform any transcervical procedure.

### High-level disinfection: three steps

*Cleaning instruments and equipment to get rid of nearly all the germs is called high-level disinfection. The following procedures could be followed to achieve it:*

- 1. Soaking:** Soak instruments for 10 minutes in 0.5% solution of bleach (chlorine). Soaking instruments in bleach solution will help protect you from infection when cleaning them. If you do not have bleach, soak your instruments in water.
- 2. Washing:** Wash all instruments with soapy water and a brush until each one looks very clean, and rinse them with clean water. Be careful not to cut yourself on sharp edges or points. Wear gloves when washing instruments; if possible, use heavy gloves.
- 3. Disinfecting:** Steam or boil the instruments for 20 minutes.
  - To steam them, you need a pot with a lid. The water does not need to cover the instruments, but use enough water to keep steam coming out of the sides of the lid for 20 minutes. Do not overload with instruments. No instruments should protrude above the rim of the pot.
  - To boil them, you do not need to fill the whole pot with water. But you should make sure the water covers all the instruments in the pot for the entire time. Put a lid on the pot.
  - For both steaming and boiling, start timing the 20 minutes after the water with the instruments is fully boiling. Do not add any new instrument to the pot once you begin to count.

**Table shows how to make a disinfection solution of 0.5%, 1% and 2% available chlorine**

Product	Chlorine available	How to dilute to 0.5%	How to dilute to 1%	How to dilute to 2%
Sodium hypochlorite – liquid bleach	3.5%	1 part bleach to 6 parts water	1 part bleach to 2.5 parts water	1 part bleach to 0.7 parts water
Sodium hypochlorite - liquid	5%	1 part bleach to 9 parts water	1 part bleach to 4 parts water	1 part bleach to 1.5 parts water
NaDCC (sodium dichlor – oisocyanurate) - powder	60%	8.5 grams to 1 liter water	17 grams to 1 liter water	34 grams to 1 liter water
NaDCC (1.5g/ tablet) - tablets	60%	6 tablets to 1 litre water	11 tablets to 1 litre water	23 tablets to 1 litre water
Chloramine - powder	25%	20 grams to 1 litre water	40 grams to 1 litre water	80 grams to 1 litre water

**Note:** Bleach solution becomes unstable rapidly, hence it needs to be freshly prepared daily or changed on becoming dirty/turbid. Chlorine bleach can be corrosive. Protect metal instruments by thoroughly rinsing them with water after soaking for 10 minutes.

### Cleaning of the health centers

Wet mopping must clean patient care areas. Only dry sweeping is not recommended. Any areas visibly contaminated with blood or body fluids should be cleaned immediately with detergent and water.



**Table: Common disinfectants used for environmental cleaning in health centers**

Disinfectants	Recommended use	Precautions
<b>Sodium hypochlorite</b> 1% In-use dilution, 5% solution to diluted 1:5 in clean water	Disinfections of material contaminated with blood and body fluids	<ul style="list-style-type: none"> <li>• Should be used in well-ventilated areas</li> <li>• Protective clothing required while handling and using undiluted solutions</li> <li>• Do not mix with strong acids to avoid release of chlorine gas</li> <li>• Corrosive to metals</li> </ul>
<b>Bleaching powder</b> 7g/litre with 70% available chlorine (Table shows dilutions for bleach)	Toilets/bathrooms – If liquid bleach is not available, this may be used	<ul style="list-style-type: none"> <li>• Same as above</li> </ul>
<b>Alcohol</b> (70%) Isopropyl, ethyl alcohol, methylated spirit	Smooth metal surfaces, tabletops and other surfaces on which bleach cannot be used	<ul style="list-style-type: none"> <li>• Flammable, toxic, to be used in well-ventilated area, avoid inhalation</li> <li>• Kept away from heat source, electrical equipment, flames, hot surfaces</li> <li>• Allow it to dry completely, particularly when using diathermy as it can cause diathermy burns</li> </ul>

**Note:** A neutral detergent and warm water solution should be used for all routine and general cleaning. When a disinfectant is required for surface cleaning, e.g. after spillage or contamination with blood or body fluids, the manufacture's recommendation for use and occupational health and safety instruction should be followed.



## Annexure - I

### REFERENCES AND SOURCE

We gratefully acknowledge the use of material that has been adapted from the following sources:

Source	Publication	Year
AVSC International	Sexually Transmitted and Other Reproductive Tract Infections	2000
Pathfinder International	Comprehensive Reproductive Health and Family Planning Training Curriculum (Module 12)	2000
World Health Organisation	Guidelines for the Management of Sexually Transmitted Infections	2003
World Health Organisation	Sexually Transmitted and Other Reproductive Tract Infections – A Guide to Essential Practice	2005
World Health Organisation	Draft Global Strategy for the Prevention and Control of Sexually Transmitted infections	2005
Engender Health	Sexually Transmitted Infections – Online minicourse	2006
Government of India	National Guidelines on Prevention, Management and Control of Reproductive Tract Infections including Sexually Transmitted Infections	2006

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