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Sexually Transmitted Diseases (STD)

Diverse pathogens that have the ability to cause disease in genital system (for women and men), but there are diseases common in both sexes as it can be to one that passed on to others through sexual contact, for example, gonorrhea, and syphilis and chlamydia infection which include urethritis, or inflammation cervix and others in although the involvement of etiology and disease, but shows the difference in severity of symptoms as well as the site of injury and sampling between the two sexes. On the basis of gender divide venereal diseases to:

- **Genital infection in women**

Consists vaginal secretions normal of water and salts, epithelial cells, fatty acids, proteins, sugars, and pH is (4.5) also contains the secretions of normal flora and the most important and most abundant are *Lactobacillus acidophilus* and other species belonging to this species in addition to *E. coli* and Coagulase-ve *Staph* and *Gardnerella vaginalis* and Non hemolytic *Streptococci* and *Prevotella* and *Bacteroid fragilis* as well as the yeast *Candida*.

The imbalance of microorganisms between normal flora in the vagina and increase a certain type of those normal flora is higher than the normal level on the one hand and reducing the number of members of the sex *Lactobacillus* on the other hand is the main factor to get vaginal infections, or called (Vaginitis), which may encourage a range of factors paved the other such as pregnancy,age, use of contraceptic pregnancy, fluctuation of the immune response, the incidence of diabetes *Diabetes mellitus*, poor nutrition, as well as the random use of antibiotics.

A-Type of vaginitis

Vaginal infections include the following:

1-Bacterial vaginosis and called Non specific vaginitis

It is the most species and the spread of the main cause of *Gardnerella vaginalis* and *Mycoplasma hominis* and some kinds of anaerobic bacteria such as *Bacteriodes fragilis*.

The most important symptoms that may appear in this injury is the smell of fish emanating from the vaginal secretions, especially after adding a drop of solution KoH concentration of 10%, and these secretions sticky textures of white color yellowish or greenish and pH higher than 4.5 observed in microscopic examination of the appearance of cells clue.

Clue cells: Epithelial cells lining the wall of the vagina is noted on the outer enveloping of a large number of adhesion of bacteria causing the inflammation and not invade these bacteria to epithelial cells, so cells do not consist abscess (pus cell) and not see the microscopic examination of the so-called screening non specific, disappear as *Lactobacillus* bacteria that resides in the case of natural abundance.

Is a take swab of the high vaginal swab (HVS) and add to it (1-2) ml of saline solution and preparation, two slides, one examined directly and the other pigmentation gram stain and watch the epithelial cells (Clue cells) in, two slides as

well as the presence and no presence bacteria *Lactobacillus* and usually for diagnosed this type of injury depending on the direct examination and there is no need for culture laboratory only when the frequency of injury and not see the pus cell. Diagnose bacteria and forms through the slide stained gram.

2- Vulvo Vaginal Candidiasis

The main cause is yeast candida, their kinds, the most currency *Candida albicans* and *Candida tropicalis* and exposed most of the women during the fertile period (from the age of puberty to menopause) to infection with the type of inflammation, especially after the treatment phase as antibiotics affect on the bacteria of normal flora in the vagina and especially drugs Metronidazol as inhibits the growth of bacteria, *Lactobacillus* and allowing increasing numbers of yeast and cause injury and invasion of the lining of the vagina.

Diagnosed symptoms by irritation and itching and the appearance of vaginal secretions cheese like dischary and be attached to the wall of the vagina in a thick layer and a yellowish white color and pH less than (4.5). And increase the severity of symptoms during pregnancy or oral contraceptives.

Taken swab (HVS) works including wet film and examine direct and see the budding yeast cells in addition to pseudomycelium and true with the presence of pus cell.

For the purpose of isolating yeast *Candida* using Central Sabouroud agar and add him antibiotic chloramphenicol with 0.5 g / liter to inhibit the growth of bacteria. Most probably do not need to have the culture because the previous specifications microscopic sufficient evidence of injury.

3- Trichomoniasis

The main cause is the parasite *Trichomonas vaginalis* as infect patient be very irritation and secretions to yellow-green and mixed strings of blood and unpleasant smell and pH equal to (4.5).

Samples collect vaginal swabs and swabs from the urethra and cervix for microscopic examination and normal saline is added to the swabs and examined directly and watch the parasite with the presence of pus cells and cell RBC.

B- Cervisitis

The most important causes of this disease, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

For the purpose of the examine of this type of injury take swabs of the endocervical because to avoid microbiological contamination of normal flora found in this region.

C- Uterine sepsis

The most important causes of *Mycoplasma hominis* and *Bacteroides* and *Staphlococcus aureus* and *Streptococcus pyogens* for the purpose of examine the injury taken swabs of the endocervical swabs.

D- Genital ulceration

The most important causes are the bacterium *Treponema pallidum* and *Haemophilus ducreyi.*

E- Tuberculosis of uterus

The main causative bacteria *Mycobacterium tuberculosis.* Samples collect in cases genital ulceration and tuberculosis of uterus either by a biopsy, from the area of injury or swab from the cervix or the lining of the uterus.

F- Viral diseases

Viruses infect the genital area where the virus causes Cytomegatovirus case congenital infection. And the virus causing human papilloma virus status cervical cancer (Viral wart). And the virus Human Immunodeficiency virus (HIV) cause a condition Acquired Immunodeficiency Syndrome (AIDS).

In the case of gonorrhea infection swabs taken from the cervix are not taken (HVS), because of the gonorrhea bacteria can grow in high pH, and sometimes to grow, but weak growth of the bacteriological culture while the normal flora in the vaginal secretions overcomes on them. The direct examination notes epithelial cells and within pairs of bacteria may be outside the cells in after times.

- **Genital Infection in Man**

It is the most important diseases of the male genitalia:

1- Urethritis

Divided into two depending on the type of causes:

- **Gonococcal urithritis**

Cause of injury when the bacteria *Neisseria gonorrhoeae* appear in the direct examination or during the culture bacteriological on selective media (Thayer martin agar) where the cases disease is characterized by pus secretion from genital urinary tract. The second type Non Gonococcal urithritis the main cause of it is *Chlamydia trachomatis.*

2- Prostatitis

And most cases are caused by infection with gonococci or *Chlamydia*. As some cases of chronic, or at least severe from acute (sub acute), especially among elderly men goes back to the infection with bacteria of fecal coliform bacilli bacteria or intestinal Enterococci which is the bacterium *Enterococcus faecalis*.

3- Ulceration

The main cause is the virus Herpes simplex (type2), as well as bacteria, *Treponema pallidum* and *Haemophilus ducreyi* and *Chlamydia*.

- **Collection of specimens and Urethral & Vaginal Exudate Cultures**

These tests focus on existence of microorganisms that cause sexually transmitted diseases and caused mostly by:

***Neisseria gonorrhoeae* and *Chlamydia species* and *Tryponema pallidum* and symptoms suffer patients with gonorrhea pain when painful urination in addition to emergence urethral secretion**, collected secretions from infections genital directly from opening urethra by a glass slide sterile and clean or using swab cotton in discharge or secretions. for purpose of culturing sample may be used among transport media to transport samples to laboratory.

When staining exudate by Gram stain indicates show bacteria Gram negative kidney bean shaped diplococcal of injury gonorrhea disease, and difficult to diagnosis in event of females infection because large numbers bacteria in addition to diversity of normal flora specific to vaginal tract and to investigate ***Neisseria gonorrhoeae*** using swabs from **cervix and urethra** and cultured especially media is **Thayer martin agar** and incubating of 5-10% of CO_2 and using media such as blood agar and **Horse blood agar** and **Chocolate agar** and **Macconkey agar** and **Sabouraud agar** to investigate for microorganisms pathogens such as ***Haemophilus ducreyi*, and *Streptococcus pyogenes* and *Staphylococcus aureus* and *Candidia albicans***.

As for bacteria ***Tryponema pallidum*** is bacteria belonging to the group of bacteria Spirochetes and causing syphilis there cannot growth on culture media laboratory, but examine microscopically using special techniques such as **drake-field microscopy** as possible to see shape of morphology and is diagnosed using **serological tests**, but at same time care must be taken of wrong diagnosis because it is likely presence of spirochetes bacteria other than not pathogenic in same sample.

For detection of Herpes simplex virus infection used screening **ELISA or immunofluorescence Abs** and **PCR**.

- **Respiratory Tract Infection**

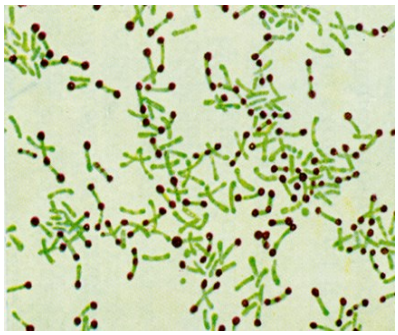
Diphtheria is a rare disease the infection with toxin-producing strains of a Gram-positive bacterium, ***Corynebacterium diphtheriae***, causes the disease. Sites of infection are primarily the respiratory mucosa (**respiratory diphtheria**) and the skin (cutaneous diphtheria). Rarely, extra-respiratory mucosal sites (e.g., eye, ear, genitals) may be affected. Humans are the only known reservoir of C. diphtheriae. The disease is transmitted from person to person by respiratory droplets or direct contact with respiratory secretions, discharges from skin lesions or, rarely, fomites.

The onset of respiratory diphtheria is insidious and begins after an incubation period of 2–5 days (range 1–10 days). Initial symptoms of **illness include a sore throat, difficulty in swallowing, malaise, and low-grade fever.** The characterization of respiratory diphtheria is the presence of a **tough, grayish-white pseudomembrane over the tonsils, nasopharynx, or larynx**. The **pseudomembrane** is strongly adherent to the underlying tissue, and attempts to dislodge it usually result in bleeding. Inflammation of the cervical lymph nodes and swelling of the surrounding soft tissue of the neck can give rise to a "**bull-neck**" appearance, which is a sign of severe disease. **Diphtheria toxin** may be **absorbed from the site of infection and result in systemic complications, including damage to the myocardium, nervous system, and kidneys**.

Non–toxin-producing strains of *C. diphtheriae* can also cause disease. It is generally less severe, potentially causing a mild sore throat and, rarely, a membranous pharyngitis, although invasive disease, including bacteremia and endocarditis, has been reported.

Identification Key Tests

- Albert staining identifies *C. diphtheriae*. *C. diphtheriae* produces characteristic metachromatic granules that appear bluish black and the body appears green or bluish green. Methylene blue is also used for staining, which makes the granules appear red.



***Corynebacterium diphtheriae* bacteria**

- Polymerase chain reaction (PCR) testing is performed by CDC, which can be done to confirm a toxigenic strain infection. The PCR test detects the regulatory gene for toxin production and the diphtheria toxin gene.

- Other diagnostic tests include isolation of *C. diphtheriae* on culture, biotype (i.e., substrain) determination, and toxigenicity testing. **Toxigenicity testing is performed by using the Elek test**, which determines whether the isolated *C. diphtheriae* produces toxin.

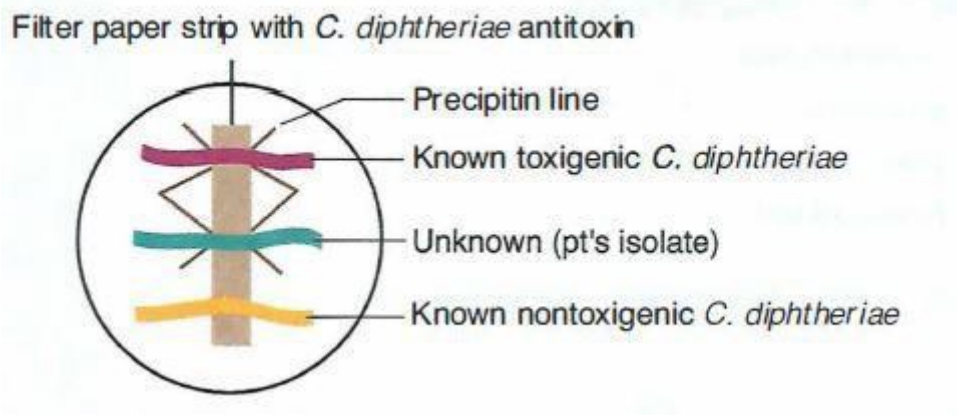
- **ELEK TEST**

The Elek culture plate precipitin test is routinely used for the detection of **exotoxin from toxigenic strains of *Corynebacterium diphtheriae***. The test for toxigenicity, which detects the **potent exotoxin**, **a phage-encoded protein**, is the most important test and should be done without delay on any suspect isolate that is found by routine screening or while investigating a possible case of diphtheria. The toxigenic species *C. diphtheriae* acquire this characteristic when infected by the family of β-phages or other families of corynephages.

- **The Elek test principle**

The Elek test principle **A filter paper strip impregnated with diphtheria antitoxin** is buried just beneath the surface of a special agar plate before the agar hardens. Strains to be tested, known positive and negative toxigenic strains are streaked on the agar's surface in a line across the plate, and at a right angle to the antitoxin paper strip. After 24 hours of incubation at 37° C, plates are for the presence of **fine precipitin lines at a 45-degree angle to the streaks**. **The presence of precipitin lines**

indicated that the strain produced toxin that react with the antitoxin.



Diphteria toxin – mode of action

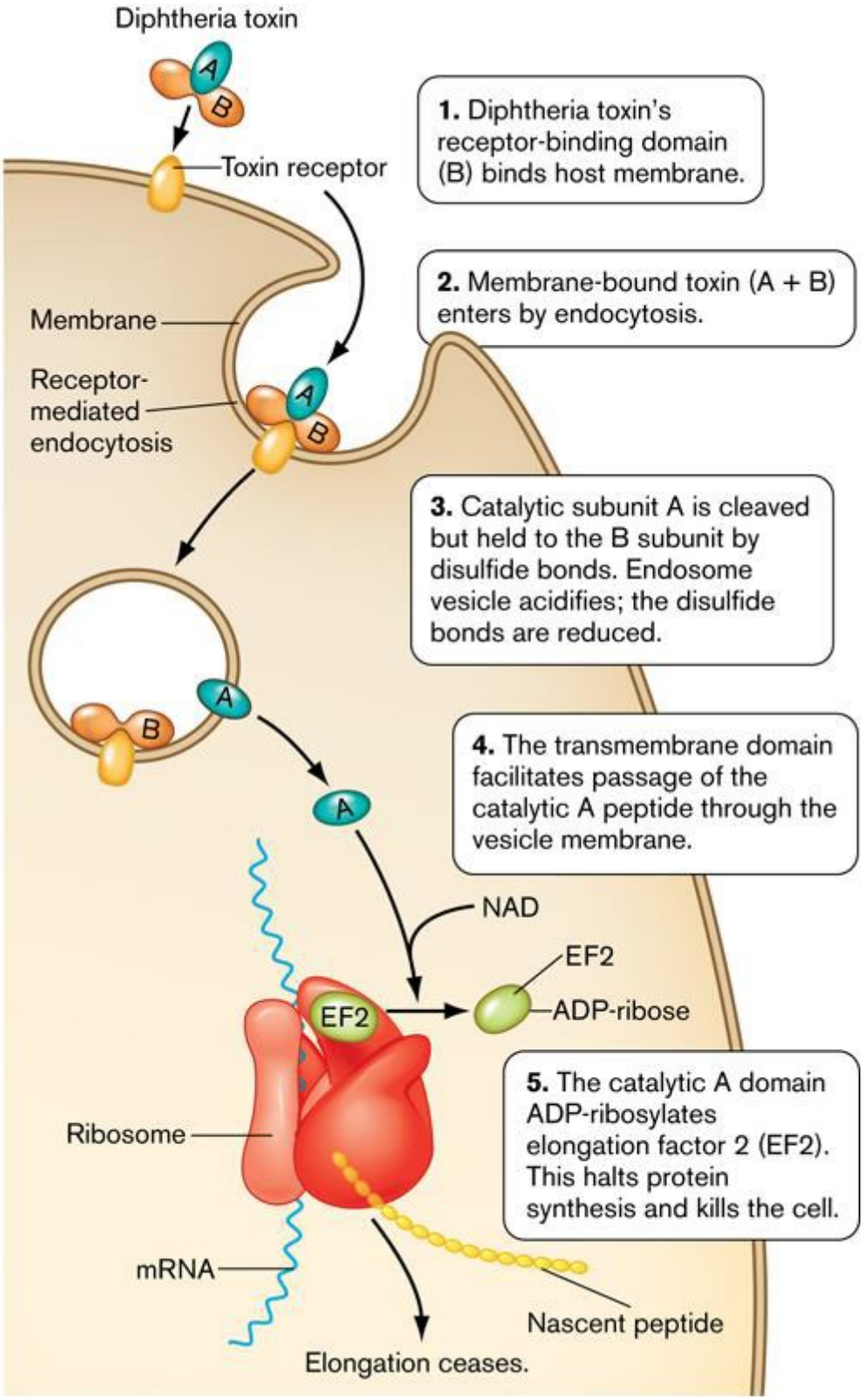
Diphtheria toxin (DT) is an extracellular protein of *Corynebacterium diphtheriae* that inhibits **protein synthesis and kills susceptible cells**. Diphtheria toxin is a single polypeptide chain consisting of two subunits linked by disulfide bridges, known as an **A-B toxin**. Binding to the cell surface of the **B subunit** (the less stable of the two subunits) allows the **A subunit (the more stable part of the protein) to penetrate the host cell.**

The diphtheria toxin **catalyzes the transfer of NAD+ to a diphthamide residue in eukaryotic elongation factor-2 (eEF2)**, **inactivating this protein**. It does so by **ADP-ribosylating the unusual amino acid diphthamide.** In this way, it acts as a **RNA translational inhibitor**. The **acceptor is diphthamide, a unique modification of a histidine residue in the elongation factor found in archaebacteria and all eukaryotes, but not in eubacteria**.

The catalysed reaction is as follows:
NAD+ + peptide diphthamide nicotinamide + peptide N-(ADP-D-ribosyl) diphthamide

Diphtheria toxin has also been associated with the development of myocarditis. **Myocarditis secondary to diphtheria toxin is considered one of the biggest risks to non-immunized children**. Diphtheria toxin is extraordinarily potent. The lethal dose for humans is about 0.1 µg of toxin per kilogram of bodyweight. A massive release of toxin into the body will likely cause **lethal necrosis of the heart and liver**.

The toxin is labile, prolonged storage, incubation at 37° C for 4-6 weeks, treatment with **0.2-0.4% formalin, acid pH converts it to toxoid**.


Diphtheria toxin
A
B
Toxin receptor
1. Diphtheria toxin's receptor-binding domain (B) binds host membrane.
2. Membrane-bound toxin (A + B) enters by endocytosis.
Membrane
Receptor-mediated endocytosis
3. Catalytic subunit A is cleaved but held to the B subunit by disulfide bonds. Endosome vesicle acidifies; the disulfide bonds are reduced.
4. The transmembrane domain facilitates passage of the catalytic A peptide through the vesicle membrane.
NAD
EF2
ADP-ribose
5. The catalytic A domain ADP-ribosylates elongation factor 2 (EF2). This halts protein synthesis and kills the cell.
Ribosome
mRNA
Nascent peptide
Elongation ceases.

Cytogenetic: is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes. It includes routine analysis of G-banded chromosomes, other cytogenetic banding techniques, as well as molecular cytogenetics such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH).

G-banding or Giemsa banding is a technique used in cytogenetics to produce a visible karyotype by staining condensed chromosomes. It is useful for identifying genetic diseases through the photographic representation of the entire chromosome complement. The metaphase chromosomes are treated with trypsin (to partially digest the chromosome) and stained with Giemsa. Dark bands that take up the stain are strongly A, T rich. Banding can be used to identify chromosomal abnormalities, such as translocations, because there is a unique pattern of light and dark bands for each chromosome.

Comparative genomic hybridization is a molecular cytogenetic method for analyzing copy number variations (CNVs) relative to ploidy level in the DNA of a test sample compared to a reference sample, without the need for culturing cells. The aim of this technique is to quickly and efficiently compare two genomic DNA samples arising from two sources, which are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or sub-chromosomal regions (a portion of a whole chromosome). Ploidy is the number of sets of chromosomes in the nucleus of a cell

Fluorescence in situ hybridization (FISH): is a cytogenetic technique that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets (mRNA) in cells, circulating tumor cells, and tissue samples. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues.

A karyotype: (Greek karyon = kernel, seed or nucleus): is the number and appearance of chromosomes in the nucleus of a eukaryotic cell. The term is also used for the complete set of chromosomes in a species, or an individual organism. **Karyotypes describe the number of chromosomes**, **and what they look like under a light microscope**. Attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes, and any other physical characteristics. The preparation and study of karyotypes is part of cytogenetics. The basic number of chromosomes in the somatic cells of an individual or a species is called the somatic number and is designated 2n. Thus, in humans 2n = 46. In the germ-line (the sex cells) the chromosome number is n (humans: n = 23). So, in normal diploid organisms, autosomal chromosomes (not a sex chromosome)

are present in two copies. Polyploid cells have multiple copies of chromosomes and haploid cells have single copies. The study of karyotypes is important for cell biology and genetics, and the results may be used in evolutionary biology (karyosystematics) and medicine. Karyotypes can be used for many purposes; such as to study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events.

Chromosome Preparation includes:

- Culture blood in media and bovine calf serum
- Phytohemagglutinin (PHA) - a mitogen is added to stimulate white blood cells to divide in culture
- Centrifuge cells - white blood cells go to the bottom of the tube (red cells on top)
- Hypotonic solution - causes white blood cells to swell and ruptures the red cells
- Fixation of cells - stabilizes cells and chromosomes
- Drop cells onto slides - cells burst open and chromosomes fall out
- Band chromosomes by staining with Giemsa and analyze under the microscope.

How are chromosomes classified? By size and position of centromere:

1- Metacentric – centromere in the middle of the chromosome.
2- Submetacentric – centromere divides the chromosome into 1/3 and 2/3.
3- Acrocentric – centromere near the end of the chromosome.

Cytogeneticists use three things to tell chromosomes apart:

1- chromosome size.
2- the position of the centromere.
3- characteristic banding patterns of alternating light and dark bands (caused by staining the chromosomes with dyes).