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Genital system infection

Normal flora (Microbiome) of the genital tract:

Vagina	Anterior urethra
Lactobacillus (G+ve bacilli) – **Aerobic lactobacillus (Doderlein's bacilli) → main bacilli** – **Present only in the reproductive female life (Not present at prepuberty and diminishes after menopause).** – **Responsible for acidic PH of vagina d2 fermentation of glycogen to lactic acid.** – **High acidic PH is inhibitory to some bacteria (Staph, Strept, yeast 'candida').** **Anerobic streptococci (G+ve cocci), Diphteroid (G+ve bacilli), Coliform (G-ve bacilli) and yeast** – **Are also present as normal flora**	– **Staph epidermidis (G+ve cocci), علي الجلد** – **Enterococcus faecalis and some alpha-hemolytic streptococci. مش كتتتير** – **Some enteric bacteria (e.g. *E. coli*, Proteus) and** – **Corynebacteria, which are probably contaminants from the skin, vulva, or rectum.**

Classification of genital system infections and causative organisms in each type:

<table>
<tr><th>Sexually transmitted diseases, STDs (Venereal diseases):</th><th colspan="2">Non-venereal diseases:</th></tr>
<tr><td rowspan="2">a. السيلان Gonorrhea caused by N. gonorrheae.
b. الزهري Syphilis caused by T. pallidum.
c. Soft sore caused by Haemophilus ducreyi (H. ducryii).
d. Lymphogranuloma venereum caused by Chlamydia.
e. Nongonococcal uretheritis (NGU) caused by Chlamydia and Mycoplasma genitalium مهم يعني حاجة غير السيلان.
f. 3 H HIV, HBV & HSV-2.</td><td>In males: Prostatitis & urethritis.</td><td>In female: Salpingitis, oopharitis & endometritis</td></tr>
<tr><td colspan="2">Causative organisms:
– S. aureus
– S. pyogenes
– E. coli.
– P. pyocanea.
– Proteus.</td></tr>
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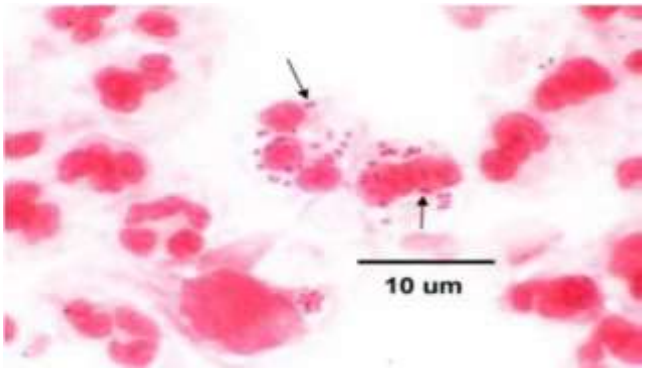
Neisseria gonorrhea

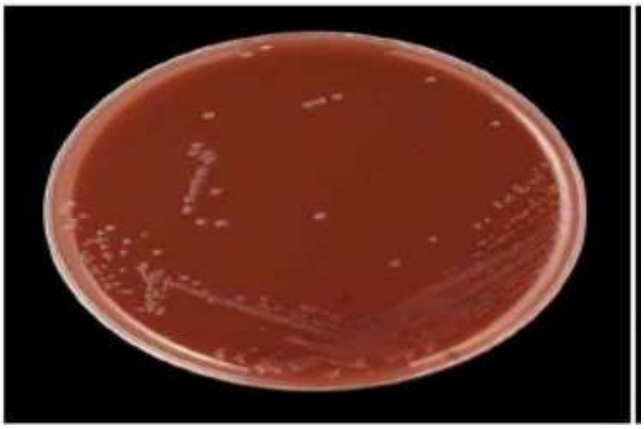


Morphology:

- **G-ve intracellular diploccoci (arrangement)** kidney-shaped
- Not spore forming, Non motile, Not capsulated.

Culture characters:

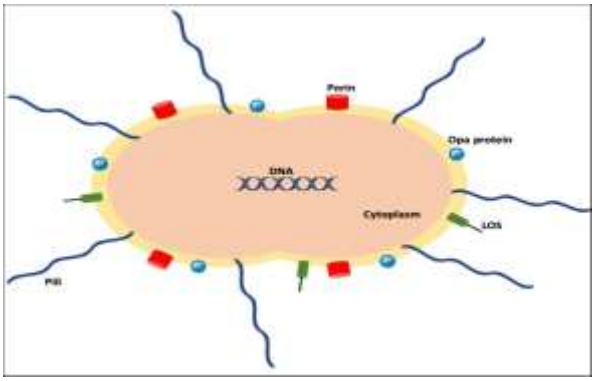
- O2: **Aerobic**
- Optimum temperature is 37°C
- CO2: **10% CO2** is required for growth.



Culture media:

- **Ordinary media: Cannot grow on it**
- **Enriched media: Grow on chocolate agar**
- **Selective media: Grow on Thayer-Martin media**

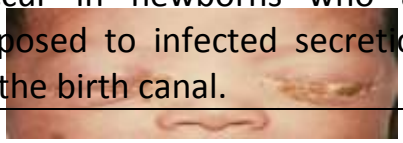
هن بتحب الشيكولاتة والعربيات (سيارة مارتن)



Virulence factors

- **Pili (fimbriae) and outer membrane proteins:** adhesion.
- **Endotoxin:** Lipooligosacchrides.
  زي أي جرام نيجتف
- **IgA proteases:** inactivate human IgA.

Manifestations: (Gonorrhea)

Male:	**Female:**	**Newborn: Ophthalmia neonatorum:**
Characterized by profuse purulent discharge	**Cervicitis:** Characterized by profuse vaginal mucopurulent discharge & sometimes**.** **Acute urethritis & dysuria.**	Occur in newborns who are exposed to infected secretions in the birth canal. 

	Vulvovaginitis: Occurs in girls 2-8 years of age.	

Laboratory diagnosis of *N. gonorrhea*:

1. **Sample:**

In acute disease	**In chronic disease:** (Scanty or no discharge)	
Profuse pus & secretions from the urethra or cervix.	**a. In females**: Cervical secretion is obtained after cervical irritation.	**b. In males**: 1. morning drop from the urethra 2. centrifuged deposit of urine 3. prostatic massage

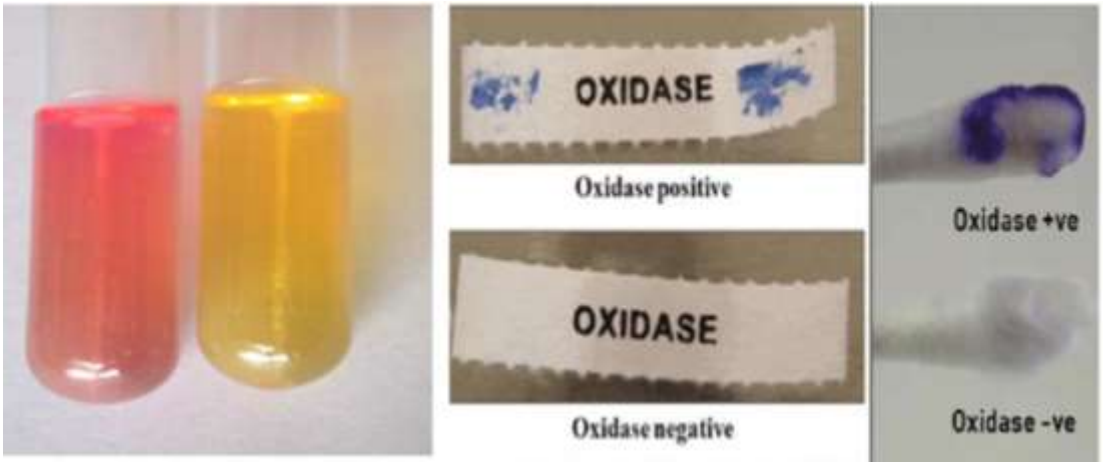
2. **Direct film stained with Gram stain:**

   Detection of intracellular Gram negative diploccoci is **diagnostic**

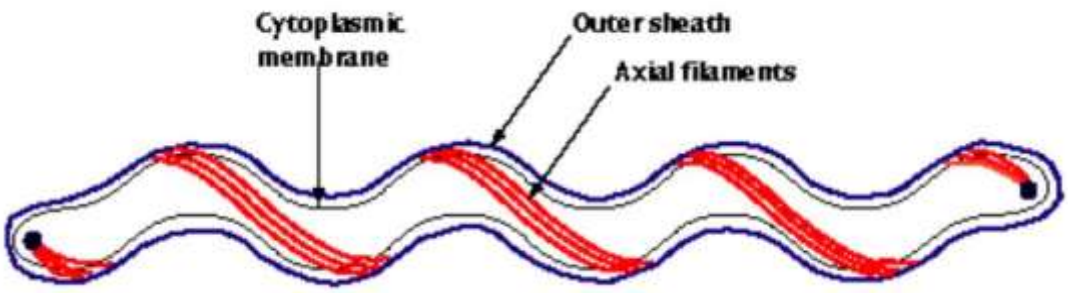
3. **Culture:** As before

4. **Identification of the obtained 'colonies' by**

- Film stained with Gram stain to show characteristic morphology (Gram negative diplococci, Non motile, Non spore forming).
- **Oxidase test: positive.**
- Sugar fermentation: *N. **g**onorrhea* ferment **g**lucose only **(with acid production only).**



Treponema pallidum (نوع من أنواع ال Spirochetes)



Morphology:

Slender, **Spiral** filaments with regular coils (coils are small and large in numbers), motile **فيها O**.	Cannot be seen by light microscopy but can be seen unstained by **dark field microscopy**.
Cannot be stained by Gram stain, can be stained by **Giemsa and Fontana stains**.	In **tissues** can be visualized by **silver** impregnation methods.
– Have axial filaments, which are otherwise **similar to bacterial flagella** – Filaments enable **movement** of bacterium by rotating in place.	

Culture characters:

- Pathogenic Treponema **UN cultivable**.
- Pathogenic strains: can grow **in testicles of rabbit or cultured rabbit epithelial cells**
- The cells have **a high lipid content (cardiolipin, cholesterol)**, which is unusual for most bacteria.
- **Microaerophilic**, Fastidious organism with narrow optimal ranges of pH **(7.2 to 7.4)**, and temperature **(30 to 37°C)**.

Pathogenesis of Syphilis:

- **Causative agent:** *T. pallidum*, strictly a human disease (Less than **10** organisms are capable of producing infection).
- **Mode of transmission: by sexual contact.**

The disease passes into 3 clinical stages:

Primary Syphilis: chancre
(Painless, indurated ulcer) develops at the site of inoculation and associated with **regional lymphadenopathy.** This lesion is filled with treponemes and is, therefore, **highly contagious.**

Secondary Syphilis:
Mucocutaneous rash. Mucous patches on mucous membranes and **wart-like lesions called condyloma lata** in moist intertriginous areas.
All these lesions are **highly contagious.**

Tertiary syphilis:

Is characterized by long-term complications: **gumma (granulomatous-like lesion), cardiovascular and neurosyphilis.**

Can affect all areas of the body and **be fatal.**

Congenital syphilis:

through placenta: fetus may die or borne live with congenital anomalies

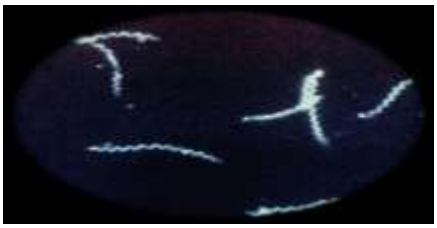
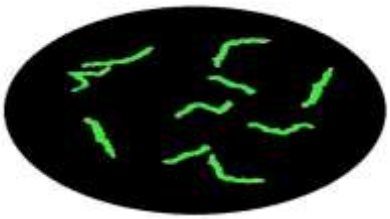
Laboratory diagnosis of Syphilis:

1. **Sample:**

Primary stage:	**Secondary stage:**	**Blood**
Exudate from **chancre**	**Mucous patches and skin eruptions**	for serology (Antibodies)

2. **Direct film:**

Unstained 'fresh wet' film	**Direct immune fluorescence (IF)**
Examined with dark field microscope for motile *treponemas*	Using fluorescein-labelled anti-*treponemal* antibodies examined by flurescent microscope

3. **Culture:** non cultivable
4. **Serology:**

Syphilitic patients produce 2 types of antibodies:

1st (specific): reacts only with treponemal antigens	2nd (Nonspecific): reagin antibodies react with lipid antigens e.g., cardiolipin

1. *Treponemal* antigen tests	**2. Non *treponemal* antigen tests**
Detect specific antibodies (antibodies directed against protein constituents of *T. pallidum*).	Which measure nonspecific antibodies (directed against lipid antigens, principally cardiolipin)
Examples are: a. Fluorescent *T. pallidum* antibody absorption **(FTA-ABS)**. b. *Treponema pallidum* immobilization **(TPI)** test c. Micro-hemagglutination *for T. pallidum **(MHA-TP)**.*	a. Venereal Disease Research Laboratory **(VDRL)** b. Rapid plasma reagin test **(RPR)** c. Wassermann test **[Complement fixation test]**
Use: Confirmation.	– Screening. – Epidemiological purposes. – Evaluate the effect of treatment
The treponemal tests often **remain reactive for life.**	The results of non-treponemal tests usually **parallel the extent of infection**; titers tend to be **highest during secondary syphilis** and **subside during subclinical infection (latency) or following antibiotic therapy.**

Treatment:

Penicillin is the drug of choice in the treatment of syphilis.

(Penicillin was found to be effective in eradicating syphilis of all clinical stages as well as the congenital infection).

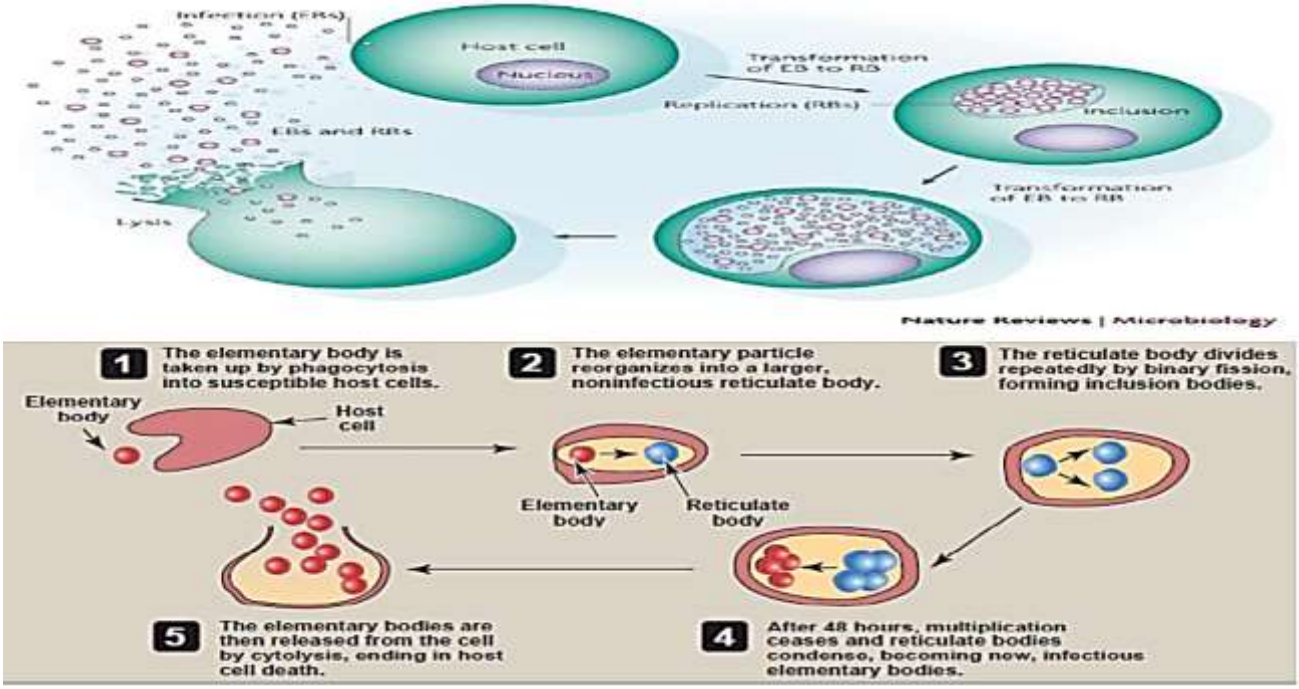
Chlamydia trachomatis

Chlamydial infections in genital system:

Lymphogranuloma venereum, is a venereal disease spread sexually characterized by genital lesions and **regional lymph node involvement (buboes)**	**Non gonococcal urethritis** in men and **acute salpingitis and cervicitis** in women.

***Chlamydiae* resemble viruses in the following:**	***Chlamydiae* resemble bacteria in the following:**
- Obligate intracellular - Cannot make ATP - Grow in cell culture - No peptidoglycan	- Have DNA, RNA, ribosome. - Have Inner and outer membrane & cell wall. - Susceptible to Antibiotics - Multiply be binary fission.

Life cycle of *chlamydiae*:



Elementary body	Reticulate body (RB)
– Small – Extracellular – Infectious form – Non-replicating – Released from ruptured infected cell	– Large – Intracytoplasmic – Non-Infectious form – Metabolically active and replicating – Within cells, the site of replication – appears as an inclusion body, which can be stained and visualized microscopically

Laboratory diagnosis of *chlamydia*:

1. **Sample:**

Scrapping from the urogenital tract.	Urethral or Cervical exudates.

2. **Microscopic examination:**

   Inclusion bodies in scraped tissue cells are detected by staining with **Giemsa or iodine** or by staining with **fluorescent monoclonal antibodies.**

3. **Culture:**

On McCoy cells	Yolk sac of embryonated egg

Isolation of chlamydia is possible by **yolk-sac** inoculation method and tissue culture in **McCoy cells (synovial carcinoma cell line)**

***Chlamydia trachomatis* in McCoy cells brown colored**

4. **Serological tests:**

   Detection of **chlamydial antigen** or **anti-Chlamydia antibodies**

5. **Serological tests:**

   DNA probes or PCR

Treatment:

- There is no available vaccines

- It is important to take precautions against sexually transmitted infections because chlamydia is so common and it doesn't produce symptoms.
- **Tetracycline and Erythromycin are the drugs of choice.**
- **Penicillin is not effective**

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Viral Genital Infections

Herpes viruses

- **Eight** human herpesvirus species are known.
- **All** have the ability to **enter a latent state** following primary infection and to be **reactivated** at a later time.
- **Structure:**
  - ☒ **Virion:** icosahedral.
  - ☒ **Genome:** Double-stranded DNA, linear.
  - ☒ **Envelope:** Contains glycoprotein spikes.
- **Classification of Herpesviruses:**
  - ☒ **They have been divided into three subfamilies:**

Alpha herpesvirinae	Beta herpesvirinae	Gamma herpesvirinae
Herpes simplex virus type 1 (HSV-1 or HHV-1).	**Cytomegalovirus** (HHV-5).	**Epstein-Barr virus** (HHV-4).
Herpes simplex virus type 2 (HSV-2 or HHV-2).	Human herpes virus type 6 (HHV-6).	**Kaposi's sarcoma-associated herpes virus** (KSHV) or (HHV-8).
Varicella – Zoster virus (VZV or HHV-3).	Human herpes virus type 7 (HHV-7).	

Herpes simplex viruses:

- There are 2 distinct herpes simplex viruses, type 1 and type 2.
- The two viruses cross-react serologically but some unique proteins exist for each type.
- **Transmission and Pathogenesis:**

	HSV-1	HSV-2
Mode of transmission	Primarily in saliva (kissing)	By sexual contact
Multiplies locally in the mucous membrane or abraded skin causing vesicular lesions.		
Site of lesion	Mainly orofacial lesions	Genital lesions
However, both types of HSV can infect oral or genital mucosa depending on regions of contact.		

- **Laboratory diagnosis:**
  - ☒ **Sample:** vesicular fluid.
  - ☒ Detection of **viral particles** by electron microscope.
  - ☒ Detection of **viral antigens** by immunofluorescence in vesicular fluid.
  - ☒ Detection of **HSV DNA by PCR.**
  - ☒ **Virus isolation** from herpetic lesions.
  - ☒ **Serologic diagnosis** (Detection of IgM or 4-folds rising titer of IgG).
  - ☒ **Histological staining (Giemsa stain)** of scrapings or swabs from the base of skin lesions.

Treatment

- ☒ **Acyclovir, Famciclovir, and Valacyclovir** are the treatment of choices.
- ☒ It shortens the duration of the lesion and decreases shedding of the virus.
- ☒ No drug treatment prevents recurrences.
- ☒ No effect on the latent state.

Human papilloma virus (HPV)

Morphology:

- ☒ HPV is a small, Icosahedral **non-enveloped** virus with a circular **double**-stranded DNA genome.
- ☒ There are more than 100 different types of HPV.
- ☒ The **most common** sexually transmitted virus.

Mode of Transmission:

- ☒ It requires **direct contact** with infected individuals e.g. sexual contact or contaminated surfaces e.g. common bathroom floors.

Clinical significance:

HPV type	Clinical lesion	Benign/ Malignant
Type-1	Planter wart.	Benign
Type- 2, 4	Skin warts.	Benign
Type-16, 18	Carcinoma (cervix, penis) (70%).	**Malignant**

Type-6, 11	Anogenital condylomas (90%) and laryngeal papilloma.	**Low oncogenic** potential

☒ **Condylomata (genital wart)** are irregular, soft, pink growths that are found on external genitalia or the anus.

- **HPV and cancer:**

☒ HPV virulence factors include proteins **(E6 and E7)** that are capable of **inactivating** tumor suppressor proteins (**P53**) → uncontrolled cell division and the development of cancer.

- **Laboratory Diagnosis:**

☒ Virus has not been isolated in cell line.

☒ Serologic tests rarely done (large number of serotypes).

☒ PCR to detect virus DNA is available.

☒ Screening by Pap smear for all women above 30 in USA (koilocytes).

- **HPV vaccine:**

☒ Virus-like particle (VLP) vaccine. (L1 and/ Or L2 protein).

☒ There are **3 versions** of the L1-only HPV vaccine.

- **Gardasil:** HPV16, 18, 6 and 11.
- **Cervarix:** HPV 16, 18.
- **Gardasil 9:** HPV16, 18, 6, 11, 31, 33, 45, 52, and 58.

☒ Given to females **10 - 25** years of age.

- **Treatment:**

☒ Most HPV infections resolve **spontaneously**.

☒ **Topical medications:**

- **imiquimod** (which stimulates the production of **interferon**).

☒ **Cryotherapy or surgery,** but these approaches are less effective for genital warts than for other types of warts.

☒ **Electrocauterization and carbon dioxide laser therapy.**

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Infection in Pregnancy

Congenital Viral Infections

Viral infections in pregnancy are major causes of maternal and fetal morbidity and mortality.

- **The clinical manifestations vary depending on:**
  - ☒ **Viral agent** (Some viruses results in congenital malformation).
  - ☒ **Gestational age at infection:** the early is the infection, the more dangerous is the outcome.
- **Mode of transmission:**
  - ☒ **Infections that develop in the neonate can be transmitted through:**

Trans-placental Through placenta	Perinatal (from vaginal secretions or blood)	Postnatal (from breast milk or other sources)

- **Causative organisms:**
  - ☒ **Infections causing congenital defects are described with "TORCH"**

    T (Toxoplasma), O (Others), R (Rubella), C (Cytomegalovirus), H (Herpes).
  - ☒ **Other viruses** now known to cause congenital infections include:

- Parvovirus B19 (B19V). - Varicella-zoster virus (VZV). - Adenovirus.	- Human immunodeficiency virus (HIV). - Zika virus. - Hepatitis E virus.

Prevention
General Measures: - Hygiene measures (washing hands). - Do not sharing drinking glasses or eating utensils with young children. - Do not kissing young children on the mouth or cheek. - Affected infants require regular follow-ups to monitor for hearing loss, ophthalmological abnormalities, and developmental delays.
Specific Measures: Primary (vaccination for varicella and rubella (prior to pregnancy).

Outcomes			
Spontaneous abortion.	**Premature birth.**	**Intrauterine growth restriction (IUGR).**	**Abnormalities in the CNS, the skeletal and endocrine systems, and the internal organs (e.g., cardiac defects, vision and hearing loss).**

Cytomegalovirus (CMV)

- **Structure:**
  - ☒ **Virion:** icosahedral
  - ☒ **Genome:** Double stranded DNA, linear
  - ☒ **Envelope:** Contains
  - ☒ **Glycoprotein spikes.**

- **Transmission and pathogenesis:**

Early in life:	Later in life:
• **Trans-placental** • **Within birth canal** • **Via breast milk.**	• **Saliva (most common route).** • **Sexually.** • **Blood transfusion.** • **Organs transplant.**

Clinical Significance:

A) Primary infection:
☒ **In healthy individuals may cause:** • **Asymptomatic infection** with intermittent shedding in saliva and urine. • **Infectious mononucleosis-like syndrome:** similar to EBV infection but heterophil antibodies negative.
☒ **Infection of immunodeficient patients:** • **Hepatitis and pneumonia** are common, In AIDS, (diarrhea and retinitis may occur). • **Congenital infections: the most common intrauterine viral infection:** • **In-utero, it causes:** abortion, still birth or cytomegalic inclusion disease: (Mental retardation, microcephaly, blindness and deafness). • **Perinatal infection** from the birth canal or from the milk: usually subclinical infection.
B) Latency and Reactivation:
Latency is established in monocytes, macrophages and kidney. Reactivation: repeated episodes of asymptomatic virus shedding over prolonged periods of times.

Laboratory diagnosis:

Direct Detection:	Virus isolation in cell culture:	Serological test
1. **Fluorescent antibody & histological staining** of inclusions in giant cells in urine and in tissue. 2. **PCR** for detection of CMV nucleic acid in tissues or body fluid as CSF.	**CPE is 2-3 weeks** (Typical swollen and translucent cells with intranuclear inclusion bodies (Owl eye) appearance.	**detect rising IgG titer or IgM.**

Diagnosis of CMV during pregnancy:

☒ **Mother:**

- Positive IgM
- PCR.

☒ **Fetus:**

- **Amniotic fluid** sample is taken and CMV is demonstrated either by PCR or isolation on tissue culture.

Prevention and treatment:

☒ **Prevention:** General preventive measures for intra-uterine viral infections.

☒ **Treatment:** Ganciclovir: is effective in immunocompromised patients.

Parvoviruses

- **Structure:**
  - ☒ **Virion:** icosahedral
  - ☒ **Genome:** Single-stranded DNA, linear
  - ☒ **Non-enveloped**
- **Clinical Significance:**

Dependo-viruses:	**Autonomous parvoviruses:**
They require helper virus for their replication which usually an adenovirus (e.g. Adeno-associated virus).	They are capable of independent replication (e.g. human parvovirus B19).

Human B19

- **Human B19 virus targets immature cells of the erythroid lineage (RBCs).**
- **Diseases caused:**
  - ☒ Transient aplastic crisis in patients with sickle cell disease.
  - ☒ **Erythema infectiosum** (**fifth disease or slapped cheek disease**).
    - A common childhood disease.
    - Associated with bright red rash of the cheeks.
    - Exacerbation of rash by sunlight, heat and stress.
  - ☒ Hydrops fetalis and fetal death:
    - Due to severe anemia if Infection occurs during pregnancy

- May leads to miscarriage (spontaneous abortion) if occurs before 20th week. Or stillbirth after 20th week of gestation.

Laboratory diagnosis: PCR

Prevention:

☒ The best approach is to recommend all pregnant women to avoid contact with children with current symptoms of infection.

☒ The risk to the fetus is reduced by:

- Correct diagnosis of the anemia (by ultrasound scans)
- Correct treatment (blood transfusions).

Rubella virus

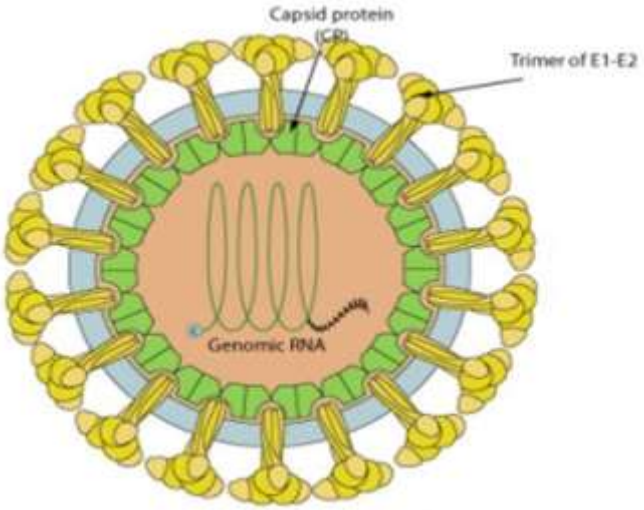
Structure:

☒ **Virion:** icosahedral nucleocapsid. Spherical shape.

☒ **Genome:** positive-sense, single stranded RNA

☒ **Enveloped with** hemagglutinin-containing spike-like projections.

☒ Belongs to **Togavireadea family**



Transmission:

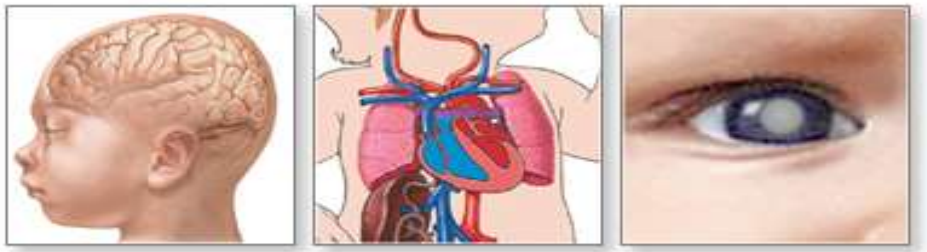
- ☒ The virus is transmitted via respiratory droplets.
- ☒ Initial replication of the virus occurs in the oropharynx and local lymph nodes.
- ☒ From there it spreads via the blood to the internal organs (including placenta) and skin.
- ☒ I.P. 14-25 days after infection, patient is infectious for most of this time.

Clinical Significance

- ☒ **The disease is characterized by:**
  - In 95% of adolescent patients 14-25 days (average: 18 days) after infection
  - Characteristic pink continuous maculopapular rash appears.
  - Posterior auricular lymphadenopathy are characteristic of the disease
  - In children, a mild febrile illness - less severe than measles.
  - In adults other than pregnant women: symptoms are rare.

Congenital rubella syndrome:

- ☒ Virus crosses placenta and multiplies in the fetus.
- ☒ Up to 85% of infants infected in the first trimester of pregnancy get congenital rubella syndrome (CRS) with low birth weight, deafness, CNS involvement, cardiac abnormality, eye abnormality and abortion.
- ☒ The earlier in pregnancy infection occurs, the worse the prognosis becomes.
- ☒ Fetus is persistently infected (presumably due to immature immune response) and continues to excrete virus after birth (a risk to doctors, nurses and other patients).



Prevention and control:

- ☒ **MMR vaccine:**
  - Trivalent live attenuated vaccine (MMR)
  - Usually given by sub cutaneous injection.
  - It is given at 12 – 15 months.
  - A single dose of the MMR vaccine gives around
  - 90% protection against measles and mumps and
  - 95-99% against rubella.
  - (For women infected during 1st trimester of pregnancy, therapeutic abortion is recommended).

Diagnosis of genital system infections

Q11: identify this film.

Q12: state type of microscope.

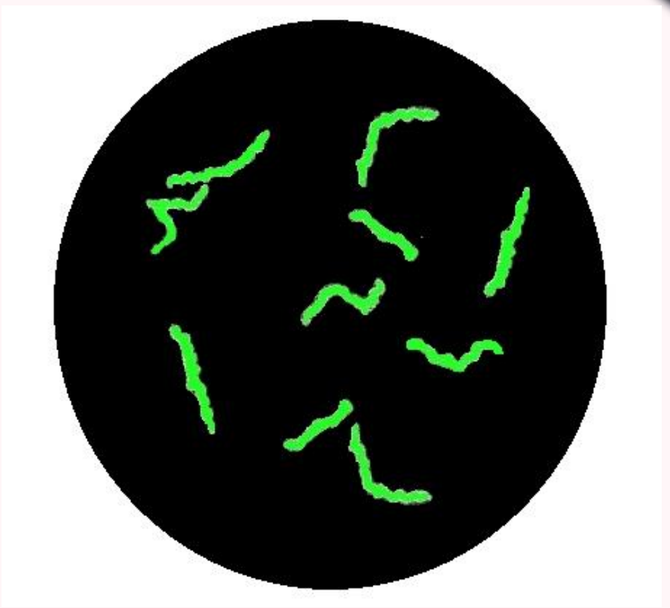
- Unstained ‘fresh wet’ film

Examined with dark field microscope for motile treponemas.

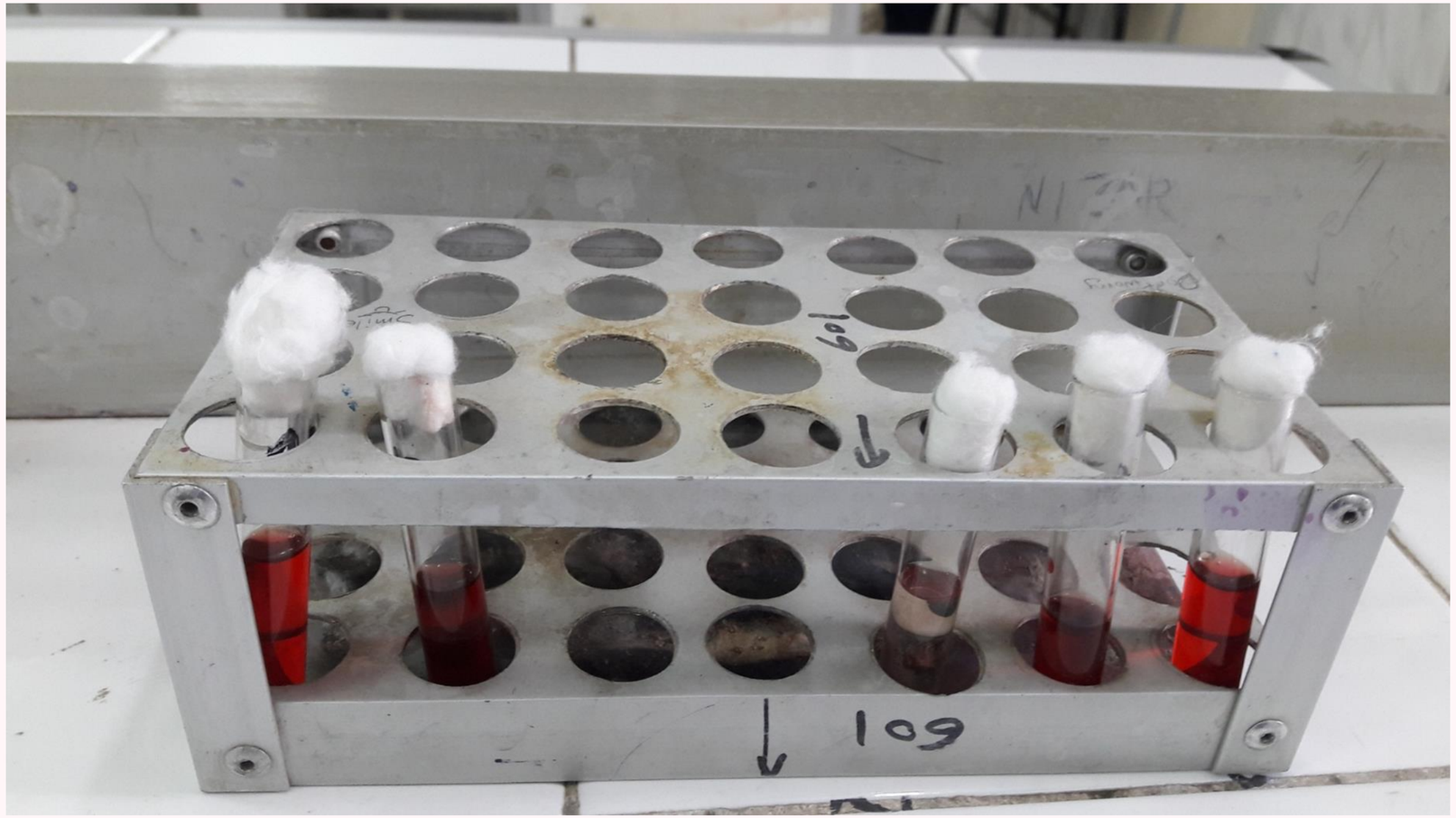
Q13: identify this film.

Q14: state type of microscope.

- Direct immune fluorescence (IF) Using fluorescein-labelled anti-treponemal antibodies examined by florescent microscope

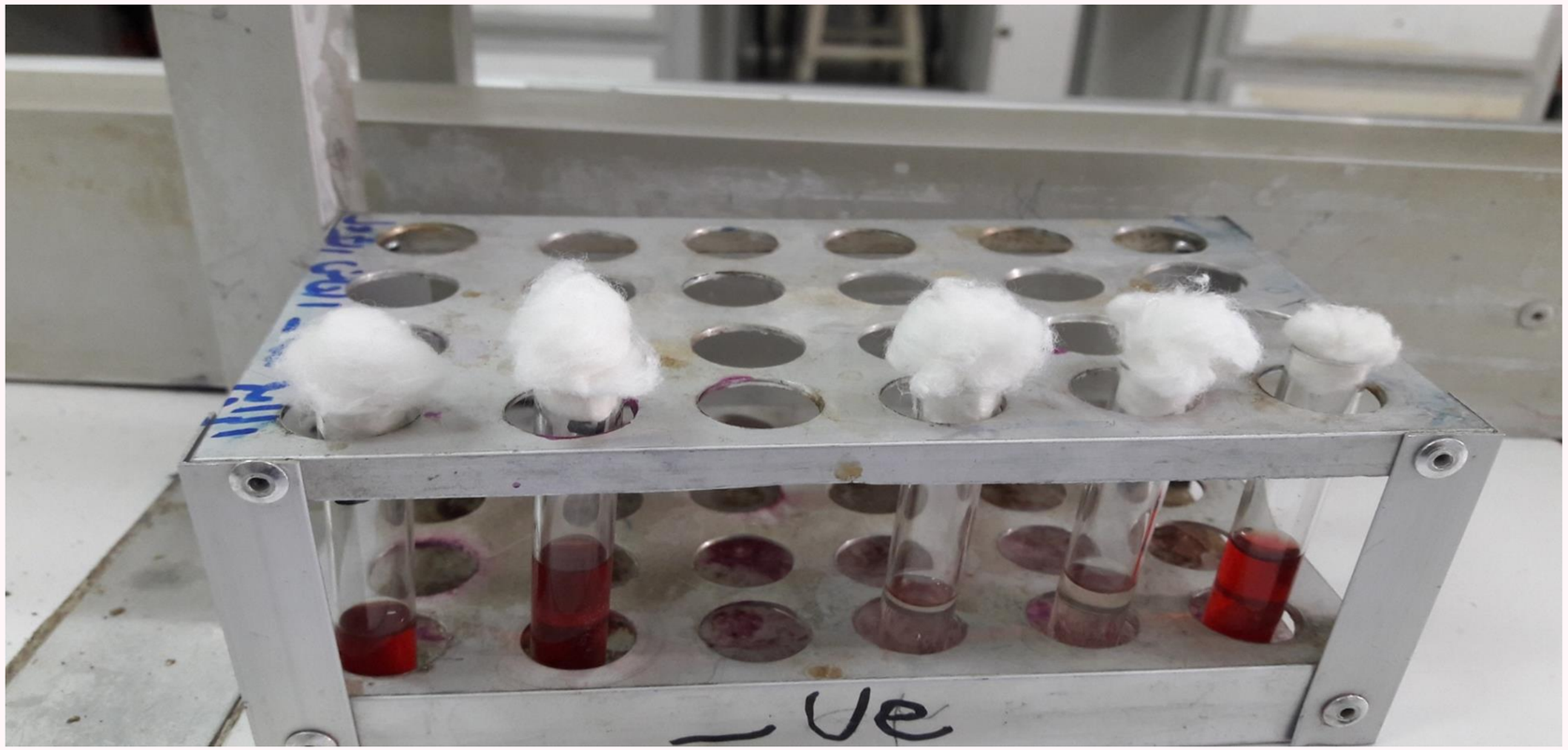


Diagnosis of genital system infections



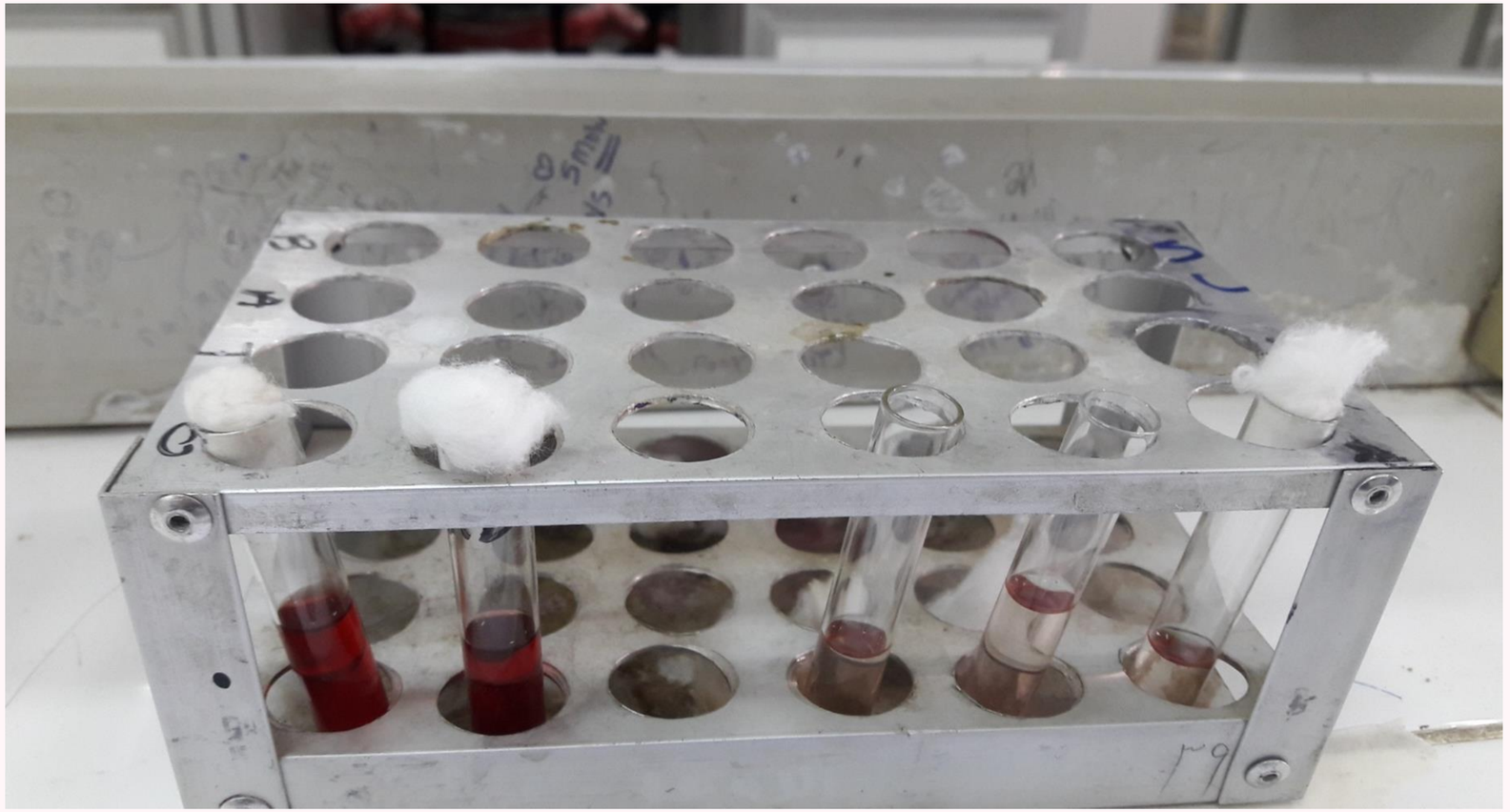
Wassermann test
(Mild positive)

Diagnosis of genital system infections



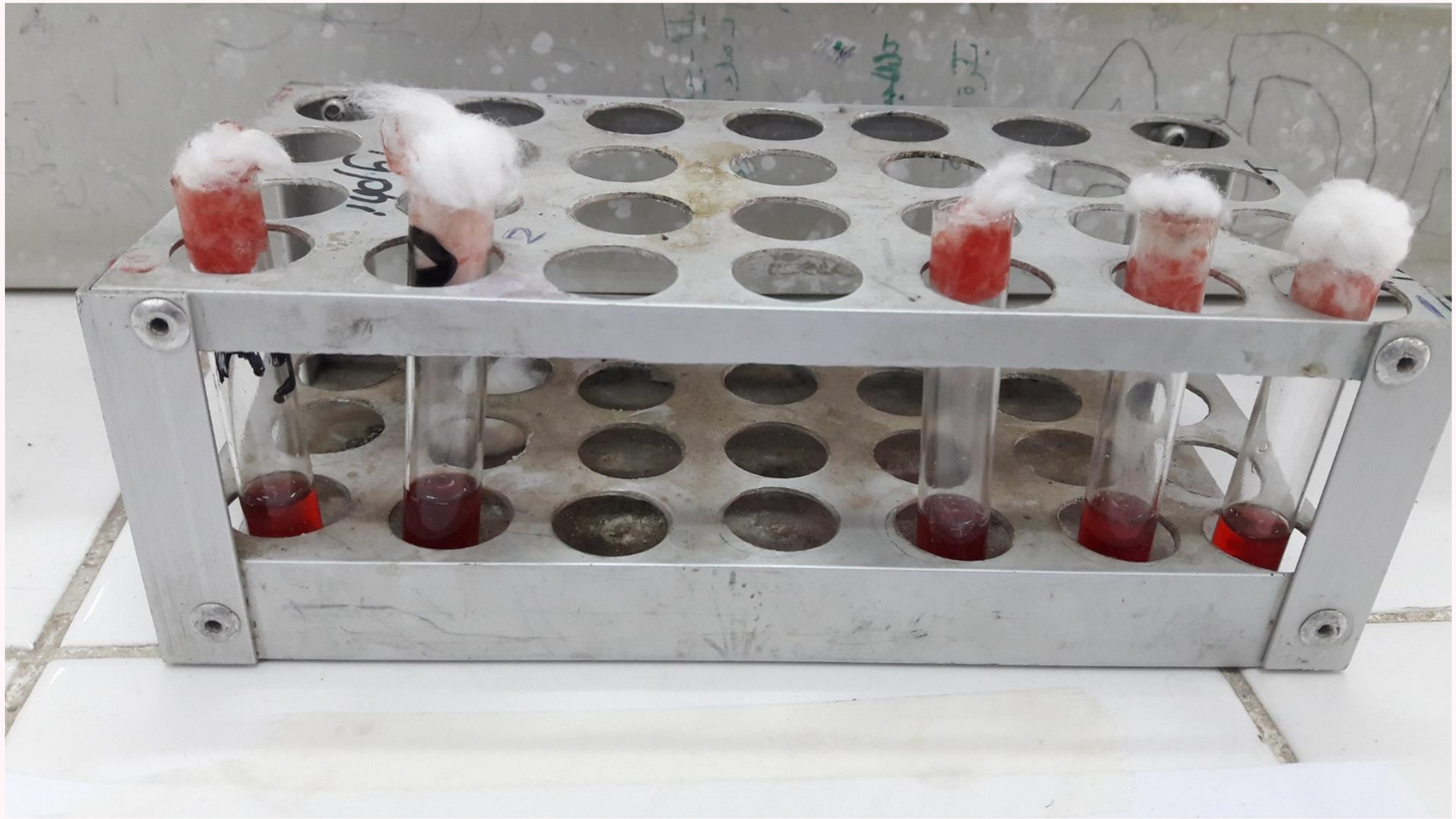
Wassermann test (Moderate positive)

Diagnosis of genital system infections



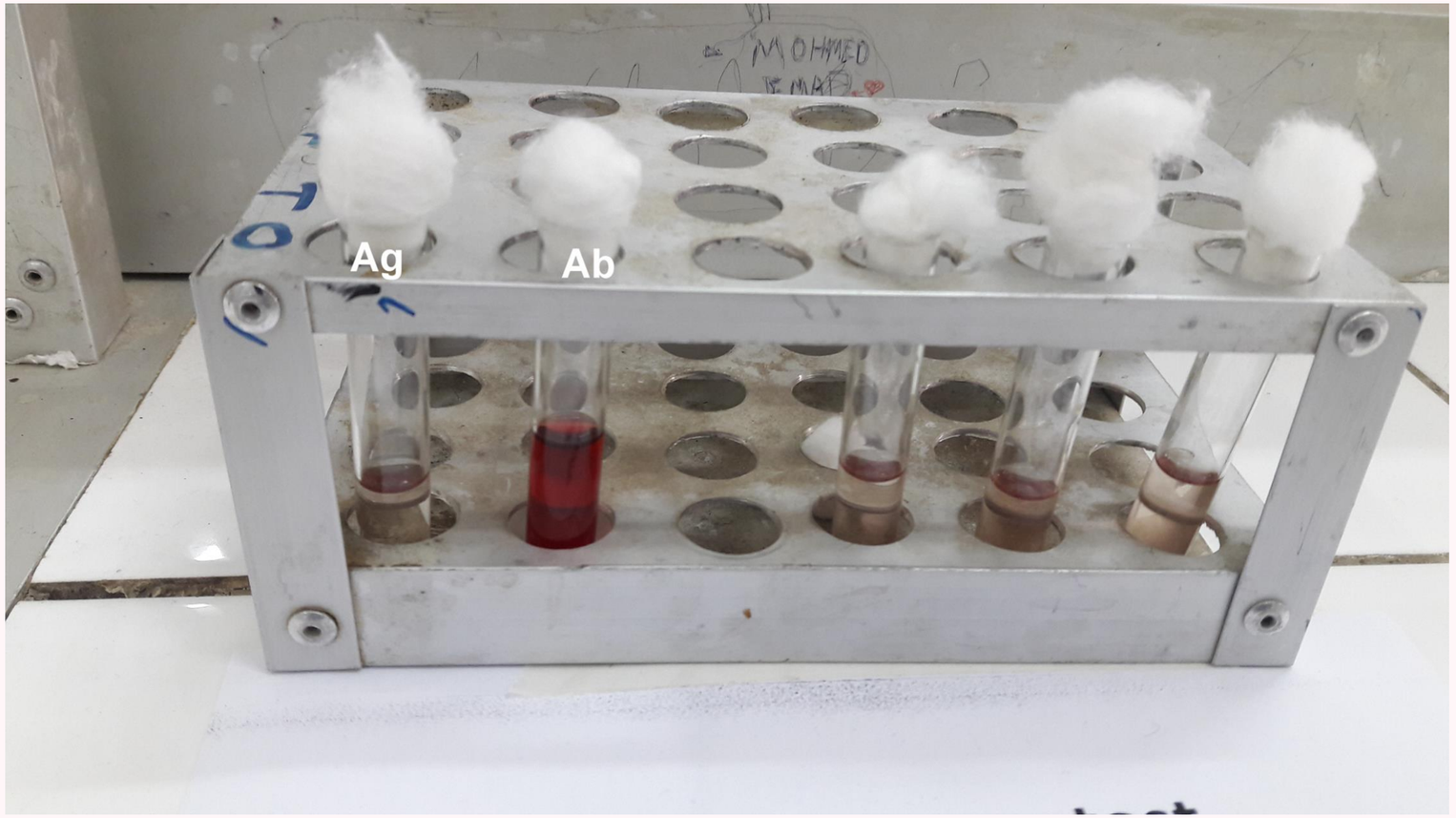
Wassermann test
(Strong positive)

Diagnosis of genital system infections



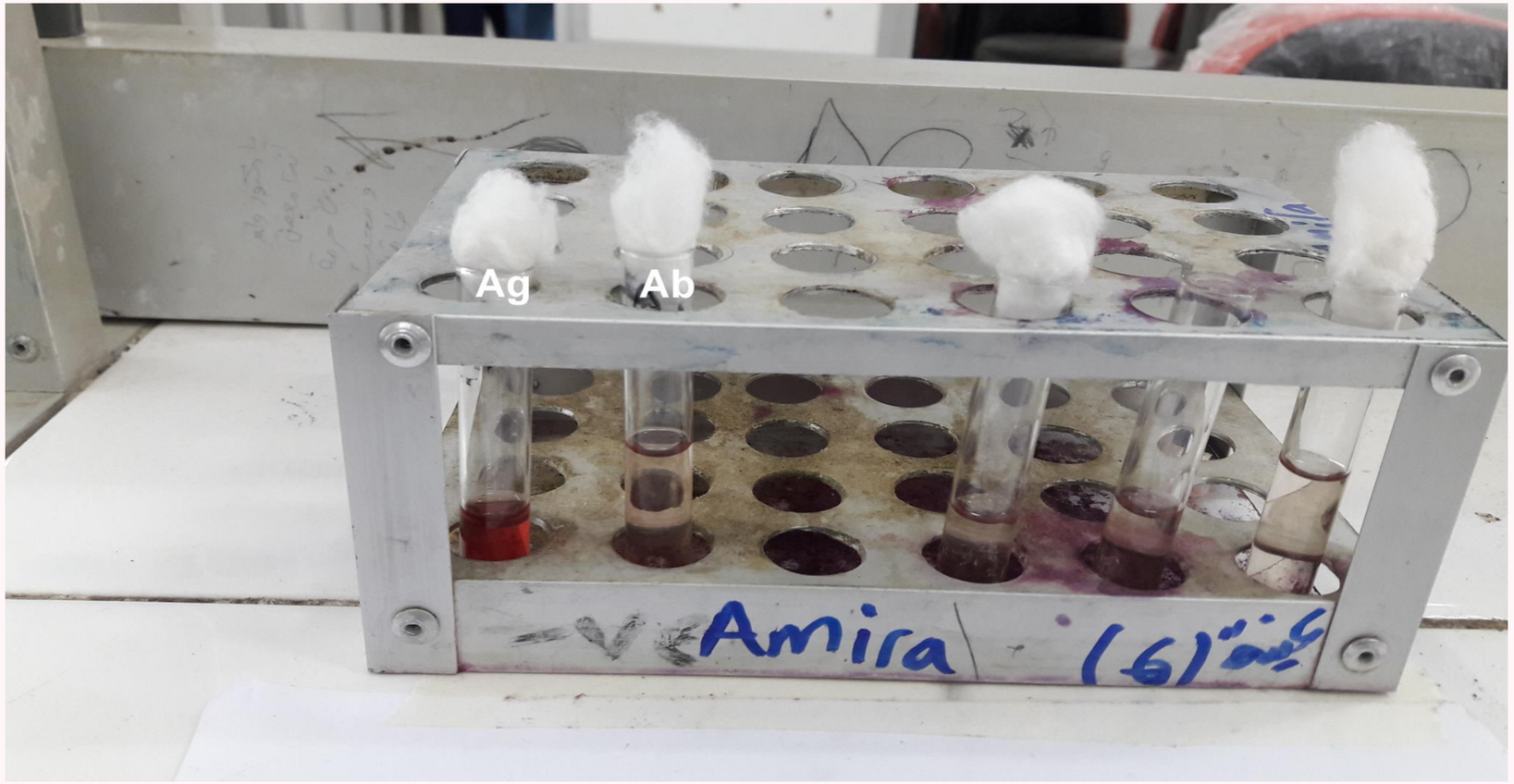
Wassermann test (Negative)

Diagnosis of genital system infections



Wassermann test (Ag anticomplementary)

Diagnosis of genital system infections



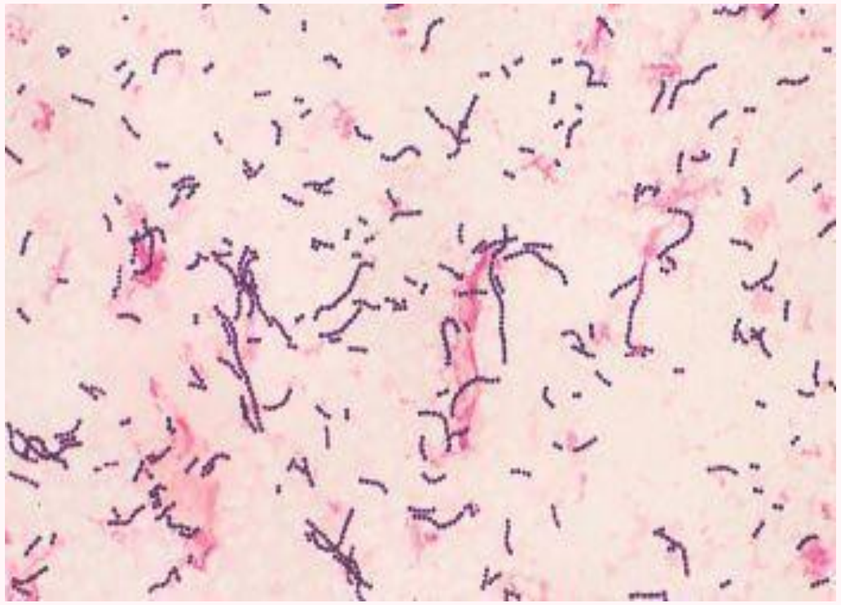
Wassermann test (Ab anticomplementary)

Diagnosis of puerperal sepsis

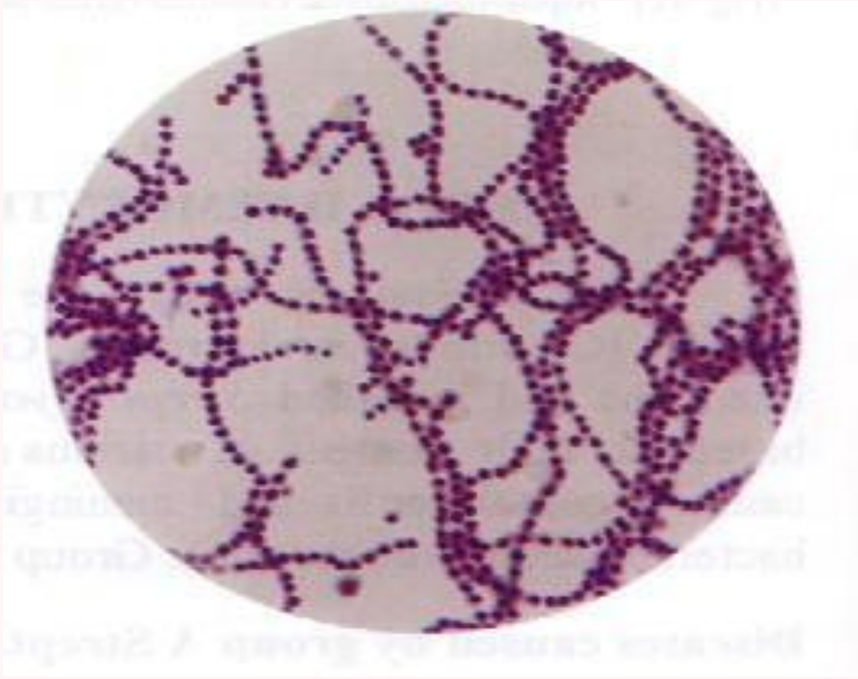
Q1:Identify this stained film.

Q2: Describe morphology of this organism.

- Gram positive cocci arranged in long chains, Non spore forming, non motile and capsule of hyaluronic acid.



Streptococcus pyogenes by gram stain in pus

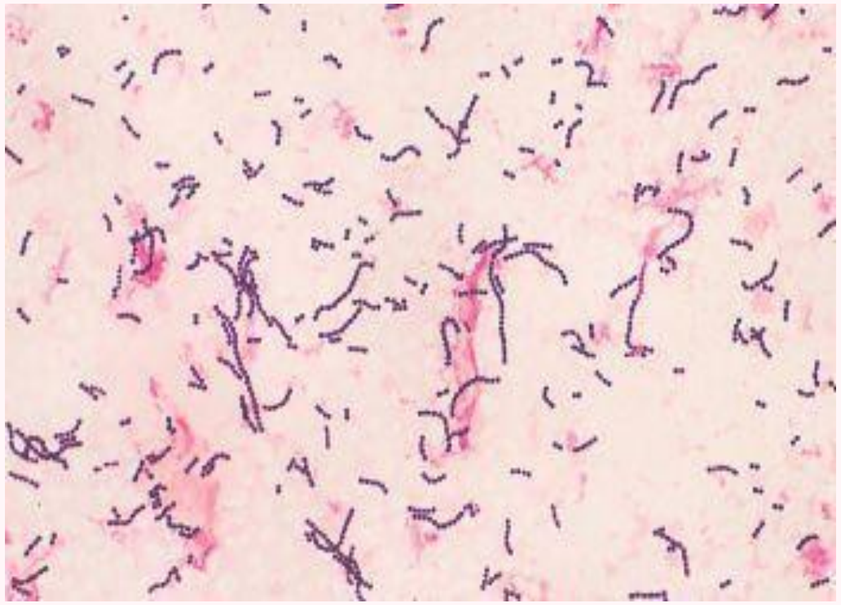


Streptococcus pyogenes by gram stain in culture

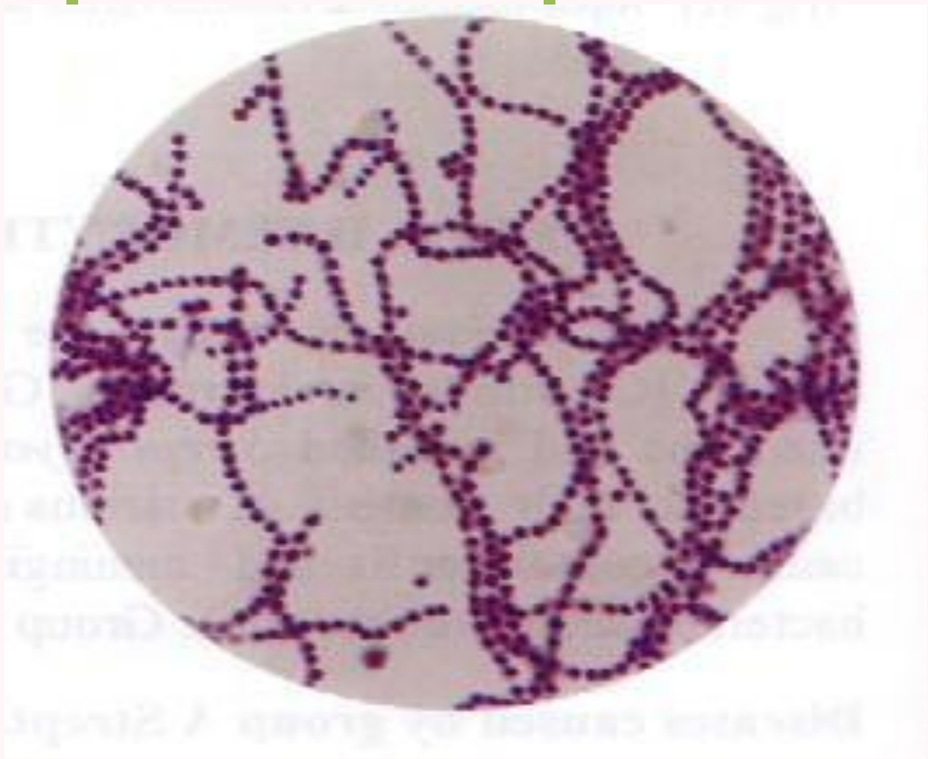
Diagnosis of puerperal sepsis

Q1: Describe cultural characters of the organism isolated in this stained film.

Q2: Describe culture media of the organism isolated in this stained film.



Streptococcus pyogenes by gram stain in pus



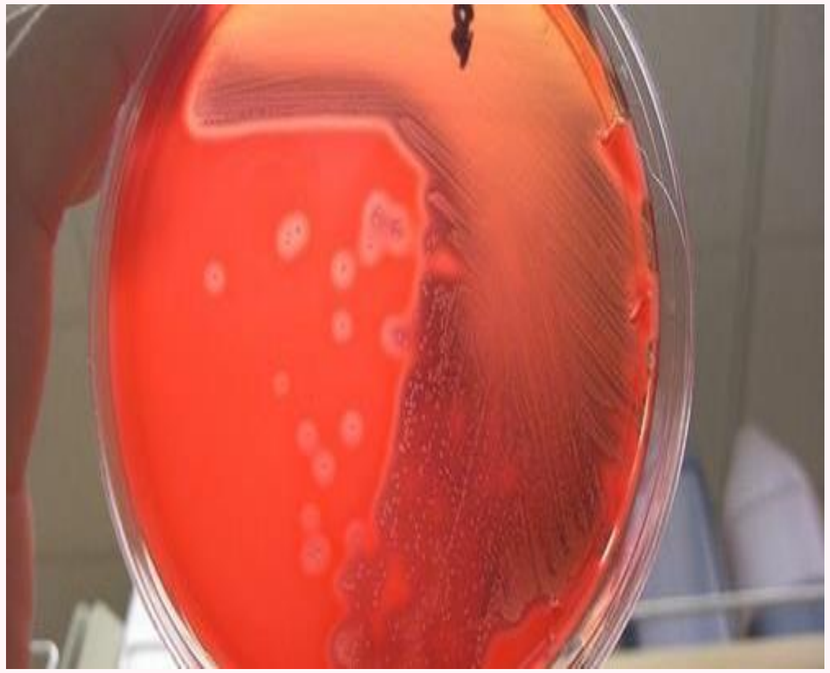
Streptococcus pyogenes by gram stain in culture

Diagnosis of puerperal sepsis

Q1: Identify colonial growth on this plate.

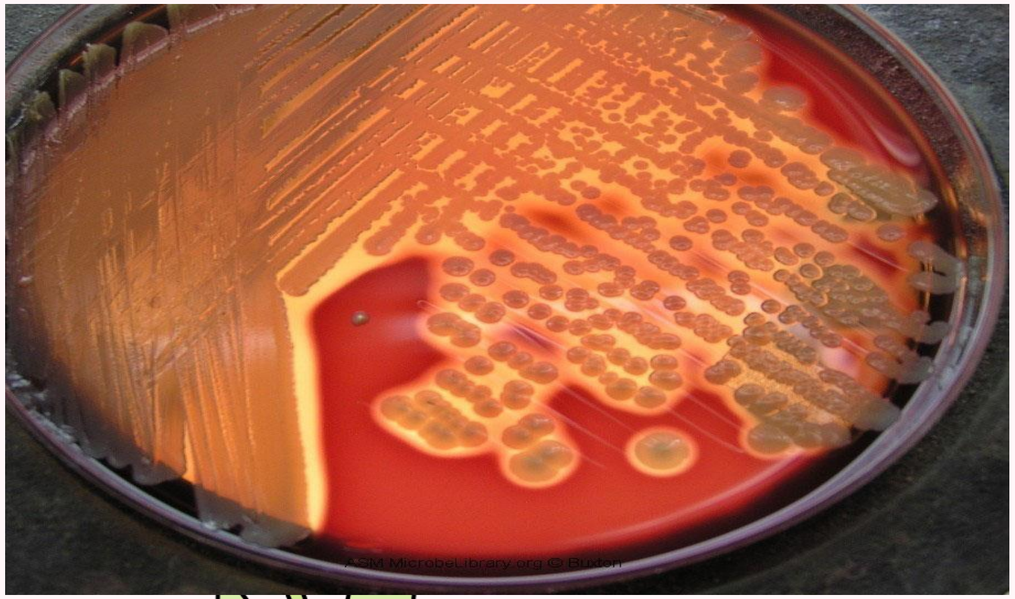
- Beta hemolysis on blood agar plate.

Q2: State 2 organisms causing this reaction on blood agar plate.



Streptococcus pyogenes **on Blood agar**

Staphylococcus aureus **on Blood agar**



Diagnosis of puerperal sepsis

Q1: identify this test.

- Catalase test.

Q2: State principle of this test.

- Some organisms produce catalase enzyme which breakdown the hydrogen peroxide to oxygen and water.

Q3: List use of this test.

- Differentiate bacteria that produce catalase enzyme, Staphylococci, from non catalase producing bacteria such as Streptococci.

Diagnosis of puerperal sepsis

Q1: Name this test.

- Bacitracin sensitivity test.

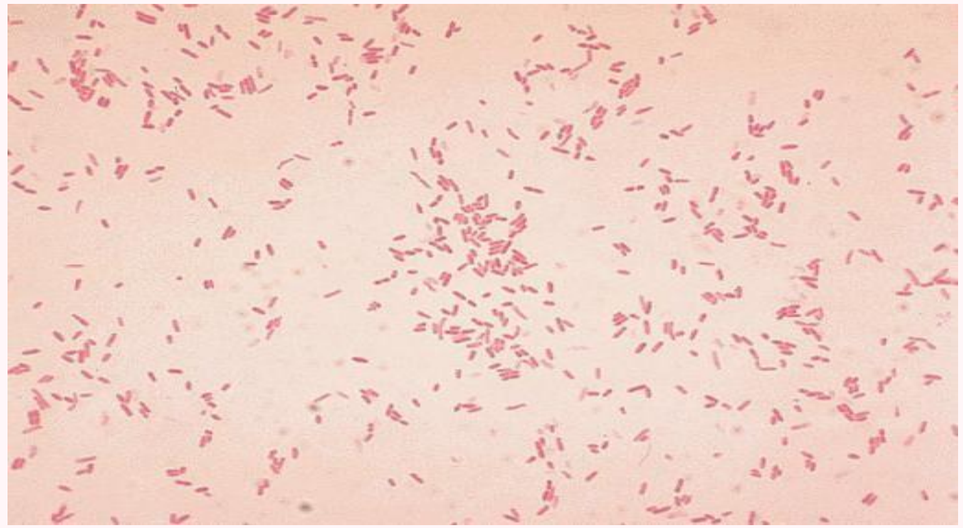
Q2: Illustrate use of this test.

-A zone of inhibition of growth around the disc. differentiate *streptococous pyogenes* from other beta hemolytic streptococci which are bacitracin resistant.

Diagnosis of puerperal sepsis

Q1: Identify this stained film.

- Gram negative bacilli in culture.



Q2: State type of this media

- Indicator media.

Q4: Describe colonial growth on this media.

- LF colonies on MacConkey agar plate

Diagnosis of puerperal sepsis

Q1: identify organism causing this reaction.

Biochemical reactions of Ecoli

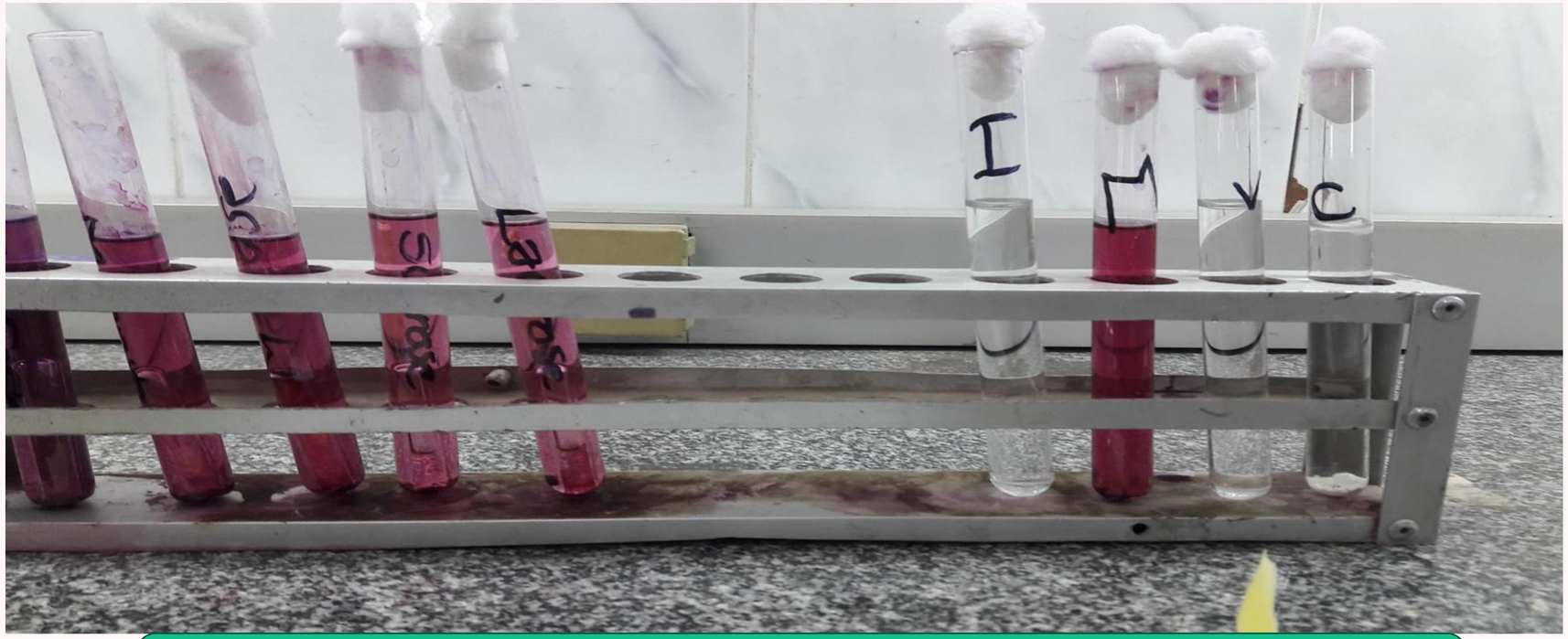
Diagnosis of puerperal sepsis

Q1: identify organism causing this reaction.

Biochemical reactions of Klebsiella

Diagnosis of puerperal sepsis

Q1: identify organism causing this reaction.



Biochemical reactions of Citrobacter