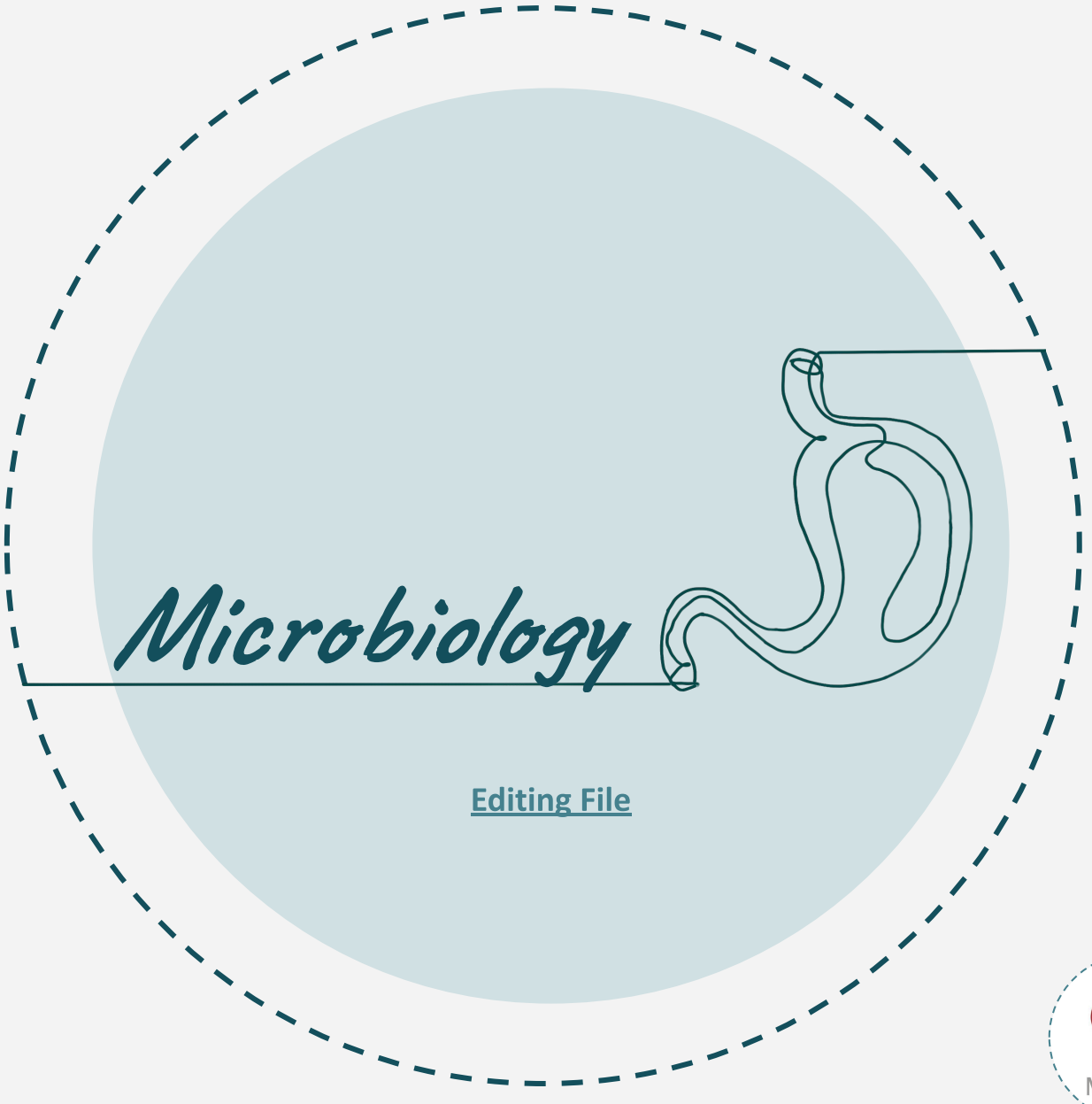
Practical



Editing File

Color index

- Girls' slides
- Boys' slides
- Main content
- Extra
- Important
- Drs' notes

Objectives:

- **Part 1 (Hepatitis):**
  - Understand the use of viral serological studies for the diagnosis of hepatitis A, B & C infections.
  - To know measures to prevent hepatitis A & B infections.
  - To know the viral serological tests used to screen blood donors.
  - Risk of transmission of HBV
- **Part 2 (Blood parasites):**
  - We have not found them so we are just making things up
  - To fill the space that we have made for the objectives
  - And we hope that you do not notice
  - We have one objective, as a team
  - To provide you with the best teamwork possible

Case 1: Hepatitis

Scenario

Mohammed Khan is a 20 year-old male who has recently arrived from India to work as a food handler in a restaurant in Riyadh. Three weeks after his arrival he was seen in A&E Dept. of KKUH because of **repeated vomiting**, **abdominal pain and fever**. On examination, his temperature was **38° C**, his pulse rate 110/min and BP 120/80 mmHg, he was **jaundiced** and had **tenderness in the right upper quadrant** of his abdomen.

Investigation Results :

CBC, Blood film & Culture	
Hb	14.2 g/L (Normal, so it excludes anemia and hemolysis)
WBCs	6100 mm^3 (Normal)
Platelet	271 g/L (Normal)
ESR	4mm/h (Normal)
Blood film for Malaria	Negative (so we can exclude malaria)
Blood Culture	Negative (So we can exclude salmonella typhi)

Liver Function Tests (LTFs)	
AST (12-37 U/L)	1557 U/L (significantly elevated)
ALT (20-65 IU/L)	1879 IU/L (significantly elevated)
ALP (175-476 IU/L)	441 IU/L
Albn (30-50 g/L)	42.3 g/L
Bilirubin (3-17 µmol/L)	86 µmol/L (high & indicated jaundice)

Case 1: Hepatitis

Q1- What are the possible causes for his presentation?

- Viral hepatitis
- Acute Cholecystitis
- Malaria
- Leptospirosis
- Typhoid
- Alcohol
- Hemolytic anemia

Q2- What investigations would you like to order for him?

- Explain how these investigations would help you.

Test	How this investigation will help you?
CBC [1] & ESR	Shows non-specific signs of infections or inflammation.
Blood film for malaria	To exclude malaria. (Thick & Thin films)
Liver Function Test (LFT)	To assess liver function.
Viral hepatitis screening	To exclude viral hepatitis.
Blood culture	To exclude typhoid fever.

Q3- Based on the findings what is the most likely diagnosis?

Viral hepatitis A, B or C

Q4- What further investigations would you like to order?

Hepatitis serology

Serological Tests		
Test	**Result**	**Interpretation [2]**
Anti-HAV-IgM A marker for acute infection	**Positive**	**Patient is infected with acute Hepatitis A infection.**
Anti-HAV-IgG A marker for immunity	**Negative**	**Patient has NO IMMUNITY against Hepatitis A**
HBsAg	Negative	Patient is not infected with Hepatitis B infection.
Anti-HCV (IgG)	Negative	Patient is not infected with Hepatitis C infection.
Anti-HEV IgM	Negative	Patient is not infected with Hepatitis E infection.

[1] To exclude anemia

[2] Dr. Malak: If we ask for interpretation, you have to comment on whether the person is infected or not on all markers results. However, if we ask about the diagnosis you only comment on the positive markers that the patient infected by. For example, in table above the interpretation is as written in the table but the diagnosis is that the patient is infected with Hepatitis A infection only

Case 1: Hepatitis

Q5- Based on the serologic results, what is the diagnosis?

Acute Viral hepatitis A

Q6- Briefly outline the management of this patient.

- Supportive (with painkiller + antipyretic drug for the fever)
- Contact tracing (close contacts should receive the vaccine)
- Not working
- Follow up (clinical and laboratory)

Extra

Q7- How can we prevent this disease?

- Hig (Human immunoglobulin):
  - Given before or within 2 weeks of exposure
  - Indication: Travellers
- Vaccine: inactivated (Killed):
  - Given IM in two doses, >1 Y of age.
  - Indication: Patients at high risk of infection and severe disease

Extra

Q8- How is this organism transmitted?

- Fecal-oral route (Mainly)
- Sexual contact
- Blood transfusions (Rare)

Extra

Q9- What does positive Anti-HAV IgG indicate?

Previous infection or immunity

Case 2: Hepatitis

Scenario

Mohammed Abdullah is a 34 year old married Saudi male who has donated two units of blood at KKUH for a relative undergoing an operation. Two days later, the Blood Bank called him because of abnormal blood test results and advised him to see his physician. On arrival to the blood bank, the doctor informed him that his blood is not suitable for transfusion because of the **presence of infection**..

Investigation Results :

The next day Mohammed came to see his general practitioner with a letter from the blood bank. The letter revealed the result shown below

Serology test		
Test	**Result**	**Interpretation** [1]
HBsAg	Negative	Patient is not infected with Hepatitis B infection.
Total Anti-HBc (IgG & IgM)	Negative	Patient was not exposed to hepatitis B virus
Anti-HCV A marker for infection (we need further tests such as RIBA / PCR to distinguish between acute, chronic, resolved infection)	Positive	Patient is **infected** with hepatitis C
HIV-Ag/Ab	Negative	Patient is not infected with HIV
Anti-HTLV	Negative	Patient is not infected with HTLV

Laboratory Test (All are normal)	
Test	**Result**
ALT (20-65 IU)	49 IU
AST (12-37 IU)	29 IU
Bilirubin (3-17 mol/L)	4 mol/L

[1] Dr. Malak: If we ask for interpretation, you have to comment on whether the person is infected or not on all markers results. However, if we ask about the diagnosis you only comment on the positive markers that the patient infected by.

Case 2: Hepatitis

Q1- What type of infectious agents can be transmitted through blood transfusion? (List 4 infections)?

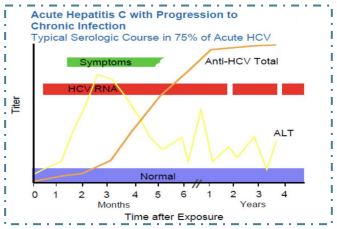
- Hepatitis B
- Hepatitis C
- HIV
- HTLV[1]
- Syphilis

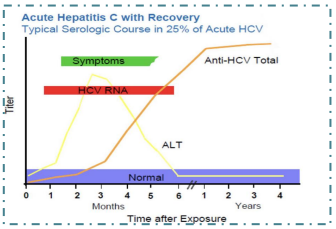
Q2- What is your interpretation based on the serology results?

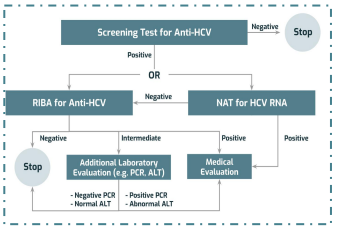
- **HBsAg :** Patient is not infected for Hepatitis B infection.
- **Anti-HBc :** Patient is not infected for Hepatitis B infection.
- **ANTI-HCV :** Patient is **infected** with Hepatitis C infection.
- **HIV-Ag/Ab :** Patient is not infected for HIV infection.
- **Anti-HTLV :** Patient is not infected for HTLV infection.

Q3- What do you do next?

- Repeat tests in duplicate and Serology (RIBA)[2] or secondary (PCR)[3] to confirm diagnosis.
- Liver Function Tests (LFTs)







Q4- How would you interpret the laboratory results based on the table?

- **ALT :** Normal
- **AST :** Normal
- **Bilirubin :** Normal

Q5- How do you diagnose HCV infection?

- **Serological assay**
  - **Screening** for (Anti-HCV) by ELISA (highly sensitive, but it may give false positive or false negative)
  - **Confirmatory test**[4] by recombinant immunoblot assay (RIBA) or Line Immunoassay INNO-LIA HCV
- **Molecular assay**[5] for HCV-RNA in blood using: RT-PCR

[1] Human T-cell Lymphotropic Virus
[2] RIBA - used to confirm presence of Antibodies
[3] PCR - used to confirm presence of Nucleic acid
[4] To exclude any false result of ELISA
[5] Molecular assays are recommended in certain situations; in cases of immunodeficiency hemodialysis or early acute hepatitis cases)

Case 2: Hepatitis

Extra

Q6- How do you confirm HCV infection?

By RIBA and RT-PCR

Q7- What other laboratory test are needed?

- The General practitioner arrange for him to see hepatologist who examine him and review his results. He further added PCR with genotype for Hepatitis C.

Test	Significance	How it can help?
RT-PCR	1- Qualitative: - or + (HCV-RNA) 2- Quantitative: Viral load[1]	1- Confirm the Diagnosis (Dx) 2- Monitor response to Treatment (Rx)
Genotype[2]	identify the genotype of HCV	Guide the choice & duration of therapy.

Extra

Q8- What's the appropriate treatment for this case?

Combined therapy: Pegylated alpha interferon and Ribavirin

Extra

Q9- How are HCV and HBV transmitted?

- Parenterally (e.g. Direct exposure to infected blood)
- Sexually (Homosexuals)
- From mother to child perinatally (During delivery, Breastfeeding)

Extra

Q10-Mention 3 risk factors for HBV & HCV infection?

- IV drug users
- Tattoo
- Hemodialysis

Q11- What is the outcomes of hepatitis C?

80% will develop chronicity Remember hepatitis C with Chronic

20% of patients with hepatitis C will resolve.

[1] To monitor the response to antiviral therapy

[2] There are 8 genotypes for hepatitis C. The dominant in SA is genotype 4. Genotype detection is important because some types tend to be less sensitive than others, and this will affect the prognosis and treatment plan

Case 3: Hepatitis

Scenario

A 15-weeks pregnant Saudi woman was seen for the first time at the antenatal clinic at KKUH. As part of the antenatal screening, the doctor arranged for blood screening for viral serology.
The results are in the table below.

After 25 weeks the mother is admitted in labour and you were among the staff involved in the delivery. During a repair of the episiotomy, you accidentally prick your finger with a needle stained by the patient blood.

Investigation Results :

Serology Test		
Test	**Result**	**Interpretation**[1]
HBsAg A marker for infection of HBV	**Positive**	Indicates that **patient is infected with hepatitis B virus** either acute or chronic
HBeAg A marker for replication & infectivity	Negative	Negative result of this **antigen** indicates low infectivity due to absence of viral replication
Anti-HBe A marker for LESS replication & infectivity	**Positive**	Positive results of this **antibody** indicates low infectivity because positive anti-HBe means that there are antibodies against the antigen that responsible for infectivity
Total Anti-HBc (both IgG+IgM)	**Positive**	Since Anti-HBc IgM is negative and Total Anti-HBc, this indicates that the patient has been exposed to HBV a long time ago (Chronic or recovered from previous acute infection)
Anti-HBc IgM A marker acute infection	Negative	
HIV Ag/Ab	Negative	Patient is not infected with HIV
Anti-HCV	Negative	Patient is not infected with hepatitis C infection

[2]

[3]

[1] Dr. Malak: If we ask for interpretation, you have to comment on whether the person is infected or not on all markers results. However, if we ask about the diagnosis you only comment on the positive markers that the patient infected by.

[2] Note that we have negative antigen but positive antibody, so we can say that the infectivity of this patient is low which is good. However, if we have it the other way around, ie. the antigen was positive and while the antibody was negative, we can say that the infectivity of this patient is high which is bad.

[3] When a patient is infected with hepatitis B infection, s/he will develop IgM against it first (so it is considered to be a marker for acute infection). Later on **throughout life,** the patient will develop IgG immunity. Thus, **Positive total Anti-HBc in the absence of IgM tells us that the we can exclude acute infection**, and that patient was **exposed** to HBV long time ago **or** s/he has developed the **chronic** form.

Case 3: Hepatitis

Q1- How would you interpret the results of the mom in the 15 weeks?

- **Chronic Hepatitis B1 with low infectivity (Diagnosis)**
- Patient is negative for HIV infection
- Patient is negative for Hepatitis C

Q2- On the lights of these Laboratory results how would you manage the newborn?

- **Post-exposure prophylaxis:**[1]
  - Hepatitis B immunoglobulin (HBIG) within 12 hours of birth.
  - First dose of HBV vaccine.

Q3- Is there a risk of transmission of HBV to the newborn?

- HBsAg (+ve) & HBeAg (-ve) Mother → 10-20%
- HBsAg (+ve) & HBeAg (+ve) Mother → 90%

Q4- What further management would you offer to the mother?

- **Pregnant Hepatitis B carriers should be advised to:**
  - Not donate blood, body organs, other tissue.
  - Not share any personal items that may have blood on them (e.g., toothbrushes).
  - Obtain vaccination against hepatitis viruses A as indicated.
  - Be seen at least annually by their regular medical doctor.
  - Discuss the risk for transmission with their partner and need for and testing.

Q5- What should you do when you accidentally prick your finger with a needle stained by the patient blood? ?

- Report occupational exposures immediately.
- The hepatitis B vaccination status and the vaccine-response status (if known) should be reviewed:

1- HBsAg 2- Anti-HBc. 3- Anti-HBs

Q6- What is the risk of infection to you?

- The risk of developing serologic evidence of HBV infection:
  - HBsAg (+ve) & HBeAg (+ve) Blood → 37-62%
  - HBsAg (+ve) & HBeAg (-ve) Blood → 23-27%

Q7- What is the outcomes of HBV?

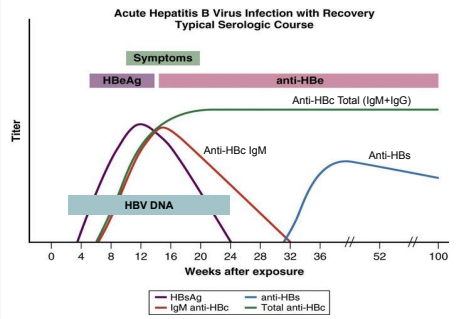
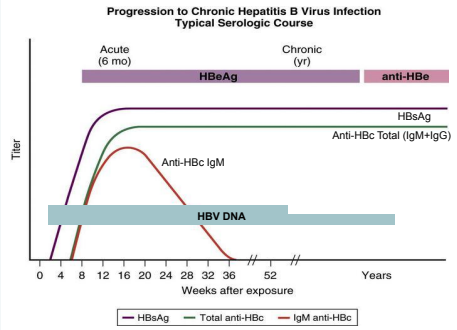
9-10% of adults develop chronicity

[1] Newborn at risk has to take both the vaccine AND the immunoglobulin at the same time but in different sites. Why? to prevent neutralization (because the vaccine is actually a modified surface antigen, and the immunoglobulin is an antibody. If we give them together at the same site, they will cancel each other out).

Q1: How do you confirm HBV infection? By neutralization
Q2: What's the appropriate treatment for HBV in general? Pegylated alpha interferon

Interpretation of the Hepatitis B1 panel tests

Click here for a helpful table from 438

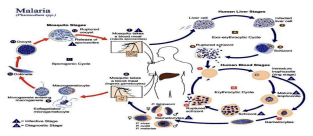
Test	Result	Interpretation
HBsAg	Negative	Susceptible
Anti-HBc	Negative	
Anti-HBs	Negative	
HBsAg	Negative	Immune due to natural previous infection (positive **Anti-HBs** and **Anti-HBc** together)
Anti-HBc	**Positive**	
Anti-HBs	**Positive**	
HBsAg	Negative	Immune due to hepatitis B vaccination (positive **Anti-HBs** but negative **Anti-HBc**)
Anti-HBc [1]	Negative	
Anti-HBs	**Positive**	
HBsAg	Positive	Acutely infected 
Anti-HBc	Positive	
Anti-HBs	Negative	
anti-HBc IgM	Positive	
HBsAg	Positive	Chronically infected 
Anti-HBc	Positive	
Anti-HBs	Negative	
anti-HBc IgM	Negative	
HBsAg	Negative	**Four interpretations are possible:** - May be recovering from acute HBV infection. - May be distantly immune and test not sensitive enough to detect very low level of anti-HBs in serum. - May be susceptible with a false positive anti-HBc. - May be undetectable level of HBsAg present in the serum and the person is actually a carrier.
Anti-HBc	Positive	
Anti-HBs	Negative	

[1] In vaccinated people, you will not find Anti-HBc as it can only be acquired after previous natural infection.

Malaria

Introduction

- Vector : Anopheles Mosquito
- Infective stage of human: Sporozoites
- infective stage of anopheles: Gametocytes
- Main pathology : penetrate the Red Blood Cell



Laboratory diagnosis

Malaria Can Be Diagnosed Commonly By:

1- Microscopy (Light Microscopy): Gold standard

-Uses a blood smear to identify whether parasites are present in the patient's blood.

-Thick film: for screening

-Thin film: for different spices identification and stages (ring stage , gametocyte…)

2 - Rapid Diagnostic Tests (Rdts): used to look for malarial antigen not parasite

- RDTs are quick tests for screening that use a drop of blood from the finger tip to identify whether parasites are present in the patient or no.

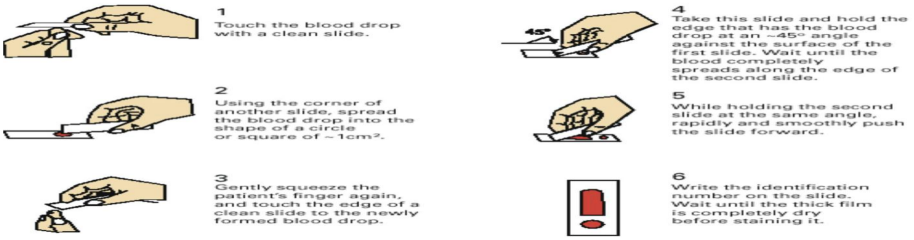
3- Serology

4- PCR

Light microscopy

Gold standard

Preparing blood film (Thick & Thin)



Thin film

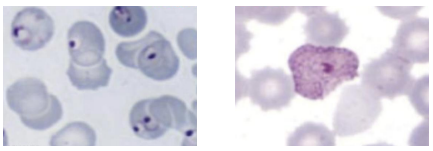
- Fixed RBCs, single layer
- Smaller volume
- Good for species differentiation
- Require more time to read

Thick film

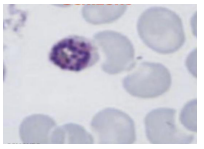
- Lysed RBCs
- Large volume
- Good screening test
- Positive or negative

Three developmental stages seen in blood films:

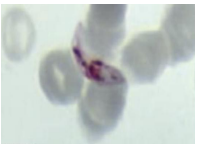
1. Trophozoite or ring stage
2. Schizont
3. Gametocyte



Trophozoites



Schizont



Gametocyte

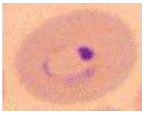
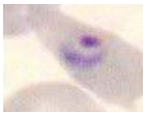
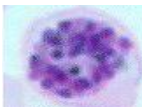
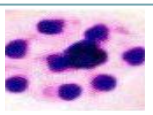
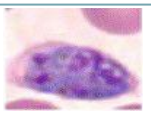
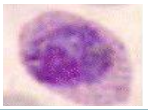
Q1: What stain do we use for malaria blood film? Giemsa stain
Q2: Mention 4 complications of severe malaria? Cerebral malaria, Acute renal failure, Generalized convulsions, Severe normocytic anemia.
Q3: How is malaria transmitted? By Female Anopheles mosquitoes

Malaria

Dr. Mona: in malaria ospe, we will only ask you about plasmodium falciparum
مارح أسألكم عن شي غيرها + in the exam we use thin film picture only

Species (Extra)

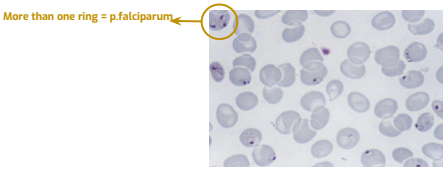
Species of Malaria (Plasmodium Spices) is identified by its characteristic microscopic appearance

Species / Stage	Falciparum	Vivax	Malariae	Oval
Ring stage				
Trophozoite				
Schizont				
Gametocyte				

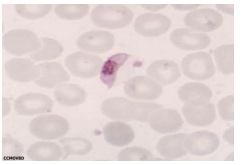
Light Microscopy [1]

Thin film (Plasmodium falciparum)

Trophozoite / ring stage in thin smear

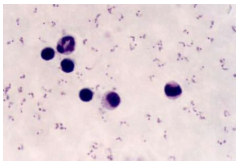


Banana-shaped or crescent-shaped gametocyte stage (infective stage of anopheles) **in thin smear**



Thick film (Dr: we will not show you thick smear in exam)

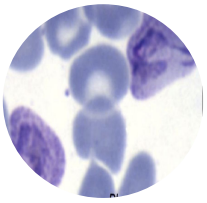
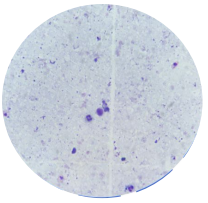
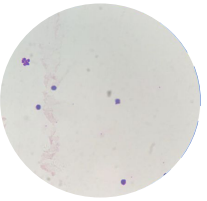
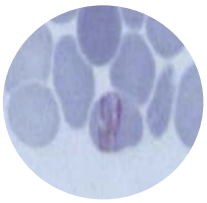
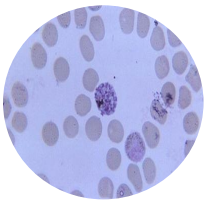
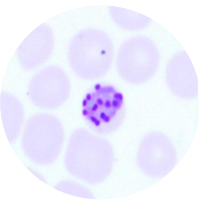
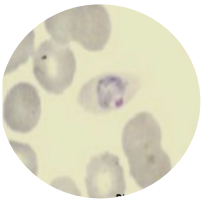
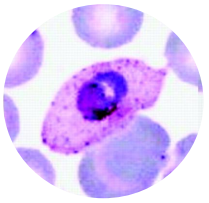
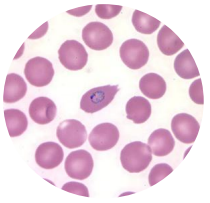
trophozoite stage in thick smear



[1] In the exam, if we ask you about diagnosis of malaria. mention that we have thin smear for differentiation & thick smear for screening.

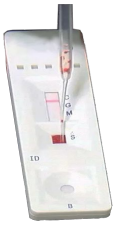
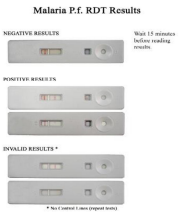
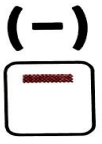
Malaria

Light Microscopy

Plasmodium Vivax		 Extra	 Extra
Plasmodium Malariae		 Extra	 Extra
Plasmodium Ovale		 Extra	 Extra

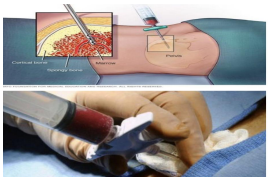
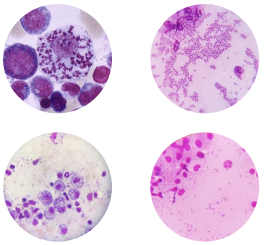
Rapid diagnostic tests (RDTs)

Rapid diagnostic tests detect malaria antigens

The RDTs Test (for screening)	 Card	 Plastic cassette	 Hybrid cassette-dipstick	 Dipstick
RDTs Result	 Positive	 P.f.  P.v. P.m. (Po)  P.f. or mixed	 Negative	 Neg.

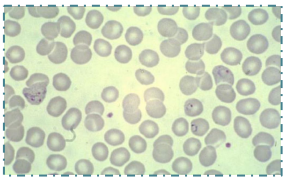
Leishmania

Extra **except** the picture is from the slides

Introduction	- Vector: sand flies - Infective stage: Promastigote - Diagnostic stage: Amastigote	
Types of leishmaniasis	**Cutaneous** - Leishmania Major > Zoonotic > Wet type lesion - Leishmania Tropica > Anthroponotic > Dry type lesions	• **Oriental sore (most common) classical self-limited ulcer** Starts as **painless** papule on exposed parts **(face).** Leishmania Major > Zoonotic > Wet type lesion Leishmania Tropica > Anthroponotic > Dry type lesions lesion ulcerates few months lager producing an leishmaniasis Ulcer with an **indurated** margin
	Mucocutaneous Leishmania Braziliensis	Pustular swelling in the **mouth** or on the **nostrils** >become ulcerative after many months and > extend fL into the naso-pharyngeal mucous membrane. Secondary infection is very common with destruction of the nasal cartilage and the facial bone
	Visceral Leishmaniasis (Kala-Azar) - Leishmania infantum mainly affect children - Leishmania donovani mainly affects adult	-Early symptoms: low-grade fever, malaise, sweating & anemia -In later stages: fever becomes intermittent then hepatomegaly, splenomegaly or hepatosplenomegaly due to hyperplasia of the lymphoid-macrophage system and bone marrow.
Diagnosis	1- Microscopy (light microscopy). 2- Culture in NNN medium.	
Samples	1- Bone marrow aspirate (most important) 2- Splenic aspirate 3- Lymph node 4- Biopsy	
Findings	- Visceral leishmaniasis = Bone marrow aspirate (Amastigotes stage)	

Cases

Case1 : A 25 year-old male from India, who came 3 months ago was admitted in KKUH with a history of severe anaemia and intermittent high grade fever for the last two months not responding to antibiotics.



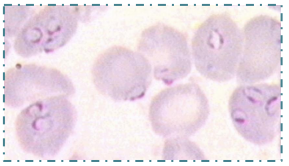
Q1- What's the diagnosis?

Plasmodium vivax (malaria)

Q2- In what stage?

Trophozoite (Ring stage)

Case2 : A student in KSU who returned three weeks from vacation in Africa, he developed intermittent fever last week and lost consciousness a short time ago. (if things like renal failure, coma, lost of consciousness, black water fever etc.. were mentioned in the case, we know it is malignant malaria and you have to write malignant in the answer)



Q1- What Is The Diagnosis?

Malignant/severe Malaria

Q2- What is the most possible pathogen?

Plasmodium Falciparum

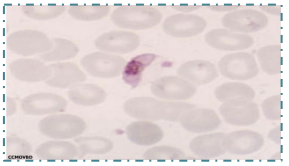
Q3- In what stage?

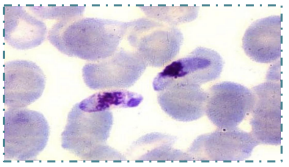
Trophozoite (Ring stage)

Q4- What are some severe complications?

Renal failure, cerebral malaria, anemia, hypoglycemia, shock, and generalized convulsions.

The patient was then treated with schizontocidal antimalarial drugs, a follow-up blood film is shown.





Q1- Name the parasite?

Plasmodium Falciparum

Q2-In what stage?

Gametocyte (Infective stage for anopheles)[1]

Q3-Describe the shape?

Banana-shaped or crescent-shaped gametocytes

How can you diagnose malaria?

1. **Light microscopy (Gold standard)**
   - Thin film for differentiation + Thick film for screening
2. **Rapid Diagnostic Tests (Rdts):**
   - used to look for malarial antigen
3. **PCR**
4. **Serology**

What is the vector of malaria?

- Female anopheles mosquito

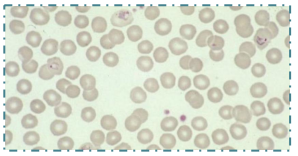
What is the main pathology of malaria?

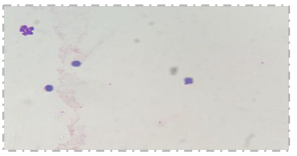
- Blood hemolysis and anemia due to rupture blood schizonts

[1] **In the exam,** if the stage in the given picture gametocyte, **you have to also mention** that it is the infective stage of anopheles mosquito)

Cases

Case3 : A businessman who makes frequent trips to Thailand, presents with intermittent fever





Extra Picture

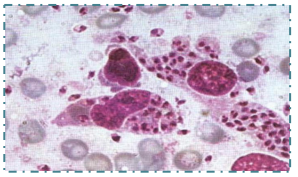
Q1- What Is The Diagnosis?

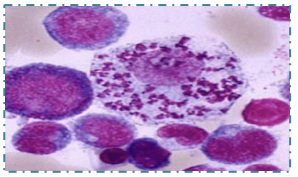
Malaria or plasmodium vivax.

Q2- What's the stage?

Trophozoite

Case4 : A 7 year old child presented with anemia, hepatosplenomegaly and fever. Not responding to antimalarials and antibiotics. Bone marrow aspirate smear is shown:





Q1- What's the diagnosis?

Visceral Leishmaniasis (kala-azar)

Q2- What's the stage?

Macrophage full with Amastigotes

Q3- What's the vector?

Sandfly

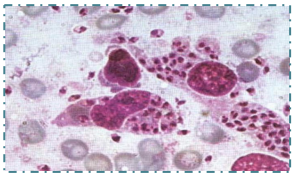
Q4- What is the infective & diagnostic stage?

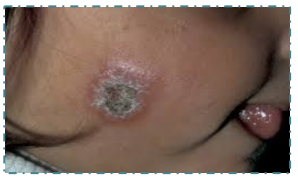
Infective stage: Promastigote **Diagnostic stage:** Amastigote

Q5- What are the causative parasites of Kala-Azar?

1- Leishmania infantum mainly affect children 2-Leishmania donovani mainly affects adult

Case5 [1] : A 7 year old child presented with ulcer on his face and fever. Not responding to antimalarials and antibiotics. Skin biopsy taken from the margin of the ulcer's smear is shown:





Q1- What's the diagnosis?

Cutaneous Leishmaniasis

Q2- What's the stage?

Macrophage full with Amastigotes

Q3- What's the vector?

Sandfly

Q4- What is the infective & diagnostic stage?

Infective stage: Promastigote **Diagnostic stage:** Amastigote

Q5- What are the causative parasites of Cutaneous leishmaniasis?

1- Leishmania Major > Zoonotic > Wet type lesion 2- Leishmania Tropica > Anthroponotic > Dry type lesions

[1] Note that microscopy of visceral & cutaneous is the same, but the case history and the sample obtained are different, For example:

- Hepatospleenomegaly in the case → visceral leishmania
- Face ulcer → cutaneous leishmania
- Nose ulcer with cartilage erosion → mucocutaneous leishmania

Members Board

Team Leaders

Muneerah Alsadhan

Shuaa Khdary

Abdurahman Addweesh

Team Member

Rand Alrefaei

Organizer

Sarah Alquwayz

Note takers

Duaa Alhumoudi

Faisal Alomari