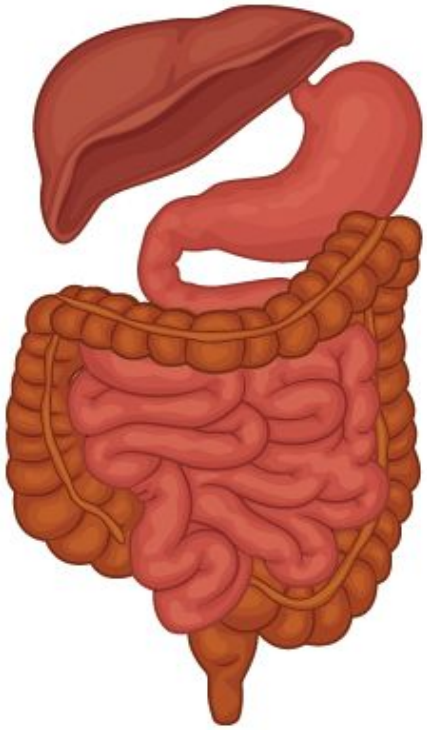
MEDICINE
KING SAUD UNIVERSITY

MICROBIOLOGY 436

MICROBIOLOGY PRACTICAL

Gastrointestinal & Nutrition Block



This file include the two microbiology practical lectures
Good luck!

- **Doctor's notes**
- Extra explanation
- Answers
- Headings

"لاحول ولا قوة الا بالله العلي العظيم" وتقال هذه الجملة اذا داهم الانسان امر عظيم لا يستطيعه، او يصعب عليه القيام به.

First

PRACTICAL ON BLOOD PARASITES

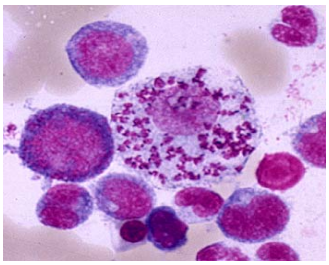
Malaria	
Definition	**Malaria** is the most important of all tropical parasitic disease, causes death and debility and is endemic throughout the tropics and subtropics.
Clinical signs	Fever – chills – sweating – anemia – anorexia – headache
Pathogen	Plasmodium falciparum , vivax, ovale, malariae, knowlesi.
Life cycle	Asexual = Anopheles Mosquito > Sporozoites > go to the hepatocytes > Merozoites > penetrate the Red Blood Cell **(ring stage)** > hemolysis and anemia > some Merozoites develop into male and female Gametocyte. Sexual = male and female Gametocyte > taken up by another mosquito > Sporozoites.
Infective stage	to human = Sporozoite. Mosquito = Gametocyte.
Complications	Cerebral malaria, Hypoglycaemia, pulmonary edema in pregnancy, blackwater fever, Tropical splenomegaly, anemia.
Diagnosis	• **light Microscopy** (*the gold standard method*) = Used for identify parasite density, species diagnosis & monitoring response to treatment. • **Rapid diagnostic tests** = To detect malaria antigens.

Leishmania	
Definition	Intra-cellular parasite which can survive within the macrophages in the human body.
Route of transmission	Sandfly
Life cycle	Sandfly bites > promastigote > phagocytosed by macrophages > immediately becomes amastigote > replicates until cell explode > go to other macrophages.
Infective stage	promastigotes
Diagnostic stage:	amastigotes
types	• **Cutaneous Leishmaniasis (Oriental sore)** = *Leishmania tropica & Leishmania major.* • **Mucocutaneous Leishmaniasis** = *Leishmania braziliensis.* • **Visceral Leishmaniasis** = *Leishmania donovani & Leishmania infantum*.
Clinical presentation	• **Cutaneous Leishmaniasis** > Starts as a painless papule on exposed parts > ulcerates > In some cases dry-type-lesion > In other cases wet-type-lesion • **Muco-cutaneous leishmaniasis** > starts as a pustular swelling in the mouth or on the nostrils > become ulcerative > extend into the naso-pharyngeal mucous membrane. • **Visceral Leishmaniasis** (kala-azar) > Hepatosplenomegaly, intermittent fever, Anemia.
Parasitological diagnosis	**cutaneous & muco-cutaneous leishmaniasis** = The parasite can be isolated from the margin of the ulcer **Visceral Leishmaniasis** = bone marrow aspiration ● Smear: Giemsa stain (microscopy for LD bodies "**amastigotes**"). ● biopsy : culture in NNN medium for **promastigotes**[1].

1- NNN media we put specimen contain Amastigote in the media will result to Promastigote**.**

The previous schedules are just to give you a better understanding to answer any possible questions.

1. Leishmania

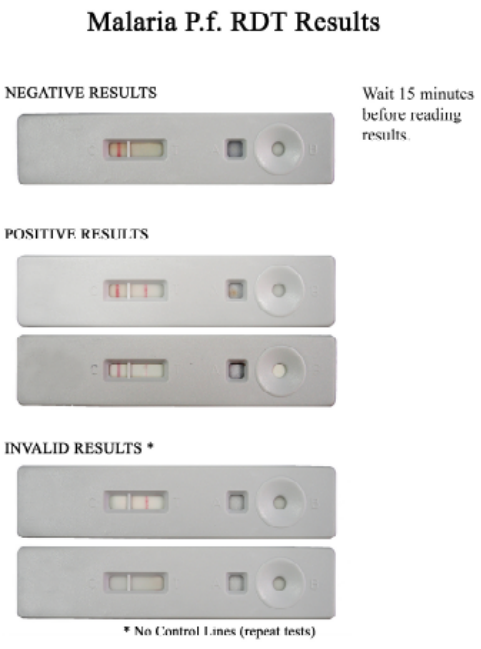


amastigote stage in Bone marrow smear

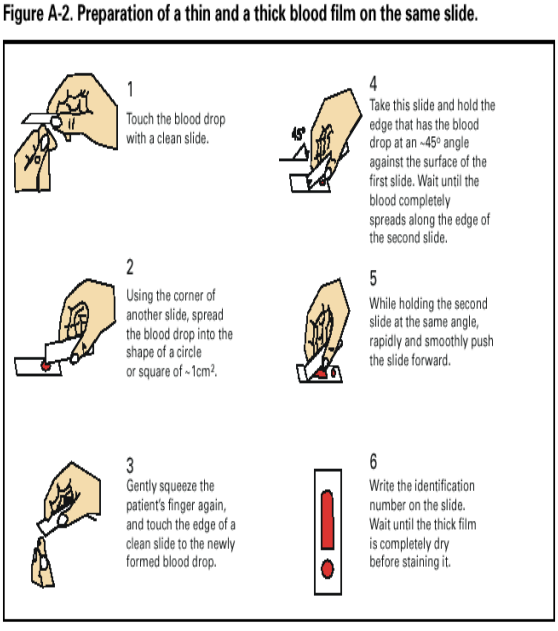
Bone marrow aspiration

2. Malaria

we have two common methods for parasitological diagnosis of malaria:



Rapid diagnostic tests (RDTs).



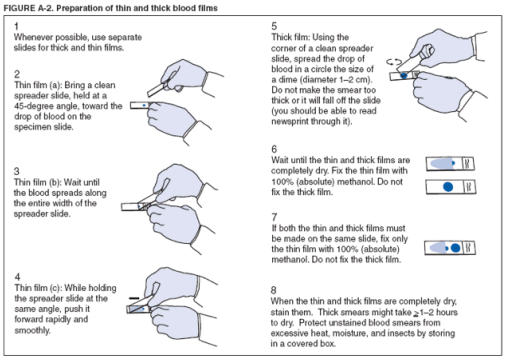
Light microscopy

A. Rapid diagnostic tests

- detects malaria antigen.
- Products come in a number of formats:
- Plastic cassette.
- Card.
- Dipstick.
- Hybrid cassette-dipstick.

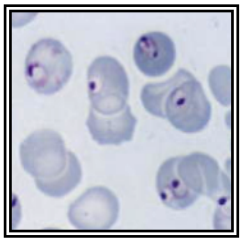
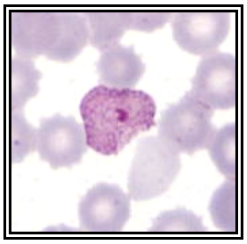
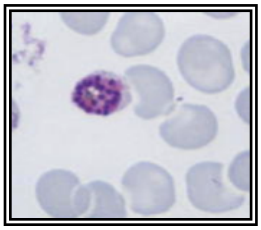
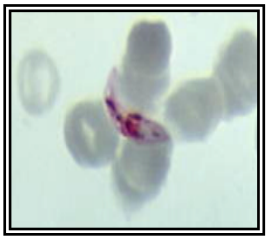
B. Light microscopy

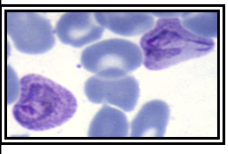
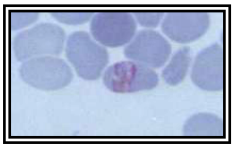
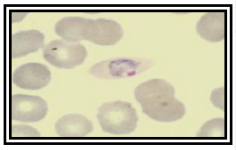
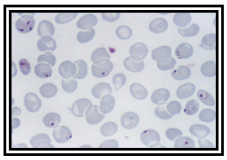
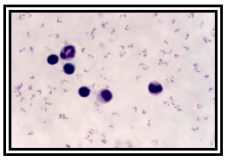
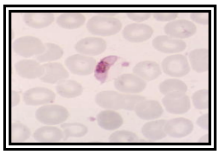
the most important gold diagnosis is light microscopy ”thin & thick film”



You don’t have to memories the procedure

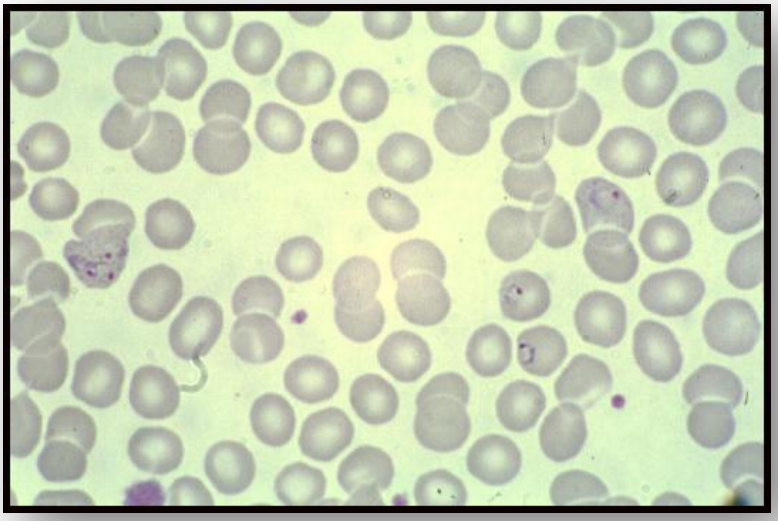
Blood film (thin & thick)

Three developmental stages seen in blood films:		
 		
Trophozoite (ring shaped)	Schizont	Gametocyte (banana shaped)

Plasmodium vivax	*Plasmodium malariae*	*Plasmodium ovale*	*Plasmodium falciparum*		
			trophozoite stage in thin smear	trophozoite stage in thick smear	banana-shaped gametocyte stage
					

Case 1

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



Q1: What is the diagnosis?

Malaria.

Q2: What is the most possible pathogen?

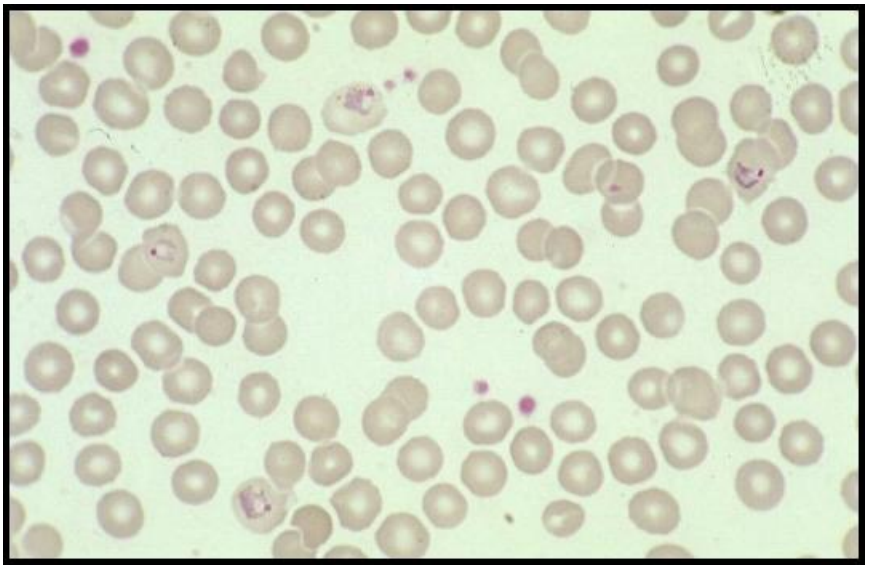
plasmodium Vivax. You just need to mention *Plasmodium* with any type and identify the stage.

Q3: at what stage are the parasites?

Ring stage.

Case 2

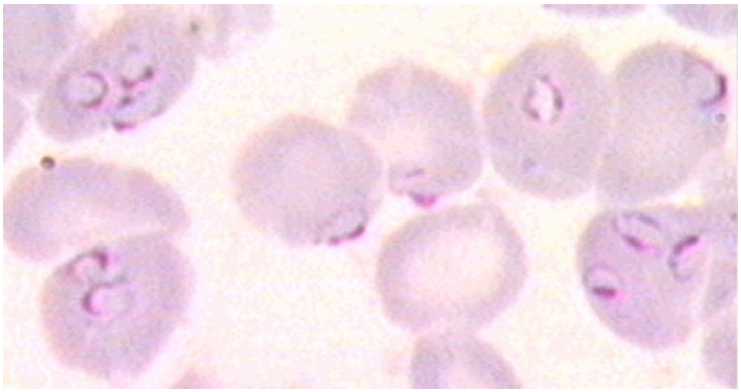
- A businessman who makes frequent trips to Thailand , presents with intermittent fever.



Q1: What is the diagnosis?
Malaria.
Q2: What is the most possible pathogen?
plasmodium Vivax.
Q3: at what stage are the parasites?
Ring stage.

Case 3

- 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.



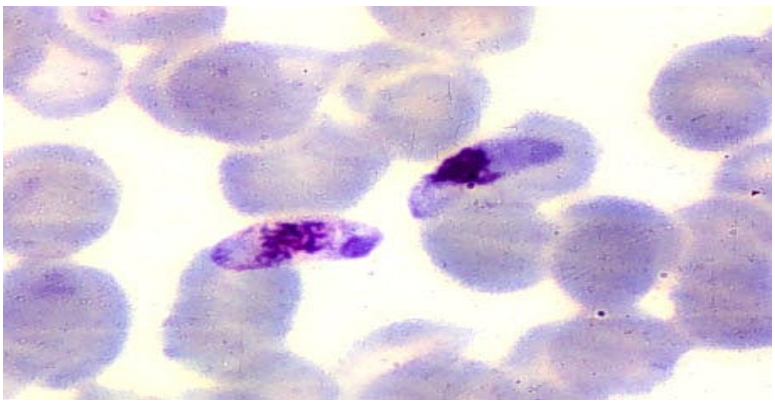
Q1: What is the diagnosis?

Malaria.

Q2: What is the most possible pathogen?

plasmodium flaciparum. Because the symptoms are sever

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



Q3: are there any parasites?

Yes, plasmodium flaciparum.

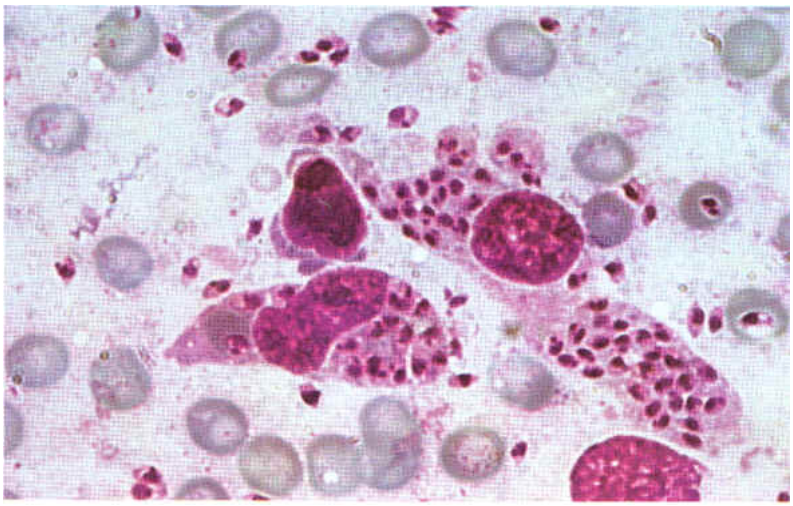
Q4: at what stage?

Gametocyte stage

Case 4

- A 7 year old child presented with anemia, hepatospenomegaly and fever. Not responding to antimalarials and antibiotics .

Bone marrow smear is shown



Q1: are there any parasites ?

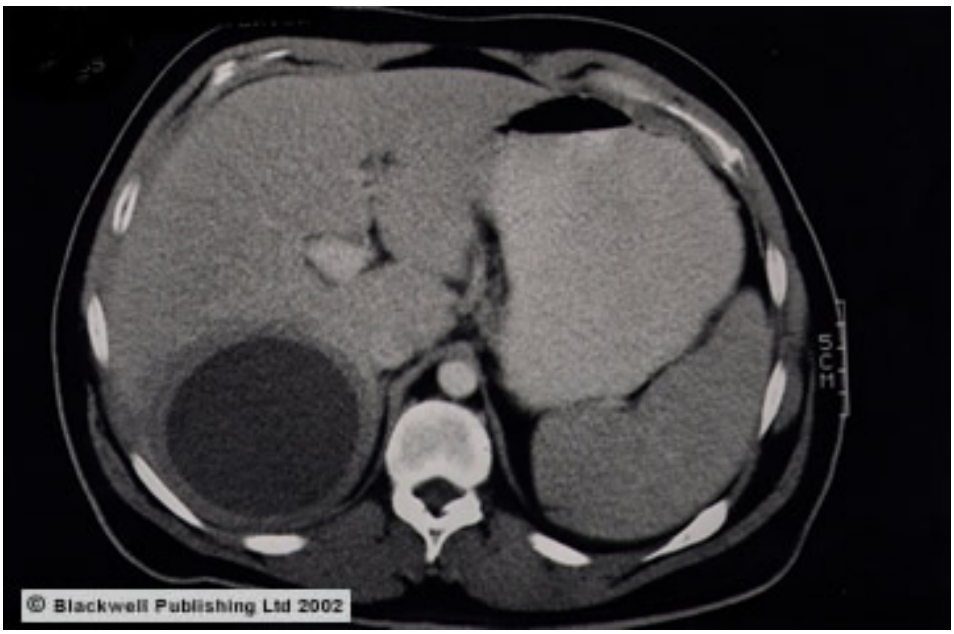
Yes, ***Leishmania***.

Q2: at which stage?

Amastigote stage.

Extra-intestinal Amoebiasis

A 30-year-old male experienced diarrhea for two weeks with fever of 39° C, nausea, vomiting, malaise and right upper abdominal pain.
Physical examination revealed hepatomegaly 6 cm below the right costal margin.
CT scan showed a single hypodense mass in the right lobe of 7.8 x 5.2 cm, round, with well defined borders.



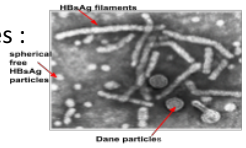
Q1: what is your diagnosis?
Amebic liver abscess
Q2: what is the most likely cause?
Entamoeba histolytica.
Q3: what test would you like to perform?
Serology to look for *Entamoeba histolytica.*
Q4: what is the main drug for treatment?
Metronidazole

Second

Hepatitis

Hepatitis A	
Characteristics	Non-enveloped ssRNA with positive polarity.
Transmission	Contaminated food & water (Faecal-oral route).
Age	Children.
Pathogenesis	ingestion of contaminated food → replicates in the intestine, and then spread to the liver → multiplies in hepatocytes → cellular mediated immunity attack the infected hepatocytes → Damage → Increase in ALT, AST and Bilirubin levels.
Manifestation	• Asymptomatic. • symptoms: - Pre-icteric phase = fever, fatigue, nausea, vomiting & pain in the right upper abdominal quadrant. - Icteric phase = jaundice. Dark urine and pale Stools.
Diagnosis	Markers by ELISA: IgM = indicates current infection. IgG = indicates previous infection or immunity.
Treatment	Supportive therapy ,Not specific.
Prevention	• Sanitation & hygiene measures • Human Immunoglobulin (HIg) within two weeks of exposure. • Inactivated virus vaccine. • Twinrix vaccine.

Hepatitis E	
Characteristics	Non-enveloped ssRNA with positive polarity.
Transmission	Contaminated food & water (Faecal-oral route) - Zoonotic foodborne.
Age	Young adults.
Pathogenesis	ingestion of contaminated food → replicates in the intestine, and then spread to the liver → multiplies in hepatocytes → cellular mediated immunity attack the infected hepatocytes → Damage → Increase in ALT, AST and Bilirubin levels.
Manifestation	Similar to HAV infection.
Diagnosis	Markers by ELISA: Anti-HV IgM = Current infection.
Treatment	Supportive therapy ,Not specific
Prevention	• Sanitation & hygiene measures. • No HIg. • No vaccine.

Hepatitis B	
Characteristics	- Virion consists of: • Outer envelope containing hepatitis B surface antigen (HBsAg)& envelope antigens (HBeAg). • Internal core (HBcAg) • DsDNA genome. • 8 genotypes (A to H), genotype D is common in KSA. - The serum of infected individual contains 3 types of hepatitis B Particles : • free HBsAg Particles • filament • complete HBV particles (Dane particles) 
Transmission	• **Parentally:** Direct exposure to infected blood & Using and sharing contaminated tools,needles, razors, or tooth brushes. • **Sexually:** close personal contact with body fluids of infected individuals. • **Perinatally:** during delivery or breastfeeding.
The clinical outcome	• About 90%of infected the adults will develop **acute hepatitis B** infection and recover completely. • less than 9% of the infected adult, 90%of infected infants and 20% of infected children may progress to **chronic hepatitis B**. • less than 1% may develop **fulminant hepatitis B.**
Diagnosis	1-Markers by ELISA: HBsAg, Anti-HBs , Anti-HBe , IgM Anti-HBe and Anti-HBc. 2- Neutralization test for confirmation. 3- Liver function test.
Treatment	• Pegylated alpha interferon. • Lamivudine (antiviral drug). • Adefovir (antiviral drug: nucleoside analogue).
vaccine	HBsAg particles vaccine.

• Hepatitis B surface antigen (HBsAg):
A protein on the surface of hepatitis B virus, it can be detected in high levels in serum during acute or chronic hepatitis B virus infection. The presence of HBsAg indicates that the person is infectious. The body normally produces antibodies to HBsAg as part of the normal immune response to infection. HBsAg is the antigen used to make hepatitis B vaccine.
• Hepatitis B surface antibody (anti-HBs):
The presence of anti-HBs is generally interpreted as indicating recovery and immunity from hepatitis B virus infection. Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B.
• Hepatitis core antigen (HBcAg):
A Hepatitis B viral protein that Cannot be detected in the serum because it is intracellular in the hepatocytes, it's indicates that The person is infectious.
• Total hepatitis B core antibody (anti-HBc):
Appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with hepatitis B virus in an undefined time frame.
• IgM antibody to hepatitis B core antigen (IgM anti-HBc):
Positive indicates recent infection with hepatitis B virus. It's presence indicates acute infection.
• Hepatitis B envelope antigen (HBeAg):
Detected During acute and chronic Hepatitis B infection, and indicates that The virus is replicating and the infected person has high levels of it.
• Hepatitis B envelope antibody (Anti-HBe):
During recovery from acute hepatitis B, HBeAg level declines and becomes undetectable in the serum, while hepatitis B e-antibody (anti-HBe) appears and becomes detectable in the serum. Anti-HBe usually remains detectable for many years after recovery from acute HBV infection.

Hepatitis C		
Characteristics	• Consists of an outer envelope icosahedral core and linear positive polarity ss-RNA gemone. • There are 6 major genotypes, genotype 4is the dominant in Saudi patients.	
Transmission	**Similar to HBV:** Parenterally - Sexually- From mother to child perinatally	
The clinical outcome	• About 80%of the infected will progress to chronic hepatitis C. • About 20%of the infected individuals will develop self-limiting acute hepatitis C and recover completely. • less than 1% may develop **fulminant hepatitis C,** liver failure and death.	
markers	1- Hepatitis C virus RNA	Is the **1st marker** that appears in circulation, it appears as early **as 2-3 weeks** after exposure. It is a marker of infection.
	2- hepatitis C core antigen	The **2nd marker** that appears in the blood, usually 3-4 weeks after exposure. Marker of infection.
	3- IgG Hepatitis C antibody	is the **Last marker** that appears in the blood, usually appear **50 days after exposure**.
Manifestation	Similar to HAV infection.	
Diagnosis	• Markers by ELISA. HCV-RNA, Anti-HCV, HCcAg, and IgG Hepatitis C antibody. • RIBA or PCR for confirmation.	
Treatment	**combined** therapy using: Pegylated alpha interferon and ribavirin.	
Prevention	No vaccine available.	

Hepatitis D	
Characteristics	• defective virus that cannot replicate by its own. • requires a helper virus is HBV • small ss-RNA genome, surrounded by delta antigen.
Types of infection	1- **Co-infection:** The patient is infected with HBV and HDV at the same time leading to sever acute hepatitis . 2- **Super infection:** delta virus infects those who are already have chronic hepatitis B leading to severe chronic hepatitis.

The previous schedules are just to give you a better understanding to answer any possible questions.

This table is NOT Extra:

Interpretation of the Hepatitis B Panel Tests Results Interpretation:		
Test	**Results**	**Interpretation**
HBsAg	negative	susceptible The patient not infected
anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	immune due to natural infection[1]
anti-HBc	positive	
anti-HBs	positive	
HBsAg	negative	immune due to hepatitis B vaccination
anti-HBc	negative	
anti-HBs	positive	
HBsAg	positive	acutely infected Note that IgM indicates acute infection
anti-HBc	positive	
IgM anti-HBc	positive	
anti-HBs	negative	
HBsAg	positive	chronically infected[2] Note that the absence of IgM indicates chronic infection
anti-HBc	positive	
IgM anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	Interpretation unclear, four possibilities: 1- Resolved infection (most common). 2- False-positive anti-HBc, thus susceptible. 3- 'low level' chronic infection. 4- Resolving acute infection.
anti-HBc	positive	
anti-HBs	negative	

1- Anti-HBs = indicates immunity
Anti-HBc (+) = indicates immunity due to previous exposure.
Anti-HBc (-)= indicates immunity due to vaccination.

2- if a case come with history of positive HBsAg more than six months
This is a CHRONIC infection couldn't be acute whether he mention IgM or not

Case 1

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 department of KKUH because of repeated vomiting, abdominal pain and fever. On examination, his temperature was 38°C, his pulse rate 110/min and BP 120/80mmHg, he was jaundiced and had tenderness in the right upper quadrant of his abdomen.

Q1: What are the possible causes for his presentation?

- Viral hepatitis
- Acute Cholecystitis
- Malaria
- Leptospirosis. Leptospirosis is a bacterial disease that affects humans and animals.
- It is caused by bacteria of the genus *Leptospira,* common in India and it causes jaundice
- Typhoid fever.
- Alcohol.
- Drug-induce hepatitis.
- Autoimmune hepatitis.
- Hemolytic disorders (sickle cell 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?
1	CBC & ESR Complet blood count&erythrocyte sedimentation rate	Shows non-specific signs of infections or inflammation
2	Blood Film for Malaria Thin & thick Giemsa stain smear.	To exclude malaria
3	Liver function test	To asses liver function
4	Viral Hepatitis screening	To exclude viral hepatitis
5	Blood Culture	To exclude typhoid fever
6	Viral hepatitis screening serology test for all types	To exclude viral hepatitis
7	Viral hepatitis confirming serology test for a specific type	To confirm diagnosis if screening was positive.

- Investigation of our patient:

Complete Blood Count[3]		Liver Function Test[4]	
Hb So we exclude anemia	**14.2** g/L	AST	**1557** (Normal = 12-37 IU/L)
WBCs No infection	**6100** mm3	ALT	**1879** (Normal = 20-65 IU/L)
Platelet	**271** g/L	ALP	**441** (Normal = 175-476 IU/L)
ESR	**4** mm/h	Albumin	**42.3** (Normal = 30-50 g/L)
Blood film for Malaria	**NEGATIVE**	Bilirubin	**86** (Normal = 3-17 µmol/L)
Blood culture	**NEGATIVE**		

3- all are normal
4- HIGH liver enzymes levels

Q3: Based on these findings what is the most likely diagnosis?
Viral hepatitis A, B, C, or E

Q4: What further investigations would you like to order?
Hepatitis serology.

- The serologic results were as follows:

TEST	RESULT
Anti-HAV-IgM	Positive
HBsAg	Negative
Anti-HCV	Negative
Anti-HEV IgM	Negative

What's your interpretation? You have to comment on each one explain the positive and negative findings.

Q5:Interpret the serology results found above?
Anti-HAV-IgM positive = indicates an acute13 hepatitis A infection.
HBsAg negative = excludes hepatitis B infection.
Anti-HCV negative = excludes hepatitis C infection.
Anti-HEV IgM negative = excludes acute hepatitis E infection.

Q6: Based on the findings above, what is your final diagnosis?
Acute Hepatitis A Infection.

Q7: Briefly outline the management of this patient?
- Supportive.
- Not working. For about one week
- Contact tracing.
- Follow up (Clinical and laboratory).

Case 2

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.
On arrival to the blood bank, the doctor informed him that his blood is not suitable for transfusion because of the presence of infection and advised him to see his physician

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

- Hepatitis B Virus (HBV).
- Hepatitis C Virus (HCV).16
- Human Immunodeficiency Virus (HIV).
- Human T-Lymphotropic Virus (HTLV).
- Malaria.

TEST	RESULT
HBsAg	Negative
Anti-HBc	Negative
Anti-HCV	Positive
Anti-HBs	Negative
HIV-Ag/Ab	Negative
Anti-HTLV	Negative
ALT	49 (Normal = 20-65 IU)
AST	29 (Normal = 12-37IU)
Bilirubin	4 (Normal = 3-17mol/L)

Q2: The next day Mohammed came to see his general practitioner with a letter from the Blood Bank. The letter revealed the result shown below.
what is your interpretation?

- **HBsAg** negative = Not infected with HBV.
- **Anti-HBc** negative = never exposed to HBV.
- **Anti-HCV** Positive = Infected with HCV and is asymptomatic.
- **HIV-Ag/Ab** negative = Not infected with HIV.
- **Anti-HTLV** negative = Not infected with HTLV.
- **ALT & AST & Bilirubin** = Liver enzymes are normal, this indicates a chronic presentation.

Q3: What is your diagnosis, and how do you define it?
Chronic Hepatitis C infection.

Q4: What do you do next?

- Repeat tests and Serology.
- liver function tests.

Q5: How do you diagnose HCV infection?

A. Serological assay:
- Screening test for Anti-HCV by ELISA.
- Confirmatory test by recombinant17 immunoblot assay (RIBA) or PCR.

If the ELISA test was positive we need to confirm it with RIBA.
ELISA is highly specific and sensitive, however it may cause

- False positive = the patient not infected but the result is positive.
- OR False negative = the patient is infected with the virus but the result is negative.

So we have to confirm by RIBA test.
Because RIBA is highly specific and it will exclude the false results.

B. Molecular assay for detection of RNA (For early acute HCV).
Used to diagnosis of HCV in immunocompromised patients.
Presents of hepatitis C RAN with anti-HCV more than 6 months indicates CHRONIC hepatitis C.

Q6: How do you confirm HCV infection?
RIBA with PCR.

Q7: What is the significance of these tests and how they can help in the management?

Test	Significance	How it can help?
PCR	1- Qualitative: -ve or +ve (HCV-RNA). 2- Quantitative: viral load Viral load: is a numerical expression of the quantity of virus in a given volume. It is often expressed as viral particles, or infectious particles per mL depending on the type of assay	1- Confirm the Diagnosis. 2-Monitor response to treatment.
Genotype	Identify the genotype of HCV Because different genotype shows different respond to the treatment.	Guide the choice antiviral & duration of therapy.

Case 3

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.

test	Result
HBsAg	positive
HBeAg	negative
Anti-HBe	positive
Anti-HBc IgM	negative
Total Anti-HBc	positive
HIV Ag/Ab	negative
Anti-HCV	negative

Q1: How would you interpret these results?

- **HBsAg & Anti-HBe & Total Anti-HBc are positive** = The patient is infected with Hepatitis B.
- **HBeAg negative** = The patient's rate of infectivity is low.
- **Anti-HBc IgM negative** = The patient's infection is chronic.
- **HIV Ag/Ab & Anti-HCV are negative** = The patient is not infected with HCV or HIV.

Q2: what's your diagnosis?

Chronic hepatitis B infection with low infectivity.

Q3: Which HBV marker indicate the rate of infectivity?

HBeAg and Anti-HBe.

Q4: How can you confirm HBV?

Neutralizing test.

Q5: On the lights of these Laboratory results how would you manage the newborn?

Post-exposure prophylaxis:

A- Hepatitis B immune globulin (HBIG) within 12 hours of birth.

B- First dose of recombinant HBV vaccine.

Q6: Is there a risk of transmission of HBV to the newborn?

- **Seropositive for HBsAg with no immunoprophylaxis:** vertical transmission21 = 10-20%.
- **Seropositive for both HBsAg and HBeAg:** vertical transmission = 90%.
- **Acute hepatitis B occurs in the first trimester:** vertical transmission = 10%.
- **Acute hepatitis B occurs in the third trimester:** vertical transmission = 90%.

Q7: What further management would you offer to the mother?

- No donation of blood, body organs, or other tissues.
- No sharing of personal items (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 her partner and need for testing.

Today 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.

Q1: What should you do?

1. report occupational exposures immediately
2. Review the hepatitis B vaccination status and the vaccine-response status.

Q2: What is the risk of infection to you?

- **If the blood was positive (HBsAg & HBeAg):** risk of serological evidence is 37-62%.
- **If the blood was positive HBsAg & negative HBeAg:** risk of serological evidence is 23-37%.

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