## Problem 5 – Unit 6 – Microbiology: lab

### Station 1:

- **Note**: it is very important to know the history of the patient especially if he is coming or traveling to an endemic area of malaria.
- Lab test for malaria:
  - Blood film (gold standard).
  - <u>Thin blood film:</u>
    - ✓ It is used to study the morphology of RBCs (RBCs are equally distributed on the slide).
    - ✓ To detect the species of parasites and to know the percentage of parasitized red cells.
    - ✓ To detect co-infections.
  - Thick blood film:
    - ✓ Blood + one drop of water → breakdown of RBCs with hemoglobin background.
    - ✓ Severity of infection cannot be detected.
    - ✓ Also, co-infection cannot be detected.
    - It is a rapid test (qualitative) to detect if parasites are present or not.

# - Procedure of preparing thin and thick blood film:

- Thin blood film:
  - ✓ Add one drop of blood on a slide.
  - ✓ Using another slide, put it on the first slide (with the blood drop) in a 45 angle.
  - $\checkmark$  Then spread the blood on one side in a quick motion.
  - ✓ Let the slide dry.
  - ✓ Fix the slide in methanol for 30 seconds.
  - ✓ Add eosin stain without washing the slide.
  - ✓ Add methylene blue without washing the slide.
  - $\checkmark$  Rinse with water.
  - ✓ Let it dry.
  - ✓ Examine it under the microscope.
- Thick blood film:
  - ✓ Add one drop of blood on a slide.
  - ✓ Mix the blood on the slide using a stick.
  - ✓ Let it dry.
  - ✓ Then add one drop of water.
  - ✓ Let it dry.
  - ✓ Add eosin stain.
  - ✓ Add methylene blue (without washing the slide).
  - ✓ Rinse with water.
  - ✓ Let it dry.
  - ✓ Examine under the microscope.

### Station 2:

## Malaria: it is caused by protozoa parasite:

- Genus: Plasmodium.
- <u>Species</u>:
  - ✓ P.vivax.
  - ✓ P.malariae.
  - ✓ P.falciparum.
  - ✓ P.ovale

**Note**: these are sporozoa parasites (because of a stage known as sporozoites in their life cycle. This is the infectious stage).







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### - Life cycle:

- Infected female Anopheles mosquito with sporozoites in its salivary glands will inject them in the circulation of a human.
- Sporozoites will move to the liver (infected hepatocytes: within 30 minutes).
- Sporozoites will grow to schizonts in liver cells.
- Schizonts will rupture releasing merozoites.
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- Merozoites are the infective stage of RBCs. The first erythrocytic cycle will start by merozoites invading the red cells.
- Merozoites will grow to trophozoites and then to schizonts.
- Schizonts will rupture releasing merozoites which will infect other RBCs again and again.
- After several cycles, some of these merozoites will grow to gametocytes. This is considered as the second erythrocytic cycle.
- Gametocytes will be taken by the feeding female Anopheles mosquito where they will grow to macrogametocytes (females) and microgametocytes (males).
- Zygote will be produced and then it will be converted to oocyst which will rupture releasing sporozoites that will travel to the salivary glands of the mosquito.



### - Plasmodium:

- P.vivax:
  - It is a common cause of malaria.
  - ✓ It is difficult to identify it from the ring stage.
  - ✓ Trophozoites: ameboid form with schuffner's dots.
  - ✓ Schizonts: large RBCs with scattered merozoites and schuffner's dots.
  - ✓ P. vivax infects RBCs in the reticulocyte stage.
  - ✓ It has a dormant cycle in the liver producing a latent form (hypnozoite) which will cause relapse of the disease.
  - ✓ The timing of the fever cycle is every 48 hours (benign tertian malaria).
  - ✓ The receptor of P.vivax is the duffy blood group antigen. More than 90% of black west Africans and many of their American descendants do not produce the Duffy antigen and are thereby resistant to vivax malaria.



- <u>P.ovale:</u>
  - ✓ Trophozoites: characterized by the presence of schuffner's dots and the RBCs look like a broken egg.
  - Schizonts: RBCs are oval in shape merozoites are arranged like a flower with the presence of schiffner's dots.
  - ✓ It has a dormant cycle in the liver producing a latent form (hypnozoites) which will cause relapse of the disease.
  - ✓ The timing of the fever cycle is every 48 hours (benign tertian malaria).
- <u>P.malariae:</u>
  - ✓ Trophozoites: band-shape .
  - Schizonts: merozoites arranged like a flower with no schuffner's dots.
  - ✓ It is a cause of nephrotic syndrome.
  - ✓ It affects mature RBCs.
  - ✓ It causes quartan malaria: fever cycle is every 72 hours.
- <u>P.falciparum:</u>
  - Ring shape: more than two rings can be found in the same RBC. Also, marginal form might be found. Rings are characterized by double chromatin.
  - ✓ Trophozoites: rarely seen in peripheral blood.
  - Schizonts: rarely seen in peripheral blood merozoites are scattered.
  - ✓ It is a common cause of malaria which will be more severe than that caused by other plasmodia.
  - ✓ It is characterized by infection of far more red cells (high level of paracytemia).
  - ✓ Occlusion of the capillaries with parasitized red cells will lead to the lifethreatining condition (cerebral malaria).
  - ✓ Also, there will be extensive hemolysis and kidney damage with resulting hemoglobinuria (blackwater fever).
  - ✓ It causes malignant tertian malaria: fever every 48 hours.
  - ✓ People with G6PD deficiency are protected against severe effects of P.falciparum malaria.

**Note**: individuals with sickle cell trait (HbA/HbS) are protected against malaria because their red cells have too little ATPase activity and cannot produce sufficient energy to support growth of the parasite. Also, there is efflux of  $K^+$  from sickled RBCs (potassium is needed for the growth of parasites).

# - Gametocytes:

- P.vivax is characterized by large RBCs with the presence of schuffner's dots.
- P.ovale is characterized by RBCs (oval in shape) with the presence of schiffner's dots.
- P.malariae has no schuffner's dots.
- P.falciparum is banana-shaped or crescent-shaped.



# - Treatment:

- Chloroquine is the drug of choice for acute malaria caused by sensitive strains (they kill merozoites and thus reducing parasitemia).
- Hypnozoites of P.vivax and P.ovale in the liver are killed by primaquine. Note that primaquine should not be given if the patient is G6PD deficient because it induces severe hemolysis.
- In areas where chloroquine-resistant strains of P.falciparum exist, the drug of choice is malarone (atovaquone + proguanil).
- In severe cases, especially of falcuparum malaria, IV quinidine plus doxycycline should be used.







RARELY SEEN IN PERIPHERAL BLOOD



