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| | **KULLIYYAH OF MEDICINE & HEALTH SCIENCES** |
| **Course/Course Code** | Medical Parasitology/ PPB62303 |
| **Topic** | Parasitology Practical 3: Blood Protozoa |
| **Year/Semester/Session** | 2 (Cohort 5) / 3 / 2018 (3) |
| **Date** | |
| **Time** | |
| **Student's Name/ ID** | |
| **Lecturer's Name** | Dr. Lee Ii Li |

**At the end of the practical session, students should be able to:**

| TLO No | Topic Learning Outcome (TLO) | Course Learning Outcome (CLO) |
|---|---|---|
| 1 | Identify blood protozoa by stages and their distinctive morphological features | 5 |

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| **References** | 1. Franklin A.N. & Harold W. (1998). **Basic and Clinical Parasitology** (6th Edition) New York Prentice Hall. |
| | 2. Viqar, Z., & Loh, A.K. (1996) **Handbook of Medical Parasitology** (3rd Edition). |
| | 3. Mak, J.W. & Choong, M.F. (2012). **Atlas of Medically Important Parasites** (3rd Edition) UY Printers. |

## Introduction:

Diagnosis of parasitic infections of the blood is mainly based on the identification of parasitic stages in blood samples. Thus, it is important for students to have knowledge of collecting diagnostic stages of protozoa and identifying the parasites based on morphology of the parasites. Most often, parasites are not visible even under the microscope, therefore students will be taught some basic techniques of preparation and staining of samples.

## Principle:

Identification is based on the morphology of stained parasites.

## Material/Reagents Preparation:

### Materials:

1. Glass slides
2. Cover glass
3. Microscope
4. Lens paper
5. Needles for pricking fingers /lancet
6. Methanol
7. 70% ethanol
8. Dropping pipette
9. Gloves

## Procedures:

1. Prepare thick and thin blood smear.
2. Observe the slides.
3. Differentiate thick blood film from thin.
4. Draw and label the morphological features of the parasites.

## ACTIVITIES

### Blood collection for thick or thin blood smears

In general, there are 2 blood collection methods. Namely, capillary blood obtained by fingerstick and venous blood obtained by venipuncture. For this practical, we will concentrate on the former.

### Capillary blood obtained by fingerstick

1. Label pre-cleaned slides (preferably frosted-end) with patient's name (or other identifier), date and time of collection.
2. Wear gloves.
3. Clean slides with 70 to 90% alcohol and allow to dry. Do not touch the surface of the slide where the blood smear will be made.
4. Select the finger to puncture, usually the middle or ring finger. In infants, puncture the heel.
5. Clean the area to be punctured with 70% alcohol; allow to dry.
6. Puncture the ball of the finger, or in infants puncture the heel.
7. Wipe away the first drop of blood with clean gauze.
8. Touch the next drop of blood with a clean slide. Repeat with several slides (at least two thick and two thin smears should be made). If blood does not well up, gently squeeze the finger.

4.

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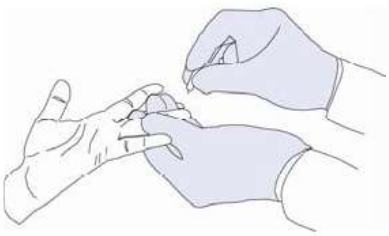

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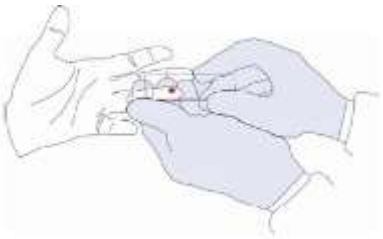
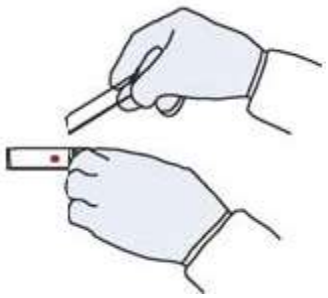
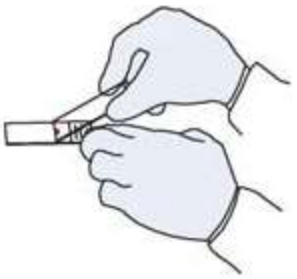


## Making thick and thin blood smears

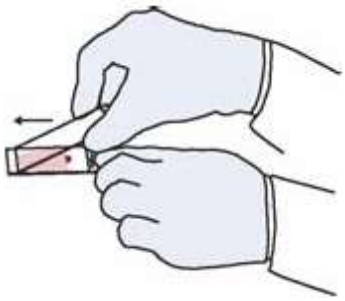
1. Whenever possible, use separate slides for thick and thin smears.
2. **Thin film** (a): Bring a clean spreader slide, held at a 45° angle, toward the drop of blood on the specimen slide.



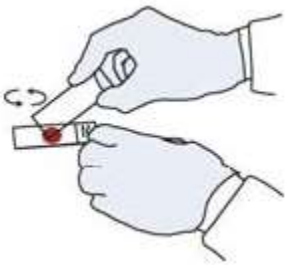
3. Thin film (b): Wait until the blood spreads along the entire width of the spreader slide.



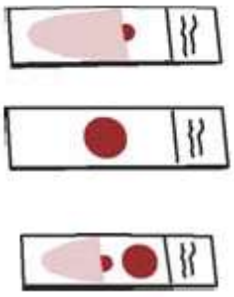
4. Thin film (c): While holding the spreader slide at the same angle, push it forward rapidly and smoothly.



5. **Thick film**: Using the corner of a clean slide, spread the drop of blood in a circle the size of a dime (diameter 1-2 cm). Do not make the smear too thick or it will fall off the slide. (You should be able to read newsprint through it.)



6. Wait until the thin and thick films are completely dry before staining. Fix the thin film with methanol (100% or absolute) and let it dry completely before staining. The thick film should not be fixed.
7. If both thin and thick films need to be made on the same slide, fix only the thin film with methanol. The thick film should not be fixed.



*Reference:*
*https://www.cdc.gov/dpdx/resources/pdf/benchAids/malaria/Malaria_procedures_benchaid.pdf*

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| **TLO1:** | **Identify blood protozoa by stages and their distinctive morphological features** |

Note:
Diagnostic technique for the following parasites is through thin blood smear

| | Description on intestinal protozoa | | Morphology |
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| 1. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium falciparum*<br>Late trophozoite<br>Thicker cytoplasm. A few reddish spots called Maurer's clefts may be seen in the RBC's stroma. The size of the infected RBC is similar to that of uninfected RBC *(*similar to P.m)* | |
| 2. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium falciparum*<br>Schizont<br>Rarely seen in peripheral blood. Contain 8 – 24 merozoites. Dark pigment clumped in one mass | |
| 3. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium falciparum*<br>Gametocyte<br>Unique crescentric or banana-shaped. Dense chromatin at the centre of parasite. Malaria pigment may be scattered throughout the cytoplasm or closely aggregated around the nucleus | |
| 4. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium vivax*<br>Late trophozoite<br>Infected RBC appears enlarged. Amoeboid cytoplasm. The presence of Schuffner's dots that stained pinkish-orange | |

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| 5. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium vivax*<br>Schizont<br>Contains 12 – 24 merozoites (usually 16), filling the enlarged RBC | |
| 6. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium vivax*<br>Gametocyte<br>Large rounded parasite, which fills or nearly fills the RBC. The cytoplasm is blue and fairy homogenous. Nuclear chromatin is single, well-defined purplish mass. | |
| 7. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium malariae*<br>Late trophozoite<br>The cytoplasm extends across the RBC, giving rise to 'band-form' trophozoite. The size of infected RBC is similar to that of uninfected RBC *(*similar to P.f)* | |
| 8. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium malariae*<br>Schizont<br>Contains 6 – 12 large merozoites (usually 8) arranged in a distinct 'rosette' form. Normal size on infected RBC | |
| 9. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium knowlesi*<br>Late trophozoite<br>*similar to *P. malariae,* but, has double chromatin dots, two or three parasites per erythrocyte | |

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| 10. | **Example**<br>**Stage**<br>**Feature** | *Plasmodium knowlesi*<br>Schizont<br>*similar to *P. malariae,* but, has 16 merozoites | |
| 11. | **Example**<br>**Stage**<br>**Feature** | *Trypanosoma evansi*<br>Trypomastigote<br>Presence of nucleus, a kinetoplast and a flagellum that stems from the kinetoplast and runs through the remainder of the parasite and also extends beyond it | |