LABORATORY BIOSAFETY GUIDELINES FOR HANDLING AND PROCESSING SPECIMENS

General Guidelines (for working with potentially infectious materials)

1. All laboratories should perform a site-specific and activity-specific risk assessment to identify and mitigate risks. Risk assessments and mitigation measures are dependent on:

   - The procedures performed
   - Identification of the hazards involved in the process and/or procedures
   - The competency level of the personnel who perform the procedures
   - The laboratory equipment and facility
   - The resources available

2. Follow Standard Precautions when handling clinical specimens, all of which may contain potentially infectious materials. Standard Precautions include but not limited hand hygiene and the use of personal protective equipment (PPE), such as laboratory coats or gowns, gloves, and eye protection.

3. Follow routine laboratory practices and procedures for decontamination of work surfaces and management of laboratory waste.

4. Any procedure with the potential to generate fine-particulate aerosols (e.g., vortex or sonication of specimens in an open tube) should be performed in a Class II Biological Safety Cabinet (BSC). Appropriate physical containment devices (e.g., centrifuge safety buckets; sealed rotors) should be used for centrifugation.

   I. After centrifuge, wait after 15 minutes before opening the centrifuge.

   II. Ideally, rotors and buckets should be loaded and unloaded in a BSC. Perform any procedures outside a BSC in a manner that minimizes the risk of exposure to an inadvertent sample release.

5. After specimens are processed, decontaminate work surfaces and equipment and transportation box with appropriate disinfectants.

6. All clinical disposable waste should be put into appropriate biohazard bag and sealed to be autoclaved or incinerated.

A. Routine Diagnostic Testing Other Than Respiratory Samples

Routine diagnostic testing procedures, are recommended can be handled in a BSL-2 laboratory using Standard Precautions: For example:

- Initial processing of specimens or sample preparation.
- Testing specimen using automated instruments and analyzers.
- Staining and microscopic analysis of fixed smears (Staining and reading BFMP, FBP). LAMPIRAN A
- Examination of bacterial cultures.
- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues (HPE)
- Packaging of specimens for transport to diagnostic laboratories for additional testing
- Using inactivated specimens, such as specimens in nucleic acid extraction buffer
- Performing electron microscopic studies with glutaraldehyde-fixed grids
- Performing routine antibody or antigen detection tests

NOTE: The packaging of specimen and transportation to the laboratory FOLLOWS THE STANDARD PRACTICE OF THE LABORATORY.

B. The following activities involving manipulation of potentially infected specimens should be performed as above and, in a Class II, BSC:

- Aliquot and/or diluting specimens
- Inoculating bacterial or mycological culture media (Sample processing) PLEASE PROCESS POSITIVE BLOOD CULTURES AND ANY SAMPLE IN THE BSC.
- Performing diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
- Nucleic acid extraction procedures involving potentially infected specimens
- Preparation and chemical- or heat-fixing of smears for microscopic analysis. ie: AFB Smear

C. Procedures with a High Likelihood of Generating Droplets or Aerosols

- Many routine laboratory procedures can generate aerosols and droplets that are often undetectable. For example, the following laboratory procedures have been associated with the generation of infectious aerosols and droplets: centrifugation, pipetting, vortexing, mixing, shaking, sonicating, removing caps, decanting liquids, preparing smears, flaming slides, aliquoting and loading specimens, loading syringes, manipulating needles, syringes or sharps, aspirating and transferring blood and body fluids, subculturing blood culture bottles, spilling specimens, and cleaning up spills.

- Procedures with a high likelihood of generating aerosols or droplets should be conducted using either a certified Class II BSC or additional precautions to provide a barrier between the specimen and personnel.

- Site- and activity-specific biosafety risk assessments should be performed to determine if additional biosafety precautions are warranted based on situational needs, such as high testing volumes.

D. Virus Isolation

- Virus isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens should only be conducted in a Biosafety Level 3 (BSL-3) laboratory.

RISK ASSESSMENT FOR COVID-19

Site- and specific biosafety risk assessments should be performed to determine if additional biosafety precautions are warranted based on situational needs.

a) Risk assessment is a systematic process of gathering information and evaluating the likelihood and impact of exposure to or release of workplace hazard(s) and determining the appropriate risk control measures to reduce the risk.

i. Diagram 1: Risk assessment process

b) Risk assessment is carried out by gathering information and identifying hazards (Diagram 1) in a situation that may expose operators to infections when conducting test. Factors to be taken into account are the use of personnel protective equipment, facility design, ventilation of testing area, level of health of personnel conducting tests, standard operating procedures in place and operators' competency. After information are gathered, risks are evaluated.

c) After considering the above factors, the inherent risk can be qualitatively determined using a two-dimensional graph as below:

Consequences of exposure/ release	Severe	Medium	High	Very high
	Moderate	Low	Medium	High
	Negligible	Very low	Low	Medium
		Unlikely	Possible	Likely
		Likelihood of exposure/release		

d) The likelihood of exposure will depend on control measures that are already in place. Very low or low risk level indicates that existing controls are sufficient to protect operators from infection during the testing. For moderate, high or very high risk level, additional mitigation measures should be carried out. Risk should be reduced to a level that is acceptable.

e) A control strategy must be developed but must take in consideration sufficient resources to secure and maintain potential risk control measures. Additional mitigation, for example, is to provide engineering controls such as the addition of ventilation exhaust fans to increase room ventilation, use of additional personal protective devices or any other strategy considered feasible.

f) Risk assessment should be a continuous process and should be performed whenever changes take place i.e. personnel, facility, equipment, methods and regulations.

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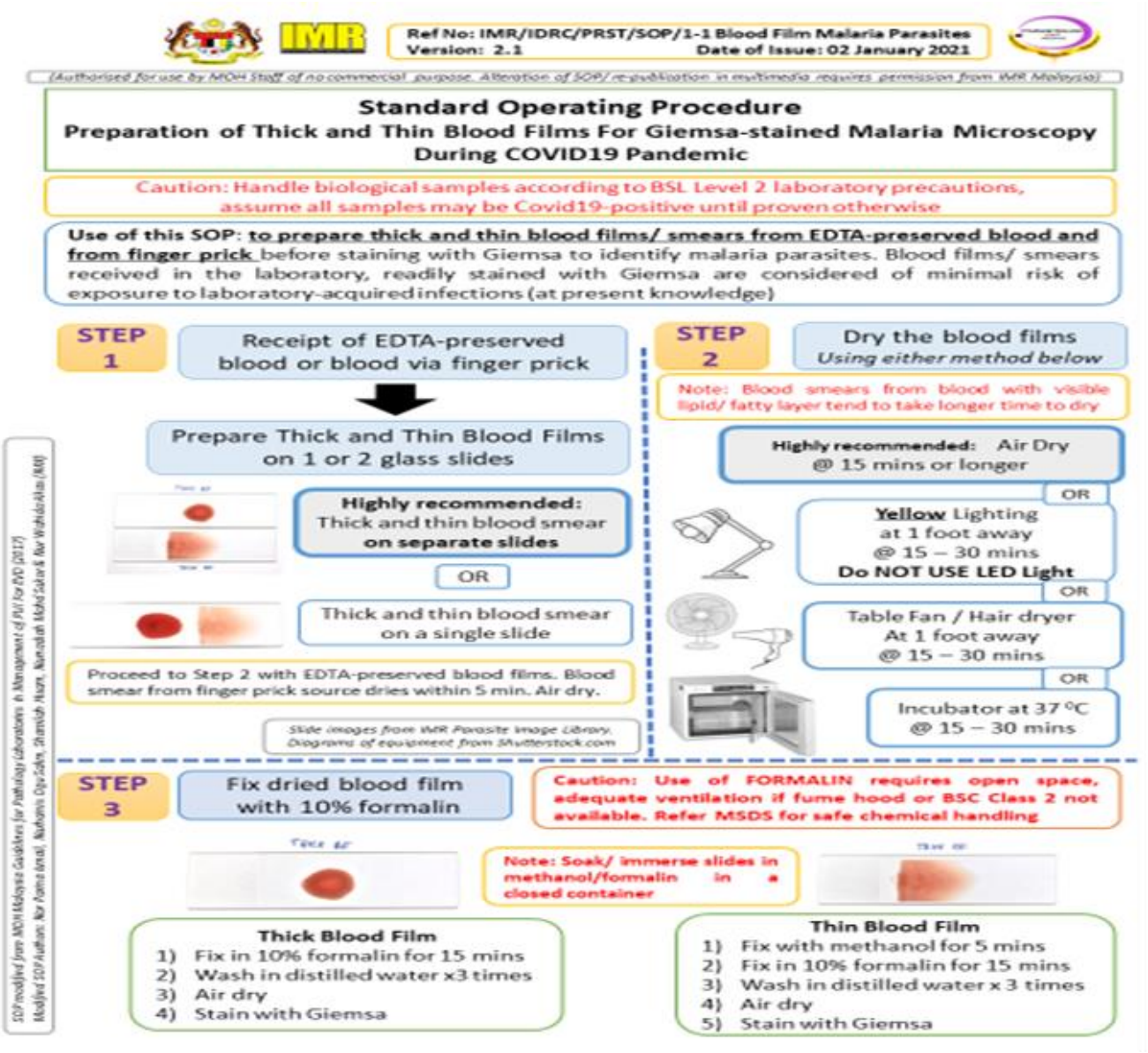
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Standard Operating Procedure
Preparation of Thick and Thin Blood Films For Giemsa-stained Malaria Microscopy During COVID19 Pandemic
Caution: Handle biological samples according to BSL Level 2 laboratory precautions, assume all samples may be Covid19-positive until proven otherwise
Use of this SOP: to prepare thick and thin blood films/ smears from EDTA-preserved blood and from finger prick before staining with Giemsa to identify malaria parasites. Blood films/ smears received in the laboratory, readily stained with Giemsa are considered of minimal risk of exposure to laboratory-acquired infections (at present knowledge)
STEP 1
Receipt of EDTA-preserved blood or blood via finger prick
Prepare Thick and Thin Blood Films on 1 or 2 glass slides
Highly recommended: Thick and thin blood smear on separate slides
OR
Thick and thin blood smear on a single slide
Proceed to Step 2 with EDTA-preserved blood films. Blood smear from finger prick source dries within 5 min. Air dry.
Slide images from IMR Parasite Image Library. Diagrams of equipment from Shutterstock.com
STEP 2
Dry the blood films
Using either method below
Note: Blood smears from blood with visible lipid/ fatty layer tend to take longer time to dry
Highly recommended: Air Dry @ 15 mins or longer
OR
Yellow Lighting at 1 foot away @ 15 – 30 mins
Do NOT USE LED Light
OR
Table Fan / Hair dryer At 1 foot away @ 15 – 30 mins
OR
Incubator at 37 °C @ 15 – 30 mins
STEP 3
Fix dried blood film with 10% formalin
Caution: Use of FORMALIN requires open space, adequate ventilation if fume hood or BSC Class 2 not available. Refer MSDS for safe chemical handling
Note: Soak/ immerse slides in methanol/formalin in a closed container
Thick Blood Film
1) Fix in 10% formalin for 15 mins
2) Wash in distilled water x3 times
3) Air dry
4) Stain with Giemsa
Thin Blood Film
1) Fix with methanol for 5 mins
2) Fix in 10% formalin for 15 mins
3) Wash in distilled water x 3 times
4) Air dry
5) Stain with Giemsa