SOP

Preparation of Saline and Iodine Wet Mounts for Direct Microscopy of Stool Samples

Department of Parasitology
Faculty of Medicine
UWUSL

Standard Operating Procedure (SOP) for Preparation of Saline and Iodine Wet Mounts for Direct Microscopy of Stool Samples

Issued By
Faculty of Medicine, Uva Wellassa University of Sri Lanka

1. Purpose
The purpose of this SOP is to establish a standardized procedure for the preparation and examination of saline and iodine wet mounts for direct microscopic identification of intestinal parasites in stool samples, ensuring accuracy, reliability, and consistency in laboratory practice.

2. Scope
This SOP applies to all academic staff, non-academic staff and students involved in parasitology practical sessions and diagnostic laboratory work related to stool microscopy within the Faculty of Medicine.

3. Responsibilities
3.1 Academic Staff / Demonstrators
- Supervise and guide students during slide preparation and examination
- Ensure adherence to laboratory safety and procedural standards
- Verify accuracy of observations and interpretations

3.2 Students
- Prepare wet mount slides according to the standard procedure
- Ensure proper labeling and handling of specimens
- Maintain cleanliness and avoid contamination
- Perform systematic microscopic examination

3.3 Technical officer
- Ensure availability of required materials and reagents
- Maintain quality assurance in laboratory procedures
- Prepare and standardize reagents and protocols

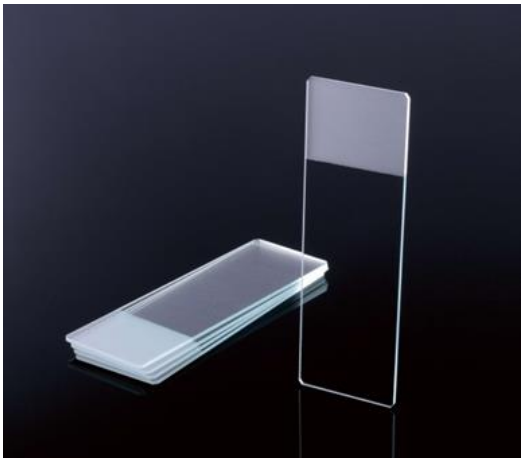
3.4 Laboratory assistant
- Clean and maintain laboratory equipment
- Arrange materials for practical sessions
- Dispose of waste properly

4. Safety Considerations
When working with stool specimens face potential risks including ingestion of eggs or cysts, skin penetration by infective larvae, and infection by nonparasitic agents found in stool. These risks can be minimized by adopting universal precautions as well as standard microbiological laboratory practices (Biosafety Level 2).
These include:
- Wear protective safety glasses, gloves and laboratory coat when processing specimens.
- Use biological safety cabinets as needed.
- Do not eat, drink, smoke, apply cosmetics or manipulate contact lenses in work area.
- Decontaminate work surface at least once a day and after any spill of potentially infectious material.
- If you have cuts or abrasions on the skin of your hands, cover them with adhesive dressing.

- If you use any sharp instruments, dispose of them in a “sharps” container for decontamination.
- Remove gloves and wash your hands after completing any task involving the handling of fecal material.
- Dispose of biological waste according to laboratory guidelines

5. Materials and reagents need,

- Clean glass slides (75 × 25 mm)



- Cover slips

- Wooden applicator sticks or wire loops

- Dropping bottles containing:
    - Physiological saline (0.85% NaCl)
    - Lugol's iodine solution (1% solution)

- Functional light microscope with 10X, 40X and 100X objectives

- Marker for labeling

- Gloves

6. Procedure

6.1 Specimen Collection

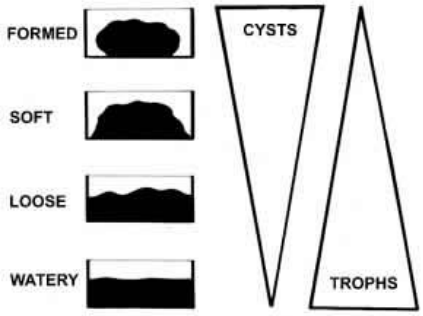
- Collect approximately 10g of fresh faeces (approximately equivalent to 1 to 2 table spoons) uncontaminated by urine or water, using wooden spatula.
- Place in a clean, leak proof, wide mouthed container with a screw cap.

- The container should be free from antiseptics and disinfectants.
- Label the sample clearly with the patient's name, reference number, date and time of collection.
- Samples with patients with infections (e.g.: - AIDS, hepatitis) should be labeled as 'Biohazard'.
- Because parasites may be shed irregularly, 3 stool samples on alternative days are usually examined.
- In highly suspected cases, up to 6 samples may be required.

6.2 Storage and Preservation

- Most viable parasites are sensitive to desiccation and temperature changes.
- So, if examination is delayed, preservatives may be required, to retain the morphology of the parasites as near as original as possible.
- Various preservatives are available.
- The most commonly used being 10% aqueous formalin.

6.3 Delivery and transportation



- Formed stool samples without evidence of blood or mucus, should be examined on the same day of passage. Can be stored up to 4 hours at 4 °C.
- Soft, unformed or liquid stool samples and samples with blood or mucus, may contain vegetative, trophozoite forms of protozoa. These samples should reach the lab within 30 minutes – 1 hour of collection

6.4 Saline and Iodine wet mount preparation

i. Slide Labeling

- Label the slide with the patient's name or identification number and date on the left-hand end using a marker.

ii. Preparation of Reagents on Slide (Figure 1)

- Place a drop of saline in the center of the left half of the slide
- Place a drop of iodine solution in the center of the right half of the slide

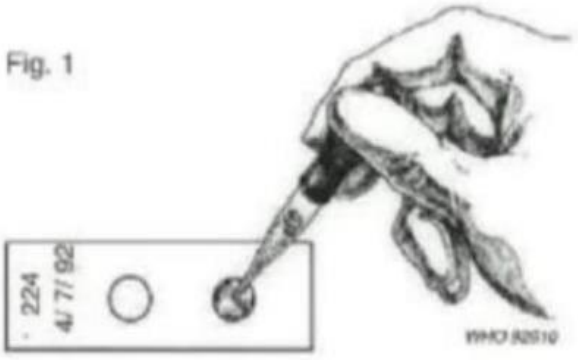


Figure 1

iii. Sample Application (Figure 2)

- Using an applicator stick, pick a small portion of stool (approximately 2 mg, about the size of a match head)
- Mix one portion with the saline drop
- Mix another portion with the iodine drop
- Ensure a uniform suspension is formed

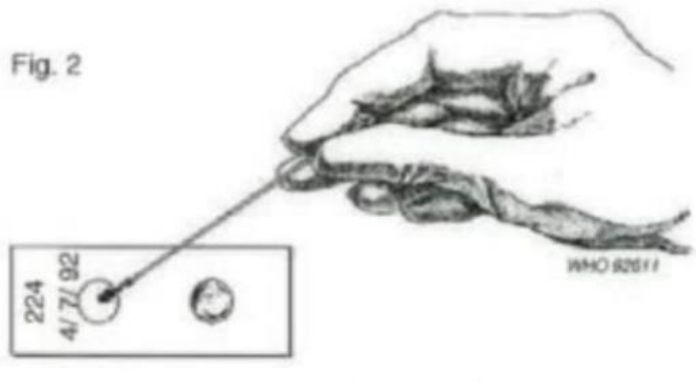


Figure 2

iv. Cover Slip Placement (Figure 3)

- Place a cover slip over each preparation carefully
- Lower it gently at an angle to avoid air bubbles

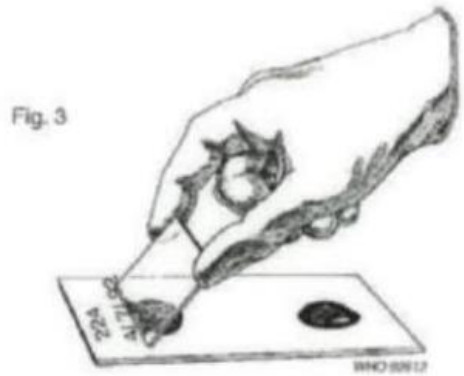


Figure 3

- Ensure the preparation is neither too thick nor too thin (Thickness check: Slide should be translucent enough to read text beneath) (Figure 4)

Figure 4

v. Microscopic Examination

- Examine the saline wet mount slide using the 10× objective lens initially

- Examine the entire cover slip area by moving the slide systematically backwards and forwards or up and down. (Figure 5)

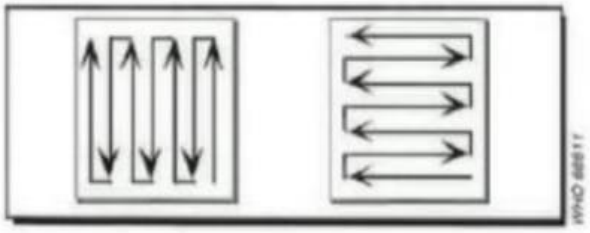


Figure 5

- Switch to the 40× objective lens when suspected parasites are seen for detailed examination and identification.
- Examine the iodine wet mount similarly.

7. Records and Documentation

- Record observations accurately – If parasites are present, record as positive for specific parasite and their stage
- Report findings to the supervising academic staff

8. Post-Examination Procedure

- Universal precautions should be followed when handling stool specimens.
- All contaminated materials (slides, coverslips, applicator sticks, specimen containers) should be disposed of as biohazardous waste according to the laboratory's safety guidelines and relevant regulations.
- Clean microscope stage, stage knobs, coarse and fine adjustment knobs using 70% ethanol
- Clean the objective lenses and condenser lens using lens paper only

Prepared By:

Snr. Prof. Channa Yahathugoda
Chair Professor of Parasitology
Department of Parasitology
Faculty of Medicine
University of Ruhuna

Dr. Samira Jayasinghe
Senior Lecturer
Head of the Department
Department of Parasitology
Uva Wellassa University of Sri Lanka

Dr. Samantha De Silva
Senior Lecturer,
Department of Parasitology
Faculty of Medicine
Uva Wellassa University of Sri Lanka

Dr. Sudath Abeyawickrama
Senior Lecturer
Department of Pediatrics
Faculty of Medicine
Uva Wellassa University of Sri Lanka

Snr. Prof. Muditha Vidanapathirana
Dean
Faculty of Medicine
Uva Wellassa University of Sri Lanka

Dr. Prabhavi Jeewarathne
Temporary Demonstrator
Department of Parasitology
Faculty of Medicine
Uva Wellassa University of Sri Lanka

Date of Implementation
09/04/2026