PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20100 – Acridine Orange Stain	Policy Number: 15-152-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 18/03/2024	Next Review Date: 18/03/26
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 18/03/2024
Accreditation Canada Applicable Standard: NA	

GUIDING PRINCIPLE:
The acridine orange stain is an optional stain that can be helpful in detecting organisms not visualized by Gram stain. This may be due to the nature of the organism's cell wall or due to organisms being hidden in cellular debris. It is also useful for positive blood culture specimens, where no bacteria are seen in the Gram-stained smear.

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the acridine orange stain.

SCOPE/APPLICABILITY:
This standard operating procedure applies to Medical Laboratory Technologists (MLTs) performing the acridine orange stain.

SAMPLE INFORMATION:

Type	• Sterile fluids, including CSF • Blood culture specimens where no bacteria are seen in the Gram-stained smear

REAGENTS and/or MEDIA:
- Methanol
- Acridine orange stain

SUPPLIES:
- Glass microscope slide
- QC slide
- Immersion oil
- Slide storage tray

EQUIPMENT

- Hot plate
- Fluorescent microscope

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

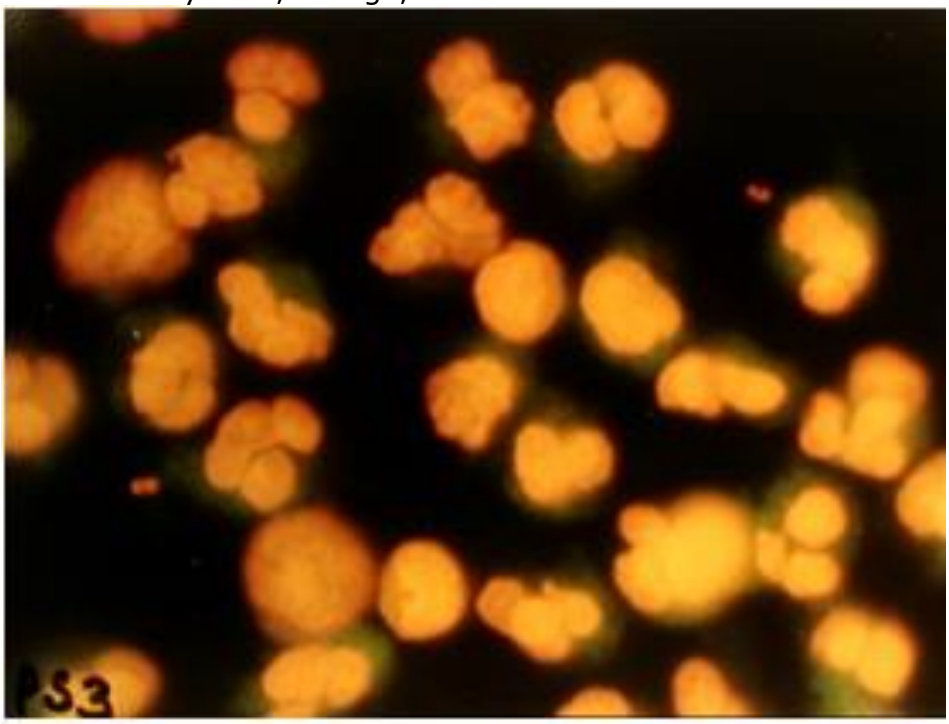
QUALITY CONTROL:

- Quality control is performed as tested
- A TQC order is automatically generated when test is ordered
- Refer to MIC60060-Microbiology Stain Quality Control

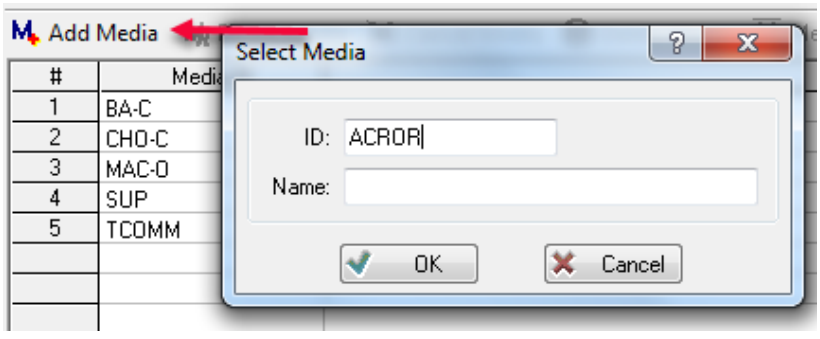
PROCEDURE INSTRUCTIONS:

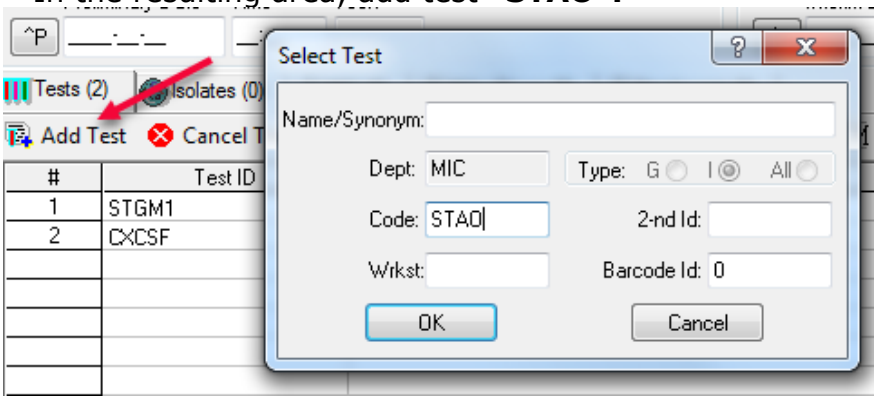
Step	Action
Performing the acridine orange stain	
1	Turn on the fluorescent component of the microscope and allow the lamp to warm up for at least 15 minutes.
2	Prepare a smear of the specimen to be stained. Refer to MIC10000-Microbiology Specimen Handling for slide preparation instructions.
3	Place slide on the slide warmer in the BSC until dry.
4	Once dry, fix smears with methanol for 1 minute. After 1 minute, drain off remaining methanol without rinsing, and allow the slide to air dry again.
5	Flood slide with acridine orange stain for 2 minutes.
6	Drain the excess stain and rinse thoroughly with tap water.
7	Allow to air dry. The slide may be gently blotted on a clean sheet of filter paper or paper towel to decrease drying time.
8	Examine with fluorescent microscope at 400X or 1000X (oil immersion lens). Look for distinct morphology of bacteria or fungi. No coverslip is needed.

INTERPRETATION OF RESULTS:

Step	Action
1	• Bacteria and fungus stain bright orange • Background appears black to yellow-green • WBC will stain yellow, orange, and red 
2	If no organisms were seen on the initial Gram stain but acridine orange stain is positive for organisms, review the Gram-stained smear again to see if organisms can be recognized.

REPORTING INSTRUCTIONS:

IF	REPORT
Acridine orange stain was used to verify bacteria seen on Gram-stained smear	• Report Gram stain • In the media resulting plate log, add the media **"ACROR"**:  • Result the media using the **"ACROR"** keypad • Do **NOT** report results of acridine orange stain on the final report

No organisms were seen on Gram-stained smear but acridine orange stain is positive for organisms	• In the resulting area, add test **"STAO":**  • Result the test using the **"STAO"** keypad • Add test comment **}ACRO** to state: **"Culture positive for bacteria by acridine orange stain; bacteria not seen by Gram stain"** • Organisms seen in sterile fluids or blood cultures are considered critical results. Phone ordering location to give result • Document call in the **"Call"** box • If unable to reach ordering location, consult the hospital wide policy "Laboratory: Critical Values-Responsible Party"

LIMITATIONS:

1. Nuclei or granules from disintegrated activated leukocytes and certain types of debris may fluoresce in acridine orange-stained smears. These may be differentiated from microorganisms on the basis of morphology.
2. Acridine orange staining does not distinguish between Gram-negative and Gram-positive organisms. The Gram stain may be determined by Gram staining directly over the acridine orange after removal of the immersion oil. Acridine orange staining may also be done over Gram stain (after removal of oil) if necessary.
3. Intracellular organisms may be more difficult to see by the acridine orange stain, due to the staining of cellular nuclei.
4. The sensitivity of the acridine orange smear is approximately 10^4 bacteria/mL.

CROSS-REFERENCES:

- MIC10000-Microbiology Specimen Handling
- MIC60060-Microbiology Stain Quality Control
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
2. BD Acridine Orange Stain package insert, 2024-03

APPROVAL:

March 18, 2024
Date

Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	07 Feb 17	Initial Release	L. Steven
2.0	31 Mar 22	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	19 Feb 24	Procedure reviewed	L. Steven
4.0	02 Mar 26	Procedure reviewed	L. Steven