**PROCEDURE: ACRIDINE ORANGE**

1. **USE**

This product can be used to stain microorganisms in direct smears from clinical specimens and blood cultures. Use in this lab will be for blood cultures that have been detected as positive by the BC instrument, are no growth, and are detected as positive upon re-incubation, or multiple positive bottles that have negative gram stains.

1. **PRINCIPLE**

Fluorochrome acridine orange binds to nucleic acids of bacteria. The low pH of the buffer solution results in an orange staining of bacteria and a green to yellow staining of human cells and tissue debris.

1. **TEST CODE**

ACRO

1. **STORAGE**
   1. Store in its original container at 20-25°C until used.
   2. Do not freeze or overheat.
   3. Protect from light.
2. **PRODUCT DETERIORATION**
   1. This product should not be used if any of the following apply:
      1. Evidence of dehydration
      2. Color has changed
      3. Expiration date has passed
      4. Other signs of deterioration
3. **PROCEDURE**
   1. Fix the prepared smears in methanol for 2 minute and allow to air dry.
   2. Flood with Acridine Orange and allow to stain for 2 minutes.
   3. Rinse with tap water and air dry. Scan the smears with the 100% objective utilizing a fluorescent illuminator. Smears stained with Acridine Orange can subsequently be stained with the gram stain procedure.
4. **INTERPRETATION**
   1. Background: Black, yellow, or pale green
   2. Leukocytes: pale apple-green
   3. RBC's: non-fluorescent
   4. Bacteria and yeast: red-orange
   5. Inclusion Leukocytes: Apple-green, yellow, orange, or red
5. **QUALITY CONTROL**
   1. Quality control is performed each time of use:

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| QC Organism | Expected Result |
| *Escherichia coli* ATCC 26922 | Bright red-orange fluorescent rods |
| *Staphylococcus aureus* ATCC 25923 | Bright red-orange fluorescent cocci |

1. **REFERENCES**
   1. Kronvall, G., and E. Myhre, 1977. Differential Staining of Bacteria in Clinical Specimens Using Acridine Orange Buffered at a Low pH. Acta. Path. Microbiol. Scand., Section B. 85:249-254.
   2. Senne, J.E. and J.R. McCarthy, 1979. Comparison of a Fluorescent Staining Method to Conventional Blind Subculture Methods for Detection of Microorganisms in Blood Cultures. P. 312 in Abstr. Annu. Meet. Am. Soc. Microbiol.
   3. Remel BactiDrop Acridine Orange PI, Revised September 11, 2014**.**
2. **REVISIONS**
   1. 02/10/2020 Updated procedure and interpretation criteria to reflect revised package insert.