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Section: Technical Manual	Subject Title: Acridine Orange Stain	
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ACRIDINE ORANGE STAIN

Principle

Acridine orange is a fluorescent dye which will bind to the nucleic acid of bacteria and other cells. It is recommended for use for the detection of microorganisms in direct smears. It is useful for the rapid screening of specimens from normally sterile sites (eg. CSF) and blood smears, or smears containing proteinaceous material where differentiation of organisms from background material may be difficult.

Reagents

Acridine Orange spot test dropper. Stored at room temperature.
Absolute Methanol

Procedure

1. Prepare a smear of the specimen to be stained.
2. Allow to air dry.
3. Fix with methanol for 1 to 2 minutes.
4. Hold the dropper upright and squeeze gently to crush the glass ampoule inside the dispenser.
5. Flood the slide with the acridine orange and stain for 2 minutes.
6. Rinse thoroughly with tap water and allow to air dry.
7. Examine with a fluorescent microscope using low and oil immersion objectives.

Interpretation

Bacteria and fungus stain bright orange. The background appears black to yellow green. Leukocytes will stain yellow, orange and red.

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Quality Control

Stain a smear of *Streptococcus pneumoniae* (ATCC 6303) each time the test is performed.

References

1. Difco Spot Test Acridine Orange Stain package insert, 1984.