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| Acridine Orange Stain |
| **Purpose** | This procedure provides instruction for performing Acridine Orange Stain.  |
| **Principal and Clinical Significance** | BD Acridine Orange is recommended for use in the fluorescent microscopic detection of organisms in direct smears prepared from clinical materials, particularly normally sterile specimens where few organisms may be present. Acridine orange, a vital stain, is a fluorochromatic dye that binds to nucleic acids of bacteria and other cells. Under UV light, Acridine orange stains RNA and single-stranded DNA orange; double-stranded DNA appears green. This staining method can be used to confirm the presence of bacteria in blood cultures when Gram stain results are difficult to interpret or when the presence of bacteria is suspected but none are detected by Gram stain. The low pH of the buffer solution results in an orange staining of bacteria and fungi, and green to yellow staining of human epithethial and inflammatory cells and background debris. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture plate reading. |
| **Test Code** | AO |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** |
|  | * Acridine Orange Stain
 | * Glass slide
 | * Fluorescent microscope filtered for FITC
* Inoculating loop
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
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| **Storage** | * 1. Store in original container at 15-30ºC until expiration date.
	2. Do not use if the color has changed from an orange-yellow, clear liquid.
	3. Do no use if there is evidence of a precipitate or the solution shows other signs of deterioration.
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| **Quality Control** | 1. **Positive:** Prepare smear from a known positive blood culture with staphylococci or streptococci.
2. **Negative:** Prepare smear from a known negative blood culture.
3. Prepare QC smears by placing a drop of a known positive and negative blood culture on a slide. Heat fix and store at RT.
4. **Perform QC with each patient run**. Record results on daily QC log.
5. Perform QC with each new lot or shipment before put into service. Record results in QC manual.
6. If there is a QC failure, document observation, notify supervisor and call BD Technical Service at 1-800-638-8663 or [www.bd.com](http://www.bd.com).
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| **Procedure** | 1. Prepare slide and air dry.
2. Heat fix slide.
3. Flood slide with Acridine Orange stain. Allow to stain for 2 minutes.
4. Rinse with tap water.
5. Stand slide to air dry.
6. Avoid excessive exposure to light as it may lower the intensity of fluorescence of the organisms.
7. Scan slide under 400X magnification using a fluorescent microscope. Confirm using 1000X under oil immersion.
8. Blot excess oil from slide and store frozen for one week.
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| **Interpretation** | * Bacteria and yeast will fluoresce bright orange against a green-fluorescing or dark background. The nuclei of blood cells may also fluoresce. PMNs will stain pale green. Red cells may not stain or stain pale green.
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| **Limitations** | 1. The presence of organisms in smears stained by Acridine Orange must be confirmed by culture.
2. The Acridine Orange stain does not distinguish between gram-positive and gram-negative organisms. The Gram stain can be performed by staining over the Acridine Orange stain after removing the oil with xylene.
3. Certain debris may fluoresce yellow, orange or red. Examination at a higher magnification will differentiate on the basis of morphology.
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| **Method Performance Specifications** | 1. The Acridine Orange stain can be used for the detection of cell-wall deficient bacteria such as Mycoplasma that are incapable of staining with the Gram stain.
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| **Result Reporting** | 1. Record culture work-ups in Sunquest MRE, Work-ups. code **AO**

Workups: Wkup # 1 Workup components Med : BPNK GMS : NOS Desc : POS AO : POS Id : NOS |
| **References** | 1. Acridine Orange, BD package insert, revised 9/2017, Becton, Dickinson and Company, 7 Loveton Circle Sparks, MD 21152.
2. Bacto® Acridine Orange Stain, Difco Manual, 11th edition, 1998, Difco Laboratories, Division of Becton Dickinson and Company, Sparks, MD, 21152, pg 597-598.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Pat Ackerman | 6/1980 | Initial Version |
| 1.1 | Pat Ackerman | 09/18/2003 |  |
| 1.2 | Pat Ackerman | 02/03/1992 |  |
|  | 1.3 | Pat Ackerman | 09/18/2003 | Updated Sunquest reporting information |  |  |
| 1.4 | Pat Ackerman | 06/02/2007 | Added warning re: carcinogenic information per ASM |
| 1.5 | Jessica Craig | 09/29/2010 | Updated into online format. |
|  | 2 | Becky Carlson | 4/18/2015 | Re-numbered from MC 702 for CMS load |
|  | 3 | Susan DeMeyere | 8/28/2019 | Changed perform QC with each patient run. |
|  | 4 | Susan DeMeyere | 8/31/2020 | Change product storage requirements to 15-30ºC |
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