| TITLE: KOH StringTest | Revision Date: 20-April-2018 | Issue Date: 20-April-2016 |
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| Document Number: MIC51100 | Status: Approved | |
| Distribution: Microbiology Test Manual | Page: 1 of 4 | |
| Approved by: S. Asmussen, Manager of Diagnostic Services | Signed by:  | |

## PURPOSE:

A visible loopful of cells from a single, well-isolated colony is emulsified into a drop of 3% KOH. If the mixture becomes viscous and "strings" within 60 seconds of mixing (KOH-positive) then the colony is considered gram-negative. The reaction depends on the lysis of the gram-negative cell in the dilute alkali solution releasing cellular DNA to turn the suspension viscous. The formation of a string (DNA) in 3% KOH indicates that the isolate is a gram negative organism although it can be non-reactive while testing anaerobes.

## REAGENTS and/or MEDIA:

- 40% KOH

## SUPPLIES:

- 50mL conical tube
- Sterile blue loop
- Pipette
- Eppendorf

## SPECIAL SAFETY PRECAUTIONS:

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

- 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 where there is a known or potential risk of exposure to 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.

## QUALITY CONTROL:

Performed each time the test is performed:

- Positive control: *P.aeruginosa* ATCC2783
- Negative control: *S.aureus* ATCC25923

A QC order is generated in the TQC system: Resulting Worklist → MICS → 3STR

## PROCEDURE INSTRUCTIONS:

| Step | Action |
|---|---|
| **Preparing the 3% KOH Solution** | |
| **1** | Using the equation: **C1V1=C2V2**<br>Calculate the amount of 40% KOH solution needed for a 3 mL solution with a final concentration of 3%<br>(40)X=(3)(3)<br>X=9/40<br>X=0.225mLs of KOH needed<br>Water needed: 3mLs-0.225mLs=2.8mLs<br>Using an Eppendorf pipettor set to 225µL – pipette 40% KOH into a conical tube |
| **2** | Add 2.8 mL of sterile water and mix |

## PROCEDURE INSTRUCTIONS:

| Step | Action |
|---|---|
| **Performing the String Test** | |
| 1 | Label 3 slides as follows:<br>1. **TEST**<br>2. **POS Control**<br>3. **NEG Control** |
| 2 | Add 1 drop of 3% KOH to each slide |
| 3 | Emulsify a loopful of organism in the KOH |
| 4 | Stir for a maximum of 60 seconds and slowly lift the loop observing for string formation |

## EXPECTED RESULTS:

| | |
|---|---|
| **Positive** | Formation of a string occurs  |
| **Negative** | No string formation occurs  |

## PRECAUTIONARY NOTES:

- False negatives can occur when using too light of an inoculums and/or testing anaerobes
- False positives can occur when testing mucoid organisms and using too heaving of an inoculum

## REFERENCES:


- Mount Sinai Microbiology Manual. (n.d.). Retrieved October 23, 2013, from http://microbiology.mtsinai.on.ca/manual/tech/tech22.pdf

- Scott Sutton, P. (n.d.). *The Gram Stain*. Retrieved October 23, 2013, from The Microbiology Network: http://www.microbiol.org/resources/monographswhite-papers/the-gram-stain/


## REVISION HISTORY:

| REVISION | DATE | Description of Change | REQUESTED BY |
|---|---|---|---|
| 1.0 | 31Dec2013 | Initial Release | A.Darrach |
| 2.0 | 31Mar2016 | Update of “Special Safety Precautions” to reflect risk assessment recommendations. | C. Russell |
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