PURPOSE: The ALA (Aminolevulinic Acid) test is used to rapidly detect porphyrin as a means of speciating *Haemophilus* species.

SAMPLE INFORMATION:

Type	Few, well isolated colonies that are: • Tiny, gram-negative bacilli or coccobacilli • Growing only on Chocolate agar • Possess typical *Haemophilus* colonial morphology • Do not grow on Blood agar • 18 to 24 hours old

REAGENTS and/or MEDIA:

Type	remel A.L.A. Disk
Stability and Storage Requirements	• Store at 2°C to 8°C. • Allow to come to room temperature before use. • Protect disks from moisture by removing from the vial only those disks necessary for testing. • Protect disks from light, as the substrate is highly light sensitive. • Promptly replace the cap and return the vial to 2°C to 8°C.

PLEASE NOTE:

- **The A.L.A test is only to be used on small, Gram-negative bacilli resembling *Haemophilus* spp. and must be confirmed with Vitek 2 NH card or API NH if from sterile site.**
- **Any positive result from a sterile site for *Haemophilus influenzae* must be sent immediately to Provincial Lab Edmonton for typing as soon as identification is confirmed. Assure there is a purity plate made that can be used for this purpose and can be sent out the day the identification is confirmed. Refer to MIC10510.**

SUPPLIES:

- Forceps
- Wooden sticks
- Disposable loops
- Blood agar
- Filter paper
- Sterile water
- 35° ambient air incubator
- Long wave ultraviolet lamp

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 when 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.

All patient specimens are assumed to be potentially infectious. Universal precautions 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 weekly:
  - Positive: *Aggregatibacter aphrophilus* ATCC 7901
  - Negative: *Haemophilus influenzae* ATCC 10211
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the ALA test	
1	Verify the test isolate resembles *Haemophilus* spp. by gram-stain and colonial morphology.
2	Place the ALA disk, with the "A" side down, on the agar surface of a Blood agar plate.
3	Inoculate the disk with a heavy, visible inoculum from a pure 18 to 24 hour culture of the test isolate.
5	Place a piece of filter paper saturated with water on the lid of the agar plate.
6	Incubate for up to 6 hours in the O_2 incubator.
7	Examine the disk at 1 hour under the ultraviolet light in the Tech2 office for orange fluorescence. If negative, re-incubate the test and examine hourly for up to 6 hours before reporting as negative.

INTERPRETATION OF RESULTS:

IF	THEN
Bright orange fluorescence	**ALA = Positive**
No fluorescence	**ALA = Negative**

LIMITATIONS/PRECAUTIONS:

1. Examine for fluorescence in a darkened room.
2. The ALA test is used for differentiating *Haemophilus* spp. only.
3. Best results are obtained using a heavy inoculum. False negative reactions may occur if the inoculum is insufficient or if the culture is greater than 24 hours old.
4. *Aggregatibacter aphrophilus* was previously known as either *Haemophilus parainfluenzae* or *Haemophilus aphrophilus*.
5. Many organisms will give a positive reaction. If test is performed only on Gram-negative bacillus colonies that grow well on Chocolate agar within 24 hours and not on Blood agar, results are for *Haemophilus* spp.
6. The ALA test will not separate *Haemophilus influenzae* from *Haemophilus haemolyticus*. The latter is rare and not pathogenic. It will sometimes grow on Blood agar without a "staph streak" if it is able to hemolyze the blood to supply it with V factor.
7. Organisms that are strongly oxidase positive or catalase positive may give a false positive result. Such organisms make heme and its precursors from ALA in the process of synthesizing oxidase or catalase. Verify that the test organism resembles *Haemophilus* both by Gram stain and colonial morphology before testing.
8. Product should not be used if the colour has changed from white, the expiration date has passed, the desiccant has changed from blue to pink or there are other signs of deterioration.
9. If the filter paper is not kept moist during incubation, the reaction can be falsely negative.

REFERENCES:

- remel A.L.A. Disk package insert, July 26, 2010
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	8 APR 19	Initial Release	L. Steven