PURPOSE:

The ALA test is a rapid test used to determine the growth requirement for hemin (X factor) in the identification of *Haemophilus* species.

SAMPLE INFORMATION:

Type	Tiny Gram-negative rods or coccobacilli growing only on chocolate agar with the typical Haemophilus colonial morphology and which do not grow on BAP
Source	18-24h culture

REAGENTS and/or MEDIA:

Type	A.L.A. Reagent Disk
Source	Remel
Volume	1 disk
Stability	Stable until date of expiration indicated on the container
Storage Requirements	Store at 2-8°C Protect disks from moisture. Protect from light, as the substrate is highly light sensitive.
Criteria for rejection and follow up action	Do not use if: • The disk color has changed from white • The expiration date has passed • The desiccant has changed from blue to pink • There are other signs of deterioration

SUPPLIES:

- Wooden applicator sticks
- Wood's lamp
- O_2 incubator

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential 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 of 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:

Quality control is set up each day the test is performed using the following control organisms:

Positive Control: A*ggregatibacter aphrophilus* ATCC 7901
Negative Control: *Haemophilus influenzae* ATCC 10211

A TQC order is automatically generated to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action	
Performing an ALA test		
1	In the plate log - order ^ALA.	
2	Prior to inoculation, allow product to equilibrate to room temperature.	
3	Place ALA disk, with “A” side down, on the agar surface of the culture plate.	
4	Inoculate the disk with a heavy, visible inoculum removed from a pure, 18-24h culture of the test isolate.	
5	Incubate for up to 6 hours at 35°C in ambient air incubator.	
6	Examine disk at 1h under the ultraviolet light for reddish-orange fluorescence. If negative, re-incubate the test and examine periodically for up to 6h before reporting as negative.	
7	**If:**	**Then:**
	Reddish-orange fluorescence production	Positive
	No fluorescence production	Negative

INTERPRETATION OF RESULTS:

Result	Interpretation
Positive	The presence of red fluorescence indicating that the organism does not require X factor or hemin and that the ALA has been utilized **NOT** indicative of *Haemophilus influenzae*
Negative	The lack of red fluorescence indicating that the organism requires X factor and that the ALA has not been utilized **INDICATIVE** of *Haemophilus influenzae*

PROCEDURAL NOTES:

- Use for differentiating *Haemophilus species* only.
- Best results are obtained using a heavy inoculum.
- Examine for fluorescence in a darkened room.
- False negative reactions may occur if the inoculum is insufficient or if the culture is greater than 24hr old. Cultures being tested must not be older than 24hr.
- *Aggregatibacter aphrophilus* was previously known as either *Haemophilus paraphrophilus* or *Haemophilus aphrophilus.*

LIMITATIONS:

- Many organisms will give a positive reaction. If test is performed only on Gram-negative coccobacilli colonies that growth well on CHOC in 24h and not on BAP, results are for Haemophilus species.
- The ALA test will not separate *Haemophilus influenzae from Haemophilus haemolyticus*. The latter is rare and not pathogenic. It will sometimes grow on BAP without a “staph streak” if it is able to hemolyze the blood to supply it with V factor.
- Organisms that are strongly oxidase positive or catalase positive may give a false positive test. 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.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- Remel ALA Disk™ package insert, revised July 26, 2010

REVISION HISTORY:

REVISION	DATE	Description of Change	BY
1.0	28 October, 2016	Initial Release – Test Implementation	L. Steven