TITLE: Porphyrin	Revision Date: 20-April-2018	Issue Date: 20-April-2016
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Approved by: S. Asmussen, Manager of Diagnostic Services	Signed by: 	

PURPOSE:

The porphyrin test is used as a method to differentiate *Haemophilus* species based on their ability to synthesize heme.

PRINCIPLE:

Certain Haemophilus species produce the enzyme porphobilinogen synthase. These organisms are capable of synthesizing heme (factor X) and, therefore, do not require an exogenous source of X factor for growth. The test substrate, delta-aminolevulinic acid, is the precursor molecule for which porphobilinogens, porphyrins, and heme are synthesized. Organisms grown in this substrate and possessing the enzyme will produce porphobilinogen and porphyrins as by-products. These breakdown products can be detected by the addition of Kovacs reagent or by the appearance of fluorescence under ultraviolet light. *Haemophilus parainfluenzae* produce these enzymes but *Haemophilus influenza* do not.

SAMPLE INFORMATION:

Type	Tiny Gram-negative cocco-bacilli, growing on chocolate agar, satellitic on Blood Agar with a staphylococcus streak
Source	18-24 hour culture

REAGENTS and/or MEDIA:

Type	Porphyrin Test Substrate	Kovac's reagent
Source	Oxoid	Dalynn Biologicals
Volume	~1mL	30 mL
Stability	Stable until date of expiration indicated on the tube	Stable until date of expiration indicated on the tube
Storage Requirements	Store at 2-8°C	Store at 2-8°C in the dark
Criteria for rejection and follow up action	Do not use if there are signs of contamination or deterioration (evaporation or discoloration).	Do not use if there are signs of deterioration.

SUPPLIES:

- Wooden sticks or inoculating loop/wire
- 35°C incubator
- Wood's lamp
- Kovac's reagent

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:

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

Positive: *Haemophilus parainfluenzae* ATCC # 7901

Negative: *Haemophilus influenzae* ATCC # 10211

A TQC order is automatically generated to record the QC results. To result: Resulting Worklist → MICS → PORPH

PROCEDURE INSTRUCTIONS:

Step	Action	
Performing a Porphyrin Test		
1	In the plate log – Order **^PORPH**	
2	Prior to inoculation, the substrate should be brought to room temperature.	
3	Suspend a loopful of organisms into the enzyme substrate.	
4	Incubate at 35°C in the O2 incubator for 4 hours	
5	Observe in a darkened room using a Wood's lamp	
6	**IF**	**THEN**
	Tube shows red fluorescence	Test is positive, see result interpretation
	Tube shows no fluorescence	Re-incubate for 18hrs, proceed to step 7
7	Remove tubes from incubator and proceed to step 8	
8	**Method #1:**	**Method #2:**
	After incubation, add an equal volume of Kovacs reagent and vortex the mixture.	After incubation, observe for fluorescence under a Wood's lamp.
	Allow the substrate and reagent to separate and observe for colour change.	

INTERPRETATION OF RESULTS:

Result	Method #1	Method #2
Positive	The formation of a **red (pink)** colour in the lower aqueous phase	Red Fluorescence
Negative	No colour change	No fluorescence

EXPECTED RESULTS:

Result	Interpretation
Positive	NOT indicative of *H.influenzae,* consult SOP for further work-up
Negative	INDICATIVE of *H.influenzae,* consult SOP for further work-up

NOTES AND PRECAUTIONS:

1. Care must be taken when interpreting the result after the addition of Kovac's reagent that the colour change occurs in the aqueous phase. Some *Haemophilus* species are indole positive but do not produce the enzyme. With these strains, a pink colour will be produced in the alcohol phase, but the aqueous phase will remain clear.
2. Use for differentiating *Haemophilus* species only.
3. Best results are obtained using a heavy inoculum.
4. This test will not differentiate *H.influenzae* from *H.haemolyticus*; the latter is rare and non-pathogenic. It will sometimes grow on BAP without the staphylococcal streak; if it is able to hemolyze the blood to supply it with V factor.

REFERENCES:

- Garcia, L. S. (n.d.). ALA Test for Porphyrin Synthesis. *Clinical Microbiology Procedures Handbook, 3rd Ed* , pp. 3.17.3.1-3.17.3.3
- Oxoid. (n.d.). Porphyrin Substrate Package Insert.

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