PORPHYRIN (d-Ala) (ALA)

REF No. 57321

Contain delta-aminolevulinic acid for the detection of hemin (X-factor) requirement in the **differentiation of *Haemophilus influenzae* from *Haemophilus parainfluenzae***. This enzymatic test is rapid and independent of several factors affecting the usual tests for growth factor requirements (e.g. presence of X-or V-factor in the test medium, "carry over" of X-factor with the inoculum from chocolate agar, lack of other essential nutrients in the test medium).

Principle of the Test

Haemophilus parainfluenzae does not require hemin (X-factor) for growth because it possesses enzymes for the biosynthesis of heme (Fig. 1). When supplied with delta-aminolevulinic acid, *Haemophilus parainfluenzae* strains synthetize porphobilinogen and porphyrins, which are detected in the test.

Porphyrins show characteristic **red fluorescence** when exposed to long wave UV-light (360 nm). Porphobilinogen that contains a pyrrole ring produces a **red colour with Kovacs' reagent** (92031) (in the lower water phase).

Kilian (1974) tested 134 Haemophilus strains and found a perfect agreement between the two methods of reading.

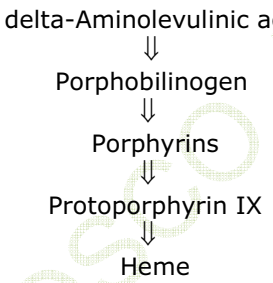

Fig. 1. Main steps of the heme biosynthesis.

Haemophilus influenzae that requires hemin for growth lacks the enzymes for heme synthesis and consequently does neither produce porphyrins nor porphobilinogen from delta-aminolevulinic acid (negative reaction).

Procedure

Prepare a dense bacterial suspension (at least McFarland No. 4) of fresh colonies (18-24 hours) of the haemophilus strain to be tested in 0.25 ml saline in a tube. Add one Porphyrin (ALA) diagnostic tablet and close the tube. Incubate at 35-37 °C for **4-6 hours** or in case of negative or doubtful reactions for **up to 24 hours**.

Reading of the test

The test can be read in two ways:

a) Add **4 drops of Kovacs' reagent** (92031), shake and wait for up to 10 minutes.

Positive reaction:	**Red/pink** colour in the **lower water phase**
The strain does not require X-factor:	***Haemophilus parainfluenzae***
Negative reaction:	**Colourless** water phase
The strain requires X-factor:	***Haemophilus influenzae***

After the addition of Kovacs' reagent the tube cannot be reincubated for re-reading. For rapid results it is advisable to incubate two tubes for each strain: one for addition of reagent after 4 hours, and another for later confirmation (re-incubation), if a negative test result is obtained.

b) Expose to long wave UV light, 360 nm (Wood's lamp).

Positive reaction: **Red fluorescence**
The test strain does not require X-factor: ***Haemophilus parainfluenzae***

Negative reaction: **No red fluorescence**
The test strain requires X-factor: ***Haemophilus influenzae, Haemophilus haemolyticus***

In case of doubtful or negative reactions, the tube should **be re-incubated for 18-24 hours**.

Other haemophilus

	ALA
H. ducreyi (usualliy genital sources)	0
H. aegyptius (conjunctivitis)	0
H. haemolyticus (oral flora)	0
Aggregatibacter aphrophilus	+ or 0
Aggregatibacter segnis	0

Other bacteria

	ALA (Kovacs')
Staphylococci (CAT +)	+
Staphylococci (CAT 0)	+
Streptococci	0
Aerococci	0
Rothia mucilaginosa	+

ALA = Porphyrin D.T.

1) Differentiation of *Aggregatibacter* spp. (ALA +^{0}, NO_3 +, Alk P +, non-haem)

	ALA	**CAT**	**ONPG**	**SUC**	**TRE**	**γ-GLU**	**LACT**
A. actinomycetemcomitans	+	+	0	0	0	+	0
A. aphrophilus	+/0	0	+	+	+	+	+
A. segnis	+	V	V	+	0	0	0
Haemophilus parainfluenzae	+	·	·	+	0	0	0

CAT = Catalase, ONPG = ONPG D.T. SUC = Sucrose D.T., TRE = Trehalose D.T. , γ-GLU = Gamma-Glutamyl Aminopeptidase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Porphyrin (d-Ala) (d-Aminolevulinic acid)	*H. parainfluenzae* ATCC 7901	*H. influenzae* ATCC 49247

References

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