

INTENDED USE

The PRO-LAB HAEMOPHILUS RING PL.970 is offered as a procedure for the identification of *Haemophilus* species isolated from clinical specimens.

SUMMARY AND EXPLANATION

Members of the genus *Haemophilus* are found as part of the normal flora of the respiratory tract of humans and many animals. The type species *H. influenzae* is responsible for a variety of diseases which include chronic respiratory infections, meningitis, otitis media and purulent contagious conjunctivitis. *Haemophilus* species are not readily distinguishable by their colonial morphology or gram stain appearance. Oral flora grown from routine sputum cultures often contains organisms which resemble *Haemophilus* species. *H. influenzae*, which is the principal human pathogen, can be distinguished from other *Haemophilus* species and oral flora by determining the need for essential factors for growth, specifically Haemin (X factor) and Nicotinamide-adenine dinucleotide (V factor). *H. influenzae* requires both factors for growth where as some of the other species require only one.¹

In addition to the X and V test the HAEMOPHILUS RING also has a porphyrin test incorporated in the V tip. The porphyrin test is a modification of that which was originally described by Kilian.² It provides an additional means of distinguishing *H. parainfluenzae* and *H. parahaemolyticus* which manufacture their own X factor from X factor dependent species i.e. *H. influenzae*. This is particularly useful because of the difficulty of preparing solid medium which is completely free of X and V factors.

PRINCIPLE

PRO-LAB HAEMOPHILUS RING PL.970 is a paper ring with three hydrophobically isolated tips equidistantly spaced around the outer circumference of the support ring. Included on the ring is X factor (Haemin), V factor (Nicotinamide-adenine dinucleotide) and a tip with both X and V. The V tip also incorporates a test for porphyrin synthesis.

Each ring is clearly labeled using letter codes:

X	Haemin
V	NAD and d-aminolevulinic acid (ALA)
XV	Haemin and NAD

REAGENTS

PRO-LAB HAEMOPHILUS RING PL.970 is supplied 50 rings per tin, each ring is sufficient for one test. Each tin includes a drying agent to protect the rings from moisture.

PRECAUTIONS

- Do not use reagents after expiry date shown on product label.
- PRO-LAB HAEMOPHILUS RINGS are for in vitro diagnostic use only.
- During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard if mishandled.

STABILITY AND STORAGE

Store at 2°-8°C and ensure that the container is tightly sealed at all times to keep out moisture. Avoid extended exposure to direct sunlight. PRO-LAB HAEMOPHILUS RINGS have a shelf life of 12 months when stored under these conditions and can be used until the expiry date on the label.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

For specific procedures regarding specimen collection and preparation of primary cultures refer to a standard microbiology text.

MATERIALS REQUIRED BUT NOT PROVIDED

- Trypticase Soy Broth (TSB), Peptone Water or sterile saline.
- Sterile Swabs.
- Culture plate containing TSB, Peptone Agar or Columbia Agar Base with no X or V factors.
- Sterile forceps.
- Inoculation Loop (available from PRO-LAB).
- 37°C incubator.
- Ultra violet light (360nm).

TEST PROTOCOL

- The organism should be prepared by making a suspension from the primary isolation medium in Trypticase Soy Broth (TSB), Peptone Water, or sterile saline. The final suspension should be slightly turbid, corresponding to a McFarland No. 1 or No. 2.
- Using a sterile swab dipped in the suspension, spread the organism evenly over the surface of a TSA, Peptone Agar or Columbia Agar Base plate which does not contain any X or V factors.
- With sterile forceps carefully place the HAEMOPHILUS RING in the centre of the plate and gently press each tip to ensure direct contact between the tip and the agar. The porphyrin test is accomplished by using an inoculation loop to smear a heavy inoculum (5-6 colonies) directly on the V tip which is on the agar plate.
- Incubate the plate at 37°C for 18-24 hours in an aerobic atmosphere.

INTERPRETATION OF RESULTS

After the incubation period the plate should be examined for signs of growth factor requirement. A clearly defined zone of growth around one or more of the tips identifies the species of *Haemophilus* as shown in the following table:

The tip of the V ring should be examined under ultra violet light (360nm). Any non-haemin requiring *Haemophilus* will synthesize haemin precursors from d-ALA resulting in an orange/red fluorescence on the filter paper tip.

	Growth Factor Requirements	Synthesis of porphyrins	Haemolysis on Horse Blood
	x	v	
<i>H. aegyptius</i>	+	+	-
<i>H. haemolyticus</i>	+	+	+
<i>H. influenzae</i>	+	+	-
<i>H. parahaemolyticus</i>	-	+	+
<i>H. parainfluenzae</i>	-	+	-

REFERENCES

- Kilian M. 1985. *Haemophilus*, in Lennette, E.H., Balows, A., Hausler, W.J., and Shadomy, H. J. (eds.) Manual of Clinical Microbiology, 4th ed. American Society for Microbiology. Washington, D.C. pp. 387-393.
- Kilian, M. 1974. Acta Path. Microbiol. Scand., Sect. B 82:835-842.

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