

**INTENDED USE**

PRO-LAB Microring AC PL.985 is offered as a supplementary test to the Pro-Lab Anaerobic Microring AN PL.980, as a means of presumptively identifying non-sporing anaerobes. It offers further tests to enable the identification of *Peptococcus niger* and individual species of *Peptostreptococcus* particularly *Peptostreptococcus anaerobius*.

**SUMMARY AND EXPLANATION**

Current improvements in collection and cultivation of anaerobic strains have increased the isolation of non-sporing anaerobes. Publications by Wren et al<sup>1</sup>, Graves et al<sup>2</sup>, Wideman et al<sup>3</sup>, Kazue<sup>4</sup> and Takayuki et al<sup>5</sup> demonstrate the use of metronidazole, novobiocin and sodium polyanethol sulphionate in the identification of *Peptostreptococcus niger* and individual species of *Peptostreptococcus* particularly *Peptostreptococcus anaerobius*.

**PRINCIPLE**

PRO-LAB Microring AC PL.985 is a ring with three hydrophobically isolated disc shaped projections equidistantly spaced around the outer circumference of the support ring. Each disc area is identified by an alphabetic system as well as by colour coding, and functions independently of the other two test zones on the ring. The following table identifies the configuration of the ring:

Alphabetical Code	Antibiotic	Quantity	Colour Code
MZ	Metronidazole	5 ug	White
SPS	Sodium Polyanethol Sulphonate	1 mg	Pink
NO	Novobiocin	5 ug	Blue

**REAGENTS**

PRO-LAB Microring AC PL.985 is supplied 50 rings per pack, each ring sufficient for one test. Each pack includes a drying agent to protect rings from moisture.

**PRECAUTIONS**

1. PRO-LAB Microring AC PL.985 are for in vitro diagnostic use only.
2. 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 bio-hazard if mishandled.

**STABILITY AND STORAGE**

Store at 2° to 8°C and ensure that the container is tightly sealed at all times to keep out moisture. Avoid extended exposure to direct sunlight. When stored under these conditions, the Pro-Lab Microring AC PL.985 can be used until the expiry date on the product 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**

1. Thioglycollate broth or sterile saline.
2. Sterile swab.
3. Culture plates with 20-25 mls solid medium e.g. Columbia Agar with defibrinated blood.
4. Sterile forceps.
5. 35° - 37°C anaerobic incubator

**TEST PROTOCOL**

1. Suspend the isolated organism in thioglycollate broth or sterile saline. The final suspension should be slightly turbid, corresponding to a McFarland # 0.5.
2. Using a sterile swab, uniformly spread the suspension over the surface of a culture plate containing 20-25 ml solid medium e.g. Columbia Agar with defibrinated blood. To avoid the appearance of excessively large zones of inhibition, do not use plates with less than 20 ml of medium.
3. Use sterile forceps to carefully place a PRO-LAB Microring AC onto the centre of the plate and gently press each tip to ensure direct contact between the tip and the agar.
4. Incubate the plate at 35°-37°C for 24-48 hours under anaerobic conditions.

**INTERPRETATION OF RESULTS**

After the incubation period the plate should be examined for signs of growth inhibition around each of the tips.

1. Metronidazole: Obligate anaerobes are susceptible. Microaerophilic and facultative anaerobic cocci are resistant -no zones of inhibition
2. Novobiocin: Susceptible organisms produce zones of inhibition of 15 mm or greater in diameter. Resistant organisms e.g. *Peptococcus niger*, produce no zones of inhibition.

**3. Sodium Polyanethol Sulphonate:**

*Peptostreptococcus anaerobius* susceptible -producing zones of inhibition of 12 - 30 mm in diameter.

All other Peptostreptococci are resistant (occasional exceptions, see footnotes of Microring AC table).

**Microring AC Identification Table**

	MZ 5 ug	NO 5 ug	SPS 1 mg	Indole
<i>Peptococcus niger</i>	S	R	R	-
<i>Peptostreptococcus</i>				
<i>asaccharolyticus</i>	S	R	R	+
<i>indolicus</i>	S	R	R	+
<i>magnus</i>	S	R	R	-
<i>prevotii</i>	S	R	R(a)	-
<i>anaerobius</i>	S	S	S	-
<i>micros</i>	S	S	R(b)	-
<i>productus</i>	S	S	R	-
<i>tetradius</i>	S	*	R	-
<i>Streptococcus parvulus</i>	S	S	R	-
<i>Staphylococcus saccharolyticus</i>	R/S	R	R	-
<b>Other microaerophilic streptococci</b>				
<i>Streptococcus</i>				
<i>intermedius</i>	R	S	R	-
<i>morbillorum</i>	R	S	R	-
<i>constellatus</i>	R	S	R	-

S=Susceptibility

R=Resistant

R/S=aerotolerant strains resistant

(a)=Occasional strains may give a zone size >12 mm but these organisms are easily distinguishable from *Peptostreptococcus anaerobius* as they are resistant to Novobiocin.

(b)=Occasional strains may give a small zone to SPS ≤12 mm in diameter.

\* = Unpublished

**REFERENCES**

1. Wren, M.W.D., Eldon, C.P. and Dakin, G.H. 1977. J. Clin. Path. **30**: 620-622.
2. Graves, M.H., Morello, J.A. and Kocka F.E. 1974. Appl. Microbiol. **27(6)**: 1131-1133.
3. Wideman, P.A., Vargo, V.L. and Citrobaum, D., et al. 1976. J. Clin. Microbiol. **4**: 330-333.
4. Kazue Ueno, Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Gifu, Japan. Proceedings of the 2nd International Symposium on Anaerobes Tokyo, Japan. June 1985. 109-122.
5. Takayuki Ezaki, Naoki Yamamoto, Keiu Ninomiya, Schoichiro Suzuki and Eiko Yabuuchi. 1983. Int. J. Sys. Bact. **33(4)**: 683-698.

Revision: 2002 03