

INTENDED USE

PRO-LAB Anaerobic Microring AN PL.980 is offered as a means of presumptively identifying gram negative non-sporing anaerobes.

SUMMARY AND EXPLANATION

Current improvements in collection and cultivation of anaerobic strains have increased the isolation of non-sporing anaerobes. Much time and cost may be involved in identification. Sutter and Finegold¹ offered a combination of six antibiotic concentrations which they demonstrated as having definite patterns of susceptibility for different non-sporing gram negative anaerobes. Peach² confirmed the value of this range of antibiotics as an identification procedure with emphasis on *B. fragilis* and also determined that all non-sporing gram positives were sensitive to Vancomycin. Leigh³ demonstrated that these six antibiotics, when presented in a multiple point ring system comparable to PRO-LAB Anaerobic Microring AN, was well suited to the characterization of the most commonly isolated gram negative anaerobe, *B. fragilis*.

PRINCIPLE

PRO-LAB Anaerobic Microring AN PL.980 is a hexagonal paper ring with six 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 five test zones on the ring. The following table identifies the configuration of the ring:

Alphabetical Code	Antibiotic	Quantity	Colour Code
E	Erythromycin	60 ug	Red
RP	Rifampicin	15 ug	Dark red
CO	Colistin	10 ug	White
PG	Penicillin G	2 units	Pink
K	Kanamycin	1000 ug	Salmon
VA	Vancomycin	5 ug	Blue

REAGENTS

PRO-LAB Anaerobic Microring AN PL.980 is supplied 50 rings per pack, each ring sufficient for one test. Each tin includes a drying agent to protect rings from moisture.

PRECAUTIONS

1. PRO-LAB Anaerobic Microring AN PL.980 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 time 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 Anaerobic Microring AN PL.980 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 No. 1 or No. 2.
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 mls of medium..
3. Use sterile forceps to carefully place a PRO-LAB Anaerobic Microring AN 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. Overall zone diameters exceeding 15 mm, are interpreted in the following table as sensitive, those less than 15 mm are interpreted as resistant:

Anaerobe Ring Identification Table

	E 60 ug	RP 15 ug	CO 10 ug	PG 2 units	K 1000 ug	VA 5 ug
<i>Bact. fragilis</i> group	Sr	S	Rs	R	R	R
<i>Prevotella melaninogenicaloralis</i>	S	S	Sr	Sr	R	R
<i>Porphyromonas</i> spp.	S	S	S	Sr	Rs	Sr
<i>Bact. ureolyticus</i>	S	V	S	S	S	R
<i>Fuso. mortiferum</i> / <i>varium</i>	R	R	S	S	S	R
Other Fusobacteria	Rs	Rs	S	S	S	R
Gram positive cocci	S	S	R	Sr	V	S
<i>Clostridia</i> sp.	S	S	R	Sr	V	S
Gram positive Bacilli (NSGPB)	S	Sr	R	Sr	V	S
Gram negative cocci	S	S	S	S	S	R

S=Sensitive
 R=Resistant
 Sr=Majority are sensitive, occasional strains are resistant
 Rs=Majority are resistant, occasional strains are sensitive
 V=variable

Where there may be occasional atypical results or difficulty in differentiating *P. oralis* and *P. melaninogenica*, supplementary tests may be required e.g. growth in 0.1% desoxycholate, pigment production on lysed blood agar, indole production, nitrate reduction and aesculin hydrolysis¹.

REFERENCES

1. Sutter, V.L. and Finegold, S.M. 1971. Appl. Microbiol. 24: 13-20.
2. Peach, S. 1975. J. Clin. Path. 28: 388-391.
3. Leigh, D. A. 1977. J. Clin. Path. 30: 991-992.

The product PL.985, Pro-Lab Microring AC is available to assist in the presumptive identification of non-sporing anaerobes. Microring AC offers further tests to enable the identification of *Peptococcus niger* and individual species of *Peptostreptococcus* particularly *Peptostreptococcus anaerobius*.

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