ANAEROBE IDENTIFICATION USING SPECIAL-POTENCY DISKS

Principle

Special potency antimicrobial disks of vancomycin (5 μg), kanamycin (1,000 μg) and colistin (10 μg) are used as an aid in determining the Gram reaction of anaerobes as well as in preliminary categorization of some anaerobic genera and species (Table 1). In general, gram positive organisms are resistant to colistin and susceptible to vancomycin, while most gram negative organisms are resistant to vancomycin. This difference is especially useful with some Clostridia that consistently stain gram negative.

Table 1 - Anaerobic Identification by Means of Special Potency Disks

	Response[1] to Disk:		
Type of Organism	**Vancomycin (5 μg)**	**Kanamycin (1,000 μg)**	**Colistin (10 μg)**
Gram negative	R	V	V
Gram positive	S	V	R
B. fragilis group	R	R	R
B. ureolyticus group	R	S	S
Fusobacterium spp.	R	S	S
Porphyromonas spp.	S	R	R
Veillonella spp.	R	S	S

[1] R - resistant; S - susceptible; V - variable

Materials

A. Reagents

1. Special potency antibiotic disks:

Vancomycin	5 μg
Kanamycin	1,000 μg
Colistin	10 μg

Store a small supply of disks (one carton each) in a tight container with desiccants at 4^0C.

2. Brucella or other anaerobic blood agar plate.

B. Supplies

1. Single disk dispenser or forceps
2. Ruler (divided into millimeters)

Procedure

1. Allow the container with disks to reach room temperature before opening it.
2. Subculture the isolate on a BAP. To ensure an even, heavy lawn of growth, streak the first quadrant back and forth several times. Streak the other quadrants to yield isolated colonies.
3. Place the three antibiotic disks on the first quadrant will apart from each other.
4. If you have several organisms to test, first streak all the plates and then add the disks to them at the same time.
5. Incubate the plate(s) anaerobically for 48-72 hours at 35-37^0C.
6. Examine for zones of inhibition of growth around the disks.

Interpretation

A. Susceptible: Zone of inhibition of ≥ 10 mm
B. Resistant: Zone of inhibition of < 10 mm

Quality Control

A. Test special potency antibiotic disks by lot when initially received and weekly thereafter.

B. Test *Bacteroides fragilis* (ATCC 25285), *Clostridium perfringens* (ATCC 13124), and *Fusobacterium necrophorum* (ATCC 25286) as described below under Procedure. The results should show the following:

1. *B. fragilis*: resistant to all three antibiotics
2. *F. necrophorum*: resistant to vancomycin; susceptible to colistin and kanamycin
3. *C. perfringens*: susceptible to vancomycin and kanamycin and resistant to colistin

C. Record the results on a QC log.

SPS Disk for Differentiation of Anaerobic Cocci

Principle

Sodium polyanethol sulfonate (SPS), a commonly used anticoagulant, inhibits certain bacteria such as *Peptostreptococcus anaerobius* and the aerobe *Gardnerella vaginalis*. Paper disks impregnated with 5% SPS can be used as a tool for differentiating *P. anaerobius* from other anaerobic cocci.

Materials

A. Reagents

1. SPS Disks

   a. Combine the following in a flask.

SPS	5 g
Distilled water	100 ml

   b. After dissolving SPS, sterilize the mixture by filtration (0.22 μm pore size filter).
   c. Dispense 20 μl onto sterile 1/4-inch diameter filter paper disks that are spread inside empty, sterile petri dishes. Allow these to dry for 72 hours at room temperature.
   d. Store the disks at room temperature, and label with an expiration date of 6 months.

2. SPS disks are also commercially available (Anaerobe Systems, Difco, Oxoid, Remel). Store as indicated by the manufacturers.
3. Brucella or other anaerobic blood agar plate.

B. Supplies

1. Single-disk dispenser or forceps
2. Ruler (divided into millimeters)

Procedure

1. Allow the container with disks to reach room temperature before use.
2. Subculture the isolates on a BAP. To ensure an even, heavy lawn of growth, streak the first quadrant back and forth several times. Streak the other quadrants to yield isolated colonies.

3. Place the SPS disk on the first quadrant.
4. If you have several organisms to test, first streak all the plates and then add the disks to them at the same time. You can use one plate for up to four tests.
5. Incubate the plate(s) anaerobically for 48-72 hours at 35-37^{0}C.
6. Examine for a zone of inhibition of growth around the disk.

Interpretation

A. Susceptible: Zone of inhibition of ≥ 12mm

P. anaerobius usually gives a very large zone of inhibition (≥ 16 mm), whereas other anaerobic cocci that appear susceptible to SPS give smaller zones. To presumptively identify *P. anaerobius*, you must also consider the Gram stain, typical colonial morphology, and odor. Some strains of *P. micros* may be susceptible to SPS. Examine the Gram stain for the small cell size of *P. micros* and chaining characteristic of *P. anaerobius.*

B. Resistant: Zone of inhibition of <12 mm.

Quality Control

A. Test each lot upon receipt and monthly thereafter.
B. Test *P. anaerobius* ATCC 27337 and *Peptostreptococcus asaccharolyticus* ATCC 29745 as described below under Procedure. The results should show the following:
   1. *P. anaerobius*: susceptible to SPS
   2. *P. asaccharolyticus*: resistant to SPS
C. Record the results on a QC log.