

Anaerobe Special Potency Antibiotic Disk Identification Procedure

I. Principle

Susceptibility to special potency antibiotic disks (vancomycin 5 μg , kanamycin 1000 μg , and colistin 10 μg) may be used as an aid in determining the Gram reaction and in separating different anaerobic species and genera. Generally, gram-positive organisms are sensitive to vancomycin and resistant to colistin, whereas the gram-negative organisms are resistant to the vancomycin disk. The vancomycin susceptibility is especially helpful with those clostridia that consistently stain gram-negative.

The addition of a bile disk further aids in the identification of organisms that are able to grow in the presence of 20% bile (most commonly *Bacteroides fragilis* Group).

II. Specimen Information

Ideally, the organism in question should be isolated in order to obtain a pure inoculum for testing. Aerotolerance testing should occur simultaneously to verify the growth characteristics of the isolate.

III. Reagents & Equipment

- A. Brucella Agar w/5% Sheep Blood, Hemin, and Vitamin K1
- B. Sterile saline
- C. Sterile swab

IV. Procedure

- A. Prepare a saline suspension of the isolate equivalent to a 0.5 McFarland standard.
- B. Using a sterile swab, inoculate a brucella blood agar plate with the bacterial suspension. Streak the entire plate in 3 directions to help ensure confluent growth.
- C. Using sterile forceps, place the antibiotic disks and bile disk on the inoculated plate. Place disks at least 20 mm apart from each other to allow for visualization of individual zones of inhibition.
- D. Incubate the plate anaerobically for 24-48 h at $35 \pm 2^\circ\text{C}$. Adequate growth must be present to interpret results.
- E. Examine for a zone of inhibition of growth around each disk.

V. Interpretation

- A. Vancomycin, kanamycin, and colistin disks
 - i. Sensitive (S): zone of inhibition is ≥ 10 mm.
 - ii. Resistant (R): zone of inhibition is < 10 mm.

B. Bile disk

Note: A large zone of hemolysis typically surrounds the bile disk and the organisms that grow in this zone often produce a cloudy precipitate in the agar medium.

- i. Sensitive (S): zone of inhibition > 10 mm.
- ii. Resistant (R): growth up to disk.

C. See procedure and textbook charts for expected susceptibility patterns of anaerobes.

VI. Quality Control

Quality control testing should be performed with each new lot or shipment of disks. See table below for set-up and expected results.

Control Organism	Bile Disk	Colistin 10 µg	Kanamycin 1000 µg	Vancomycin 5 µg
<i>F. nucleatum</i> (25586)	≥ 10 mm S	≥ 10 mm S	≥ 10 mm S	≤ 10 mm R
<i>B. fragilis</i> (25285)	Growth up to disk R	≤ 10 mm R	≤ 10 mm R	≤ 10 mm R
<i>Peptostreptococcus</i> (29743)	ND	ND	ND	≥ 10 mm S

VII. Limitations

- A. Slow growing organisms may require additional incubation in order to visualize zones of inhibition.
- B. Results yielding double zones of inhibition are likely due to a mixed inoculum. Gram stains should be prepared from both areas of growth to help identify mixed growth.

VIII. References

- A. Summanen, P., E.J. Baron, D.M. Citron, C.A. Strong, H.M. Wexler, S.M. Finegold. 1993. Wadsworth Anaerobic Bacteriology Manual, Fifth edition. Star Publishing Company, Belmont, CA.
- B. Package insert: REMEL Bile Disk, IFU 21122. Revised December 15, 2004.

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Updates and Revisions: