



BILE DISKS

- For in vitro use only -

Catalogue No. DB65

Our Bile Disks are used to aid in the differentiation and identification of the *Bacteroides fragilis* group (BFG).

One distinguishing characteristic of members of the BFG is their ability to grow in the presence of bile, while other anaerobic bacilli are actively inhibited. In 1976, Draper and Barry were the first individuals to report the use of bile disks to rapidly identify *Bacteroides fragilis*.

Other anaerobes that demonstrate resistance to bile include: *Fusobacterium varium*, *Fusobacterium mortiferum*, and *Bilophila* species, but differentiation from the *B. fragilis* group is possible since these organisms are all sensitive to kanamycin.

Kanamycin Disks (Dalynn DK15) should be used in conjunction with our Bile Disks, since members of the *B. fragilis* group are the **only** commonly encountered anaerobes that can grow in the presence of bile and exhibit resistance to kanamycin. When used in conjunction with our other special-potency antibiotic disks (colistin 10- μ g, kanamycin 1000- μ g, and vancomycin 5- μ g), Bile Disks, can rapidly provide presumptive identification of the *Bacteroides fragilis* group.

Recommended Procedure

1. Obtain a pure, fresh culture of the test organism in thioglycollate broth with turbidity equivalent to a 0.5 McFarland standard.
2. Using a sterile swab, streak a sample of the organism onto a Brucella Blood Agar Plate in three directions to obtain a heavy, confluent growth.
3. Aseptically place a Bile Disk onto the agar surface.
4. Incubate the blood agar plate anaerobically at 35°C for up to 48 hours.

Interpretation of Results

Resistant: Growth up to the edge of the disk (<16-mm)

Sensitive: A zone of inhibition around the disk (\geq 16-mm)

Species	Bile	K	V	C	Catalase
<i>B. fragilis</i> group	R	R	R	R	V
Other <i>Bacteroides</i>	S	R	R	V	- ⁺
<i>Bilophila</i> sp.	R	S	R	S	+
<i>Sutterella</i> sp.	R	S	R	S	-
<i>Desulfomonas</i> sp.	V	S	R	R	-
<i>Desulfovibrio</i> sp.	V	S	R	R	-
<i>Fusobacterium</i> sp.	V	S	R	S	-
<i>F. nucleatum</i>	S	S	R	S	-
<i>F. necrophorum</i>	S	S	R	S	-
<i>F. varium</i>	R	S	R	S	-
<i>F. mortiferum</i>					

Headings:

K = Kanamycin (1000- μ g) C = Colistin (10- μ g)

V = Vancomycin (5- μ g)

Results:

R = resistant S = susceptible V = variable

+ = positive reaction for majority of strains

- = negative reaction

-⁺ = most strains negative, some strains positive

- Typically, all members of the *B. fragilis* group are resistant to bile and show good growth after 24 hours, although *B. uniformis* may exhibit a weak reaction
- The presence of the bile disks will cause a large zone of hemolysis on the blood plate, and this is not a indication of growth. Organisms that grow within this zone often produce a cloudy precipitate in the agar making zone interpretation easier

- *Resistant organisms, such as BFG, rarely exhibit zone sizes greater than 10-mm*
- *The use of a turbidity standard to ensure a consistent inoculum is recommended since a straight inoculum results in smaller and less distinct zones*

5. MacFaddin JF. Biochemical tests for the identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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Quality Control

Organism	Expected Result
<i>Bacteroides fragilis</i> ATCC 25285	Resistant (R) Growth up to the disk
<i>Fusobacterium nucleatum</i> ATCC 25586	Sensitive (S) Zone around disk

Storage and Shelf Life

Our Bile Disks should be stored at 4 to 8°C. At this temperature they have a shelf life of 52 weeks from the date of manufacture.

References

1. Finegold SM, Harada NE, Miller LG. Antibiotic susceptibility pattern as aids in classification and characterization of gram-negative anaerobic bacilli. J Bacteriol 1967; 4:1443-50.
2. Sutter VL, Finegold SM. Antibiotic disk susceptibility tests for rapid presumptive identification of gram-negative anaerobic bacilli. Appl Micro 1971; 21:13-20.
3. Draper DL, Barry AL. Rapid Identification of *Bacteroides fragilis* with bile and antibiotic disks. J Clin Micro 1977; 5:439-43.
4. Weinberg LG, Smith LL, McTighe AH. Rapid identification of the *Bacteroides fragilis* group by bile disk and catalase tests. Lab Med 1983; 14:785-8.