


remel

Anaerobe Identification Disks

INTENDED USE

Remel Anaerobe Identification Disks are antibiotic-impregnated disks recommended for use in qualitative procedures to confirm the Gram stain reaction of anaerobic bacteria and presumptively identify anaerobic, gram-negative bacilli.

SUMMARY AND EXPLANATION

In 1971, Sutter and Finegold proposed a method for identification of anaerobic, gram-negative bacilli based on differences in response to antibiotics.¹ The organisms could be categorized into groups based on their characteristic patterns of inhibition when tested with erythromycin, rifampin, colistin, penicillin, kanamycin, and vancomycin.^{2,3}

PRINCIPLE

Special potency antibiotic disks can be used in the presumptive identification of anaerobic bacteria.^{3,4} Colistin and vancomycin confirm the Gram stain reactions which are especially helpful with the clostridia that stain gram-negative. Penicillin, rifampin, and kanamycin separate *Bacteroides* and *Fusobacterium* species.

REAGENTS

Colistin Disk 10 µg (REF R21123)
Kanamycin Disk 1000 µg (REF R21126)
Penicillin G Disk 2 U (REF R21136)
Rifampin Disk 15 µg (REF R21139)
Vancomycin Disk 5 µg (REF R21156)

PRECAUTIONS

These products are For *In Vitro* Diagnostic Use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

These products are ready for use and no further preparation is necessary. Store products in their original containers at 2-8°C until used. Allow products to come to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

These products should not be used if (1) the color has changed from white, with the exception of Rifampin which is light orange, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) there are other signs of deterioration. Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁵

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Anaerobic Reducible Blood Agar (REF R01060), Brucella Blood Agar w/ Hemin & Vitamin K (REF R01252), or alternative nonselective anaerobic blood agar, (5) Quality control organisms, (6) Forceps, (7) Ruler or caliper.

PROCEDURE

1. Use established laboratory procedures for processing anaerobic organisms.
2. Allow disk(s) to equilibrate to room temperature before use.
3. Select the test isolate from a primary anaerobic culture or use a pure subculture.
4. Select one well-isolated colony and streak the first quadrant of the plate back and forth several times to ensure an even lawn of growth. Streak the remaining quadrants for isolation.
5. Using forceps, place the Colistin, Kanamycin, and Vancomycin disks in the first quadrant, well separated from each other. Subsequent disks for presumptive identification of anaerobes may be placed in the second quadrant of the plate. Consult appropriate laboratory procedures or texts.^{3,4}
6. Aerotolerance testing of the test isolate should be performed at this time if not already established.
7. Incubate plate anaerobically at 35-37°C for 24-48 hours or longer until adequate growth is evident.
8. Measure zone of inhibition and record.

INTERPRETATION

Sensitive - Zone of inhibition greater than 10 mm

Resistant - No zone of inhibition or zone less than 10 mm

EXPECTED VALUES^{2,6}

	VAN 5 µg	KAN 1000 µg	COL 10 µg	PEN 2 U	RIF 15 µg
Gram-positive	S	V	R	-	-
Gram-negative	R	R ^S	S*	-	-
<i>Bacteroides fragilis</i> gp.	R	R	R	R ^S	-
Other <i>Bacteroides</i> spp.	R	R	V	S	-
Pigmented <i>Porphyromonas</i> spp.	S	R	R	-	-
<i>Fusobacterium</i> spp.	R	S	S	-	**
<i>Bacteroides ureolyticus</i> gp.	R	S	S	-	-

S = sensitive, R = resistant, R^S = some strains sensitive, V = variable

*Unusual gram-negative anaerobes may be resistant. *Porphyromonas* is one of the exceptions; it is a gram-negative bacillus with a gram-positive disk pattern.

***Fusobacterium mortiferum varium* group is resistant to rifampin as opposed to other *Fusobacterium* species.⁴

QUALITY CONTROL

All lot numbers of Colistin Disk, Kanamycin Disk, Penicillin Disk, Rifampin Disk, and Vancomycin Disk have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Colistin Disk		
<i>Fusobacterium mortiferum</i> ATCC® 25557	Anaerobic, 24-48h @ 35-37°C	Sensitive
<i>Bacteroides fragilis</i> ATCC® 25285	Anaerobic, 24-48h @ 35-37°C	Resistant
Kanamycin Disk		
<i>Fusobacterium mortiferum</i> ATCC® 25557	Anaerobic, 24-48h @ 35-37°C	Sensitive
<i>Bacteroides fragilis</i> ATCC® 25285	Anaerobic, 24-48h @ 35-37°C	Resistant
Penicillin Disk		
<i>Peptostreptococcus anaerobius</i> ATCC® 27337	Anaerobic, 24-48h @ 35-37°C	Sensitive
<i>Bacteroides fragilis</i> ATCC® 25285	Anaerobic, 24-48h @ 35-37°C	Resistant
Rifampin Disk		
<i>Bacteroides fragilis</i> ATCC® 25285	Anaerobic, 24-48h @ 35-37°C	Sensitive
<i>Fusobacterium mortiferum</i> ATCC® 25557	Anaerobic, 24-48h @ 35-37°C	Resistant
Vancomycin Disk		
<i>Clostridium perfringens</i> ATCC® 13124	Anaerobic, 24-48h @ 35-37°C	Sensitive
<i>Bacteroides fragilis</i> ATCC® 25285	Anaerobic, 24-48h @ 35-37°C	Resistant

PERFORMANCE CHARACTERISTICS

An evaluation of 42 strains of anaerobic bacteria showed 100% agreement with expected values.⁷

LIMITATIONS

1. These disks are for Gram stain confirmation and for presumptive identification purposes. Further biochemical testing may be necessary for definitive identification.
2. Anaerobe Identification Disks are not intended to be used for susceptibility testing for therapeutic purposes.

3. Virginia Polytechnic Institute recommends that nichrome loops not be used in processing anaerobes.^{5,6}




BIBLIOGRAPHY

1. Sutter, V.L. and S.M. Finegold. 1971. Appl. Microbiol. 21:13-20.
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3. Forbes, B.A., D.F. Sahn, and A.S. Weissfeld. 2002. Bailey and Scott's Diagnostic Microbiology. 11th ed. Mosby, St. Louis, MO.
4. Summanen, P., E.J. Baron, E.M. Citron, C.A. Strong, H.M. Wexler, and S.M. Finegold. 1993. Wadsworth Anaerobic Bacteriology Manual. 5th ed. Star Publ. Co., Belmont, CA.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover. 2003. Manual of Clinical Microbiology. 8th ed. ASM, Washington, D.C.
6. Holdeman, L.V., E.P. Cato, and W.E.C. Moore. 1977. Anaerobe Laboratory Manual. 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, VA.
7. Data on file. 1991. Remel Inc., Lenexa, KS.

PACKAGING

REF R21123, Colistin Disk 10 µg25 Disks/Vial
 REF R21126, Kanamycin Disk 1000 µg25 Disks/Vial
 REF R21136, Penicillin Disk 2 U25 Disks/Vial
 REF R21139, Rifampin Disk 15 µg.....25 Disks/Vial
 REF R21156, Vancomycin Disk 5 µg25 Disks/Vial

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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