BACTEROIDES FRAGILIS ISOLATION AGAR
(Bacteroides Bile Esculin) (BBE)

INTENDED USE
Remel Bacteroides Fragilis Isolation Agar is a solid medium recommended for use in qualitative procedures for the isolation and presumptive identification of *Bacteroides fragilis* group.

SUMMARY AND EXPLANATION
Members of the *B. fragilis* group are frequently isolated from human clinical infections.[1] Because clinical specimens for anaerobic culture frequently contain mixed flora, the use of selective media is recommended.[2] Livingston et al. formulated a selective and differential medium, Bacteroides Esculin Agar, which provided recovery and presumptive identification of the *Bacteroides fragilis* group [3]

PRINCIPLE
The selectivity of this medium is provided by addition of gentamicin and bile. Gentamicin inhibits most facultative anaerobes and bile inhibits anaerobic gram-negative bacilli with the exception of *B. fragilis* group and other bile resistant *Bacteroides* and *Fusobacteria*. Differentiation is facilitated by the hydrolysis of esculin to esculetin by the *B. fragilis* group. Ferric ammonium citrate is added to the medium to react with esculetin and produce a brown-black complex surrounding the colony. Vitamin K is a growth factor. Hemin also is a growth factor and allows testing for catalase production.[4]

REAGENTS (CLASSICAL FORMULA)*

Bile (Oxgall)	20.0	g	Ferric Ammonium Citrate	0.5	g
Casein Peptone	15.0	g	Gentamicin	0.1	g
Meat Peptone	5.0	g	Vitamin K	10.0	mg
Sodium Chloride	5.0	g	Hemin	5.0	mg
Esculin	1.0	g	Agar	15.0	g
			Demineralized Water	1000.0	ml

pH 7.0 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prior to use, reduce the plates for a minimum of 24 hours by placing them in an anaerobic environment at room temperature.
2. Inoculate specimens for anaerobic culture on both selective and non-selective media as soon as possible after receipt in the laboratory; streak plates for isolation.
3. Incubate anaerobically at 33-37°C for 48-72 hours.
4. Confirm anaerobic growth by subculture to an aerobic blood agar plate.

QUALITY CONTROL
All lot numbers of Bacteroides Fragilis Isolation Agar 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
Bacteroides fragilis ATCC® 25285	Anaerobic, up to 48 h @ 33-37°C	Growth, blackening of media
Bacteroides thetaiotaomicron ATCC® 29148	Anaerobic, up to 48 h @ 33-37°C	Growth, blackening of media
Clostridium perfringens ATCC® 13124	Anaerobic, up to 48 h @ 33-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Pseudomonas aeruginosa ATCC® 27853	Aerobic, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, 18-24 h @ 33-37°C	Inhibition (partial to complete)

LIMITATIONS
1. Fusobacteria are resistant to 20% bile and may grow on Bacteroides Fragilis Isolation Agar; however, *Fusobacterium* spp. are catalase-negative.[4]
2. Some enterococci and enteric gram-negative bacilli may grow, but the colonial morphology produced is not characteristic of *B. fragilis* group.[4]
3. Some penicillin-resistant strains of *Bacteroides* are inhibited by 20% bile and may not grow on this medium.[4]
4. Yeast is not inhibited on this medium; however, if growth occurs the colonies will not cause blackening of the agar.[4]

BIBLIOGRAPHY

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4. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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