

ANAEROBIC REDUCIBLE BLOOD AGAR w/ and w/o ADDITIVES

INTENDED USE

Remel Anaerobic Reducible Blood Agar w/ and w/o Additives are solid media recommended for use in qualitative procedures for the primary isolation and cultivation of fastidious anaerobic bacteria.

SUMMARY AND EXPLANATION

Ellner et al. used Columbia Agar Base to formulate a reducible media for improved recovery of anaerobic bacteria.¹ The reducing tendency, or redox potential (Eh), of media is a measure of its capacity to give up electrons. Media, prepared and sterilized by conventional methods, was modified by addition of the reducing agents, cysteine hydrochloride, palladium chloride, and dithiothreitol.² After exposure to an anaerobic environment, the Eh of such media was shown to be further reduced. Colistin and nalidixic acid are added to the base to produce a selective medium for isolation of gram-positive bacteria. Finegold added kanamycin and vancomycin as selective agents for recovery of gram-negative anaerobes, especially *Bacteroides* spp.³

PRINCIPLE

Anaerobic Reducible Blood Agar is a nutritious medium containing peptone, hemin, vitamin K, and sheep blood which supply anaerobic organisms with required growth factors. Colistin and nalidixic acid are inhibitory to most gram-negative bacilli. Kanamycin and vancomycin inhibit gram-positive organisms and facultative anaerobic bacteria. Neomycin facilitates the isolation of anaerobic organisms, such as *Bacteroides* and *Clostridium* spp. and inhibits enteric gram-negative bacilli. Laked blood is added to enhance pigment formation by anaerobic bacteria.

REAGENTS (CLASSICAL FORMULAE)*

Casein Peptone.....	12.0 g	Palladium Chloride	0.33 g
Meat Peptone.....	5.0 g	Dithiothreitol	0.1 g
Sodium Chloride.....	5.0 g	Vitamin K	10.0mg
Beef Extract.....	3.0 g	Hemin	5.0mg
Yeast Extract.....	3.0 g	Sheep Blood.....	5 %
Corn Starch.....	1.0 g	Agar.....	13.5 g
Cysteine HCl.....	0.5 g	Demineralized Water.....	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

The following combination of optional ingredients are available per liter of media:

1. Colistin	10.0 mg	3. Neomycin.....	100.0 mg
Nalidixic Acid.....	10.0 mg		
2. Laked Sheep Blood.....	5 %		
Kanamycin	100.0 mg		
Vancomycin.....	7.5 mg		

*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 nonselective 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 Anaerobic Reducible Blood Agar w/ and w/o Additives have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.⁴ 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

Anaerobic Reducible Blood Agar:

**Bacteroides fragilis* ATCC® 25285
**Clostridium perfringens* ATCC® 13124
Escherichia coli ATCC® 25922
**Fusobacterium nucleatum* ATCC® 25586
**Peptostreptococcus anaerobius* ATCC® 27337
Prevotella melaninogenica ATCC® 25845
Staphylococcus aureus ATCC® 25923

*CLSI recommended organism

INCUBATION

Anaerobic, 48-72 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C

RESULTS

Growth
Growth, beta hemolysis
Growth
Growth
Growth
Growth
Growth

CONTROL

Anaerobic CNA Blood Agar:

Bacteroides fragilis ATCC® 25285
Escherichia coli ATCC® 25922
Peptostreptococcus anaerobius ATCC® 27337
Staphylococcus aureus ATCC® 25923

Anaerobic LKV Blood Agar:

Bacteroides fragilis ATCC® 25285
Escherichia coli ATCC® 25922
Prevotella melaninogenica ATCC® 25845
Staphylococcus aureus ATCC® 25923

Anaerobic Neomycin Blood Agar:

Bacteroides fragilis ATCC® 25285
Clostridium perfringens ATCC® 13124
Escherichia coli ATCC® 25922
Prevotella melaninogenica ATCC® 25845

INCUBATION

Anaerobic, 48-72 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C

Anaerobic, 48-72 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C

Anaerobic, 48-72 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C
Anaerobic, 48-72 h @ 33-37°C

RESULTS

Growth
Inhibition (partial to complete)
Growth
Growth

Growth
Inhibition (partial to complete)
Growth
Inhibition (partial to complete)

Growth
Growth, beta hemolysis
Inhibition (partial to complete)
Growth

BIBLIOGRAPHY

1. Ellner, P.D., C.I. Stoessel, E. Drakeford, and F. Vasi. 1966. *Am. J. Clin. Pathol.* 45:502-504.
2. Ellner, P.D., P.A. Granato, and C.B. May. 1973. *Appl. Microbiol.* 26:904-913.
3. Finegold, S.M. 1965. International Mikrobiologisches Symposium, Potsdam, Germany, *Ehrnahrungsforschung*, 10:517-528.
4. Clinical and Laboratory Standards Institute (CLSI). 2004. *Quality Control for Commercially Prepared Microbiological Culture Media*; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.

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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