

Wilkins-Chalgren Agar • Anaerobe Broth MIC

Intended Use

Wilkins-Chalgren Agar is used for susceptibility testing of anaerobes and for isolating and cultivating anaerobes.

Anaerobe Broth MIC is used for susceptibility testing of anaerobes by the broth dilution technique.

Summary and Explanation

Wilkins-Chalgren Agar was designed by Wilkins and Chalgren¹ for use in determining the minimal inhibitory concentration (MIC) of antibiotics for anaerobic bacteria by the agar dilution procedure. This medium was recommended in the protocol used in the CLSI Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.² More recently, Wilkins-Chalgren Agar has been replaced by Brucella Agar supplemented with laked sheep blood, hemin and vitamin K₁ as the recommended reference medium.³

Anaerobe Broth MIC is a modification of the formula described by Wilkins and Chalgren.¹ In Anaerobe Broth MIC the agar has been omitted.

The preferred medium for agar dilution tests with anaerobes is Wilkins-Chalgren Agar or Brucella Agar.¹ For broth microdilution tests Anaerobe Broth MIC has been used successfully.⁴ Supplements must be added to these media to support the growth of certain fastidious anaerobes, including *Bacteroides gracilis*, *Bilophila wadsworthia*, *Prevotella* species, *Fusobacterium* species and anaerobic cocci.⁴ Defibrinated sheep blood, 5% or lysed sheep blood is an adequate supplement for many fastidious anaerobic organisms.²

Principles of the Procedure

Peptones provide the nitrogen and amino acids in Wilkins-Chalgren Agar and Anaerobe Broth MIC. Yeast extract is the vitamin source in the media formulations. Dextrose is the carbon source, and sodium chloride maintains the osmotic balance of the media. L-arginine and sodium pyruvate are added to provide the proper environment for anaerobic growth. Hemin and vitamin K₁ are growth factors. Agar is the solidifying agent in Wilkins-Chalgren Agar.

User Quality Control

Identity Specifications

Difco™ Wilkins-Chalgren Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 4.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.

Prepared Appearance: Light to medium amber, slightly opalescent.

Reaction of 4.8%

Solution at 25°C: pH 7.1 ± 0.1

Difco™ Anaerobe Broth MIC

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 3.3% solution, soluble in purified water upon warming. Solution is light amber, clear, may have a slight precipitate.

Prepared Appearance: Light amber, clear, may have a slight precipitate.

Reaction of 3.3%

Solution at 25°C: pH 7.1 ± 0.1

Cultural Response

Difco™ Wilkins-Chalgren Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under anaerobic conditions for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Bacteroides fragilis</i> *	25285	10 ² -10 ³	Good
<i>Bacteroides thetaiotaomicron</i> *	29741	10 ² -10 ³	Good
<i>Clostridium perfringens</i>	13124	10 ² -10 ³	Good
<i>Eubacterium lentum</i>	43055	10 ² -10 ³	Good

Minimal Inhibitory Concentration (MIC) Assay: Prepare plates and inoculate as described by CLSI.³ Test organisms marked (*) and compare the MIC (lowest concentration of antimicrobial that inhibits growth of the test bacterium) of the antimicrobials tested to the CLSI standard.³

Difco™ Anaerobe Broth MIC

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Bacteroides fragilis</i> *	25285	10 ² -3 × 10 ²	Good
<i>Bacteroides thetaiotaomicron</i> *	29741	10 ² -3 × 10 ²	Good
<i>Eubacterium lentum</i>	43055	10 ² -3 × 10 ²	Good

Minimal Inhibitory Concentration (MIC) Assay: Prepare broth microdilution trays and inoculate as described by CLSI.³ Test organisms marked (*) and compare the MIC (lowest concentration of antimicrobial that inhibits growth of the test bacterium) of the antimicrobials tested to the CLSI standard.³

Formulae

Difco™ Wilkins-Chalgren Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	10.0 g
Peptone	10.0 g
Yeast Extract	5.0 g
Dextrose	1.0 g
Sodium Chloride	5.0 g
L-Arginine	1.0 g
Sodium Pyruvate	1.0 g
Hemin	5.0 mg
Vitamin K ₁	0.5 mg
Agar	15.0 g

Difco™ Anaerobe Broth MIC

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
Difco™ Wilkins-Chalgren Agar – 48 g;
Difco™ Anaerobe Broth MIC – 33 g.
Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on susceptibility testing of anaerobic bacteria refer to appropriate procedures outlined in the references.²⁻⁵

Expected Results

Refer to appropriate references for acceptable ranges.

Limitation of the Procedure

Anaerobe Broth MIC is supplemented to a final concentration of 0.5 µg per mL of vitamin K₁ and 5.0 µg of hemin per mL. CLSI changed their recommendations to include use of broth with a final concentration of 1 µg of vitamin K₁ per mL.² To follow CLSI recommendations, the concentration of vitamin K₁ should be increased accordingly. A final concentration of 0.5 µg of vitamin K₁ per mL is sufficient, but some fastidious anaerobes may need a higher concentration of vitamin K₁.⁵

References

1. Wilkins and Chalgren. 1976. *Antimicrob. Agents Chemother.* 10:926.
2. Clinical and Laboratory Standards Institute. 1993. *Methods for antimicrobial susceptibility testing of anaerobic bacteria.* Approved standard M11-A3. CLSI, Wayne, Pa.
3. Clinical and Laboratory Standards Institute. 2001. *Methods for antimicrobial susceptibility testing of anaerobic bacteria.* Approved standard M11-A5. CLSI, Wayne, Pa.
4. Wexler and Doern. 1995. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
5. Isenberg (ed.). 1995. *Clinical microbiology procedures handbook*, vol 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Wilkins-Chalgren Agar

CCAM

Cat. No. 218051 Dehydrated – 500 g

Difco™ Anaerobe Broth MIC

CCAM

Cat. No. 218151 Dehydrated – 500 g