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BD BBL™ Prepared Plated Medium for Susceptibility Testing of *Neisseria gonorrhoeae*

GC II Agar with IsoVitaleX™ Enrichment



8809031JAA
2010/07

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INTENDED USE

GC II Agar with IsoVitaleX™ Enrichment is used for antimicrobial disc diffusion susceptibility testing of *Neisseria gonorrhoeae*.

SUMMARY AND EXPLANATION

Because of the growing problem of resistance of gonococci to penicillin and other antimicrobial agents, the Centers for Disease Control and Prevention (CDC) issued, in 1987, policy guidelines for the detection, management and control of antibiotic-resistant *N. gonorrhoeae*.¹ The medium recommended for the disc diffusion method of antimicrobial susceptibility testing was GC agar base with a defined supplement, such as IsoVitaleX Enrichment.

The National Committee for Clinical Laboratory Standards (NCCLS) also recommends the use of GC Agar with a defined supplement (equivalent to IsoVitaleX Enrichment) for antimicrobial disc diffusion susceptibility testing of *N. gonorrhoeae*.^{2,3}

PRINCIPLES OF THE PROCEDURE

GC II Agar Base contains casein and meat peptones as sources of nutrients, phosphate buffer to control pH and corn starch to neutralize toxic fatty acids that may be present in the agar. IsoVitaleX Enrichment is a defined supplement that provides V factor (nicotinamide adenine dinucleotide, NAD), vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors that improve the growth of pathogenic *Neisseria*.

REAGENTS

GC II Agar with IsoVitaleX Enrichment

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein.....	7.5 g
Selected Meat Peptone.....	7.5 g
Corn Starch.....	1.0 g
Dipotassium Phosphate.....	4.0 g
Monopotassium Phosphate.....	1.0 g
Sodium Chloride.....	5.0 g
Agar.....	12.0 g
IsoVitaleX Enrichment.....	10.0 mL

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

IsoVitaleX Enrichment

Approximate Formula* Per Liter Purified Water

Vitamin B ₁₂	0.01 g
L-Glutamine.....	10.0 g
Adenine.....	1.0 g
Guanine Hydrochloride.....	0.03 g
p-Aminobenzoic Acid.....	0.013 g
Nicotinamide Adenine Dinucleotide.....	0.25 g
Thiamine Pyrophosphate.....	0.1 g
Ferric Nitrate.....	0.02 g
Thiamine Hydrochloride.....	0.003 g
L-Cysteine Hydrochloride.....	25.9 g
L-Cystine.....	1.1 g
Dextrose.....	100.0 g

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

Warnings and Precautions

For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 to 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 to 8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

SPECIMEN COLLECTION AND HANDLING

This medium is not intended for direct use with clinical specimens or mixed cultures. The disc diffusion susceptibility test is designed for use with pure cultures. A Gram stain and a presumptive identification of *N. gonorrhoeae* are required.

PROCEDURE

Material Provided: GC II Agar with IsoVitaleX Enrichment

Materials Required But Not Provided:

1. Inoculum broth tubed in 5-mL amounts, such as Mueller Hinton Broth (20 x 112 mm tube) or Mueller Hinton II Broth (16 x 102 mm tube), for preparation of standard inoculum.
2. A 0.5 McFarland barium sulfate standard for adjustment of inoculum (prepared by adding 0.5 mL of 0.048 M BaCl₂ [1.175% w/v BaCl₂·2 H₂O] to 99.5 mL of 0.18 M [0.36 N] H₂SO₄ [1% v/v]).
3. A photometric device for comparing the turbidity of the barium sulfate standard with the inoculum suspension.
4. Control culture - *Neisseria gonorrhoeae* ATCC™ 49226.
5. Paper discs impregnated with specified amounts of antimicrobial agents, such as BBL™ Sensi-Disc™ susceptibility test discs.
6. Disc dispensing device, such as the Sensi-Disc Self-Tamping 12-Place Dispenser.
7. Device for measuring or interpreting zone diameters, such as the Sensi-Disc Zone Interpretation Set.
8. A reagent or device for performing a rapid β-lactamase test, such as BBL™ Cefinase™ Discs.
9. An incubator that produces an atmosphere containing 5 to 7% CO₂, or another device that produces a CO₂-enriched aerobic atmosphere.
10. Ancillary culture media, reagents, and laboratory equipment as required.

Test Procedure

The direct colony suspension procedure should be used when testing *N. gonorrhoeae*. Observe aseptic techniques. Agar surfaces should be smooth and moist but without excessive moisture.

1. Prepare a Gram stain and perform an oxidase test before starting susceptibility testing to confirm culture purity and to confirm tentative identification of *N. gonorrhoeae*.
2. Use several well-isolated colonies taken directly from an overnight chocolate agar plate as the source of the inoculum.
3. A rapid β-lactamase test (Cefinase disc) should be utilized to assess the clinical utility of penicillin. This rapid test provides the clinician with valuable antimicrobial susceptibility information sooner than the disc diffusion test.
4. Using colonies taken directly from an overnight chocolate agar plate, prepare a suspension of the test organism in Mueller Hinton Broth, Mueller Hinton II Broth or 0.9% saline and dilute with more broth or saline to a turbidity equivalent to that of the 0.5 McFarland standard (see Materials Required But Not Provided). This suspension will contain approximately 1 to 2 x 10⁸ CFU/mL. Care must be exercised in preparing this suspension since higher inoculum concentration may lead to false-resistant results and too light an inoculum can result in false-susceptible results. Periodically perform dilutions and plate counts of inoculum suspensions to confirm that the adjustment method employed produces an inoculum containing approximately 1 to 2 x 10⁸ CFU/mL.
5. Within 15 min of adjusting the turbidity of the inoculum, dip a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
6. Inoculate the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation.
7. Replace the lid of the plate and hold the plate at room temperature for 3 to 5 min, but no longer than 15 min, to allow any surface moisture to be absorbed before applying the drug-impregnated discs.
8. Apply the discs by means of an antimicrobial disc dispenser, using aseptic precautions. Most antimicrobial agents produce larger zones of inhibition when tested against *N. gonorrhoeae* compared with other organisms, in some cases limiting the number of discs to no more than 9 discs per 150 mm plate. After discs have been placed on the agar, tamp them with a sterile needle or forceps to make complete contact with the medium surface. This step is not necessary if the discs are deposited using the Sensi-Disc Self Tamping 12-Place Dispenser (tamper will not descend from holes lacking cartridges).
9. Within 15 min after the discs are applied, invert the plates and incubate for 20 to 24 h at 35°C in an aerobic atmosphere enriched with 5 to 7% carbon dioxide.

User Quality Control:

1. Examine the plates for signs of deterioration as described under "Product Deterioration."
2. The control culture, *Neisseria gonorrhoeae* ATCC 49226, should be included each time a susceptibility test is performed. Control limits for zones of inhibition are given in Table 1 and indicate the correct performance of the entire procedure.

TABLE 1. Quality Control Limits for *N. gonorrhoeae**

Antimicrobial Agent	Disc Content	<i>N. gonorrhoeae</i> ATCC™ 49226
Cefepime	30 µg	37-46
Cefixime	5 µg	37-45
Cefotaxime	30 µg	38-48
Cefotetan	30 µg	30-36
Cefoxitin	30 µg	33-41
Cefpodoxime	10 µg	35-43
Ceftazidime	30 µg	35-43
Ceftizoxime	30 µg	42-51
Ceftriaxone	30 µg	39-51
Cefuroxime	30 µg	33-41
Ciprofloxacin	5 µg	48-58
Enoxacin	10 µg	43-51
Gatifloxacin	5 µg	45-56
Lomefloxacin	10 µg	45-54
Ofloxacin	5 µg	43-51
Penicillin	10 units	26-34
Spectinomycin	100 µg	23-29
Trovafloxacin	10 µg	42-55

*From NCCLS publication M100-S13, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved standard. With permission.

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

RESULTS

- Examine the plates after 20 to 24 h of incubation. A confluent "lawn" of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the test should be repeated.
- Measure the diameter of the zone of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the Petri plate, which is held over a black, non-reflecting background and illuminated from above.
The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies that can be detected with difficulty near the edge of the obvious zone of inhibition.
- Refer to the Zone Diameter Interpretive Standards chart (Table 2) for interpretation of results obtained with clinical isolates of *N. gonorrhoeae*. Results may be reported as resistant, intermediate or susceptible depending on the zone diameters obtained. Organisms testing positive for β-lactamase production should be considered resistant to penicillin regardless of the zone diameters obtained.

TABLE 2. Zone Diameter Interpretive Standards for *N. gonorrhoeae**

Antimicrobial Agent	Disc Content	Zone Diameter, nearest whole mm		
		Resistant	Intermediate ^c	Susceptible
Cefepime ^a	30 µg	–	–	≥31
Cefixime ^a	5 µg	–	–	≥31
Cefotaxime ^a	30 µg	–	–	≥31
Cefotetan	30 µg	≤19	20-25	≥26
Cefoxitin	30 µg	≤23	24-27	≥28
Cefpodoxime ^a	10 µg	–	–	≥29
Ceftazidime ^a	30 µg	–	–	≥31
Ceftizoxime ^a	30 µg	–	–	≥38
Ceftriaxone ^a	30 µg	–	–	≥35
Cefuroxime	30 µg	≤25	26-30	≥31
Ciprofloxacin	5 µg	≤27	28-40	≥41
Enoxacin	10 µg	≤31	32-35	≥36
Gatifloxacin	5 µg	≤33	34-37	≥38
Lomefloxacin	10 µg	≤26	27-37	≥38
Ofloxacin	5 µg	≤24	25-30	≥31
Penicillin ^b	10 units	≤26	27-46	≥47
Spectinomycin	100 µg	≤14	15-17	≥18
Trovafloxacin ^a	10 µg	–	–	≥34

* From NCCLS publication M100-S13, Performance Standards for Antimicrobial Susceptibility Tests; Approved standard. With permission.

- For these antimicrobial agents, the current absence of resistant strains precludes defining any results categories other than "Susceptible." Strains yielding results suggestive of a "Nonsusceptible" category should be submitted to a reference laboratory for further testing.
- Gonococci with 10 U-penicillin disc zone diameters of ≤19 mm are likely to be β-lactamase-producing strains. However, the β-lactamase test remains preferable to other susceptibility methods for rapid, accurate recognition of this plasmid-mediated penicillin resistance.
- An intermediate result for an antimicrobial agent indicates either a technical problem that should be resolved by repeated testing or a lack of clinical experience in treating organisms with these zones. The latter seems to be the case for cefotetan, cefoxitin and spectinomycin (see M2-A8). Strains with intermediate zones to the other agents have a documented lower clinical cure rate (85 to 95%) compared with >95% for susceptible strains.

LIMITATIONS OF THE PROCEDURE

The ability to detect resistance to cefixime, cefotaxime, cefotetan, cefoxitin, cefpodoxime, ceftazidime, ceftizoxime, ceftriaxone, cefuroxime, ciprofloxacin, enoxacin, lomefloxacin, and ofloxacin among *Neisseria gonorrhoeae* is unknown because resistant strains were not available at the time of testing.

With some organism-antimicrobial agent combinations, the inhibition zone may not have a sharply demarcated edge, which could lead to incorrect interpretation.

Due to inconsistent performance of cefmetazole, and greater than 10 minor errors with tetracycline, the use of these two antimicrobics on this medium is contraindicated.

Incorrect inoculum concentration may produce inaccurate results. Zones of inhibition may be too small if the inoculum is too heavy and they may be too large and difficult to measure if the inoculum is too light.

Improper storage of antimicrobial discs may cause a loss of potency and a falsely resistant result.

In vitro susceptibility of an organism to a specific antimicrobial agent does not necessarily mean that the agent will be effective *in vivo*. Consult appropriate references for guidance in the interpretation of results.^{4,5}

PERFORMANCE CHARACTERISTICS

Prior to release, all lots of GC II Agar with IsoVitaleX™ Enrichment are tested for performance characteristics. Representative samples of the lot are tested with a cell suspension of *Neisseria gonorrhoeae* ATCC 43069, by inoculating with a normal saline suspension diluted to yield 1x10³ to 1x10⁴ CFUs/plate. Samples are also tested with *Neisseria gonorrhoeae* ATCC 49226 by swabbing the surface of the medium with a suspension equivalent to a 0.5 McFarland standard. Discs containing specified amounts of antimicrobial agents are added. All plates are incubated at 35 – 37°C for one day in a CO₂-enriched aerobic atmosphere. Moderate to heavy growth is observed with *N. gonorrhoeae* ATCC 43069. Correct zones of inhibition are observed around discs with *N. gonorrhoeae* ATCC 49226.

AVAILABILITY

Cat. No.	Description
221240	BBL™ GC II Agar with IsoVitaleX™ Enrichment, Pkg. of 100 size 8 plates (150 x 15 mm-style)

REFERENCES

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- Jorgensen, J.H. and J.D. Turnidge. 2003. Susceptibility test methods: dilution and disk diffusion methods, p. 1108-1127. In Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenover (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.



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