



BD™ Chocolate Agar (GC II Agar with IsoVitaleX™) • BD Chocolate Agar (Blood Agar No. 2 Base)

INTENDED USE

BD Chocolate Agar (GC II Agar with IsoVitaleX) and **BD Chocolate Agar (Blood Agar No. 2 Base)** are non-selective media for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* and *Haemophilus* species, from a variety of clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Carpenter and Morton described an improved medium for the isolation of gonococcus in 24 h.¹ The efficiency of this medium, GC Agar supplemented with hemoglobin and yeast concentrate, was demonstrated in a study of twelve media in use for the isolation of this organism.² Later on, several improvements of the medium were made.³⁻⁵ In **BD Chocolate Agar (GC II Agar with IsoVitaleX)** nutrients are provided from the GC II base which contains casein and selected meat peptones as nitrogen sources, phosphates to maintain pH, and corn starch, to neutralize toxic fatty acids that may be present in the agar. Hemoglobin provides X factor (hemin). **BD IsoVitaleX** Enrichment is a defined supplement which provides V factor (nicotinamide adenine dinucleotide, NAD), essential for *Haemophilus* species, and vitamins, amino acids, coenzymes, glucose, ferric ion, and other factors which improve the growth of pathogenic *Neisseria* and other fastidious organisms. Pyridoxal and specific growth factors have been added to improve the growth of Gram positive bacteria and pyridoxal has been added to support growth of *Granulicatella* and *Abiotrophia* species (=nutritionally variant streptococci).⁶⁻⁹

Chocolate Agar (Blood Agar No. 2 Base) is an alternative medium for the isolation of fastidious organisms from clinical specimens.⁷ **BD Chocolate Agar (Blood Agar No. 2 Base)** contains Proteose peptone, liver digest and yeast extract as sources of nitrogen and vitamins. Sodium chloride maintains the osmotic stability. Heated (heat-denatured) horse blood supplies both the X factor (heme) and, since it does not contain NADase, the V factor (nicotinamide adenine dinucleotide, NAD) necessary for the growth of *Haemophilus influenzae* and supplies additional nutrients.

REAGENTS

Formulas* Per Liter Purified Water

BD Chocolate Agar (GC II Agar with IsoVitaleX)		BD Chocolate Agar (Blood Agar No. 2 Base)	
Pancreatic Digest of Casein	7.5 g	Proteose Peptone No.3	15.0 g
Selected Meat Peptone	7.5	Liver Digest	2.5
Corn Starch	1.0	Yeast Extract	5.0
Dipotassium Phosphate	4.0	Sodium Chloride	5.0
Monopotassium Phosphate	1.0	Horse Blood (heated)	7%
Sodium Chloride	5.0	pH 7.4 +/- 0.2	
Agar	12.0		
Hemoglobin	10.0		
IsoVitaleX Enrichment	12.0 ml		
Pyridoxal	0.01 g		
Growth Factors	0.5		
pH 7.2 +/- 0.2			

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

BD IsoVital[®]enrichment contains the following growth factors (formula* per liter purified water):

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

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

PRECAUTIONS

IVD . For professional use only. 

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

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Read plates after 18 to 24 and after 42 to 48 hours of incubation.

Strains*	BD Chocolate Agar (GC II Agar with IsoVital [®] enrichment) and BD Chocolate Agar (Blood Agar No. 2 Base)
<i>Haemophilus influenzae</i> ATCC™ 10211	Good to excellent growth
<i>Neisseria gonorrhoeae</i> ATCC 43069	Fair to excellent growth
<i>Neisseria meningitidis</i> ATCC 13090	Good to excellent growth
<i>Streptococcus pneumoniae</i> ATCC 6305	Good to excellent growth
Uninoculated	Chocolate brown, opaque, may be slightly inhomogeneous

* Additionally, **BD Chocolate Agar (GC II Agar with IsoVital[®]enrichment)** may be tested with *Granulicatella (Abiotrophia) adiacens* DSM 9848 to reveal growth of nutritionally variant streptococci.^{8,9}

PROCEDURE

Materials Provided

BD Chocolate Agar (GC II Agar with IsoVital[®]enrichment) or **BD Chocolate Agar (Blood Agar No. 2 Base)**, both provided in 90 mm **Stacker™** plates. Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

These media can principally be used for all types of specimens from infections suspected to contain fastidious organisms, especially but not only for specimens from primarily sterile body sites (e.g., cerebrospinal fluid, abscesses). Their main use is for the nonselective isolation of *Neisseria*, *Haemophilus* and other bacteria that may not grow on routinely used blood agar media, such as Columbia Agar with 5% Sheep Blood. In addition, **BD Chocolate Agar (GC II Agar with IsoVitaleX)** may be used for the isolation of *Abiotrophia* and *Globicatella* species (=nutritionally variant streptococci).^{8,9} The media are also used as subculture media from blood cultures. (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Specimen Collection and Transport

Neisseria gonorrhoeae, *N. meningitidis*, *Haemophilus* and other fastidious organisms are sensitive to adverse environmental conditions. Therefore, appropriate transport media must be used for all specimens. Specimens must be sent to the laboratory as fast as possible and must not be older than 24 hours, even if transport media are used. The optimal transport temperature is 20 to 25° C. Do not refrigerate!^{10,11}

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory onto **BD Chocolate Agar (GC II Agar with IsoVitaleX)** or **BD Chocolate Agar (Blood Agar No. 2 Base)**. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

If the specimen has been collected from a body site containing normal flora, it should also be inoculated onto appropriate selective media, depending on the pathogenic agent to be isolated. For *Neisseria gonorrhoeae*, a **BD Martin-Lewis Agar, modified** or **BD GC-Lect™ Agar** plate, and for *Haemophilus*, a **BD Chocolate Agar with IsoVitaleX and Bacitracin** plate should be included.

Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide. Read plates after 18 to 24 and after 42 to 48 hours of incubation.

Results

Typical colonial morphology is as follows:

Organisms	BD Chocolate Agar (GC II Agar with IsoVitaleX) and BD Chocolate Agar (Blood Agar No. 2 Base)
<i>Haemophilus influenzae</i>	Small (1mm), moist, pearly with a characteristic "mousy" odor
<i>Neisseria gonorrhoeae</i>	Small, grayish-white to colorless, mucoid
<i>Neisseria meningitidis</i>	Medium to large, blue-gray, mucoid
<i>Streptococcus pneumoniae</i>	Small, flat or larger mucoid greenish colonies, medium surrounding colonies may be greenish
<i>Granulicatella (Abiotrophia) adiacens*</i>	Small gray-greenish colonies, medium surrounding colonies may be greenish

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Chocolate Agar (GC II Agar with IsoVitaleX) and **BD Chocolate Agar (Blood Agar No. 2 Base)** are enriched, non-selective media on which fastidious and non-fastidious bacteria, including normal flora, will grow. Therefore, it is recommended to inoculate specimens from body sites containing normal flora also onto appropriate selective media.

The term "fastidious bacteria" relates to bacteria that do not grow or do not grow well on normally used primary isolation media containing sheep blood, e.g. *Haemophilus*, pathogenic *Neisseria*, and several other organisms. For detailed descriptions of the type of specimens that must be inoculated onto these media and of the type of organisms for which these media are used for isolation, consult the references.^{8,9,11,12}

The number and types of bacterial species occurring as infectious agents is very large. Therefore, before these media are routinely used for rarely isolated or newly described microorganisms, their suitability must first be tested by the user by cultivating pure cultures of the organism in question.

BD Chocolate Agar (Blood Agar No. 2 Base) has not been tested to support the growth of *Abiotrophia* species and other nutritionally variant streptococci.

For discussion of nutritionally variant streptococci, consult the references. ^{6,8,9,13}

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PACKAGING/AVAILABILITY

BD Chocolate Agar (GC II Agar with IsoVitalEX)

Cat. No. 254060	Ready-to-use Plated Media, cpu 20
Cat. No. 254089	Ready-to-use Plated Media, cpu 120

BD Chocolate Agar (Blood Agar No. 2 Base)

Cat. No. 257011	Ready-to-use Plated Media, cpu 20
Cat. No. 257456	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

For further information please contact your local BD representative.



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