BD™ Chocolate Agar with IsoVitaleX™ and Bacitracin • BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin

INTENDED USE

BD Chocolate Agar with IsoVitaleX and Bacitracin and **BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin** are selective media for the isolation of *Haemophilus influenzae* from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.
Although *Haemophilus* species are a normal part of the upper respiratory tract flora of man, certain serotypes of *H. influenzae*, such as b and f (and much rarer also c), are an important cause of meningitis, sepsis, epiglottitis and many other severe infections. Until the implementation of vaccination in many countries, *H. influenzae* was one of the three leading causes of bacterial meningitis worldwide.[1] On regular media with sheep blood agar, *H. influenzae* and certain other *Haemophilus* species are unable to grow since they need X (=heme) and V (=NAD) factors.
BD Chocolate Agar with IsoVitaleX and Bacitracin contains hemoglobin which supplies heme and **BD IsoVitaleX** which supplies NAD (=nicotinamide adenine dinucleotide) and other vitamins. General nutrients are provided by the GC II Chocolate Agar base.
In **BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin**, the general nutrients are supplied by Brain Heart Infusion Agar, and the horse blood supplies heme (X factor) and NAD (V factor) to support the growth of *H. influenzae*. Growth of this organism may be enhanced by placing V factor strips (e.g. **BD Taxo™** V factor strips) on the medium after its inoculation.

Bacitracin is added to both media to inhibit most of the normal flora whereas *H. influenzae* is resistant to this antimicrobial agent.[1-4]

BD Chocolate Agar with IsoVitaleX and Bacitracin and **BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin** are used in the isolation of *Haemophilus influenzae* from clinical specimens containing normal flora.

REAGENTS

Formulas* Per Liter Purified Water

BD Chocolate Agar with IsoVitaleX and Bacitracin		BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin	
Pancreatic Digest of Casein	7.5 g	Brain Heart, Infusion from Solids	8.0 g
Selected Meat Peptone	7.5	Peptic Digest of Animal Tissue	5.0
Corn Starch	1.0	Pancreatic Digest of Casein	16.0
Dipotassium Hydrogen Phosphate	4.0	Sodium Chloride	5.0
Potassium Dihydrogen Phosphate	1.0	Glucose	2.0
Sodium Chloride	5.0	Disodium Hydrogen Phosphate	2.5
Agar	14.0	Agar	13.5
Bacitracin	50,000 IU	Bacitracin	16,500 IU
Hemoglobin	10.0	Horse Blood, defibrinated	50.0 ml
IsoVitaleX	10.0 ml	pH 7.4 +/- 0.2	
pH 7.2 +/- 0.2			

*Adjusted and/or supplemented to meet the performance criteria.

BD IsoVitaleX Enrichment, included in **BD Chocolate Agar with IsoVitaleX and Bacitracin**, 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 for 24 to 48 hours in a aerobic atmosphere enriched with carbon dioxide.

Strains	**BD Chocolate Agar with IsoVitaleX and Bacitracin**	**BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin**
Haemophilus influenzae ATCC™ 10211	Acceptable to good growth; small transparent to grey colonies	Satisfactory growth; transparent, small colonies; good to excellent growth around V factors strips placed on the medium
Staphylococcus aureus ATCC 25923	Inhibition partial to complete	Inhibition partial to complete
Streptococcus pneumoniae ATCC 6305	Inhibition partial to complete	Inhibition partial to complete
Streptococcus pyogenes ATCC 19615	Inhibition partial to complete	Inhibition partial to complete
Streptococcus mitis ATCC 6249	Inhibition partial to complete	Inhibition partial to complete
Uninoculated	Chocolate brown, opaque	Red (blood color)

PROCEDURE

Materials Provided

BD Chocolate Agar with IsoVitaleX and Bacitracin or
BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin, both provided in 90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required; **BD Taxo™** V Factor strips (for **BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin** only).

Specimen Types

These media are intended to be used for the isolation of *Haemophilus influenzae* from respiratory tract specimens, e.g. throat swabs, or other specimens containing normal flora, e.g., conjunctival swabs (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Specimen Collection and Transport

Haemophilus influenzae is 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!

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. 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. A V-factor strip may be placed on **BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin** after inoculation for improved growth.
Incubate the plates for 24 to 48 hours in a moist environment. Although *H. influenzae* is not dependent on an elevated CO_2 concentration, the incubation in a **BD GasPak™** jar equipped with a **BD GasPak** carbon dioxide generator envelope may enhance the growth of the organism. For the isolation of all agents present in the specimen, **BD Chocolate Agar (GC Agar with IsoVitaleX)** or **BD Chocolate Agar (Blood Agar No. 2 Base)** should also be inoculated.

Results

BD Chocolate Agar with IsoVitaleX and Bacitracin: After the incubation, inspect plates for transparent to grey colonies with a diameter of less than 2 mm after 48 hours of incubation. *Haemophilus* species other than *H. influenzae* may also grow on this medium.

BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin: After the incubation, inspect plates for small, transparent colonies after 48 hours of incubation. Growth of *H. influenzae* is enhanced around the V-factor strip. Other *Haemophilus* species may grow without the V- factor supplementation. *H. influenzae* is non-hemolytic, *H. haemolyticus* is usually beta hemolytic.

Additional biochemical or serological tests are needed for a definitive identification of the isolates from these media.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

These media are used for the isolation of *Haemophilus influenzae* from clinical specimens from body sites that contain normal flora. These media must not be used for the primary isolation of *Haemophilus* species from cerebrospinal fluid or from specimens from other primary sterile body sites. Instead, use non-selective media such as **BD Chocolate Agar (GC Agar with IsoVitaleX)** or **BD Chocolate Agar (Blood Agar No. 2 Base)** for these specimens.

Although **BD Chocolate Agar with IsoVitaleX and Bacitracin** and **BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin** are selective, organisms other than *Haemophilus influenzae* might grow. The media inhibit most of the bacterial species of the oral flora, but are not completely inhibitory to oral *Neisseria* species, *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens* and to certain staphylococci.
Gram stains and further biochemical and serological tests are necessary to confirm the isolation of *H. influenzae* or other *Haemophilus* species.
Certain *Haemophilus* strains and species, including *H. influenzae* var. *aegyptius* (=*H. aegyptius*) and *H. ducreyi* are known to be inhibited by bacitracin and will therefore not grow on these media.
To obtain a full isolation of all pathogens involved, a nonselective medium such as **BD Chocolate Agar (GC Agar with IsoVitaleX)** or **BD Chocolate Agar (Blood Agar No. 2 Base)** must also be inoculated with the specimen.
Since *Haemophilus* species are a regular part of the respiratory tract flora, their presence in specimens from the upper respiratory tract such as throat swabs and sputa does not necessarily indicate that these organisms are the cause of an infection. Serological tests are required to demonstrate that the isolate belongs to serogroups often involved in infections.[1,2]

REFERENCES

1. Kilian, M. 2003. *Haemophilus*. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
2. Mannheim, W. et al.: *Pasteurellaceae*. *In:* Mikrobiologische Diagnostik (ed. F. Burkhardt). Thieme Verlag, Stuttgart 1992.
3. Vastine, D.W., C.R. Dawson, I. Hoshiwara, C. Yonega, T. Daghfous, and M. Messadi. 1974. Comparison of media for the isolation of *Haemophilus* species from cases of seasonal conjunctivitis associated with severe endemic trachoma. Appl. Microbiol. 28:688-690.
4. Chapin, K., and G.V. Doern. 1983. Selective media for recovery of *Haemophilus influenzae* from species contaminated with upper respiratory tract microbial flora. J. Clin. Microbiol. 17:1163-1165.

PACKAGING/AVAILABILITY

BD Chocolate Agar with IsoVitaleX and Bacitracin
Cat. No. 254046 Ready-to-use Plated Media, cpu 20

BD Brain Heart Infusion Agar with 5% Horse Blood and Bacitracin
Cat. No. 256182 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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