# **Brain Heart Agar**

A high-quality full culture medium to be used both for the cultivation of fastidious pathogens and as a base medium for the production of various special culture media.



In Vitro Diagnostic Medical Device –

For professional use only

CE

Version 06-01-2011 Merck KGaA, 64271 Darmstadt

## **General Information**

In 1919, ROSENOW described the isolation of difficult-to-cultivate bacteria from infections of the oral cavity using dextrose broth to which he added brain tissue. Brain-heart broth/agar is a modification of the medium described by ROSENOW in which the brain tissue has been replaced by brain extract and the calcium carbonate by di-sodium hydrogen phosphate (MacFADDIN, 1985; ATLAS, 1997). Brain-heart agar additionally contains agar-agar.

## Principle

Microbiological method.

#### **Mode of Action**

The culture medium is suitable for the cultivation of many fastidious bacteria such as streptococci, pneumococci and meningococci. Gonococci can be cultivated after the addition of ascites.

The culture substrate of brain-heart extract and peptones provides a broad spectrum of organic nitrogen compounds, carbon sources, sulfur, vitamins and trace elements. Glucose serves as an additional carbon and energy source. The pH is adjusted and stabilized by di-sodium hydrogen phosphate.

The nutrient supply is increased by the addition of blood (5 - 10%). Thus DOUGHERTY et al. (1996) successfully used brainheart blood/agar to isolate fastidious bacteria such as *Mycobacterium avium, Bartonella henselae* or *Cryptococcus neoformans* from the blood of AIDS patients.

Brain-heart agar is also described as a base culture medium for various selective media.

QUEIROZ et al. (1987) developed a selective medium for the detection of *Campylobacter pylori* on the basis of brain-heart agar (Belo Horizonte medium/BHM).

MacKENZIE et al. (2002) determined the antibiotic sensitivity of *Staphylococcus* isolates from blood cultures on brain-heart agar to which vancomycin or teicoplanin had been added.

The isolation of *Brachyspira aalborgi* from feces on brain-heartblood agar to which spectinomycin and polymyxin B had been added was described for the first time in 2003 (BROOKE et al.).

By adding 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan to brain-heart agar, *Pseudomonas aeruginosa* could be isolated from sputum, urine and feces (ARAJ, 1984).

Apart from its use in bacteriology, brain-heart agar is also suitable for the isolation of pathogenic fungi from clinical material such as specimens from eye infections, cerebrospinal fluid, blood, bone marrow, urine, secretions from the vagina and respiratory tract, as well as specimens from the upper respiratory tract such as ears, nose and mouth (ROBERTS et al., 1985). To See also General Instruction for Use "How to use Dehydrated Culture Media"

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inhibit the growth of accompanying microorganisms it is recommended to add a mixture of gentamycin (5 mg/l) and chloramphenicol (16 mg/l) or penicillin (20 mg/l) and streptomycin (40 mg/l) to brain-heart agar.

Alternatively, gentamycin can be added to brain-heart agar in a concentration of 50 mg/l without addition of further antibiotics.

Cycloheximide (0.5 mg/l), too, can be added to brain-heart agar, also in combination with the above-named antibiotics. As some pathogenic fungi are inhibited by cycloheximide, a parallel specimen should also always be tested on a medium without cycloheximide.

For the detection of highly fastidious fungi, a blood-containing brain-heart agar (5 - 10% sheep blood) should also be inoculated in parallel. This may contain the above-named antibiotics.

# Typical Composition (g/litre)

Culture substrate (brain-heart extract and peptones) 27.5, D(+)glucose 2.0, sodium chloride 5.0, di-sodium hydrogen phosphate 2.5, agar-agar 15.0.

#### Preparation

Completely dissolve 52 g of brain-heart agar in 1 liter of deionized water while heating in a steam pot, autoclave (15 minutes at  $121^{\circ}$ C), cool on a water bath to 45 - 50°C and pour plates.

With addition of blood (5% blood): homogeneously mix 95 ml of sterile base culture medium at 45 - 50°C with 5 ml of blood and pour plates.

The ready-to-use culture medium has a pH of 7.4  $\pm$  0.2 at 25°C.

The prepared plates with base culture medium are clear to slightly opalescent and yellow-brown. With the addition of blood they are light red and non-hemolytic.

## Specimen

e. g. Sputum, urine, feces or blood.

## **Application and Eveluation**

#### Application

Streak out the clinical specimens on the brain-heart agar directly after sampling using the surface method. Incubation temperatures and times depend on the intended application. Bacteria are normally detected after incubation at 35°C for 1 to 2 days. Fungal cultures are usually incubated at 30°C for up to 4 weeks. In the case of longer incubation times the plates must be protected against drying out.

#### Evaluation

Each growth counts as a positive result. The isolated colonies are subsequently identified by appropriate tests.

## **Analytical Specificity**

The base culture medium contains no inhibitors or dyes; a broad spectrum of bacteria and fungi grow on it. Differentiation is only possible on the basis of typical colony morphology.

Also after the addition of blood, differentiation is difficult due to the form of hemolysis, since hemolysis is inhibited by the glucose content.

Brain-heart agar becomes a selective agar by the addition of inhibitors (antibiotics).

Further tests for the identification of isolates are necessary.

#### Literature

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#### **Ordering Information**

Product	Ordering. No.	Pack size
Brain Heart Agar	1.13825.0500	500 g

#### **Quality control**

The base culture medium contains no inhibitors. A broad spectrum of bacteria and fungi grow on it. At an incubation temperature of 35°C the following bacteria grow within 24 hours; the fungi Candida albicans and Aspergillus niger grow at 28°C within 3 days.

Test strains	Inoculum	Recovery rate %		
	(CFU/ml)	(without blood)	(with blood)	
Escherichia coli ATCC 25922	10 <sup>3</sup> -10 <sup>5</sup>	>70	>70	
Staphylococcus aureus ATCC 25923	$10^3 - 10^5$	>70	>70	
Streptococcus pyogenes ATCC 12344	10 <sup>3</sup> -10 <sup>5</sup>	>70	>70	
Streptococcus pneumoniae ATCC 6301	10 <sup>3</sup> -10 <sup>5</sup>	>70	>70	
Enterococcus faecalis ATCC 19433	10 <sup>3</sup> -10 <sup>5</sup>	>70	>70	
Bacillus cereus ATCC 11778	10 <sup>3</sup> -10 <sup>5</sup>	>70	>70	
Candida albicans ATCC 10231	10 <sup>3</sup> -10 <sup>5</sup>	>70	>70	
Aspergillus brasiliensis, formely A. niger,		growth good/very good		

ATCC 16404









Lactobacillus acidophilus ATCC 4356

Staphylococcus aureus ATCC 25923

Streptococcus pneumoniae ATCC 6301

Streptococcus pyogenes ATCC 12344