

For Identification and Differentiation of Bifidobacterium species

HiCrome™ Bifidobacterium Agar

Recommended for the differentiation of *Bifidobacterium* and *Lactobacillus* species.

M1960

Composition **

Ingredients	Grams/Litre
Peptone special	23.00
Sodium chloride	5.00
M Protein powder#	5.00
Chromogenic mixture	10.48
Agar	16.00

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

#Equivalent to Milk Protein

Directions

Suspend 59.48 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

The genus *Bifidobacterium* is the third most numerous bacterial populations found in the human intestine after *Bacteroides* and *Eubacterium*. It is an anaerobic bacteria that makes up the gut microbial flora. It resides in the colon and have health benefits for their hosts. *Bifidobacteria* are also associated with lower incidences of allergies (1, 2). *Bifidobacterium* Agar is used for the cultivation and maintenance of *Bifidobacterium* species (3).

Peptone special provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride maintains osmotic balance. M Protein powder aids in detecting casein hydrolysis activity which is exhibited by *Bifidobacterium breve*. A halo zone is observed around the colony in case of casein hydrolysis. The indicator system in the chromogenic mixture helps in distinguishing between *Lactobacillus* and *Bifidobacterium* species. *Lactobacillus* species usually produce green colonies with opaque background. *Bifidobacterium infantis* produces dark blue to bluish green colonies. Agar serves as an solidifying agent.

Type of specimen

Clinical, Dairy : Milk & Milk products samples

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4, 5).

For Clinical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6, 7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

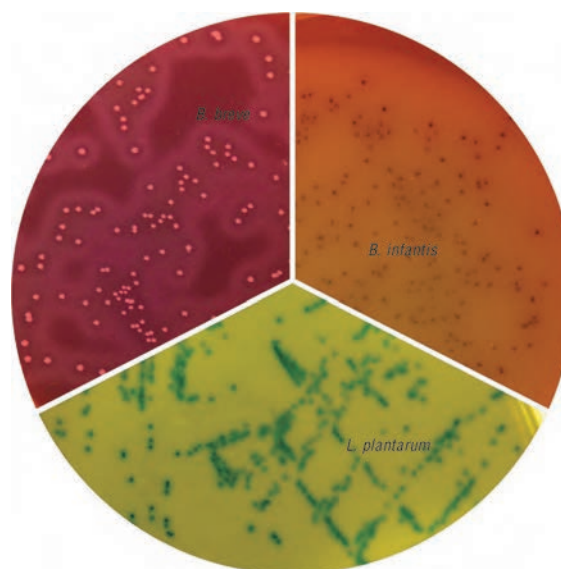
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. *Bifidobacterium* species are strict anaerobes, hence condition must be appropriately maintained

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.



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Quality Control

Appearance of Powder	: Cream to yellow homogeneous free flowing powder
Gelling	: Firm, comparable with 1.6% Agar gel
Colour and Clarity of prepared medium	: Reddish orange coloured clear to slightly opalescent gel forms in Petri plates
Reaction	: Reaction of 5.95% w/v aqueous solution at 25°C. pH :7.2±0.2
Cultural Response	: Cultural characteristics observed after an incubation at 35-37°C for 48 hours in an anaerobic conditions.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of the colony
<i>Bifidobacterium infantis</i> (25962)	50-100	good-luxuriant	≥50%	Dark blue - bluish green
<i>Bifidobacterium breve</i> (15698)	50-100	good-luxuriant	≥50%	Red-pink with halo zone
<i>Lactobacillus plantarum</i> (8014)	50-100	good-luxuriant	≥50%	Green colonies w/ hazy background
<i>Lactobacillus fermentum</i> (9338)	50-100	good-luxuriant	≥50%	Pink without halo zone

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

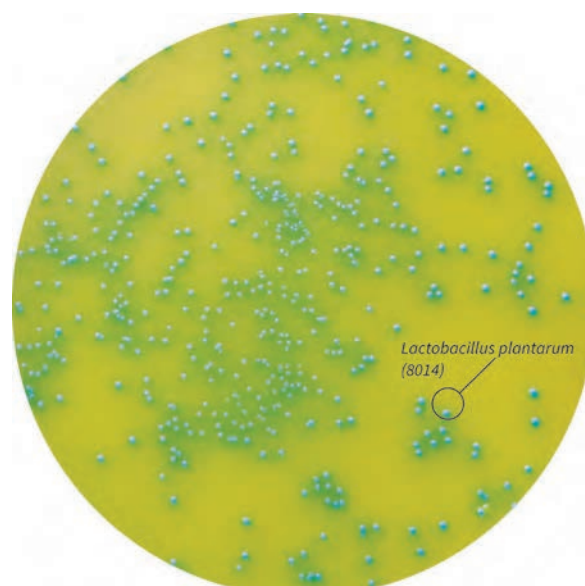
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6, 7).

References

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6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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