



BD™ Bifidobacterium Agar, Modified

INTENDED USE

BD Bifidobacterium Agar, Modified is a partially selective medium for the isolation of the *Bifidobacterium* flora in human stool specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Bifidobacterium species are Gram positive, anaerobic, branched or pleomorphic rods that can be isolated from a variety of materials such as human and animal feces, sewage and from the oral cavity. Their main habitat in humans is the large intestine where they are among the major groups of normal intestinal bacteria, reaching 10^9 to 10^{11} per gram of feces in healthy adults.¹ When the composition of the normal flora is disturbed by internal or external factors, e. g. by antimicrobial or antineoplastic therapy, they might be overgrown by *Enterobacteriaceae*, pseudomonads, or yeasts.¹⁻³ The overgrowth state may be responsible for chronic diarrhea and other intestinal and digestive disorders. Since their pathogenicity is low, bifidobacteria and lactobacilli are increasingly used as probiotics to improve the composition of the intestinal flora in case of disorders. Additionally, use of probiotics has been discussed to improve certain extraintestinal disorders or syndromes, e.g. vaginitis, *Helicobacter pylori* infection, and cystic fibrosis.³ Administration of bifidobacteria has recently been claimed to improve weight gain and intestinal abnormality in preterm infants.⁴

Several media have been devised for the elective or selective isolation of bifidobacteria.^{1,2,5-9}

Since the genus consists of more than 25 known species with a considerable heterogeneity in resistance to antimicrobial agents and other inhibitors, it is difficult to design a single medium with a good selectivity while maintaining a good recovery. In several investigations, it is claimed that Bifidobacterium Medium as described by Beerens^{1,5} allows good recovery of *Bifidobacterium* species present in the human intestinal tract. Recent studies show that most of the strains are recovered in higher counts than on comparable selective media for these bacteria.^{1,2}

Bifidobacterium Medium as described by Beerens is based on Columbia Agar base, supplemented with propionic acid, at pH 5.0.⁵ Propionic acid has been shown to inhibit fungi and many bacteria other than bifidobacteria, such as intestinal *Bacteroides* and *Enterobacteriaceae*. The low pH of the medium further contributes to inhibit other predominating organisms of human feces, such as *Bacteroides* and *Eubacterium* species. Cystein is a reducing agent. **BD Bifidobacterium Agar, Modified** is a slight modification of the original medium, supplemented with lactulose, a sugar used as a prebiotic that is preferably fermented by bifidobacteria. Glucose as a universal sugar has been added to accelerate initial growth. Riboflavin is a vitamin for many bifidobacteria.⁸ The pH has been slightly increased from 5.0 to 5.5 to improve gel strength of the agar and better growth of *Bifidobacterium*.

REAGENTS

BD Bifidobacterium Agar, modified

Formula* Per Liter Purified Water

| | |
|--------------------|--------|
| Columbia Agar Base | 42.5 g |
| Glucose | 2.5 |
| Lactulose | 2.5 |
| Cystein-HCl | 0.5 |
| Riboflavin | 0.01 |
| Propionic Acid | 5.0 ml |

pH 5.5 +/- 0.2

*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 anaerobically for 2 to 3 days at 35 to 37° C.

| Strains | Growth Results |
|---|--|
| <i>Bifidobacterium longum</i> DSM 20219 | Growth good to excellent; creamy white colonies, acid odor |
| <i>Bifidobacterium bifidum</i> DSM 20082 | Growth good to excellent; creamy white colonies, acid odor |
| <i>Bacteroides fragilis</i> ATCC™ 25285 | Inhibition (partial to) complete |
| <i>Lactobacillus acidophilus</i> ATCC 314 | Inhibition partial to complete |
| <i>Escherichia coli</i> ATCC 25922 | Inhibition (partial to) complete |
| Uninoculated | Light amber |

PROCEDURE

Materials Provided

BD Bifidobacterium Agar, Modified (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is used for the isolation and quantitative determination of *Bifidobacterium* species from stool specimens of patients that suffer from chronic diarrhea and other intestinal and digestive disorders (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Stool specimens (ideally 10 to 15 grams of stool) must not be older than 24 hours. The use of an anaerobic transport medium is recommended.

Test Procedure

Before use, **BD Bifidobacterium Agar, Modified** may be prerduced in an anaerobic atmosphere for at least 24 hours, kept at room temperature. This procedure may increase the viable counts of the organisms to be detected. The **BD GasPak™** Anaerobic system may be used for this purpose.

For the study of the fecal flora, fresh human stool specimens should be suspended in sterile saline or anaerobic saline (saline containing 0.1 g of cystein-HCl per liter), followed by tenfold dilutions in the same suspension medium. Samples of 20 to 50 µl of the highest dilutions (e.g. 10⁻⁴ to 10⁻⁷) should be pipetted onto **BD Bifidobacterium Agar, Modified** which is then spread-inoculated and incubated anaerobically, e.g., by using the **BD GasPak** Anaerobic system.

Other media (e.g., for the determination of total counts and possibly for detection of other bacterial groups, e.g. *Lactobacillus*, *Bacteroides*, *Clostridium*, *Enterobacteriaceae*) should also

be inoculated from the appropriate fecal dilutions and incubated according to the requirements of the media and the bacterial groups.

Results and Interpretation

After the incubation, plates are inspected for growth. Appropriate colonies of must be tested microscopically (Gram stains) for the presence of typical bifid, gram positive rods. Colonies may then be counted, and the number of colonies is then multiplied by the dilution factor of the sample to obtain the CFU per gram feces. Subcultures and biochemical tests must be performed for a final identification of the organisms isolated.

In the feces of healthy individuals, bifidobacteria shall be present in high counts while their absence or low counts may be a hint for intestinal disorder.^{1-3,5,9}

Reduced occurrence of bifidobacteria in normal flora does not imply treatment of patients with antimicrobial agents or medications other than probiotics unless specific infectious agents have been detected as the cause of the disorder.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

This medium is used for the determination of the *Bifidobacterium* flora in human feces.

The Beerens formulation of Bifidobacterium Agar and similar media have been found to be superior to media with a higher degree of selectivity for the isolation of bifidobacteria from the human gut.^{1,2,5,6}

There exist bifidobacteria that are extremely fastidious and do not grow sufficiently on this or other selective media. Therefore, a nonselective medium (e.g. **BD Schaedler Agar with Vitamin K1 and 5% Sheep Blood**) should be included.^{1,6,7}

Due to its low pH and the addition of propionic acid, **BD Bifidobacterium Agar, Modified** inhibits lactobacilli, *Eubacterium* species, clostridia, *Enterobacteriaceae* and many others.

Inhibition may be partial or complete, depending on the organisms. If undiluted human feces are inoculated, organisms other than *Bifidobacterium* may grow on this medium.

BD Bifidobacterium Agar, modified should not be used for the isolation of *Bifidobacterium* species from feces of species other than man.¹

REFERENCES

1. Hartemink, R., and F.M Rombouts. 1999. Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from faecal samples. J. Microbiol. Meth. 36: 181-192.
2. Hartemink, R. et al. 1996. Raffinose-Bifidobacterium (RB) agar, a new selective medium for bifidobacteria. J. Microbiol. Meth. 27: 33-43.
3. Gorbach, S.L. 2002. Probiotics in the third millenium. Dig. Liver Dis. 34 (suppl. 2):S2-S7.
4. Kitajima, H., et al. 1997. Early administration of *Bifidobacterium breve* to preterm infants: randomized controlled trial.. Arch. Dis. Child. Fetal Neonatal Med. 76: F101-F107.
5. Beerens, H. 1990. An elective and selective isolation medium for *Bifidobacterium* spp. Lett. Appl. Microbiol. 11: 155-157.
6. Dave, R.I., and N.P. Shah. 1995. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and bifidobacteria. J. Dairy Sci. 79: 1529-1536.
7. Munoa, F.J., and R. Pares. 1988. Selective medium for isolation and enumeration of *Bifidobacterium* species. Appl. Env. Microbiol. 54: 1715-1718.
8. Nebra, Y., and A.R. Blanch. 1999. A new selective medium for *Bifidobacterium* spp. Appl. Env. Microbiol. 65: 5173-5176.
9. Silvi, S. et al. 1996. An assessment of three selective media for bifidobacteria in faeces. J. Appl. Bacteriol. 81: 561-564.

PACKAGING/AVAILABILITY

BD Bifidobacterium Agar, Modified

Cat. No. 254546

Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.



BD Diagnostic Systems

Tullastrasse 8 – 12

D-69126 Heidelberg/Germany

Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16

Reception_Germany@europe.bd.com

BD Diagnostic Systems Europe

Becton Dickinson France SA

11 rue Aristide Bergès

38800 Le Pont de Claix/France

Tel: +33-476 68 3636 Fax: +33-476 68 3292 <http://www.bd.com>

BD, BD logo, Stacker and GasPak are trademarks of Becton, Dickinson and Company.

ATCC is a trademark of the American Type Culture Collection.

© 2003 Becton, Dickinson and Company