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. 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. 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. Administration of bifidobacteria has recently been claimed to improve weight gain and intestinal abnormality in preterm infants.

Several media have been devised for the elective or selective isolation of bifidobacteria. 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 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.

Bifidobacterium Medium as described by Beerens is based on Columbia Agar base, supplemented with propionic acid, at pH 5.0. 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. 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

<table>
<thead>
<tr>
<th>Formula* Per Liter Purified Water</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Columbia Agar Base</td>
<td>42.5 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactulose</td>
<td>2.5</td>
</tr>
<tr>
<td>Cystein-HCl</td>
<td>0.5</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.01</td>
</tr>
<tr>
<td>Propionic Acid</td>
<td>5.0 ml</td>
</tr>
</tbody>
</table>

pH 5.5 +/- 0.2

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

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.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium longum DSM</td>
<td>Growth good to excellent; creamy white colonies, acid odor</td>
</tr>
<tr>
<td>Bifidobacterium bifidum DSM</td>
<td>Growth good to excellent; creamy white colonies, acid odor</td>
</tr>
<tr>
<td>Bacteroides fragilis ATCC</td>
<td>Inhibition (partial to) complete</td>
</tr>
<tr>
<td>Lactobacillus acidophilus ATCC</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>Escherichia coli ATCC</td>
<td>Inhibition (partial to) complete</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>Light amber</td>
</tr>
</tbody>
</table>

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 prereduced 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

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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**


**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.

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