



BD™ LBS Agar (Lactobacillus Selection Agar)

INTENDED USE

BD LBS Agar (also known as Rogosa Agar) is a semi-defined, partially selective medium for the isolation and enumeration of lactobacilli from foods and from intestinal, vaginal, and dental flora.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Lactobacilli are among the predominant organisms of the intestinal tract and vaginal floras. 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 The overgrowth state in the intestinal tract may be responsible for chronic diarrhea and other intestinal and digestive disorders. Also, a reduction of the *Lactobacillus* flora has been found to be associated with vaginitis or vaginosis in premenopausal women.²⁻⁴ Since their pathogenicity is low, lactobacilli and bifidobacteria are increasingly used as probiotics to improve the composition of the normal flora in case of disorders such as acute and chronic diarrhea and vaginitis.^{5,6}

Rogosa et al. developed LBS Agar as a selective medium for the isolation and enumeration of oral and fecal lactobacilli.^{7,8} They reported that LBS Agar was more selective in preventing the overgrowth of moulds, streptococci and spreading organisms than the previously used tomato juice agar. **BD LBS Agar** is used for the isolation and enumeration of lactobacilli from foods, dairy products, and human intestinal, vaginal, and dental floras.^{9,10}

In **BD LBS Agar**, casein peptone, yeast extract, and ammonium salt provide nitrogen. Polysorbate 80 supplies fatty acids necessary for the growth of lactobacilli. Manganese and magnesium are growth factors. Glucose is a universal energy and carbon source. Ammonium citrate, sodium acetate, acetic acid and ferrous sulfate act as inhibitors of streptococci and other contaminating organisms and provide the low pH which is tolerated by lactobacilli but not by many other organisms. Phosphate, together with acetate and acetic acid, stabilizes the pH.

REAGENTS

BD LBS Agar (Lactobacillus Selection Agar)

Formula Per Liter Purified Water

Pancreatic Digest of Casein	10.0 g	Sodium Acetate Hydrate	25.0 g
Yeast Extract	5.0	Acetic Acid	1.3 ml
Potassium Dihydrogen Phosphate	6.0	Magnesium Sulfate	0.575 g
Ammonium Citrate	2.0	Manganese Sulfate	0.12
Glucose	20.0	Ferrous Sulfate	0.034
Polysorbate 80	1.0	Agar	15.0

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 the plates at 35 to 37° C in an anaerobic atmosphere for 2 to 3 days.

Strains	Growth Results
<i>Lactobacillus acidophilus</i> ATCC™ 4356	Growth good to excellent
<i>Lactobacillus plantarum</i> ATCC 8014	Growth good to excellent
<i>Proteus mirabilis</i> ATCC 43071	Inhibition complete
<i>Escherichia coli</i> ATCC 25922	Inhibition complete
Uninoculated	Clear, light amber to amber

PROCEDURE

Materials Provided

BD LBS Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This medium is used in studies on the presence of the *Lactobacillus* flora of patients that suffer from chronic diarrhea and other intestinal and digestive disorders [use stool specimens (ideally 10 to 15 grams of stool) that are not older than 24 hours] and for testing the presence of lactobacilli in vaginal and dental flora (use vaginal or dental swabs). (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). The medium is also used to determine the lactobacilli present in foods. The use of an anaerobic transport medium is recommended for all types of specimens and samples.

Test Procedure

For the study of the intestinal 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 LBS Agar** which is then spread-inoculated and incubated anaerobically, e.g., by using the **BD GasPak™** anaerobic system. The same procedure may be used for food samples.

If material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak for isolation from this inoculated area.

Other media (e.g., for the determination of total counts and possibly for detection of other bacterial groups, e.g., *Bacteroides*, *Clostridium*, *Enterobacteriaceae*) should also be inoculated and incubated according to the requirements of the media and the bacterial groups.

Optimal incubation times and temperature are 2 to 3 days at 35 to 37° C. A longer incubation of the medium should be avoided if subcultures of the isolates must be made because the viability of the colonies may decrease thereafter.

Results and Interpretation

On **BD LBS Agar**, lactobacilli appear as medium-sized to large, white colonies. If specimens or samples had been plated quantitatively, colonies may be counted, and the number of colonies is then multiplied by the dilution factor of the sample to obtain the CFU per gram specimen or material. Growth must be further differentiated by microscopic and biochemical tests.

In feces or vaginal specimens of healthy individuals, lactobacilli are present in high counts while their absence or low counts may be a hint for intestinal disorder or vaginitis, respectively.^{1,2,4} Reduced occurrence of lactobacilli 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 diarrhea or vaginitis.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD LBS Agar is a standard medium used for the isolation of *Lactobacillus* from human flora and from foods.^{1,7-10}

The presence and counts of lactobacilli in vaginal flora are age dependent; high counts are only expected in healthy, premenopausal women.²

BD LBS Agar must not be used as a maintenance media for lactobacilli.

The medium is not completely inhibitory to streptococci, enterococci, and lactococci. Gram stain and microscopy is an easy means for differentiation of lactobacilli from these Gram positive cocci.

Due to its high salt content, the medium is not suitable for the isolation of *Lactobacillus lactis* and *L. bulgaricus* which are usually isolated from dairy products.⁹

REFERENCES

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PACKAGING/AVAILABILITY

BD LBS Agar

Cat. No. 255011

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>

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