



BD™ Group B Streptococcus Differential Agar (Granada Medium)

INTENDED USE

BD Group B Streptococcus Differential Agar (Granada Medium) is used for the isolation and identification of *Streptococcus agalactiae* (Group B *Streptococcus*) from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Streptococcus agalactiae is a cause of severe newborn infections, including septicemia, meningitis, and multiple organ infections.¹⁻³ The newborn is infected from the mother who can asymptomatically carry the organism in the vaginal flora. The incidence is about 1.5 per 1000 live births, and the mortality is about 8.7%.³ It has been shown that appropriate detection of the agent in the vaginal flora of pregnant women followed by appropriate therapy of the newborn can significantly reduce the risk of infection.^{2,3} The standard isolation technique for the isolation from vaginal specimens is the use of blood agar or selective blood agar and detection of the characteristic beta hemolysis, followed by biochemical or serological identification.¹ Recent investigations have shown that also low numbers of *S. agalactiae* in the vaginal flora may pose risk to the newborn of getting infected. Therefore, enrichment techniques such as LIM Broth have been recommended.⁴ However, this enrichment medium is not totally selective for *S. agalactiae*, and other Gram positive organisms may as well be enriched by this method, possibly hiding *S. agalactiae*.

In the recent years, modifications of the Islam medium, described in 1977, have been tested for suitability to detect and isolate the organism.^{5,6} On these media, like on New Granada medium, which is a recent modification of Islam medium, beta-hemolytic strains of *S. agalactiae* produce orange to salmon colonies.^{7,8} The colony coloration is due to the organism's own pigment, granadaene, an ornithine rhamno-polyene.¹⁶

The pigmentation is very specific and does not occur with streptococci other than group B or other organisms. However, the stability of New Granada medium is limited.^{9,10}

BD Group B Streptococcus Differential Agar (Granada Medium), which is a modification of New Granada medium with improved stability and selectivity, Proteose Peptone no. 3 is a protein source and provides precursors necessary for pigment production and growth. Starch is a nutrient and acts as a stabilizer of the pigment. Glucose, pyruvate, and cysteine are nutrients. Magnesium is a trace element. The combination of MOPS and phosphate acts as a pH buffer. Crystal violet inhibits staphylococci. Inhibitors and inducers have been added to suppress the accompanying flora, such as Gram negative bacteria and strict anaerobes and to enhance pigment formation.

REAGENTS

BD Group B Streptococcus Differential Agar (Granada Medium)

Formula* Per Liter Purified Water

Proteose Peptone No. 3	25.0 g	Disodium Hydrogen Phosphate	10.7 g
Corn Starch	14.0	Crystal Violet	0.0005
Glucose	2.5	Inhibitors and Inducers	0.021
Pyruvic Acid Sodium Salt	1.0	Agar	15.2
Cysteine Hydrochloride	0.1		
Magnesium Sulfate	0.3		
MOPS (3-Morpholinopropane sulfonic acid), Hemisodium Salt	11.0		

pH 7.4 ± 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 18 to 24 hours at 35 ± 2° C.

Strains	Growth Results
<i>Streptococcus agalactiae</i> ATCC 12386	Growth good to excellent, small to medium-sized orange colonies with or without colorless borders
<i>Streptococcus pyogenes</i> ATCC 19615	Growth; very small colorless to grey colonies
<i>Enterococcus faecalis</i> ATCC 29212	Growth; medium-sized colorless to grey colonies
<i>Staphylococcus aureus</i> ATCC 25923	Complete inhibition
<i>Bacteroides fragilis</i> ATCC 25285	Partial to complete inhibition
<i>Proteus mirabilis</i> ATCC 43071	Partial to complete inhibition, swarming completely inhibited, colorless colonies
Uninoculated	White to light grayish, opaque

PROCEDURE

Materials Provided

BD Group B Streptococcus Differential Agar (Granada Medium) (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

BD Group B Streptococcus Differential Agar (Granada Medium) can be used for the isolation of *S. agalactiae* (group B streptococci) from all types of human clinical specimens. Frequent specimens include swabs from the female genital tract or swabs and other specimens from newborns. Apply appropriate techniques for specimen collection and transport.^{1,11}

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory onto **BD Group B Streptococcus Differential Agar (Granada Medium)**. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.

Alternatively, 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.

Incubate anaerobically for 18 to 24 hours at 35 ± 2° C. If negative, plates may be incubated for additional 18 to 24 hours, although this is usually not necessary. In order to recover all pathogens involved in an infection or colonization, the specimen must also be streaked onto a blood agar plate, e.g. **BD Columbia Agar with 5% Sheep Blood** which should be incubated in a CO₂ enriched atmosphere for 18 to 48 hours at 35 ± 2° C. If liquid pre-enrichment media such as Lim Broth are used, they may be subcultured onto **BD Group B Streptococcus Differential Agar (Granada Medium)** with a loopful of the broth after 18 to 24 hours incubation and incubated as described above.

Results

After incubation, beta-hemolytic strains of *S. agalactiae* will produce small to medium-sized, pale to strong orange, or salmon-orange colored colonies which may or may not be surrounded by colorless borders. In order to detect weakly pigmented strains, the plates must be read on a white surface. Any intensity of orange pigmentation is considered positive. Do not hold plates in front of a light source for reading! Staphylococci, Gram negative rods, and strict anaerobes will usually be completely inhibited on the medium. Most other streptococci and enterococci will grow without inhibition, but will produce colorless to gray colonies. Nonhemolytic strains of *S. agalactiae* will also produce gray to gray-blue colonies. Their occurrence is rare (up to 4% in pregnant women).

To differentiate nonhemolytic B streptococci from enterococci or non-group B streptococci, a PYR test (use **BD DrySlide PYR kit, cat. no. 231747**) can be performed directly from gray to gray-blue colonies on **BD Group B Streptococcus Differential Agar (Granada Medium)**: enterococci, *Streptococcus pyogenes* and several other streptococci are PYR positive (=red to purple color within 1 min), while *S. agalactiae* and a variety of other streptococci are PYR negative (=yellowish to colorless).¹⁵ Isolates with PYR-negative results from gray or gray-blue colonies must be further tested to confirm the presence of a nonhemolytic *S. agalactiae* strain, e.g. by serological typing.

The orange pigmentation on this medium is very specific for *S. agalactiae*, and serological or biochemical identification is not necessary for confirmation. However, serological typing may be performed directly from **BD Group B Streptococcus Differential Agar (Granada Medium)** without further subculture. The **BBL™ Streptocard™ Enzyme Latex Test Kit** (cat. no. 240950) may be used for this purpose.

Also, the **BD Columbia Agar with 5% Sheep Blood** plate must be inspected for the presence of nonhemolytic strains of *S. agalactiae* and of additional pathogens.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Group B Streptococcus Differential Agar (Granada Medium) is used for the isolation and identification of *Streptococcus agalactiae* (Group B streptococci) from all types of human clinical specimens. The orange colony coloration on this medium is highly specific for *S. agalactiae* (see **Performance Characteristics**); therefore, confirmatory tests are not necessary. In case of doubt or with weakly pigmented strains, serological grouping and/or a PYR test should be performed directly from the isolation plate.

It has been reported that the genes responsible for the pigment production and for the production of the hemolysin of *S. agalactiae* are genetically linked.^{12,13} About 1 to 2% of *S. agalactiae* strains are nonhemolytic¹⁴ and, therefore, may not be pigmented on **BD Group B Streptococcus Differential Agar (Granada Medium)**. However, it has been determined internally that weak hemolysin producers that appear nonhemolytic on most blood agar media may still be weak pigment producers. The hemolysin has been reported to be one of the major pathogenicity factors of *S. agalactiae*.^{12,13}

In order to detect all pathogens involved in an infection, including nonhemolytic strains of B streptococci, a blood agar medium, such as **BD Columbia Agar with 5% Sheep Blood** must also be inoculated with the specimen.

BD Group B Streptococcus Differential Agar (Granada Medium) is not suitable for the isolation of streptococci other than *S. agalactiae* or other pathogens that may produce similar infections (e.g. *Listeria monocytogenes*).

Performance of this medium with veterinary specimens has not been determined.

Performance Characteristics

A performance evaluation was done with 151 clinical specimens (vaginal and cervical swabs, various specimens from newborns) determined positive for *S. agalactiae* by standard plating onto a Blood Agar plate followed by serological typing of the isolates.¹⁵ Of these 151 specimens, 148 were positive upon second culture on **BD Columbia Agar with 5% Sheep Blood** and yielded one nonhemolytic and 147 beta-hemolytic isolates.

On **BD Group B Streptococcus Differential Agar (Granada Medium)**, incubated anaerobically for 18 to 24 hours, 149 of the 151 specimens yielded growth of B streptococci; of these, 148 yielded any type of orange colonies (sensitivity 98%) which were all identified serologically as B streptococci. Of these 148 cultures, three were scored "very pale orange". If these three cultures are subtracted, 96% sensitivity is obtained.

In this study, inoculated plates of **BD Group B Streptococcus Differential Agar (Granada Medium)** were also incubated in an aerobic atmosphere enriched with carbon dioxide. Under these incubation conditions, the sensitivity was 96.8%. On these plates, 14 cultures were scored "very pale orange". If these are subtracted, sensitivity is 87.4%.

When incubated anaerobically, much more isolates with a strong orange colony coloration were obtained than after the incubation in an aerobic atmosphere enriched with carbon dioxide ($P < 0.005$). Additionally, colonies of many strains were larger when incubated anaerobically.

Therefore, the anaerobic incubation significantly improves the detection of orange colonies.

In this evaluation, 52 specimens previously determined negative for *S. agalactiae* were also included. No false positives were obtained when cultured on **BD Group B Streptococcus Differential Agar (Granada Medium)** (specificity = 100%).

The use of **BD Group B Streptococcus Differential Agar (Granada Medium)** improves and accelerates the diagnosis of B streptococci and reduces costs and turnaround time since further identification tests from orange colonies are unnecessary.

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PACKAGING/AVAILABILITY

BD Group B Streptococcus Differential Agar (Granada Medium)

REF 257079 Ready-to-use plated media, 20 plates

FURTHER INFORMATION

For further information please contact your local BD representative.



Becton Dickinson GmbH

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

<http://www.bd.com>

<http://www.bd.com/europe/regulatory/>

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