

# BD™ Group B Streptococcus Differential Agar (Granada Medium)

For the isolation and identification of *Streptococcus agalactiae* (Group B *Streptococcus*)

## Reference to Reading the Plates

BD Group B Streptococcus Differential Agar (Granada Medium) - catalog number 257079 - is a modification of New Granada Medium with improved stability and selectivity.

### Incubation

Incubate anaerobically for 18 to 24 hours at  $35 \pm 2^\circ \text{C}$ .

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 specimens must also be streaked onto a blood agar plate, e.g. BD Columbia Agar with 5% Sheep Blood plate which should be incubated in a  $\text{CO}_2$  enriched atmosphere for 18 to 48 hours at  $35 \pm 2^\circ \text{C}$ .

The blood plate must be inspected for the presence of nonhemolytic strains of *S. agalactiae* and of additional pathogens. 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.



*S. agalactiae*: medium sized orange colonies



*S. agalactiae*: small orange colonies



*S. agalactiae*: pale orange colored colonies



Gray to gray-blue colonies, produced by non-beta hemolytic *S. agalactiae* and by *Enterococcus*

### Examples how to read the plates

- Plates must be read on a white surface in order to detect weakly pigmented strains.
- Do not hold plates in front of a light source for reading!
- Beta-hemolytic strains of *S. agalactiae* will appear orange on the BD™ Group B Streptococcus Differential Agar
- Any intensity of orange pigmentation is considered positive, such as pale to strong orange, or salmon-orange colored.
- Pigmentation may or may not be surrounded by colorless borders.
- Colonies are small to medium-sized
- Staphylococci, Gram negative rods, and strict anaerobes will usually be completely inhibited on the medium.
- Other streptococci and enterococci will grow without inhibition, but will produce colorless to gray or gray-blue colonies.
- Occasional strains of *S. agalactiae* that are nonhemolytic, will grow on the medium but will not appear as orange colonies.



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The orange colony coloration is due to the organism's own pigment. The pigmentation on this medium is very specific for *S. agalactiae*, and does not occur with streptococci other than group B or other organisms. Therefore, 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.

Nonhemolytic strains of *S. agalactiae* 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 which all may produce gray or gray-blue colonies, a PYR test (use BD DrySlide PYR kit, cat. no. 231747) can be performed directly from BD Group B Streptococcus Differential Agar (Granada Medium).

A negative test (=yellow or colorless) indicates the probable presence of a *S. agalactiae* strain. Such isolates should be confirmed by biochemical tests or by serological typing (see picture). A red to pink coloration (=PYR positive) indicates the presence of *Enterococcus spp.* or *Streptococcus pyogenes*.

The BBL™ Streptocard™ Enzyme Latex Test Kit (cat. no. 240950) may be used for serological testing (see picture).



BD DrySlide PYR kit, cat. no. 231747  
*Enterococcus* (=PYR positive): left top test field; *S. agalactiae* (=PYR negative): right bottom test field (remaining fields not inoculated)



The BBL™ Streptocard™ Enzyme Latex Test Kit cat. no. 240950



BD™ Group B Streptococcus Differential Agar (Granada Medium) cat. no. 257079 Intensification of orange pigment of *S. agalactiae* under a cover slide incubated in a CO<sub>2</sub> enriched aerobic atmosphere

Incubation of BD Group B Streptococcus Differential Agar (Granada Medium) in an aerobic atmosphere enriched with carbon dioxide results in weaker formation of the orange pigment and, possibly, in the loss of weak pigment producers and is therefore not recommended. Additionally, colonies of many strains are larger when incubated anaerobically. Therefore, the anaerobic incubation significantly improves the detection of orange colonies.

**Cover slide method:** This method allows incubation of inoculated plates without using an anaerobic atmosphere. The medium is inoculated as usual, and directly afterwards a cover slide is placed on the agar surface, preferably in the first or second streak area. Gently press the slide on the agar by means of a loop or forceps. Afterwards, incubate the plates in a CO<sub>2</sub> enriched aerobic atmosphere for 18 to 24 hours at 35-37°C. If *B-streptococci* are present, they will grow on the medium and will produce light orange colonies. Under the cover slide, however, anaerobic conditions occur which lead to an intensification of the orange pigment, thus indicating the presence of *S. agalactiae* on the plate. Note that even with this method, nonhemolytic strains of *S. agalactiae* will not produce orange colonies.

*This reference sheet does not substitute the 'Instruction for Use' or any of its content.*



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