GRANADA MEDIUM

Cat. no. G123	Granada Medium, 15x100mm Plate, 19ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Granada Medium is used for the primary isolation and screening of beta-hemolytic group B streptococci (*Streptococcus agalactiae*) from clinical specimens.

SUMMARY

Approximately 10-35% of women are asymptomatic carriers of group B streptococci (GBS) in the genital and gastrointestinal tracts. [1] GBS remains a leading cause of serious illness and death in newborn populations, and therefore, the detection of GBS in the vaginal-anorectal area is critical to the prevention of neonatal GBS disease. Several surveys have been conducted that show the incidence of neonatal sepsis and meningitis due to GBS is currently 0.5-3 cases per 1,000 live births, although there are substantial geographical and racial differences. [2] The case-fatality ratios are now declining due to prompt recognition and proper treatment. [3]

The Center of Disease Control (CDC) recommends the screening of all pregnant women for vaginal and rectal GBS colonization between 35 and 37 weeks of gestation using an enrichment broth followed by subculture to a Blood Agar plate (Cat. no. A10) or other appropriate media. [4] Although widely utilized and considered the gold-standard method, alternative methods have emerged with the goal of improving sensitivity and specificity while reducing the incubation time and need for additional plated media. [5,7,9-10] More recently, chromogenic agars that undergo color change in the presence of beta-hemolytic colonies of GBS have become available. As with pigmented enrichment broths, these chromogenic agars can facilitate detection of beta-hemolytic GBS, but the majority will not detect non-hemolytic strains. These non-hemolytic strains can be further tested using Hardy Diagnostics GBS Detect™ Agar plate (Cat. No. A300)

Production of an orange carotenoid pigment on Granada medium is unique to the beta-hemolytic group B streptococci isolated from humans. [5] The use of Granada Medium as a primary isolation medium, can enable the detection of those organisms that possess the ability to produce pigment. Confirmation of colonies can be achieved by using latex agglutination test methods. [6,7] There is no significant difference in the recovery of group B streptococci when compared to Selective Columbia Blood Agar, or procedures using LIM or Todd-Hewitt Broth, however, the pigmented colonies can be immediately recognized. [5,7,9] These features make Granada Medium a highly sensitive, accurate, and faster method of detecting beta-hemolytic group B streptococci.

Granada Medium contains peptones, starch, and horse serum which provide nutrients necessary for growth of bacteria. The medium contains MOPS and sodium phosphate as buffers. Methotrexate enhances the pigment production of group B streptococci. Dextrose and sodium pyruvate prevent the formation of inhibitory compounds in the media. The selective agents present in the media are metronidazole (to inhibit gram-negative anaerobes), colistin sulfate (gram-negatives), and crystal violet (gram-positives). Agar is used as the solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

Peptone	25.0gm
Soluble Starch	20.0gm
Morpholinepropanesulfonic acid (MOPS)	11.0gm
Disodium Phosphate	8.5gm
Dextrose	2.5gm
Sodium Pyruvate	1.0gm
Metronidazole	1.0gm
Magnesium Sulfate	20.0mg
Methotrexate	6.0mg
Colistin Sulfate	5.0mg
Crystal Violet	0.2mg
Horse Serum	50.0ml
Agar	11.0gm

Final pH 7.45 +/- 0.1 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store media at 2-8ºC. away from direct light. Media should not be used if there are any signs deterioration, discoloration, contamination, or if the expiration date has passed. Product is extremely light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

Always store media in a refrigerated atmosphere. Do not use media after the expiration date, sensitivity is not optimal after expiration date or if inadequately stored.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious

agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

1. Collect vaginal or rectal or other suspect specimen following the appropriate guidelines for swab sample collection.

2. Submit to laboratory in Amies, Stuart's or other appropriate transport media without delay. If the specimen is to be delayed more that 24 hours, it must be refrigerated a 2-6ºC. [12]

3. Streak a plate of Granada Medium with the swab used to collect the sample.

Note: Follow the anaerobic incubation instructions (4a) or the aerobic incubation instructions (4b). Both methods yield similar results with regard to sensitivity and recovery of group B streptococci.

4a. Incubate the inoculated Granada Medium plate in an anaerobic atmosphere for at least 18 hours at 35ºC.

-OR-

4b. Flame-sterilize and cool a glass cover slide and place it over the inoculum on the Granada Medium plate. Incubate aerobically for at least 18 hours at 35ºC.

5. Examine plates for orange or red pigmented colonies typical of group B streptococci.

6. Negative colonies (not orange) can be further tested on GBS Detect™ Agar plates (Cat. No. A300) to determine if they are non-hemolytic strains of *S. agalactiae* ATCC ® 13813.

Consult appropriate references for more information regarding specimen collection and handling. [1-4,6,7]

INTERPRETATION OF RESULTS

On Granada Medium, colonies of beta-hemolytic group B streptococci appear red to orange in color. The pigment makes the colonies readily distinguishable from other organisms that may be growing on the plate. Any degree of orange development would be considered a positive result.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Granada Medium was found to be 89% to 91% sensitive, which was of comparable sensitivity to Selective Columbia Blood Agar. [7] Non-hemolytic group B streptococci are infrequently (approximately 4%) found in clinical specimens. These strains do not produce the orange pigment and should be identified by other techniques (e.g. antigen detection by latex agglutination (Cat. No. PL032HD), camp test, or GBS Detect Agar (Cat. No. A300)). For this reason, do not use the non-hemolytic strain, *S. agalactiae* ATCC ® 13813, for quality control purposes because it will not produce the characteristic orange pigment.

For pigment development, Granada Medium must be incubated anaerobically or aerobically with a cover slide covering a portion of the inoculum. Plates incubated aerobically may yield false-negative results.

Repeat quality control testing is recommended if there is any question about the performance of the media or the duration of the shelf life.

Granada Medium is not completely selective for *S. agalactiae* . Other bacteria can grow, mainly from rectal specimens (e.g. enterococci), but do not produce orange or red colonies after 18-48 hours.

Granada Medium plates are very heat labile and must remain refrigerated and kept away from light. Warm only those plates that will be used the same day.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
*Streptococcus agalactiae*** ATCC ® 12386	A	18-24hr	35°C	**	Growth; orange to red pigmented colonies
Streptococcus pyogenes ATCC ® 19615	A	18-24hr	35°C	**	Growth; white to off-white colonies
Escherichia coli ATCC ® 25922	B	18-24hr	35°C	**	Inhibited; no orange to red pigment
Bacteroides fragilis ATCC ® 25285	B	18-24hr	35°C	**	Inhibited; no orange to red pigment

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** See "Procedure" section for information regarding incubation/atmosphere conditions.

*** Do not use *Streptococcus agalactiae* ATCC ® 13813 for quality control purposes. This organism is non-hemolytic and will not produce the characteristic orange pigment.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Granada Medium should appear slightly opalescent to opalescent, with a flocculent precipitate, and light tan in color.

Streptococcus agalactiae (ATCC ® 12386) colonies growing on Granada Medium (Cat. no. G123). Incubated anaerobically for 24 hours at 35ºC.

Streptococcus pyogenes (ATCC ® 19615) growth inhibited on Granada Medium (Cat. no. G123). Incubated anaerobically for 24 hours at 35ºC.

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