IFU

Instructions for Use

GRANADA MEDIUM

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

INTENDED USE

Granada Medium is a selective and differential agar which is intended for the qualitative detection of Group B Streptococcus (GBS) from LIM Broth enrichment cultures of vaginal/rectal swabs from antepartum women following 18-24 hours of incubation.

Recovery of orange colored colonies on Granada Medium is a positive result for presence of β-hemolytic GBS. Results can be interpreted after18-24 hours of anaerobic incubation. Due to the properties of Granada Medium, white colonies recovered on Granada Medium must undergo additional testing to confirm absence of GBS. Subculture of GBS colonies must be performed for conducting susceptibility testing as recommended for penicillin- allergic women. A lack of growth or the absence of orange colonies on Granada Medium does not preclude the presence of GBS. Granada Medium is not intended to diagnose infection, or to guide or monitor treatment for infections.

SUMMARY AND PRINCIPLES

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 Centers for Disease Control and Prevention (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 (Cat. no. A10) plate 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.[5,7,8-9]

More recently, selective and differential agars that undergo color change in the presence of beta-hemolytic colonies of GBS have become available. As with pigmented enrichment broths, such as Carrot Broth (Cat. no. Z40) these agars can facilitate the detection of beta hemolytic GBS, but may have difficulty in detecting non-hemolytic strains. The production of light orange to orange to red-orange pigment on Granada Medium is a unique characteristic of beta-hemolytic GBS.[5] Confirmation of colonies can be achieved by using latex agglutination test methods (Cat. no. PL030HD).[6,7]

There is no significant difference in the recovery of group B streptococci when compared to blood agar, however, the pigmented colonies can be immediately recognized.[5, 7, 8] This feature makes Granada Medium a highly sensitive, accurate, and faster method of detecting beta-hemolytic group B streptococci.

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
Magnesium Sulfate	20.0mg
Crystal Violet	0.2mg
Inhibitors and Inducers	24.0mg
Agar	11.0gm

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

Final pH 7.45 +/- 0.1 at 25°C

PHYSICAL APPEARANCE

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

STORAGE AND SHELF LIFE

Storage: Product is temperature sensitive. Upon receipt store at 2-8°C until use; protect from light, excessive heat, moisture, and freezing. Media should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed.

Product is extremely light sensitive: protect against damage from excessive illumination and store away from any direct light source.

Do not use media after the expiration date. Sensitivity is not optimal after expiration date or if the product has been stored inadequately.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed.

Refer to the document "Storage" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as 10µL inoculating loops, specimen transport materials, other culture media (LIM Broth, Cat. no. L57), swabs, incubators, etc., as well as serological and biochemical reagents are not provided.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.

Federal law restricts this device to sale by or on the order of a licensed practitioner.

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 handled observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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.

PROCEDURE

Clinical Procedure

Specimen Transport and Storage:

Please refer to CDC, or other appropriate, guidelines for transport and storage conditions for LIM Broth (Cat. No. L57) enrichments of vaginal/rectal swab specimens. [4] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated into an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection and storage.[2-5]

Method of Use:

1. After an overnight incubation of vaginal/rectal swab specimen in LIM Broth (Cat. no. L57), subculture the enriched LIM Broth to a Granada Medium plate using a 10µL loop. Use a four-quadrant streak for isolation.
2. Incubate the Granada Medium for 18-24 hours at 35°C in an anaerobic environment.
3. Examine the Granada Medium plates for orange colonies typical of Group B Streptococci.

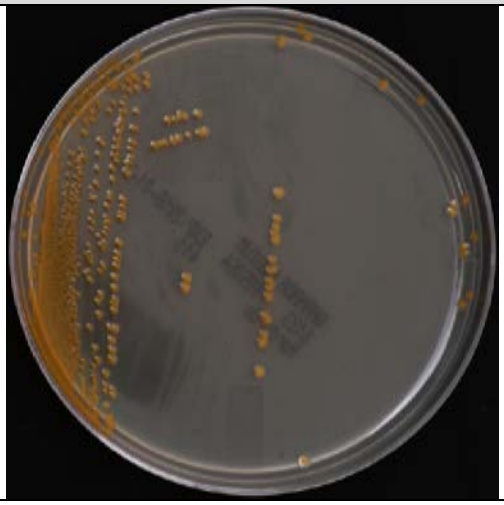
INTERPRETATION OF RESULTS

Growth of beta-hemolytic Group B Streptococci on Granada Medium results in the development of a light orange to orange to red-orange colored colonies within 18 to 24 hours. Any degree of orange color development would be considered a positive result.

18-24 hour Incubation	Interpretation/Recommended Action
Light to dark orange colonies	Positive – GBS (β-hemolytic Group B *Streptococcus* detected).
White colonies*	Presumptive Negative-Additional testing to rule-out presence of weakly β-hemolytic or non-hemolytic GBS.
Colonies that are not light to dark orange colonies or white colonies	Negative – No GBS (β-hemolytic Group B *Streptococcus* and non-hemolytic GBS not detected.
No growth	Negative –No GBS detected

*Non-hemolytic Group B *Streptococcus* represent a small percentage of GBS and produce white colonies on Granada Medium.

VARIATION IN COLOR DEVELOPMENT

	Light Orange	**Orange**
Color		

Note: Color-blind individuals may encounter difficulty in distinguishing color differences on Granada Medium.

LIMITATIONS

1. Do not incubate plates in a CO_2 or ambient air atmosphere.

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

3. Although rare, a small percentage of GBS may not produce beta-hemolysis. GBS detection on Granada Medium is only possible with beta-hemolytic colonies on a Blood Agar plate. Beta-hemolytic, pigment producing GBS occurs with 95.3-99.5% of all GBS strains isolated from clinical specimens. [10-12] Non-hemolytic GBS will result in white colonies. Additional testing (e.g. biochemical testing) is required for white colonies that appear on Granada Medium to rule-out the presence of non-hemolytic GBS.

4. Clinical testing of Granada Medium has shown that *Streptococcus salivarius* subsp. *salivarius* may be recovered as yellow to pale orange colored colonies. However, analytical testing of Granada Medium has shown recovery of white colonies for ATCC strains of *Streptococcus salivarius* subsp. *salivarius* and *S. salivarius* subsp. *thermophilus*.

5. Subculture to non-selective media should be performed as needed for susceptibility testing or typing.

6. Color-blind individuals may encounter difficulty in distinguishing color differences in Granada Medium

7. Storage of vaginal/rectal specimens at room temperature is not recommended over 24 hours as this significantly impacts the recovery of GBS from LIM enrichment.

8. GBS serotypes VII, VIII, and IX have not been evaluated with the Granada Medium.

9. The clinical performance of Granada Medium has not been established with swab transport systems other than those referenced in the clinical studies.

10. Analytical testing of Granada Medium showed that *Enterococcus faecalis* inhibited recovery of GBS at high concentrations. When *E. faecalis* concentration was reduced to $1.5x10^6$ CFU/mL, target GBS was recovered.

11. Analytical testing of Granada Medium showed that colony size, but not recovery, of GBS was affected by *E. faecalis* (ATCC 51299), *E. avium* (ATCC 14025), *E. gallinarum* (ATCC 49573), *E. saccharolyticus* (ATCC 43076), *Lactococcus lactis* (ATCC 19435), *Morganella Morganii* (ATCC 25830), *Proteus mirabilis* (ATCC 43071), and *Serratia marcescens* (ATCC 13880) when present at a high concentration ($1.5x10^8$ CFU/mL). When the concentration of these organisms was reduced to $1.5x10^7$ CFU/mL, colony size of target GBS was as expected.

12. Analytical testing of Granada Medium showed that colony size and color, but not recovery, of GBS was affected by *Vibrio parahaemolyticus* (ATCC 17802) when present at a high concentration ($1.5x10^8$ CFU/mL). GBS colonies appeared as light orange in the presence of high concentrations of *V. parahaemolyticus*. When *V. parahaemolyticus* concentration was reduced to $1.5x10^7$ CFU/mL, colony morphology of target GBS was a darker orange, as expected.

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

EXPECTED VALUES

In the prospective clinical evaluation described below, the overall prevalence of Group B Streptococci by reference method was 21.1% (163/771). Of these, 4.3% (7/163) were non-hemolytic Group B Streptococci.

PERFORMANCE CHARACTERISTICS

The performance of Granada Medium was evaluated at four geographically diverse hospitals with routine GBS specimen in the form of vaginal/rectal swabs. In Table 1, the detection of Group B Streptococci (β-hemolytic GBS and non-hemolytic GBS) by the reference method was compared to the recovery of orange colonies on Granada Medium. In Table 2, the detection of β-hemolytic GBS by the reference method was compared to the recovery of orange colonies on Granada Medium. The reference method was defined as the selective enrichment of specimen in LIM Broth followed by subculture to blood agar. Organisms that grew on Granada Medium or blood agar were confirmed to be Group B Streptococci by looking at hemolytic reaction on blood agar, gram-stain, catalase test, and StrepPRO™ latex agglutination. For the clinical study, vaginal/rectal swab specimens were collected in ESwab™ Liquid Amies, sponge based Liquid Amies (ex: Cat. no. 4140BX), and Sponge based Stuart's liquid (ex: Cat. no. 4432BX).

All discrepant isolates were frozen in CryoSavers™ with Brucella Broth and returned to Hardy Diagnostics for testing. As part of discrepant analysis, the identity of each isolate was confirmed (β Group B Streptococci, non-hemolytic (NH) Group B Streptococci, or non-Group B Streptococci). Once the identity was confirmed, positive organisms (β Group B Streptococci or NH Group B Streptococci) were tested at $1.5x10^2$ CFU/mL in donated negative-vaginal/rectal matrix (equivalent to an inoculum of 4.5 CFU (1 - 9 CFU tested) and evaluated for their recovery from LIM Broth reference method and color development on Granada Medium.

A total of 884 specimens were tested against routine culture, 113 specimens did not meet enrollment criteria, and were therefore excluded from the analysis. Of the remaining 771 valid samples tested, a total of 154 specimens showed orange color development on Granada Medium after 18-24 hours of incubation at 35°C that were positive for Group B Streptococci by the LIM reference method. Results are shown in Table 1.

Table 1. LIM Reference Method vs. Granada Medium Color reaction

Site	TP	FP[1]	FN[2]	TN	Sensitivity	95% CI		Specificity	95% CI	
1	46	1	5	141	**90.2**	79.0	95.7	**99.3**	96.1	99.9
2	41	7	2	133	**95.3**	84.5	98.7	**95.0**	90.0	97.6
3	27	1	1	82	**96.4**	82.3	99.4	**98.8**	93.5	99.8
4	40	3	1	240	**97.6**	87.4	99.6	**98.8**	96.4	99.6
Overall	**154**	**12**	**9**	**596**	**94.5**	**89.8**	**97.1**	**98.0**	**96.6**	**98.9**

[1]There were 12 False Positives observed after 18 to 24 hours of incubation. All isolates were re-tested and confirmed by the discrepant analysis protocol above. Ten isolates recovered from Granada Medium were confirmed to be β-Group B Streptococci. Two isolates were recorded as pale orange on Granada Medium, but were not identified as Group B Strep. During discrepant analysis, both isolates were confirmed to be *Streptococcus salivarius* subsp. *salivarius*. One *Streptococcus salivarius* subs. *salivarious* isolate exhibited yellow colonies on Granada Medium and the other isolate grew as pale orange colonies.

[2]There were 9 False Negatives observed after 18 to 24 hours of incubation. All isolates were re-tested and confirmed by the discrepant analysis protocol above. Of these nine GBS isolates recovered by the reference method, three were β-hemolytic and six were non-hemolytic. For the β GBS isolates identified by the reference method and evaluated by discordant analysis, all three isolates grew as orange colonies on Granada Medium where originally the colony color was white. Samples where the six NH Group B Streptococci were identified by the reference method showed white colonies on Granada Medium as expected (no discordant analysis was performed for these isolates).

Because NH GBS cannot be detected by colony color, the performance of Granada Medium (orange color development) was compared to the recovery of only β-hemolytic GBS by the reference method. In Table 2, the overall sensitivity value increased to 98.1% (95% CI: 94.5-99.3%) while the overall specificity value, at 97.9% (95% CI: 96.4-98.8%), did not significantly change.

Table 2. LIM Reference Method (beta hemolytic Group B *Streptococcus*) vs. Granada Medium Color reaction

Site	TP	FP[1]	FN[2]	TN	Sensitivity	95% CI		Specificity	95% CI	
1	45	2	2	144	**95.7**	85.8	98.8	**98.6**	95.1	99.6
2	41	7	0	135	**100.0**	91.4	100.0	**95.1**	90.2	97.6
3	27	1	1	82	**96.4**	82.3	99.4	**98.8**	93.5	99.8
4	40	3	0	241	**100.0**	91.2	100.0	**98.8**	96.4	99.6
Overall	**153**	**13**	**3**	**602**	**98.1**	**94.5**	**99.3**	**97.9**	**96.4**	**98.8**

[1]12 of the 13 False Positives are identical to the False Positives listed in Table 1. The additional False Positive was originally recorded as non-hemolytic Group B Strep on blood agar and orange on Granada Medium. During discrepant analysis, the isolate was later confirmed as beta-hemolytic on blood agar.

[2]The 3 False Negatives are the 3 β-hemolytic GBS described in Table 1.

RECOVERY RATE

To determine the recovery [Limit of Detection (LoD)] of Granada Medium, the media was challenged with two beta-hemolytic ATCC® strains of Group B Streptococci at 10-fold decreasing concentrations and evaluated for color reaction. The lowest concentration at which a positive reaction was seen, indicated by orange colonies, was determined to be the LoD. The LoD was confirmed by testing Granada Medium with five replicate dilutions of the determined LoD concentration. Granada Medium, by direct inoculation, was able to recover both *S. agalactiae* ATCC® 12386 and *S. agalactiae* ATCC® 12403 at a LoD of $1.5x10^2$ CFU/mL, or 15 CFU (3-30 CFU) inoculated directly to Granada Medium. Blood agar plates were used to determine the concentrations of organisms present in each dilution.

To evaluate the performance of Granada Medium with a sample of overnight enriched culture, LIM Broth was challenged with two beta-hemolytic ATCC® strains of Group B Streptococci at 10-fold decreasing concentrations in GBS-negative specimen matrix. After overnight enrichment, LIM Broth culture was subcultured to Granada Medium and evaluated for color reaction. The lowest GBS concentration (previously spiked in LIM Broth and incubated overnight) yielding expected results on Granada Medium was then confirmed with five replicate dilutions of the lowest concentration. Following overnight enrichment in LIM Broth, Granada Medium was able to recover both *S. agalactiae* ATCC® 12386 and *S. agalactiae* ATCC® 12403 from a flocked vaginal/rectal swab specimen with $1.5x10^2$ CFU/mL (1-9 CFU tested). Blood agar plates were used to determine the concentrations of organisms present in each dilution.

ANALYTICAL REACTIVITY

Fifty-four selected ATCC, NCIMB, NCTC reference and clinical GBS strains representing seven of the nine different serotypes were directly inoculated to Granada Medium at the determined LoD of $1.5x10^2$ CFU/mL, corresponding to an inoculum of 15 CFU (3-30 CFU). Granada Medium was able to recover all GBS strains tested at the LoD with the expected color development. The GBS serotypes included in this study were serotypes Ia, Ib, II, III, IV, V, and VI. Serotypes VII and VIII are rare and were not available for testing. Four strains that were non-typable against the nine known serotypes were also included. Of the 54 GBS strains tested, 48 were beta-hemolytic strains (88.9%) and six were non-hemolytic strains (11.1%). All beta-hemolytic GBS strains produced the expected orange colony color reaction and all non-hemolytic GBS strains produced the expected white colony color reaction on Granada Medium after 24 hours of incubation.

INCUBATION

In order to determine a recommended incubation time range, Granada Medium was evaluated using nine beta-hemolytic strains of GBS by direct inoculation from a $1.5x10^2$ CFU/mL suspension, corresponding to an inoculum of 15 CFU (3-30 CFU). Growth and color reaction were recorded every 2 hours from 18 to 24 hours.

All organisms tested grew with visible orange colonies by 18 hours. The incubation range for Granada Medium was set from 18-24 hours.

ANALYTICAL SPECIFICITY

Eighty-four organisms that are phylogenetically-related to Group B Streptococci or potentially encountered in a vaginal-rectal swab were tested on Granada Medium following overnight enrichment in LIM Broth. A $1.5x10^8$ CFU/mL suspension of each organism was inoculated to a LIM Broth tube, corresponding to an inoculum of $1.5x10^6$ CFU. After incubation, each LIM broth tube was subcultured to a Granada Medium plate and evaluated for growth and color reaction after 24 hours of anaerobic incubation. Organisms tested either produced a negative color reaction (39/84, 46.4%) or were not recovered (45/84, 53.6%) on Granada Medium after LIM Broth enrichment.

Non-target Organisms Tested in Analytical Specificity		
Acinetobacter baumannii	*Enterococcus hirae*	*Providencia alcalifaciens*
Aeromonas hydrophila	*Enterococcus malordoratus*	*Pseudomonas aeruginosa*
Bacillus cereus	*Enterococcus mundtii*	*Pseudomonas fluorescens*
Bacillus subtilis	*Enterococcus pseudoavium*	*Salmonella enterica (typhii)*
Bacteroides fragilis	*Enterococcus raffinosus*	*Salmonella enterica arizonae*
Bifidobacterium breve	*Enterococcus saccharolyticus*	*Serratia marcescens*
Campylobacter jejuni	*Enterococcus sulfureus*	*Shigella boydii*
Candida albicans	*Escherichia coli*	*Shigella flexneri*
Candida glabrata	*Gardnerella vaginalis*	*Shigella sonnei*
Candida parapsilosis	*Geotrichum candidum*	*Staphyloccocus aureus*
Candida tropicalis	*Klebsiella oxytoca*	*Staphylococcus epidermidis*
Citrobacter freundii	*Klebsiella pneumoniae*	*Staphylococcus saprophyticus*
Clostridium difficile	*Lactobacillus acidophilus*	*Stenotrophomonas maltophilia*
Clostridium novyi	*Lactobacillus fermentum*	*Streptococcus anginosus*
Clostridium perfringens	*Lactobacillus gasseri*	*Streptococcus bovis*
Clostridium sporogenes	*Lactobacillus leichmannii*	*Streptococcus dysgalactiae*
Enterobacter aerogenes	*Lactococcus lactis*	*Streptococcus equi subsp. zooepidemicus*
Enterobacter cloacae	*Leuconostoc mesenteroides*	*Streptococcus gallolyticus*
Enterococcus avium	*Listeria monocytogenes*	*Streptococcus mitis*
Enterococcus casseliflavus	*Moraxella cartarrhalis*	*Streptococcus mutans*
Enterococcus cecorum	*Morganella morganii*	*Streptococcus pneumoniae*
Enterococcus columbae	*Neisseria gonorrhoeae*	*Streptococcus pyogenes*
Enterococcus dispar	*Pediococcus acidilacti*	*Streptococcus salivarius subsp. salivarius*
Enterococcus durans	*Pediococcus damnosus*	*Streptococcus salivarius subsp. thermophilus*
Enterococcus faecalis	*Peptostreptococcus anaerobius*	*Streptococcus uberis*
Enterococcus faecium	*Plesiomonas shigelloides*	*Vibrio parahaemolyticus*
Enterococcus flavescens	*Proteus mirabilis*	*Yersinia enterocolitica*
Enterococcus gallinarum		

MICROBIAL INTERFERENCE

Granada Medium was challenged to determine if target organism at low concentration could be recovered in the presence of non-target organisms at a high concentration. All organisms that were recovered on Granada Medium in the Analytical Specificity study were tested in the Microbial Interference study. Non-target organisms at a high concentration ($1.5x10^8$ CFU/mL) were mixed 1:1 with each target organism (near $1.5x10^4$ CFU/mL) and inoculated directly to Granada Medium with a 10µL loop. If the target organism was not recovered on Granada Medium, the concentration of the non-target organism was lowered 10-fold until the target organism was recovered.

Granada Medium was able to produce the expected orange color reaction and recover target organisms when in the presence of high concentrations of all but one of the non-target organisms used in this study. The only organism found to affect recovery of GBS was *Enterococcus faecalis* (ATCC 29212). At $1.5x10^8$ CFU/mL, this organism inhibited recovery of GBS on Granada Medium, however when reduced to $1.5x10^6$ CFU/mL, target GBS was recovered.

Several organisms did not affect recovery, but did affect colony size of target organisms when inoculated to Granada Medium at $1.5x10^8$ CFU/mL: *E. faecalis* (ATCC 51299), *E. avium* (ATCC 14025), *E. gallinarum* (ATCC 49573), *E. saccharolyticus* (ATCC 43076), *Lactococcus lactis* (ATCC 19435), *Morganella Morganii* (ATCC 25830), *Proteus mirabilis* (ATCC 43071), and *Serratia marcescens* (ATCC 13880). However, when these non-target organisms were reduced to $1.5x10^7$ CFU/mL, GBS colony size was larger and more clearly visible on Granada Medium. Colony size and color, but not recovery, of GBS was affected by *Vibrio parahaemolyticus* (ATCC 17802) when present at a high concentration ($1.5x10^8$ CFU/mL). When *V. parahaemolyticus* concentration was reduced to $1.5x10^7$ CFU/mL, colony morphology of target GBS was as expected.

SPECIMEN STABILITY

Various types of specimen transport swabs were evaluated to determine the storage conditions that allowed for recovery of GBS on Granada Medium after LIM Broth enrichment. Swabs were spiked with beta-hemolytic Group B Streptococci strains in vaginal/rectal matrix and were kept under both room temperature and refrigerated conditions. The swabs were inoculated to LIM Broth at 0, 24, 48, 72, 96, and 120 hours. TransPRO™ swabs with Liquid Amies (liquid-based transport system) and five types of Healthlink swabs were used in this study: Liquid Amies, Amies Gel, Liquid Stuart's, Stuart's Gel, and Amies Charcoal. After an overnight incubation at 35°C, LIM Broth cultures were subcultured to Granada Medium.

Granada Medium was able to recover 2/2 (100%) of GBS strains from TransPRO™ Liquid Amies and Healthlink swabs in Liquid Amies, Amies Gel, Liquid Stuart's, Stuart's Gel, and Amies Charcoal when stored at room temperature up to 24 hours and at 2-8°C for up to 120 hours. GBS was recovered from all time points and storage conditions on Granada Medium from each method.

REPRODUCIBILITY

Prior to initiating the study, a panel of 22 blinded isolates (set of 11 organisms tested in duplicate) provided by Hardy Diagnostics was tested at three distinct study sites on five work days to demonstrate reproducibility and to document proficiency in the performance of the test. Agreement of >95% with known test results was required before proceeding with the study. The testing was done with at least one operator and two readers, blinded to each other's results, per site. Strains in the reproducibility panel produced the expected color results with Granada Medium $\geq$ 95% of the time after 24 hours. All beta-hemolytic GBS isolates tested (100%) were recovered by Granada Medium with the expected orange color reaction of isolated colonies on all days of the reproducibility study.

INTERFERENCE

Commonly used or encountered endogenous and exogenous substances that may be present in vaginal/rectal specimens were evaluated for potential interference of growth or color reaction on Granada Medium. The substances tested are listed in the table below. No interference was observed with any substance at the highest clinically relevant concentration in the GBS-negative specimen matrix.

Interfering Substances		
Category	**Substance/Supplier**	**Concentration in Sample Matrix[1]**
Anti-diarrheal Medication	Pepto-Bismol® (Bismuth subsalicylate solution)	1% v/v
	Imodium A-D® (Loperamide HCl)	2% w/v
Body Oil	Neutrogena Body Oil	2% v/v
Body Powder	Gold Bond Body Powder	1% w/v
Contraceptive Gel	Options Gynol II® (Nonoxynol-9)	0.59% w/v
Enema Solution	Physiological saline	0.25% v/v
Lubricating Gel	K-Y® Jelly	0.57% w/v
Oral Laxative	Milk of Magnesia	1.78% v/v
	Dulcolax® (Sodium picosulfate solution)	1% w/v
Polysorbate 80	Tween®80	10% v/v
Rectal Laxative	Fleet® Glycerin Suppositories	10% v/v
Topical Hemorrhoid Ointment	Preparation-H®	0.26% w/v
Vaginal Anti-Itch Medication	Vagisil® Cream	0.41% w/v
Vaginal Anti-Fungal Medication	Monistat® (Miconazole nitrate)	0.29% w/v
	Lotrimin® (Clotrimazole)	0.29% w/v
Endogenous Substances		
Human Amniotic Fluid	Medfusion	2% v/v
Human Feces	Central Coast Pathology	2% v/v
Human Meconium	LEE Biosolutions	2% v/v
Human Urine	Central Coast Pathology	2% v/v
Human Whole Blood	In-house	2% v/v
Mucin	Sigma, M2378	0.05% w/v

[1]Specific amounts of substance added to vaginal/rectal specimen matrix calculated using $C_1V_1=C_2V_2$ with the assumption that 1g=1mL.

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			Expected Results
		Temp	Atmosphere	Time	
*Streptococcus agalactiae** ATCC® 12386	A	35ºC	Anaerobic	18-24hr	Growth; bright orange to red color change
Streptococcus pyogenes ATCC® 19615	A	35ºC	Anaerobic	18-24hr	Growth; no color change
Escherichia coli ATCC® 25922	B	35ºC	Anaerobic	18-24hr	Partial to complete inhibition; no color change
Bacteroides fragilis ATCC® 25825	B	35ºC	Anaerobic	24hr	Partial to complete inhibition; no color change

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

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

Inoculation Method

METHOD A
Suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (TSB) and incubate for 4 to 5 hours. Adjust the turbidity to match that of a 0.5 McFarland standard. Dilute the cell suspension to 1:100 in TSB or normal saline. Inoculate the test media with a 10uL calibrated loop of the diluted suspension. This will provide approximately 10^3 to 10^4 CFU per tube.

METHOD B
Use the same cell suspension (equivalent to a 0.5 McFarland standard) described in "Method A" and dilute to 1:10 in Tryptic Soy Broth (TSB). Inoculate the media as described in "Method A" with a 10uL calibrated loop. This should result in 10^4 to 10^5 CFU per plate. A non-inhibitory plate (e.g. TSA) is inoculated at the same time to serve as a positive control.

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.

REFERENCES

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