IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

STREP B CARROT BROTH™ ONE-STEP CE

REF

Cat. no. Z40	Strep B Carrot Broth™ One-Step, 13x100mm Tube, 4ml	20 tubes/box
Cat. no. Z44BX	Strep B Carrot Broth™ One-Step, 12x80mm Tube, 4ml	100 tubes/box
Cat. no. Z46BX	Strep B Carrot Broth™ One-Step, 16x100mm Tube, 6ml	100 tubes/box

INTENDED USE

Strep B Carrot Broth™ One-Step is a selective and differential medium which is intended for the detection of Group B *Streptococcus* (GBS) from anovaginal specimen collected from pregnant women. The medium is used as an aid in the qualitative determination of GBS colonization in pregnant women. The color change reaction from white to orange is representative of a positive result for presence of GBS. The medium requires 24 hours of incubation but positive results can be interpreted and reported as early as 16 hours. Due to the properties of Strep B Carrot Broth™ One-Step, non-hemolytic GBS cannot be detected by the medium's color change and requires subculture for identification. Any presumptive negative indicated by lack of color change at the end of the incubation period must be subcultured to a non-selective medium (e.g., Tryptic Soy Agar with 5% Sheep Blood) to confirm absence of GBS. Subculture must also be performed to recover isolates for conducting susceptibility testing as recommended for penicillin-allergic women.

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. The use of a selective and differential enrichment broth, such as Strep B Carrot Broth™, has been included in the CDC's Prevention of Perinatal Group B Streptococcal Disease.[4] In certain studies, Strep B Carrot Broth™ has been shown to reduce incubation time and need for additional plated media to identify GBS when compared to traditional culture methods.[5-9, 23-26]

The production of light orange to orange to red-orange pigment is a unique characteristic of hemolytic GBS due to reaction with substrates such as starch, peptone, serum, and folate pathway inhibitors. Since the original description of starch serum agar by Islam in 1977, there have been many improvements to the original formula.[10]

Hardy Diagnostics Strep B Carrot Broth™ One-Step contains the necessary components for pigment detection of beta-hemolytic GBS, including peptone, starch and buffers which are supplied in the Strep B

Carrot Broth™ One-Step (Z40A, Z44A, or Z46A). The advantage to this medium is that it will produce positive results in as little as sixteen hours and does not require subculture to a blood agar plate unless the results are negative. Enrichment broth procedures are known to be more sensitive than plate methods in their ability to detect GBS colonization. Beta-hemolytic, pigment producing GBS occurs with 95.3 to 99.5% of all GBS strains isolated from clinical specimens.[18-20] Two separate studies by Block, et. al. and Czerepuszko et al. have shown that Strep B Carrot Broth™ can be successfully used in conjunction with broth-enhanced PCR procedures or PNA FISH™ respectively.[24, 26]

FORMULA

Ingredients per liter of deionized water:*

Strep B Carrot Broth™ One-Step (Z40, Z44BX, Z46BX):	
Peptone	25.0gm
Starch	10.0gm
Selective Agents	38.8gm
Morpholinepropanesulfonic Acid (MOPS)	11.0gm
Disodium Phosphate	8.5gm
Dextrose	2.5gm
Sodium Pyruvate	1.0gm
Magnesium Sulfate	0.2gm

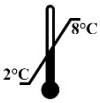
* Adjusted and/or supplemented as required to meet performance criteria.

Final pH 7.0 +/- 0.2 at 25°C

PHYSICAL APPEARANCE

Strep B Carrot Broth™ One-Step should appear hazy to cloudy, colorless; may or may not have a white precipitate at the bottom of the tube.

STORAGE AND SHELF LIFE



Storage: Product is temperature sensitive. Upon receipt store at 2-8°C; 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" on the Hardy Diagnostics Technical Document website for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as 10µL inoculating loops, specimen transport materials, other culture media, swabs, incubators, etc., as well as serological and biochemical reagents are not provided.

Anovaginal specimens preserved in Liquid Amies, Amies Gel, Liquid Stuart's, and Stuart's Gel (Cat. no. TP3F, 4140BX, 4108BX, 4432BX, or 4111BX) have been validated as appropriate specimens for use in Strep B Carrot Broth™ One-Step.

PRECAUTIONS

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

U. S. 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." The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdcgov/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" on the Hardy Diagnostics Technical Document website for more information.

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

PROCEDURE

Clinical Procedure

Specimen Transport and Storage:

The medium has been evaluated for use with anovaginal swab specimens preserved in Liquid Amies, Amies gel, Stuart's liquid, and Stuart's Gel (Cat. no. TP3F, 4140BX, 4108BX, 4432BX, or 4111BX). An internal investigation found that target GBS strains produced the expected orange coloration from Healthlink swabs in Liquid Amies, Liquid Stuart's, Amies Gel, and Stuart's Gel when stored at 2-8°C for up to four days and from flocked swab TransPRO™ Liquid Amies stored at 2-8°C for up to five days. All transport systems tested saw a decline in recovery of GBS after 24 hours of room temperature storage. 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.[2-5]

Method of Use:

1. Allow the Strep B Carrot Broth™ One-Step tube to equilibrate to room temperature prior to inoculation.
2. Insert the specimen swab into the Strep B Carrot Broth™ One-Step tube. If using a gel-based transport medium, rotate the swab in the broth to emulsify the gel in the broth. Do not shake, agitate, or vortex. Carefully break the swab shaft, leaving the swab in the tube. Replace the tube cap and screw down **tightly**. It is important that the caps be tightly sealed in order to create the necessary anaerobic conditions at the bottom of the tube.
3. Incubate the inoculated Strep B Carrot Broth™ One-Step tube at 35°C.
4. Examine tubes after 16 hours for a light orange to orange to red-orange color change and/or spots typical of Group B Streptococci. See "Interpretation of Results" section below. If no orange color is present, re-incubate the tube until 24 hours.
5. If no orange color is present after 24 hours, subculture the tube to a Blood Agar plate (Cat. no. A10). Incubate Blood Agar plates 18-24 hours at 35°C in a CO_2 enriched environment. Examine the Blood

Agar plates for hemolytic colonies and/or non-hemolytic colonies typical of Group B Streptococci. Perform group specific latex testing on colonies suspicious of being GBS.

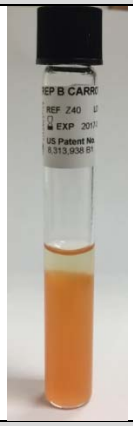
Study results show no adverse events if the incubation of Strep B Carrot Broth™ tubes occurs without the addition of the transport swab, provided users swirl swabs in the broth for no less than three seconds to ensure appropriate inoculation from the specimen. Alternatively, 30µl of the transport medium from a flocked swab may be aliquoted to the Strep B Carrot Broth™ tube and incubated as appropriate without issue.

INTERPRETATION OF RESULTS

Growth of beta-hemolytic Group B Streptococci in Strep B Carrot Broth™ One-Step results in the development of a light orange to orange to red-orange pigment within 16 to 24 hours. Visualization of any light orange to orange to red-orange pigment in the Strep B Carrot Broth™ One-Step is indicative of the presence of beta-hemolytic Group B Streptococci in the specimen.

In cases where the GBS count is low in the specimen, development of small orange to red spots (colonies) or streaks on the swab or within the tube can be observed rather than the entire tube turning orange-red. These should also be considered a positive result for GBS.

24 hour Incubation	Interpretation/Recommended Action
Light orange to orange to red-orange color development	Positive for GBS*
No change in color	Presumptive Negative: Subculture to Blood Agar to rule-out presence of weakly β-hemolytic or non-hemolytic GBS

Color	**Light Orange**	**Orange**	**Red-Orange**
			
Growth Characteristic	**Complete Color**	**Spheres**	**Streaks**
			

** Subculture to 5% Sheep Blood must be done to recover isolates for performing susceptibility testing as this is recommended for penicillin-allergic women.*

LIMITATIONS

1. It is recommended that biochemical, immunological, molecular, or mass spectrometry testing is performed on colonies from pure culture for complete identification.
2. Although rare, a small percentage of GBS may not produce beta-hemolysis. GBS detection with Strep B Carrot Broth™ One-Step is only possible with beta-hemolytic colonies. Beta-hemolytic, pigment producing GBS occurs with 95.3-99.5% of all GBS strains isolated from clinical specimens. [18-20] For this reason, do not use *S. agalactiae* ATCC® 13813 for quality control purposes because it will not produce the characteristic orange pigment.
3. Failure to properly emulsify gel-based transport medium, as outlined in the procedure section, may inhibit proper color development and recovery of Group B Streptococci.
4. The performance of Strep B Carrot Broth™ One-Step has not been evaluated with transport swabs containing charcoal.
5. The performance of Strep B Carrot Broth™ One-Step has not been evaluated in the presence of Human DNA.
6. Some strains of *E. faecalis,* when at concentrations above 10^5 CFU/mL in the vaginorectal swab specimen, have been shown to be inhibitory to the detection and recovery of GBS strains.
7. Invert negative tubes to mix prior to subculture to Blood Agar (Cat. no. A10).
8. Strep B Carrot Broth™ One-Step does not provide susceptibility results. Subculture to non-selective media should be performed as needed for susceptibility testing.
9. Color-blind individuals may encounter difficulty in distinguishing color differences in Strep B Carrot Broth™.
10. Storage of anovaginal specimens at room temperature is not recommended over 24 hours as this significantly impacts color development and the recovery of GBS.
11. GBS serotypes VII, VIII, and IX have not been evaluated with the Strep B Carrot Broth™ One-Step.
12. The clinical performance of Strep B Carrot Broth™ One-Step has not been established with swab transport systems other than those referenced in the clinical studies.

Refer to the document "Limitations of Procedures and Warranty" on the Hardy Diagnostics Technical Document website 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) and of these, 4.3% (7/163) were non-hemolytic Group B Streptococci.

PERFORMANCE CHARACTERISTICS

Performance of Strep B Carrot Broth™ One-Step was evaluated at four geographically diverse hospitals with routine GBS specimen in the form of an anovaginal swab. The detection of Group B Streptococci by orange color development in Strep B Carrot Broth™ One-Step was compared to routine culture, defined as the selective enrichment of specimen in LIM Broth, followed by subculture to Blood Agar and confirmed by biochemical testing. Additionally, the recovery of Group B Streptococci from Strep B Carrot Broth™ One-Step that had been subsequently subcultured to Blood Agar was also compared to LIM broth routine culture. Organisms that grew on Blood Agar were confirmed to be Group B Streptococci using gram-stain, catalase test, and latex agglutination.

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 143 specimens were positive for Group B Streptococci by orange color development in Strep B Carrot Broth™ One-Step after 24 hours of incubation at 35-37 ºC and were concordant with results obtained by the LIM reference method. Those results are shown in Table 1. The specimens included in the evaluation of performance were obtained in three different types of transport swabs/media—Liquid Stuart's Sponge (n=284), Liquid Amies Sponge (n=111), and Liquid Amies ESwab (n=376).

All discrepant isolates were frozen in CryoSavers™ with Brucella Broth and returned to Hardy Diagnostics for testing. The identity of each isolate was confirmed (β Group B Streptococci, 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 LoD (10^3 CFU/mL) in donated negative vaginal rectal matrix for their recovery from the LIM reference method, color development in Carrot Broth™ One-Step, and recovery from the Carrot Broth™ One-Step to Blood Agar System.

Table 1. LIM Broth vs. Strep B Carrot Broth™ One-Step Color reaction

Site	TP	FP[1]	FN[2]	TN	Sensitivity	95% CI		Specificity	95% CI	
1	47	1	4	141	**92.2**	81.5	96.9	**99.3**	96.1	99.9
2	35	4	8	136	**81.4**	67.4	90.3	**97.1**	92.9	98.9
3	26	0	2	83	**92.9**	77.4	98.0	**100.0**	95.6	100.0
4	35	3	6	240	**85.4**	71.6	93.1	**98.8**	96.4	99.6
Overall	**143**	**8**	**20**	**600**	**87.7[3]**	81.8	91.9	**98.7[3]**	97.4	99.3

[1] There were 8 False Positives observed. All isolates were re-tested and confirmed at Hardy Diagnostics using the discrepant analysis protocol described above. Of these, six isolates were originally negative by LIM reference method, showed a positive color reaction in Strep B Carrot Broth™ One-Step, and were confirmed to be β-hemolytic when subcultured on Blood Agar. One was not able to be confirmed because no GBS isolate was frozen and the remaining specimen had β-hemolytic colonies present on the blood agar plates; however, *Proteus* swarmed the plates, preventing the technician from isolating the β-hemolytic colonies for identification.
[2] There were 20 False Negatives observed. All isolates were re-tested and confirmed at Hardy Diagnostics using the discrepant analysis protocol described above. Fourteen of the β Group B Streptococci isolates recovered from LIM, but originally gave a negative Strep B Carrot Broth™ One-Step color reaction, were confirmed as β Group B Streptococci and subsequently confirmed to have a positive Strep B Carrot Broth™ One-Step color reaction at LoD. Two isolates were identified as very weak β Group B Streptococci and did not produce the expected color reaction in Strep B Carrot Broth™ One-Step. Four isolates were confirmed as non-hemolytic Group B Streptococci with a negative color reaction in Strep B Carrot Broth™ One-Step™.
[3] Considering that non-hemolytic GBS cannot be detected by the medium's color change and require subculture for identification, there were 5 specimens that were found to be non-hemolytic. Considering those results as true negatives, the overall Sensitivity and Specificity values observed when comparing the recovery of β-hemolytic GBS by the LIM reference method to the Strep B Carrot Broth™ One-Step color reaction were 90.4% (141/156) [95% CI: 84.7-94.1] and 98.4% (605/615) [95% CI: 97.0-99.1], respectively.

When comparing the number of Group B Streptococci positive specimens recovered by the LIM reference method to the number identified by Strep B Carrot Broth™ One-Step color change in conjunction with the subculture of presumptive negatives to the Blood Agar, an additional 18 specimens showed concordant positive results for a total of 161 true positive results. The LIM reference method included the identification of both β-hemolytic and non-hemolytic GBS from samples by culture. Those results are shown in Table 2.

Table 2. LIM Reference Method vs. Strep B Carrot Broth™ One-Step (color), plus Subculture of Presumptive Negatives to Blood Agar with Biochemical Testing

Site	TP	FP[1]	FN[2]	TN	Sensitivity	95% CI		Specificity	95% CI	
1	50	0	1	142	**98.0**	89.7	99.7	**100.0**	97.4	100.0
2	43	6	0	134	**100.0**	91.8	100.0	**95.7**	91.0	98.0
3	28	0	0	83	**100.0**	87.9	100.0	**100.0**	95.6	100.0
4	40	5	1	238	**97.6**	87.4	99.6	**97.9**	95.3	99.1
Overall	**161**	**11**	**2**	**597**	**98.8**	95.6	99.7	**98.2**	96.8	99.0

[1] There were eleven False Positives observed. All isolates were re-tested and confirmed at Hardy Diagnostics using the discrepant analysis protocol described above. All isolates recovered from the Strep B Carrot Broth to Blood Agar system were confirmed to be β Group B Streptococci.

[2] There were two False Negatives observed. Both isolates were re-tested and confirmed at Hardy Diagnostics using the discrepant analysis protocol described above. Both of the β Group B Streptococci isolates recovered from LIM were confirmed as β Group B Streptococci.

RECOVERY RATE

To determine the recovery [Limit of Detection (LoD)] of Strep B Carrot Broth™ One-Step, the media was challenged with two beta-hemolytic ATCC® strains of Group B Streptococci at 10-fold decreasing concentrations and evaluated for color change. The lowest concentration at which a positive reaction was seen, indicated by an orange color, was determined to be the LoD. The determined LoD was confirmed by testing Strep B Carrot Broth™ One-Step with 20 replicate dilutions of the determined LoD concentrations. Strep B Carrot Broth™ One-Step was able to recover both *S. agalactiae* ATCC® 12386 and *S. agalactiae* ATCC® 12403 at a LoD of 10^3 CFU/mL of the fluid from a flocked anovaginal swab specimen (30 CFU/tube when using a 30µL inoculum). Variable recovery was seen at lower concentrations. Blood agar plates were used to determine the concentrations of organisms present in each dilution.

ANALYTICAL REACTIVITY

Fifty-four ATCC reference and clinical Group B Streptococci strains representing seven of the nine known serotypes were recovered in Strep B Carrot Broth™ One-Step when inoculated at the limit of detection concentration of 10^3 CFU/mL. The GBS serotypes included in this study were serotypes Ia, Ib, II, III, IV, V, and VI. Four strains that were non-typable against the nine known serotypes were also included. All the beta-hemolytic strains of Group B Streptococci produced the expected orange color in Strep B Carrot Broth™ One-Step. The non-hemolytic strains showed no orange color in Strep B Carrot Broth™ One-Step but were successfully recovered upon subculture to Blood Agar.

ANALYTICAL SPECIFICITY

Seventy-eight non-target organisms that are phylogenetically-related to Group B Streptococci or potentially encountered in an anovaginal swab were tested in Strep B Carrot Broth™ One-Step at a concentration of 10^8 CFU/mL. All organisms tested are listed in Table 3 below. After 24 hours of incubation, all Carrot Broth tubes were evaluated for color reaction. In order to determine if Strep B Carrot Broth™ One-Step supported the growth of non-target organisms in the absence of a color reaction, all negative tubes were subcultured to an appropriate medium for the non-target organism. All organisms tested produced a negative color reaction in Strep B Carrot Broth™ One-Step and 45/78 (57.7%) of the organisms were recoverable when subcultured after enrichment.

Table 3. List of non-target organisms tested in Analytical Specificity

Organism		
Acinetobacter baumannii	*Enterococcus durans*	*Proteus mirabilis*
Aeromonas hydrophila	*Enterococcus faecalis*	*Providencia alcalifaciens*
Aspergillus brasiliensis	*Enterococcus faecium*	*Pseudomonas aeruginosa*
Bacillus cereus	*Enterococcus flavescens*	*Pseudomonas fluorescens*
Bacillus subtilis	*Enterococcus hirae*	*Saccharomyces cerevisiae*
Bacteroides fragilis	*Enterococcus raffinosus*	*Salmonella enterica (typhii)*
Bifidobacterium breve	*Enterococcus saccharolyticus*	*Salmonella enterica arizonae*
Campylobacter coli	*Escherichia coli*	*Serratia marcescens*
Campylobacter jejuni	*Gardnerella vaginalis*	*Shigella boydii*
Candida albicans	*Geotrichum candidum*	*Shigella flexneri*
Candida glabrata	*Hafnia alvei*	*Shigella sonnei*
Candida parapsilosis	*Klebsiella oxytoca*	*Staphyloccocus aureus*
Candida tropicalis	*Klebsiella pneumoniae*	*Staphylococcus epidermidis*
Citrobacter brakkii	*Lactobacillus acidophilus*	*Staphylococcus saprophyticus*
Citrobacter freundii	*Lactobacillus gasseri*	*Stenotroph. maltophilia*
Citrobacter koseri	*Lactobacillus leichmannii*	*Streptoccocus mutans*
Clostridium difficile	*Lactococcus lactis*	*Streptococcus anginosus*
Clostridium novyi	*Legionella pneumophila*	*Streptococcus bovis*
Clostridium perfringens	*Listeria monocytogenes*	*Streptococcus dysgalactiae*
Clostridium sporogenes	*Micrococcus luteus*	*Streptococcus mitis*
Corynebacterium jekeium	*Moraxella cartarrhalis*	*Streptococcus pneumoniae*
Enterobacter aerogenes	*Morganella morganii*	*Streptococcus pyogenes*
Enterobacter cloacae	*Neisseria gonorrhoeae*	*Streptococcus salivarius*
Enterococcus casseliflavus	*Pediococcus acidilacti*	*Streptococcus uberis*
Enterococcus cecorum	*Peptostreptococcus anaerobius*	*Vibrio parahaemolyticus*
Enterococcus dispar	*Plesiomonas shigelloides*	*Yersinia enterocolitica*

MICROBIAL INTERFERENCE

Strep B Carrot Broth™ One-Step was challenged to determine if target organisms at low concentration could be recovered in the presence of non-target organisms at a high concentration. All organisms that were recovered upon subculture from Strep B Carrot Broth™ One-Step in the Analytical Specificity study were used in this Microbial Interference study. Non-target organisms at a high concentration (1.5×10^8 CFU/mL) were mixed with each target organism at the LoD concentration and inoculated into Strep B Carrot Broth™ One-Step. If the target organism was not recovered, the concentration of the non-target organism was lowered 10-fold until the target organism was recovered.

Strep B Carrot Broth™ One-Step was able to produce the expected color reaction with target organisms and allowed the recovery of both GBS strains in the presence of high concentrations (1.5×10^8 CFU/mL) of all but one of the non-target organisms used in this study. The only organism found to affect recovery was *E. faecalis* ATCC 29212. *S. agalactiae* ATCC 12386 produced the expected color reaction when the concentration of *E. faecalis* ATCC 29212 was 10^5 CFU/mL or lower. *S. agalactiae* ATCC 13813, a non-hemolytic strain, was recovered upon subculture when the concentration of *E. faecalis* ATCC 29212 was 10^6 CFU/mL or lower.

INTERFERENCE

Commonly used or encountered endogenous and exogenous substances that may be present in anovaginal swabs were evaluated for potential interference of growth or chromogenic reaction in Strep B Carrot Broth™ One-Step. 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 anovaginal specimen matrix calculated using $C_1V_1=C_2V_2$ with the assumption that 1g=1mL.

INCUBATION

In order to determine a recommended incubation time range, the performance of Strep B Carrot Broth™ One-Step was evaluated using nine beta-hemolytic strains of GBS and one non-hemolytic strain of GBS at the LoD from 12 to 24 hours at 35°C. The enrichment broth was subcultured to a Tryptic Soy Agar plate with 5% sheep blood every two hours to confirm the presence of GBS. All hemolytic organisms tested produced some kind of orange color reaction by 16 hours and a definitive orange color reaction by 20 hours. All organisms, including the non-hemolytic strain tested, were recovered upon subculture of Strep B Carrot Broth™ One-Step at 12 hours. The incubation range for Strep B Carrot Broth™ was set from 16-24 hours.

SPECIMEN STABILITY

Various types of specimen transport swabs were evaluated to determine the acceptable storage conditions required to recover GBS from Strep B Carrot Broth™ One-Step. Swabs were spiked with Group B Streptococci and a contrived matrix consisting of organisms commonly found in vaginal flora, kept at both room temperature and refrigerated conditions, and were inoculated to Carrot Broth at 0, 24, 48, 72, 96, and 120 hours. Eight different GBS strains were used in this study and were spiked into the contrived matrix near LoD. The contrived matrix containing non-target organisms consisted of *E. faecalis*, *E. coli*, *C. albicans*, and *L. acidophilus*. TransPRO™ swabs with Liquid Amies (flocked swab liquid-based transport system) and four types of Healthlink transport systems: Sponge-based Liquid Amies and Liquid Stuart's, and Gel-based: Amies Gel and Stuart's Gel were used in this study.

Strep B Carrot Broth™ One-Step was able to recover 8/8 (100%) of GBS strains and produce orange coloration from Healthlink swabs in Liquid Amies, Stuart's liquid, Amies Gel, and Stuart's Gel when stored at 2-8°C for up to 96 hours. 100% of GBS strains also produced the expected orange color reaction from the flocked swab TransPRO™ Liquid Amies stored at 2-8°C for up to 120 hours. All transport systems tested saw a decline in recovery of GBS after 24 hours of room temperature storage.

REPRODUCIBILITY

Prior to initiating the study, a panel of 12 blinded isolates provided by Hardy Diagnostics was tested at three distinct study sites in triplicate 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 Strep B Carrot Broth™ One-Step $\geq$ 95% of the time at 24 hours. All non-hemolytic GBS isolates tested (100%) were recovered upon subculture to TSA with 5% Sheep's Blood.

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	Growth	Reaction
Streptococcus agalactiae ATCC® 12386	A	35ºC	Aerobic	16-24hr	Positive	Growth; bright orange to red color change
Streptococcus agalactiae Clinical strain	A	35ºC	Aerobic	24hr	Positive	Growth; light orange color change
Streptococcus pyogenes ATCC® 19615	A	35ºC	Aerobic	24hr	Positive	Growth; no color change
Escherichia coli ATCC® 25922	B	35ºC	Aerobic	24hr	Negative	Partial to complete inhibition; no color change
Proteus mirabilis ATCC® 12453	B	35ºC	Aerobic	24hr	Negative	Partial to complete inhibition; no color change

Refer to the document "Inoculation Procedures for Media QC" on the Hardy Diagnostics Technical Document website for more information.

*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 documents on the Hardy Diagnostics Technical Document website for more information on QC: "Introduction to Quality Control" and "Finished Product Quality Control Procedures," or see reference(s) for more specific information.

REFERENCES

1. Regan, J.A., Klebanoff, M.A., Nugent, R.P. 1991. *The epidemiology of group B streptococcal colonization in pregnancy.* Vaginal Infections and Pregnancy Study Group. Obstet. Gynecol.; 77:604-10.

2. Schrag, S.J., E.R. Zell, R. Lynfield, et al. 2002. *A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates.* N. Engl. J. Med. 25;347(4):233-9.

3. Schuchat, A. 2001. *Group B streptococcal disease: from trials and tribulations to triumph and trepidation.* Clin. Infect. Dis. 5;33(6):751-6.

4. The Centers for Disease Control and Prevention. 2010. *Prevention of Perinatal Group B Streptococcal Disease.* Revised Guidelines. MMWR 59 (RR-10). Internet: http://www.cdc.gov/mmwr/pdf/rr/rr5910.pdf

5. Overman, S.B., D.D. Eley, B.E. Jacobs, J.A. Ribes. 2002. *Evaluation of methods to increase the sensitivity and timeliness of detection of Streptococcus agalactiae in pregnant women.* J. Clin. Microbiol.; 40(11):4329-31.

6. de la Rosa, M., M. Perez, C. Carazo, L. Pareja, J.I. Peis and F. Hernandez. 1992. *New Granada Medium for detection and identification of group B streptococci.* J. Clin. Microbiol.; 30:1019-1021.

7. Garcia Gil, E., M.C. Rodriguez, R. Bartolome, B. Berjano, L. Cabero and A. Andreu. 1999. *Evaluation of the Granada Agar plate for detection of vaginal and rectal group B streptococci in pregnant women.* J. Clin. Microbiol.; 37:2648-2651.

8. Rosa-Fraile, Manuel, J. Rodriguez-Granger, M. Cueto-Lopez, A. Sampedro, E. Biel Gaye, J.M. Haro and A. Andreu. 1999. *Use of Granada Medium to detect group B streptococcal colonization in pregnant women.* J. Clin. Microbiol.; 37:2674-2677.

9. Rosa-Fraile, Manuel, A. Sampedro, J. Varela, M. Garcia-Pena, and G. Gimenez-Gallego. 1999. *Identification of a peptide pigment from mammal albumins responsible for enhanced pigment production by group B streptococci.* Clin. Diag. Lab. Imm.; 6:425-426.

10. Islam, AKMS. 1977. *Rapid recognition of group B streptococci.* Lancet 309(8005):256-257.

11. B. Spellerberg, B. Pohl, G. Haase, S. Martin, J. Weber-Heynemann and R. Lütticken. 1999. *Identification of Genetic Determinants for the Hemolytic Activity of Streptococcus agalactiae by ISS1 Transposition.* J. Bacteriol.; 181: 3212-3219.

12. Hardy Diagnostics and Fleury Medical Diagnostic Center. 2004. *Evaluation of Three Methods for Recovery of Group B Streptococci*. A poster presentation at American Society for Microbiology 104th General Meeting, New Orleans, LA.

13. National Center for Infectious Diseases, et al. 2002. Revised Guidelines from CDC: *Prevention of Perinatal Group B Streptococcal Disease.* MMWR Recommendations and Reports/51(RR11);1-22. Internet: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5111a1.htm.

14. Rosa-Fraile, M. et al. 2005. *Specimen Storage in Transport Medium and Detection of Group B Streptococci by Culture.* J. Clin. Microbiol.; 43:928-930.

15. Schreckenberger et al. 2005. *Evaluation of Strep B Carrot Broth™ and LIM Broth Methods for Recovery of Group B Streptococci (GBS): Results of a Multi-Center Trial.* A poster presentation at American Society for Microbiology, 105th General Meeting, Atlanta, GA.

16. DiPersio, J. 2005. *GBS Preservation by Strep B Carrot Broth™ Inoculated with Patient Specimens.* Unpublished data.

17. Facklam, R. et al. 2006. *Evaluation of Accuracy of Strep B Carrot Broth™ in the Detection of Different Seryotypes of Group B Streptococci (GBS).* A poster presentation at American Society for Microbiology, 106th General Meeting, Orlando, FL.

18. Young, Uh et al. 1998. *Serotypes and Biochemical Reaction Patterns of Group B Streptococci.* Korean J. Clin. Path.; 18:386-390.

19. Merrit, K. and Jacobs, N. 1978. *Characterization and Incidence of Pigment Production by Human Clinical Group B Streptococci.* J. Clin. Microbiol.; Vol. 8, No. 1, p. 105-107.

20. Noble, M., Bent, J., West, A. 1983. *Detection and identification of group B streptococci by use of pigment production.* J. Clin. Path.; 36:350-352.

21. de la Rosa, M., R. Villareal, D. Vega, C. Miranda, and A. Martinezbrocal. 1983. *Granada Medium for Detection and Identification of Group B Streptococci.* J. Clin. Microbiol.; Vol. 18, No. 4, p.779-785.

22. Rosa-Fraile, Manuel, J. Rodriguez-Granger, A. Haidour-Benamin, J.M. Cuerva, and A. Sampedro. 2006. *Granadaene: Proposed Structure of the Group B Streptococcus Polyenic Pigment.* J. Clin. Microbiol.; Vol. 72, No. 9, p.6367-6370.

23. da Gloria Carvalho, M., R. Facklam, D. Jackson, B. Beall, and L. McGee. 2009. *Evaluation of Three Commercial Broth Media for Pigment Detection and Identification of a Group B Streptococcus (Streptococcus agalactiae).* J. Clin. Microbiol.; Vol. 47, No. 12, p.4161-4163.

24. Block, T., E. Munson, A. Culver, K. Vaughan, and J. Hryciuk. 2008. *Comparison of Carrot Broth- and Selective Todd-Hewitt Broth-Enhanced PCR Protocols for Real-Time Detection of Streptococcus agalactiae in Prenatal Vaginal/Anorectal Specimens.* J. Clin. Microbiol.; Vol. 46, No. 11, p.3615-3620.

25. Church, D.L., H. Baxter, T. Lloyd, B. Miller, and S. Elsayed. 2008. *Evaluation of Strep B Carrot Broth™ versus Lim Broth for Detection of Group B Streptococcus Colonization Status of Near-Term Pregnant Women.* J. Clin. Microbiol.; Vol. 46, No. 8, p.2780-2782.

26. Czerepuszko, D.J., and M.J. Lewis. 2010. *Comparison of LIM Broth with PNA FISH to Carrot Broth with PNA FISH for Identification of Group B Streptococcus in Prenatal Vaginal/Rectal Specimens.* A poster presentation at American Society for Microbiology, San Diego, CA.

MDSS GmbH
Schiffgraben 41
30175 Hanover, Germany

HARDY DIAGNOSTICS
1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · Florida · New York · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.