510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K173903

B. Purpose for Submission:

To obtain a substantial equivalence determination for Granada Medium for the qualitative detection of Group B Streptococcus (GBS)

C. Measurand:

Group B Streptococcus (GBS)

D. Type of Test:

Detection of GBS using a selective and differential chromogenic medium

E. Applicant:

Hardy Diagnostics

F. Proprietary and Established Names:

Granada Medium

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.2360

2. Classification:

   Class I (non-exempt)

3. Product code:

   PQZ

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   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.

2. Indication(s) for use:

   Same as the Intended Use.

3. Special conditions for use statement(s):

   Prescription Use only

   White colonies on Granada Medium after 18-24 hours incubation are considered presumptive negative for GBS and must be confirmed by additional testing to rule-out presence of weakly β-hemolytic or non-hemolytic GBS.

4. Special instrument requirements:

   Not Applicable

I. Device Description:

Granada Medium is a selective and differential medium used for the detection of *Streptococcus agalactiae* (*S. agalactiae,* GBS) colonization in pregnant women by testing LIM Broth enriched cultures of vaginal/rectal swabs. Color development (light orange to dark orange color) is a unique characteristic of β-hemolytic GBS strains resulting from colored pigment production in the presence of substrates such as starch, peptone, serum, and folate pathway inhibitors. Non-hemolytic GBS cannot be detected by color production, but can be recovered from Granada Medium by evaluating white colonies growing on the medium. Growth of microorganisms belonging to other species is either inhibited, or if there is growth, the colonies do not produce the expected color reaction.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   chromID Strepto B Agar

2. Predicate 510(k) number(s):

   K163042

3. Comparison with predicate:

Similarities		
Item	Granada Medium (K173903)	chromID Strepto B Agar (K163042)
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.	chromID Strepto B agar is a selective chromogenic medium that is intended to aid in the qualitative determination of Group B Streptococcus (GBS) colonization in pregnant women. This medium supports the growth of, but does not differentiate between, hemolytic and non-hemolytic GBS strains. The test is performed on 18-24 hour LIM broth enrichments of vaginal/rectal swabs obtained from pregnant women. chromID Strepto B agar results can be interpreted after 24 hours incubation with confirmation of characteristic GBS colonies from the media. chromID Strepto B agar is not intended to diagnose infection nor to guide or monitor treatment for infections. chromID Strepto B agar does not provide susceptibility results. Subculture to non-selective media should be performed as needed for susceptibility testing. chromID Strepto B agar is intended for use by laboratory health practitioners in a clinical laboratory.
Specimen Type	18-24 hour LIM broth enrichments of vaginal/rectal swabs	18-24 hour LIM broth enrichments of vaginal/rectal swabs

Similarities		
Item	Granada Medium (K173903)	chromID Strepto B Agar (K163042)
Interpretation	Manual/visual, subculture colonies for susceptibility	Manual/visual, subculture colonies for susceptibility
Culture Media Type	Selective and differential media	Selective and differential media

Differences		
Item	Granada Medium (K173903)	chromID Strepto B Agar (K163042)
Interpretation	Orange colonies representative of β-hemolytic GBS. Subculture white colonies to rule-out weakly β-hemolytic and non-hemolytic GBS.	Growth/color development (pale pink to red colonies) does not distinguish between hemolytic and non-hemolytic GBS.

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

Granada Medium is a selective, differential medium for the detection of GBS. The ability to detect β-hemolytic GBS is based on the presence of selective agents that suppress growth of organisms other than GBS, and the presence of components necessary for pigment production (color development) that allow the detection of β-hemolytic GBS. Granada Medium also supports the growth of non-hemolytic GBS, which are identified by additional testing of white colonies growing on the culture medium. After vaginal/rectal swabs from antepartum women are enriched in LIM Broth, an aliquot of culture is inoculated directly onto Granada Medium and incubated anaerobically at 35°C for 18-24 hours. Plates are examined after incubation for the development of light orange to dark orange colored colonies. Plates with no growth, or where testing of white colonies ruled-out the presence of GBS, are considered negative.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility was demonstrated at three sites using an 11-member panel, which included 5 hemolytic GBS strains, 1 non-hemolytic GBS strain, and 5 non-GBS strains (as the negative controls). At each site, panel members were tested in duplicate on Granada Medium each day for five days—GBS strains (near 10^3 CFU/ml) and non-targets strains (10^7 CFU/ml) resulting in 660 total test results.

Plates were observed for growth and color development after 24 hours at 35°C. The study was conducted with at least one operator and two readers per site who were blinded to study results. All GBS strains produced the expected results with Granada Medium at 24 hours. Isolates were also plated onto Tryptic Soy Agar with 5% sheep blood to ensure viability and purity of cultures.

b. *Linearity/assay reportable range:*

Not Applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Quality Control (QC) Testing
QC testing was performed to examine growth and color development with Granada Medium. Three QC organisms were tested at each study site with Granada Medium for each day of testing. QC testing results provided expected reactions across each testing site (Table 1). The submitted QC data are acceptable.

Table 1. QC Data Summary

QC Strain	Time of Incubation	Expected Results after 18-24 hrs at 35°C	QC Results (all sites) Observed/Expected
Streptococcus agalactiae ATCC 12386	18-24 hrs	Growth, orange colonies	100/100
Streptococcus pyogenes ATCC 19615	18-24 hrs	Growth, white to off-white colonies	100/100
Escherichia coli ATCC 25922	18-24 hrs	Partial to complete inhibition; no color change	100/100

d. *Detection limit:*

Recovery Study
A Recovery Study was performed with two GBS strains (ATCC 12386 and ATCC 12403). After preparing a dilution series per strain in LIM broth, Granada Medium plates were inoculated and incubated anaerobically between 18-24 hours at 35°C before evaluating color development. The lowest dilution at which the strain grew with the expected color reaction on Granada Medium was determined to be the LoD. The LoD concentration for the two GBS strains was confirmed by testing Granada Medium with five replicate dilutions with both strains. The LoD was reported as 10^2 CFU/ml.

Recovery on Granada Medium Upon Subculture from LIM Broth Enrichment

To evaluate the performance of Granada Medium after an overnight enrichment in LIM Broth, LIM Broth was spiked with two β-hemolytic ATCC strains of Group B Streptococci (*Streptococcus agalactiae* ATCC 12386 and *Streptococcus agalactiae* ATCC 12403) prepared in GBS-negative vaginal/rectal specimen matrix at 10-fold decreasing concentrations. After overnight enrichment, the 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 GBS strains originally spiked at approximately 4.5 CFU (1 - 9 CFU). Blood agar plates were used to determine the concentrations of organisms present in each dilution.

Analytical Reactivity

A study was conducted to demonstrate the sensitivity of Granada Medium in detecting various GBS strains at a concentration of 10^2 CFU/ml. A 100 µl aliquot of bacterial suspension prepared in saline was plated onto Granada Medium. The study included 54 ATCC reference and clinical GBS strains representing seven of the nine different serotypes; forty-eight strains were hemolytic and six were non-hemolytic. Plates were read at 24 hours of incubation for colony counts and orange color development (indicative of β-hemolytic GBS). The inclusivity panel and the observed colony color on Granada Medium are shown in Table 2.

Table 2. GBS Panel for Inclusivity Testing

Strain	Source	Hemolysis	Serotype[1]	Observed Colony Color on Granada Medium
BAA-611	ATCC	β	V	Orange
12403	ATCC	β	III	Orange
12386	ATCC	β	II	Orange
8017	NCTC	β	III	Orange
1	Clinical	β	V	Orange
2	Clinical	β	II	Orange
KPWP	Clinical	β	III	Light Orange
P003-001	Clinical	β	Ia	Light Orange
7	Clinical	β	III	Orange
10	Clinical	β	1a	Orange
11	Clinical	β	V	Orange
13	Clinical	β	VI	Orange
3	Clinical	β	II	Orange
4	Clinical	β	VI	Orange
QOVHI	Clinical	β	Ia	Light Orange
27	Clinical	β	III	Orange
28	Clinical	β	III	Orange

14	Clinical	β	IV	Orange
15	Clinical	β	III	Orange
18	Clinical	β	1b	Orange
19	Clinical	β	1b	Light Orange
24	Clinical	β	II	Orange
26	Clinical	β	1b	Orange
29	Clinical	β	II	Orange
30	Clinical	β	1b	Orange
MS2	Clinical	β	III	Orange
MS3	Clinical	β	1b	Orange
MS4	Clinical	β	1a	Orange
MS5	Clinical	β	1b	Orange
MS6	Clinical	β	V	Orange
MS7	Clinical	β	1b	Orange
MS8	Clinical	β	1a	Orange
MS9	Clinical	β	1b	Orange
MS10	Clinical	β	1a	Orange
MS11	Clinical	β	NT[1]	Orange
MS12	Clinical	β	III	Orange
French	Clinical	β	1a	Orange
MS13	Clinical	β	NT	Orange
MS14	Clinical	β	1a	Orange
MS15	Clinical	β	1a	Orange
MS17	Clinical	β	1a	Orange
MS18	ATCC	β	1b	Orange
MS19	ATCC	β	NT	Light Orange
MS26	Clinical	β	1b	Orange
MS27	Clinical	β	III	Orange
MS28	Clinical	β	III	Orange
MS29	Clinical	β	II	Orange
MS30	Clinical	β	1b	Orange
13813	ATCC	Non-hemolytic	II	White
701348	NCIMB	Non-hemolytic	II	White
MS20	Clinical	Non-hemolytic	III	White
MS21	Clinical	Non-hemolytic	III	White
MS22	Clinical	Non-hemolytic	III	White
MS23	Clinical	Non-hemolytic	NT	White

[1] NT= Non-Typable against the nine known serotypes.

On Granada Medium, all 48 β-hemolytic strains produced the expected orange color, and all six non-hemolytic GBS strains showed a negative color reaction. Results also

demonstrated that Granada Medium was able to grow all non-hemolytic GBS strains tested at the LoD (10^2 CFU/ml).

Incubation Study

An Incubation Study was conducted to determine the effect of various incubation times on the performance of Granada Medium when tested with ten GBS strains (hemolytic and non-hemolytic) at 10^2 CFU/ml. A 100 µl aliquot of bacterial suspension prepared in saline was plated onto Granada Medium. The recovery of organisms and development of characteristic color on Granada plates were evaluated every 2 hours from 18-48 hours. At the earliest time point (18 hours), all β-hemolytic organisms produced an orange color reaction. The one non-hemolytic strain tested showed white colonies at each incubation time from 18-48 hours. The earliest incubation time for positive detection of GBS by color development was set at 18 hours. The incubation range for Granada medium was reported as 18-24 hours.

e. Analytical specificity:

Cross-Reactivity Study

In order to evaluate the performance of Granada Medium with microorganisms potentially encountered in vaginal/rectal swabs or related to GBS, a Cross-Reactivity Study was completed with 69 non-target organisms (gram negative bacteria, gram positive bacteria, and yeast). Using a 10 µl loop, non-target organisms were streaked onto Granada Medium from a 10^8 CFU/ml suspension of cells. Results showed that all 69 organisms from the cross-reactivity panel produced a negative result with Granada Medium (no growth or non-orange colonies). A total of 35 organisms (50.7%) were recovered on Granada Medium after 24 hours incubation. The cross-reactivity panel is shown in Table 3.

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

Organism		
Acinetobacter baumannii	*Enterococcus faecium*	*Pseudomonas aeruginosa*
Aeromonas hydrophila	*Enterococcus flavescens*	*Pseudomonas fluorescens*
Bacillus cereus	*Enterococcus saccharolyticus*	*Salmonella enterica (typhii)*
Bacillus subtilis	*Escherichia coli*	*Salmonella enterica arizonae*
Bacteroides fragilis	*Gardnerella vaginalis*	*Serratia marcescens*
Bifidobacterium breve	*Geotrichum candidum*	*Shigella boydii*
Campylobacter jejuni	*Klebsiella oxytoca*	*Shigella flexneri*
Candida albicans	*Klebsiella pneumoniae*	*Shigella sonnei*
Candida glabrata	*Lactobacillus acidophilus*	*Staphyloccocus aureus*
Candida parapsilosis	*Lactobacillus gasseri*	*Staphylococcus epidermidis*
Candida tropicalis	*Lactobacillus leichmannii*	*Staphylococcus saprophyticus*
Citrobacter freundii	*Lactococcus lactis*	*Stenotroph. maltophilia*
Clostridium difficile	*Legionella pneumophila*	*Streptococcus anginosus*
Clostridium novyi	*Listeria monocytogenes*	*Streptococcus bovis*

Organism		
Clostridium perfringens	*Moraxella cartarrhalis*	*Streptococcus dysgalactiae*
Clostridium sporogenes	*Morganella morganii*	*Streptococcus mitis*
Enterobacter aerogenes	*Neisseria gonorrhoeae*	*Streptococcus mutans*
Enterobacter cloacae	*Pediococcus acidilacti*	*Streptococcus pneumoniae*
Enterococcus casseliflavus	*Pediococcus damnosus*	*Streptococcus pyogenes*
Enterococcus cecorum	*Peptostreptococcus anaerobius*	*Streptococcus salivarius*
Enterococcus dispar	*Plesiomonas shigelloides*	*Streptococcus uberis*
Enterococcus durans	*Proteus mirabilis*	*Vibrio parahaemolyticus*
Enterococcus faecalis	*Providencia alcalifaciens*	*Yersinia enterocolitica*

Cross-Reactivity from LIM Broth Enrichment

In a separate study, 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. $1.5x10^6$ CFU of each organism was inoculated into a LIM Broth tube. After 24 hours incubation, each LIM Broth culture tube was subcultured to a Granada Medium plate using a 10 µl loop and evaluated for growth and color reaction after 24 hours of anaerobic incubation. All organisms tested were considered to produce no cross reaction. 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.

Interference Study

The purpose of this study was to evaluate the impact of potentially interfering substances commonly found in vaginal/rectal swab specimens on the detection of six hemolytic GBS strains. Twenty-one substances (Table 4) were tested at physiologically or biologically relevant concentrations and mixed with bacterial suspensions in GBS-negative specimen matrix. A 30 µl aliquot of GBS suspension (9-90 CFU) was inoculated into LIM Broth and incubated at 35°C. After overnight incubation, a 10 µl aliquot of LIM Broth culture was inoculated onto Granada Medium. Growth and development of color were evaluated after 18 hours and 24 hours incubation on Granada Medium. No interference on Granada Medium was observed for any substance (at the highest clinically relevant concentration) when GBS was prepared in negative specimen matrix and enriched in LIM Broth in the presence of potentially interfering substances.

Table 4. List of Potential Interfering Substances

Category	Substance/Active Ingredient or Supplier	Concentration in Sample Matrix
Exogenous Substances		
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	LEE Biosolutions	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

Microbial Interference Study

A Microbial Interference Study was conducted to demonstrate that high levels of non-target organism would not suppress color development and recovery of GBS *(Streptococcus agalactiae*, ATCC 12386 and *Streptococcus agalactiae*, ATCC 12403). Organisms that were recovered from Granada Medium in the Cross-Reactivity Studies were tested in the Microbial Interference Study. Non-target organisms at a concentration of 3 $x10^8$ CFU/ml were mixed 1:1 with 1.5 $x10^4$ CFU/ml of target organism and streaked for isolation onto Granada Medium with a 10 µl loop. If the target organism was not recovered, the concentration of the non-target organism was lowered 10-fold until the target organism was recovered. A total of 51 non-target organism strains were included in the study.

At 24 hours, both GBS strains gave expected results (orange colonies of the appropriate size) in the presence of 42 non-target organisms in the Microbial Interference Study. However, GBS in the presence of the following 10 non-target organisms produced unexpected results: *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecalis* (ATCC 51299), *Enterococcus avium* (ATCC 14025), *Enterococcus gallinarium* (ATCC 49573), *Enterococcus saccharolyticus* (ATCC 43076), *Lactococcus lactis* (ATCC 19435), *Morganella morganii* (ATCC 25830), *Proteus mirabilis* (ATCC 43071), *Serratia marcescens* (ATCC 13880), and *Vibrio parahaemolyticus* (ATCC 17802). In the presence of $1.5x10^8$ CFU/ml of *Enterococcus faecalis* (ATCC 29212), both strains of GBS were not recovered on Granada Medium; however, when *Enterococcus faecalis* (ATCC 29212) was reduced to $1.5x10^7$ CFU/ml, GBS strain ATCC 12403 was recovered (a further reduction to $1.5x10^6$ CFU/ml allowed for the recovery of GBS strain ATCC 12386). For the remaining (9) non-target strains producing unexpected results in the

Microbial Interference Study, it was observed that the colony size of one or both GBS was affected when the non-target organism was tested at $1.5x10^8$ CFU/ml. When these non-target organisms were inoculated onto Granada Medium at $1.5x10^7$ CFU/ml in the presence of GBS, it was reported that GBS colony size was larger and more clearly visible in the mixed culture. Also, in the presence of $1.5x10^8$ CFU/ml *Vibrio parahaemolyticus*, GBS colonies appeared as light orange colonies. The expected color (dark orange) was observed when the *Vibrio parahaemolyticus* concentration was reduced to $1.5x10^7$ CFU/ml. Inhibition of growth and changes in colony size/color with these specific non-target organisms are noted as Limitations in the product labeling.

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable—Compared to Standard Reference Method

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

Granada Medium was evaluated at four clinical sites. A total of 884 vaginal/rectal swabs from pregnant women were prospectively collected and inoculated per protocol into LIM broth, followed by subculture onto Granada Medium. Due to protocol deviations (enrollment criteria not met), 108 specimens were excluded from the study, leaving 776 swab specimens for evaluating GBS detection by Granada Medium. An additional 5 samples were excluded due to improper storage (time of set-up) as specified by the clinical study protocol. In total, 771 protocol compliant specimens were included in the final performance calculations.

To evaluate performance of Granada Medium, LIM Broth enriched cultures (vaginal/rectal swabs inoculated and incubated in LIM Broth overnight) were subcultued onto Granada Medium plates and streaked for isolation. Granada plates were incubated under anaerobic atmosphere for 18-24 hours at 35-37°C. For the Reference Culture Method, vaginal/rectal specimens were tested by inoculating LIM Broth with 30 µl of specimen from the transport swab system and incubating for 24 hours at 35-37°C. LIM Broth cultures were subcultured to Tryptic Soy Agar with 5% Sheep Blood, and all colonies with characteristic appearance suggestive of GBS

were screened to confirm the presence of GBS (both hemolytic and non-hemolytic strains) using established laboratory methods: gram stain, catalase, latex agglutination.

Results of Granada Medium from 18-24 hours incubation were compared to the Reference Culture Method. Growth and color development on Granada Medium were observed after 18-24 hours. Growth of orange colonies was representative of a positive result for the presence of GBS. White colonies growing on the media were subject to biochemical testing to identify non-hemolytic GBS. Tables 5-7 below show the clinical performance data for Granada Medium vs the Reference Culture Method (all sites). Performance (sensitivity and specificity) of Granada Medium was calculated based on color development and the recovery of GBS from the medium and compared to the recovery of β-hemolytic GBS and total GBS strains by the Reference Culture Method.

All isolates with discrepant results were frozen in CryoSavers with Brucella Broth and returned to Hardy Diagnostics for testing. The identity of each isolate was confirmed (β-hemolytic GBS, non-hemolytic GBS, or non-GBS isolate). Once the identity was confirmed, positive organisms (β-hemolytic GBS or non-hemolytic GBS) were tested in donated GBS negative-vaginal/rectal matrix for their recovery from the LIM Broth Culture Reference Method and color development on Granada Medium.

Table 5. Comparison between Granada Medium+ Biochemical Testing (Recovery of all GBS) vs Reference Culture Method (all GBS)

Granada Medium (18-24 hrs)	**Reference Culture Method**		
	Positive	Negative	Total
Positive	162	10	172
Negative	1	598	599
Total	163	608	771
Sensitivity: 99.4% (161/163), 95% CI (96.6%-99.9%) Specificity: 98.4% (598/608), 95% CI (97.0%-99.1%)			

Table 6. Comparison between Granada Medium (orange color) vs Reference Culture Method (all GBS)

Granada Medium (18-24 hrs)	**Reference Culture Method**		
	Positive	Negative	Total
Positive	154	12[1]	166
Negative	9[2]	596	605
Total	163	608	771
Sensitivity: 94.5% (154/163), 95% CI (89.8%-97.1%) Specificity: 98.0% (596/608), 95% CI (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-hemolytic GBS. Two isolates were recorded as pale orange on Granada Medium by the clinical site, but were not identified as GBS. During discrepant analysis, both isolates were confirmed to be Streptococcus salivarius subsp. salivarius. Upon*

additional analysis, one Streptococcus salivarius subs. salivarious isolate exhibited yellow colonies on Granada Medium and the other isolate grew as pale orange colonies on Granada Medium.

[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-hemolytic 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 observed as white at the clinical site. Samples where the six non-hemolytic 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).

Table 7. Comparison between Granada Medium (orange color) vs Reference Culture Method (only β-hemolytic GBS)[1]

Granada Medium (18-24 hrs)	**Reference Culture Method**		
	Positive	Negative	Total
Positive	153	13	166
Negative	3	602	605
Total	156	615	771
Sensitivity: 98.1% (153/156), 95% CI (94.5-99.3%) Specificity: 97.9% (602/615), 95% CI (96.4-98.8%)			

[1]Considering that non-hemolytic GBS cannot be detected by the medium's color change and require additional identification, there were six specimens that were found to contain non-hemolytic GBS strains upon subculture and identification by the Reference Method. If these specimens are included as negatives, then the overall sensitivity and specificity values observed when comparing the recovery of β-hemolytic GBS by the LIM Reference Method to the Granada Medium color development can also be evaluated.

b. Clinical specificity:

See above

c Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

The overall prevalence of GBS (both hemolytic and non-hemolytic strains) by the Reference Culture Method was 21.1% (163/771).

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.