



C-316

# Evaluation of Accuracy of StrepB Carrot Broth™ in the Detection of Different Serotypes of Group B Streptococci (GBS)

R. Facklam<sup>1</sup>, J. Elliott<sup>1</sup>, A. Y. Hsiung<sup>2</sup>, R. Clasen<sup>2</sup>, G. Peterson<sup>2</sup>, S. Strickler<sup>2</sup>, J. Hardy<sup>2</sup>

<sup>1</sup>Centers for Disease Control and Prevention, Atlanta, GA | <sup>2</sup>Hardy Diagnostics, Santa Maria, CA

## Abstract

Detection of GBS in the vaginal-anorectal area is critical for the prevention of neonatal GBS disease. Several microbiological assays employing different methods have been developed worldwide with increased sensitivity, reduced costs, and shorter turnaround time for the detection of GBS. However, very little has been documented concerning the accuracy of each methodology in the detection of different serotypes of GBS. The goal of this study was to evaluate StrepB Carrot Broth™ against several serotypes of GBS and a few rare pigmented strains of *Enterococci* and *Streptococci*. A total of 50 isolates (45 GBS of several serotypes, four pigmented *Enterococcus*, and one *Streptococcus porcinus*) were retrieved from Centers for Disease Control and Prevention's collection and inoculated into StrepB Carrot Broth™ in order to evaluate the ability of this medium to accurately identify GBS. Overall, StrepB Carrot Broth™ demonstrated 100% sensitivity and 100% specificity against all the beta-hemolytic GBS, and produced no false positives against pigmented *Enterococci* and *S. porcinus* which is known to cross-react with group B antisera. As expected, all non-hemolytic GBS produced negative results. Based on these findings, StrepB Carrot Broth™ can be employed as a reliable method for the detection of beta-hemolytic Group B Streptococci.

## Introduction

Approximately 10% to 30% of pregnant women are colonized with GBS in the vaginal-rectal region which can be transmitted to the neonate during delivery resulting in sepsis, pneumonia, or other complications. GBS is the most frequent cause of systemic infection in neonates under 7 days of age.<sup>(1)</sup> To prevent GBS infections in neonates, the CDC recommends screening all pregnant women for vaginal and rectal GBS between 35 and 37 weeks of gestation. During birth GBS infections can be prevented by administering interpartum antibiotics to all pregnant women identified as carriers of GBS. Due to these measures, the incidence of GBS illness in neonates has been greatly reduced.<sup>(2)</sup>

The current gold standard method for GBS detection is incubation in LIM broth followed by subculture onto a blood agar plate.<sup>(2)</sup> Alternative methods have recently been developed to reduce laboratory costs and decrease turnaround time. On Granada medium<sup>(5)</sup> GBS will produce a distinct orange-red pigment

## Introduction (continued)

making identification easy. StrepB Carrot Broth™ (Hardy Diagnostics, Santa Maria, CA) is a test tube assay based on this principle. StrepB Carrot Broth™ is designed to detect GBS directly from a patient specimen without the need for subculture or pre-incubation. StrepB Carrot Broth™ is a selective and differential media and will detect GBS within 6 to 24 hours after inoculation.

Previous studies have evaluated StrepB Carrot Broth™ for sensitivity and specificity.<sup>(3)</sup> The purpose of this study is to determine the accuracy of StrepB Carrot Broth™ in the detection of different serotypes of GBS. GBS has been classified into nine serotypes: Ia, Ib, II, III, IV, V, VI, VII, and VIII. The majority of isolates from pregnant women and infected neonates in the United States are serotypes Ia, II, III, and V.<sup>(1)</sup> Serotypes VI and VII are becoming more predominant in Japan<sup>(9)</sup> while serotype IV is emerging in Europe.<sup>(6)</sup> Serotype III GBS is associated with more invasive disease in infants than is any other serotype.<sup>(4)</sup> Due to the variety of serotypes commonly isolated it is vital that detection methods for GBS accurately identify all serotypes of GBS.

## Materials and Methods

All of the strains tested were retrieved from the Center for Disease Control and Prevention's collection.

- 50 total strains were tested.
- All 9 serotypes were tested.
- 9 non-hemolytic strains were tested.
- 5 Non-GBS strains (4 pigmented *Enterococcus* and 1 *S. porcinus*) were tested.

### Suspension Preparation and Inoculation

- Swabs were used to inoculate the StrepB Carrot Broth™ as per the manufacturer's instructions.
- Cultures were incubated at 35°C for 24 hours.

### Interpretation

- All tubes that turned orange were considered positive.
- All tubes that formed small orange to red spots on the swab were considered weakly positive.
- All tubes which showed no color change were considered negative.

## Results

**Table 1:** Accuracy of StrepB Carrot Broth™ in the detection of different serotypes of Beta Hemolytic *Streptococcus agalactiae*.

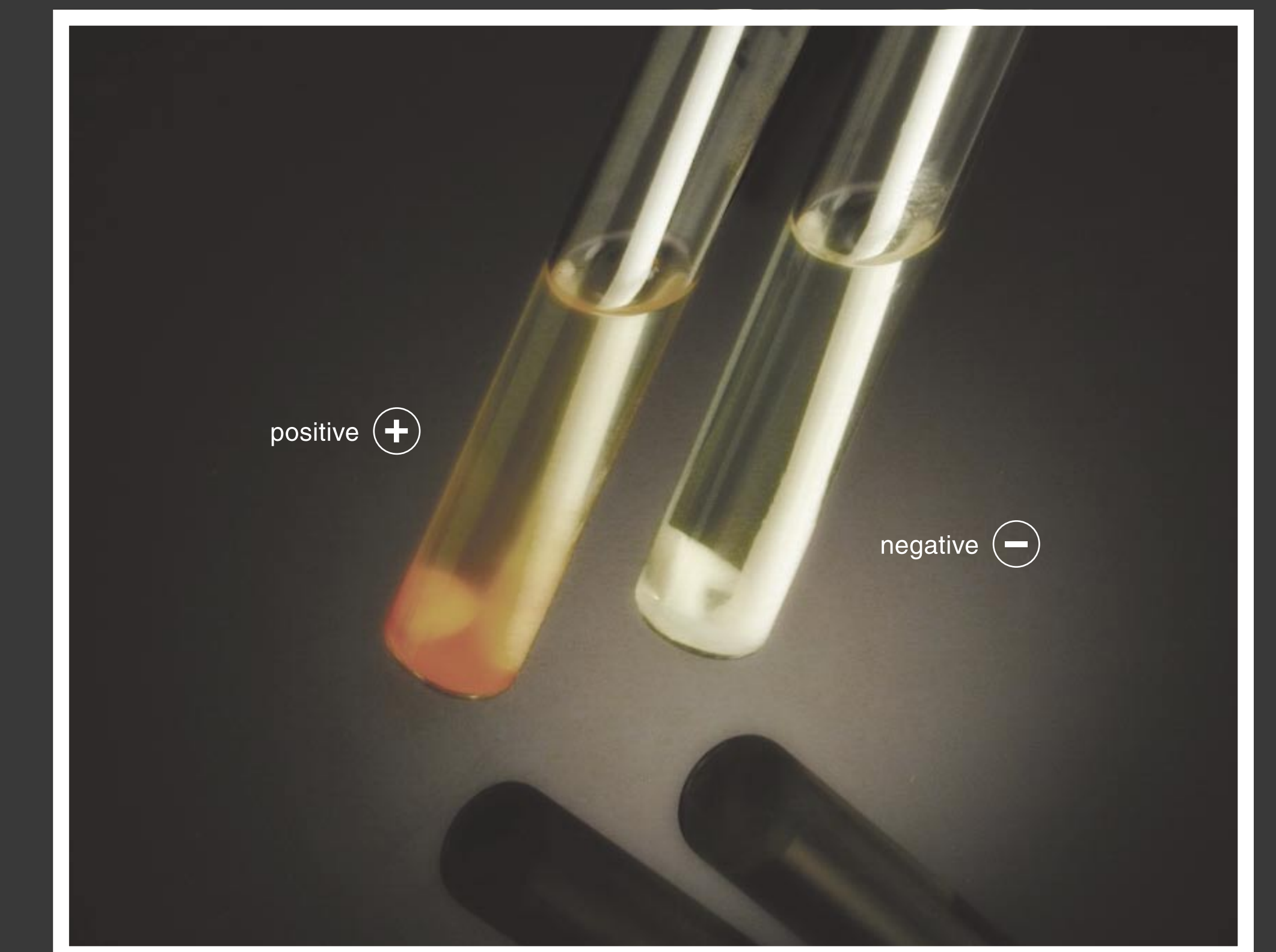
Strain	Serotype	Number of Isolates Tested	Number of Isolates StrepB Carrot Broth™ positive	% Isolates Positive
<i>S. agalactiae</i>	Ia	6	6	100%
<i>S. agalactiae</i>	Ib	4	4	100%
<i>S. agalactiae</i>	II	4	4	100%
<i>S. agalactiae</i>	III	4	4	100%
<i>S. agalactiae</i>	IV	4	4	100%
<i>S. agalactiae</i>	V	8	8	100%
<i>S. agalactiae</i>	VI	1	1	100%
<i>S. agalactiae</i>	VII	1	1	100%
<i>S. agalactiae</i>	VIII	2	2	100%
<i>S. agalactiae</i>	Non-typeable	2	2	100%
<b>Total</b>		<b>36</b>	<b>36</b>	<b>100%</b>

**Table 2:** Results of StrepB Carrot Broth™ against non-hemolytic *Streptococcus agalactiae*, *Streptococcus porcinus*, and pigmented *Enterococcus* strains.

Species	Number of Isolates Tested	Number of Isolates StrepB Carrot Broth™ positive	% Isolates Positive
<i>S. agalactiae</i> (non-hemolytic)	9	0	0%
<i>S. porcinus</i>	1	0	0%
<i>S. casseliflavus</i>	1	0	0%
<i>S. faecalis</i> (variant)	3	0	0%
<b>Total</b>	<b>14</b>	<b>0</b>	<b>0%</b>

## Discussion

- StrepB Carrot Broth™ detected all beta-hemolytic GBS regardless of serotype.
- None of the non GBS strains tested positive using StrepB Carrot Broth™, including *S. porcinus* which is known to cross-react with group B antisera.<sup>(15)</sup>
- As expected none of the non-hemolytic GBS strains tested positive.
- StrepB Carrot Broth™ was 100% sensitive and specific against all reference isolates tested.
- Based on these findings StrepB Carrot Broth™ can be considered a reliable test for detection of all beta-hemolytic GBS.



## References

- Hickman M, Rench M, Ferrieri P, Baker C. Changing Epidemiology of Group B Streptococcal Colonization. *Pediatrics*, 1999. 104:2:203-209
- Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of Perinatal Group B Streptococcal Disease. Revised guidelines from CDC. *MMWR Recommendations and Reports*, 2002. 6:51(RR-11):1-22
- Schreckenberger et al. (2005) Evaluation of StrepB Carrot broth™ and LIM broth methods for recovery of group B streptococci (GBS). Results of a multi-center trial. Abstracts of the Annual Meeting of the ASM (C-109).
- Davies H, Jones N, Whittam T, Elsayed S, Bisharat N, Baker C. Multilocus Sequence Typing of Serotype III Group B Streptococcus and Correlation with Pathogenic Potential. *J Infect Dis*, 2004. 181:1097-1102
- de la Rosa M, Perez M, Carazo C, Pareja L, Peis JI, Hernandez F. New Granada Medium for Detection and Identification of Group B Streptococci. *J Clin Microbiol*, 1992. 30:1019-1021.
- Jelinkova J, Motolova J. Worldwide distribution of two new serotypes of group B streptococci: type IV and provisional type V. *J Clin Microbiol*, 1985. 21:361-362
- Juncosa T, et al. [Usefulness of the Granada Culture Media in the Detection of Pregnant Women Carrying Streptococcus agalactiae]. *Enferm Infecc Microbiol Clin*, 1995. 13(9):572-3.
- Kelly VN, Garland SM. Evaluation of new Granada medium (modified) for the antenatal screening of group B streptococcus. *Pathology*, 1994. 26(4):487-9.
- Lachenauer CS, Kasper DL, Shimada J, et al. Serotypes VI and VII Predominate Among Group B Streptococci Isolated From Pregnant Japanese Women. Presented at the Program and Abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1997. 318:K80
- Overman SB, et al. Evaluation of Methods to Increase the Sensitivity and Timeliness of Detection of Streptococcus Agalactiae in Pregnant Women. *J Clin Microbiol*, 2002. 40(11):4329-31.
- Picard FJ, Bergeron MG. Laboratory Detection of Group B Streptococcus for Prevention of Perinatal Disease. *Eur J Clin Microbiol Infect Dis*, 2004. 23(9):665-71.
- Regan JA, Klebanoff MA, Nugent RP. Vaginal Infections and Prematurity Study Group. The Epidemiology Of Group B Streptococcal Colonization In Pregnancy. *Obstet Gynecol*, 1991. 77:604-10.
- Rousset A, Tytgat F, Minck R. [The Importance of Lancefield Group B Streptococci (S. agalactiae) in Gynecology and obstetrics.] *J Gynecol Obstet Biol Reprod (Paris)*, 1979. 8(3):219-21
- Schuchat, Anne. Epidemiology of Group B Streptococcal Disease In The United States: Shifting Paradigms. *Clin Microbiol Rev*, 1998. 2:3:497-513
- Thompson T, Facklam R. Cross-reactions of Reagents from Streptococcal Grouping Kits with Streptococcus porcinus. *J Clin Microbiol*, 1997. 35(7):1885-6.