Modified GC Medium for Identification of Group B Streptococci

Somchai Santiwatanakul, PhD*, Chantana Mekseepralard, PhD**,
Wongsawat Sawatpunyachote, BSc (MT)***, Sarayuth Susasin, BSc (MT)***

** Department of Pathology, Faculty of medicine, Srinakharinwirot University*
*** Department of Microbiology, Faculty of medicine, Srinakharinwirot University*
**** Medical technology student at Faculty of Align Health Science, Chulalongkorn University*

The modified GC medium (MGC) was developed for identification of beta-hemolytic group B streptococci. This medium was developed on the basis of enhancingñpigment production of group B streptococci. Three hundred and thirty isolates were tested including 180 isolates of beta-hemolytic group B streptococci, 102 isolates of beta-hemolytic non-group B streptococci, and 48 isolates of Enterococcus faecalis. All isolates of group B streptococci gave carotenoid pigment by this medium. On the other hand, all of non-group B streptococci and E. faecalis did not show pigment after 72 h incubation. The specificity and sensitivity of MGC was 100 %. There were no false positive and false negative in this medium. The MGC may be the alternative of choice for the presumptive identification of group B streptococci.

Keywords: *MGC, Carotenoid pigment, Group B streptococci, GBS, Streptococcus lactiae, beba-hemolytic streptococci*

J Med Assoc Thai 2005; 88(Suppl 1): S1-4
Full text. e-Journal: *http://www.medassocthai.org/journal*

Group B streptococci (GBS; Streptococcus agalactiae) are the most important pathogens in peripartum women and their newborn infants[1]. This organism can cause neonatal morbidity and mortality[2]. Among pregnant women, the prevalence of colonization with GBS in cervical range from 10-40%[3,4]. It also transfers from mother to newborn infant range from 40-73%[3,5]. The incidence of sepsis in newborns is 2-3 cases per 1000 live births and the death rate is 15%[6,7]. In Thailand, Yossuck and Preedisripipat[8] reported that the incidence of neonatal group B streptococcal bacteremia from 1996 to 2001 was very low but with a very high mortality.

In 2002 the Centers for Disease Control and Prevention (CDC) published guidelines designed to minimize the risk of neonatal GBS disease[9]. To detect GBS carriers, CDC advises that two separate swabs of the distal vagina and anorectum or a single vaginoarectal swab are cultured prenatally. The use of selective broth, such as Todd-Hewitt broth, supplemented with nalidixic and either gentamicin[10] or colistin[11] was also specified. Specimens should be incubated in the broth and subcultured onto blood agar plates that are screened for beta-hemolytic colonies, which can then be identified as GBS by antigen detection, with genetic probes, or by CAMP test.

CAMP test[12,13] has been long used in many countries as a presumptive identification of GBS in pregnant women. In 1977 Islam[14] developed a new medium to detect GBS by its property of carotenoid pigment production which is closely linked to the beta-hemolysin characteristic[15]. This medium is composed of horse serum and soluble starch for enhancing pigment production of GBS. Sukroongreung et al [16] developed a PPR medium by using proteose peptone No. 3 (Difco) and rice powder as well as the buffer system to enhance the carotenoid pigment production of beta-hemolytic group B streptococci. This medium, which is inexpensive

Correspondence to : Santiwatanakul S, Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Thaveewattana, Bangkok 10300, Thailand. Phone: 0-2260-2122 ext 4401, Fax: 0-2623-0292, E-mail: somchaii@swu.ac.th

and easy to prepare, is used in most of the laboratories in Thailand. However, proteose peptone No. 3 is not routinely used in the microbiology laboratory and it may expire before it can all be used. Therefore, the authors tried to develop another medium from the routine media as an alternative media for detection of GBS. GC agar base (Oxoid) is one of the routine media chosen in the present study.

Material and Method

Bacterial strain collection

Three hundred and thirty isolates of beta-hemolytic streptococci and *Enterococcus faecalis* were obtained from clinical specimens, which were from Vajira Hospital, Bangkok, Thailand and Sirindhorn Medical Center, Ong-Karag, Thailand from April 2003 to May 2005. These isolates were composed of 180 isolates of beta-hemolytic group B streptococci, including 152 vaginal or cervical specimens and 28 perianal regions, and 102 isolates of beta-hemolytic non-group B streptococci from various sites of human sources as well as 48 isolates of *E. faecalis*. All isolates were serologically identified by latex agglutination (Slidex Strepto-Kit, BioMårieux) and by CAMP test. Their ability to grow on 6.5% NaCl, and bile aesculin reactions were recorded accordingly.

Medium for Enhancing pigment formation

Medium for enhancing pigment formation was prepared by using GC agar base (Oxoid) as a base medium. This medium (MGC, modified GC medium) contained 3.26% GC agar base, 5% rice powder, and 0.5% NaCl (pH 7.2). MGC medium was distributed into 13 x 75 mm test tubes and sterilized by autoclaving. They were allowed to cool and kept in the refrigerator overnight before further use.

Pigmentation test

Each isolate was heavily stabbed by the side of the tube down to the bottom of the MGC tube. The tubes were incubated at 35^0C aerobically. The pigment producing ability was noted after 24, 48, and 72 h incubation.

Results

The carotenoid pigment production ability of 180 beta-hemolytic strains of group B streptococci on the MGC medium was 100% (Table 1). The 102 isolates of non-group B streptococci and 48 isolates of E. faecalis did not produce any pigment at up to 72 h incubation. One hundred and seventy eight isolates (99%) of pigment producing GBS gave positive results in 24 h. The other isolates produced pigment at 48 h and 72 h.

Discussion

GBS have been recognized as a significant cause of neonatal and adult disease[1,2]. Although CAMP test has been used as the detection for beta-hemolytic group B streptococci, alternative tests has been developed on the basis of pigment production[14,16-18]. The development of MGC medium was also an alternative choice for testing the pigment production of GBS. The heavy stabbing of these isolates by the side of the tube down to the bottom of medium enhanced anaerobiosis and pigmented colonies were easily seen[19-21]. The special peptone in GC agar base was able to support the growth of 180 isolates of GBS as orange colonies within 24 h (99%) and after 24 h (1%). This result was similar to PPR medium[16].

The specificity and sensitivity of MGC medium were 100% for beta-hemolytic group B streptococci, which compared well with other effective pigment enhancing media[14,16-18]. There were no false positive results among 102 isolates of non-group B streptococcal types and 48 isolates of E. faecalis other than GBS.

In conclusion, MGC medium may be an alternative choice for the presumptive identification

Table 1. The carotenoid pigment production in MGC medium of 282 isolates of beta hemolytic streptococci and 48 isolates of *Enterococcus fecalis*

	Latex agglutination grouping				Non group A,B,C or G	*Enterococcus fecalis*
	B	A	C	G		
Strain beta-lysis	180	45	10	12	35	48
Pigment production	180	0*	0	0	0	0

* no pigment observed

of GBS. It can be easily prepared and used in most of microbiology laboratories.

References

1. Schuchat A. Epidemiology of group B streptococci disease in the United States: shifting paradigms. Clin Microbiol Rev 1998; 11: 497-513.
2. Shet A, Ferrieri P. Neonatal & maternal group B streptococcal infections: A comprehensive review. Indian J Med Res 2004; 120: 141-50.
3. Hafner E, Sterniste W, Rosen A, et al. Group B streptococci during pregnancy: a comparison of two screening and treatment protocols. Am J Obstet Gynecol 1998; 179: 1-8.
4. Regan JA, Klenbanoff MA, Nugent RP. The epidemiology of group B streptococcal colonization in pregnancy. Obstet Gynecol 1991; 77: 604-10.
5. Boyer KM, Gotoff SP. Prevention of early onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. N Eng J Med 1986; 314: 1665-9.
6. ACOG Committee on Obstetric Practice. Prevention of early-onset group B streptococci disease in new borns. ACOG committee opinion. No.173. Washington, DC: American College of Obstertricians and Gynelogists, 1996.
7. Morales WS, Dickey SS, Bornick P, Lim DV. Change in antibiotic resistance of group B streptococcus: impact on intrapartum management. Am J Obstet Gynecol 1999; 181:1-8.
8. Yossuck P, Preedisripipat K. Neonatal group B streptococcal infection: incidence and clinical manifestation in Siriraj Hospital. J Med Assoc Thai 2002; 85(Suppl 2): S479-87.
9. Centers for Disease Control and Prevention. 2002. Prevention of perinatal group B streptococcal disease: a public health perspective. Morb Mortal Wkly Rep; 51(RR-11): 1-24.
10. Baker CJ, Clark DJ, Barret FF. Selective broth medium for isolation of group B streptococci. Appl Microbiol 1973; 26: 884-5.
11. Jones DE, Friedl EM, Kannarek KS, Williams JK, Lim DV. Rapid identification of pregnant women heavily colonized with group B streptococci. J Clin Microbiol 1983; 18: 558-60.
12. Wilkinson HW. CAMP-disk test for presumptive identification of group B streptococci. J Clin Microbiol 1977; 6: 42-5.
13. Darling CL. Standardization and evaluation of the CAMP reaction for the promt, presumptive identification of *Streptococcus agalactiae* (Lancifield group B) in clinical material. J Clin Microbiol 1975; 1: 171-4.
14. Islam AKMS. Rapid recognition of group of group B hemolytic streptococci. Lancet 1977; i: 256-7.
15. Tapsall JW. Pigment production by Lancefield-group-B streptococci (*Streptococcus agalactiae*). J Med Microbiol 1986; 21: 75-81.
16. Sukroongreung S, Nilakul C, Tuntimavanich S, Santiwatanakul S. New medium for enhancing pigment production of group B streptococci. J Clin Pathol 1984; 37: 1310-1.
17. Noble MA, Bent JM, West AB. Detection and identification of group B streptococci by use of pigment production. J Clin Pathol 1983; 36: 350-2.
18. de la Rosa M, Villareal R, Vega D, Miranda C, Martinezbrocal A. Granda medium for detection and identification of group B streptococci. J Clin Microbiol 1983; 18: 779-85.
19. Merritt K, Treadwell TL, Jacobs NJ. Rapid recognition of group B streptococci by pigment production and counterimmunoelectrophoresis. J Clin Microbiol 1976; 3: 287-90.
20. Merritt K, Jacobs NJ. Improved medium for detecting pigment production by group B streptococci. J Clin Microbiol 1976: 379-80.
21. Merritt K, Jacobs NJ. Characterization and incidence of pigment production by human clinical group B streptococci. J Clin Microbiol 1978; 8: 105-7.

การพัฒนา GC medium เพื่อการตรวจหาเชื้อ Group B streptococci

สมชาย สันติวัฒนกุล, จันทนา เมฆสีประหลาด, วงศ์สวัสดิ์ สวัสดิ์ปัญญาโชติ, สรายุทธ์ สุสะสินธิ์

การตรวจหาเชื้อ beta-hemolytic group B streptococci ได้รับการพัฒนาขึ้นโดยใช้ modified GC medium (MGC) ซึ่งอาศัยพื้นฐานการผลิตรงคหวัตถุของเชื้อ group B streptococci เชื้อจากสิ่งส่งตรวจ 330 ตัวอย่าง ได้แก่ เชื้อ beta-hemolytic group B streptococci 180 ตัวอย่าง เชื้อ beta-hemolytic non-group B streptococci 102 ตัวอย่าง และ Enterococcus faecalis 48 ตัวอย่าง ได้รับการทดสอบกับ MGC medium ผลการทดสอบพบว่าเชื้อ group B streptococci ทั้งหมดจะสร้างรงคหวัตถุแบบ carotenoid ใน MGC medium ในขณะที่เชื้อ non-group B streptococci และ E. faecalis ไม่สร้างรงคหวัตถุใน MGC medium หลังการอบเพาะนาน 72 ชั่วโมง ความไวและความจำเพาะในการใช้อาหารเลี้ยงเชื้อดังกล่าวพบร้อยละ 100 และไม่พบผลบวกลวงและผลลบลวง ดังนั้น MGC medium น่าจะเป็นทางเลือกอีกทางในการตรวจวินิจฉัยเบื้องต้นของเชื้อ group B streptococci