IMPROVEMENT OF TRANSPORT CONDITION OF SWABS FOR GROUP B STREPTOCOCCAL (GBS) SCREENING

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ABSTRACT

Background For the screening-based strategy for prevention of perinatal GBS disease, CDC Guidelines as many others recommends use of appropriate transport media (Amies, Stuart, e.g.) and processing of specimen as soon as possible within 1 to 4 days. False negative cultures occur for several causes including lost of GBS viability during transport. Could Lim broth, recommended for the selective enrichment, and Granada tubes be used as transport media for swab? Simulating conditions of routine practice, Lim broth and Granada tubes, were evaluated in vitro as transport media.

Methods Tubes of 3 brands of Lim broth (Becton Dickinson, bioMérieux, Copan) and Granada tubes (bioMérieux) were inoculated with low inocula of 10-100 CFU of GBS. Each type of tubes was incubated at 4°C, room T° (RT) and 35°C. GBS were enumerated from each tube by quantitative subculture on blood agar after 1, 2, 3 and 4 days of storage at the different T°. All tests were processed in triplicates with 3 strains of GBS belonging to serotype Ia, III and V.

Results Per type of media, no difference of survival was observed between the 3 strains. T° had significant impact on GBS recovery for each type of tubes. At 4°C the viability was hardly sustained along the 4 days. At RT and 35°C, an increase >6 log of the inocula was observed. The increase of GBS density was sustained at least 4 days for the 3 brands of Lim broth. For the Granada broth, such increase was also observed but at day 3 for tubes incubated at 35°C, viability decreased and for some tubes, GBS subcultures were negative at day 3 or 4.

Conclusions To improve sensitivity of GBS screening cultures, Lim broth could be recommended as a strong transport media and the advisable storage condition would be RT to 35°C up to 4 days. In this way, initiating selective enrichment culture at the time of collection of specimen would provide higher sensitivity even for low density of colonization. Transport at 4°C should be avoided in favour with RT to 35°C. Studies in clinical setting are expected. For Granada tubes, storage at RT was fine but improvement seemed restricted in time at 35°C as there was a loss of viability after 3 days. For Granada tubes, extended evaluation and delimitation of use are needed.

BACKGROUND

In 2013, neonatal GBS diseases remain a notable global public health concern. From the 1990s to the present, where guidelines for prevention of perinatal GBS disease have been widely implemented, the incidence of neonatal early onset disease (EOD) has dramatically decreased to <0.5 cases per 1,000 live births but has not been eradicated. Despite preventive strategies, cases still occur and continues to be an important cause of neonatal sepsis and meningitis.

The screening-based strategy for prevention of perinatal GBS disease recommends that all pregnant women should be screened between 35-37 week's gestation for GBS vaginal and rectal colonization. Antibiotics should be given at time of delivery to women identified GBS positive. Among crucial criteria to control in this strategy are the type of specimen collected, the time of collection, transport conditions and microbiological procedures. CDC Guidelines as many others recommends use of appropriate transport media (Amies, Stuart, e.g.) and processing of specimen as soon as possible within 1 to 4 days. False negative cultures occur for several causes including lost of GBS viability during transport. Several studies showed the negative impact of the length of time that has elapsed between collection and inoculation to the recovery of GBS. In Belgium the recommended procedure for culture includes a selective enrichment step in Lim broth further sub-cultured on selective differential agar as Granada medium or on a specific chromogenic agar.

Aiming improvement of transport conditions to sustain GBS viability, could Lim broth, recommended in the culture procedure, and Granada tubes be used as swab transport media or would their selective antimicrobial content impair viability of GBS after prolonged exposure ?

OBJECTIVES

This study was designed for the evaluation of GBS viability in Lim broth and Granada tubes in varied conditions simulating transport/ storage in routine practice, aiming to recommend they use as transport media.

- **Lim broth from 3 different manufacturers and a Granada biphasic broth from bioMérieux.**
- **Storage during 96 hours.**
- **Storage at 4-8°C, at room temperature (RT) and at 35°C.**

METHODS

GBS Strains

Three clinical strains of GBS isolated from invasive diseases were selected from the collection of the NRC for GBS. They represented the most frequent serotypes identified in perinatal infections: capsular serotypes Ia, III and V.

Sored at -80°C, they were subcultured on sheep blood Columbia agar before testing.

Evaluated media for transportation/storage

Two types of media in ready to use tubes were evaluated:

- **Lim broth**
  - BBL™ Lim broth, Becton Dickinson, Ireland.
  - Todd Hewitt broth + antibiotics, bioMérieux, France.
  - Todd Hewitt CNA Broth, Copan., Italy.
- **Granada** biphasic **broth**, bioMérieux, France.

Processing

Inoculation

From a fresh culture of each strain, a suspension was prepared in sterile saline and further diluted to achieve a density of approximately 10^3 CFU/ml.

For each suspension, 100 µl were inoculated in the different tested tubes in triplicate for each condition of temperature to evaluate.

Storage

Sets of 3 tubes per strain, per type of medium and per condition of temperature were stored 4 days in aerobic condition:

- at 4-8°C to simulate refrigerator as recommended in guidelines for Amies/ Stuart transport media.
- at 18-24°C to simulate room temperature.
- at 35°C to simulate warm weather condition.

Quantitative cultures

At time of inoculation and further after each 24 hours intervals, 1 µl of broth from each tube was subcultured on Columbia sheep blood agar incubated 18-24h hours at 35°C in air + CO_2. GBS growth was recorded in CFU per plate. Any negative culture was further incubated for 24 hours more, before reporting a negative result.

RESULTS

Recovery of GBS after different periods of storage in different conditions.

No difference of survival was observed between the 3 strains whatever were the combinations of the tested conditions, except at 35°C for the Granada broth. Therefore, results were aggregated per medium and condition apart for Granada broth at 35°C.

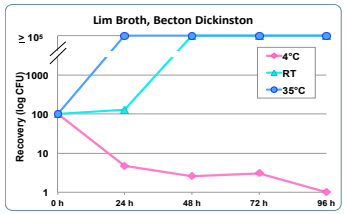

Figure 1: Recovery of GBS from Becton Dickinson's Lim broth after 24 hours intervals following inoculation and stored refrigerated, at RT and at 35°C.

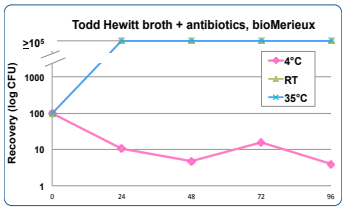

Figure 2: Recovery of GBS from bioMerieux's "Lim broth" after 24 hours Intervals following inoculation and stored refrigerated, at RT and at 35°C.

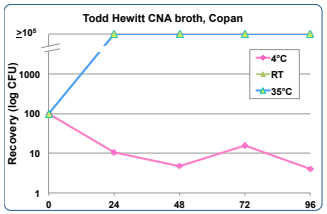

Figure 3: Recovery of GBS from Copan's "Lim broth" after 24 hours intervals following inoculation and stored refrigerated, at RT and at 35°C.

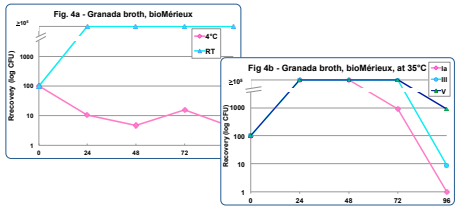

Figure 4 a & b: Recovery of GBS from bioMérieux's "Granada broth" after 24 hours intervals following inoculation and stored (a) refrigerated and at RT and (b) at 35°C for the different strains (Types Ia, III and V).

Lim broth (<3 manufacturers) and Granada broth stored at 4-8°C

- Refrigeration of bacterial samples had a negative impact on recovery with a decrease of minimum 1 log after 24 hours. A slow continuous decrease was showed by following observations at 48, 72 and 96 hours after inoculation.

Lim broth (<3 manufacturers) and Granada broth stored at RT

- Storage at RT generated an increase of minimum 3 log of the initial inoculum of GBS after 24 or 48 hours and was sustained for the 4 days .

Lim broth (<3 manufacturers) stored at 35°C

- As at RT, the 35°C condition generated also an amplification of minimum 3 log of the initial inoculum of GBS after 24 hours and was sustained for the 4 days .

Granada broth stored at 35°C

- For the 3 strains, initially the inoculum was also amplified of minimum 3 log but after 48-72 hours an abrupt lost of viability was observed.

DISCUSSION & CONCLUSION

- A key improvement for screening GBS cultures at 35-37 weeks gestation would be the preservation of GBS viability during long-term transportation aiming a reduction of the rate of false negative cultures.
  - Currently use of non-nutritive transport media stored at 4°C is recommended even if progressive lost of viability is well known after 24 hours.
  - But during transport and storage before transfer to the lab, specimens are often exposed to various temperatures sometimes for extended period of several days.
- This study aimed to demonstrate the robustness of alternatives to non-nutritive media for transportation of swabs collected for GBS screening culture.
  - Use of a selective enrichment broth, as **Lim broth**, already recommended for culture as transport media could represent a true improvement
    - Viability was not only sustained, but the initial inoculum of GBS was also amplified resulting in increase of culture sensitivity even for low initial inoculum.
    - Furthermore, the improvement was demonstrated for a wide range of temperature up to 35°C and storage will not require anymore refrigeration.
    - Cost-effective, as the broth routinely used for selective enrichment will spare use of non-nutritive transport device.
  - For Granada broth as alternative, management would be probably more difficult as following an initial increase of GBS, a dramatic lost of viability was observed after 2-3 days.
- Clinical evaluation is necessary to confirm all these benefits without interference of the commensal flora.

REFERENCES

- Prevention of perinatal group B streptococcal diseases: Revised guidelines from CDC 2010. *MMWR* 2010;59 (RR-10), 1-32
- Guideline from the Belgian Health Council, 2003 (SHC 7721): Prevention of perinatal group B.streptococcal infections http://www.health.fgov.be/CSH_HGR/
- Rosa-Fraile M, Camacho-Muñoz E, Rodíguez-Granger J and Liébana-Martos C. Specimen storage in transport medium and detection of group B streptococci by culture. *J Clin Microbiol 2005; 43: 928-930.*
- Trotman-Grant A, Raney T and Dien Bard J. Evaluation of optimal storage temperature, time and transport medium for the detection of Group B streptococcus in StrepB carrot broth. *J clin Microbiol 2012; 50(7): 2446-2449.*