To Evaluate Impact on Detection Rate of Streptococcus Agalactiae in the Third Trimester of Pregnancy

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Research Article

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Abstract

Background: The streptococcus agalactiae infectious leading to early neonatal morbidity and mortality, the streptococcus agalactiae screen became an important examination for pregnant women. The Centers for Disease Control and Prevention (CDC) recommends whole detection protocols of isolation and culture on streptococcus agalactiae. However, the essential factors including incubation time of agar plates, enrichment time of broth, and transport media storage conditions be ignored. This study was designed to understand above easy ignored point and to provide scientific proof for optimization detection method, revision of health standards and prevention and control of disease.

Methods: The transport medium without and with enrichment were directly inoculated onto Sheep blood agar plate for 24~48 hours. The positive detection rate of streptococcus agalactiae on different incubation time, with and without enrichment were compared, respectively. The transport medium with enrichment were inoculated for 24~48 hours. The positive detection rate of streptococcus agalactiae on different enrichment time were compared. The transport medium were respectively stored at 2℃~8℃ and 20℃~25℃. Interval 24 hours within 5 days, The recovery rate of streptococcus agalactiae on transport medium different storage temperature were compared. The growth of streptococcus agalactiae colonies were examined for pale pink to red, round and pearly colonies. The isolates colonies were identified by matrix assisted laser desorption ionization-time of flight mass spectrometry to confirm negative or positive.

Results: The overall results shown that the detection rate were significantly higher at 48 hours with directly culture methods (P 0.01) or after enrichment culture methods (P 0.05). The detection rate have no difference after enrichment for 24 hours or 48 hours (P 0.05). The recovery rate of streptococcus agalactiae transport medium (stored at 2℃~8℃) were 100% within 4 days and 83.33% at 5th day. However, the recovery rate of streptococcus agalactiae transport medium (stored at 20℃~25℃) were 100% within 2 days, 97.22% at 3th day, 52.78% at 4th day and 33.33% at 5th day, respectively.

Conclusions: In conclusion, the results suggested that the enrichment culture time was 24 hours, the transport media stable best time is 2 days stored at 20℃~25℃ or 4 days stored at 2℃~8℃, and the best incubation time is 48 hours for streptococcus agalactiae clinical testing in pregnant women.

1. Background

Streptococcus agalactiae (also known as group B streptococcus or GBS) is beta-hemolytic, catalase-negative, and facultative anaerobe which can be intermittently, transiently or persistently colonized in the digestive tract and reproductive tract[1, 2]. Streptococcus agalactiae colonization of pregnant women refers to the positive result of streptococcus agalactiae in vaginal, rectal or perianal sampling culture during pregnancy, as kind of conditional pathogenic bacteria, which can change from colonization to pathogenic bacteria under certain conditions[3]. So then leading to invasive streptococcus agalactiae disease in pregnant women or newborns[4, 5].
Nowadays, the streptococcus agalactiae colonization rate of pregnant women in different countries and regions is different, the meta-analysis of 391 studies from 86 countries shown that overall the streptococcus agalactiae colonization rate of pregnant women was 18% (95% CI: 17%~19%) in USA and Europe, such as: 12.5% (95% CI: 10%~15%) in South Asia, 11% (95% CI: 10%~12%) in East Asia, In addition, 11.3% (95% CI: 10%~12%) in China[6–13], respectively. Approximately 50% streptococcus agalactiae colonization pregnant women maybe transmit the bacteria by delivery process to their newborns, thereby how to reduce the streptococcus agalactiae infectious leading to early neonatal morbidity and mortality[14–18]. The third trimester of pregnancy(35 ~ 37 weeks) was correlate more closely with streptococcus agalactiae colonization at term delivery. The Centers for Disease Control and Prevention (CDC) in the United States of America has recommended that all pregnant women be screened for carriage of streptococcus agalactiae at between 35 ~ 37 weeks of gestation. For streptococcus agalactiae colonization positive pregnant women will be received antibacterial intervention,and prior to delivery or caesarean birth[19], To minimize the risk of mother-to-child transmission.

For department of clinical laboratory, how to maximize detection rate and accuracy of streptococcus agalactiae in pregnant women become an important research subject. Recently, the studies focus on anatomic site of sampling, culture media and culture methods ect.yields[20–24]. The essential factors such as: incubation time of agar plates, enrichment time of broth, and transport media storage conditions were easy to be ignored. This study was designed to understand above easy ignored point and to provide scientific proof for optimization detection method, revision of health standards and prevention and control of disease.

2. Materials And Methods

2.1 Study design

The study was approved by the research ethics committee of the Peking University First Hospital, Beijing, China (No.2021/191), and the informed consent were written for all participation in pregnant women. During February, 2020 to June, 2021, the rectovaginal Swab samples were collected from pregnant women at 35 ~ 37 weeks of gestation. To evaluate the positive detection rate effect of streptococcus agalactiae on culture time, with and without enrichment, enrichment time and transport media storage conditions.

2.2 Materials

The transport media were obtained from Tianjin Jinzhang Technology Development Co., Ltd. in China; and the Sheep blood agar plate (BAP) and Group B Streptococcus enrichment broth (Todd-Hewitt broth) were obtained from bioMérieux, SA in France. Matrix assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Germany) with software (MALDI Biotyper 3.0).

2.3 Clinical Specimens Collection
All rectovaginal Swab samples were collected by midwife and transported to the Diagnostic Microbiology Laboratory of Peking University First Hospital within 2 hours. Pregnant women age was from 22 to 46 years old. The 1764 cases collection samples divided into two parts were stored at 2°C~8°C or 20°C~25°C. The 1427 cases collection samples were stored at 2°C~8°C. All collection samples were immediately treated when diagnostic microbiology laboratory receive them.

2.4 Specimens Culture Treatment

2.4.1 The Different Culture Time Were Evaluated

The transport medium of 1764 cases stored at 2°C~8°C were directly inoculated onto sheep blood agar plate with 5% sheep blood incubated at 37°C in 5% CO₂ for 24~48 hours.

The transport medium of 1427 cases stored at 2°C~8°C were immediately inoculated onto 9 mL Group B Streptococcus enrichment broth with colistin (10 mg/L) and nalidixic acid (15 mg/L) for 24 hours or 48 hours at 35°C in aerobic conditions, after vortexed with blender, and then 10µl vortexed culture medium were directly inoculated onto sheep blood agar plate with 5% sheep blood incubated at 37°C in 5% CO₂ for 24~48 hours.

2.4.2 The Different Storge Temperature of Transport Medium Were Evaluated

The 1764 cases collection samples divided into two parts were respectively stored at 2°C~8°C or 20°C~25°C. Interval 24 hours within 5 days, the transport medium stored at different temperature were directly inoculated onto Sheep blood agar plate with 5% sheep blood incubated at 37°C in 5% CO₂, respectively. The recovery rate of streptococcus agalactiae transport medium was statistically analyzed for every day[25].

The growth of streptococcus agalactiae colonies were examined for pale pink to red, round and pearly colonies. The isolates colonies from Sheep blood agar plate were identified by Matrix assisted laser desorption ionization-time of flight mass spectrometry as described previously[26] to confirm negative or positive.

2.5 Statistical Analysis

Statistical calculations were performed using Chi-Square test using SPSS software version 16.0. Values of \( P < 0.05 \) were considered statistically significant.

3. Results

3.1 The Effect of Different incubation Time on Detection Rate
The positive detection of streptococcus agalactiae were observed at 24 hours and 48 hours with directly and after enrichment culture methods. The directly culture methods results shown that streptococcus agalactiae positive were respectively 57 subject of 1764 cases (3.23%) at 24 hours and were 72 subject of 1764 cases (4.08%) at 48 hours. The detection rate were significantly higher at 48 hours time point than 24 hours time point ($P \leq 0.01$). The after enrichment culture methods results shown that streptococcus agalactiae positive were respectively 121 subject of 1427 cases (8.48%) at 24 hours and were 127 subject of 1427 cases (8.90%) at 48 hours. The detection rate were statistically significant at 48 hours time point than 24 hours time point ($P \leq 0.05$). The data was shown in Table 1. The results suggested that the best positive detection time was after incubation sheep blood agar plate 48 hours, and the positive detection rate was higher compared with enrichment than without enrichment.

### 3.2 The Effect of Different Enrichment Culture Time on Detection Rate

After different enrichment culture time (24 hours or 48 hours), and then were directly inoculated onto Sheep blood agar plate with 5% sheep blood for 48 hours. The 24 hours enrichment culture time results shown that streptococcus agalactiae positive were respectively 120 subject of 1427 cases (8.41%) at 24 hours and were 126 subject of 1427 cases (8.83%) at 48 hours. The 48 hours enrichment culture time results shown that streptococcus agalactiae positive were respectively 121 subject of 1427 cases (8.48%) at 24 hours and were 127 subject of 1427 cases (8.90%) at 48 hours. The data was shown in Table 2. The results shown that the positive detection rate has no statistical significance within different enrichment culture time ($P > 0.05$).

### 3.3 The Effects of Different Transport Medium Storage Temperature and Time on Detection Rate

For different storage temperature of transport medium, the results shown that recovery rate of streptococcus agalactiae transport medium (stored at 2°C~8°C) were 100% within 4 days and 83.33% at 5th day. The results shown that recovery rate of streptococcus agalactiae transport medium (stored at 20°C~25°C) were 100% within 2 days, 97.22% at 3th day, 52.78% at 4th day and 33.33% at 5th day, respectively. The data was shown in Table 3. The results shown that streptococcus agalactiae transport medium was stable stored at 2°C~8°C within 4 days and stored at 20°C~25°C within 2 days. The false positives identified shown all resembling colonies were streptococcus agalactiae by matrix assisted laser desorption ionization-time of flight mass spectrometry.

### 4. Discussion

Because of the streptococcus agalactiae infectious leading to early neonatal morbidity and mortality, the streptococcus agalactiae examination is important for pregnant women. Many meta-analysis studies shown that approximately 50% streptococcus agalactiae colonization pregnant women maybe transmit the bacteria by delivery process to their newborns now[27–29]. The populations in developing or underdeveloped countries have different lifestyles, live in different geographical locations, different ages,
socioeconomic levels, schooling, and gestational ages and have different importance for streptococcus agalactiae infectious[11, 12, 15, 18, 30–34]. it is essential to improve and extend streptococcus agalactiae screening performance and accuracy in pregnant women, especially underdeveloped countries. The Centers for Disease Control and Prevention of the United States of America revised consensus guidelines for the prevention of earlyonset streptococcus agalactiae disease. The guidelines recommended universal screening of all pregnant women at 35 ~ 37 weeks gestation for vaginorectal colonization by a broth enrichment method[16, 19].

Now, many studies focus on specimen type, collection time, specimen storage temperature and time, transport medium, type of culture medium, enrichment broth type etc. But the essential factors such as: incubation time of agar plates, enrichment time of broth, and transport media storage conditions were easy to be ignored.

We should be attention to each detail of detection procedure and to ensure detection accuracy. This study was conducted to be ignored problem and to make up for the lack of data in this field. The results shown that it is higher positive detection rate of streptococcus agalactiae with enrichment comapared with without enrichment method. As mentioned in CDC revised consensus guidelines were as many as 50% of streptococcus agalactiae carriers culture results are false negative in without enrichment[35]. Our results shown that the streptococcus agalactiae colonization rate of pregnant women were 8.41%~8.90% with enrichment culture. However, the without enrichment incubation results were 3.23%~4.08%. Other studies also found that the results were the same for enrichment and without enrichment culture[14, 21, 36, 37]. Therefore, our results strongly recommended streptococcus agalactiae colonization by a broth enrichment method. For different incubation time, our results shown that the positive detection rate of streptococcus agalactiae pregnant women were meanwhile increased at 48 hours compared with 24 hours in enrichment and without enrichment culture. Moderately prolonging the incubation time can improve the streptococcus agalactiae detection rate. Our results recommended that the best time is 48 hours.

For enrichment culture time, our results shown that the detection rate of streptococcus agalactiae pregnant women have no statistically significant at 48 hours compared 24 hours in enrichment culture method. We recommended that the enrichment culture best time is 24 hours. We can issue test report for patient as soon as. In terms of transport medium storge temperature and time, our results shown that recovery rate of streptococcus agalactiae transport medium (stored at 2°C~8°C) were 100% within 4 days and 83.33% at 5th day, however, the recovery rate of streptococcus agalactiae transport medium (stored at 20°C~25°C) were 100% within 2 days, 97.22% at 3th day, 52.78% at 4th day and 33.33% at 5th day, respectively. There was few research in this area, the other studies were unable to provide accurate storage temperature and stabilization time about transport media. Therefore, we recommended that the stable best time is 2 days stored at 20°C~25°C or 4 days stored at 2°C~8°C.

5. Conclusions
In conclusion, the results suggested that the enrichment culture time was 24 hours, the transport media stable best time is 2 days stored at 20°C~25°C or 4 days stored at 2°C~8°C, and the best incubation time is 48 hours for streptococcus agalactiae clinical testing in pregnant women.

**Abbreviations**

GBS
Group B streptococcus
CDC
The Centers for Disease Control and Prevention
CI
Confidence interval
BAP
Sheep blood agar plate

**Declarations**

7.1 **Ethics approval and consent to participate:**

All experiments performed in this study are in accordance with the relevant guidelines and regulations by the research ethics committee of the Peking University First Hospital, Beijing, China (No.2021/191). All participants signatured informed consent form.

7.2 **Consent for publication:**

Not Applicable.

7.3 **Availability of data and materials:**

All data generated or analysed during this study are included in this published article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

7.4 **Competing interests:**

All authors declare that they have no competing interests.

7.5 **Funding:**

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7.6 **Authors' contributions:**
YP.J and LY.S designed the experiment; YP.J, J.Z, ZY.L and LY.S performed experimental operation, statistical analysis and interpretation of the data; YP.J wrote the manuscript; All authors reviewed and approved the final manuscript.

7.7 Acknowledgements:

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References


Tables

Tables 1 to 3 are available in the Supplementary Files section.

Supplementary Files

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- Tables.pdf
- RawData.xlsx