Author's response to reviews

Title: Evaluation of chromID Strepto B as a screening media for Streptococcus agalactiae

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Author's response to reviews: see over
Dr. Philippa Harris  
Executive Editor  

Dear Dr. Philippa Harris

Thank you for reviewing our manuscript and giving us an opportunity for revision. We have modified our original manuscript according to the reviewers’ comments and the changes are listed below. We hope that the revised version is now suitable for publication in your journal.

Sincerely,

Dongyun FENG

Responses to Dr. Philippa Harris
Thank you for your comments. We added “These swabs were taken as part of standard patient care.” (Red letters) in page 6, line 2. We also found some English defects and spelling mistakes. We corrected these in blue letters.

Responses to REVIEWER 1,
Thank you for your comments. We have answered them as described below.

Spelling mistake: Methods, Page 6: “The two different media were assessed by two different institutions for the bind (blind) test”
→ We corrected the word “bind” to “blind” and modified the sentence slightly (Red letters).

The problem of false positive detection of STRB has been reported by other studies also this study. It is not clear whether there were false positive growth of non-GBS organisms as pink/red colonies on STRB identified in this study, and how they were further identified as non-GBS organism by biochemical tests or latex cases detected in this study.
→ We added the following sentence to page 8. 4th para, line 4.

The target organisms are characterized by enzyme systems that metabolize the substrate to release the chromogen, which enables the organisms to display a typical color. STRB includes three chromogenic substrates, and three enzymes (phosphatase, esterase and β-cellobiosidase) react with these substrates such that the color of the organisms results from the combination of these three substrates. Each substrate gives a pink, blue, and purple color for phosphatase activity, esterase activity, and β-cellobiosidase activity, respectively. GBS are colored by only one of these three substrates, and thus show only one color. On the other hand, other species are colored by one to three enzymatic reactions so that various color combinations and variable colors can be seen. A given occurrence or enzymatic activity for each substrate can differ between organisms. In our study, some *Streptococcus* spp. and *Enterococcus* spp. strains showed blue, white and purple colonies, while others showed pink colonies. *Enterococcus* spp. and *Streptococcus* spp. on STRB was sub-cultured on blood agar. *Enterococcus* spp. was identified by Gram stain, 6.5% NaCl, bile esculin and EF agar. *Streptococcus* spp. was identified by Gram stain, 6.5% NaCl, bile esculin, EF agar, hemolysis and Optochin test.
Responses to REVIEWER 2,
Thank you for your comments. Our responses are given below.

Major Compulsory Revisions
1. Table1. The calculation of sensitivity would require the evaluation of the method(s) under test against an independent gold standard method. In this case the gold standard is a composite of the two test methods, and is not independent, so we would be unable to calculate a true sensitivity. I suggest to revise or delete table 1. It is reasonable, however, to conclude that the STRB method is more sensitive than the BA method.
   →We deleted Table 1, and also have changed the following related sections.

   Deleted:
   1. Statistical methods
   2. The corresponding sensitivities were 99.7% for STRB and 93.7% BA (Table 1). The GBS detection rate was significantly higher than the detection rate on BA (McNemar test, p < 0.05)
   3. The results of this study show that the sensitivity of chtomID StreptoB (STRB) is higher than that of BA (99.7% vs. 93.7%).

   Changed:
   1. Page3, 3rd para, line 2, STRB had a calculated sensitivity of 99.7 (1 false negative) and BA of 93.7% (20 false negatives).
      →One false negative was observed on STRB, and 20 false negatives were observed on BA (Red letters).
   2. Page 7. 2nd para, line 5.
      The one positive that was missed following the 24-hour incubation on STRB eventually displayed pink colonies upon a 48-hour incubation.
      →In our study, only one positive result that was missed following the 24-hour incubation on STRB eventually displayed pink colonies upon a 48-hour incubation (Red letters).
      the sensitivity of
      →the positive rate of (Red letters)

2. Page 6. 2nd para, line 15. The different methods were assessed in different institutions. How can we be sure that differences in the quality of processing did not the differences in yield between the two methods?
   →We added the following sentence to Page 6. 2nd para, line 17. All assessments were performed at each institution by well-trained staff members who were blinded to the results of the other (Red letters).

As mentioned above, all members of staff at both institutions were well-trained, and both institutions received the same high score from domestic surveillance organized by the Japanese Association of Medical Technologists during 2009 to 2013.

Minor Essential Revisions
1. Page1, 3rd in institutions, spelling of “Obstetrics”
   →We corrected the word “Obsterics” to “Obstetrics” (Red letters)
2. Page 3, 1st para, last line. I think need to mention that the agar cultures followed a selective enrichment broth.
   →We added “followed by a selective enrichment broth.” to page 3, 1st para, last line (Red letters).
3. Page 3, 2nd para, line 13. Suggest to revise wording to clarify meaning. I think that the staff in the two institutions were blinded to the results in the other?
   →We have added “…by well-trained staff members who were blinded to the results of the other” to page 6, last line (Red letters).

4. Page 9, 2nd para, line 1. I suggest to explain what is meant by a “conventional” agar. I assume this would mean non selective or non differential?
   →We added “non-selective” to page 9, 2nd para, line 1(Red letters).

Discretionary Revisions
1. Page 3, 1st para, 1st line. Suggest “Streptococcus” rather than “Streptococci”
   →Streptococci was corrected to Streptococcus (Red Letters)

2. Page 3, 1st para, 4th line. The use of the word “critical” would suggest that there is no alternative strategy for prevention of GBS. But the debate of universal vs. risk-based screening continues. It could be said that the detection of colonization is “a major strategy” in the prevention of neonatal GBS, whilst leaving the possibility that there may be other strategies.
   →We corrected “critical” to “a major strategy” (Red Letters)

   →We added “selective (Red letters)” to page 3, 2nd para, line 3