Identification of Group B Streptococcus on Chromogenic Medium CHROMagar StrepB by MALDI-ToF Mass Spectrometry

*Nicolas GERMAIN, Camille CORDIER, Malo PENVEN, Olivier GAILLOT**

Department of Bacteriology, Lille University Hospital, F-59000 Lille, France

BACKGROUND

Screening for *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) on pre-partum vaginal samples is critical in order to prevent neonatal infection[(1)].

GBS-selective chromogenic media used in this setting are not 100% specific, thus requiring further confirmation by Lancefield serotyping or phenotypic identification.

MALDI-ToF mass spectrometry (MTMS) is a fast and accurate tool for this purpose, but some reports have initially pointed out that it might not be able to identify all GBS colonies grown on chromogenic media[(2)].

OBJECTIVES

1. To compare the spectral data provided by MALDI-ToF mass spectrometry (MTMS) when applied to *Streptococcus agalactiae* (GBS) colonies grown on CHROMagar StrepB, CNA blood agar, and non-supplemented Müller-Hinton agar.

2. To determine on a large collection of clinical isolates the capability of (i) MTMS, and (ii) latex streptococcal agglutination, to identify GBS colonies grown on CSB and CNA.

METHODS

A collection of 150 clinical GBS isolates were grown overnight on CHROMagar StrepB agar (CSB), colistin-nadixic acid 5% blood agar (CNA) and Müller-Hinton agar (MH). All were tested for Lancefield serogroup B antigen with the Prolex™ Streptococcal Grouping Latex Kit (Pro-Lab diagnostics, Bromborough, UK). **Three colonies** from each medium were then smeared (one spot per colony), overlaid with cyano-4-OH-cinnamic acid matrix **without formic acid extraction,** and analyzed with a MTMS Bruker microflex LT apparatus and BioTyper 4.1 system software. Results were expressed as log(score) values and the highest score from each triplicate analysis was kept for comparison. When a score value was <2.0, *sodA* gene sequence identification was performed.

Then, **200 vaginal swabs** from preterm pregnant women were plated on CSB and grown overnight. When primary culture of mauve colonies was obtained, 3 colonies per plate were randomly chosen and identified as above.

The presence, value (m/z) and intensity of the 3 main peaks characteristic of GBS were assessed on each MTMS spectrum.

For each isolate, the best log(score) value obtained from CSB-grown colonies was compared to that of CNA-grown colonies by a Student t-test.

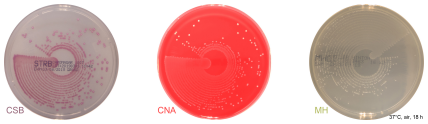
RESULTS

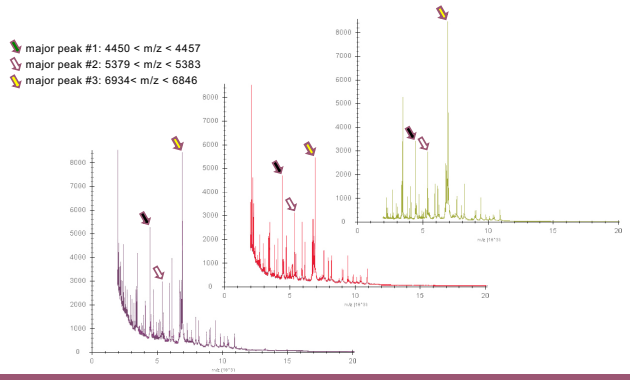
For all **150 collection isolates** on all media, ***S. agalactiae* was the best MTMS match in the Biotyper database**, with log(score) values >1.8 and a difference with the 2nd highest ranking species >0.25 (*i.e., S. dysgalactiae*, *S. equi*, *S. urinalis S. parauberis* or *S. pyogenes*).

146 isolates (97.3%) on CSB, 147 (98%) on CNA and 149 (99%) on MH were unambiguously identified as GBS **with log(score) values ≥2.0**. Three isolates from CNA and CSB and another one from CSB yielded GBS log(score) values <2.0, although >1.8, with a difference with the 2nd highest ranking species >0.4.

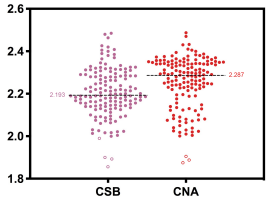
Vaginal swabs : 31/200 yielded primary cultures of mauve colonies, 29 of which were unambiguously identified as GBS by latex agglutination or MTMS [log(score) values ≥2.0]. The remaining 2 were *S. pyogenes*, a known false positive on CSB, readily differentiated from GBS by MTMS or Lancefield seroagglutination.

Microflex LT spectral analysis:





Distribution of log(score) values from CNA- and CSB-grown GBS colonies:



MT-MS identification scores of 150 GBS isolates grown on CSB (left, mauve spots) and CNA (right, red spots). Empty circles indicate log(score) values <2.0. Dotted lines represent median score values.

- scores range was almost identical regardless of the culture medium used
- however, scores obtained were higher from CNA-grown colonies (median values : 2.287 and 2.193, respectively, P<0.005). This might be due to the higher number of additional peaks of lower mass ranges generated when using CSB as compared to CNA.
- regardless of the culture medium used, all scores were >1.85, a significant improvement from preliminary reports[(2)]

CONCLUSIONS

- No GBS MTMS misidentification was observed among the isolates grown from CSB.

- Without any extraction procedure, score values were higher than previously reported, possibly due to the upgrade of the Biotyper 4.1 database.

- CHROMagar StrepB medium can be safely used to identify of *S. agalactiae* by MALDI-ToF MS, as well as Prolex™ GBS latex agglutination.

- As previously recommended for other microbial groups[(3)], applying slightly less restrictive log(score) value criteria (>1.8 and difference in values with the 2nd highest ranking species >0.25) would have allowed certain identification of all GBS isolates on both media.

REFERENCES

1. Verani. 2010. *Morbidity Mortality Weekly Report.*
2. Joubrel *et al.* 2014. *Diagnostic Microbiology and Infectious Disease*
3. Schulthess *et al.* 2016. *Journal of Clinical Microbiology.*

The authors wish to thank Alberto Lerner (CHROMagar, Paris, France) for providing the chromogenic medium.