Diagnostic Microbiology and Infectious Disease 101 (2021) 115427

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Performance of PhenoMatrix for the detection of Group B *Streptococcus* from recto-vaginal swabs

Claudio Foschi[a,b,*], Gabriele Turello[a], Tiziana Lazzarotto[a,b], Simone Ambretti[b]

[a] *Microbiology, DIMES, University of Bologna, Bologna, Italy*
[b] *Microbiology Unit, IRCCS S.Orsola-Malpighi Hospital, Bologna, Italy*

ARTICLE INFO

Article history:
Received 24 March 2021
Revised in revised form 3 May 2021
Accepted 6 May 2021
Available online 13 May 2021

Keywords:
PhenoMatrix
WaspLab
GBS
Group B *Streptococcus*

ABSTRACT

We assessed the performance of PhenoMatrix digital imaging software in detection of Group B *Streptococcus* from recto-vaginal swabs plated on a specific chromogenic medium, using the WASP automated processor. PhenoMatrix algorithm showed a sensitivity of 100% and a specificity of 64.5%. False-positive results were mainly due to commensal viridans streptococci.

Streptococcus agalactiae (Group B Streptococcus – GBS) is a Gram-positive bacterium that colonizes the gastrointestinal and genital tract of about 15-40% of pregnant women (Russell et al., 2017). Although GBS colonization usually remains asymptomatic, it can be passed from the woman to her newborn during the process of labor, potentially leading to pneumonia, septicaemia, and meningitis in the infant (Seedat et al., 2018).

To prevent neonatal infection, pregnant women are usually screened for GBS colonization at the third trimester of pregnancy (Filkins et al., 2020). In the case of GBS colonization and risk factors for an early onset GBS infection, intrapartum antibiotic prophylaxis is prescribed (Kolkman et al., 2020).

Thus, a rapid and accurate detection of GBS is crucial for a correct management during pregnancy and childbirth.

Bacterial culture from recto-vaginal swabs of LIM broth is the gold-standard for the detection of GBS colonisation (Berg et al., 2013). Recently, the use of selective and chromogenic media has proved to increase the sensitivity of culture for GBS isolation (Salem et al., 2015; Verhoeven et al., 2014).

In this context, the use of WaspLab system followed by PhenoMatrix digital imaging analysis software can significantly improve the management of GBS culture in terms of diagnostic performances, hands-on time, and turn-around time (TAT).

The WASPLab system (Copan Diagnostics) is composed of an up-front specimen processing unit and an integrated track line to automatically move inoculated culture plates from processing to incubation to bench. The system also includes a digital imaging system to record images of plates at programmable time points (Foschi et al., 2020).

PhenoMatrix software (Copan Diagnostics) is an advanced artificial intelligence module with the capacity to develop algorithms able to pre-assess and pre-sort culture plates based on its 'reading' of digital plate images. The software can segregate culture plates according to whether they show growth or no growth, and the color detection module can recognize and differentiate colony colors on chromogenic media (Van et al., 2019; Faron et al., 2020).

Here, we assessed the performance of the PhenoMatrix software used with CHROMagar StrepB chromogenic medium (Kima Meus) for GBS detection. Routine visual inspection of plates was used to check software analyses.

From December 2020 to February 2021, a total of 1068 recto-vaginal swabs in LIM broth submitted to the Microbiology Unit of S. Orsola-Malpighi Hospital (Bologna, Italy) for GBS detection were prospectively analyzed.

LIM broths were incubated at 35-37°C for 18-24 hours, and, afterwards, 1 μL of LIM broth was inoculated by the WaspLab onto CHROMagar StrepB medium. Cultures were incubated in aerobic atmosphere at 35°C and digital images were captured at 16 hours after plating.

Digital images of plates were automatically screened by PhenoMatrix. This software analyses the plates to identify differences in growth and colony colour. A specific protocol was developed evaluating the medium type and the incubation time used by the laboratory for GBS surveillance cultures. By means of this internal algorithm, the

* Corresponding author: Tel: +39 051 2144513; Fax: +39 051 307397
E-mail address: claudio.foschi2@unibo.it (C. Foschi).

https://doi.org/10.1016/j.diagmicrobio.2021.115427

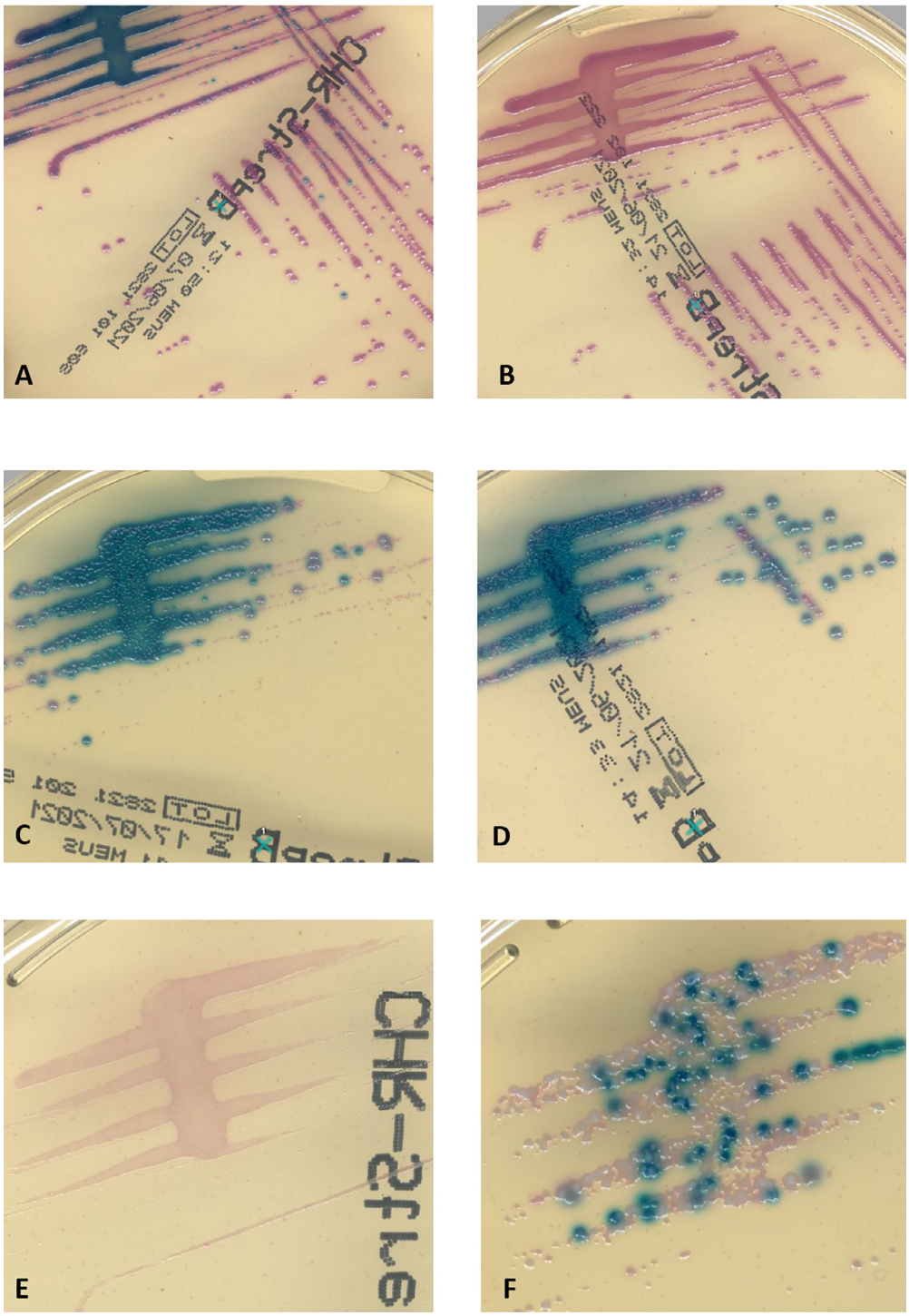

Fig. 1. . Images of representative cultures on CHROMagar StrepB medium. (A-B) Streptococcus agalactiae - GBS; (C) Streptococcus anginosus; (D) Enterococcus faecalis; (E) Streptococcus oralis; (F) Staphylococcus epidermidis.

software automatically sorted the cultures into the categories 'negative for GBS' and 'potential-positive for GBS'. In our protocol, plates with no bacterial growth or with blue/colourless colonies are marked as GBS-negative, whereas plates with pink/light red/purple colonies are defined as presumptive GBS-positive.

Cultures were also reviewed manually by a technologist to check software analyses and scored for the presence or absence of colonies resembling GBS.

Colonies consistent with GBS were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) identification.

A specimen was considered a true-positive if a colony was confirmed to be GBS by MALDI-TOF.

A total of 587 plates (55%) were correctly segregated as GBS-negative by PhenoMatrix, with no false-negative results. The automatic reading system marked 481 plates (45%) as potentially positive; 158 of them were confirmed as GBS by MALDI-TOF.

The remaining cases (323; 30.2% of the total) sorted positive by PhenoMatrix were characterized by the absence of pink colonies at visual inspection (212 cases) or by a MALDI-TOF identification different from GBS (111 cases). The first cases were due to

the time lapse (approximately 4–8 hours) between the digital and manual reading, leading to a change in colony color. Indeed, we noticed that blue/green colonies of enterococci, when small, can be characterized by a light-pink halo, leading to a positive sorting by Phenomatrix.

The latter cases showed the following identifications: *Streptococcus mitis/oralis* (38.7%), *Streptococcus anginosus* (19.8%), *Lactobacillus* spp. (9%), *Streptococcus salivarius* (8.1%), *Enterococcus avium* (6.3%), *Enterococcus faecalis* (6.3%), *Streptococcus parasanguinis* (4.5%), *Enterococcus faecium* (4.5%), *Staphylococcus epidermidis* (0.9%), *Streptococcus constellatus* (0.9%) and *Streptococcus dysgalactiae* (0.9%).

Representative images of positive and negative cultures on chromogenic medium are presented in Fig. 1.

PhenoMatrix software used with CHROMagar StrepB medium showed a sensitivity and a specificity for GBS detection of 100% and 64.5%, respectively. Negative and positive predictive values were as follows: NPV = 100%, PPV = 32.8%.

Overall, our results agree with a recent report showing a sensitivity of 95.5% and a specificity of 63%, when PhenoMatrix software was combined with a chromogenic medium for GBS detection (Baker et al., 2020).

Considering that an accurate detection of GBS in pregnant women by screening cultures is crucial for the prevention of neonatal infections, the algorithm of PhenoMatrix is tuned to detect all presumptive GBS colonies, avoiding the risk of false negative results. In line with this idea, the proposed protocol showed an excellent sensitivity and negative predictive value for GBS detection.

In previous studies, algorithmic detection on chromogenic media demonstrated 100% sensitivity for the detection of presumptive positive cultures for methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and group A *Streptococcus* (Faron et al., 2016; Cherkaoui et al., 2019; Van et al., 2019).

As noted in our experience, false-positive results can occur, due to a combination of factors: (1) incomplete specificity of the chromogenic medium, (2) PhenoMatrix algorithm, set to sort as positive all 'pink' colonies, irrespective of the color intensity.

Most false-positive results were characterized by bacterial colonies of light pink color (e.g., commensal viridans streptococci) instead of mauve pink. To improve specificity, PhenoMatrix algorithm can be modified, by means of a finer setting of the intensity/type of color for plate sorting.

It should be remembered that the primary goal of utilizing chromogenic media plus PhenoMatrix is to identify all the negative GBS cultures rapidly and effectively, maximizing the negative predictive value. Only the potentially positive cultures are then subjected to MALDI-TOF, a rapid, cost-saving, and high-performing technique.

Further studies are needed to evaluate the cost-benefit ratio of the proposed algorithm compared to other protocols, including PCR-based methods. The use of NAAT for GBS detection is recently increasing, thanks to the excellent sensitivity and speed and the reduced hands-on time. However, PCR-based methods are often more expensive and may have slightly lower specificity than culture (Shin et al., 2019).

In conclusion, our protocol represents an excellent and cost-effective method for GBS detection from recto-vaginal swab cultures. The use of digital imaging analysis and the implementation of dedicated algorithms can increase laboratory automation and standardization, reducing hands-on time and TAT for surveillance cultures.

Author contributions

Claudio Foschi: Conceptualization, Methodology, Validation, Data curation, Writing - Original Draft, Writing - Review & Editing; **Gabriele Turello:** Formal analysis, investigation; **Tiziana Lazzarotto:** Conceptualization, Supervision; **Simone Ambretti:** Conceptualization, Methodology, Validation, Writing - Original Draft, Supervision, Project Administration

Acknowledgments

We would like to thank all the technical staff of the Microbiology Unit for the excellent assistance during the study.

Financial support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

This study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest. All the authors declare the absence of any dual or conflicting interest.

References

Baker J, Timm K, Faron M, Ledeboer N, Culbreath K. Digital image analysis for the detection of group B streptococcus from ChromID Strepto B medium using phenomatrix algorithms. J Clin Microbiol 2020;59: e01902-19. 10.1128/JCM.01902-19.

Berg BR, Houseman JL, Garrasi MA, Young CL, Newton DW. Culture-based method with performance comparable to that of PCR-based methods for detection of group B Streptococcus in screening samples from pregnant women. J Clin Microbiol 2013;51:1253–5 10.1128/JCM.02780-12.

Cherkaoui A, Renzi G, Charretier Y, Blanc DS, Vuilleumier N, Schrenzel J. Automated incubation and digital image analysis of chromogenic media using copan WASP Lab enables rapid detection of vancomycin-resistant enterococcus. Front Cell Infect Microbiol 2019;9:379. 10.3389/fcimb.2019.00379.

Faron ML, Buchan BW, Vismara C, Lacchini C, Bielli A, Gesu G, et al. Automated scoring of chromogenic media for detection of methicillin-resistant staphylococcus aureus by use of WASP Lab image analysis software. J Clin Microbiol 2016;54:620–4. doi: 10.1128/JCM.02778-15.

Faron ML, Buchan BW, Relich RF, Clark J, Ledeboer NA. Evaluation of the WASPLab segregation software to automatically analyze urine cultures using routine blood and MacConkey agars. J Clin Microbiol 2020;58: e01683-19. 10.1128/JCM.01683-19.

Filkins L, Hauser JR, Robinson-Dunn B, Tibbetts R, Boyanton BL, Revell P. American Society for Microbiology Provides 2020 guidelines for detection and identification of Group B streptococcus. J Clin Microbiol 2020;59: e01230-20. 10.1128/JCM.01230-20.

Foschi C, Gaibani P, Lombardo D, Re MC, Ambretti S. Rectal screening for carbapenemase-producing Enterobacteriaceae: a proposed workflow. J Glob Antimicrob Resist 2020;21:86–90. doi: 10.1016/j.jgar.2019.10.012.

Kolkman DGE, Rijnders MEB, Wouters MGAJ, Dommelen PV, de Groot CJM, Fleuren MAH. Adherence to three different strategies to prevent early onset GBS infection in newborns. Women Birth 2020;33:e527–34 10.1016/j.wombi.2019.12.004.

Russell NJ, Seale AC, O'Driscoll M, O'Sullivan C, Bianchi-Jassir F, Gonzalez-Guarin J. GBS Maternal Colonization Investigator Group. Maternal colonization with group B streptococcus and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis 2017;65:S100–11 10.1093/cid/cix658 .

Salem N, Anderson JJ. Evaluation of four chromogenic media for the isolation of Group B Streptococcus from vaginal specimens in pregnant women. Pathology 2015;47:580–2. doi: 10.1097/PAT.0000000000000299.

Seedat F, Brown CS, Stinton C, Patterson J, Geppert J, Freeman K, et al. Bacterial load and molecular markers associated with early-onset Group B Streptococcus: a systematic review and meta-analysis.. Pediatr Infect Dis J 2018;37:e306–14 10.1097/INF.0000000000002050 .

Shin JH, Pride DT. Comparison of 3 nucleic acid amplification tests and culture for detection of Group B streptococcus from enrichment broth. J Clin Microbiol 2019;57: e01958-18. 10.1128/JCM.01958-18.

Van TT, Mata K, Dien Bard J. Automated detection of streptococcus pyogenes pharyngitis by use of colorex strep A CHROMagar and WASP Lab artificial intelligence chromogenic detection module software. J Clin Microbiol 2019;57:. doi: 10.1128/JCM.00811-19 e00811-19.

Verhoeven PO, Noyel P, Bonneau J, Carricajo A, Fonsale N, Ros A, et al. Evaluation of the new brilliance GBS chromogenic medium for screening of Streptococcus agalactiae vaginal colonization in pregnant women. J Clin Microbiol 2014;52:991–3. doi: 10.1128/JCM.02926-13.