Can image analysis automatically screen for *Streptococcus agalactiae* in specimens collected from pregnant women?

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Background

Group B streptococcus (GBS) is an asymptomatic colonizer of the gastrointestinal tract in up to 30% of healthy adults, and is the main risk factor for neonatal GBS infection. About 50 to 75% of newborns exposed to intravaginal GBS become colonized, and 1 to 2% of newborns of carrier mothers will develop early-onset invasive disease. In the mother, GBS may cause abortion, urinary infection, preterm birth, chorioamnionitis or puerperal endometritis. GBS screening (between the 35th and 37th gestational weeks) in pregnant women and antimicrobial prophylaxis (when indicated) may reduce neonatal morbidity and mortality.

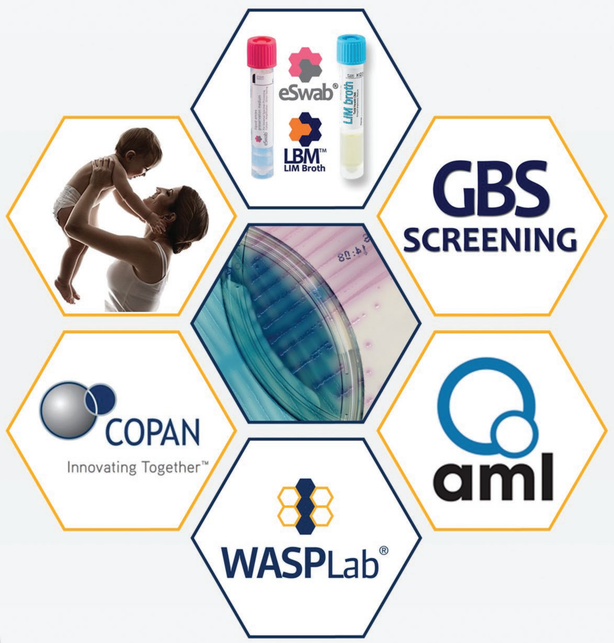
COPAN developed a software, named Chromogenic Detection Module (CDM), that analyses plates images and links colony target color with the medium used by the laboratory.

We used CDM to discriminate between positive and negative chromogenic GBS media through the automatic recognition of pigmented colonies, and WASPLab™(COPAN WASP S.R.L.).

Objectives

The objectives of this study were to:

1. Validate the performance of CDM to be used to discriminate between positive and negative chromogenic GBS media.
2. Implement CDM for GBS Screening in order to automate and optimize the current screening procedure.
3. Reduce the time spend to report negative cultures in order to focus on the positive results instead.



Materials & Methods

Genital and rectovaginal specimens (N=5337) collected from pregnant women were analyzed between June 15th and September 29th 2018. ESwab™ samples were loaded on the WASP™ and 30 µL of each sample was used to inoculate a tube of LIM broth (COPAN) an enrichment medium for isolation of GBS. After 18 - 24 hours incubation at 37°C (± 2°C), the LIM broth tubes were loaded onto the WASP™ and 10 µL was used to streak a Brilliance GBS agar (OXOID) using WASP™. Agar plates were incubated at 37°C (± 2°C) in WASPLab™. After 18 hours of incubation, a picture was acquired by WASPLAB™ and examined by both CDM and trained technicians.

CDM performed a colorimetric analysis of the digital images by converting RGB pixels into a bubble-shape tolerance composed of Hue, Saturation, and Value (HSV). Specimen with HSV within the set tolerance level were reported as automation positive (AP), while the others were reported as automation negative (AN).

The technicians, blinded to the CDM results, read the plate images on a HD monitor. Interpretation of GBS agar was mainly based on absence/presence of growth and if growth was present, the color and colony appearance were then recorded. Specimens with chromogenic color and colony appearance were reported as Manual Positive (MP), the others were reported as Manual Negative (MN). Suspected colonies are confirmed by latex agglutination and/or VITEK® MS (Mass Spectrometry).

Results

With the 5337 samples processed a final positivity rate of 23.55% was obtained. Raw comparison (before review) of automated and manual reading showed a 91.87% agreement, a specificity of 89.60%, and a sensitivity of 99.67%. Discrepant analysis of the 430 AP/MN demonstrated that 57 (13.26%) MN where positive after review, 79 (18.37%) had borderline colors and 294 (68.37%) contained colorimetric reactions due to residual matrix or growth of contaminants. The table below (*Table 1*) is showing an overview of all the result (before and after review by a senior lab technician).

Method Result (without review)		Total n (%)		After review by a senior lab technician			
Automated by CDM colorimetric analysis	Manual by a trained lab technician			Positive n (%)		Negative n (%)	
Positive	Positive	1200	(22.48)	1200	(95.47)	-	(-)
Positive	Negative	430	(8.06)	57	(4.53)	373	(9.14)
Negative	Positive	4	(0.07)	-	(-)	4	(0.10)
Negative	Negative	3703	(69.38)	-	(-)	3703	(90.76)
Total:		5337	(100.00)	1257	(100.00)	4080	(100.00)

Table 1, Result overview

From the data gathered we were able to calculate the agreement, sensitivity, specificity. false positivity rate, and false negativity rate for both automated reading by CDM and manual reading by a trained lab technician (*Table 2*).

	Automated by CDM colorimetric analysis	Manual by a trained lab technician	Differences
Agreement (%)	93.01	98.86	- 5.85
Sensitivity (%)	100.00	95.47	+ 4.53
Specificity (%)	90.86	99.90	- 9.04
False positivity rate (%)	9.14	0.10	+ 9.04
False negativity rate (%)	0.00	4.53	- 4.53

Table 2, Result calculations

With automated reading by CDM we find a final sensitivity of 100.00%, a specificity of 90.86% and an overall agreement of 93.01%. With CDM the sensitivity increases with 4.53% to 100.00% and the false negativity rate decreases with 4.53% to 0.00%.

Discusion

At first, it should be worth to mention that, depending on the medium used you will be able to achieve a higher or lower specificity based on the specificity of the colorimetric reactions of the particular medium.

Secondly, MP results that have borderline colors and/or a specific colony appearance are confirmed by serological tests, therefor we detected 4 false positive results in the manual reading. These specimen where reported as negative in the end while the screening gave "suspected colonies" based on colorimetric reactions.

Analysis of 430 AP/MN results by reviewing the digital images, and the incubated medium (where needed) showed:

- 57 (13.26%) GBS positive results, missed on manual reading. The colonies observed where "hidden" underneath confluence growth of contaminants like *Enterococcus faecalis*, *Lactobacilli,* and other organisms, easy to miss for the naked eye.
- 79 (18.37%) had borderline colors close to the target color of the medium used. This is a negative side effect of achieving a 100.00% sensitivity and/or the specificity of the colorimetric reaction of the medium used. Using a different, more specific medium could help but might also raise the false negativity rate.
- 294 (68.37%)where showing growth with colors within the defined target color range but a different colony appearance (most of them being *Lactobacilli*). Again this might be a side effect of achieving a high sensitivity not only for automated reading by CDM but even more related to the medium used. A more selective medium could help but might also raise the false negativity rate.

Conclusions

- Automated reading on WASPLAB™ using CDM software is highly sensitive for the detection of GBS on Brilliance GBS Agar (OXOID).
- CDM software detects colonies that the naked eye misses, as a result to this, the false negativity rate was lowered to zero.
- Using the automated reading by CDM software the time spend on negative screenings are reduced to a minimum which indirectly reduces the cost for GBS screening and enabling the technicians to focus on (suspected) positive cultures instead.
- The use of CDM is not limited to GBS Screening only but, can be recommended for all kind of microbial screenings being performed in the laboratory (e.g. MRSA, CPE, VRE).

References

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