Digital Detection of Group A Streptococcus using Colorex Strep A CHROMagar and WASPLab Chromogenic Detection Module

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REVISED BACKGROUND: Despite the availability of several diagnostics tools for the diagnosis of Group A *Streptococcus* (GAS) pharyngitis, culture remains one of the primary methods in use today and is still considered the gold standard for the detection of GAS from pharyngeal samples. This is likely because culture has high sensitivity compared to rapid antigen tests and low cost compared to molecular approaches. However, in larger volume laboratories, screening for GAS by culture can be cumbersome and streamlined approaches using automated plating instrumentation, smart incubation and image analysis could be helpful.

This study evaluates the capability of the WASPLab™ Total Laboratory Automation System (TLA) (**Figure 1**) (Copan Diagnostics, Murrieta, CA) PHENOMatrix™ Chromogenic Detection Module (CDM) to automatically detect and interpret orange GAS colonies on a novel chromogenic agar called Colorex Strep A Agar (CHROMagar, Paris, France). To date the Colorex Strep A Agar is not Food and Drug Administration (FDA) cleared.

REVISED MATERIALS/METHODS: A total of 250 remnant pharyngeal samples, during the period of September 2017 through January 2018, from pediatric patients presenting to the Emergency Department at Children's Hospital Los Angeles with presumed bacterial pharyngitis were enrolled in this study. Samples were collected using the ESwab Transport System (Copan Diagnostics) and initially tested for the presence of GAS by PCR (Lyra Direct Strep Assay, Quidel, San Diego, CA). The Lyra Direct Strep Assay detects GAS and Groups C and G streptococci but does not differentiate C from G. The Lyra assay was performed at Children's Hospital Los Angeles as standard of care per the manufacturer recommendations.

Remnant Eswab samples (that were either positive or negative for GAS by Lyra PCR) were inoculated onto a blood agar plate (BAP) and a Colorex Strep A agar, using the WASP specimen plater (30 µl loop) and incubated in the WASPLab™ (Copan Diagnostics) (**Figure 1**) for 24 hours (CO_2). Incubation atmosphere and time for Colorex Strep A agar were per manufacturer recommendations.

At 24 hours the medical technologists examined the plates for the presence of orange colonies on the Colorex Strep A agar (**Figures 2 & 3**). Negative, or non-GAS colonies are colorless as seen in **Figure 4**. The results from the visual examination of the chromogenic agar by the medical technologists were then compared to the results from the PHENOMatrix™ Chromogenic Detection Module (CDM). Orange colonies that were observed on the Colorex Strep A Agar were also confirmed via Matrix Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS). Performance of the Colorex Strep A Agar was also compared to growth on the BAP.

REVISED RESULTS: After 24 hours incubation the Colorex Strep A agar was manually examined by the medical technology staff on a total of 250 cultures included in the study. The WASPLab™PHENOmatrix™ CDM system after secondary manual review had a sensitivity of 100% and a specificity of 96.5% in detecting orange colonies on the Colorex Strep A agar (**Table 1**). When plates were examined manually by a technologist (not assisted by the CDM software) after secondary manual review the sensitivity for detecting orange colonies was 96.5% with a specificity of 100% (**Table 2**).

Figure 1. The WASPLab - Total Laboratory Automation system consisting of the WASP (Walk Away Specimen Processor) plating instrument, track for moving petri dishes, and a smart incubator with a 27-megapixel camera for imaging analysis.

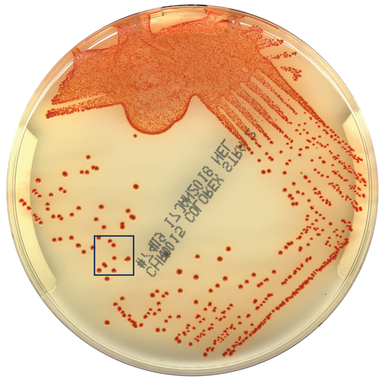

Figure 2. Colorex Strep A Agar, CHROMagar. Orange colonies represent Group A Streptococcus.

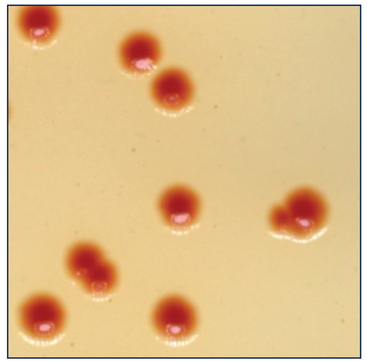

Figure 3. Colorex Strep A Agar, CHROMagar. Orange colonies represent Group A Streptococcus (enlarged view of orange colony)

Additionally, to validate that the orange colonies seen on the Colorex Strep A Agar were GAS, MALDI-TOF MS was performed on each colony. Of the 57 cultures that grew orange colonies on the Colorex Strep A Agar, 51 were confirmed as GAS by MALDI-TOF MS. There were 4 cultures that grew orange colonies that were not confirmed by MALDI-TOF MS and were identified as the following: 2 were identified as *Staphylococcus simulans*, 1 was identified as *Staphylococcus aureus* and 1 was identified as *Kocuria rhizophila*. It is important to note that one of the *S. simulans* and the *S. aureus* grew individually from samples that were initially PCR positive by the Quidel Lyra assay. The other *S. simulans* and the *K. rhizophila* grew from specimens that were initially PCR negative. It is unclear if there was contamination when the colony was picked for MALDI-TOF MS \ resulting in the lack of confirmation of GAS growth on the plate. Unfortunately, 2 of the cultures that grew orange colonies were not able to be tested by MALDI-TOF MS. There were 5 specimens that were initially PCR positive that did not grow in culture on the Colorex Group A Strep Agar.

Table 3 highlights the suboptimal performance of the BAP in the isolation of GAS with a sensitivity of 78.9% and specificity of 73.6% compared to Colorex Strep A Agar. It is important to note that even though the Colorex Strep Strep A Agar is not FDA cleared fewer colonies would require confirmation compared to the BAP plate.

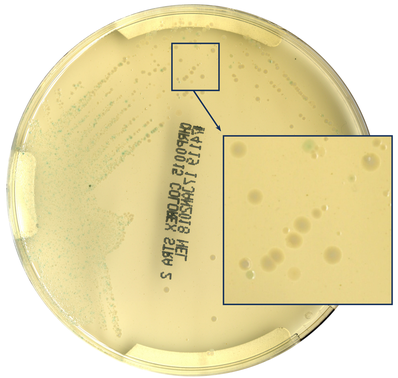

Figure 4. Colorex Strep A Agar, CHROMagar. Colorless colonies are non-GAS.

Table 1. Manual examination of Colorex Strep A Agar after 24 hours incubation with secondary manual review

		Orange Colony	
		Pos	Neg
CHROMagar at 24 hours (visual)	Pos	55	0
	Neg	2	193
		Sensitivity: 55/55+2 = **96.5%**	Specificity: 193/193+0 = **100.0%**

PPV = 55/55 + 0 = 100% ; NPV = 193/193+2 = 98.9%

Table 2. WASPLab examination of Colorex Strep A agar after 24 hours incubation using CDM software with secondary manual review

		Orange Colony	
		Pos	Neg
CHROMagar at 24 hours (CDM algorithm)	Pos	57	7
	Neg	0	186
		Sensitivity: 57/57 + 0 = **100%**	Specificity: 186/186 + 7 = **96.4%**

PPV = 57/57 + 7 = 89.1%: NPV = 186/186 + 0 = 100%

Table 3. Comparison of manual examination of BAP versus Colorex Strep A Agar (with secondary manual review)

		Orange Colony	
		Pos	Neg
Beta Hemolysis Present on BAP	Pos	45	51
	Neg	12	142
		Sensitivity: 45/45 +12 = **78.9%**	Specificity: 142/142 + 51 = **73.6%**

CONCLUSION: To date, there have been 2 excellent published studies demonstrating the ability of the WASPLab™ CDM software in detecting and sorting positive and negative cultures based on pigmentation production on chromogenic media[1,2]. This proof of concept study validates the ability of the CDM software in the detection of GAS using a novel chromagar, Colorex Strep A Agar. This study also demonstrated that the Colorex Strep A Agar has good sensitivity and specificity compared to an FDA cleared PCR assay for GAS and better overall performance compared to culture on a BAP.

The WASPLab™ CDM software can segregate cultures based on whether they are negative or positive for orange colonies. Negative cultures can be grouped together and quickly confirmed as negative and resulted as such by the medical technologist. Positive cultures can be sent for confirmation, or if the media is ever FDA cleared for direct detection of GAS, resulted as positive.

Since culture is still widely used in the clinical laboratory for the diagnosis of GAS, WASPLab™ CDM Software in combination with the Colorex Strep A Agar has the ability to dramatically improve workflow by reducing turn-around-time and redirecting laboratory personnel to other more complex tasks. In addition, given the improved sensitivity of the CDM software in identifying positive orange colonies presumptive for GAS critical positive specimens that might be missed by technologist review can now be captured with the use of Copan's digital imaging software. The data from this study is very promising. Additional studies would be beneficial to corroborate the performance of this new medium.

1. Faron et al., 2016. Automatic Digital Analysis of Chromogenic Media for Vancomycin Resistant Enterococcus Screens Using Copan WASPLab. J Clin Microbiol. 54:2464 – 2469
2. Faron et al., 2016. Automated Scoring of Chromogenic Media for Detection of Methicillin-Resistant Staphylococcus aureus by use of WASPLab Image Analysis Software.

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