



PRIME SELECTION OF PUBLICATIONS BASED ON COPAN AUTOMATION SOLUTIONS

COPAN | 2020 EDITION



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ACKNOWLEDGEMENTS:

Foremost, we would like to express our sincerest gratitude to the authors whose works have been devotedly arranged in the booklet. They rendered insight and expertise that greatly assisted the prime selection.

Furthermore, we would like to thank all experts throughout the world for the trust placed in our products. Their esteem is the best acknowledgment ever.

This booklet comprises the main accomplished studies on WASP®, WASPLab® and Colibri® Systems and the main LBM® (Liquid Base Microbiology™) products.

With a valuable collection of the most representative papers and posters from several authors throughout the world, the aim was to show and tell the use of Copan Automation in combination with LBM®.

For the materials other than “open access” we have used a QR code that provides a quick link to the webpage where the original work can be viewed/purchased.

WASP®

Copan WASP®: Walk-Away Specimen Processor™ is a truly revolutionary instrument for specimen processing for Microbiology. WASP® provides a comprehensive system encompassing all aspects of automated specimen processing, planting and streaking, Gram slide preparation and enrichment broth inoculation.

WASPLab®, PhenoMATRIX™ & AST

WASPLab® is the natural evolution of the WASP® project and Copan philosophy, and it takes care of the incubation/imaging of the plates and brings the customer into the world of Digital Bacteriology. Before and during the incubation, the unique WASPLab® vision system acquires images that will be available in high-quality digital format to the lab technician to perform the reading phase. Moreover, WASPLab® enables the fully automated dispensing of antibiotic discs onto plates, for an Antibiotic Susceptibility Testing (AST) with no risk of contamination.

PhenoMATRIX™ uses artificial intelligence combined with clinical information from the LIS system to automatically read, interpret, and segregate bacterial cultures with the click of a button. Adding PhenoMATRIX™ suite of algorithms to WASPLab® automation system ease the interpretation of patient results and give to microbiology labs the ability to shorten the time to results.

Colibri™

Colibri® is a microbiology system that automatically picks colonies previously selected by an operator on the WASPLab® reading station. The instrument spots targets for microbial identification through MALDI-TOF technology and prepares microbial suspensions for Antibiotic Susceptibility Testing (AST).

WASP® and WASPLab® sample workflow

Rapid identification by MALDI-TOF/MS and antimicrobial disk diffusion susceptibility testing for positive blood cultures after a short incubation on the WASPLab®

Cherkaoui A, Renzi G, Azam N, Schorderet D, Vuilleumier N, Schrenzel J

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Copan WASPLab® automation significantly reduces incubation times and allows earlier culture readings

Cherkaoui A, Renzi G, Vuilleumier N, Schrenzel J

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Preneshni Naicker, Keitha Mentoor, Clive Misland, V. Mischka Moodley, Pascal Musoni, Chad Centner, Clinton Moodley, Janet Scholtz, Mark Nicol
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WASP® and STOOL SAMPLES with FecalSwab™

Evaluating the preservation and isolation of stool pathogens using the COPAN FecalSwab™ transport system and walk away specimen processor

Goneau LW, Mazzulli A, Trimi X, Cabrera A, Lo P, Mazzulli T
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Microbiology meets molecular diagnostics evolving in flexible sample to result workflow

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Implementation of Copan FecalSwab™ and Copan Selenite™ on WASP® for the Automated Processing of Stool Specimens

Ann Lemmens, Truus Goegebuer, and Luc Hendrickx
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Accelerated Time-to-Diagnosis by Automated Processing of Positive Blood Cultures on Copan WASPLab®

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Use of Copan's WASPLab® PhenoMATRIX™ Artificial intelligence to improve the efficiency of Urine Culture interpretation

Susan M. Poutanen, Jessica Bourke, Pauline Lo, Karen Pike, Katherine Wong, Tony Mazzulli

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Faron ML, Buchan BW, Relich RF, Clark J, Ledebøer NA

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Segregation and WASPLab® automation impact on MRSA screening at LTHT

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Vittorio Sambri, Maria Federica Pedna

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Evaluation of WASPLab® Software To Automatically Read chromID CPS Elite Agar for Reporting of Urine Cultures

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Clinical performance of WASPLab® AI/IA-PhenoMATRIX™ software in detection of GBS from LIM enriched culture plated to CROMID strepto B Chromogenic media

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M. Gaskin, D. Yamamura, J. Korver

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J. Bayette, A. Roche, T. Bayol, J-F. Bessel, P. Rolland, P. Mion, R. Fournier, G. Teissier

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Multicenter Evaluation of the WASPLab® Digital Image Analysis Software to Segregate Significant Growth of Urine Cultures on Blood and MacConkey Agar

M.L. Faron, B. W. Buchan, R.F. Relich, A. Phillips, D. Kahn, J. Clark, and Nathan. A. Ledebøer

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

Jennifer Dien Bard, Jacob Nelson, Kenneth Mata, Darnell Thorpe, SusanNovak-Weekley

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My-Lien Pham, Ken Van Horn

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COLIBRÍ®

Copan Colibrí®, an innovative fully automated instrument for Clinical Microbiology Laboratory

A. Bielli, V. Lepera, S. Torri, A. Nava, G. Lombardi, C.F. Perno, C. Vismara

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Colibrí® and Bruker MALDI-TOF: Does the identification performance change when different chromogenic media are used for Urine culture?

Michela Paolucci, Laura Navarria, Santina Castriciano

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Evaluation des performance du preleveur automatique de colonies COLIBRI®

A. Roché, G. Teissier, R. Fournier, P. Mion, T. Bayol, J. Bayette

RICA1 2019

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**WASPLab® and ANTIMICROBIAL
SUSCEPTIBILITY TESTING****Disc diffusion AST automation: one of the last pieces missing for full microbiology laboratory automation**

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ECCMID 2016

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WASP[®] and WASPLab[®]

SAMPLE WORKFLOW



Rapid identification by MALDI-TOF/MS and antimicrobial disk diffusion susceptibility testing for positive blood cultures after a short incubation on the WASPLab®

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KEYWORDS

WASPLab®

| blood culture

| AST

| MALDI/TOF

| TAT



This study evaluates the shortest incubation times on the WASPLab® for MALDI-TOF/MS-based identification and inoculum preparation for AST in a wide panel of common pathogens in bloodstream infections.

4 hours of incubation allowed the accurate identification of 73% of the strains; this rate was increased to 85% after 6 hours and to 100% after 8 hours. AST by disk diffusion required times ranging between 3 and 8 hours depending on the bacterial species analyzed.



Copan WASPLab® automation significantly reduces incubation times and allows earlier culture readings

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KEYWORDS

WASPLab®

shortened TAT

automated vs manual diagnostic

performances



This study evaluates whether lab automation may help to reduce the turn-around times, comparing WASPLab® automation to WASP®-based automated inoculation with conventional incubation and manual diagnostic. The results show how WASPLab® reduces the culture reading time for several types of specimens without affecting sensitivity and confidence performances. Implementing this technology could shorten turn-around times with a beneficial effect on the patient's outcome.



Impact of the introduction of WASPLab® on the reporting time of blood cultures

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KEYWORDS

Colibri® | AST | ID | blood samples | reliability | time saving

INTRODUCTION

Aims of automation of bacteriological cultures:

- improve quality (e.g. standardization, traceability)
- improve efficiency
- reduce time to reporting of results

Impact of introduction of WASPLab® (Copan, Italy) on reporting time of identification (ID) and antimicrobial susceptibility testing (AST) results of blood cultures (BC) in real-life laboratory setting.

MATERIALS AND METHODS

- Belgian tertiary care hospital UZ Leuven
- Opening hours of bacteriology laboratory on weekday: 8.30 am – 6.00 pm
- Streaking of positive blood cultures during the night at 22.00 pm and 2.00 am
- Manual streaking of plates on at least a blood agar and a MacConkey agar
- Identification when sufficient visual growth with MALDI-TOF MS (Bruker)
- Antimicrobial susceptibility testing with Vitek® 2 (BioMérieux) or disk diffusion (Neo-Sensitab, Rosco)
- Automation of incubation and reading of plates: WASPLab® (period 2)

Period 1 (June-October 2017)	Period 2 (June-October 2018)
Conventional incubation and reading	During opening hours bacteriology lab: WASPLab® incubation and reading (photographs of plates at 4,6,10,16,24 and 48 hours)
Reading of agar at 8.30 am, 2 pm and 5 pm	During night: conventional incubation and reading

RESULTS

	period 1	period 2
method of incubation and reading of BC	conventional	WASPLab®
number of positive BC	4905	4310
median time between BC positivity and reporting ID	20h 00 min	17 h 42 min
median time between BC positivity and reporting AST	40h 54 min	37 h 54 min

CONCLUSION

A real-life laboratory introduction of WASPLab® resulted in faster reporting of both ID and AST results of positive BC compared to conventional incubation and reading, while maintaining the same number of laboratory technicians and the same opening hours of the laboratory.



Utilizing Digital Imaging to Determine Optimal Incubation Times for Routine Urine Cultures

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KEYWORDS

Urines | reduced TAT | WASPLab® | Optimal Incubation | Customized Imaging

ABSTRACT

Background: Full laboratory automation has the potential to image cultures at defined period times to expedite the re-release of results while maximizing efficient use of laboratory staff. This study focused on TAT for urine culture results using Copan's WASPLab® digital imaging to determine optimal reading times. We compared reading urine cultures from Blood and MacConkey agars every 2 hours starting at 11/12/13 hours and up to 23/24 hours using the WASPLab®.

Materials and Methods: Urine specimens collected during the fall and winter months of 2017 were included in the study. A total of 946 specimens were analyzed by observing digital images taken with the WASPLab® every two hours. A total of 184 (19.5%) of the specimens were negative throughout the study period.

Results: Of the remaining 762 positive cultures, 109 (14.3%) had optimal growth at 11/12 hours of incubation, 133 (17.5%) after 13/14 hours, 84 (11.0%) after 15/16 hours, 412 (54.1%) after 17/18 hours, 10 (1.3%) after 19/20 hours and 14 (1.8%) after 23/24 hours of incubation. Thus, 31.8% of all positive urine cultures could be read optimally at 14 hours of incubation, with a total of 96.9% of all positive urine cultures having optimal reading times at or before 18 hours. In addition, all 24 urine specimens showing optimal growth after 18 hours of incubation had growth detected on blood agar on or before 18 hours. As there was a concern for possibly missing slower growing organisms after only 18 hours of incubation, such as yeast and alpha-hemolytic colonies, these cultures were looked at individually. All specimens that contained yeast (25 cultures) or small alpha-hemolytic colonies (27 cultures) were also detected at or before 18 hours of incubation.

Conclusions: In summary, based on our patient population, including reading times at 14 and 18 hours, would allow for approximately 1/3 of positive urine cultures to be worked up after only 14 hours of incubation (including over half of the cultures that contained gram negative bacilli) and the other approximately 2/3 of positive urine cultures to be worked up after 18 hours of incubation. The 3.1% of cultures that had optimal reading times after 18 hours would not be missed as growth was detected at or before 18 hours of incubation. Instituting a single 18 hour read time would allow a more optimized work flow in the laboratory, better utilization of microbiology staff, and have a positive impact to clinicians and patients.

INTRODUCTION

Nearly 1 in 3 women have a clinically significant urinary tract infection (UTI) by the age of 24, almost half of women will experience a UTI in their lifetimes, and almost half of women who get one UTI experience a recurrence within 6-12 months. In the United States, UTIs account for more than 8-10 million office visits, 1-3 million emergency department visits and 100,000 hospitalizations each year. Therefore, it is not surprising that urine constitutes the most common type of specimen submitted to the microbiology laboratory. When treated promptly and properly, lower UTIs (infections of the bladder and urethra) rarely lead to complications. However, if left untreated, a UTI can have serious consequences such as spread of the infection from the bladder to one or both kidneys. When bacteria infect the kidneys, they can cause damage that may permanently reduce kidney function, and in people who already have kidney problems this can lead to kidney failure. Thus, the efficient detection of the causative agent of a UTI along with the antimicrobial susceptibility testing of the pathogen can be critical for patient care. Full laboratory automation has the potential to image microbiological cultures at defined periods of time and can lead to expedited release of results while maximizing the efficient use of laboratory staff. This study focused on utilizing Copan's WASPLab® digital imaging to determine the optimal time for urine culture incubation in order to accurately detect significant urinary pathogens.

METHODS

The laboratory's currently protocol was to process all urine specimens on the Copan WASP® automated processing instrumentation and read urine culture images taken with the WASPLab® on both blood agar and MacConkey agar at 2-time frames: 14 hours of incubation and again at 24 hours of incubation. Previous data showed that the 14-hour image read resulted in the ability to work up approximately 20% of the all clinically significant culture growth. In order to determine the optimal time for reading urine cultures, we compared images taken from 946 patient urine specimens every 2 hours on both media starting at 11/12 hours of incubation up to 23/24 hours. Results for each specimen were recorded indicating the presence or absence of growth, and if growth was present the colony count and type of colonies present.

RESULTS

A total of 184 or 19.5% of the specimens were negative at each time frame images were read. Of the remaining 762 positive cultures, 109 (14.3%) had optimal growth at 11/12 hours of incubation, 133 (17.5%) after 13/14 hours, 84 (11.0%) after 15/16 hours, 412 (54.1%) after 17/18 hours, 10 (1.3%) after 19/20 hours and 14 (1.8%) after 23/24 hours of incubation.

TABLE 1.

Hours of incubation	11/12	13/14	15/16	17/18	19/20	21/22	23/24
Percentage of cultures w/optional reading	109 (14.3%)	133 (17.5%)	84 (11.0%)	412 (54.1%)	10 (1.3%)	0 (0.0%)	14 (1.8%)

RESULTS

A total of 242 (31.8%) of all positive urine cultures could have images read optimally at 14 hours of incubation. A total of 96.9% of all positive urine cultures had optimal image reading times at or before 18 hours of incubation. A total of 24 (2.5%) urine specimens showed optimal growth after 18 hours of incubation, however, 100% (24/24) of these cultures had growth detected on the blood agar on or before this time frame. Although they had not yet had their optimal incubation, they would not have been called negative; these would be continued to be incubated until such time that appropriate work up could be performed. All urine specimens that showed slower growing organisms, such as yeast (25 cultures) or small alpha-hemolytic colonies (27 cultures) also had growth detected at or before 18 hours of incubation.

CONCLUSIONS

Based on our patient population, changing our image reading times from 14 and 24 hours to 14 and 18 hours, would allow for approximately 1/3 of positive urine cultures to be worked up after only 14 hours of incubation (including over half of the cultures that contained gram negative bacilli). The other approximately 2/3 of positive urine cultures could be worked up after 18 hours of incubation rather than needing to wait until a full 24 hours of incubation. The 3.1% of cultures that had optimal reading times after 18 hours would not have been missed as growth was detected at or before 18 hours of incubation in all of these cultures. These cultures would continue to be incubated until they could be appropriately worked up. In addition, as there was a concern for possibly missing slower growing organisms, such as yeast and small alpha-hemolytic colonies after only 18 hours of incubation, these cultures were looked at individually. All specimens growing yeast (25 cultures) or small alpha-hemolytic colonies (27 cultures) were also detected at or before 18 hours of incubation. The data from this study indicates that the laboratory's current 24-hour incubation time could be shortened to 18 hours without negatively impacting culture accuracy and reliability. In addition, the possible elimination of the 14-hour image read would allow for streamlining the culture process and allow for more specimens to be processed accommodating increased capacity as needed. In our facility, instituting a single 18-hour image read would allow a more optimized work flow in the laboratory, effective utilization of microbiology staff, and have a positive impact to clinicians and patients delivering urine culture results hours sooner.



The patient is in the centre: integrated management of results with WASPLab® System

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KEYWORDS

WASPLab® | integrated management | automation | general medicine | precision clinical microbiology

ABSTRACT

In the last decade automation introduced innovation in the bacteriology laboratories since plates can be automatically streaked, incubated and growth digitally recorded and evaluated.

AIM OF THE STUDY

In this study, we present an additional feature introduced in our laboratory through WASPLab® (Copan, Italy) that consists in the contextual evaluation and interpretation of all the patient specimens by a single microbiologist. Possibility of working by patient rather than material: "precision clinical microbiology». We present two clinical cases optimally managed employing this strategy:

- ICU patient
- General Medicine patient

MATERIALS AND METHODS

The first case was represented by a patient admitted in ICU after abdominal surgery: a rectal swab (RS), nasal swab (NS), Bronchial Aspirate (BAS) and Blood Cultures (BCs) were collected. The second patient was admitted to general medicine for heart failure: a rectal swab, a urine and BC samples were ordered. All the samples were processed on WASP® and WASPLab® automation.

RESULTS

At day 1, microbiologists simultaneously evaluated the bacterial growth on agar plates for all samples incubated in WASPLab®. For both patients, the plates interpretation suggested a *K. pneumoniae* Carba-R rectal colonization and the Gram stain from positive BC indicated Gram negative bacilli. For the first patient the nasal swab reported *P. aeruginosa*, BAS indicated KES, the second patient urine was positive for KES. Since the Carba-R colonization status, a molecular test was immediately and properly ordered to define if these pathogens were the cause of the systemic infection. In the first patient, a *K. pneumoniae* KPC was confirmed and notified two days earlier than the previous routine procedure based on single material analysis approach. In the second case the aetiology was identified as multi-drug sensitive *E. aerogenes* (Figures 1 & 2).

CONCLUSION

In our context, the possibility for a clinical microbiologist to analyse all samples for each patient is consistent with a powerful overall vision and integrated management of results. This strategy could be named "precision clinical microbiology" because offers an additional value in complex cases and encourages the construction.



Medical value of WASPLab® automation solution in a 24/7 clinical microbiology laboratory: some preliminary results

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KEYWORDS

WASPLab® | manual vs automated | standardization | reduced time-to-results

ABSTRACT

According to lab automation manufacturers, their solutions improve standardization, decrease time to obtain results (TTR) and reduce lab workforces. A WASPLab® solution was introduced in the 24/7 Lyon Hospitals lab early November 2017 ensuring automatization of approximately 88% of the samples in September 2018. To assess the medical value of lab automation solution at this stage, TTR of urine, blood cultures (BC) and multi drug resistant organism screenings (MDRO) were compared before and after automation.

MATERIALS AND METHODS

Based on laboratory information system data (GLIMS, MIPS), the time between sample streaking to final confirmation by lab technicians (LT): TTR "final confirmation" and validation by medical microbiologists: TTR "final validation" were measured on three types of samples: urine, MDRO and BC. Data from matched periods of 2017 without automation versus 2018 with WASPLab® (Copan/bioMérieux) were compared whilst the other instruments remained unchanged. Whereas the LT number and MDRO workflow was unaffected between 2017 and 2018, the implementation of WASPLab® allowed a new lab organization with an extended D1 lab technicians activities from 8am-4pm to 8am-8pm for urine and BC samples. For positive MDRO, off line tests (Etest, MIC) were added in 2018. For BC, an additional visual reading of pictures after 8h of incubation was also introduced.

Table start at next page.

CONCLUSIONS

These preliminary results reveal that automation improve standardization of incubation/reading steps. Without workflow change, the TTR of MDRO was slightly lengthened (due to increase of positive rate and addition of off line tests). In contrast, the association of WASPLab®, extension of reading time to 8pm by LT, and for BC an additional reading time point, induced a TTR shortening : until 5 hours (≈339min) for urine, and 3 to 8 hours (≈504min) for BC samples. These promising results need to be confirmed on larger sample flow.

URINE WORKFLOW

TECHNICAL TTR FOR TECHNICAL STEP		TOTAL TTR FOR TECHNICAL AND MEDICAL VALIDATION	
SAMPLE ARRIVAL - SAMPLE PROCESSING - READING PHASE - ID/AST			
2017	D0 TASKS 24/7 D1 - 8AM > 4PM	POSITIVITY RATE	2017: 32% 2018: 39%
2018	D0 TASKS 24/7 D1 - 8AM > 8PM		% over 20h of incubation 2017: 78% 2018: 99%

URINE SAMPLE

Sample type and results per sample	Number of samples 2017 vs 2018	Technical TTR by LT Δ [2018 – 2017 in minutes]	Total TTR for Technical and medical validation Δ [2018 – 2017 in minutes]
Global	2015 vs 2093	-142	-110
Negative & non-significant	842 vs 785	-7	-23
Contamination	466 vs 484	-332	-254
Positive	707 vs 827	-337	-339

MDRO WORKFLOW

TECHNICAL TTR FOR TECHNICAL STEP		TOTAL TTR FOR TECHNICAL AND MEDICAL VALIDATION	
SAMPLE ARRIVAL - SAMPLE PROCESSING - READING PHASE - ID/AST			
2017	D0 TASKS 24/7 D1 - 8AM > 4PM	POSITIVITY RATE	2017: 7% 2018: 12%
2018	D0 TASKS 24/7 D1 - 8AM > 4PM		% over 20h of incubation 2017: 85% 2018: 98%

MULTI RESISTANT BACTERIA SCREENING

Sample type and results per sample	Number of samples 2017 vs 2018	Technical TTR by LT Δ [2018 – 2017 in minutes]	Total TTR for Technical and medical validation Δ [2018 – 2017 in minutes]
Global	1089 vs 921	+39	+14
Negative	1008 vs 816	-33	-62
Positive	81 vs 105	+460	+377

MDRO WORKFLOW

TECHNICAL TTR FOR TECHNICAL STEP		TOTAL TTR FOR TECHNICAL AND MEDICAL VALIDATION	
SAMPLE ARRIVAL - SAMPLE PROCESSING - READING PHASE - ID/AST			
2017	D0 TASKS 24/7 D1 - 8AM > 4PM	POSITIVITY RATE	2017: 9.5%
2018	D0 TASKS 24/7 D1 - 8AM > 8PM + 8H READING		2018: 6.1%

BLOOD CULTURE

Sample type and results per sample	Number of samples 2017 vs 2018	Technical TTR by LT Δ [2018 – 2017 in minutes]	Total TTR for Technical and medical validation Δ [2018 – 2017 in minutes]
Global	3821 vs 8293	-218	-259
Negative	3438 vs 7788	Automatic validation	Automatic validation
Positive	363 vs 504	-242	-504



Competence Assessment, Training and Teaching In a Total Automated Microbiology Laboratory

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KEYWORDS

Gram stain

| slide preparation

| Standardization

| WASP®

INTRODUCTION

Competence assessment, training and teaching are integral in developing knowledgeable competent staff and essential in meeting accreditation requirements for a Microbiology Laboratory. The introduction of WASP®/WASPLab® automation in the laboratory supports effective methodologies to provide the required consistency in specimen processing, incubation and digital imaging. The objective of this study was to use digital culture imaging and gram slide preparations generated by the WASP®/WASPLab® automated system as well as automated staining for competence assessment, training and teaching of staff, students and residents.

MATERIALS AND METHODS

WASP®/WASPLab® automation and automated staining were used to provide competence assessment to 49 Medical Laboratory Technologists (MLT) and 13 Medical Laboratory Assistants (MLA). MLTs analyzed digital images on the WASPLab® from various patient specimens. All MLTs looked at the same images. MLTs also read gram stain slides prepared by the WASP® and stained with an automated stainer. MLAs performed daily maintenance procedures on the WASP® and WASP® slide preparation module.

RESULTS

Upon direct observation, all 13 MLAs performed the appropriate maintenance tasks on the WASP® slide prep module as well as demonstrating problem solving skills. Using WASP®/WASPLab®, 5 urine specimens were processed and analyzed by 49 MLTs. Images were available in the plate browser and MLTs were given a simulated choice of button selections in the screening/reading modules. All MLTs were successful in their selections, image analysis and reporting. The same MLTs were given 5 gram slide preparations of positive blood cultures made by the WASP® and stained with the automated stainer. All MLTs were correct in their smear interpretation.

CONCLUSION

Laboratory automation provides standardized sample preparation, processing and digital imaging analysis which aligns itself perfectly for competence assessment as well as training and teaching opportunities. Digital images are stored and available for viewing over long periods of time which helps overcome the challenge of assessing staff over a range of different shifts for many weeks. Laboratory automation can help to develop highly skilled competent personnel who provide consistent, predictable and high quality results. Stored WASPLab® images can be used for training new staff as well as teaching students and residents.

Comparison of Copan WASP® versus BD Kiestra™ Inoqula™ in Isolating Colonies from Positive Urine Culture Specimens

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KEYWORDS

Comparative study

WASP® vs BD kiestra inoqula™

Urines

Saving

INTRODUCTION

Laboratory automation in microbiology has evolved over the last decade to include sophisticated plating instruments, which improves the quality of plating and reduces the associated labor. Positive patient identification is also another added quality feature non-existent in the plating process prior to automation. There have been no publications comparing the Copan WASP® (Copan Diagnostics, Murrieta, CA) plating instrument to the Becton Dickinson Kiestra™ Inoqula™ (BD, Sparks, MD). In this study a total of 294 turbid urine specimens were plated using both instruments. The ability of each instrument to produce enough isolated colonies for immediate culture work up and the time it took to read a subset of cultures were analyzed. Results show that though isolation is equivalent with both systems, cultures containing $\geq 100,000$ CFU/mL were better isolated with the WASP® (1 μ l whole plate) versus the Kiestra™ (10 μ l whole plate). In addition, a subset of images screened by technologists and their reading time was recorded. Reading times were less for both the whole and bi-plate inoculated on the WASP®. The ability of the WASP® to inoculate a smaller volume of urine provides more isolated colonies resulting in less time needed for examination and fewer subcultures.

MATERIAL & METHODS

Routinely collected (non-invasive) turbid urine specimens received at Sutter Health Shared Laboratory (SHSL) were used to ensure we achieve the objectives of the study, and higher chance for significant growth. The two instruments were located at two different affiliates 45 miles apart, SHSL and CPMC (California Pacific Medical Center). To eliminate the effect of transport time between plating and incubation, specimens were divided in two batches and each batch received the same amount wait time to incubation equally.

Specimen Processing: At Sutter Health System urine samples are collected remotely in a sterile cup and immediately transferred to a boric acid (BD Vacutainer Gray-top) tube for transport to SHSL. After receipt 294 turbid specimens were chosen and inoculated by the WASP® onto a Blood-Agar/MacConkey (BAP/MAC) bi-plate using a dual 1 μ L loop and Copan VST2 streaking pattern (WB1), and by the WASP® on BAP and MAC whole plates using a 1 μ L loop and Copan SST6 streaking pattern (WW1). The identical samples were plated using the Kiestra™ Inoqula™ (Becton Dickinson) by pipetting 10 μ L onto BAP and MAC whole plates using Kiestra™ streaking Pattern 5 (KW10). It is important to note that the Kiestra™ Inoqula™ is not able to provide 1 μ l volumes for plating, hence only 10 μ l volume was used. All plates were incubated in the WASP Lab® (Copan Diagnostics, Murrieta, CA) in CO₂ for 18 hours. After incubation for 18 hours images were taken by the WASPLab® and stored for future analysis. **Reading time:** To record the difference in average read times a set of 150 plates was analyzed. The average technologist salary at SHSL (excluding benefits) is \$58.59/hr and was used to calculate the cost of labor needed to examine 100 urine cultures.

RESULTS

Colony Quantitation: Total of 211, 213, and 207 urine cultures had growth using plate and streaking combinations WB1, WW1 and KW10, respectively. WB1 positive samples had a total of 272 isolates present of which 234 isolates had enough colonies for work-up (86%). WW1 positive samples had a total of 282 isolates present of which 250 had enough colonies for work-up (89%). KW10 positive samples had a total of 270 isolates present of which 224 had enough colonies for work-up (83%). To estimate the difference in colony isolation ability, a score was given (1colony= 1 score, >6 colony= 7 score): no difference was found between WW1 vs KW10 ($p= 0.404$) and in analyzing WB1

vs KW10 a p value of 0.402 was observed for isolates with colony counts between $\geq 10,000$ CFU/mL and $< 100,000$ CFU/mL. A significant difference was found in samples with a concentration of $\geq 100,000$ CFU/mL: the number of isolated colonies was higher in WW1 in comparison with KW10 ($p= 0.001$). No statistical difference was found between KW10 and WB1 ($p= 0.921$).

Reading time: Average technologist read times for each inoculated set of 150 plates was 17.47 minutes (WB1), 19.04 minutes (WW1), and 28.78 minutes (KW10). When compared to the KW10 set, WB1 set was read 11.31 minutes faster and the WW1 set 9.74 minutes faster.

CONCLUSIONS

- There is no statistical difference between the two systems when the colony count is light ($< 100K$ CFU/mL), and they both provide acceptable isolated colonies for culture workup.
- Sample inoculation via the WASP[®] produced better isolation when the colony count was heavy ($> 100K$ CFU/mL). This may result in better urine culture turn-around-time, which could have a positive impact on patient care. We believe the higher inoculation volume (10 μ L) of Inoqua[®] is the main reason for lower yield of isolated colonies.
- Based on our data WASP[®] 1 μ L inoculation volume also provides easier and faster reading time per positive plates. This may have a major impact on high volume laboratories such as SHSL with averaging about 700 urine cultures per day. At SHSL the impact will be an added \$22.09 per 100 positive urine samples. If considering only one-fifth of total cultures are positive this will amount to annual savings of about \$11,000 per year.

Instrument	Vol.	Streak Pattern	Description	Enough Colonies	WASP [®] vs Inoqua [®] Light growth ($< 100K$ CFU/mL)	WASP [®] vs Inoqua [®] Heavy growth ($> 100K$ CFU/mL)	50 plate-Ave. reading time	Δ in minutes	Cost savings/ 100 Urine Cult.
Copan WASP [®]	1 μ L	WB1	Bi-plate BAP/MAC	86%	No difference ($p= 0.402$)	No difference ($p= 0.921$)	17.47 min	Fastest	\$22.09
	1 μ L	WW1	Whole plates BAP + MAC	89%	No difference ($p= 0.404$)	Significant difference ($p= 0.001$)	19.04 min	+1.57	\$19.02
BD Kiestra [®] Inoqua [®]	10 μ L	KW10	Whole Plates (BAP + MAC)	83%	-	-	28.78 min	+11.31	-

Comparison of WASP® and WASPLab® and Inoqula™ for Primary Specimens Streaking Quality

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KEYWORDS

WASP®

| Streaking Pattern

| Isolation

| MALDI-TOF

| AST

INTRODUCTION

This study evaluated the ability of Copan WASP® and BD Kiestra™ Inoqula™ streaking automations to generate sufficient single colonies on primary agar plates for further processing, i.e. identification and susceptibility testing in various clinical specimen types. Overall, comparable numbers of single colonies were yielded applying both devices. A practically important difference was seen in the mean distance and average size of grown colonies, both of which were greater using the WASP® 5-quadrant-streaking pattern as compared to the Inoqula™ 4-quadrant streaking pattern, thereby facilitating colony picking for further processing.

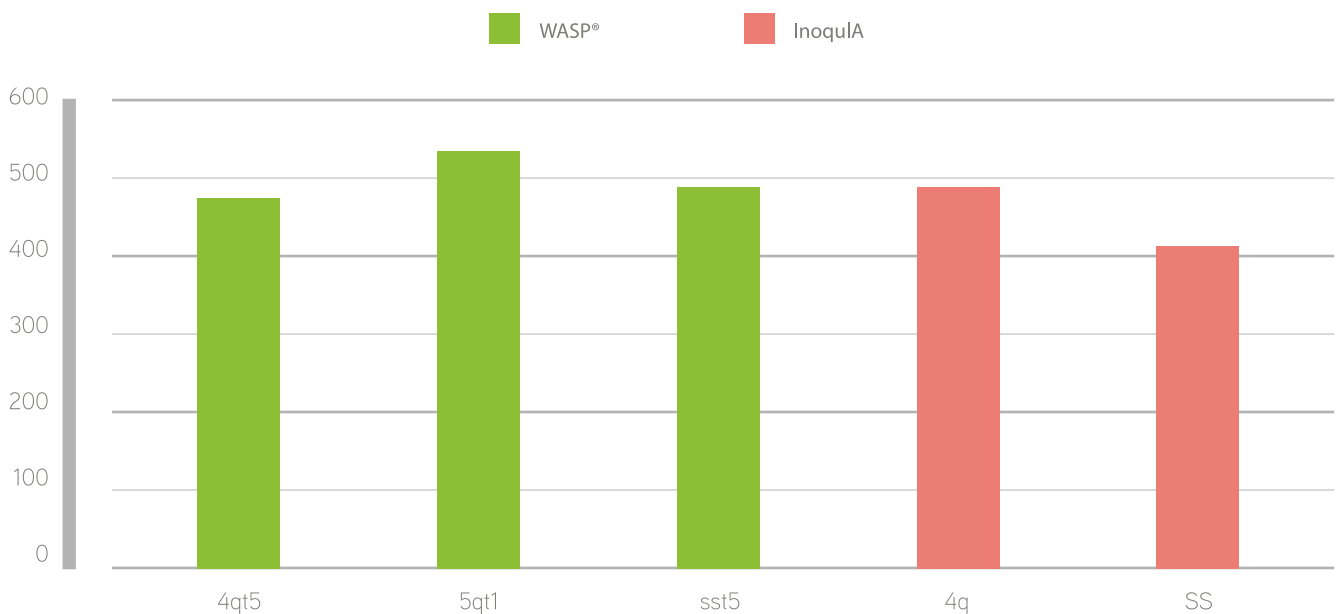
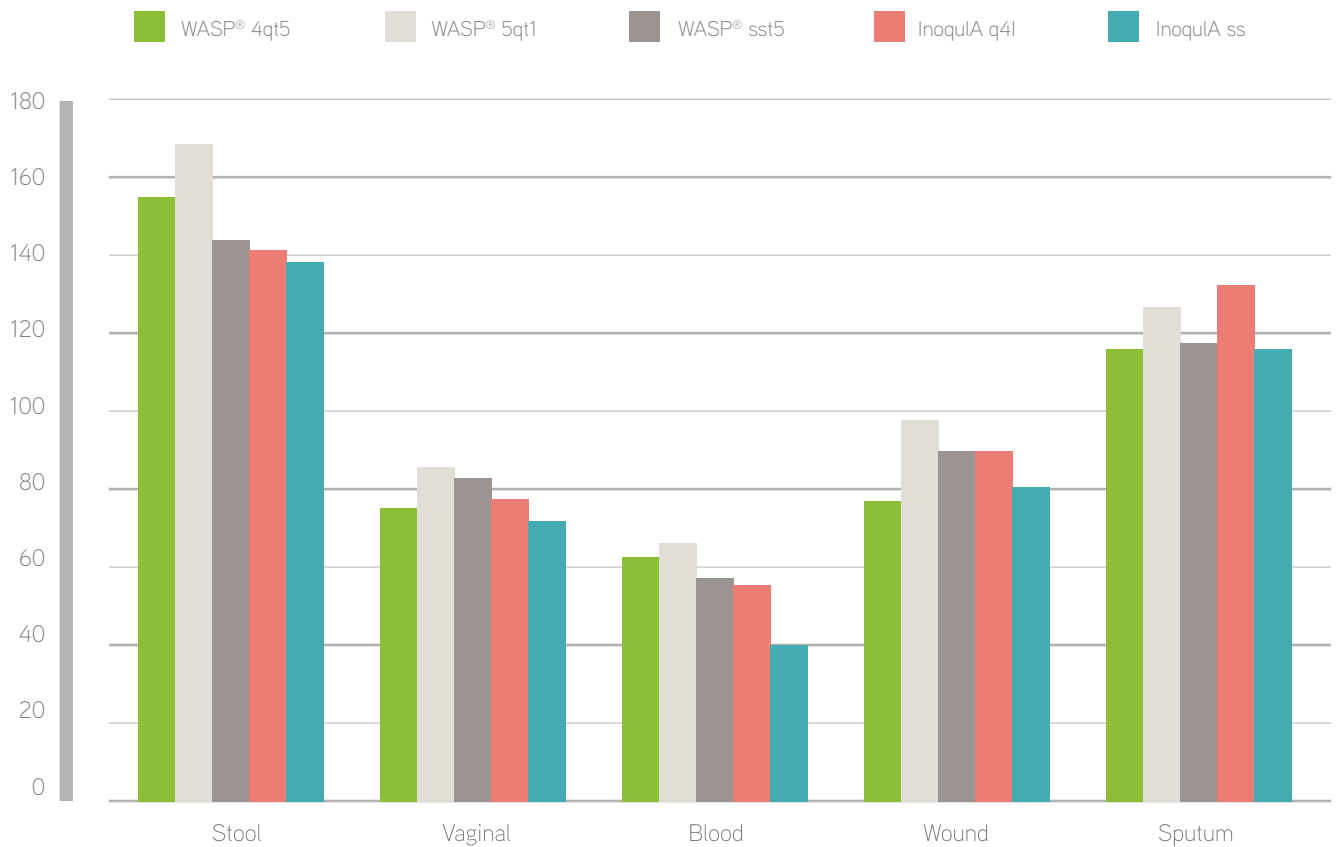
Automation in clinical microbiology increasingly enters clinical laboratories, and the prospects for improved streaking accuracy and related diagnostic accuracy are promising. The Copan WASP® and WASPLab® automation system uses a “loop concept” for sample streaking, while the Inoqula™ (BD Kiestra™) uses a “ball concept”. To date, scientific studies comparing the two systems were done using single bacterial strains and urine clinical samples. In order to evaluate the ability of the two systems to generate well-streaked primary agar plates with sufficient single colonies for further processing in various specimen types, high impact and complex clinical specimens were selected and streaked with WASP® and Inoqula™ benchtop (BD) in parallel. The objectives of this study were to: i) Compare the WASP® “loop based” and the “ball-based” benchtop Inoqula BT™ to inoculate sputum, wound, vaginal, and stool specimens and positive blood culture broth (PBC); ii) Compare both systems ability to generate single colonies from all specimens; iii) Assess the feasibility of streaking results for a complete diagnostic workup (MALDI-TOF identification, susceptibility testing [AST]).

MATERIALS & METHODS

Consecutive clinical sputum, wound, vaginal, and stool specimens and PBC were included in this study. Sputum samples pre-treated with SLSolution™ (1:1 ratio), wound and vaginal specimens in eSwab®, and stool samples in FecalSwab™ were loaded on the WASP® and automatically streaked using a 10 µl loop (WASP®) and 4-quadrant streaking pattern (4Q5 SP); PCB were transferred into BC+™ tubes (Copan) and streaked using both 1 µl and 10 µl loops and a 4Q3 SP. For the Inoqula BT™ 10 µl of each sample were manually dispensed on the appropriate agar plates using a manually calibrated pipettor and streaked with the ball using similar SP similar to the WASP®, or SP as suggested by the manufacturer. All plates were incubated in the automated WASPLab® incubators and images were taken by WASPLab® after various periods of incubation. Growth, streaking pattern, CFU counts, and feasibility for further processing were evaluated for all specimen types by experienced personnel on-screen and results were assessed by scores. Points were given for the possibility to perform a MALDI-TOF analysis (1 single colony) and/or additional AST (3 additional single colonies) for each morphology.

RESULTS

Overall, comparable numbers of single colonies were present in all WASP® “loop based” and “ball-based” benchtop Inoqula BT™ processed specimens. Single colonies feasible for further processing in appropriate numbers were obtained in all specimen types allowing MALDI-TOF based identification, AST and additional testing. WASP® was superior as compared to the Inoqula BT™ regarding the mean distance and average size of grown colonies leading to, WASP® produced SP being considered more user-friendly by technical personnel for plate reading and colony picking.



CONCLUSIONS

WASP® and InoqlA BT™ both were able to produce sufficient numbers single colonies for MALDI-TOF based identification and AST. WASP® 5-quadrant-SP paves the path to fully automated colony picking both for identification and AST since mean distance and average size of grown colonies will most probably be crucial for precision of picking robots.

A Comparative Evaluation of Automated (Copan WASP®) Versus Manual Methods for Plate Streaking and Specimen Processing



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KEYWORDS

WASP®

| Plate Streaking

| eSwab®

| Efficiency

| Quality

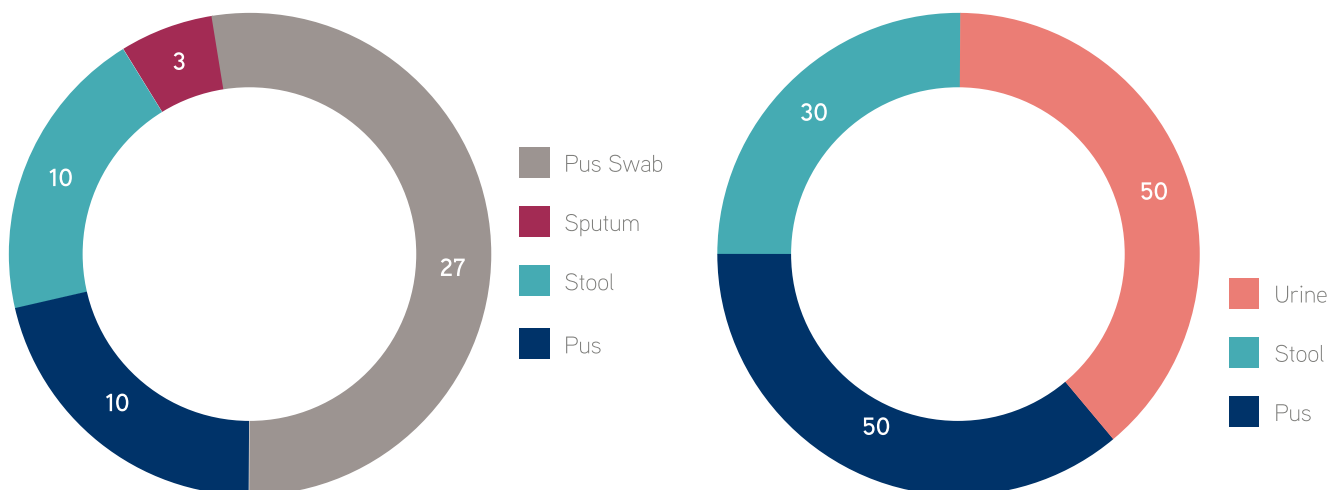
INTRODUCTION

Laboratories are under pressure to produce quality results, with shorter turn-around-times using fewer materials and staff. The Copan Walk-Away Specimen Processor™ (WASP®) is an automated total solution for microbiology specimen processing.

Our aim was to assess the performance and efficiency of the WASP® versus manual processing in a high throughput clinical diagnostic microbiology laboratory.

MATERIAL & METHODS

The plate-streaking ability of the WASP® was assessed using 50 specimens (27 pus swabs, 10 sputa, 10 stools and 3 pus samples). Specimens were inoculated manually onto plates and were then either streaked by the WASP® or manually by a technologist. The second part of the evaluation assessed the WASP® for complete specimen processing (inoculation and streaking) using 50 urines, 50 stools and 30 pus specimens. All specimens except pus were tested in duplicate for both methods (twice on WASP® and twice manually). Efficiency and quality parameters were assessed including: organism recovery, grading of growth, single colony adequacy and crosscontamination. A stress run was performed using 100 urine specimens to test robustness. Reproducibility and accuracy was calculated. Productivity was assessed by calculating full time employee (FTE) "hands-on-time" saved.



RESULTS

Reproducibility and accuracy was not calculated for pus specimens. There was zero crosscontamination observed with the WASP®. The stress test plated 100 urines on a bi-plate within 20 minutes, which was found to be highly efficient. Overall, the WASP® showed better single colony adequacy and grade 3 growth of colonies which allows for better interpretation of plates and more colonies for further investigations. There was a time-saving with all methods using the WASP®, which was more evident for specimen processing than streak-only mode. Time saving was also increased when using the WASP® for pus swabs which included gram stain preparation and broth inoculation. The major challenges related to the specifications of the specimen containers and media plates currently in use by the laboratory.

	Reproducibility (WASP®)	Reproducibility (Manual)	Accuracy (WASP®)	Accuracy (Manual)
Streak only mode	94%	94%	100%	95%
Urine	94%	86%	97%	94%
Stool	82%	96%	80%	95%

Reproducibility and accuracy of Copan Walk-Away-Specimen Processor™ (WASP®) vs. manual methods

	FTE "hands on time" saved
Streak only mode	25 minutes per 100 plates
Urine	63 minutes per 100 urines
Stool	105 minutes per 100 stools
Pus	190 minutes per 100 pus (+gram stain + broth)

Full time employee (FTE) "hands on time" saved

CONCLUSIONS

The WASP® showed comparable efficiency and quality to the existing manual processing method, with significant time saving. The WASP® displayed better reproducibility and accuracy for urines compared to stool specimens. Technical problems associated with the WASP® settings for stool processing may have contributed to this reduction in accuracy. To fully automate specimen processing using the WASP® system, standardized media (size and quality) and high quality specimen containers and a move towards liquid-based microbiology specimen collection (e.g. ESwab® collection and transport system) is recommended. The WASP® is a promising tool for automated specimen processing in high throughput microbiology laboratories.

WASP[®] and STOOL
SAMPLES with



FecalSwab[®]



Evaluating the preservation and isolation of stool pathogens using the COPAN FecalSwab™ transport system and walk-away specimen processor

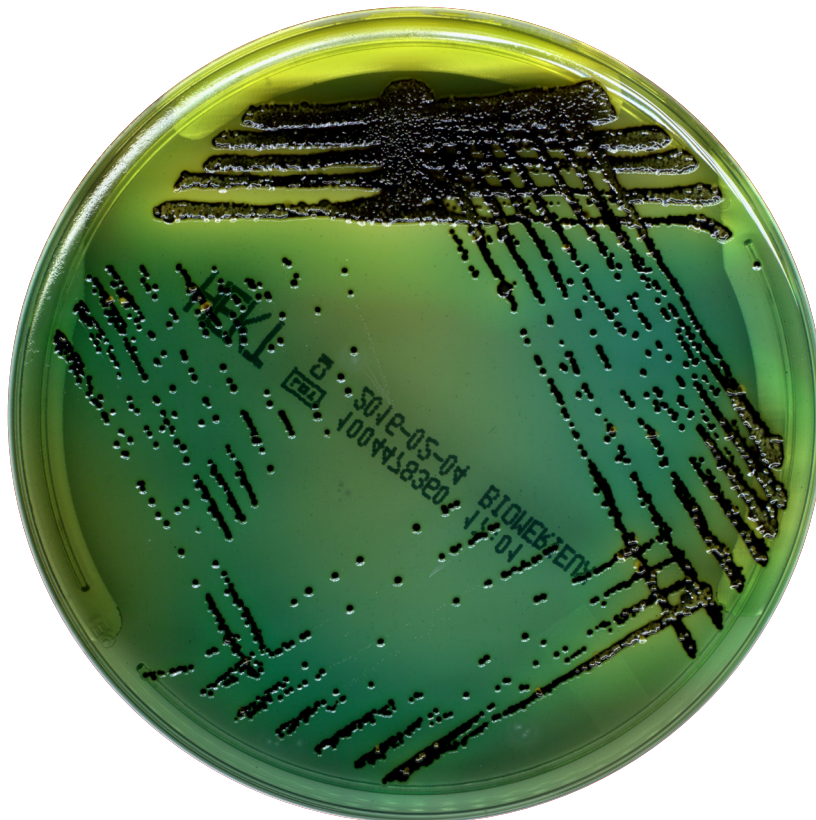
Goneau LW¹, Mazzulli A², Trimi X², Cabrera A², Lo P², Mazzulli T²

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KEYWORDS

Enteric pathogen | Stool culture | FecalSwab™ | WASP® | Sample stability



This study describes how FecalSwab™ media increases stool samples stability (viability and abundance) compared to modified Cary-Blair media and how an additional pre-incubation in COPAN's Selenite™ media significantly enhance the yield of Salmonella enterica serovar Typhimurium. Moreover, WASP® planting improves the isolation of individual pathogen colonies.



Microbiology meets molecular diagnostics evolving in flexible sample to result workflow

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KEYWORDS

FecalSwab™ | Molecular biology | WASP® | Integration | Automated & simplified workflow

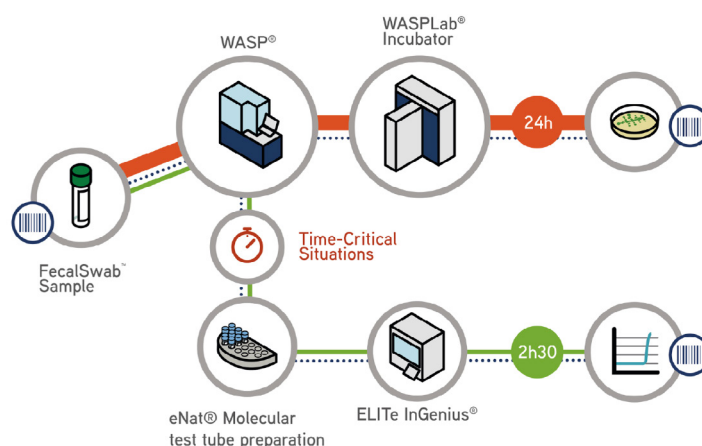
INTRODUCTION

Beneficial interactions between traditional culture methods and molecular diagnostic can significantly improve current management of Carbapenem-Resistant Enterobacteriaceae (CRE) positive patients in health care facilities. According to CDC guidelines CRE-colonized patients should be placed on contact precaution to prevent transmission of resistant bacteria reducing mortality rates¹. An innovative approach integrates WASP® and ELITe InGenius® systems to contemporaneously support culture and molecular diagnostic methods in a standardized, traceable and time-saving automated workflow for CRE analysis simultaneously performed from the same collection device: Copan FecalSwab™. Aim of the study was to compare MANUAL versus WASP® automated liquid handling for the preparation of ready-to-process primary tubes for molecular diagnostics testing.

MATERIALS & METHODS

Workflow analysis was carried out through detailed measurement of the multiple steps performed by a skilled technician to process a complete run of 12 FecalSwab™ samples using ELITe InGenius® sample-to-result molecular platform. Analytical approach has evaluated two phases: the baseline where all the pre-analytical steps were manually performed and after the integration of WASP® liquid handling module. For each FecalSwab™ sample, WASP® has performed the streaking on chromogenic media (dual plate CHROMID™ CARBA SMART bioMérieux Italia) and the transfer of 500µL of Cary Blair media in eNAT® tube for molecular analysis (Fig.1). After the samples selection, the complete molecular diagnostic workflow consists of the following automated steps: Sample Preparation (SP), Instruments Set up (IS), Instrument Loading (IL), Extraction and Amplification (EA) and Data Analysis (DA). Technician hands on time (HOT), risk of errors and total number of analytical steps were compared before and after the integration. In addition, the performance of WASP® liquid handling module has been evaluated considering three parameters:

1) cross contamination between a group of positive and negative alternating samples (24 positive and 24 negative), 2) the accuracy of aspiration volume (Ct value of each eNAT® tube inoculated with 500µL of positive FecalSwab™ media spiked with stools contaminated with *Klebsiella pneumoniae* KPC resistant and *E.coli* OXA 48 clinical strains) and 3) the time to perform dual plate streaking plus eNAT® inoculation.



RESULTS

For baseline, a total of 252 steps from samples to results were identified: 152 manual SP, 8 IS, 82 IL, 7 EA and 3 DA. The total HOT was 23 mins with 17 points of error risks while the total laboratory lead time was 3 h and 10 min. After the integration of WASP® liquid handling module a total of 320 steps were identified with a total HOT of 20 mins. The major simplification was observed in the primary tube selection and preparation phase: 28 steps versus 152 steps, with a reduction of 81,6% of manual handling (Figure 2).

Regarding the performance of WASP® liquid handling module: no cross contamination between positive and negative alternating samples has been detected. The DCt value of the samples inoculated with the same bacterial load was between 1 and 1,5. The total time to perform one run of 12 FecalSwab™ with a protocol of dual plate streaking (10µL) and eNAT® inoculation was 23 mins and 44 sec.

CONCLUSIONS

COPAN WASP® liquid handling module and ELITe InGenius® sample-to-result systems provide an innovative solution to integrate molecular diagnostics and automated bacteriology processing for CRE Screening and allow to improve standardization, traceability and reduce sample-hands on time (HOT) to support clinical decisions and patient management.

1 CDC Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase- Producing, *Klebsiella* spp. and *E. coli* from Rectal Swabs.



Implementation of Copan FecalSwab™ and Copan Selenite™ on WASP® for the Automated Processing of Stool Specimens

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KEYWORDS

WASP® | FecalSwab™ | Selenite™ | Standardization | Manual Comparison

INTRODUCTION

Automated processing of stool specimens is difficult due to different sample consistency, volume availability and variety of primary containers. Appropriate specimen collection and transportation systems can standardize the stool sample processing, enhancing the diagnostic process. Copan FecalSwab™ (FS), a tube with 2ml CaryBlair medium and a flocked swab, can be used for culturing most relevant enteric pathogens from both rectal swabs and stool samples. Copan Selenite™ broth, available in a 2 ml tube, can be used for selective enrichment of *Salmonella* spp.

The objective of this study was to validate the implementation process of FecalSwab™ and LBM® Selenite™ broth on WASP® for the clinical microbiology laboratory in order to convert stool processing from the manual streaking process to an automated procedure.

MATERIALS & METHODS

Spiked negative stools and clinical stools were used for this study (n=97). Aliquots (3 grams) of the negative stools were spiked with 300 µl of diluted *Y. enterocolitica* serovar 3 biotype 4, *S. typhimurium* (ATCC 25241), *S. flexneri* (clinical strain) and *Campylobacter jejuni* (ATCC 33560) to obtain final bacterial concentrations/stool of 108 CFU/g, 107 CFU/g, 106 CFU/g. All clinical stool samples (n=61) and spiked stools in triplicate (n=36) were transferred in FecalSwab™ medium tubes using the flocked swab. All samples were manually plated onto the first quadrant of McConkey, XLD, CIN, Campyloset agar plates using a swab and streaked with a 10 µl loop, while the FecalSwab™ stools were loaded on WASP® and processed using a 10 µL loop and a 4 quadrants streaking pattern. All clinical stool samples and the negative ones spiked with *S. typhimurium* (n=73), were also inoculated in LBM® Selenite™ broth, from FecalSwab™ and from the sample directly, and then plated on SS-agar after overnight incubation.

RESULTS

For the spiked samples we found 100% concordance for *S. flexneri* and *C. jejuni*. Discrepant results were found in the stools spiked with the lowest concentration of *S. typhimurium* and *Y. enterocolitica*, negative when manually plated but positive from FecalSwab™ and LBM® Selenite™ broth. We found 100% concordance in the clinical samples with *S. typhimurium* and *Y. enterocolitica*, three *Campylobacter coli* were not isolated, maybe due to sampling bias. Culture via FecalSwab™ yielded two extra *Aeromonas* species possibly because WASP® streaked FecalSwab™ samples had more isolated colonies to perform successive analysis. LBM® Selenite™ broth detected all the *Salmonella* spp. and the *Yersinia* spp. with both WASP® and manual streaking methods.

Isolates	Spiked Samples (n=36)	
	Manual Streaking (Swab)	WASP® Streaking (10µ)
Salmonella	6	6
Shigella	9	9
Campylobacter	9	9
Yersinia	2	2
Negative	10	10

Isolates	Clinical Samples (n=61)	
	Manual Streaking (Swab)	WASP® Streaking (10µ)
Salmonella	8	8
Shigella	0	0
Campylobacter	26	23
Yersinia	6	6
Aeromonas	2	2
Negative	18	21

Isolates	Clinical Samples (n=61) after LBM® Selenite™ Enrichment	
	Manual Streaking (Swab)	WASP® Streaking (10µ)
Salmonella	14	14
Shigella	0	0
Campylobacter	0	0
Yersinia	3	4
Aeromonas	0	0
Negative	44	43

CONCLUSIONS

FecalSwab™ and LBM® Selenite™ broth are facilitating WASP® automation stool processing and are reliable devices for diagnosis of gastric infections. Automatic processing of FecalSwab™ and LBM® Selenite™ broth allows standardization and time reduction of sample processing.

**WASP® and POSITIVE
CULTURES with**



BC+™



Evaluation of the Copan BC+™ for the Automated Management of Positive Blood Cultures: Microscope Slides and Subcultures Preparation

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Clinical Chemistry and Microbiology Laboratory, Niguarda Ca' Granda Hospital, Milan, Italy

KEYWORDS

WASP®

BC+™

Blood Culture

Automation

Standardization

INTRODUCTION

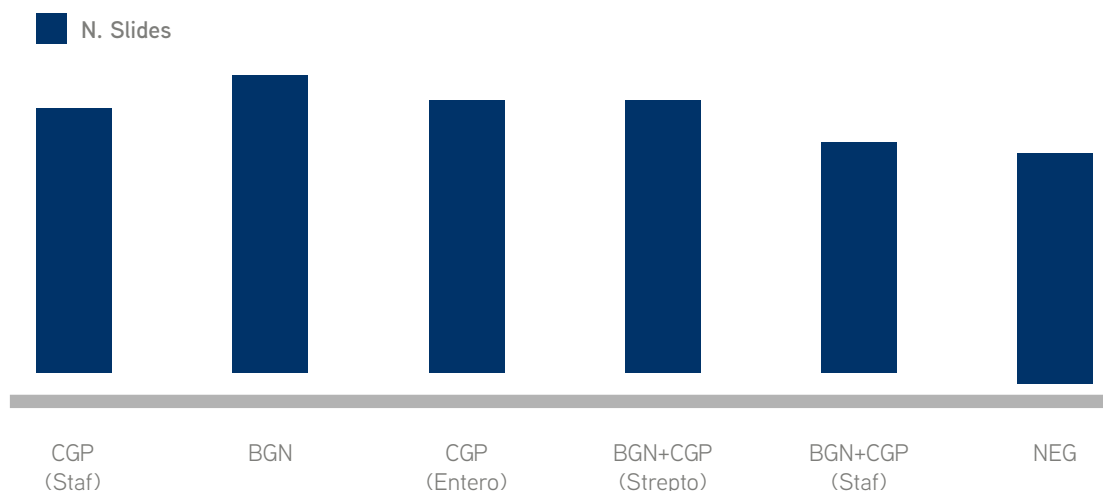
The pre-analytical and analytical phase of Microbiology Laboratory has undergone a major change over the last few years through the development of robotic systems, capable of automating many activities. The use of WASPLab® (COPAN ITALIA, Brescia, Italy) in our Laboratory allowed for a better standardization of the management of different samples. Blood cultures are not easy to manage in an automatic way. The aim of the study was to introduce and evaluate the performance of an automatic method for the preparation of slides and subcultures from positive blood culture by the means of a new device: Copan BC+™ (COPAN ITALIA, Brescia, Italy).

MATERIALS & METHODS

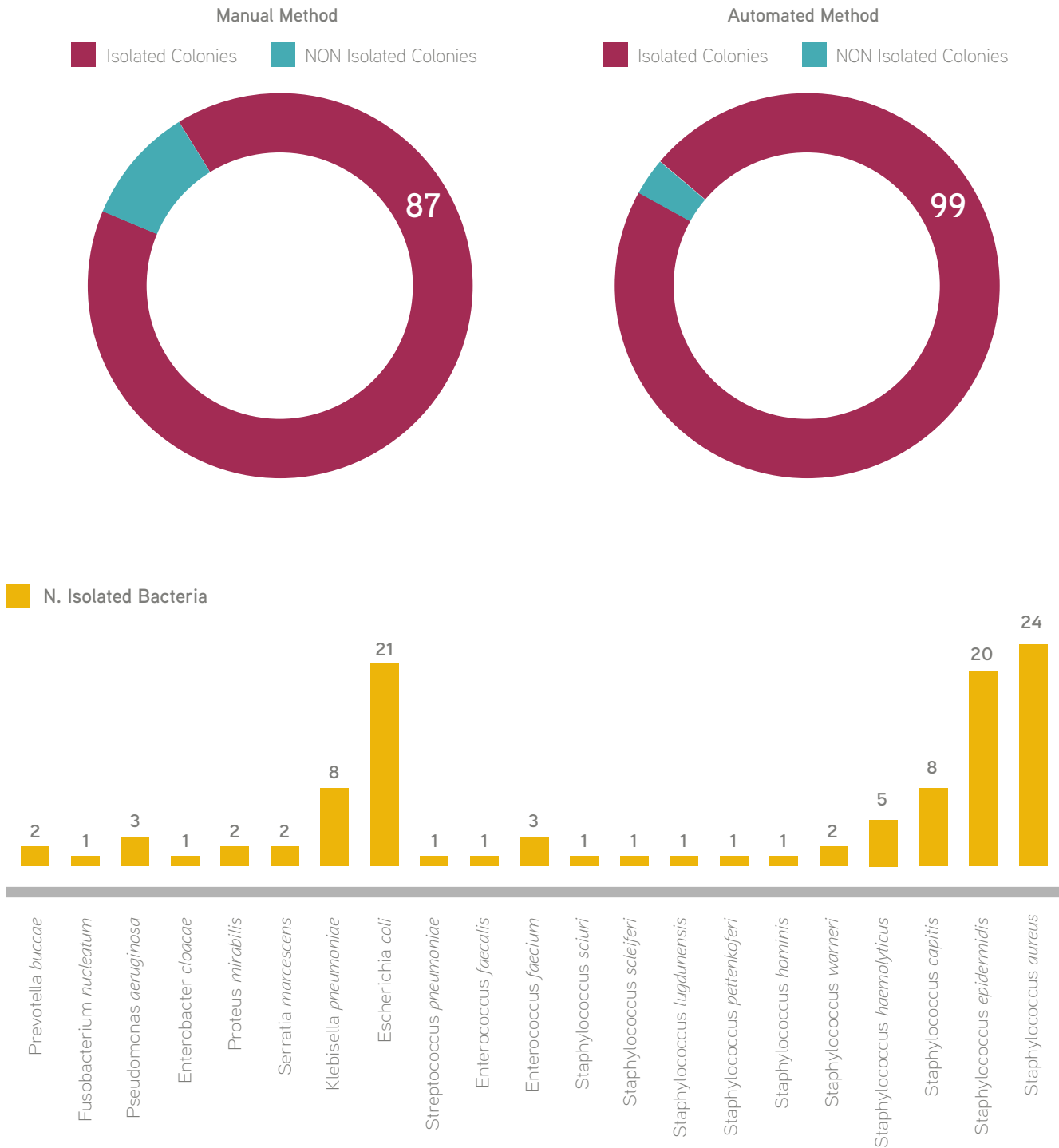
A total 91 positive blood cultures were included in this evaluation at the Microbiology Laboratory of Niguarda Hospital, Milan, Italy. Copan BC+™ is a device allowing to aspire a specific aliquot of blood (2.5 mL). All samples were manually transferred to the CopanBC+™ and loaded onto the Wasplab® System. The automated system streaked 10 µL of blood on agar plates (blood agar and Mac Conkey) and then prepared one smear for microscopic examination. The results were compared with those obtained by the routine manual method using the same protocol as for the automated method. The plates were incubated at 35°C for 16-48 hours.

RESULTS

Our data demonstrate that the quality and interpretation of microscopic examination, prepared with automated method are comparable in 100% of cases (91/91) with the traditional method. The quality of the inoculum and the presence of isolated bacterial colonies were considered to assess the reliability of the automated method. The routine manual method achieved isolated colonies in 79/91 cases (87%). The



automated procedure, instead, achieved a better quality in isolation of bacterial colonies. In fact, in 90/91 samples (99%) it was possible to obtain isolated colonies, where as in 1 sample (1%) the growth was too abundant.



CONCLUSIONS

This work demonstrated that Copan BC+™ is an innovative device to manage positive blood cultures in an almost all-automated way. The quality of smear and the microscopic examination were comparable to the traditional method, thus confirming the reliability of the automated method. Moreover, the automated method allowed for a better achievement of isolated bacterial colonies, also improving safety for the operator during the transfer of blood cultures in Copan BC+™. On the base of these results, we decided to manage the positive blood cultures by the WaspLab® System using the Copan BC+™ device, allowing for a better tracking of the sample and standardization of the workflow.



Accelerated Time-to-Diagnosis by Automated Processing of Positive Blood Cultures on Copan WASPLab®

Marion Jetter, Martina Marchesi, Peter M. Keller, Michael Hombach
Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

KEYWORDS

WASPLab® | Blood Culture | Faster Result | Sepsis | Shorter Incubation

INTRODUCTION

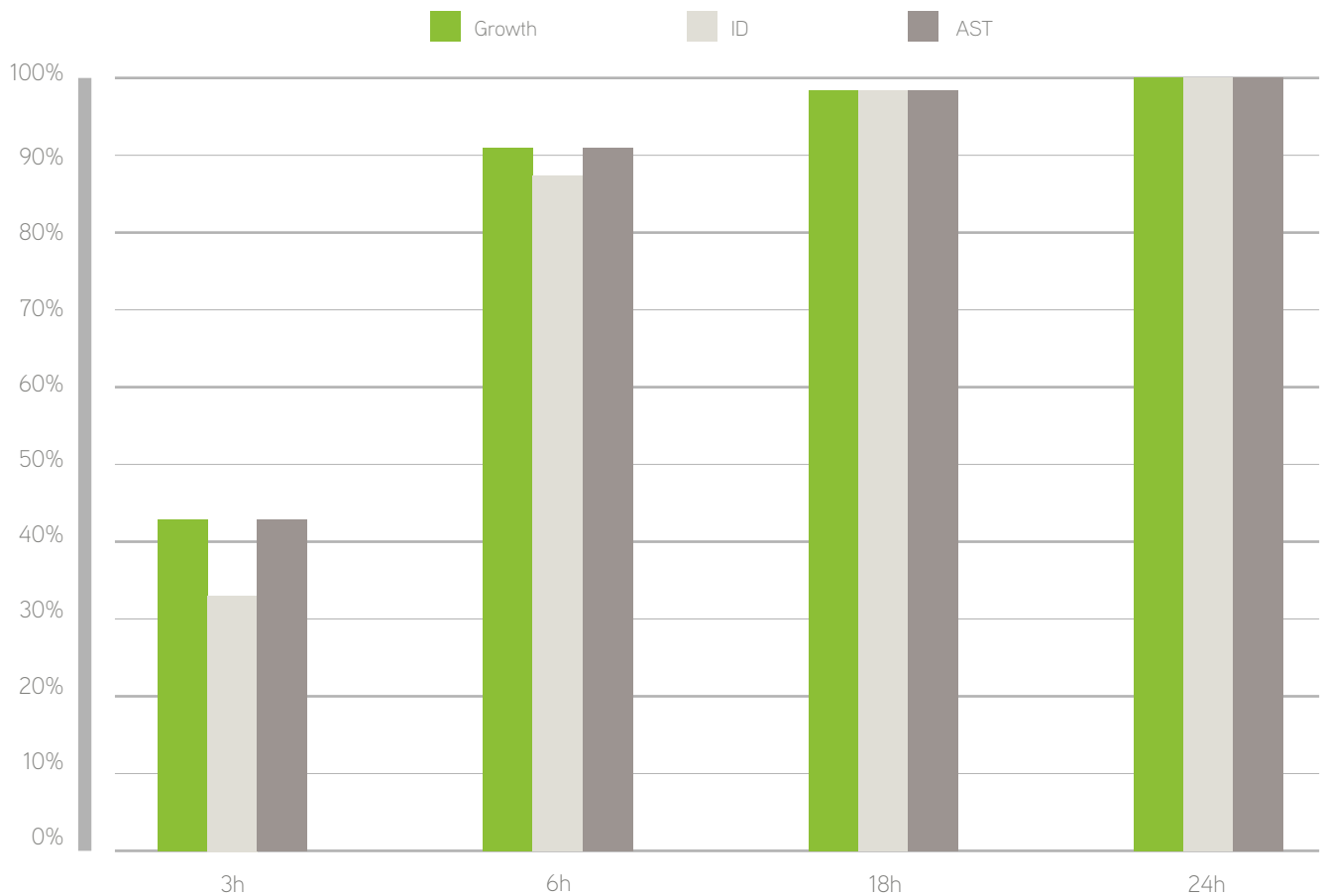
Fast identification of pathogens causing sepsis and rapid antimicrobial susceptibility testing are crucial for ensuring an adequate therapy and the survival of patients. Manual growth monitoring of incubated plates from positive blood cultures in short time intervals after primary plate inoculation is very time consuming and can hardly be integrated in a regular routine workflow. The present study demonstrates that the fully automated WASP®/WASPLab® enables microbiological laboratories to follow a distinctive accelerated time-to-diagnosis algorithm based on early automated plate reading and therefore poses an essential contribution to decrease mortality rates of sepsis patients. Time-to-diagnosis is critical in sepsis patients as an early targeted treatment significantly decreases morbidity and mortality (Kumar A. et al. Crit Care Med. 2006;34[6]:1589-96.). The aim of this study was to speed-up pathogen identification and antimicrobial susceptibility testing (AST) from positive blood culture samples. We validated a novel diagnostic algorithm integrating fully automated WASP®/WASPLab® (Copan, Brescia, Italy) sample processing and early MALDI-TOF-MS identification.

MATERIALS & METHODS

For this study broths of 63 individual positive blood cultures from 54 patients consecutively collected from September to November 2015 in our clinical laboratory were analyzed. Broth from blood culture bottles that were reported as positive by the BacT/ALERT® system (bioMérieux, Marcy L'Étoile, France) were immediately transferred into vacuum tubes (Copan, Italy) using an adapter (Greiner, Austria) and processed in the WASP®/WASPLab® automation system, which comprised automated streaking by WASP® (Copan) on appropriate agar plates, incubation of plates in automated WASPLab® incubators, and automated image taking of the plates by WASPLab® after 3h, 6h, 18h, 24h, and 40h of incubation. Images were subsequently checked for growth by a technician on-screen. If growth was detected, MALDI-TOF-MS using a Bruker Biotyper (Bruker Daltonics, Bremen, Germany) and AST was performed from the colonies detected on the plates. If MALDI-TOF MS and/or AST were not possible or did not yield appropriate results due to lack of colony material, plates were re-incubated and the next scheduled imaging-time-points were waited for. Results were compared to those of the routine work-up of our clinical laboratory, i.e. a classical manual workup including an overnight incubation of subcultures from blood culture broth.

RESULTS

After 3h of incubation the overall growth rate was 45% for all samples, and MALDI-TOF MS identification and disk diffusion AST were possible for 37% and 45% of samples, respectively. After 6h of incubation MALDI identification and AST rates both increased to 93%. At 18h and 24h of incubation MALDI-TOF MS identification and AST-rates of 97% and 100% were yielded. Discrepancies in successful MALDI-TOF MS identification and AST rates were observed mainly for coagulase-negative staphylococci and streptococci at early time-points of incubation.



Incubation Time	Growth	ID	AST
3h	45%	37%	45%
6h	93%	86%	93%
18h	97%	97%	97%
24h	100%	100%	100%

Overall growth, identification and susceptibility rate after 3h, 6h, 18h, and 24h of incubation

CONCLUSIONS

Automated periodic plate-screening can accelerate time-to-diagnosis of blood cultures providing same-day results in contrast to the classical manual work-up that uses an 18-24h incubation period before identification and AST will be done. The accelerated work-up facilitates bacterial identification by MALDI-TOF MS superseding additional extraction procedures and, in addition, enabling species specific AST at the same time. Copan WASPLab® automation facilitated the implementation of same-day MALDI-TOF MS identification and AST for blood cultures with a potentially significant impact on expected patient morbidity and mortality.

WASP[®] and



eSwab[®]



Evaluating WASPLab® against manual processing for infection control screening swabs - An Australian perspective

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KEYWORDS

WASPLab®

| Infection control

| Manual VS automated processing

| Improve TAT

INTRODUCTION

Automation is expected to have a great impact on how microbiology will process samples, read culture plates and report results; with a key factor being improving turn-around-times (TAT) for results to doctors which will (likely) improve patient care.

The NSW Health Pathology microbiology department located at John Hunter Hospital, NSW is examining the use of the Copan WASPLab® automated system and undertook an evaluation to determine how it performed against manual processing. Of the 4 sample types evaluated, one was infection control screening (ICS) swabs.

MATERIALS & METHODS

The aim was to assess how automated processing and examination of infection control swabs using WASPLab® compared to using conventional manual techniques for plating, streaking and culture reading for those same samples.

A random sampling of 276 infection control screening swabs were processed through the WASP® and incubated in air in the WASPLab®. Only samples collected using the E-swab (containing 1mL liquid Amies transport media) were processed. The same samples (using the swab from the e-swab collection system) were processed by conventional manual techniques.

WASP® Inoculated 10µL of the liquid Eswab® sample onto a bioMerieux VRE, MRSA or ESBL chromogenic agar plate plate, or combination thereof according to required protocol. All plates were incubated in WASPLab® in O2 at 35°C and were imaged at 0, 18 and 38 hrs.

Scientists examined digital images produced by WASPLab® and recorded those culture results. These were compared against those obtained from manual examination of culture plates of manually processed and incubated samples.

RESULTS

Of the 276 samples processed, 29 (11%) were considered positive (i.e. MRSA, VRE or Multi-resistant GNR [MRGNR] organisms were isolated) using WASPLab. There was greater than 99% concordance between methods.

There were 2 discrepant results:

- 1 x MRSA was recovered manually which was not recovered through WASPLab®
- 1 x VRE was recovered via WASPLab®, but not recovered via manual culture methods.

It was noted that 34% (11/29) of positive samples were recovered a day earlier by WASPLab® than by manual methods.

CONCLUSIONS

Using WASPLab® we found we were able to quickly and easily screen out “negative” culture results, which represented up to 90% of all culture plates, providing significant time savings. There were only 2 discrepancies. The MRSA not recovered from WASPLab® could be explained by low numbers as only 1 colony was recovered from manual culture set up.

Overall the WASP®/WASPLab® combination proved to be equal to, or better than, manual processing, exhibiting equal or better colony isolation when compared with manually processed samples, improving the readability of culture plates.

The fact that the WASPLab® is a closed system, providing consistent, temperature controlled incubation conditions, probably accounts positive cultures being recovered a day earlier than by manual methods in 34% of cases. Added advantages included standardisation of culture streaking and incubation times, storage of images and traceability of samples as they move through the system.

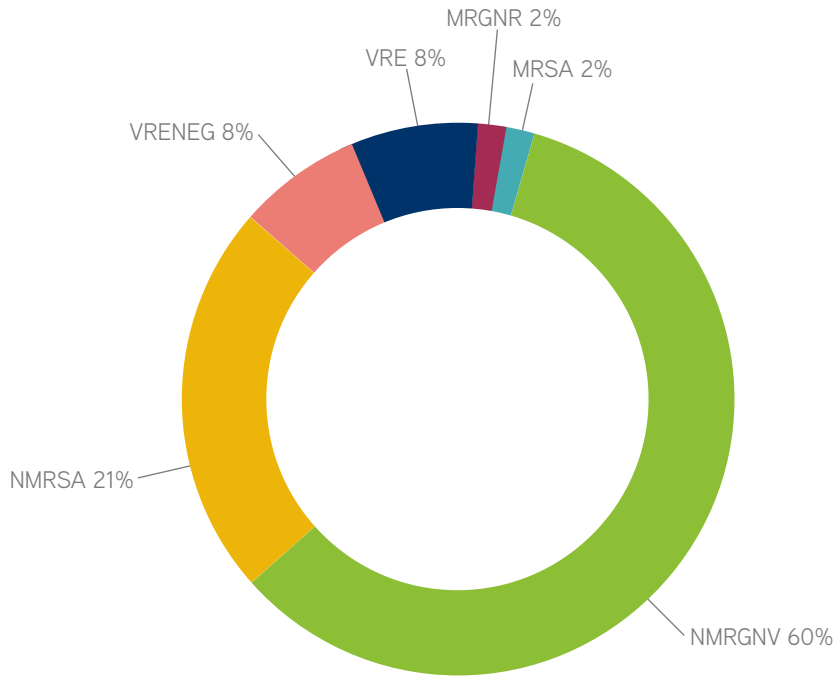
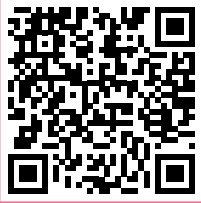


TABLE 1

<i>Culture Result</i>	<i>Manual count</i>	<i>WASPLab® count</i>
No MRGNR's	166/192	166/192
No MRSA	59/253	59/253
No VRE	22/216	22/216
MRGNR	4/192	4/192
MRSA	4/253	3/253
VRE	21/216	22/216
Total positive	29	29



Comparison of Five Media for Detection of Extended-Spectrum Beta-Lactamase by Use of the WASP® Instrument for Automated Specimen Processing

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KEYWORDS

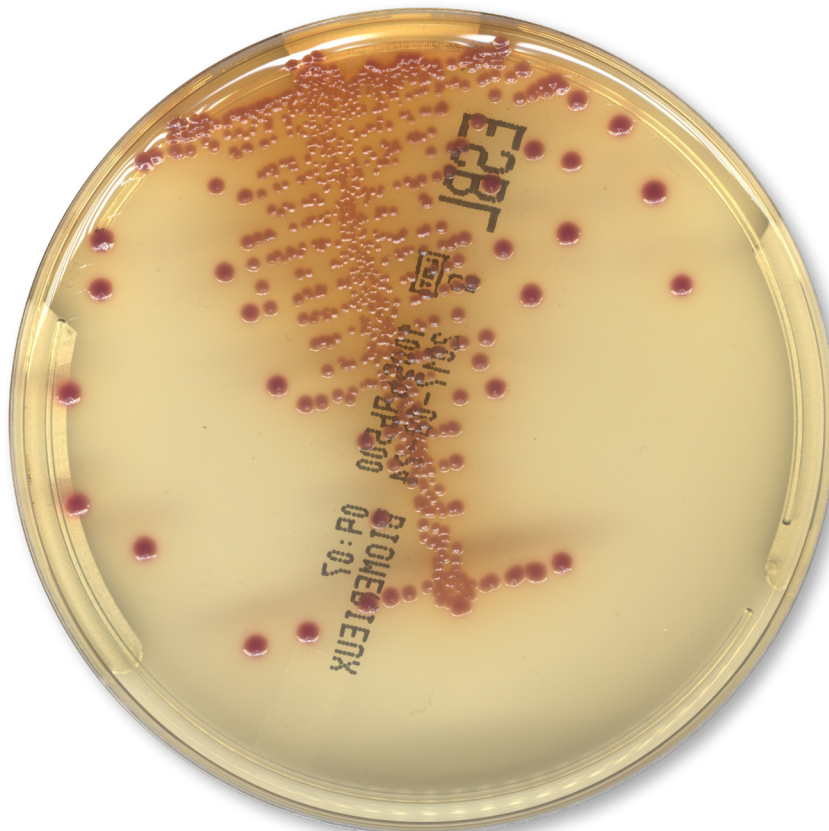
WASP®

ESBL

eSwab®

Standardization

Bacteriology



WASP® instrument is suitable for the routine screening of nosocomial infection samples, meanwhile supporting the seeding of a large amount of selective media for ESBL detection.

Comparison of Automated Processing of Flocked Swabs with Manual Processing of Fiber Swabs for Detection of Nasal Carriage of *Staphylococcus aureus*



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KEYWORDS

WASP®

|

Flocked Swabs

|

eSwab®

|

Efficiency

|

MRSA



*The use of eSwab® together with automatic streaking with WASP® turn out to be the ideal combination for the detection of a *S. aureus* colonisation compared with the manual use of a fiber swab showing an increase of sensitivity of the method.*



Pheno**MATRIX**TM

and IMAGE ANALYSIS



Use of Copan's WASPLab® PhenoMATRIX™ Artificial Intelligence to Improve the Efficiency of Urine Culture Interpretation

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KEYWORDS

WASPLab®

PhenoMATRIX™

AI

GBS

ABSTRACT

Background: Urine cultures are one of the most common cultures set up in clinical bacteriology laboratories. The person time spent sorting and discriminating between plates with no growth, no significant growth, mixed growth, and significant growth is significant. We evaluated the use of Copan's WASPLab® PhenoMATRIX™ artificial intelligence to improve the efficiency of culture interpretation.

Methods: From July 2017 through December 2017, the WASPLab® PhenoMATRIX™ trained on a random selection of 2000 voided urine culture plates (set up by WASP® automated specimen processor using 1µL loops using Oxoid Brilliance UTI clarify/CNA biplates) that had been read by routine clinical laboratory technologists using the WASPLab® App in a single tertiary care academic institution's microbiology laboratory. The trained system was then tested for its performance on 2872 consecutive voided urine culture plates from April 19, 2018 through May 9, 2018. Percent classified and accuracy of plates classified were calculated.

Results: WASPLab® PhenoMATRIX™ interpreted voided urine cultures as no growth, no significant growth, significant counts of *Escherichia coli*, or significant counts of growth other than *Escherichia coli*. In addition, it segregated those plates belonging to females of child-bearing age to facilitate closer investigation for Group B streptococci. A total of 1767 out of 2872 (61.5% CI 59.7-63.3%) voided urine culture plates were classified in this way. Of those classified, 99.4% (95% CI 98.9%-99.7%) were accurately classified as positive (significant counts of *Escherichia coli*, or significant counts of growth other than *Escherichia coli*) or negative (no growth, no significant growth, or mixed growth). The overall sensitivity of those classified was 94.8% (95%CI 89.9%-97.5%) and specificity was 99.8% (95%CI 99.4%-100%).

Conclusions: The use of Copan's WASPLab® PhenoMATRIX™ artificial intelligence streamlined segregation of a significant proportion of voided urine cultures with high accuracy. Combining the use of this technology with expertise in the laboratory has the potential to significantly improve microbiology laboratory efficiency. Work on the efficiencies gained by introducing this promising technology and expansion beyond the utility with urine cultures is ongoing.

INTRODUCTION

- Urine cultures are one of the most common cultures set up in clinical bacteriology laboratories. The person time spent sorting and discriminating between plates with no growth, no significant growth, mixed growth, and significant growth is significant.
- We evaluated the use of Copan's WASPLab® PhenoMATRIX™ artificial intelligence to improve the efficiency of culture interpretation.

METHODS

- From July 2017 through December 2017, the WASPLab® PhenoMATRIX™ trained on a random selection of 2000 voided urine culture plates (set up by WASP® automated specimen processor using 1µL loops using Oxoid Brilliance UTI clarify/CNA biplates) that had been read by routine clinical laboratory technologists using the WASPLab® App in a single tertiary care academic institution's microbiology laboratory.
- The trained system was then tested for its performance on 2872 consecutive voided urine culture plates from April 19, 2018 through May 9, 2018.
- Percent classified and accuracy of plates classified were calculated. Discrepancies were reviewed manually by experienced technologists.

RESULTS






- WASPLab® PhenoMATRIX™ interpreted voided urine cultures as no growth, no significant growth, significant counts of Escherichia coli, or significant counts of growth other than Escherichia coli.
- In addition, it segregated those plates belonging to females of child-bearing age to facilitate closer investigation for Group B streptococci.
- A total of 1767 out of 2872 (61.5% CI 59.7-63.3%) voided urine culture plates were classified in this way.
- Of those classified, 99.4% (95% CI 98.9%-99.7%) were accurately classified as positive (significant counts of Escherichia coli, or significant counts of growth other than Escherichia coli) or negative (no growth, no significant growth, or mixed growth). On discrepancy analysis, a number of human errors in interpretation were detected that were accurately interpreted by PhenoMATRIX™.
- The overall sensitivity of those classified was 94.8% (95%CI 89.9%-97.5%) and specificity was 99.8% (95%CI 99.4%-100%). All missed “significant growth” cultures were lactobacilli on MALDI and were interpreted as no significant growth by the laboratory.

TABLE 1. Comparison of PhenoMATRIX™ Interpretation of 2872 consecutive urine cultures compared to the clinical laboratory interpretation

NSG = no significant growth, F12-60 = female of child-bearing age, ID AST = refers to plates with significant growth other than E. coli that require identification (ID) and antimicrobial susceptibility testing (AST); BP AST refers to significant growth of E. coli that require ID and AST, POS = significant growth

	No Growth	NSG	Mixed	POS	Total
No Growth	827	2		1	830 28.9%
NSG	16	367	1		384 13.4%
NSG F12-60	3	395		7	405 14.1%
ID AST		1		40	41 1.4%
ID AST F12-60		1		13	14 0.5%
BP AST				68	68 2.4%
BP AST F12-60		1		24	25 0.9%
Review	12	185	45	361	603 21.0%
Review F12-60	14	274	12	202	502 17.5%
Total	872	1226	58	716	2872

- 62% of urine cultures (n=105/d) read by PhenoMATRIX™ 38% (n=65/d) to be manually reviewed
- 59% (n=100/d) (NG, NSG, BP AST) auto-reported with the push of one button
- 56% (n=95/d) (NG, NSG) have no hands-on plate time

	Agreement	1734 60.4%
	False Positive	3 0.1%
	False Negative	8 0.3%
	Acceptable Discrepancy	22 0.8%
	For Manual Review	1105 38.5%

FOR THOSE CLASSIFIED BY PhenoMATRIX™:

	LAB POS	LAB NEG	
PhenoMATRIX™ POS	145	3	148
PhenoMATRIX™ NEG	8	1611	1611
	153	1614	1767
	Sn	Sp	
	94.8%	99.8%	

DISCUSSION & CONCLUSIONS

- The use of Copan's WASPLab® PhenoMATRIX™ artificial intelligence streamlined segregation of a significant proportion of voided urine cultures with high accuracy.
- Combining the use of this technology with expertise in the laboratory has the potential to significantly improve microbiology laboratory efficiency.
- Work on the efficiencies gained by introducing this promising technology and expansion beyond the utility with urine cultures is ongoing.



Evaluation of the WASPLab® Segregation Software to Automatically Analyze Urine Cultures using Routine Blood and MacConkey Agars

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KEYWORDS

WASPLab® | urine | blood agar | MacConkey | digital analysis vs manual reading | automation | segregation



In this study, WASPLab® colony segregation software was evaluated in 3 laboratories across the United States to distinguish between significant growth and no growth of urine cultures plated to blood and MacConkey agars. Urine cultures were processed on the WASP® and results were compared to those manually read by a technician working on the same image from a workstation. Overall the image analysis software proved to be highly sensitive and can be utilized to improve laboratory workflow.



Segregation and WASPLab® automation impact on MRSA screening at LTHT

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KEYWORDS

WASPLab® | MRSA | Chromogenic media | image analysis vs visual examination | segregation reliability

ABSTRACT

Methicillin Resistant Staphylococcus aureus (MRSA) is an increasing problem in Healthcare settings. It is a well-established cause of Hospital acquired infections and is also increasingly seen in community outbreaks. With emerging resistance to antibiotics, MRSA infections result in longer and more intensive hospital stays and post-operative complications. Healthcare institutes in the UK are already suffering from stretched finite resources and seasonal pressures, so (preventable) outbreaks would further stress an already struggling system. Active screening to identify carriers and manage individuals accordingly, is recommended by PHE guidelines. At the Leeds Teaching Hospitals Trust (LTHT) targeted screening is conducted on high risk individuals prior to or on admission of elective surgery and certain procedures; screening of inpatients is also carried out monthly and where clinically indicated. Therefore there is a need for a rapid reliable screening method for MRSA, capable of screening a large number of samples. Screening benefits to healthcare institutes include better infection control measures, shorter hospital stays, prevention of auto-infection, bacteraemia and ward outbreaks, monetary gains for meeting targets, and better overall patient care.

METHODS AND MATERIALS

Retrospective study compared traditional manual reading to the use of Copan WASPLab® WebApp and the use of Chromogenic Detection Module Analysis software (CDM), referred to locally as Segregation software.

SPECIMEN PROCESSING

MRSA screening swabs usually consist of triplicate swabs of anterior nares, axilla and groin swabs. In all cases MRSA screening samples taken within 48 hours using Copan eSwab® were processed on the Walk Away Specimen Processor (WASP®); Ten microliters of each specimen were seeded on Brilliance MRSA II (Oxoid, UK) by WASP® automation.

SCREENING

Automated WASPLab® WebApp and Segregation (CDM Analysis): a total of 5000 surveillance swabs for MRSA screening were enrolled in the study, collected between October 2016 to October 2017. After 18 hours incubation at 35°C ±2 in the integrated incubator, digital images were acquired by WASPLab® and examined both by a trained operator and by the Segregation software. Traditional screening method: Inoculated MRSA screening plates were ejected from the front of the WASP® for 18 hour manual incubation in O2at 35-37°C in a walk-in incubator. Numerically sequential racks held plates in three stacks of 10 plates with the time of incubation written at the front of each rack. Plates were reviewed and reported by a trained BMS with preliminary bench tests conducted during the course of screening throughout the day.

REPORTING

Negative reports were issued immediately after review by an operator in all three methods, however the timing between methods differed which again impacted on laboratory workflow.

Method of screening	Results (%concordance with Traditional Manual Reading)				(Second per plata)	Sensitivity %	Specificity %
	True Positive	True Negatives	False Positive	False Negative			
WASPLab® WebApp	100	100	0	0	15-20	100	99.9
WASPLab® Segregation (CDM)	100	99.6	0.4	0	1	100	99.6

RESULTS

Segregation (CDM) Software analysed each individual pixel per image (48 million pixels per image) against a HSV score determined by Copan CDM programming. HSV measured Hue (Colour), Saturation (intensity of Colour) and Value (Brightness of Colour). On Brilliance MRSA II media, denim blue colonies highlighted potentially positive growth. Other pigmentation was classed as negative for MRSA. The classification of 99.6 % of images studied by Segregation software corresponded with those read on the WASPLab® manually. Upon review of the discrepant images (False Positives), Segregation (CDM) software was found to be valid and the images were “potentially not negative” requiring further investigation. Although Segregation (CDM) appeared slightly less specific (99.6% Specificity), Sensitivity was still 100%. Segregation (CDM) software had 0 false negatives, therefore no positive plates were lost. Furthermore Segregation’s (CDM) rapid review of negative screens as 30 thumbnails per page, reduced screening times vastly, as the software reliably segregated negative media plates. Reporting of Negative results was also a key indicator in workflow improvement. Traditional manual reading required each report to be individually recorded and released. This process required multiple repeated movements and alternating between plate examination and manipulation and reporting results on the LIS using a keyboard. Although not a lengthy task for a single report, repetition for several hundred samples a day would accumulate and had the potential to cause strain and fatigue and potential errors. The laboratory benchmark for release of clear-cut Negatives to the LIS was to complete the task by mid-day. With the introduction of WebApp reading, (prior to the implementation of Segregation Software), the day’s negative reporting could be completed by mid-morning. With a simple scroll and click Negative results were reported to the LIS. The implementation of Segregation software further enhanced negative reporting as each set of 30 thumbnail images segregated as Negative samples were released to the LIS with one click. This meant that the majority of negative results were released by 9am coinciding with patient ward rounds and better patient management.

CONCLUSION

Copan Segregation (CDM) Software has shown to be a reliable tool for easy isolation and identification of potential target organisms on MRSA Chromogenic media. The use of automation to inoculate, incubate and image plates has improved the standardisation of screening methods at LTHT using the WASPLab®. Segregation (CDM) Software, developed in house by Copan for use on WASPLab®, has shown to reliably segregate work, thus has allowed a reduction in skilled workforce to conduct the same task in a shorter space of time providing greater skilled capacity and time availability for workflow purposes. Segregation software is an excellent screening tool particularly where large sample numbers are screened and positivity rate is low (1.6% average at LTHT for MRSA screens). It has given LTHT the ability to provide results in less than 24 hours for negative results, reducing turn-around times from 5 to 3 days and has transformed the way in which the Microbiology/Pathology CSU is able to operate. The wider impact is contribution to reduced waiting times, referral times and hospital stays, better infection control, improved workflow and better patient care at the LTHT.



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

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A.M.L. bvba Antwerpen, Belgium

KEYWORDS

WASPLab® | eSwab® | screening | image analysis vs visual examination | sensitivity | time saving

INTRODUCTION

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 AND 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 bubbleshape 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 (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).

TABLE 1. Result overview

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)

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. Result calculations

	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

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%.

DISCUSSION

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, therefore we detected 4 false positive results in the manual reading. These specimens were 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 were "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%) were 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.

CONCLUSION

Automated reading on WASPLab® using CDM software is highly sensitive for the detection of GBS o 1 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 spent 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).



Validation of image analysis software for automatic screening of all Carbapenemase-Producing Enterobacteriaceae

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KEYWORDS

WASPLab® | stool samples | image analysis vs visual examination | high sensitivity | reliability | time saving

ABSTRACT

Carbapenemase-Producing Enterobacteriaceae (CPE) are a group of gram negative bacteria which developed a resistance to the carbapenem, the main therapy for treating severe and life-threatening infections. A prompt identification of these organisms is essential to set up strategies to contain their spreading. COPAN developed two software, the Chromogenic Detection Module (CDM) along with the Growth-No-Growth (GnG), allowing the discrimination of plates showing growth associated to specific target colours. The combination of the two technologies allows to analyze bi-plate medias for the simultaneous identification of OXA-48 or the KPC strains for a complete CPE screening.

AUSL of Romagna. operates within the territorial framework to collect samples from the four main AUSLs of Romagna, Ravenna, Rimini, Forlì and Cesena (Fig.1) The population is about 1.200.000 inhabitants, of which 300.000 with an age over 65 years-old. The microbiology laboratory collect samples from 15 hospitals for a total of 3000 beds and every day receives on average 1200 samples processed o WASPLab® system (COPAN Italia). Of these samples approximately the request the CPE screening is about 180 samples per day.

Surveillance is routinely performed with collection of one rectal swab. In all patients admitted to the Hospital who have at least one risk factor to transmit CPE to others patients.

MATERIALS AND METHODS

From May to October 2018, a total of 4986 rectal swabs were collected with eSwab® (Copan, Italy). Ten microfliters of eSwab were streaked on both sides of the CHROMIO CARBA SMART bi-plate (BioMérieux SA) by the WASP®. After 16 hours of incubation at 35±2°C digital images were acquired by WASPLab® and examined both by a trained operator and by the combination of two software (Fig. 2):

- WASPLab® CDM software analyses different growth colours on the CARB agar to distinguish the KPC positive plate from the negative ones, accordingly to the colour.

- WASPLab® GnG software detects the presence of colonies on the OXA agar by comparing the final picture with the one acquired before the incubation. The whole automatic system claims Automation Positive (AP) when either COM o GnG see relevant growth, conversely the system scored the image as Automation Negative (AN) if no relevant growth is found.

By the interpretation of analysis software and manual reading Software analysis has shown an overall sensitivity of 100%, a specificity of 86,94% with an agreement rate of 87,79%.

DISCUSSION

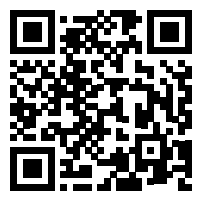
Color Collection Module (COM) Software: the imaging software, is able to differentiate colors on each single side of the selective chromogenic plate (based on rules previously defined by microbiologist). The reference target color is identified by numerical values of hue, saturation and brightness (with a tolerance range that can be chosen by the user). The software recognizes as positive the colonies with colors similar to the reference target (lighter or darker) allowing to divide the positive plates from the negative using a colorimetric output. Growth-noGrowth (GnG) Software: it is able to recognize and evaluate the growth of colonies on plates by comparing the photo at time X with the photo at time 0: it does a total count of the colonies. A single colony on OXA agar, or a green/blue or red/pink colony on CARB agar are the fixed rules considered to exclude the plate from the segregation process.

CONCLUSION

WASPLab® image software demonstrated to be fully reliable in sorting positive chromogenic bi-plates media from negatives, saving a lot of technician time by removing the 81 % of negative plates without any false negative results. The high sensitivity also shows positivity in improving the quality to the results: valuable support to increase the visibility of vary small pigmented colonies that would be otherwise difficult to be observed and captured.

RESULTS

Total samples	Positive plates	Negative plates
4.986	324	4.053
%	6,5%	81,3%



Evaluation of WASPLab® Software To Automatically Read chromID CPS Elite Agar for Reporting of Urine Cultures

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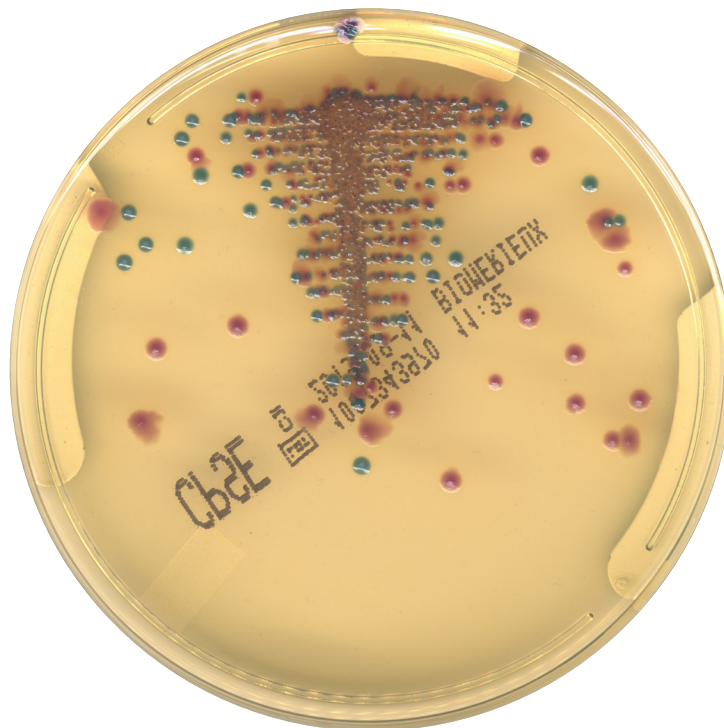
KEYWORDS

UTIs

WASPLab®

CDM

chromogenic plates



Urine cultures represent the biggest chunk of laboratory culture tests worldwide. Manual processing of urine samples on chromogenic media has allowed faster time to results and hence improved laboratory workflow and results. However, the room for improvement is still being observed as technologists struggle to detect microcolonies and handle increasing workload. Automated processing of chromogenic agar and smart incubation combined coupled with artificial intelligence algorithms allowed significant improvements in the ability of laboratories to report results faster and detect microcolonies that would go otherwise undetected even on chromogenic media.



Automated Detection of Streptococcus pyogenes pharyngitis using Colorex Strep A CHROMagar and WASPLab® Artificial Intelligence Chromogenic Detection Module Software

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KEYWORDS

PhenoMATRIX™

| GAS

| WASPLab®

| Chromogenic Plates

METHOD	% Sensitivity	% Specificity	PPV (%)	NPV (%)
Lyra Molecular assay	96.9 (93/96)	100 (384/384)	100	99.2
Manual reading of the Colorex Strep A Agar images	87.5 (84/96)	97.7 (375/384)	90.3	96.9
PhenoMATRIX™ reading of the Colorex Strep A Agar images	90.6 (87/96)	94.0 (361/384)	79.1	97.6
Manual detection of beta-hemolytic colonies on BAP images	83.3 (80/96)	69.3 (224/384)	44.7	93.3
Manual detection of beta-hemolytic colonies on BAP images with any zone of inhibition to a bacitracin disk	39.5 (38/96)	83.1 (319/384)	36.9	84.6

PhenoMATRIX™ Chromogenic Detection Module (CDM) was evaluated to detect Group A Streptococcus (GAS) from throat specimens, comparing its results with manual plate image reading. Besides, GAS PCR was performed on all samples. The software had the most accurate results detecting all suspect colonies and identifying more true positives that were missed by manual reading. The PhenoMATRIX™ CDM software can improve the detection of GAS from throat specimens and compared favorably to molecular testing.



Automated Incubation and Digital Image Analysis of Chromogenic Media Using Copan WASPLab® Enables Rapid Detection of Vancomycin-Resistant Enterococcus

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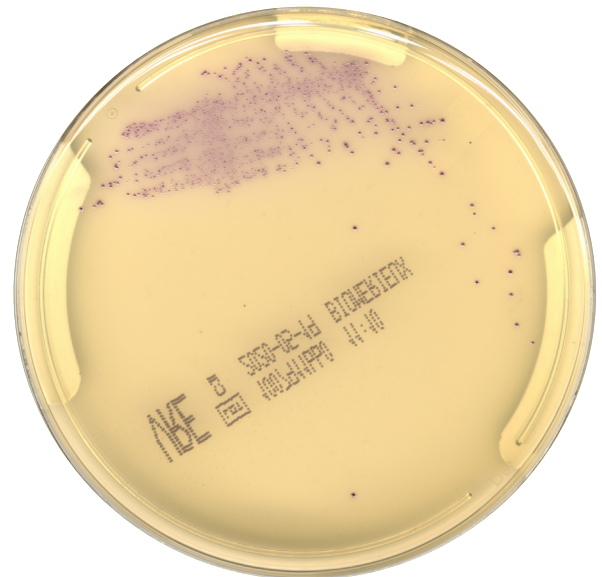
KEYWORDS

WASPLab®

VRE

manual vs automation

time saving



Strain ID, year of isolation, patient gender, patient age	VAN MIC by E-test	VAN MIC by broth microdilution	VAN (Disk-diffusion 5µg)	Growth on BHIA+6 mg/l VAN	Teicoplanin MIC by E-test	Daptomycin MIC by E-test	Linezolid MIC by E-test
E. faecium VanB (NARA-89)	6 (R)	4 (S)	R*	+	0.38 (S)	1 (S)	0.75 (S)
E. faecium VanB (NARA-388)	6 (R)	16 (R)	R*	+	1.5 (S)	2 (S)	0.75 (S)
E. faecium VanB (NARA-490)	32 (R)**	64 (R)	R*	+	1 (S)	1.5 (S)	1 (S)
E. faecium VanB (NARA-491)	96 (R)**	>64 (R)	R*	+	1 (S)	2 (S)	1 (S)
E. faecium VanB (NARA-492)	4 (S)	8 (R)	R*	+	0.75 (S)	3 (S)	0.75 (S)
E. faecium (2018, Female, 85 y) HUG	256 (R)	>64 (R)	R	+	48 (R)	3 (S)	1 (S)
E. faecium (2018, Female, 68 y) HUG	256 (R)	>64 (R)	R	+	1.5 (S)	3 (S)	1 (S)
E. faecium (2018, Female, <1 y) HUG	256 (R)	>64 (R)	R	+	12 (R)	2 (S)	0.75 (S)
E. faecium (2018, Female, <1 y) HUG	>256 (R)	64 (R)	R	+	24 (R)	3 (S)	1 (S)
E. faecium (2018, Male, 30 y) HUG	32 (R)	>64 (R)	R	+	1 (S)	2 (S)	1 (S)
E. faecium (2018, Male, <1 y) HUG	256 (R)	>64 (R)	R	+	16 (R)	3 (S)	1 (S)
E. faecium (2018, Male, 58 y) HUG	>256 (R)	>64 (R)	R	+	96 (R)	4 (S)	0.5 (S)
E. faecium (2018, Male, 84 y) HUG	128 (R)	>64 (R)	R	+	1 (S)	2 (S)	0.75 (S)
E. faecium (2018, Male, 90 y) HUG	48 (R)	>64 (R)	R	+	0.75 (S)	2 (S)	0.75 (S)
E. faecium (2018, Female, 7 y) HUG	>256 (R)	>64 (R)	R	+	64 (R)	8 (R)	0.75 (S)

VAN, Vancomycin; MIC, Minimum Inhibitory Concentration. *Fuzzy zone edges, **Presence of a resistant heteropopulation. R, resistant; S, susceptible. Daptomycin MICs were interpreted according to CLSI breakpoints.

This paper evaluates the usefulness of the WASPLab[®] automated system in the screening of vancomycin-resistant Enterococcus (VRE). Researchers analyzed negative rectal swabs, spiked with various concentrations of different strains of VRE, determining the shortest incubation times for the detection and the exclusion of the presence of VRE. Comparing the results obtained by WASP[®]-based inoculation followed by conventional incubation and manual diagnostic against the fully automated WASPLab[®] procedure, they observed a faster detection of all the VRE strains included in the study when using the automated WASPLab[®] incubation. Automation permitted then a substantial reduction of the incubation time, without affecting the analytical sensitivity as compared to the routine method used in the laboratory.



Clinical performance of the WASPLab® AI/IA-PhenoMATRIX™ software in detection of GBS from LIM-enriched cultures plated to CHROMID® strepto B Chromogenic media

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KEYWORDS

WASPLab® | chromogenic media | manual vs automated image analysis | culture vs molecular detection | sensitivity

ABSTRACT

Group B Streptococcus (GBS) can be found colonizing about 25% of all healthy, adult women and is the leading infectious cause of early neonatal morbidity and mortality in the United States. The rate of early-onset neonatal infection is ~0.22 cases per 1,000 live births (2016) and can cause sepsis resulting in neurologic sequelae such as sight/hearing loss and cerebral palsy. The CDC recommends that vaginal/rectal swabs be collected between 35 to 37 weeks gestation to test the mother for carriage and prophylactic measures taken for colonized women.

This study was undertaken to evaluate the clinical performance of the WASPLab® Artificial Intelligence/Interpretative Algorithm (PhenoMATRIX™ PM Copan Diagnostics) in detection of GBS from LIM broth enriched cultures plated on CHROMID® Strepto B (ChromGBS) Chromogenic Media (bioMérieux) after 24 and 48 hours of incubation. The performance of the PhenoMATRIX™ was compared to manual culture review of the digital images and molecular detection of GBS (BD MAX, BD Diagnostics Systems). Discrepant results were adjudicated using a second molecular method (Cepheid GeneXpert GBS, Cepheid, Inc.).

A total of 486 vaginal/rectal swab samples were collected for the study. Specimens were determined to be positive if they had a positive molecular result and/or a confirmed GBS culture result. Thus, the overall detection of GBS was considered to be 94/486 (19.3%). The ChromGBS plus PM algorithm detected 88/94 (93.6%) GBS samples at 24 hours demonstrating a sensitivity and specificity of 93.6% and 79.9%, respectively. Increasing the incubation time to 48 hours increased GBS detection to 90/94 (95.7%) for a sensitivity of the ChromGBS plus PM of 95.7%, but specificity of 66.6%. Molecular detection of GBS resulted in 90/94 (95.7%) positive samples for an overall sensitivity of 95.74% with a specificity of 98.8%. Technologist read of ChromGBS without PM, detected 84/94 (89.4%) of positive GBS colonies by 48 hours for a sensitivity and specificity of 89.4% and 99.5%, respectively.

The use of ChromGBS in combination with the PM AI/IA System was equivalent to the sensitivity of molecular detection. Further, the algorithm never called a culture negative that was determined to be positive by manual reading and identified an additional six true positive specimens that were missed by manual digital image culture reading.

INTRODUCTION

Group B Streptococcus (GBS) has been recognized as a leading cause of infectious early neonatal morbidity and mortality in the United States. Early onset GBS disease in newborns occurs within the first week of life. Patients typically present with respiratory distress, apnea or other constitutional signs of sepsis with mortality from early-onset GBS can range from 2-30%, with the highest rates among infants less than 33 weeks gestation.

The CDC recommends universal culture-based screening for GBS on all pregnant women using vaginal-rectal swabs collected at 35-37 weeks gestation. Approximately 10-30% of pregnant women are colonized with GBS during pregnancy. Following the first recommendations for screening, the rate of early-onset GBS disease has decreased from 1.7 cases per 1,000 live births (1993) to 0.22 cases per 1,000 live births (2016).

Culture of the Lim-broth enriched specimen is the gold-standard for detection of GBS. However, culture lacks sensitivity and requires 24-

48 hours following enrichment for final identification. There are several commercially available nucleic acid amplification tests available for the detection of GBS that increase sensitivity and provide a faster result, but may suffer from a lack of specificity. Based on previous studies describing the efficacy of the WASPLab® artificial intelligence interpretive algorithm, PhenoMATRIX™ (Copan Diagnostics) Chromogenic Detection Module (CDM) for detection of MRSA, VRE and Group A Streptococcus, we are evaluating the performance of the CDM on ChromID StreptoGBS (Chrom ID GBS) for the enhanced detection of GBS in enriched vaginal-rectal swabs compared to routine visual inspection and a molecular method.

METHODS

Samples: 486 residual vaginal/Rectal swabs in LIM broth were enrolled in the study. LIM broths were incubated at 35-37°C for 18-24 as per standard laboratory procedures. 1mL of enriched LIM broth was aliquoted into empty sterile Copan 12 tubes for automated processing on the WASPLab. GBS Culture: 30µl of LIM enriched cultures were inoculated by the WASP® (Copan Diagnostics) onto one ChromID StreptoB Chromogenic Media plate (bioMerieux) and a Blood Agar Plate. Cultures were incubated in the WASPLab® (Copan Diagnostics) incubator in ambient air at 35-37°C with digital images captured at initial planting (0 hours), 24 hours and 48 hours.

Culture Reading: Digital images of cultures were reviewed manually at 24 and 48 hours by a technologist and scored for the presence or absence of colonies resembling GBS. Morphologies consistent with GBS were confirmed using Gram stain, catalase reaction and latex Lancefield grouping. Algorithm-Based Detection of GBS: Acquired images were analyzed by PhenoMATRIX™ (Copan Italia) for the detection of colonies resembling GBS. Discordant Analysis: Discordant results between the PhenoMATRIX™ and manual culture review were resolved by performing identification on discordant colonies.

Molecular Detection of GBS: All enriched LIM broths were tested by BD MAX GBS (BD Diagnostics).

Data analysis: To evaluate the performance between the culture methods and NAAT, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was determined by comparing the result of each method to the consensus result.

TABLE 1. Comparison the three methods for GBS detection. Total prevalence of GBS was 19.3% (94/486)

	Total Positive	TP	FP	FN	TN	Sensitivity	Specificity	PPPV	NPV
Manual Review 24h	74	73	1	21	391	77.7%	99.7%	98.7%	94.9%
PhenoMATRIX™ 24 h	167	88	79	6	313	93.6%	79.9%	52.7%	98.1%
Manual Review 48h	86	84	2	10	390	89.4%	99.5%	97.7%	97.5%
PhenoMATRIX™ 48 h	221	90	131	4	261	95.7%	66.6%	40.7%	98.5%
BD MAX	94	90	4	4	388	95.7%	98.9%	95.7%	99.0%

TABLE 2. Comparison of PhenoMATRIX™ to manual review.

We calculated the performance of PhenoMATRIX™ compared to manual review. The sensitivity was 100% (94.7-100%) specificity was 59.0% (54.0%-63.8%), PPV was 51.4% and NPV was 100%

	VISUAL IMAGE REVIEW	
	POSITIVE	NEGATIVE
PhenoMATRIX™	POSITIVE	164
	NEGATIVE	236

TABLE 4. Identification of PhenoMATRIX™ detected/culture not detected isolates

When PhenoMATRIX™ detected an isolate that was not identified by manual review, MALDI-TOF was performed. An additional 4 *S. agalactiae* (GBS) isolates were detected by PhenoMATRIX™.

IDENTIFICATION	COUNT
<i>S. agalactiae</i>	38
Specimen artifact	51
<i>E. faecalis</i>	17
<i>S. anginosus</i>	10
<i>Lactobacillus</i> sp.	7
<i>Enterococcus</i> sp.	6
<i>S. mitis/oralis</i>	6
<i>Streptococcus</i> sp.	3
<i>E. coli</i>	2
<i>S. salivarius</i>	2
Other	5
Unable to identify	7

CONCLUSIONS

The use of ChromGBS in combination with PhenoMATRIX™ is equivalent in sensitivity to molecular detection of GBS.

ChromGBS with PhenoMATRIX™ lacks specificity due to overlap in color spectra for GBS and nonGBS isolates and color change produced by specimen artifact.

ChromID GBS plus PhenoMATRIX™ provides an efficient method for rapid screening of GBS negative cultures.



Digital detection and the use of Artificial Intelligence to detect Group A Streptococcus using a chromogenic agar

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KEYWORDS

GBS

| WASPLab®

| PhenoMATRIX™

| reduced TAT

| Higher Sensitivity

ABSTRACT

Background: Streptococcus pyogenes (Group A streptococci – GAS) is the major bacterial cause of pharyngitis occurring in people of all ages but seen most commonly among children 5 through 15 years of age. The use of antibiotics is recommended to treat GAS pharyngitis in children to shorten the duration of symptoms, reduce the likelihood of trans-mission to family members, classmates, and other close contacts, and prevent the development of complications, including acute rheumatic fever, peritonsillar abscess, and mastoiditis.

Materials and Methods: We studied the ability of a specialized Group A Strep chromogenic agar (Colorex Strep A agar –Chromagar) together with Copan's PhenoMATRIX™ artificial intelligence (AI) software (WASPLab® chromogenic detection module) to detect GAS on this media. Results were compared to manual detection of the organism on standardized SXT-blood agar and visual reading. Potential GAS organisms from both types of media were confirmed as GAS by MALDI identification. Cultures were considered positive for GAS if MALDI confirmed GAS from either the chromogenic agar plates or the SXT-blood agar plates. A pyrrolidonyl arylamidase (PYR) test was performed to confirm positive cultures for GAS from the SXT-blood agar plates.

Results: A total of 252 specimens were tested from patients presenting to our medical centers, emergency rooms and clinics, as well as 25 samples spiked with *S. pyogenes*, which resulted in a total of 42 positive GAS cultures. The SXT-blood agar detected 40/42 (95.2% sensitivity, 100% specificity) of the positive cultures, while the Group A Strep chromogenic agar/PhenoMATRIX™ AI algorithm software detected 42/42 (100% sensitivity, 97.45% specificity) of the positive cultures.

Conclusions: This study demonstrates that the utilization of Group A Strep chromogenic agar together with Copan's PhenoMATRIX™ artificial intelligence software was superior to the use of standard cultures on SXT-blood agar combined with manual reading. When undetected infections are left untreated the symptoms of Group A streptococcal pharyngitis are usually self-limited, however, patients, regardless of age, who have a positive test for GAS should receive antibiotics to prevent complications. The use of chromogenic agar together with AI software will more accurately detect these infections and allow for more appropriate patient therapy.

INTRODUCTION

Group A Streptococcus (GAS; Streptococcus pyogenes) can cause both noninvasive and invasive disease, as well as nonsuppurative sequelae. Streptococcus pyogenes is the major bacterial cause of pharyngitis occurring in people of all ages but seen most commonly among children 5 through 15 years of age. It is rare in children younger than 3 years of age but when it does occur, it rarely manifests as acute pharyngitis; instead these children usually have a subacute picture of a mucopurulent rhinitis followed by fever (rarely high fever), irritability, and anorexia called "streptococcal fever". Patients older than 3 years of age may also present with a scarlatiniform rash called scarlet fever or scarlatina. The most common risk factor for developing disease is close contact with another person with group A strep pharyngitis. Adults at increased risk for group A strep pharyngitis include parents of school-aged children and those who are of-ten in contact with children. In addition, crowding, such as found in schools, military barracks, and daycare centers, increases the risk of disease spread. The use of antibiotics is recommended to treat GAS pharyngitis in children to shorten the duration of symptoms, reduce the likelihood of transmission to family members, classmates, and other close contacts, and prevent the development of complications, including acute rheumatic fever, peritonsillar abscess, and mastoiditis. Thus, the rapid and accurate diagnosis followed by appropriate therapy is necessary to avoid transfer and complications. We studied the ability of a specialized Group A Strep chromogenic agar (Colorex Strep A agar –Chromagar) together with Copan's PhenoMATRIX™ artificial intelligence (AI) software (WASPLab® chromogenic detection module) to detect GAS on the Colorex Strep A ChromAgar. This was compared to the use of our routine throat culture protocol using a blood agar-SXT (BAP-SXT) plate to determine if times of throat cultures could be shortened and if the BAP-SXT plate could be eliminated from our culture set up.

MATERIALS AND METHODS

252 patients presenting to our medical centers, emergency rooms and clinics had throat specimens collected in eSwabs® and delivered to the laboratory processed on the WASPLab®, planted on Colorex Strep A ChromAgar and read with the PhenoMATRIX™ software. In addition, 25 spiked samples and 25 negative eSwabs® were also processed on the WASPLab®, planted on Colorex Strep A ChromAgar and read with the PhenoMATRIX™ software. These same specimens/samples were processed on the WASP® per our routine protocol using the BAP/SXT media, incubated offline and manually read. The spiked specimens were prepared by making a 0.5 McFarland suspension of a known Group A streptococcal isolate and placing 10uL into each of 25 eSwab® vials. The Colorex Strep A ChromAgar was incubated in the WASPLab® for 24 hours and then evaluated by the PhenoMATRIX™ software for positive and negative cultures. The BAP/SXT plated cultures were incubated for 48 hours and read manually. The Colorex Strep A ChromAgar/PhenoMATRIX™ software results were compared to the results of manual detection of the organisms from the BAP/SXT agar with manual reading. Potential GAS organisms from both media were con-firmed as GAS by MALDI identification. Cultures were considered true positives for GAS if MALDI confirmed GAS from either the chromogenic agar plates or the BAP/SXT agar plates. A pyrrolidonyl arylamidase (PYR) test was also performed to confirm positive cultures for GAS from the BAP/SXT agar plates.

RESULTS

From the total 252 specimens that were tested from patients, the 25 samples spiked with *S. pyogenes*, and the 25 negative eSwabs® (302 samples), a total of 42 (17 patient specimens and 25 spiked samples) were positive for GAS.

TABLE 1.

Colorex Strep A ChromAgar/PhenoMATRIX™	
Positive	
True positive	42
False negative	0
Negative	
False positive	0
True negative	260
Output	
Sensitivity	100.00%
Specificity	100.00%
Positive predictive	100.00%

TABLE 2.

Colorex Strep A ChromAgar/PhenoMATRIX™	
Positive	
True positive	42
False negative	0
Negative	
False positive	0
True negative	260
Output	
Sensitivity	100.00%
Specificity	100.00%
Positive predictive	100.00%
Negative predictive	100.00%

CONCLUSIONS

The utilization of WASPLab® and Copan’s PhenoMATRIX™ software combined with the Colorex Strep A ChromAgar out performed manual reading of routine BAP/SXT agar with regard to the detection of cultures positive for Group A Streptococcus. In addition, as the Colorex Strep A ChromAgar is designed to be read at 24 hours, we would not only be able to discontinue the use of the BAP/SXT agar in favor of the more accurate chromogenic media, but we would also be able to report out results for throat cultures sooner, decreasing our reporting time by 24 hours. It is recommended that those who have a positive test for GAS should receive antibiotics to prevent complications. The use of chromogenic agar together with AI software will more accurately detect these infections and allow for appropriate patient therapy sooner. As better and better chromogenic agars are developed which detect organisms of interest more accurately than current routine media, and as full laboratory automation in microbiology brings with it software algorithms that detect these organisms better than the ability of the naked eye, not only will we be able to detect pathogens more accurately, we will also be able to utilize software for discriminating between positive and negative chromogenic cultures. This will not only leave our technical staff the time necessary to perform other, more complex assignments, it will help fill the gaps we are currently seeing, and will continue to see, in microbiology technologist and technician positions.



Validation and implementation of Colorex™ CHROMagar™ Strep A agar on WASP®/WASPLab® for screening for Streptococcus pyogenes using the eSwab®

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KEYWORDS

WASPLab® | eSwab® | Chromogenic media | image analysis | greater sensitivity

INTRODUCTION

Streptococcus pyogenes (Group A) causes “Strep throat” which can also lead to Scarlet fever and Rheumatic fever in children. Traditional culture methods provide poor sensitivity for isolating *Streptococcus pyogenes*. The objective of this study was to validate the use of Colorex™ Strep A agar (CHROMagar™) to screen for *Streptococcus pyogenes* on throat swabs set up on the WASP® using a 30 ul loop and incubated and analyzed on the WASPLab® with digital imaging analysis. *Streptococcus pyogenes* (Group A) grows as orange to red colonies on Colorex™ Strep A agar (CHROMagar™).

MATERIALS AND METHODS

In this study 159 clinical specimens were collected with eSwab® kits and processed on a WASP® using Colorex™ Strep A agar (CHROMagar™) plates and a throat culture screening protocol with a 30 ul loop and incubated in WASPLab® in CO₂ for 20 hours at which point imaging analysis was performed. Vitek MS (Maldi-ToF) and PathoDx were performed on target and non-target colour colonies isolated. Results were compared to the same samples set up on Blood agar incubated at 35 degrees C anaerobically for 20 hours. The samples had all been tested for *Streptococcus pyogenes* by LAMP PCR.

RESULTS

Of the 159 specimens tested, 120 were positive for *S. Pyogenes* Group A by LAMP PCR. Of those 120 specimens, 116 grew on Colorex™ Strep A agar (CHROMagar™) and 109 showed beta hemolysis on blood agar. 56 target positive colonies were tested with Vitek MS (Maldi-ToF) and all 56 identified as *Streptococcus pyogenes*. The other 60 target positive colonies were tested with PathoDx using groups A and C. All tested positive with A and negative with C. White non-target colour colonies identified as other *Streptococcus* species. Colorex™ Strep A agar (CHROMagar™) showed a sensitivity of 96.7% (95%CI 0.92-0.99) and a specificity of 100% (95%CI 0.95-1) as compared to LAMP PCR. Copan analyzed the images using their PhenoMATRIX™ software algorithm and picked up a positive culture (LAMP PCR positive) that appeared negative to the naked eye.

CONCLUSION

Results showed Colorex™ Strep A agar (CHROMagar™) had a significantly greater sensitivity than Blood agar in isolating *Streptococcus pyogenes* in throat culture specimens. The use of the WASP® for set up provides efficient and consistent processing and WASPLab® imaging allows for high resolution digital imaging analysis. The software allows you to zoom in to detect scant growth in the main inoculum that might otherwise be missed. Copan's PhenoMATRIX™ software provides a sensitivity better than the human eye.



Evaluation of PhenoMATRIX™ expert system for the segregation of urine specimens on CHROMID® CPSE Elite

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KEYWORDS

WASPLab® | urine | segregation | sensitivity | specificity | productivity | time saving

INTRODUCTION

Inovie Group is the first Independent Medical-biologist's group in France. Labosud is one of the 18 Inovie's medicalbiology laboratory. Biologists serve more than 45 000 patients each day. PhenoMATRIX™ is an expert system of the WASPLab® solution (COPAN) combining plate reading algorithms and clinical information from LIS system. The algorithms detect the bacterial growth, recognize the bacteria group colors and perform enumeration on CHROMID™ CPSE Elite (bioMerieux). PhenoMATRIX™ provides a final result on the urine plates.

MATERIALS AND METHODS

To implement PhenoMATRIX™ a decisional tree was designed for sorting negative and positive specimens and for making decision on the next steps le identification and susceptibility testing.

The cut off for negative specimens (NG) was ≤ 7 colonies. Three categories of positive specimens were defined: Escherichia coli (EC), Non Escherichia coli with ≤ 2 morphotypes (NEC) and PolyGroup (PG) with ≥ 3 morphotypes. The rate of leucocytes was taken into account for each category to make the decision on the next steps. When the rate was ≤ 10.000 or not available, plates were categorized in "screening"(S). A total of 4834 samples were inoculated onto CHROMID®CPS Elite, incubated into the WASPLab® and imaged at 16h. Images were interpreted blindly by the operator and the results were compared with the PhenoMATRIX™ results. The average time of decision was also measured.

RESULTS

The 4834 specimens were segregated into 5 categories: 875 in Screening, 3959 in the other categories (NG-Fig1, EC-Fig2, NEC-Fig3, PG). The sensitivity was 100% and the specificity, 96, 6%. The 134 false positive results were due to the presence of artefacts on the plates (e.g. bubbles, dust) or the overestimation of microcolonies.

The global accuracy of PhenoMATRIX™ was 92, 8% (le comparison of the PhenoMATRIX™ results with the human interpretation). The decision average time using PhenoMATRIX™ was 1,5 s per specimen (negatives: 0,6 s; EC: 1,45 s; NEC: 3,6 s; PG: 2,73 s) whereas the average time by the operator in a manual workflow was 100 s.

CONCLUSION

The use of PhenoMATRIX™ with CHROMID®CPSE allows to segregate urine specimens with high confidence, reproducibility and productivity with an average time of decision of 1,5 s per specimen. The fully automated workflow and traceability revolutionize the daily routine management of urine specimens.



Evaluation of the performances of Color Detection Module Algorithm for the automated detection of *Streptococcus agalactiae* on CHROMID®StreptoB

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KEYWORDS

WASPLab® | chromogenic media | CDM algorithm | visual vs automated reading | workflow improvement

ABSTRACT

Streptococcus agalactiae (STRB), is a leading cause of sepsis, meningitis, and death among newborns. The Centers for Disease Control and Prevention recommends the screening for group B streptococcal colonization in pregnant women.

WASPLab® solution (COPAN) automates the culture steps for STRB screening (plat inoculation, incubation and image reading) but the plate interpretation is still made by the user.

OBJECTIVES

The aim of the study was to establish the performances of the Color Detection Module (CDM) algorithm (COPAN) for the detection of *Streptococcus agalactiae* on CHROMID® STRB (bioMérieux) in order to segregate positive from negative specimens in a fully automated workflow.

METHODS

A total of 50 vaginal eSwabs® clinical samples were inoculated by the WASP® onto CHROMID®STRB both directly and after enrichment in LIM broth (COPAN).

Among the 50 specimens, 40 were positive to *Streptococcus agalactiae* and 10 negative.

In order to cover all the methodologies used in clinical laboratories, different volumes of samples were seeded onto the plates by the WASP® using a Five Quadrant Type 1 streaking pattern (5QT1):

- 10, 30µL directly from the eSwab®
- 10, 30, 90µL after enrichment in LIM Broth

The plates were incubated into the WASPLab® at 35°C in aerobiosis with an imaging time at:

- 0, 24h, 48 h for the direct inoculation
- 0, 24h for the plates inoculated after enrichment

Images were read blindly by the operator who looked for the presence of characteristic coloration of STRB on the plate (pale pink to red, round and pearly). CDM algorithm was applied, and performances of the system were evaluated compared to results obtained by image visual reading.

RESULTS

A total of 374 images were analyzed:

- 261 after 24h of incubation
- 113 after 48h of incubation

Compared to the image visual reading, CDM algorithm showed a

- Sensitivity of 100 % at 24h and 24+48h,
- Specificity of 98,4% at 24h and 96.7% at 24+48h.

		Visual reading		
		Negative	Positive	Total
CDM Algorithm	Negative	126	0	126
	Positive	2	133	135
	Total	128	133	261

		Visual reading		
		Negative	Positive	Total
CDM Algorithm	Negative	185	0	185
	Positive	6	183	189
	Total	191	183	374

At 24h, 2 false positive results were found.

After a second visual review, one false positive result at 24h by CDM was not confirmed due to some pale pink colonies missed by the reader during the first visual reading.

The second review of all the discrepancies (2 at 24h and 6 at 24+48h) led to an improvement of the specificity to 99,2% and 97,4% respectively at 24h and 24+48h

Remaining false positive were due to:

the spontaneous coloration of the medium due to the presence of enzyme in the specimen itself (1 at 24h and 24+48h)

The presence of pink halo around the colonies (4 at 48h)

CONCLUSION

Through the WASPLab® solution, the CDM algorithm fully automates the detection of *Streptococcus agalactiae* on CHROMID® STRB medium. The high sensitivity of the algorithm enables a better detection of *S. agalactiae* compared to the visual reading even in case of very low concentration of bacteria.

The full automation of the culture and reading steps of B Streptococci screening at a high level of confidence and reproducibility constitutes a real improvement of the laboratory workflow and productivity.



Evaluation of WASPLab® Chromogenic Detection Module to Detect Significant Growth in Urine Cultures with CPSe Agar.

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KEYWORDS

WASPLab® | Chromogenic agar for urines | Image analysis | Segregation | reduced TAT

INTRODUCTION

Urine cultures are among the most common specimens received by clinical laboratories and generate a major share of the laboratory workload. Chromogenic agar can expedite culture results, but technologist review is still needed for every plate. In this study, we evaluated the WASPLab® (Copan, Brescia, IT) software to interpret urine specimens plated onto CHROMID® CPS® Elite (CPSe, bioMérieux, Marcy-l'Étoile, FR) agar.

MATERIAL & METHODS

Urine specimens submitted for bacterial culture were plated onto CPSe agar with a 1µl loop using the WASP®. Each plate was imaged after 0 and 16h of incubation. Each image was read for colony number and color by the WASPLab® software and a technologist using an HD monitor. Manual readings were blinded from the software's results. Results were reported as negative if <10 colonies/plate were detected. Time stamps created by data entry into the LIS were used to predict potential differences in time to result between manual CPSe vs Blood and MacConkey agar, and manual vs automated reporting.

RESULTS

Table 1. Performance of the WASPLab digital imaging of CPSe plates compared to manual reading.

Data set	MP/AP ^a	MN/AN ^b	MN/AP ^c	MP/AN ^d	Total	Sensitivity (95% CI)	Specificity (95% CI)
Real-time	1013	339	227	2	1581	99.8 (99-100)	59.9 (56-64)
Post second review	1186	339	54	2	1581	99.8 (99-100)	86.2 (82-89)

Table 1: The first pass of the data was only using the input from an initial read by the technologists in real time. The second review had a single technologist review each image. (a) Manual Positive Automation Positive, (b) Manual Negative, Automation Negative, (c) Manual Negative, Automation Positive, (d) Manual Positive, Automation Negative. The Majority of the MN/AP specimens were caused by missed microcolonies or technologist recording errors.

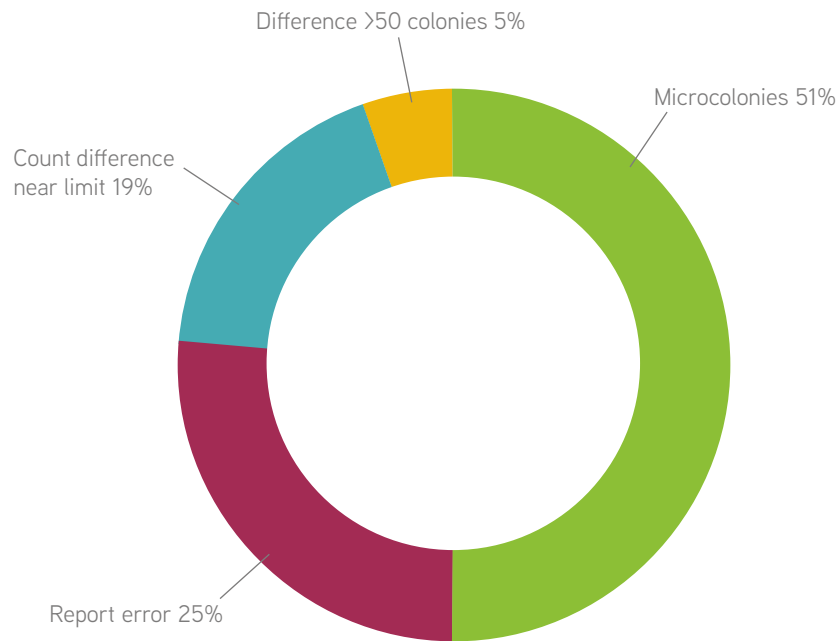


Figure 3: Analysis of each MN/AP specimen from the real-time data set was reviewed and classified into 4 errors. Microcolony discrepant is caused from technologists ignoring these organisms, report error is when a technologist failed to enter data, count difference near limit indicates specimens where minor differences between counts were detected that pushed results over positive threshold, and differences >50 are plates where the technologist and software had large differences that were > 50 colonies.

CONCLUSIONS

- The chromogenic detection module was highly sensitive correctly identifying all, but 2 specimens, which appear to be technologist error or judgement call on unknown patient data.
- Over half of the FP specimens were the result of microcolonies ignored by the technologists or due to limitations of technologists that were not able to assess the CPSe agar.
- CPSe agar was able to reduce turnaround time (TAT) on average by 3 hours before conventional testing. Conventional testing included MALDI-TOF MS for ID so laboratories using slower identification methods could see greater differences in TAT.
- Software analysis was able to further reduce TAT 7 hours for positive specimens and 5 hours for negative specimens compared to conventional culture and reduced TAT vs manual CPSe by ~5 hours.



Multicenter Evaluation of the WASPLab® Digital Image Analysis Software to Segregate Significant Growth of Urine Cultures on Blood and MacConkey Agar

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KEYWORDS

WASPLab® | Urine culture | Colony counting algorithm | Special segregation rules

INTRODUCTION

Urine culture interpretation can be complicated by several variables, including the presence of small numbers of colonies and the growth of more than one bacterial type. In general, voided-urine cultures containing $\geq 10,000$ CFU/mL should be reported as potential pathogens if there are not more than 3 pathogens or these organisms are not normal skin flora. In this study, we evaluated the accuracy of the WASPLab® (Copan, Brescia, IT) software to differentiate negative and non-negative urine cultures.

MATERIAL & METHODS

Urine specimens submitted for bacterial culture from 3 different sites were plated on sheep blood and MacConkey agars. All specimens were processed by the WASPLab® using a 1 μ L loop, and images were captured after 0 and 18 h incubation. The software quantitated each plate and reported the specimen as non-negative if any plate contained more than 10 colonies. Results were then compared to manual interpretation as either positive or negative for pathogens based on each laboratory's urine culture policy. These data were also analyzed by separating laboratory-negative specimens depending on site-specific rules no significant growth (skin or fecal contamination). Manual-positive, automation-negative cultures were reviewed by a second technologist.

TABLE 1

Overall Performance of WASPLab® segregation software compared to Manual Technologist review.

Site	MP/AP ^a	MN/AN ^b	MN/AP ^c	MP/AN ^d	Total	Sensitivity (95% CI)	Specificity (95% CI)
1	2958	1092	1149	2	5201	99.9 (99-100)	48.7 (47-51)
2	1613	3274	621	5	5513	99.7 (99-100)	84.0 (83-85)
3	1107	1232	410	2	2751	99.8 (99-100)	75.0 (73-77)
Total	5678	5598	2180	9	13465	99.8 (99-100)	72.0 (71-73)

Table 1: Reported data was after secondary review of discrepant specimens to remove technologist error. (a) Manual Positive Automation Positive, (b) Manual Negative, Automation Negative, (c) Manual Negative, Automation Positive, (d) Manual Positive, Automation Negative.

TABLE 2

Site 1/2 Breakout of MN/AP specimens based on laboratory rules.

	Lab Negative	NMW/MMO ^a	NSG/MGN ^b	Lab Positive
SW Neg	2941	602	823	7
SW Pos	176	764	830	4571

Table 2: MN/AP specimens were broken out based on the labs expert rules for calling urine specimens as negative. These reasons included (a) NMW/MMO are cultures that are called negative due to potential fecal contamination, (b) NSG/MGN are cultures that contain skin pathogens suggestive of poor collection are reported as negative.

TABLE 3

Site 3 breakout of MN/AP specimens based on Laboratory rules

	Lab Negative	GUF ^a	Lab Positive
SW Neg	2941	602	7
SW Pos	176	764	4571

Table 3: MN/AP specimens were broken out based on the labs expert rules for calling urine specimens as negative. These reasons included (a) GUF, which are cultures that contain genital urine flora and are considered negative due to poor collection.

TABLE 4

Evaluation of 9 remaining MP/AN specimens

Cause of MP/AN	# of specimens	Description
2941	602	7
176	764	4571

Table 4: The vast majority of missed positives by the software were due to growth of microcolonies. The software can detect these colonies, but a limit of 50 microcolonies were needed for positive reporting. Only 1 specimen contained a count difference that impacted results.

CONCLUSIONS

- The segregation software to count colonies is highly accurate and could be used to quickly remove negative urines, which in this study would remove 5,598 cultures.
- Specific laboratory rules could be added to improve MN/AP reporting.
- When specifically evaluating plates that did not need expert rules, sensitivity and specificity of the software increases to 99.8% and 95.0% respectively.
- Machine learning for differentiation will be necessary to create specific rule sets to removal of contaminated urine.



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

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KEYWORDS

GAS detection

| Gold standard

| Interpretation algorithm

| Chromogenic media

INTRODUCTION

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. 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) (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. 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.

MATERIALS & METHODS

- 250 remnant pharyngeal samples collected from pediatric patients during the period of September 2017 through January 2018 were enrolled in the study.
- Samples were initially tested for the presence of GAS by PCR (Lyra Direct Strep Assay) at Children's Hospital Los Angeles
- Remnant samples, either positive or negative for GAS, were inoculated onto blood agar plate (BAP) and a Colorex Strep A agar and incubated in the WASPLab® for 24 hours.
- After 24 hours, plates were examined for presence of orange colonies on the Colorex Strep A agar
- Results were compared to the results from the PhenoMATRIX™ Chromogenic Detection Module (CDM).
- Orange colonies observed on Colorex Strep A agar were 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.

RESULTS

250 cultures were manually examined by medical technology staff after 24 hours of incubation. Plates examined by the CDM software after secondary manual review had a sensitivity of 100% and a specificity of 96.4% (Table 1). Plates examined manually by technologist (not assisted by CDM software) after secondary manual review had a sensitivity of 96.5% with a specificity of 100%. Of the 57 cultures that grew orange colonies on the Colorex Strep A Agar, 51 were confirmed as GAS by MALDI-TOF MS. 6 cultures grew orange colonies; but 4 cultures were not identified as GAS and unfortunately, 2 cultures were not tested by MALDI-TOF MS. 5 specimens that were initially PCR positive did not grow in culture on the Colorex A Strep Agar. Isolation of GAS in blood agar plates had a sensitivity of 78.9% and specificity of 73.6%.

Table 1. WASPLab® examination of Colorex Strep A agar after 24 hours incubation using CDM software with secondary manual review

		ORANGE COLONY	
		POS	NEG
<i>CHROMagar at 24 h (CDM algorithm)</i>	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 2. Manual examination of Colorex Strep A Agar after 24 hours incubation with secondary manual review

		ORANGE COLONY	
		POS	NEG
<i>CHROMagar at 24 h (visual)</i>	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 3. Comparison of manual examination of BAP versus Colorex Strep A Agar (with secondary manual review)

		ORANGE COLONY	
		POS	NEG
<i>Beta Hemolysis Present on BAP</i>	Pos	45	51
	Neg	12	142
		Sensitivity: $45/45 + 12 = 78.9\%$	Specificity: $142/142 + 51 = 73.6\%$

CONCLUSIONS

This proof of concept study validates the ability of the CDM software in the detection of GAS using a novel CHROMagar, ColorexStrep 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 aBAP.

The 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.



Evaluation of A New Chromogenic Group B Streptococcus Agar and Use of Digital Analysis for Detection of Group B Streptococcus in Vaginal/Rectal Swabs

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KEYWORDS

GBS

| Image analysis VS visual examination

| Segregation

| Enhanced workflow

INTRODUCTION

Detection of Group B Streptococcus (GBS) colonization during pregnancy aids in the prevention of early onset GBS disease in newborns. A new Group B chromogenic culture medium combined with segregation software (SSW) developed for the WASPLab® system (Copan Diagnostics) may aid in the accurate detection of GBS colonization as well as enhanced laboratory workflow management. We evaluated the LIM broth/ChromID® Strepto B agar (STRB, bioMerieux) for detection of GBS along with the WASPLab SSW for automated digital analysis for separation of negative and positive culture results. The comparative method was Carrot broth/GBS Detect medium (Hardy Diagnostics). Vaginal/rectal swabs were processed by the WASPLab® system using both methods with initial inoculation into the enhancement broths which were incubated off-line at 35-37°C overnight, followed by WASP® inoculation of the plates and incubation at 35-37°C for 20 h (Detect) and 24 h (STRB). WASPLab digital images of GBS Detect (beta hemolysis) and STRB (pink to red colonies considered positive) were visually examined. STRB also had digital images analyzed by the SSW to automatically segregate negatives from positives. All positives were confirmed as GBS or not GBS by phenotypic methods. There were 245 samples acceptable for comparison with 152 negative by Detect and STRB and 87 positive by both methods. There were 4 cultures positive by Detect that were negative by STRB and 2 cultures that were negative by Detect and positive by STRB. Sensitivity was 96% and specificity 99%, however the adjusted specificity was 100% with the 2 STRB positive/ Detect negative cultures considered to be true positives. The 245 STRB plate images were analyzed by SSW after initial visual examination. There were 89 STRB visually examined and determined to be positive with 100% detected as positive by SSW with no false negatives. Of the remaining 156 STRB negative plates, 124 (79%) were negative by SSW. There were 32 positives indicated by SSW that were determined to be negative for GBS by additional phenotypic testing. These were determined to be SSW false positive most likely due to blue/purple colonies with a pink hue in the medium. The STRB is equivalent to the Detect for detection of GBS. The SSW is a powerful tool for automatic separation of positive and negative cultures to help laboratory workflow.

Streptococcus agalactiae is a colonizing microorganism in pregnant women. Genitourinary tract vaginal colonization usually occurs in late adolescence. Women of child-bearing age carry GBS at variable frequencies once colonized. The rate of GBS colonization among pregnant women usually remains stable over time. Additionally, colonization toward the end of pregnancy is a risk factor for potentially severe newborn diseases, including neonatal sepsis.

GBS infection in newborns arises by the aspiration of infected amniotic fluid or vertical transmission during delivery through the birth canal. Due to the high neonatal mortality rate due to maternal GBS colonization, the Centers for Disease Control and Prevention recommended universal intrapartum antimicrobial prophylaxis in women of high risk at 35-37 weeks of gestation and screening of anorectal and vaginal specimens at 35-37 weeks of pregnancy is recommended. In this study, we evaluated STRB for the qualitative detection of GBS in pregnant women and utilized the Copan WASPLab® digital analysis software for detection of GBS and segregation of positive from negative cultures.

MATERIALS & METHODS

A total of 245 Vaginal/Rectal swabs were processed on one WASP® line with incubation at 35-37°C, non-CO₂ in a WASPLab® incubator.

1. WASP® instrument was used to inoculate 30µL of Eswab specimen to a LIM broth. The inoculated LIM broth was removed and incubated

off-line at 35-37°C, in non-CO₂.

2. After 18-24 hours incubation, 30µL of incubated LIM broth was plated by the WASP® to a STRB chromogenic agar plate.
3. Plates were placed in a WASPLab® incubator in non-CO₂ at 35-37°C.
4. At 0 hours and after 24 hours incubation, digital imaging of all plates was performed within the WASPLab®.
 - A. All images were viewed by a CLS with positive plates removed for organism identification confirmation according to manufacturer's protocols and compared to laboratory results with Carrot Broth/Group B Detect.
 - B. All plate images of STRB were also evaluated by Copan segregation software (SSW) which may be used as a preliminary screen to segregate negative from positive plates. Image analysis results were compared to manual CLS read results.
 - A. Images of all plates were compared to results provided by the CLS read manual method.
 - B. Discrepant results were subject to image review of discrepancies by a manager/director.
 - C. Discrepancies of SSW positive, CLS negative were not considered significant. SSW would direct CLS to manually examine plate for further workup.

RESULTS

1. 245 total GBS cultures were evaluated by a CLS and by the SSW. See Tables 1 and 2.
2. There were 152 GBS cultures considered Negative and 87 Positive by both Detect and STRB.
 - a. 4 cultures positive by Detect and negative by STRB
 - b. 2 cultures negative by Detect and positive by STRB
 See Table 1
3. There were 245 STRB plate images analyzed by SSW after initial visual examination.
 - a. 89 STRB positives by both visual examination and SSW.
 - i. Zero were determined negative by SSW that grew GBS in culture.
 - b. Among 156 negative STRB by visual examination,
 - i. 124 (79%) were negative by SSW.
 - ii. 32 were determined positive by SSW.

TABLE 1. Correlation between Detect and STRB

		STRB	
		Negative	Positive
GBS Detect Medium	Negative	152	2
	Positive	4	87

TABLE 2. Correlation between Visual examination and SSW

		SSW	
		Negative	Positive
Visual Examination	Negative	124	32
	Positive	0	89

CONCLUSIONS

The STRB is equivalent to the Detect for recovery and isolation of GBS.
 The SSW is a powerful tool for automatic separation of positive and negative cultures to help laboratory workflow.



Colibrí[®]



Copan Colibrí[®], an innovative fully automated instrument for Clinical Microbiology Laboratory

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KEYWORDS

Colibrí[®] | manual vs automated picking | urine | improved safety | optimization | reduced time

INTRODUCTION

Copan Colibrí[®] is a brand-new, innovative instrument for the fully automated preparation of the MALDI-ToF target to bacterial identification, microbial suspensions for susceptibility tests and the seeding of purity plates. The aim of this study was to validate the instrument for its introduction in the laboratory routine, comparing the microbial identification of MALDI-ToF targets prepared manually and by Colibrí[®].

METHODS

A set of 130 urine samples that yielded positive results at the Microbiology Laboratory of Niguarda Hospital (Milan, Italy) were chosen for the study. One microliter of each sample was seeded on CPS[®]Elite (bioMérieux) by WASP[®] and plates incubated in WASPLab[®] at 35°C for 16h. Plates were digitalized and analyzed on WASPLab[®] working station and colonies were designed with the aid of WASPLab[®] Imaging Plug-In. Plates were then loaded on Colibrí[®] equipped with a pipetting system able to pick the colony pre-selected by the operator, transferred it on the target, and then the spot was overlaid with the matrix without the use of formic acid. Microorganism identification was performed by MALDI Biotyper system (Bruker Daltonics) and results were compared to those obtained from targets prepared by manual methods.

RESULTS

144 microorganisms were originally isolated from the 130 urine samples tested with the manual system, and used as control; 58 Gram-positive bacteria (44 Enterococcus spp, 7 Staphylococcus aureus, 6 Streptococcus spp, 1 Aerococcus urinae), 85 Gram-negative bacteria (62 Enterobacterales and 23 non-fermenting Gram-negative bacteria) and 1 Candida albicans. Usually, E.coli identification is based on the typical pink color of the colonies grown on CPS[®]Elite. The twelve E.coli strains included in the study were identified through MALDI-ToF because of their uncertain color on the chromogenic media. When assessed by Colibrí[®], an overall agreement of 94.4% (136/144) was found. In detail, the agreement was 98.8% (84/85) and 87.9% (51/58) respectively, for Gram-negative and Gram-positive bacteria. C. albicans was identified by both preparation methods. In comparison to the manual method the target prepared by Colibrí[®] reported no identification for 5/44 Enterococcus spp, 1/5 S. agalactiae, 1/2 A. urinae and 1/21 Pseudomonas aeruginosa.

CONCLUSION

Fully-automated Colibrí[®] showed a very good performance on target preparation allowing MALDI-ToF microbial identification, thus allowing a better optimization of the staff hands-on-time, the standardization of protocols, and a complete samples traceability, also contributing to an improvement of the safety of laboratory personnel.



Colibrí® and Bruker MALDI-TOF: does the identification performance change when different chromogenic media are used for urine culture?

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KEYWORDS

WASPLab®

Colibrí®

PhenoMATRIX™

MALDI-TOF

INTRODUCTION

Urine is one of the most common specimen types submitted to the clinical microbiology laboratory; the use of chromogenic agar is one method that might speed up culture results and reduce hands-on time and materials required for urine culture analysis. A major advantage of most of the chromogenic agars is the ability to directly identify *E. coli* while for the other uropathogens an identification confirmation is required by the use of MALDI-TOF MS.

The COPAN Colibrí® is a new automated system designed to automatically prepare the target for MALDI-TOF MS identification. Colibrí® adopts pipettor tips to transfer colonies from agar plates to the target and to cover them with matrix, allowing standardization of target preparation and patient's sample traceability.

The objective of this study was to evaluate the performance of Colibrí® target preparation and the following MALDI-TOF identification capability (Bruker Biotyper System) when uropathogen colonies grow on different chromogenic agar or on blood-agar-based media.

MATERIALS AND METHODS

One-hundred-thirteen urines were streaked by WASP® (COPAN) with the 10 µl loop on one non selective media: Tryptic-Soy-Agar with 5% sheep blood (Decton Dickinson), and three chromogenic media: CPSE (Biomérieux), Orientation (Becton Dickinson), UTI (Thermo Fisher Scientific).

After 18 hours of incubation at 35±2°C digital images were acquired by WASPLab® (COPAN) and examined for colonies growth. Images were analysed by PhenoMATRIX™ (COPAN) and samples were classified as:

- negative in presence of < 100 colonies
- positive in presence of one or two uropathogens with a load ≥104 CFU/ml.
- contaminated when ≥ 3 morphotypes grew at the load ≥104 CFU/ml.

For each positive sample and morphotype, two colonies were selected and the "Colibrí ID" (identification) Task was assigned in the WebApp (Graphical User Interface of WASPLab®). The media plates were loaded on Colibrí® and the designed colonies were automatically picked for Bruker Biotyper target preparation (see fig. 1). Re-usable polished steel targets were used.

Identification results obtained from the chromogenic media were compared to those obtained on blood-agar-based media.

RESULTS

Out of 113 samples, 44 were negative, 8 were classified as contaminated, and 61 were positive. In 57 samples a single uropathogen was present while two bacterial species grew in 4 samples (Fig. 2). A total of 130 spots were processed from colonies on the CPSE agar and 128 from colonies on the other agars.

A 100% concordance was obtained for the identification of Gram-negative bacteria from all media used. In addition, the ID score value for Gram-negatives was ≥ 2.0 for all media evaluated (Table 1).

The identification performance was quite different among the media tested for *E. faecalis*, the only relevant Gram-positive bacteria isolated (Table 1). *E. faecalis* was best detected on CPSE agar with 91% of cases correctly identified. UTI and ORIE were equivalent with their identification performances because both allowed 50% of *E. faecalis* diagnosis. The mean ID value registered for *E. faecalis* identification was ≤ 2.0 for all media evaluated.

CONCLUSION

The present study demonstrated that Colibrí® properly prepared MALDI-TOF targets allowing a correct identification of all Gram-negative uropathogens independently from the culture agars. Although *E. faecalis* spotting and identification were initially more critical from ORIE and UTI agars, a new cycle with the addition of formic acid was developed and evaluated and preliminary results are showing an increasing identification performance.



Evaluation des performances du préleveur automatique de colonies Colibri®

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KEYWORDS

Colibri® | urine | manual vs automated picking | reproducibility | productivity

INTRODUCTION

Labosud et Biomed34, LBM membres du groupe Inovie, regroupent 96 sites localisés dans les Bouches du Rhône, le Gard et l'Hérault. Le département de microbiologie de Labosud (Montpellier) prend en charge 2000 échantillons/jours.

OBJECTIFS

Evaluation du COLIBRI® (Copan): système automatisé qui prélève les colonies bactériennes et permet de préparer les frottis sur cibles pour l'identification par spectrométrie de masse, les suspensions microbiennes pour l'antibiogramme et les géloses de pureté.

MÉTHODES

- 175 échantillons urinaires ensemencés sur la gélose chromID®CPSE et positifs à E. coli ont été traités avec le système WASPLAB® (incubation, imagerie, tri décisionnel par PhenoMATRIX™).
- En parallèle, une suspension microbienne a été réalisée manuellement à partir de la même boîte selon la méthode de référence (Densitck) antibiogramme sur VITEK2™ (AST-N340).
- Les résultats ont été comparés en termes de concordance essentielle de concentration minimale inhibitrice (CMI) et de catégorie clinique pour l'ensemble de la carte et individuellement par antibiotique.

RÉSULTATS

Pour la concordance essentielle, un total de 2796 CMI a été comparé entre la méthode automatique et la méthode manuelle. La concordance essentielle globale était de 99.7% et individuelle allant de 97.6% pour le Mecillinam à 100% pour d'autres antibiotiques (bêta-lactamines, fluoroquinolones, aminosides, fosfomycine et nitrofurantoïne). Concernant la concordance en catégorie clinique, sur un total de 2781 données appariées, la concordance globale était de 99.1%, allant de 95.8% à 100% par antibiotique.

CONCLUSIONS

Les performances du COLIBRI® pour la préparation automatique des suspensions bactériennes pour l'antibiogramme sont équivalentes à celles de la préparation manuelle selon la norme ISO 20776-2. Le COLIBRI® permet l'automatisation totale de la réalisation de l'antibiogramme de E.coli sur le système VITEK2™. L'introduction du système COLIBRI® dans le flux du laboratoire de microbiologie a permis un gain conséquent de reproductibilité, productivité et traçabilité pour un diagnostic médical plus sûr et plus rapide.

COLIBRI® allows the full automation of bacterial suspensions for the antibiogram analysis, with performances equivalent to those of manual preparation. COLIBRI® allows a significant gain in reproducibility, and traceability for a safer and faster medical diagnosis.



WASPLab[®]

**and ANTIMICROBIAL
SUSCEPTIBILITY
TESTING**



Disc diffusion AST automation: one of the last pieces missing for full microbiology laboratory automation

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KEYWORDS

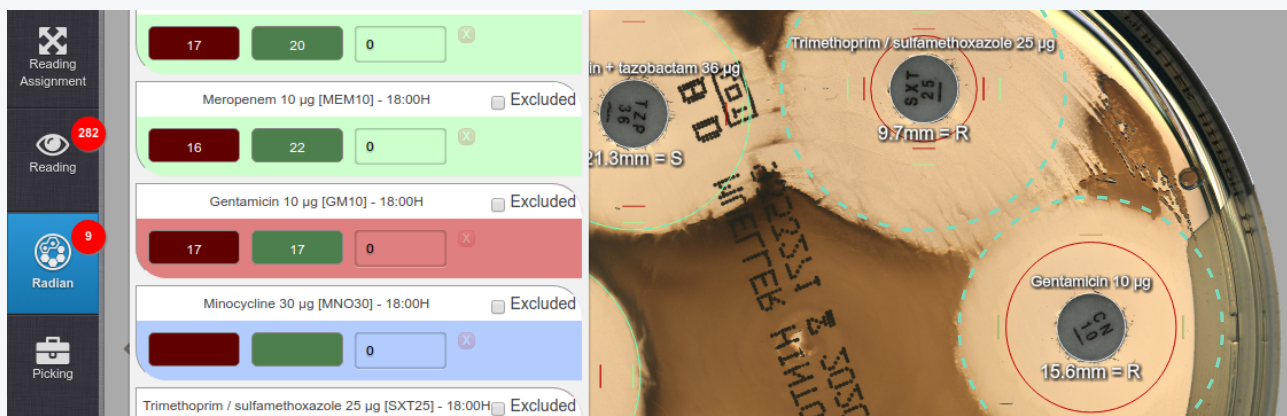
WASPLab®

AST

EUCAST

full lab automation

time-to-results



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In the past years, lab automation already improved reliability, standardization and time to results (TTR) in microbiology labs. However, the automation of the last step of the microbiology analysis, the Antibiotic Susceptibility Testing (AST), is currently supported by regulations (EUCAST). Their automation is limited at “the reading step” based on old-generation readers and it’s at the moment the slowest step of the analysis. Automated disc diffusion AST, as demonstrated by Cherkaoui et al. in 2019, will be a strategic improvement for clinical microbiology laboratories, reducing TTR without additional working time from technicians. There is still work to do before this popularization can occur in most laboratories, but numerous evolutions are planned by manufacturers to fully integrate automated disc diffusion AST. The consolidation of automatic systems, from inoculation to AST and combined with artificial intelligence, will probably lead to true full lab automation.



Tentative breakpoints and areas of technical uncertainty for early reading automated disc diffusion for Enterobacterales

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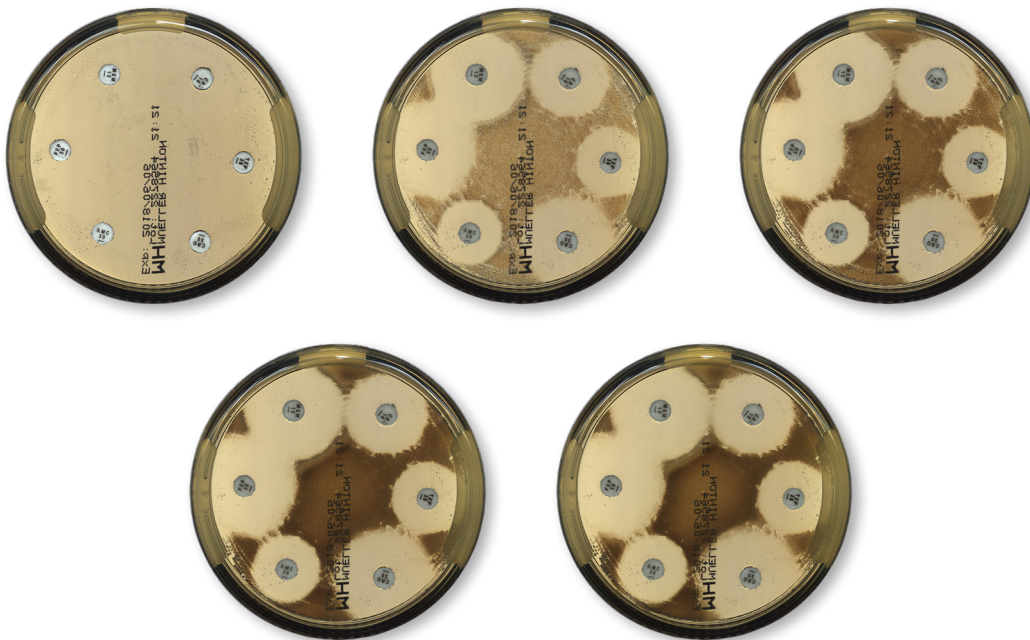
KEYWORDS

WASPLab[®]

AST

time-adapted breakpoints

time saving



Disc diffusion is the standardized method for antimicrobial susceptibility testing (AST). Although cost-effective, reliable and accurate, disc diffusion requires long incubation times. This study compares early automated AST readings (6 and 8 hours) with the standard EUCAST method (18 hours) for more than 1100 Enterobacterales with different resistance profiles. The results show how automated AST readings at 6 hours and 8 hours (complemented with time-adapted clinical breakpoints to minimize errors) give reliable results for a large number of antibiotics, ensuring a fast delivery of antibiotic therapies and improving patient outcome. Furthermore, these early readings results can be routinely confirmed after 18 hours incubation of the same plate.



Walk Away Automated Disc Diffusion Susceptibility Testing by Copan WASPLab®

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KEYWORDS

WASPLab®

| ESBL

| AST

| VITEK® 2

| Disc Diffusion

INTRODUCTION

Multi-drug resistant bacteria have become a significant public health concern which emphasizes the critical role of susceptibility testing in daily patient care. Traditionally, antimicrobial susceptibility testing (AST) has been performed by disk diffusion or microdilution, both of which are laborious procedures when performed manually. The objective of this study was to compare the Copan WASP®-WASPLab® expanded with an automated disk dispenser and inhibition zone reader (WASP®+) with the gold standard, manual disk diffusion, and with the currently used method BioMerieux VITEK® 2 (microdilution based).

MATERIALS & METHODS

198 Enterobacteriaceae, isolated from clinical specimens, were tested by VITEK® 2, WASP®+ and manually by disk diffusion for ciprofloxacin, fosfomycin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, trimethoprim and nitrofurantoin. All isolates were also screened for the presence of ESBL phenotype by cefotaxime and ceftazidime disks. Manual disk diffusion was performed conform EUCAST guidelines. For the WASP®, a 0.5 McFarland bacterial suspension was inoculated on Mueller Hinton agar plates by the WASP®+. Antibiotic disks were automatically dispensed on the inoculated agar plates and incubated in the WASP® at 35°C ambient air. Automated pictures of the plates were taken before and after 18h incubation.

	Very Major error	Major error	Minor error	Identical
AMCL	2	1	0	98%
CTX	1	0	0	99%
CAZ	0	0	2	99%
CIP	0	0	2	99%
FD	2	0	4	97%
TRIM	0	0	0	100%
STX	0	2	2	98%
Total	5	4	10	1366/1385 (99%)

WASP®+ vs Manual disk diffusion in 198 Enterobacteriaceae

	Very Major error	Major error	Minor error	Identical
AMCL	6	12	0	91%
CTX	0	0	2	99%
CAZ	0	0	16	92%
CIP	0	1	10	94%
FD	2	1	6	95%
TRIM	1	0	0	99%
STX	1	1	1	98%
Total	10	15	35	1294/1354 (96%)

WASP®+ vs VITEK® 2 in 198 Enterobacteriaceae

AMCL: amoxicillin-clavulanic acid, CTX: cefotaxim, CAZ: ceftazidime, CIP: ciprofloxacin, FD: nitrofurantoin, TRIM: trimetoprim, SXT: cotrimoxazole.

RESULTS

WASP®+ disk diffusion was in 1366/1385 susceptibility tests (98.6%) in accordance with manual disk diffusion. Ten minor discrepancies, 4 major and 5 very major discrepancies were found. WASP®+ and VITEK® 2 were in accordance in 1294/1354 susceptibility tests (95.6%). All ESBL's (n=34) detected by the VITEK® 2 were also detected by WASP®+ .

> Very major error: WASP®+ S, reference method R,

> Major error: WASP®+ R, reference method S

> Minor error: one method I

CONCLUSIONS

> The Copan WASP® -WASPLab®AST module provides an automated and standardized disk diffusion test that can reliably test up to 8 antibiotic disks on a Mueller Hinton agar plate.

> Automated cefotaxime and ceftazidime disk diffusion can be used to screen for ESBL-positive Enterobacteriaceae.

