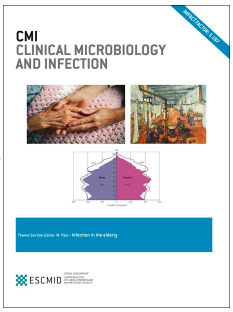
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PII: S1198-743X(19)30152-1

DOI: https://doi.org/10.1016/j.cmi.2019.04.001

Reference: CMI 1628

To appear in: *Clinical Microbiology and Infection*

Received Date: 11 January 2019

Revised Date: 3 April 2019

Accepted Date: 4 April 2019

Please cite this article as: Cherkaoui A, Renzi G, Vuilleumier N, Schrenzel J, Copan WASPLab automation significantly reduces incubation times and allows earlier culture readings, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2019.04.001.

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

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Keywords: Copan WASPLab, turn-around time, urine, genital tract specimens, non-sterile site specimens, MRSA, MSSA, ESBL, CPE

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Abstract

Objective: to evaluate whether laboratory automation (inoculation and automated incubation combined with timely-defined high-resolution digital imaging) may help reducing the time required to obtain reliable culture analysis results.

Methods: we compared the results obtained by the WASPLab automation against WASP-based automated inoculation coupled to conventional incubation and manual diagnostic on 1294 clinical samples (483 for the derivation set and 811 for the independent validation set) that included urine, genital tract and non-sterile site specimens, as well as ESwabs for screening of MRSA, MSSA, ESBL, and CPE. We used sequential routine specimens referred to the bacteriology laboratory at Geneva University Hospitals between October 2018 and March 2019.

Results: The detection sensitivity of MRSA and MSSA at 18h on WASPLab was 100% (95% confidence interval [CI], 94.48% to 100.00%). The detection sensitivity of ESBL and CPE at 16h on the WASPLab was 100% (95% confidence interval [CI], 94.87% to 100.00%). For urine specimens, the similarity was 79% (295/375) between 18h and 24h of incubation on the WASPLab. For genital tract and non-sterile site specimens, the similarity between 16h and 28h of incubation on the WASPLab were 26% (72/281) and 77% (123/159) respectively. Thus, 28h was defined as the final incubation time on WASPLab for genital tract and non-sterile site specimens.

Conclusions: The results of this study show that WASPLab automation enables a reduction of the culture reading time for all specimens tested without affecting performances. Implementing the established and duly validated incubation times will allow building appropriate laboratory workflows for improved efficiency.

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Introduction

Over the last decade, laboratory automation has improved productivity, traceability and quality in clinical chemistry, molecular biology, immunology and haematology laboratories [1, 2]. In addition, automation has significantly reduced the time required to obtain the analysis results (i.e. the turn-around time, TAT) [3]. The diversity of clinical specimens and container types, the complexity of the analytical procedures and the variety of the diagnostic methods constituted major hurdles that impaired automation in clinical microbiology laboratory. However, within less than a decade, the introduction of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) for the identification of bacteria, mycobacteria, and fungi has fundamentally modified the well-established diagnostic methods in routine microbiology to the point where MALDI-TOF has become the gold standard for microbial identification [4-6]. Nowadays, two automated instruments systems are currently available for clinical specimen streaking; inoculated media are loaded on conveyors for transfer between instruments and automated incubators where cultures are read with high-resolution digital imaging at pre-defined times. The Work Cell Automation (WCA) and the Total Lab Automation (TLA) are proposed by BD Kiestra, while the WASPLab is developed by Copan WASP srl, (Brescia, Italy). These systems are built around fundamental techniques used in clinical microbiology laboratory, namely growing bacterial colonies on agar media plates. New features are now available as the pre-sorted segregation of agar plates based on colony counts with growth and no growth discrimination, as well as automated image analysis to interpret chromogenic media plate results (e.g., WASPLab software is capable to read chromogenic plates to detect methicillin-resistant *Staphylococcus aureus* with

high sensitivity) [7, 8]. Additionally, both automation system manufacturers claim that bigger technological breakthroughs are coming soon.

Rapid microbiology diagnosis associated to antimicrobial stewardship has a perceptible effect on patient management and care [9]. One of the most important issues in clinical microbiology is therefore the rapid identification of critical antibiotic-resistant pathogens that impose infection control procedures, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), extended-spectrum beta-lactamases (ESBL), and carbapenemase-producing *Enterobacteriaceae* (CPE). Moreover, the need for rapid microbiology results to define infections that can be better managed, using narrow spectrum drugs or early oral administration, has spurred the development of products and concepts which are integrated in automated instrument systems.

In this study, we assessed whether the use of the WASPLab automation (automated inoculation, and automated incubation combined with timely-defined high-resolution digital imaging) may help reduce the time required to obtain reliable culture results.

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Material and methods

Setting

This study was conducted at Geneva University Hospitals, a Swiss tertiary care center with 1920 beds, and about 63000 yearly admissions. Hours of operation of our clinical bacteriology laboratory are 07h30 to 22h00 from Monday to Friday, 07h30 to 17h00 on Saturday and 07h30 to 13h00 on Sunday in addition to the on-call service until 22h00 during the week-end.

Study design

In order to identify the shortest incubation times for agar media plates with optimal analytical performances, we performed time-series image acquisitions on the WASPLab several hours before and up to the traditional incubation duration specific for each specimen type. We analysed selective chromogenic plates for the screening-ESwabs for methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), extended-spectrum beta-lactamases (ESBL), and carbapenemase-producing *Enterobacteriaceae* (CPE). We also assessed urine, genital tract and non-sterile site specimens to differentiate the presence of pathogens from that of a normal flora. We compared the results obtained by WASPLab against WASP-based inoculation coupled to conventional incubation and manual diagnostic, which represents the routine method used in our laboratory. Culture media analysis for each workflow was performed by trained clinical microbiologists, blinded of the results obtained by the other method. Results were compared and optimal imaging times were defined in the derivation cohort. The assessment between the two methods was then performed on an independent validation cohort, again using routine clinical samples sequentially referred to the bacteriology laboratory at Geneva University Hospitals between October 2018 and

March 2019. In the derivation set, the incubation period on the WASPLab was assessed at different incubation time points covering the full traditional incubation period, specific for each analysis and specimen type included in this study. For each incubation time assessed on the WASPLab, several high-resolution digital images were taken under different light and exposure conditions according to manufacturer's instructions, and analysed by the first author (clinical microbiologist) and one expert medical laboratory technologist. Both have been trained by the COPAN's application specialist. For the independent validation set, we performed the same analysis as for the derivation set but only on the incubation time points and imaging conditions that were selected for their optimal analytical performances.

Conventional diagnostic work-up

The identification of bacterial and yeast colonies was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; compass, Bruker Daltonics, Bremen, Germany) according to the manufacturer's instructions. The presence of ESBL was confirmed by double-disk synergy tests (DDST20 and DDST30). The presence of a carbapenemase was confirmed by the Eazyplex® SuperBug CRE system (Amplex Biosystems GmbH, Giessen, Germany). The confirmation of MRSA and MSSA strains was performed by a previously published qPCR assay targeting *femA* and *mecA* [10]. Table 1 details the incubation protocols, the agar media plates used for each sample type, and the number of samples included in the derivation set and in the independent validation set.

Results

Nasal and inguinal/perineal screening-ESwabs for MRSA and MSSA

Derivation dataset

As depicted in Table 2, all the 14 positive clinical specimens (12 MRSA and 2 MSSA) were already detected at 18h, with the specific color on CHROMID® MRSA (BioMérieux, Geneva, Switzerland) or SaSelect medium for MSSA (BioRad, Fribourg, Switzerland), when the chromogenic media were incubated on the WASPLab. No difference was observed between 18h, 22h and 48h for both, the presence and the semi-quantification of MSSA or MRSA. Among the 148 specimens used for the derivation set, 89% (132/148) were negative by the two compared methods. In two cases, the specimens were validated as MSSA negative by the manual method at either 18h, 24h or 48h. In contrast, when SaSelect medium was incubated on the WASPLab, those two cases showed a few colonies already detected at 18h with the specific color. MSSA was confirmed positive by qPCR (yielding only a *femA* positive signal).

Independent validation dataset

The cut-off point for MRSA and MSSA screening-ESwabs on the WASPLab was defined as 18h by the derivation set. This incubation time point was validated on another 181 clinical samples, of which 13% (24/181) were MRSA positive and 13% (24/181) were MSSA positive. All the positive specimens were detected at 18h on the WASPLab, except one sample validated as MSSA negative by the manual method but few colonies were detected at 18h on the WASPLab with the specific color on the SaSelect medium (Table 2). This discrepancy can be explained by the fact that the SaSelect medium is highly sensitive to light exposure, favoring the WASPLab that allows immediate incubation after streaking.

The detection sensitivity of MRSA and MSSA at 18h on WASPLab compared with the manual method for the 329 specimens included in the derivation and the validation sets was 100% (95% confidence interval [CI], 94.48% to 100.00%).

Rectal screening-ESwabs for ESBL-producer and CPE

Derivation dataset

The incubation period was assessed at 16h, 18h, 22h, and 48h on the WASPLab. Among the 84 clinical samples included in the derivation set, 62% (52/84) were ESBL and CPE negative by the two compared methods. At 16h on the WASPLab, 29 samples were detected ESBL positive and 3 CPE positive (Table 3), which reached optimal detection sensitivity (100% / 95% confidence interval [CI], 89.11% to 100.00%) as compared with the manual method.

Independent validation dataset

We selected 16h as the defined incubation period on the WASPLab. Sixty-six independent clinical specimens were included in the validation set, of which 28 samples were ESBL and CPE negative, 30 specimens were ESBL positive, and 8 specimens were CPE positive (Table 3). As for the derivation set, all the 38 ESBL and CPE positive specimens were detected at 16h on the WASPLab.

The detection sensitivity of ESBL and CPE at 16h on WASPLab compared with the manual method for the 150 specimens included in the derivation and the validation sets was 100% (95% confidence interval [CI], 94.87% to 100.00%).

Bacteriological examination of urine specimens

Derivation dataset

Among the 109 urine samples included in the derivation set, 19% (21/109) were negative by the two compared methods. The incubation period for urine samples was explored on the WASPLab on 11 different incubation time points (from 16h up to 48h). In 24 samples, the common *Enterobacteriaceae* uropathogens and *Pseudomonas aeruginosa* were detected on CHROMID® CPS® Elite at 16h. In 13 samples, it would have been necessary to wait until 24h of incubation on CHROMID® CPS® Elite to have sufficient growth for *Candida* and *Cyberlindnera fabianii* detection. The optimal incubation time to have sufficient growth of Gram positive bacteria, including uncommon uropathogens and flora, was determined as 18h (Table 4).

Independent validation dataset

We defined on WASPLab for CHROMID® CPS® Elite an intermediate incubation time at 18h and final incubation period at 24h. At 18h on the WASPLab, among the 266 specimens included in the independent validation set, 38% (100/266) were positive for common *Enterobacteriaceae* uropathogens, 24% (63/266) were positive for various Gram positive bacteria including uncommon uropathogens and flora, and 4% (11/266) were positive for *Pseudomonas aeruginosa*, *Acinetobacter* sp. or *Achromobacter xylosoxidans*. In contrast, 24h was necessary for CHROMID® CPS® Elite in order to identify *Candida albicans, Candida glabrata,* and *Aerococcus urinae* (Table 4). Therefore, 24h is validated as the cut-off point providing optimal analytical performances.

Bacteriological examination of genital tract specimens

Derivation dataset

The incubation period was divided on the WASPLab into several incubation time points (from 16h up to 48h). Among the 92 samples analysed in the derivation set, 62% (57/92) were positive with only vaginal flora as early as 18h. Three percent (3/92) were positive for MSSA, 1% (1/92) for MRSA, 7% (6/92) for *Enterobacteriaceae*, 4% (4/92) for *S. agalactiae,* and 5% (5/92) for *E. faecalis.* All these micro-organisms showed sufficient growth at 16h. One sample was positive for *Gardnerella vaginalis* with sufficient growth at 20h. However, the incubation period had to be prolonged up to 28h to permit sufficient growth for *Candida* spp. (Table 5).

Independent validation dataset

We defined on the WASPLab an intermediate incubation time at 16h and final incubation period at 28h for the 189 specimens included in the validation set. Except for *Candida* spp. and *Gardnerella vaginalis*, all potential pathogens were identified at 16h, indicating the importance of this intermediate incubation period, to provide early diagnostic information. However, we had to extend the incubation period to 28h in order to permit the reliable identification of *Candida* spp. and other pathogens like *Gardnerella vaginalis* (Table 5).

Bacteriological examination of non-sterile site specimens

Non-sterile site specimens included in this study were conjunctival-ESwab (6%, 10/159), ear-ESwab (16%, 25/159), and superficial-ESwab specimens (78%, 124/159).

Derivation dataset

The incubation period was divided on the WASPLab into several incubation time points (from 16h up to 72h). At 16h, among the 50 specimens included in the derivation set, 12% (6/50) were negative, 24% (12/50) were positive for MSSA, 16% (8/50) were positive for other common Gram positive pathogens, 12% (6/50) were positive for *Enterobacteriaceae*, and 12% (6/50) were positive for *P. aeruginosa* and *Acinetobacter baumannii* (Table 6). Two samples were positive for *Candida glabrata* with sufficient growth for reliable identification at 28h.

Independent validation dataset

As for the genital tract, we have chosen an intermediate incubation time at 16h and a final incubation period at 28h. In the validation set we included 109 specimens. As depicted in Table 6, a panel of 10 potential pathogens was identified with sufficient growth at the intermediate incubation time without any difference with the final traditional incubation period. The detection of *Candida glabrata* was validated at 28h.

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Discussion

The purpose of this study was to assess if the use of the WASPLab, and in particular that of the automated incubators coupled to digital imaging, permitted to shorten the time required for obtaining reliable culture analysis results. As summarized in Table 7, the use of the WASPLab allows reducing the length of the incubation time for urine, genital tract and non-sterile site specimens, as well as that for screening for MRSA, MSSA, ESBL and CPE without affecting the analytical performances.

In a recent study, Bielli *et al.* found a similarity of 93% when comparing the urine specimens tested by WASPLab at 16h and 24h of incubation [11]. In contrast, our derivation study found only 50% (55/109) similarity between those incubation times. Additionally, for all the urine specimens tested the similarity was 79% (295/375) between 18h and 24h on the WASPLab by using CHROMID® CPS® Elite. The selection of a 24h incubation time as the final incubation period for urine specimens was dictated by the willingness to improve the detection yield of urine specimens that are potentially contaminated prior to culture, which contributes to limit overdiagnosis of urinary tract infections. For genital tract specimens, the similarity was 31% (72/230) for detecting bacteria between 16h and 28h of incubation on the WASPLab. This similarity decreases to 26% (72/281) when we include *Candida* spp., whose slower growth requires more time to be detected. For non-sterile site specimens, the similarity was 77% (123/159) between 16h and 28h of incubation on the WASPLab. Finally, a unique 16h incubation time is sufficient for the screening-ESwabs for ESBL-producer and CPE, and 18h incubation time for the screening-ESwabs for MRSA, which enables rapid identification of critical antibiotic-resistant pathogens for adjusting infection control procedures.

Clinical microbiology laboratories rely upon highly trained and skilled personnel to process a substantial amount of clinical specimens with various complex procedures, a range of sampling devices and a variety of diagnostic methods. The advent of new technologies emphasizes the need to automate the repetitive tasks that do not require specific skills of trained medical microbiology technologists [1, 3, 12]. The impact of automation to improve laboratory workflows and efficiency in clinical microbiology laboratories was highlighted in different recent publications [13-18]. As demonstrated in this study, the time required to obtain culture results is reduced by automated incubation combined with high-resolution digital imaging. To realize the maximum time gain of reduced incubation times, the clinical microbiology laboratory would need to broaden its operating hours, and ideally shift to a 24/7 model. This schedule would ensure that the images of the early incubation times can be interpreted without any delay. Yet, operating hours should be adjusted, in each centre, to ideally match laboratory resources with the decisions that can actually be taken by the medical staff working during night or week-end shifts. Thus defined and duly validated incubation times will allow building appropriate laboratory workflows for improved efficiency.

One limitation of this study pertains to the relatively small number of some bacterial species analysed as well as the overall number of samples studied, yet the specimens analysed in this study provide a range of pathogens and flora conditions.

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Conclusion

The important benefits of the use of automated incubators combined with digital imaging is that they permit continuous and automatic monitoring of the cultured media plates, favoring optimal bacterial growth. The high-resolution digital images taken under different light and exposure conditions open the potential of customized reading times to improve the detection of the early growth. Shortening the turn-around times could positively improve the patient's outcome. This implies providing earlier medically-actionable results to the treating physician (e.g., switches from empiric to targeted drug regimens). In this study, the automation was found to reduce the incubation times for all specimens tested without compromising the analytical performances. Using defined and duly validated incubation times will allow building appropriate laboratory workflows, balancing laboratory resources and medical needs in each centre, for improved efficiency. Further studies are now needed to investigate the real impact of reduced time to results on the early adjustments of antimicrobial regimen.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval

There is no need for an informed consent, since this study was performed for quality control purposes, in agreement with the Swiss laws.

Funding

This study was performed by using internal funding only.

Acknowledgments

The authors would like to thank Stefane MARQUES MELANCIA, Didier SCHORDERET, and Marc VINCENT JACQUES for technical assistance.

Authors' contribution statements

Abdessalam CHERKAOUI designed the study, carried out the experiments, analysed the data, and wrote the manuscript.
Gesuele RENZI carried out the experiments and analysed the data.
Nicolas VUILLEUMIER aided in interpreting the results and revised the manuscript.
Jacques SCHRENZEL contributed to the design and implementation of the study, to the analysis of the results and to the writing of the manuscript.

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Table 1: The incubation protocols, the culture media used for each specimen type, and the number of specimens included in the derivation set and in the independent validation set.

CPE: Carbapenemase-producing *Enterobacteriaceae* , MRSA : Methicillin-resistant *Staphylococcus aureus* , MSSA: Methicillin-sensitive *Staphylococcus aureus* , ESBL : Extended-spectrum beta-lactamases; CNA agar: Colistin-Nalidixic Acid agar

Clinical specimen types	WASP coupled to conventional incubation and manual diagnostic		WASPLab	
	Culure media type	Routine incubation period	Number of samples included in the derivation set	Number of samples included in the independant validation set
Urine specimens	CHROMID® CPS® Elite (BioMérieux, Geneva, Switzerland)	18h-24h and 48h	109	266
Genital tract specimens	Blood agar, chocolate agar, CNA agar, and MacConkey agar	24h and 48h	92	189
Non-sterile site specimens	Blood agar, chocolate agar, CNA agar, and MacConkey agar	24h, 48h and 72h	50	109
Nasal and inguinal/perineal screening-ESwabs for MRSA and MSSA	CHROMID® MRSA (BioMérieux) and SaSelect Medium (BioRad)	18h-24h and 48h	148	181
Rectal screening-ESwabs for ESBL-producer and CPE	CHROMID® ESBL (BioMérieux) coupled to CHROMID® OXA-48 (BioMérieux)	18h-24h and 48h	84	66
		Total	483	811

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Table 2: Results of the derivation and validation sets for nasal and inguinal/perineal screening-ESwabs for Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA)

— : negative, +: positive

Derivation dataset

Nasal and inguinal/perineal screening-ESwabs for MRSA and MSSA	WASP coupled to conventional incubation and manual diagnostic		WASPLab		
	Incubation time points		Incubation time points		
Pathogenes type (N° of samples / semi-quantification)	**18h to 24h**	**48h**	**18h**	**22h**	**48h**
MRSA negative samples (114)	—	—	—	—	—
MRSA (12 / + to +++)	+	+	+	+	+
MSSA negative samples (18)	—	—	—	—	—
MSSA (2 / + to +++)	+	+	+	+	+
MSSA (2 / +)	—	—	+	+	+

Total = 148

Independent validation dataset

Nasal and inguinal/perineal screening-ESwabs for MRSA and MSSA	WASP coupled to conventional incubation and manual diagnostic		WASPLab
	Incubation time points		Incubation time
Pathogenes type (N° of samples / semi-quantification)	**18h - 24h**	**48h**	**18h**
MRSA negative samples (123)	—	—	—
MRSA (24 / + to +++)	+	+	+
MSSA negative samples (9)	—	—	—
MSSA (24 / + to +++)	+	+	+
MSSA (1 / +)	—	—	+

Total = 181

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Table 3: Results of the derivation and validation sets for rectal screening-ESwabs for ESBL-producer and CPE

ESBL : Extended-spectrum beta-lactamases, CPE: Carbapenemase-producing *Enterobacteriaceae*

— : negative, +: positive

Derivation dataset

Rectal screening-ESwabs for ESBL-producer and CPE	**WASP coupled to conventional incubation and manual diagnostic**		**WASPLab**			
	Incubation time points		**Incubation time points**			
Pathogenes type (N° of samples / semi-quantification)	**18h - 24h**	**48h**	**16h**	**18h**	**22h**	**48h**
ESBL and CPE negative samples (52)	—	—	—	—	—	—
Escherichia coli ESBL (23 / + to +++)	+	+	+	+	+	+
Klebsiella pneumoniae ESBL (5 / + to +++)	+	+	+	+	+	+
Salmonella Typhimurium ESBL (1 / +++)	+	+	+	+	+	+
Escherichia coli OXA-48 (1 / +++)	+	+	+	+	+	+
Klebsiella pneumoniae OXA-48 (1 / +++)	+	+	+	+	+	+
Klebsiella pneumoniae NDM (1 / +++)	+	+	+	+	+	+

Total = 84

Independent validation dataset

Rectal screening-ESwabs for ESBL-producer and CPE	**WASP coupled to conventional incubation and manual diagnostic**		**WASPLab**
	Incubation time points		**Incubation time**
Pathogenes type (N° of samples / semi-quantification)	**18h - 24h**	**48h**	**16h**
ESBL and CPE negative samples (28)	—	—	—
Escherichia coli ESBL (18 / + to +++)	+	+	+
Klebsiella pneumoniae ESBL (11 / + to +++)	+	+	+
Enterobacter cloacae complex ESBL (1 / +++)	+	+	+
Escherichia coli OXA-48 (1 / +++)	+	+	+
Klebsiella pneumoniae OXA-48 (4 / ++ to +++)	+	+	+
Klebsiella pneumoniae OXA-181 (2 / +++)	+	+	+
Escherichia coli NDM (1 / +++)	+	+	+

Total = 66

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Table 4: Results of the derivation and validation sets for bacteriological examination of urine specimens

— : negative, +: positive

Derivation dataset

Bacteriological examination of urine specimens	WASP coupled to conventional incubation and manual diagnostic		WASPLab				
	Incubation time points		Incubation time points				
Pathogenes type and flora (N° of samples / Quantification)	**18h - 24h**	**48h**	**16h**	**18h**	**22h**	**24h**	**26h up to 48h**
Negative samples (21)	—	—	—	—	—	—	—
Escherichia coli (21 / 100 to >100000 CFU/mL)	+	+	+	+	+	+	+
Proteus mirabilis (2 / 100000 CFU/mL)	+	+	+	+	+	+	+
Klebsiella pneumoniae (1 / 100000 CFU/mL)	+	+	+	+	+	+	+
Pseudomonas aeruginosa (1 / 100000 CFU/mL)	+	+	+	+	+	+	+
Candida albicans (9 / 1000 to >100000 CFU/mL)	—	+	—	—	—	+	+
Candida glabrata (3 / 1000 to >100000 CFU/mL)	—	+	—	—	—	+	+
Cyberlindnera fabianii (1 / 100000 CFU/mL)	—	+	—	—	—	+	+
Enterococcus faecalis (5 / 1000 to 100000 CFU/mL)	+	+	—	+	+	+	+
Streptococcus agalactiae (3 / 10000 to >100000 CFU/mL)	—	+	—	+	+	+	+
Mixt flora (9 / 1000 to >100000 CFU/mL)	+	+	+	+	+	+	+
Gram positive flora (18 / 100 to 1000 CFU/mL)	—	+	—	—	+	+	+
Gram positive flora (8 / 10000 to >100000 CFU/mL)	+	+	—	+	+	+	+
Lactobacillus sp. (5 / 10000 to >100000 CFU/mL)	—	+	—	+	+	+	+
Staphylococcus epidermidis (2 / 10000 to 100000 CFU/mL)	+	+	—	+	+	+	+

Total = 109

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Independent validation dataset

Bacteriological examination of urine specimens	WASP coupled to conventional incubation and manual diagnostic		WASPLab	
	Incubation time points		Incubation time points	
Pathogenes type and flora (N° of samples / Quantification)	**18h - 24h**	**48h**	**18h**	**24h**
Negative samples (30)	—	—	—	—
Escherichia coli (57 / 100 to >100000 CFU/mL)	+	+	+	+
Klebsiella pneumoniae (24 / 100 to >100000 CFU/mL)	+	+	+	+
Klebsiella oxytoca (2 / 1000 CFU/mL)	+	+	+	+
Klebsiella aerogenes (4 / 1000 to >100000 CFU/mL)	+	+	+	+
Proteus mirabilis (9 / 100 to 100000 CFU/mL)	+	+	+	+
Morganella morganii (1 / 100000 CFU/mL)	+	+	+	+
Citrobacter koseri (3 / 1000 to >100000 CFU/mL)	+	+	+	+
Acinetobacter sp. (1 / 1000 CFU/mL)	+	+	+	+
Achromobacter xylosoxidans (1 / 10000 CFU/mL)	+	+	+	+
Pseudomonas aeruginosa (9 / 100 to >100000 CFU/mL)	+	+	+	+
Candida albicans (8 / 100 to 10000 CFU/mL)	—	+	—	+
Candida glabrata (2 / 1000 and >100000 CFU/mL)	—	+	—	+
Methicillin-resistant *Staphylococcus aureus* (2 / 100000 CFU/mL)	+	+	+	+
Methicillin sensitive *Staphylococcus aureus* (2 / 1000 CFU/mL)	+	+	+	+
Staphylococcus epidermidi s (2 / 100000 CFU/mL)	+	+	+	+
Staphylococcus hominis (2 / 100 and 10000 CFU/mL)	+	+	+	+
Enterococcus faecalis (28 / 1000 to >100000 CFU/mL)	+	+	+	+
Enterococcus faecium (2 / 10000 CFU/mL)	+	+	+	+
Streptococcus agalactiae (6 / 100 to 10000 CFU/mL)	—	+	+	+
Aerococcus urinae (3 / 10000 to >100000 CFU/mL)	—	+	—	+
Mixt flora (13 / 1000 to >100000 CFU/mL)	+	+	+	+
Gram positive flora (36 / 100 to 1000 CFU/mL)	—	+	—	+
Gram positive flora (13 / 10000 to >100000 CFU/mL)	+	+	+	+
Lactobacillus sp. (4 / 10000 to >100000 CFU/mL)	—	+	+	+
Lactobacillus johnsonii (1 / 10000 CFU/mL)	—	+	+	+
Corynebacterium sp. (1 / 1000 CFU/mL)	—	+	+	+

Total = 266

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Table 5: Results of the derivation and validation sets for bacteriological examination of genital tract specimens

— : negative, +: positive

Derivation dataset

Bacteriological examination of genital tract specimens	WASP coupled to conventional incubation and manual diagnostic		WASPLab				
	Incubation time points		Incubation time points				
Pathogenes type and flora (N° of samples / semi-quantification)	**24h**	**48h**	**16h**	**18h**	**20h**	**28h**	**30h up to 48h**
Vaginal flora (57 / + to +++)	—	+	—	+	+	+	+
Gardnerella vaginalis (1 / +++)	—	+	—	—	+	+	+
Methicillin-sensitive *Staphylococcus aureus* (3 / + to +++)	+	+	+	+	+	+	+
Methicillin-resistant *Staphylococcus aureus* (1 / +++)	+	+	+	+	+	+	+
Enterobacteriaceae (6 / + to +++)	+	+	+	+	+	+	+
Streptococcus agalactiae (4 / + to +++)	+	+	+	+	+	+	+
Enterococcus faecalis (5 / + to +++)	+	+	+	+	+	+	+
Candida albicans (14 / + to +++)	—	+	—	—	—	+	+

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Candida glabrata (1 / +)	—	+	—	—	—	+	+

Total = 92

Independent validation dataset

Bacteriological examination of genital tract specimens	**WASP coupled to conventional incubation and manual diagnostic**		**WASPLab**	
	Incubation time		**Incubation time points**	
Pathogenes type and flora (N° of samples / semi-quantification)	**24h**	**48h**	**16h**	**28h**
Vaginal flora (86 / + to +++)	—	+	—	+
Gardnerella vaginalis (14 / +++)	—	+	—	+
Methicillin-sensitive *Staphylococcus aureus* (9 / + to +++)	+	+	+	+
Enterobacteriaceae (28 / + to +++)	+	+	+	+
Streptococcus agalactiae (9 / + to +++)	+	+	+	+
Streptococcus pyogenes (1 / +++)	+	+	+	+
Enterococcus faecalis (6 / + to +++)	+	+	+	+
Candida krusei (1 / +++)	—	+	—	+

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Candida dubliniensis (1 / ++)	—	+	—	+
Candida albicans (30 / + to +++)	—	+	—	+
Candida glabrata (4 / +)	—	+	—	+

Total = 189

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Table 6: Results of the derivation and validation sets for bacteriological examination of non-sterile site specimens

Non-sterile site specimens included in this study were conjunctival-ESwab (6%, 10/159), ear-ESwab (16%, 25/159), and superficial-ESwab specimens (78%, 124/159)

— : negative, +: positive

Derivation dataset

Bacteriological examination of non-sterile site specimens	WASP coupled to conventional incubation and manual diagnostic			WASPLab			
	Incubation time points			Incubation time points			
Pathogenes type and flora (N° of samples / semi-quantification)	**24h**	**48h**	**72h**	**16h**	**18h**	**20h**	**28h up to 72h**
Negative samples (6)	—	—	—	—	—	—	—
Mixt flora (4 / + to +++)	+	+	+	+	+	+	+
Gram positive flora (6 / + to +++)	—	+	+	—	+	+	+
Methicillin-sensitive *Staphylococcus aureus* (12 / + to +++)	+	+	+	+	+	+	+
Staphylococcus epidermidis (3 / +++)	+	+	+	+	+	+	+
Staphylococcus lugdunensis (1 / +++)	+	+	+	+	+	+	+
Escherichia coli ((2 / + to ++)	+	+	+	+	+	+	+
Klebsiella pneumoniae (1 / +)	+	+	+	+	+	+	+

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Klebsiella aerogenes (3 / +++)	+	+	+	+	+	+	+
Acinetobacter baumannii (1 / +++)	+	+	+	+	+	+	+
Pseudomonas aeruginosa (5 / +++)	+	+	+	+	+	+	+
Streptococcus pyogenes (1 / +++)	+	+	+	+	+	+	+
Streptococcus agalactiae (2 / ++)	+	+	+	+	+	+	+
Enterococcus faecalis (1 / +)	+	+	+	+	+	+	+
Candida glabrata (2 / +)	—	+	+	—	—	—	+

Total = 50

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Independent validation dataset

Bacteriological examination of non-sterile site specimens	WASP coupled to conventional incubation and manual diagnostic			WASPLab	
	Incubation time points			Incubation time points	
Pathogenes type and flora (N° of samples / semi-quantification)	**24h**	**48h**	**72h**	**16h**	**28h**
Negative samples (6)	—	—	—	—	—
Mixt flora (7 / + to +++)	+	+	+	+	+
Gram positive flora (22 / + to +++)	+	+	+	—	+
Methicillin-sensitive *Staphylococcus aureus* (18 / + to +++)	+	+	+	+	+
Staphylococcus lugdunensis (1 / ++)	+	+	+	+	+
Staphylococcus epidermidis (5 / ++)	+	+	+	+	+
Staphylococcus warneri (4 / +++)	+	+	+	+	+
Escherichia coli (6 / ++ to +++)	+	+	+	+	+
Klebsiella pneumoniae (8 / + to ++)	+	+	+	+	+
Klebsiella aerogenes (2 / +++)	+	+	+	+	+
Citrobacter koseri (1 / +++)	+	+	+	+	+

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Morganella morganii (1 / +++)	+	+	+	+	+
Serratia marcescens (1 / ++)	+	+	+	+	+
Proteus mirabilis (2 / +++)	+	+	+	+	+
Stenotrophomonas maltophilia (1 / ++)	+	+	+	+	+
Pseudomonas aeruginosa (7 / +++)	+	+	+	+	+
Streptococcus pyogenes (1 / +++)	+	+	+	+	+
Streptococcus agalactiae (2 / +++)	+	+	+	+	+
Streptococcus dysgalactiae (2 / ++)	+	+	+	+	+
Enterococcus faecalis (6 / +)	+	+	+	+	+
Candida glabrata (1 / +++)	—	+	+	—	+
Candida albicans (5 / + to +++)	—	+	+	—	+

Total = 109

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Table 7 : Definitive incubation protocoles based on the derivation and validation studies

CPE: Carbapenemase-producing *Enterobacteriaceae* , MRSA : Methicillin-resistant *Staphylococcus aureus* , MSSA: Methicillin-sensitive *Staphylococcus aureus* , ESBL : Extended-spectrum beta-lactamases

Clinical samples type	**WASPLab**		
	Incubation time		
	Picture at T0	**Intermediate incubation time**	**Final incubation time**
Urine specimens	Yes	18h	24h
Genital tract specimens	Yes	16h	28h
Non-sterile site specimens	Yes	16h	28h
Nasal and inguinal/perineal screening-ESwabs for MRSA and MSSA	Yes	No	18h
Rectal screening-ESwabs for ESBL-producer and CPE	Yes	No	16h

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