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Original article

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

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ABSTRACT

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

Methods: We compared the results obtained by 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 methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), extended-spectrum beta-lactamases (ESBLs) and carbapenemase-producing Enterobacteriaceae (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 18 hr on WASPLab was 100% (95% confidence interval [CI], 94.48–100.00%). The detection sensitivity of ESBL and CPE at 16 hr on WASPLab was 100% (95% confidence interval [CI], 94.87% to 100.00%). For urine specimens, the similarity was 79% (295/375) between 18 hr and 24 hr of incubation on WASPLab. For genital tract and non-sterile site specimens, the similarity between 16 hr and 28 hr of incubation on WASPLab were 26% (72/281) and 77% (123/159) respectively. Thus, 28 hr 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 appropriate laboratory workflows for improved efficiency to be built. **A. Cherkaoui, Clin Microbiol Infect 2019;25:1430.e5–1430.e12**

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 the 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 reference standard for microbial identification [4–6]. Nowadays, two automated instrument systems are currently available for clinical specimen streaking; inoculated media are loaded on

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conveyors for transfer between instruments and automated incubators where cultures are read with high-resolution digital imaging at pre-defined times. Work Cell Automation (WCA) and Total Lab Automation (TLA) has been developed by BD Kiestra (Drachten, The Netherlands), while WASPLab has been developed by Copan WASP srl, (Brescia, Italy). These systems are built around fundamental techniques used in the clinical microbiology laboratory, namely growing bacterial colonies on agar media plates. New features are now available such 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 of reading chromogenic plates to detect methicillin-resistant *Staphylococcus aureus* (MRSA) with high sensitivity) [7,8]. Additionally, both automation system manufacturers claim that bigger technological breakthroughs are coming soon.

Rapid microbiology diagnosis associated with 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 MRSA, vancomycin-resistant *Enterococcus* (VRE), extended-spectrum beta-lactamases (ESBL) and carbapenemase-producing Enterobacteriaceae (CPE). Moreover, the need for rapid microbiology results in defining 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 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.

Material and methods

Setting

This study was conducted at Geneva University Hospitals, a Swiss tertiary care centre with 1920 beds, and about 63 000 yearly admissions. The hours of operation of our clinical bacteriology laboratory are from 7.30 to 22.00 from Monday to Friday, 7.30 to 17.00 on Saturday and 7.30 to 13.00 on Sunday in addition to the on-call service until 22.00 during the weekend.

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 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 MRSA, methicillin-sensitive *Staphylococcus aureus* (MSSA), extended-spectrum beta-lactamases (ESBLs) and carbapenemase-producing Enterobacteriaceae (CPE). We also assessed urine, and 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 to 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 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 WASPLab, several high-resolution digital images were taken under different light and exposure conditions according to the manufacturer's instructions, and analysed by the first author (a clinical microbiologist) and one expert medical laboratory technologist. Both had been trained by 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

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

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)	18 hr to 24 hr and 48 hr	109	266
Genital tract specimens	Blood agar, chocolate agar, CNA agar, and MacConkey agar	24 hr and 48 hr	92	189
Non-sterile site specimens	Blood agar, chocolate agar, CNA agar, and MacConkey agar	24 hr, 48 hr and 72 hr	50	109
Nasal and inguinal/perineal screening-ESwabs for MRSA and MSSA	CHROMID® MRSA (BioMérieux) and SaSelect Medium (BioRad)	18 hr to 24 hr and 48 hr	148	181
Rectal screening-ESwabs for ESBL-producer and CPE	CHROMID® ESBL (BioMérieux) coupled to CHROMID® OXA-48 (BioMérieux)	18 hr to 24 h and 48 hr	84	66
		Total	483	811

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.

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)

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 (no. of samples / semi-quantification)	18 hr to 24 hr	48 hr	18 hr	22 hr	48 hr
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 (no. of samples / semi-quantification)	18 hr to 24 hr	48 hr	18 hr
MRSA negative samples (123)	—	—	—
MRSA (24 / + to +++)	+	+	+
MSSA negative samples (9)	—	—	—
MSSA (24 / + to +++)	+	+	+
MSSA (1 / +)	—	—	+
Total = 181			

—, negative; +, positive.

spectrometry (MALDI-TOF MS; compass, Bruker Daltonics, Bremen, Germany) according to the manufacturer's instructions. The presence of ESBL was confirmed by double-disc synergy tests (DDST20 and DDST30). The presence of a carbapenemase was confirmed by the Eazyplex® SuperBug CRE system (Amplex Biosystems GmbH, Giessen, Germany). 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 two MSSA) had already been detected at 18 hr, with the specific colour on CHROMID® MRSA (BioMérieux, Geneva, Switzerland) or SaSelect medium for MSSA (BioRad, Fribourg, Switzerland) when the chromogenic media were incubated on

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

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 (no. of samples / semi-quantification)	18 hr to 24 hr	48 hr	16 hr	18 hr	22 hr	48 hr
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			

ESBL, extended-spectrum beta-lactamases; CPE, carbapenemase-producing *Enterobacteriaceae*
—, negative; +, positive

WASPLab. No difference was observed between 18 hr, 22 hr and 48 hr 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 using the two compared methods. In two cases, the specimens were validated as MSSA negative by the manual method at either 18 hr, 24 hr or 48 hr. In contrast, when SaSelect medium was incubated on WASPLab, these two cases showed a few colonies had already been detected with the specific colour at 18 hr. 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 WASPLab was defined as 18 hr using 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 18 hr on WASPLab, except one sample validated as MSSA negative using the manual method but a few colonies were detected at 18 hr on WASPLab with the specific colour on the SaSelect medium (Table 2). This discrepancy can be explained by the fact that the SaSelect

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

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 (no. of samples / Quantification)	18 hr to 24 hr	48 hr	16 hr	18 hr	22 hr	24 hr	26 hr up to 48 hr
Negative samples (21)	—	—	—	—	—	—	—
Escherichia coli (21 / 100 to >100000 CFU/mL)	+	+	+	+	+	+	+
Proteus mirabilis (2 / 100 000 CFU/mL)	+	+	+	+	+	+	+
Klebsiella pneumoniae (1 / 100 000 CFU/mL)	+	+	+	+	+	+	+
Pseudomonas aeruginosa (1 / 100 000 CFU/mL)	+	+	+	+	+	+	+
Candida albicans (9 / 1000 to >100 000 CFU/mL)	—	+	—	—	—	+	+
Candida glabrata (3 / 1000 to >100 000 CFU/mL)	—	+	—	—	—	+	+
Cyberlindnera fabianii (1 / 100 000 CFU/mL)	—	+	—	—	—	+	+
Enterococcus faecalis (5 / 1000 to 100 000 CFU/mL)	+	+	—	+	+	+	+
Streptococcus agalactiae (3 / 10 000 to >100 000 CFU/mL)	—	+	—	+	+	+	+
Mixt flora (9 / 1000 to >100000 CFU/mL)	+	+	+	+	+	+	+
Gram positive flora (18 / 100 to 1000 CFU/mL)	—	+	—	—	+	+	+
Gram positive flora (8 / 10 000 to >100 000 CFU/mL)	+	+	—	+	+	+	+
Lactobacillus sp. (5 / 10000 to >100 000 CFU/mL)	—	+	—	+	+	+	+
Staphylococcus epidermidis (2 / 10 000 to 100000 CFU/mL)	+	+	—	+	+	+	+
Total = 109							

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 (no. of samples / Quantification)	18 hr to 24 hr	48 hr	18 hr	24 hr
Negative samples (30)	—	—	—	—
Escherichia coli (57 / 100 to >100 000 CFU/mL)	+	+	+	+
Klebsiella pneumoniae (24 / 100 to >100 000 CFU/mL)	+	+	+	+
Klebsiella oxytoca (2 / 1000 CFU/mL)	+	+	+	+
Klebsiella aerogenes (4 / 1000 to >100 000 CFU/mL)	+	+	+	+
Proteus mirabilis (9 / 100 to 100 000 CFU/mL)	+	+	+	+
Morganella morganii (1 / 100 000 CFU/mL)	+	+	+	+
Citrobacter koseri (3 / 1000 to >100 000 CFU/mL)	+	+	+	+
Acinetobacter sp. (1 / 1000 CFU/mL)	+	+	+	+
Achromobacter xylosoxidans (1 / 10000 CFU/mL)	+	+	+	+
Pseudomonas aeruginosa (9 / 100 to >100 000 CFU/mL)	+	+	+	+
Candida albicans (8 / 100 to 10 000 CFU/mL)	—	+	—	+
Candida glabrata (2 / 1000 and >100 000 CFU/mL)	—	+	—	+
Methicillin-resistant *Staphylococcus aureus* (2 / 100 000 CFU/mL)	+	+	+	+
Methicillin sensitive *Staphylococcus aureus* (2 / 1000 CFU/mL)	+	+	+	+
Staphylococcus epidermidis (2 / 100 000 CFU/mL)	+	+	+	+
Staphylococcus hominis (2 / 100 and 10 000 CFU/mL)	+	+	+	+
Enterococcus faecalis (28 / 1000 to >100 000 CFU/mL)	+	+	+	+
Enterococcus faecium (2 / 10 000 CFU/mL)	+	+	+	+
Streptococcus agalactiae (6 / 100 to 10 000 CFU/mL)	—	+	+	+
Aerococcus urinae (3 / 10 000 to >100 000 CFU/mL)	—	+	—	+
Mixt flora (13 / 1000 to >100 000 CFU/mL)	+	+	+	+
Gram positive flora (36 / 100 to 1000 CFU/mL)	—	+	—	+
Gram positive flora (13 / 10 000 to >100 000 CFU/mL)	+	+	+	+
Lactobacillus sp. (4 / 10 000 to >100 000 CFU/mL)	—	+	+	+
Lactobacillus johnsonii (1 / 10 000 CFU/mL)	—	+	+	+
Corynebacterium sp. (1 / 1000 CFU/mL)	—	+	+	+
Total = 266				

—, negative; +, positive

medium is highly sensitive to light exposure, favouring WASPLab, which allows immediate incubation after streaking.

The detection sensitivity of MRSA and MSSA at 18 hr on WASPLab compared with the manual method for the 329 specimens included in the derivation and the validation sets was 100% (95% CI 94.48–100.00%).

Rectal screening ESwabs for ESBL producer and CPE

Derivation dataset

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

Independent validation dataset

We selected 16 hr as the defined incubation period on 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 eight specimens were CPE positive (Table 3). For the derivation set, all the 38 ESBL- and CPE-positive specimens were detected at 16 hr on WASPLab.

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

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 WASPLab at 11 different incubation time points (from 16 hr up to 48 hr). In 24 samples, the common Enterobacteriaceae uropathogens and *Pseudomonas aeruginosa* were detected on CHROMID® CPS® Elite at 16 hr. In 13 samples, it would have been necessary to wait until 24 hr 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 18 hr (Table 4).

Independent validation dataset

We defined on WASPLab for CHROMID® CPS® Elite an intermediate incubation time at 18 hr and final incubation period at 24 hr. At 18 hr on 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, 24 hr was necessary for CHROMID® CPS® Elite in order to identify *Candida albicans, Candida glabrata* and *Aerococcus urinae* (Table 4). Therefore, 24 hr is validated as the cut-off point providing optimal analytical performances.

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

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 (no. of samples / semi-quantification)	24 hr	48 hr	16 hr	18 hr	20 hr	28 hr	30 hr up to 48 hr
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 +++)	—	+	—	—	—	+	+
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 (no. of samples / semi-quantification)	24 hr	48 hr	16 hr	28 hr
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 / +++)	—	+	—	+
Candida dubliniensis (1 / ++)	—	+	—	+
Candida albicans (30 / + to +++)	—	+	—	+
Candida glabrata (4 / +)	—	+	—	+
Total = 189				

—, negative; +, positive

Bacteriological examination of genital tract specimens

Derivation dataset

The incubation period on WASPLab was divided into several incubation time points (from 16 hr up to 48 hr). Among the 92 samples analysed in the derivation set, 62% (57/92) were positive with only vaginal flora as early as 18 hr. Three per cent (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 16 hr. One sample was positive for *Gardnerella vaginalis* with sufficient growth at 20 hr. However, the incubation period had to be prolonged up to 28 hr to permit sufficient growth for *Candida* spp. (Table 5).

Independent validation dataset

We defined an intermediate incubation time on WASPLab of 16 hr and the final incubation period of 28 hr for the 189 specimens included in the validation set. Except for *Candida* spp. and *Gardnerella vaginalis*, all potential pathogens were identified at 16 hr, indicating the importance of this intermediate incubation period, to provide early diagnostic information. However, we had to extend the incubation period to 28 hr 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 on WASPLab was divided into several incubation time points (from 16 hr up to 72 hr). At 16 hr, among the

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

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)	24 hr	48 hr	72 hr	16 hr	18 hr	20 hr	28 hr up to 72 hr
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 / +)	+	+	+	+	+	+	+
Klebsiella aerogenes (3 / +++)	+	+	+	+	+	+	+
Acinetobacter baumannii (1 / +++)	+	+	+	+	+	+	+
Pseudomonas aeruginosa (5 / +++)	+	+	+	+	+	+	+
Streptococcus pyogenes (1 / +++)	+	+	+	+	+	+	+
Streptococcus agalactiae (2 / ++)	+	+	+	+	+	+	+
Enterococcus faecalis (1 / +)	+	+	+	+	+	+	+
Candida glabrata (2 / +)	—	+	+	—	—	—	+
Total = 50							

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 / +++)	+	+	+	+	+
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					

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

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 28 hr.

Independent validation dataset

As for the genital tract, we have chosen an intermediate incubation time at 16 hr and a final incubation period at 28 hr. 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 28 hr.

Discussion

The purpose of this study was to assess if the use of 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 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.* [11] found a similarity of 93% when comparing the urine specimens tested by WASPLab at 16 hr and 24 hr of incubation. 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 18 hr and 24 hr on WASPLab by using CHROMID® CPS® Elite. The selection of a 24 hr 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 16 hr and 28 hr of incubation on 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 16 hr and 28 hr of incubation on WASPLab. Finally, a unique 16 hr incubation time is sufficient for the screening-ESwabs for ESBL-producer and CPE, and 18 hr 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. Hours of operating 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 weekend shifts. 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.

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

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

CPE, carbapenemase-producing Enterobacteriaceae; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; ESBL, extended-spectrum beta-lactamases.

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, favouring 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.

Transparency declaration

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. This study was performed by using internal funding only.

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