ABSTRACT The objective of this study was to evaluate the accuracy and robustness of a fully automated EUCAST RAST (rapid antimicrobial susceptibility test) directly from positive blood culture and to appreciate its implementation constraints. This study was conducted in two phases: (i) spiked blood culture bottles (BCs) using 779 non-duplicate clinical isolates and (ii) a prospective clinical trial including 534 positive BCs sequentially processed in routine at the Bacteriology Laboratory of Geneva University Hospitals. The RAST results were assessed against EUCAST standardized disk diffusion testing results. Our first finding was that the results of the spiked BCs precisely predicted the clinical trial results. The overall categorical agreements for all species analyzed were greater than 95% at the different time points. RAST for *Pseudomonas aeruginosa*, however, raised several challenges. The categorical agreement for imipenem was lower than 95% at 6 h and was not improved with longer incubation times. Additionally, piperacillin-tazobactam, ceftazidime, and cefepime cannot be released at 6 h due to suboptimal performances, but the categorical agreement substantially improved at 8 h. Our results establish that the performance of fully automated EUCAST RAST directly from positive blood culture bottles is consistently robust, even for the detection of extended-spectrum β-lactamase (ESBL), carbapenemase-producing bacteria, and methicillin-resistant *Staphylococcus aureus* (MRSA). The automation markedly enhanced the percentage of readable inhibition zones and reduced the percentage of isolates categorized in the area of technical uncertainty (ATU). In summary, a fully automated EUCAST RAST can substantially improve laboratory workflow by reducing hands-on time and removing the strong constraints linked to manual read-outs at precisely defined times.

KEYWORDS full automation, RAST, AST, WASPLab, Copan Radian, blood cultures, EUCAST RAST
faster microorganism identification. Regarding antimicrobial susceptibility testing (AST), however, certain obstacles must be overcome before routine implementation of faster methods. Full laboratory automation has permitted the disk diffusion method to return to center stage and strengthen its position among other AST methods currently used in clinical microbiology laboratories. Exquisite flexibility, cost-effectiveness, and accuracy for detecting new resistance mechanisms remain the major advantages of the disk diffusion method (11). Over the last 3 years, EUCAST has developed a standardized rapid method based on disk diffusion to enable susceptibility reports within 4 to 8 h and 16 to 18 h directly from positive blood culture bottles (BCs). Although this method provides faster and more accurate results, its manual setup and the imperative requirement to read the inhibition zone diameters at strictly defined time points are tremendously labor-intensive and have thus hampered its large-scale use in clinical microbiology laboratories, not to mention routine usage (12).

The main objective of this study was to evaluate the accuracy and robustness of the fully automated rapid antimicrobial disk diffusion susceptibility testing (RAST, EUCAST) directly from positive blood culture bottles and to appreciate its implementation constraints. This study was undertaken in two phases. Phase 1 consisted of spiked blood culture bottles including various resistant phenotypes, including MDR strains, according to the procedure described by Jonasson et al. for the development of the EUCAST RAST method. Phase 2 was a prospective clinical study which sequentially included positive BCs processed in our routine laboratory.

**MATERIALS AND METHODS**

**Clinical bacterial isolates.** The bacterial isolates included in the spiking phase consisted of 779 non-duplicate isolates (207 Escherichia coli, 118 Klebsiella pneumoniae, 129 Pseudomonas aeruginosa, 63 Acinetobacter baumannii, 148 Staphylococcus aureus, 66 Enterococcus faecalis, and 48 Enterococcus faecium) identified from nonconsecutive clinical samples referred to our laboratory. Carbapenemase-producing Enterobacteriales (CPE), multidrug-resistant (MDR) A. baumannii and P. aeruginosa, vancomycin-resistant E. faecalis and E. faecium (VRE), and S. aureus isolates with inducible clindamycin resistance were previously identified and stored at −80°C in skim milk with 15% glycerol. All stored isolates were passaged twice before testing. Table 1 shows the different phenotypes of the bacterial isolates analyzed in the spiking phase. Identification of strains was carried out by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF/MS) (MBT Compass 4.1, library version 11.0 [11,410 spectra], Bruker Daltonics, Bremen, Germany) according to the manufacturer’s instructions.

| Phenotype (n) | E. coli | K. pneumoniae | P. aeruginosa | A. baumannii | S. aureus | E. faecalis | E. faecium |
|--------------|---------|---------------|---------------|--------------|-----------|-------------|------------|
| ESBL         | 51      | 50            |               |              |           |             |            |
| OXA-23-like producers | 51      | 50            |               |              |           |             |            |
| OXA-48-like producers | 3       | 7             |               |              |           |             |            |
| OXA-58-like producers | 1       | 1             |               |              |           |             |            |
| OXA-181-like producers | 10      | 1             |               |              |           |             |            |
| KPC-like producers | 1       | 12            |               |              |           |             |            |
| NDM-like producers | 2       | 13            |               |              |           |             |            |
| VIM-like producers | 2       |               |               |              |           |             |            |
| MRSA         |         |               |               |              |           |             | 17         |
| Inducible clindamycin resistance | 47      |               |               |              |           |             |            |
| VanA producers |         |               |               |              |           |             |            |
| VanB producers |         |               |               |              |           |             |            |
| High-level gentamicin resistance |         |               |               |              |           |             |            |
| Total        | 207     | 118           | 129           | 63           | 148       | 66          | 48         |

*BC, blood culture; ESBL, extended-spectrum β-lactamase; MRSA, methicillin-resistant Staphylococcus aureus.

*For the isolates with the VanA phenotype, vancomycin MICs ranged between 32 and 256 mg/L.

*For the isolates with the VanB phenotype, vancomycin MICs ranged between 6 and 256 mg/L.

*Some isolates expressed more than one phenotype and are only counted once.
Inoculated bottles were immediately placed in a Bactec FX instrument (Becton, Dickinson and Company). BC bottles turned positive after 3 to 16 h of incubation in the instrument (average time: 10.5 h). BC bottles were removed with a bacterial suspension of 100 to 200 CFU of an overnight culture from a Columbia blood agar plate. Inoculated bottles were immediately placed in a Bactec FX instrument (Becton, Dickinson and Company). BC bottles turned positive after 3 to 16 h of incubation in the instrument (average time: 10.5 h). BC bottles were removed from the Bactec FX 15 min to 13 h after growth was detected (average time: 4 h) according to the working hours of the lab. Fully automated disc diffusion was performed immediately according to the EUCAST RAST method and the manufacturer’s instructions (Copan) (Fig. S1 in the supplemental material). We used 90-mm circular plates, MHE agar, (bioMérieux, Geneva, Switzerland), and i2a antibiotic disks (i2a, Montpellier, France). Several high-resolution digital images were acquired under different light and exposure conditions at defined time points (4, 6, and 8 h). Inhibition zone diameters were automatically derived by the WASPLab for confluent growth with distinctly delineated margins. All the inhibition zone diameters were validated on the WASPLab screen by microbiologists and experienced technologists. Therefore, there was no automatic release of the results. Inhibition zone diameters were modified manually whenever appropriate or necessary, which concerned about 5% of all tested disks.

### RAST results assessment.

For each reading time, the Copan expert system interpreted the inhibition zone diameters validated on the WASPLab screen by microbiologists and experienced technologists as S, R, or area of technical uncertainty (ATU) according to the RAST EUCAST Breakpoint Tables version 3.0. All RAST results, where an interpretation of S or R was defined, were rigorously compared to the results of EUCAST standardized disk diffusion (DD) testing. The number of major errors (MEs; RAST = S and standardized DD = S), major errors (MEs; RAST = R and standardized DD = R), major errors (MEs; RAST = R and standardized DD = S), and minor errors (MIs; RAST = S or R and standardized DD = intermediate, I) were established. The proportion of the tests validated as ATU and the tests with no readable inhibition zones were determined for the different time points (4, 6, and 8 h).

### Bacterial cells density.

For the spiked BCs, we deemed it relevant to assess the bacterial cell density when growth was detected by the instrument. Therefore, the final bacterial cell densities for 10 separate spiked BCs were assessed by viable-cell counts on Columbia agar after overnight incubation. The bacterial cell density of the positive BCs was determined between 30 min to 2 h after growth was detected by the Bactec FX instrument. Bacterial cell densities ranged between $1.3 \times 10^6$ and $5.6 \times 10^8$ CFU/mL. This range included all the species analyzed (Gram-positive and Gram-negative bacteria).

### Clinical trial.

The clinical trial prospectively included all patients’ positive BCs received at our lab between December 2021 and May 2022, except for polymicrobial BCs and positive BCs with other bacterial species not included in the RAST EUCAST Breakpoint Tables version 3.0. These positive BCs were excluded post-RAST setup. Based on these specifications, all positive BCs from each patient were included with the aim of evaluating the repeatability and reproducibility of the RAST method. In total, 534 positive BCs were analyzed in the clinical trial. The RAST was carried out using the positive blood culture broths when they initially signaled positive. We defined a panel of antibiotics covering all bacterial species analyzed in this study. Thereby, we performed the RAST without waiting for the microscopy results. The non-pertinent antibiotics according to the identified bacterial species were excluded from the analysis and reporting during the reading phase. The identification of organisms isolated from the positive blood culture was carried out by MALDI-TOF/MS using a previously defined procedure (16). This identification was always available before RAST interpretation. Table 2 depicts the number of positive BCs broken down by species and phenotype of the isolates tested.

### Quality control.

Quality control (QC) of the RAST method was performed by spiking patients’ negative BC bottles with E. coli ATCC 25929 and S. aureus ATCC 29213. We followed the same methodology as described above. QC was repeated, throughout the experiments, 12 and 9 times for E. coli ATCC 25929 and S. aureus ATCC 29213, respectively.

#### Detection of resistance mechanisms by RAST.

(i) **ESBL and carbapenemases.** Screening for ESBL and carbapenemases using the RAST method was carried out according to EUCAST RAST screening cut-off values at 4, 6 and 8 h (version 1).

#### Table 2: Independent positive BC broken down by species and phenotype

| Species                  | E. coli | K. pneumoniae | P. aeruginosa | A. baumannii | S. aureus | E. faecalis | E. faecium |
|--------------------------|---------|---------------|---------------|--------------|-----------|-------------|-----------|
| Isolates                 | 198     | 80            | 12            | 2            | 122       | 46          | 40        |
| ESBL                     | 18      | 3             |               |              |           |             |           |
| OXA-48-like producers    | 1       |               |               |              |           |             |           |
| MRSA                     | 3       |               |               |              |           |             |           |
| Inducible clindamycin resistance | 2 |               |               |              |           |             |           |
| VanB producers           |         |               |               |              | 1         |             |           |
| High-level gentamicin resistance |   |               |               |              |           |             |           |
| Total                    | 216     | 83            | 12            | 2            | 127       | 50          | 44        |

*BC, blood culture; ESBL, extended-spectrum β-lactamase; MRSA, methicillin-resistant Staphylococcus aureus.*

*For the isolate with the VanB phenotype, the vancomycin MIC was 16 mg/L.*

*Some isolates express more than one phenotype and are only counted once.*
We decided to systematically include a supplement MHE plate to the RAST AST panel defined for *E. coli* and *K. pneumoniae*. This was to assess the performance of the double-disk synergy test (DDST20) for the confirmation of ESBL producers at 4, 6, and 8 h of incubation using the RAST method. As for the standardized disk diffusion testing, an amoxicillin-clavulanate disk was automatically placed at 20 mm, center to center, of a cefepime disk on MHE agar. This test was considered positive when the inhibition zone around the cefepime disk was enhanced.

(ii) Inducible clindamycin resistance. To assess the performances of inducible clindamycin resistance test with the RAST method at 4, 6, and 8 h of incubation, we followed two protocols: a (i) clindamycin 2-μg disk and an erythromycin 15-μg disk placed 9 mm apart (edge to edge), and a (ii) clindamycin 2-μg disk and an erythromycin 15-μg disk placed 12 mm apart (edge to edge). These protocols were applied on 49 non-duplicate erythromycin-resistant *S. aureus* isolates, including 10 MRSA. For all of these 49 isolates, the D phenomenon was positive with standardized disk diffusion testing. Additional erythromycin and clindamycin disks were tested separately in the RAST AST panel for *S. aureus*.

**RESULTS**

Readable inhibition zones. The percentages of inhibition zones with no growth, no confluent growth, or no distinctly delineated growth at the different time points were assessed for each species during the two study phases (spiked BCs and clinical trial), as shown in Tables 3 to 14 for the different time points and the two study phases. The percentage of ATU decreased with the incubation time in both study phases. For Gram-negative bacteria, the most affected molecules were piperacillin-tazobactam, amikacin, ciprofloxacin, and cotrimoxazole. For *S. aureus*, gentamicin and norfloxacin were concerned.

Results according to the EUCAST RAST breakpoints (version 3.0). The percentage of ATU by antibiotic and species are shown in Tables 3 to 14 for the different time points and the two study phases. The percentage of ATU decreased with the incubation time in both study phases. For Gram-negative bacteria, the most affected molecules were piperacillin-tazobactam, amikacin, ciprofloxacin, and cotrimoxazole. For *S. aureus*, gentamicin and norfloxacin were concerned.

Area of technical uncertainty. According to the EUCAST RAST breakpoints (version 3.0), the percentage of ATU by antibiotic and species are shown in Tables 3 to 14 for the different time points and the two study phases. The percentage of ATU decreased with the incubation time in both study phases. For Gram-negative bacteria, the most affected molecules were piperacillin-tazobactam, amikacin, ciprofloxacin, and cotrimoxazole. For *S. aureus*, gentamicin and norfloxacin were concerned.

Results according to the EUCAST RAST breakpoints (version 3.0). *E. coli*. For the spiked BCs, incorrect categorization was very low despite the fact that we included a large number of isolates with diverse resistance phenotypes. Several MEs were observed for amikacin, but most disappeared at 6 h. This coincided with unexpected high rates of ATU for this antibiotic at 4 h. The percentage of ATU for piperacillin-tazobactam was also high (40.5% at 4 h), but it was almost halved at 6 and 8 h. Few errors were observed for this antibiotic and the categorical agreement exceeded 97% at 4 h. Categorical agreement for RAST at 4 h of incubation compared with the EUCAST standardized disk diffusion test results was >95% for all the antibiotics tested, with the exception of amikacin (Table 3). The overall categorical agreements were 98.4%, 98.5%, and 98.6% at 4, 6, and 8 h, respectively.

The same observations were made in the clinical trial, but with even lower rates of incorrect categorization. This can be partly explained by the selection of resistant phenotypes during the first phase. There were no carbapenem-resistant isolates and only a few ESBL in the second phase. The overall categorical agreements for all antibiotics tested during the clinical trial were 99.5%, 99.6% and 99.6% at 4, 6, and 8 h, respectively (Table 4).

**K. pneumoniae**. Categorical agreement for RAST at 4 h of incubation compared with the EUCAST standardized disk diffusion test results was >95% for all antibiotics tested with spiked BCs (Table 5). The overall categorical agreements with spiked BCs were 99.0%, 98.7%, and 99.0% at 4, 6, and 8 h, respectively. The few VMs and MEs were not related to any specific drug. Importantly, 100% of the inhibition zones were readable at 4 h. As noticed for *E. coli*, a high rate of ATU was observed for amikacin at 4 h, but this rate declined over time. The clinical trial results supported the observations made with the spiked BCs, with the exception of piperacillin-tazobactam. Some VMs and MEs were identified for this antibiotic at different time points during the clinical trial. Therefore, categorical agreement was <95% for the three time points. The overall categorical agreements for all antibiotics tested during the clinical trial were 98.8%, 99.1%, and 99.1% at 4, 6, and 8 h, respectively (Table 6).
| E. coli spiked BC parameters | Piperacillin-tazobactam 4h | 6h | 8h | Ceftazidime 4h | 6h | 8h | Imipenem 4h | 6h | 8h | Meropenem 4h | 6h | 8h | Ciprofloxacin 4h | 6h | 8h | Gentamicin 4h | 6h | 8h | Amikacin 4h | 6h | 8h | Co-trimoxazole 4h | 6h | 8h |
|-----------------------------|---------------------------|-----|-----|-----------------|-----|-----|-------------|-----|-----|--------------|-----|-----|----------------|-----|-----|--------------|-----|-----|--------------|-----|-----|--------------|-----|-----|
| Isolates by category (n)    |                           |     |     |                 |     |     |             |     |     |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Resistant                   | 30                         | 36  | 34  | 8               | 21  | 32  | 6            | 11  | 9   | 7            | 9   | 9   | 56            | 63  | 62  | 24            | 26  | 26  | 33            | 14  | 12  | 69            | 74  | 74  |
| ATU                         | 81                         | 54  | 51  | 23              | 13  | 7   | 3            | 6   | 7   | 8            | 5   | 4   | 20            | 23  | 16  | 5             | 3   | 2   | 128           | 83  | 54  | 2             | 1   | 2   |
| Susceptible                 | 89                         | 117 | 122 | 169             | 173 | 168 | 191          | 190 | 191 | 185          | 193 | 194 | 124           | 121 | 129 | 171           | 178 | 179 | 39            | 110 | 141 | 129           | 132 | 131 |
| Inhibition zone not readable| 7                          | 0   | 0   | 7               | 0   | 0   | 7            | 0   | 0   | 7            | 0   | 0   | 7             | 0   | 0   | 7             | 0   | 0   | 7             | 0   | 0   | 7             | 0   | 0   |
| Isolates by category (%)    |                            |     |     |                 |     |     |             |     |     |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Resistant                   | 15.0                       | 17.4| 16.4| 4.0             | 10.1| 15.5| 3.0          | 5.3 | 4.3 | 3.5          | 4.3 | 4.3 | 28.0          | 30.4| 30.0| 12.0          | 12.6| 12.6| 16.5          | 6.8 | 5.8 | 34.5          | 35.7| 35.7|
| ATU                         | 40.5                       | 26.1| 24.6| 11.5            | 6.3 | 3.4 | 1.5          | 2.9 | 3.4 | 4.0          | 2.4 | 1.9 | 10.0          | 11.1| 7.7 | 2.5           | 1.4 | 1.0 | 64.0          | 40.1| 26.1| 1.0           | 0.5 | 1.0 |
| Susceptible                 | 44.5                       | 56.5| 58.9| 84.5            | 83.6| 81.2| 95.5         | 91.8| 92.3| 92.5         | 93.2| 93.7| 62.0          | 58.5| 62.3| 85.5          | 86.0| 86.5| 19.5          | 53.1| 68.1| 64.5          | 63.8| 63.3|
| Inhibition zone not readable| 3.4                        | 0.0 | 0.0 | 3.4             | 0.0 | 0.0 | 3.4          | 0.0 | 0.0 | 3.4          | 0.0 | 0.0 | 3.4           | 0.0 | 0.0 | 3.4           | 0.0 | 0.0 | 3.4           | 0.0 | 0.0 |
| Categorical agreement (%)² |                            |     |     |                 |     |     |             |     |     |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| ATU included                | 98.5                       | 98.1| 98.6| 98.0            | 98.1| 98.6| 100.0        | 99.5| 99.5| 99.5         | 99.5| 99.5| 99.5          | 99.5| 99.5| 99.5          | 99.5| 98.1| 98.1          | 94.0| 98.1| 97.6          | 98.0| 97.6| 97.6          | 97.6| 97.6|
| ATU not included            | 97.5                       | 97.4| 98.1| 97.7            | 97.9| 98.5| 100.0        | 99.5| 99.5| 99.5         | 99.5| 99.5| 99.5          | 99.4| 99.5| 99.5          | 99.5| 98.0| 98.0          | 83.3| 96.8| 96.7          | 98.0| 97.6| 97.6          | 97.6| 97.6|
| Discordant results (n)      |                            |     |     |                 |     |     |             |     |     |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Minor error                 | 1                          | 2   | 1   | 1               | 1   | 1   |              |     |     |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Major error                 | 1                          | 2   | 2   | 3               | 3   | 3   | 2            | 3   | 2   |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Very major error            | 1                          | 3   | 3   | 3               | 3   | 2   | 3            | 3   | 2   |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Discordant results (%)      |                            |     |     |                 |     |     |             |     |     |              |     |     |               |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |              |     |     |
| Minor error                 | 0.0                        | 0.0 | 0.0 | 0.5             | 0.5 | 0.5 | 0.0          | 0.0 | 0.0 | 0.0          | 0.0 | 0.0 | 0.0           | 0.0 | 0.0 | 0.0          | 0.0 | 0.0 | 0.0          | 0.0 | 0.0 | 0.0          | 0.0 | 0.0 |
| Major error                 | 0.5                        | 1.0 | 0.5 | 0.0             | 0.0 | 0.0 | 0.0          | 0.5 | 0.5 | 0.5          | 0.5 | 0.5 | 0.0           | 0.5 | 0.5 | 0.5          | 0.5 | 0.5 | 0.5          | 4.8 | 0.5 | 0.5          | 1.9 | 2.4 |
| Very major error            | 1.0                        | 1.0 | 1.4 | 1.4             | 1.0 | 0.0 | 0.5          | 0.5 | 0.5 | 0.0          | 0.5 | 0.5 | 1.4           | 1.4 | 1.4 | 1.4          | 0.0 | 0.0 | 0.0          | 0.0 | 0.0 | 0.0          | 0.0 | 0.0 |

²BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

²Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.
| E. coli clinical trial parameters | Piperacillin-tazobactam | Ceftazidime | Imipenem | Meropenem | Ciprofloxacin | Gentamicin | Amikacin | Co-trimoxazole |
|----------------------------------|-------------------------|------------|----------|-----------|---------------|------------|----------|---------------|
|                                  | 4 h         | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Isolates by category (n)         |             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Resistant                        | 16          | 27  | 28  | 18  | 29  | 31  | 0    | 0    | 0    | 0    | 0    | 0    | 54   | 57   | 57   | 26   | 26  | 27  | 20  | 10  | 10  | 93  | 99  | 100 |     |     |     |     |     |     |     |     |     |
| ATU                              | 117         | 76  | 65  | 26  | 5   | 4   | 0    | 0    | 0    | 3    | 1    | 1    | 9    | 24   | 18   | 12   | 2    | 1   | 159 | 105 | 60  | 5   | 1   | 0   |     |     |     |     |     |     |     |     |     |
| Susceptible                      | 75          | 113 | 123 | 164 | 182 | 181 | 208  | 216  | 216  | 205  | 215  | 215  | 145  | 135  | 141  | 170  | 188 | 188 | 29  | 101 | 146 | 110 | 116 | 116 |     |     |     |     |     |     |     |     |     |
| Inhibition zone not readable     | 8           | 0   | 0   | 8   | 0   | 0   | 8    | 0    | 0    | 8    | 0    | 0    | 8    | 0    | 0    | 8    | 0   | 0   | 8   | 0   | 0   | 8   | 0   | 0   |     |     |     |     |     |     |     |     |
| Isolates by category (%)         |             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Resistant                        | 7.7         | 12.5| 13.0| 8.7 | 13.4| 14.4| 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 26.0 | 26.4| 26.4| 12.5 | 12.0| 12.5| 9.6 | 4.6 | 4.6 | 44.7| 45.8| 46.3|     |     |     |     |     |     |     |     |
| ATU                              | 56.3        | 35.2| 30.1| 12.5| 2.3 | 19  | 0.0  | 0.0  | 0.0  | 1.4  | 0.5  | 0.5  | 4.3  | 11.1 | 8.3 | 5.8  | 0.9 | 0.5 | 76.4| 48.6| 27.8| 2.4 | 0.5 | 0.0 |     |     |     |     |     |     |     |     |
| Susceptible                      | 36.1        | 52.3| 56.9| 78.8| 84.3| 83.8| 100.0|100.0 |100.0 |98.6 |99.5 |99.5 |69.7 |62.5 |65.3 |81.7 |87.0|87.0 |13.9|46.8|67.6 |52.9|53.7|53.7|     |     |     |     |     |     |     |     |     |
| Inhibition zone not readable     | 3.7         | 0.0 | 0.0 | 3.7 | 0.0 | 0.0 | 3.7  | 0.0  | 0.0  | 3.7  | 0.0  | 0.0  | 3.7  | 0.0  | 0.0  | 3.7  | 0.0 | 0.0 | 3.7 | 0.0 | 0.0 | 3.7 | 0.0 | 0.0 |     |     |     |     |     |     |     |     |

Categorical agreement (%)\(^a\)

- **ATU included**: 99.5 99.5 99.1 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.5 99.5 99.5 99.5 99.5 99.5 99.5 99.5 98.1 98.1 98.1 99.5 100.0 100.0
- **ATU not included**: 98.9 99.3 98.7 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0

Discordant results (n)

| Major error | 1 |
| Very major error | 1 |
|                  | 1 |

Discordant results (%)

- **Major error**: 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.5 0.5 0.5 1.9 0.0 0.0 0.0 0.0
- **Very major error**: 0.5 0.5 0.9 0.0 0.0 0.0 0.5 0.5 0.5 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0

\(^a\)BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.
\(^b\)Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.
### TABLE 5  
**Klebsiella pneumoniae** spiked BC results (n = 118 isolates)\(^a\)

| **K. pneumoniae** spiked BC parameters | Piperacillin-tazobactam | Ceftazidime | Imipenem | Meropenem | Ciprofloxacin | Gentamicin | Amikacin | Cotrimoxazole |
|----------------------------------|------------------------|-------------|----------|-----------|--------------|-----------|----------|---------------|
|                                  | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h | 4h | 6h | 8h |
| Isolates by category (n)          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Resistant                        | 45 | 51 | 52 | 46 | 50 | 49 | 27 | 27 | 30 | 24 | 30 | 31 | 41 | 36 | 44 | 29 | 30 | 27 | 23 | 13 | 22 | 43 | 43 | 46 |
| ATU                              | 23 | 18 | 15 | 4  | 2  | 3  | 3  | 4  | 1  | 5  | 4  | 3  | 18 | 16 | 9  | 5  | 3  | 6  | 50 | 13 | 27 | 4  | 4  | 1  |
| Susceptible                      | 50 | 49 | 51 | 68 | 66 | 66 | 88 | 87 | 87 | 89 | 84 | 84 | 59 | 66 | 65 | 84 | 85 | 85 | 45 | 92 | 69 | 71 | 71 | 71 |
| Inhibition zone not readable     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Isolates by category (%)         |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Resistant                        | 38.1 | 43.2 | 44.1 | 39.0 | 42.4 | 41.5 | 22.9 | 22.9 | 25.4 | 20.3 | 25.4 | 26.3 | 34.7 | 30.5 | 37.3 | 24.6 | 25.4 | 22.9 | 19.5 | 11.0 | 18.6 | 36.4 | 36.4 | 39.0 |
| ATU                              | 19.5 | 15.3 | 12.7 | 3.4 | 1.7 | 2.5 | 2.5 | 3.4 | 0.8 | 4.2 | 3.4 | 2.5 | 15.3 | 13.6 | 7.6 | 4.2 | 2.5 | 5.1 | 14.2 | 11.0 | 22.9 | 3.4 | 3.4 | 0.8 |
| Susceptible                      | 42.4 | 41.5 | 43.2 | 57.6 | 55.9 | 55.9 | 74.6 | 73.7 | 73.7 | 75.4 | 71.2 | 71.2 | 50.0 | 55.9 | 55.1 | 71.2 | 72.0 | 72.0 | 38.1 | 78.0 | 58.5 | 60.2 | 60.2 | 60.2 |
| Inhibition zone not readable     | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Categorical agreement (%)\(^b\)  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| ATU included                     | 96.6 | 96.6 | 96.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.2 | 99.2 | 98.3 | 100.0 | 100.0 | 100.0 | 99.2 | 100.0 | 100.0 | 100.0 | 99.2 | 100.0 | 100.0 | 98.3 | 98.3 | 98.3 |
| ATU not included                 | 95.8 | 96.0 | 96.1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.1 | 99.1 | 98.3 | 100.0 | 100.0 | 100.0 | 99.1 | 100.0 | 100.0 | 100.0 | 99.1 | 100.0 | 100.0 | 98.5 | 95.2 | 100.0 |
| Discordant results (n)           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Major error                      | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Very major error                 | 4 | 4 | 4 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Discordant results (%)           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Major error                      | 0.8 | 0.8 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.8 | 0.8 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.8 | 0.8 |
| Very major error                 | 3.4 | 3.4 | 3.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.8 | 0.8 | 4.2 | 0.0 | 0.8 | 0.8 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.8 | 0.8 |

\(^a\)BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

\(^b\)Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.
| K. pneumoniae clinical trial parameters | Piperacillin-tazobactam 4h | Piperacillin-tazobactam 6h | Piperacillin-tazobactam 8h | Ceftazidime 4h | Ceftazidime 6h | Ceftazidime 8h | Imipenem 4h | Imipenem 6h | Imipenem 8h | Meropenem 4h | Meropenem 6h | Meropenem 8h | Ciprofloxacin 4h | Ciprofloxacin 6h | Ciprofloxacin 8h | Gentamicin 4h | Gentamicin 6h | Gentamicin 8h | Amikacin 4h | Amikacin 6h | Amikacin 8h | Cotrimoxazole 4h | Cotrimoxazole 6h | Cotrimoxazole 8h |
|----------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Isolates by category (n)              |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| Resistant                             | 9                         | 13                        | 12                        | 6                         | 5                         | 6                         | 1                         | 1                         | 1                         | 1                         | 1                         | 1                         | 5                         | 4                         | 6                         | 4                         | 4                         | 4                         | 2                         | 1                         | 0                         | 13                        | 14                        | 14                        |
| ATU                                   | 22                        | 21                        | 23                        | 10                        | 8                         | 7                         | 2                         | 1                         | 1                         | 0                         | 0                         | 1                         | 17                        | 14                        | 13                        | 8                         | 5                         | 2                         | 40                        | 3                         | 20                        | 0                         | 0                         | 0                         |
| Susceptible                           | 51                        | 49                        | 48                        | 66                        | 70                        | 70                        | 79                        | 81                        | 81                        | 81                        | 82                        | 81                        | 60                        | 65                        | 64                        | 70                        | 74                        | 77                        | 40                        | 79                        | 63                        | 69                        | 69                        | 69                        |
| Inhibition zone not readable          | 1                         | 0                         | 0                         | 1                         | 0                         | 0                         | 1                         | 0                         | 0                         | 1                         | 0                         | 0                         | 1                         | 0                         | 0                         | 1                         | 0                         | 0                         | 1                         | 0                         | 0                         | 0                         | 0                         | 0                         |
| Isolates by category (%)              |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| Resistant                             | 11.0                      | 15.7                      | 14.5                      | 7.3                       | 6.0                       | 7.2                       | 1.2                       | 1.2                       | 1.2                       | 1.2                       | 1.2                       | 1.2                       | 6.1                       | 4.8                       | 7.2                       | 4.9                       | 4.8                       | 4.8                       | 2.4                       | 1.2                       | 0.0                       | 15.9                      | 16.9                      | 16.9                      |
| ATU                                   | 26.8                      | 25.3                      | 27.7                      | 12.2                      | 9.6                       | 8.4                       | 2.4                       | 1.2                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 20.7                      | 16.9                      | 15.7                      | 9.8                       | 6.0                       | 2.4                       | 48.8                      | 3.6                       | 24.1                      | 0.0                       | 0.0                       | 0.0                       |
| Susceptible                           | 62.2                      | 59.0                      | 57.8                      | 80.5                      | 84.3                      | 84.3                      | 96.3                      | 97.6                      | 97.6                      | 98.8                      | 98.8                      | 97.6                      | 73.2                      | 78.3                      | 77.1                      | 85.4                      | 89.2                      | 92.8                      | 48.8                      | 95.2                      | 75.9                      | 84.1                      | 83.1                      | 83.1                      |
| Inhibition zone not readable          | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       |
| Categorical agreement (%)             |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| ATU included                          | 92.7                      | 94.0                      | 94.0                      | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     |
| ATU not included                      | 90.0                      | 91.9                      | 91.7                      | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 98.5                      | 98.6                      | 98.6                      | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 100.0                     | 97.6                      | 100.0                     | 100.0                     | 100.0                     |
| Discordant results (n)                |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| Major error                           | 1                         | 2                         | 2                         |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| Very major error                      | 5                         | 3                         | 3                         |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| Discordant results (%)                |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |                           |
| Major error                           | 1.2                       | 2.4                       | 2.4                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 1.2                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       |
| Very major error                      | 6.0                       | 3.6                       | 3.6                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 0.0                       | 1.2                       | 1.2                       | 1.2                       | 0.0                       | 0.0                       | 0.0                       |

*aBC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

*bCategorical agreement for RAST at 4, 6, and 8 h of incubation compared to EUCAST standardized disk diffusion testing.
**A. baumannii.** The overall categorical agreements with spiked BCs were 99.3%, 99.5%, and 99.5% at 4, 6, and 8 h, respectively. The few VMEs were related to amikacin and co-trimoxazole. A high rate of ATU was observed for these two antibiotics at 4 h, but it declined drastically at 6 and 8 h (Table 7). Unfortunately, only two positive BCs were included during the clinical trial. We did not observe any discordant results (Table S1). As documented for *E. coli* and *K. pneumoniae*, the results of the spiked BCs predicted the findings from the clinical trial.

**P. aeruginosa.** The overall categorical agreements with spiked BCs were 95.1% and 97.2% at 6 and 8 h, respectively. The number of MEs was high for cefepime at 6 h, thus impacting the categorical agreement for this antibiotic, which was substantively less than 95%. However, this number deceased significantly at 8 h (Table 8). The same observation was made regarding ceftazidime, where a categorical agreement stood at above 95% at 8 h compared to that at 6 h. Importantly, the categorical agreement for imipenem did not improve with incubation time, exhibiting a decline of 3% and still being less than 90%. The VMEs observed for imipenem, ciprofloxacin, and tobramycin could be partially explained by heteroresistant populations. Few colonies were visible within the inhibition halo of standardized disk diffusion testing (Fig. S2). These colonies were not observed by RAST at 8 h because a longer incubation time was required.

During the clinical phase, only 12 independent positive BCs (9 patients) were included. We observed one VME for imipenem, ceftazidime, and piperacillin-tazobactam at the two time points (Table S2). Despite the small number of *P. aeruginosa*-positive BCs received during the clinical phase, the results supported the observation made for the spiked BCs. The overall categorical agreement for all antibiotics tested during the clinical trial was 97.2% at 6 and 8 h.

**S. aureus.** Among the *S. aureus* included in this study, there were 20 MRSA isolates (20/275; 7.3%). All these isolates were correctly identified as methicillin-resistant after 4 h using a cefoxitin disk. The overall categorical agreements with spiked BCs were 100%, 99.7%, and 99.8% at 4, 6, and 8 h, respectively. A high rate of ATU was observed for norfloxacin at 4 h, but it declined drastically at 6 and 8 h (Table 9). The results of the clinical trial were comparable with those of the spiked BCs. The overall categorical agreements for all the antibiotics tested during the clinical trial were 99.8%, 99.6%, and 99.6% at 4, 6, and 8 h, respectively (Table 10).

**E. faecalis and E. faecium.** The overall categorical agreement for *E. faecalis* with spiked BCs was 99.1% at 4, 6, and 8 h (Table 11). The few MEs observed were related to gentamicin. According to EUCAST RAST breakpoints (version 3.0), the rate of ATU for gentamicin was also high and declined only slightly at 6 and 8 h. The overall categorical agreement for *E. faecium* with spiked BCs was 100.0% at 4, 6, and 8 h (Table 12). The results of the clinical trial reinforced those of the spiked BCs (Tables 13 and 14).

Among the 30 non-duplicate VRE isolates analyzed in this study, only one (VanB phenotype) had a vancomycin-inhibition zone diameter in the ATU at 4 h (12 mm). However, it was categorized as vancomycin-resistant (<12 mm) at both 6 and 8 h. The vancomycin-inhibition zone diameters ranged from 6 to 11 mm at 4 h for the other 29 VRE isolates included in this study. Accordingly, they were all categorized as vancomycin-resistant. No discordant results were observed for vancomycin at the different time points.

For the isolates with the VanA phenotype, vancomycin MICs ranged between 32 and 256 mg/L. For the isolates with the VanB phenotype, vancomycin MICs ranged between 6 and 256 mg/L.

**Quality control.** The RAST procedure using reference ATCC strains was repeated several times throughout the experiments. QC values for the RAST method were always within the EUCAST RAST published ranges (Fig. S3).

**Screening and confirmation of ESBL by RAST.** Screening for ESBL producers using the RAST method was performed according to EUCAST RAST screening cutoff values at 4, 6, and 8 h for cefotaxime and ceftazidime. All *K. pneumoniae* isolates determined to be resistant to cefotaxime and/or ceftazidime by EUCAST standardized disk diffusion testing were categorized as resistant or in the ATU by RAST.

For *E. coli*, 3 VMEs were observed for ceftazidime at the different time points. However, all the cefotaxime-resistant isolates were categorized as resistant or in the ATU by RAST. For the 122 ESBL-producer isolates showing a positive DDST20 using standardized disk diffusion
| parameter                  | Imipenem | Meropenem | Ciprofloxacin | Levofloxacin | Gentamicin | Amikacin | Cotrimoxazole |
|---------------------------|----------|-----------|---------------|--------------|------------|----------|--------------|
|                          | 4 h      | 6 h      | 8 h           | 4 h          | 6 h        | 8 h      | 4 h          | 6 h          | 8 h          |
| **A. baumannii spiked BC**|          |          |               |              |            |          |              |              |              |
| **Resistant**             | 48       | 50       | 50            | 46           | 50         | 47       | 49           | 48           | 47           | 49           | 50         | 26          | 33          | 33          | 43          | 44          |
| **ATU**                   | 1        | 1        | 1             | 5            | 2          | 3        | 2            | 1            | 3            | 3           | 4          | 4           | 2           | 1           | 35          | 12          | 12          | 48          | 44          |
| **Susceptible**           | 12       | 12       | 12            | 10           | 11         | 13       | 11           | 12           | 11           | 12          | 11         | 12          | 18          | 18          | 13          | 16          | 15          |
| **Inhibition zone not readable** | 2       | 0        | 0             | 2            | 0          | 0        | 2            | 0            | 0            | 2           | 0          | 0           | 2           | 0           | 2           | 0           | 0           |
| **Isolates by category (%)** |          |          |               |              |            |          |              |              |              |              |            |            |              |              |              |            |            |              |
| **Resistant**             | 78.7     | 79.4     | 79.4          | 75.4         | 79.4       | 77.0     | 77.8         | 76.2         | 77.0         | 77.8        | 79.4       | 42.6       | 52.4       | 52.4       | 0.0         | 68.3        | 69.8        |
| **ATU**                   | 1.6      | 1.6      | 1.6           | 8.2          | 3.2        | 0.0      | 4.9          | 3.2          | 1.6          | 4.9         | 4.8        | 6.3        | 6.6        | 3.2         | 1.6         | 57.4        | 19.0        | 19.0        | 78.7        | 6.3        |
| **Susceptible**           | 19.7     | 19.0     | 19.0          | 16.4         | 17.5       | 20.6     | 18.0         | 19.0         | 22.2         | 18.0        | 19.0       | 17.5       | 16.4       | 19.0        | 19.0        | 0.0         | 28.6        | 28.6        | 21.3        | 25.4        | 23.8       |
| **Inhibition zone not readable** | 3.2    | 0.0      | 0.0           | 3.2          | 0.0        | 0.0      | 3.2          | 0.0          | 0.0          | 3.2         | 0.0        | 0.0        | 3.2         | 0.0         | 0.0         | 3.2         | 0.0         | 0.0         | 3.2         | 0.0         | 0.0        |
| **Categorical agreement (%)** |            |          |               |              |            |          |              |              |              |              |            |            |              |            |              |            |              |            |            |              |            |            |
| **ATU included**          | 100.0    | 100.0    | 100.0         | 100.0        | 100.0      | 100.0    | 100.0        | 100.0        | 100.0        | 100.0       | 100.0      | 100.0      | 100.0      | 98.4       | 98.4       | 98.4        | 98.4        | 98.4        | 98.4        | 98.4        | 98.4        | 98.4        | 98.4        |
| **ATU not included**      | 100.0    | 100.0    | 100.0         | 100.0        | 100.0      | 100.0    | 100.0        | 100.0        | 100.0        | 100.0       | 100.0      | 100.0      | 100.0      | 96.2       | 98.0       | 98.0        | 92.3        | 98.3        | 98.3        | 98.3        | 98.3        | 98.3        |

**Discordant results (n)**

| Major error | Very major error |
|-------------|-----------------|
| 1           | 1               |

**Discordant results (%)**

| Major error | Very major error |
|-------------|-----------------|
| 0.0         | 1.6             |

aBC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

bCategorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.

cIsolates with zone diameters greater than the ATU interval were reported "Susceptible, increased exposure."
| P. aeruginosa results | Piperacillin-tazobactam<sup>b</sup> | Ceftazidime<sup>b</sup> | Cefepime<sup>b</sup> | Imipenem<sup>b</sup> | Meropenem | Ciprofloxacin<sup>b</sup> | Levofloxacin | Amikacin | Tobramycin |
|-----------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Isolates by category (n) | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h |
| Resistant | 20 | 17 | 13 | 13 | 37 | 13 | 11 | 16 | 10 | 8 | 7 | 10 | 15 | 16 | 13 | 6 | 3 | 2 |
| ATU | 43 | 37 | 38 | 23 | 42 | 25 | 5 | 6 | 9 | 9 | 8 | 10 | 8 | 12 | 17 | 15 | 3 | 6 |
| Susceptible | 62 | 75 | 74 | 93 | 46 | 91 | 109 | 107 | 106 | 112 | 110 | 109 | 102 | 101 | 95 | 108 | 119 | 121 |
| Inhibition zone not readable | 4 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 | 0 |
| Isolates by category (%) | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h |
| Resistant | 16.0 | 13.2 | 10.4 | 10.1 | 29.6 | 10.1 | 8.8 | 12.4 | 8.0 | 6.2 | 5.6 | 7.8 | 12.0 | 12.4 | 10.4 | 4.7 | 2.4 | 1.6 |
| ATU | 34.4 | 28.7 | 30.4 | 17.8 | 33.6 | 19.4 | 4.0 | 4.7 | 7.2 | 7.0 | 6.4 | 7.8 | 6.4 | 9.3 | 13.6 | 11.6 | 2.4 | 4.7 |
| Susceptible | 49.6 | 58.1 | 59.2 | 72.1 | 36.8 | 70.5 | 87.2 | 82.9 | 84.8 | 86.8 | 88.0 | 84.5 | 81.6 | 78.3 | 76.0 | 83.7 | 95.2 | 93.8 |
| Inhibition zone not readable | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 | 3.1 | 0.0 |
| Categorical agreement (%)<sup>c</sup> | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h | 6 h | 8 h |
| ATU included | 96.0 | 98.4 | 93.6 | 96.1 | 80.8 | 97.7 | 92.8 | 89.9 | 98.4 | 99.2 | 97.6 | 96.9 | 98.4 | 98.4 | 98.4 | 99.2 | 98.4 | 98.4 |
| ATU not included | 93.9 | 97.8 | 90.8 | 95.3 | 71.1 | 97.1 | 92.5 | 89.4 | 98.3 | 99.2 | 97.4 | 96.6 | 98.3 | 98.3 | 98.1 | 99.1 | 98.4 | 98.4 |
| Discordant results (n) | Major error | 5 | 1 | 6 | 4 | 24 | 2 | 4 | 9 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 |
| Very major error | 1 | 2 | 1 | 1 | 5 | 4 | 2 | 2 | 1 | 1 | 2 | 2 |
| Discordant results (%) | Major error | 3.9 | 0.8 | 4.7 | 3.1 | 18.6 | 1.6 | 3.1 | 7.0 | 1.6 | 0.8 | 0.8 | 1.6 | 0.8 | 0.8 | 1.6 | 0.8 | 0.0 | 0.0 |
| Very major error | 0.0 | 0.8 | 1.6 | 0.8 | 0.0 | 0.8 | 3.9 | 3.1 | 0.0 | 0.0 | 1.6 | 1.6 | 0.8 | 0.8 | 0.0 | 0.0 | 1.6 | 1.6 |

<sup>a</sup>BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

<sup>b</sup>Isolates with zone diameters greater than the ATU interval were reported as “Susceptible, increased exposure” (I).

<sup>c</sup>Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.
testing, the performance of the DDST20 at 4, 6, and 8 h using the RAST method was as follows: 67% positivity at 4 h, reaching 100% at 6 and 8 h (Fig. S4).

Screening for carbapenemase production by RAST. All E. coli and K. pneumoniae isolates determined to be resistant to meropenem by EUCAST standardized disk diffusion testing were categorized as resistant or in the ATU by RAST. Few MEs were observed for meropenem at the different time points in both species without any impact on the sensitivity of CPE screening. Considering the low rate of meropenem-ATU (5%), isolates expressing this profile were also systematically screened for carbapenemase production. Hence, screen-positive and meropenem-ATU isolates were subjected to molecular confirmatory assays.

Inducible clindamycin resistance. When the clindamycin 2-μg disk and erythromycin 15-μg disk were placed 9 mm apart (edge to edge), the percentages of positive DT test among the 49 non-duplicate erythromycin-resistant S. aureus included in this study were 8.2% (4/49), 75.5% (37/49), and 83.7% (41/49) at 4, 6, and 8 h, respectively. When the clindamycin 2-μg disk and erythromycin 15-μg disk were placed 12 mm apart (edge to edge) the percentages of positive DT test were 12.2% (6/49), 85.7% (42/49), and 100% (49/49) at 4, 6, and 8 h, respectively (Fig. S5).

DISCUSSION

Despite the development of new and promising methods for antimicrobial susceptibility testing based on different technologies, standard bacterial growth-based methods relying on disc diffusion or broth dilution remain widely used in clinical microbiology laboratories. The slowness of these methods and their traditional requirement of pure cultures are largely balanced by their flexibility, cost-effectiveness, and accuracy for detecting known and even new resistance mechanisms (11). However, shorter times for delivering accurate antimicrobial susceptibility results remain one of the major objectives to be achieved. This has become urgent with the steady increase in antimicrobial resistance rates. In several instances, empirical therapy is not tailored to the microorganism causing the infection (10). Thereby, improving the duration until effective and personalized therapy is administered in patients remains a challenge. During the last few years, commercially available and in-house methods have been developed
for RAST directly from positive blood cultures. The main purpose of this study was to determine the diagnostic accuracy and implementation constraints of fully automated EUCAST RAST using the Copan system. We paid special attention to including a large number of clinical isolates expressing diverse resistant phenotypes during the spiked BC phase. Our first observation was that the results of the spiked BCs precisely predicted the results of the clinical trial. For *E. coli*, the categorical agreement ranged between 97.4% and 100% for all the antibiotics tested, except for amikacin at 4 h, which explains the MEs observed. According to spiked BC and clinical trial results, except for amikacin, all of the antibiotics tested could be released at 4 h with high confidence. Regarding amikacin, the results could be released only at 6 h. Moreover, the high rate of ATU observed for piperacillin-tazobactam and amikacin will impair their usage at 4 h for many cases. For *K. pneumoniae*, the entire drug panel tested can be released at 4 h, even for MDR isolates. Unfortunately, only 2 *A. baumannii* isolates were included in the clinical trial, but the results of the spiked BCs confirm that the entire tested drug panel can be safely released at 4 h. The high rate of ATU observed for imipenem at 4 h significantly declined from 78.7% to 63% at 6 h. Unlike the other species, *P. aeruginosa* posed several challenges. The categorical agreement for imipenem was less than 95% at 6 h and did not improve by 8 h. According to this study, using the RAST for imipenem is not accurate and this antibiotic should be removed from the panel of antibiotics to be tested for *P. aeruginosa*. Additionally, piperacillin-tazobactam, ceftazidime, and cefepime cannot be released at 6 h, so administration should be held off until the next time point because the categorical agreement is substantially improved at 8 h. Therefore, greater attention should be paid in interpreting the *P. aeruginosa* panel when it is tested by RAST. For *S. aureus*, cefoxitin enables the identification of MRSA with very high accuracy at 4 h, when the inhibition zone is readable. A high rate of ATU was observed at 4 h for norfloxacin, but it had declined from 74.9% to 4.7% by 6 h. The entire drug panel can therefore be released at 4 h. For *E. faecalis* and *E. faecium*, the categorical agreement was >95% for all antibiotics tested except for gentamicin. All the 30 VRE isolates analyzed in this study were categorized as vancomycin-resistant at 6 h. However, according to EUCAST recommendations, the vancomycin disk does not correctly predict resistance in vanB-positive isolates with low MICs, which explains the high rate of ATU observed using the RAST.

| *Staphylococcus aureus* clinical trial results (n = 127 independent positive BCs from 27 patients)
|---|---|---|---|---|---|---|---|---|
| S. aureus clinical trial parameters | Cefoxitin | Clindamycin | Norfloxacin | Gentamicin |
| | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Isolates by category (n) | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Resistant | 22 | 22 | 22 | 27 | 28 | 20 | 21 | 24 |
| ATU | 1 | 0 | 0 | 7 | 7 | 5 | 4 | 6 | 4 |
| Susceptible | 101 | 105 | 105 | 117 | 118 | 121 | 80 | 94 | 95 |
| Inhibition zone not readable | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 |
| Isolates by category (%) | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Resistant | 17.7 | 17.3 | 17.3 | 1.6 | 0.8 | 21.3 | 22.0 | 16.1 | 16.5 | 18.9 |
| ATU | 0.8 | 0.0 | 0.0 | 5.6 | 5.5 | 3.9 | 35.5 | 4.7 | 3.1 | 13.7 | 9.4 | 18.1 |
| Susceptible | 81.5 | 82.7 | 82.7 | 94.4 | 92.9 | 95.3 | 64.5 | 74.0 | 74.8 | 70.2 | 74.0 | 63.0 |
| Inhibition zone not readable | 2.4 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 | 2.4 | 0.0 | 0.0 |
| Categorical agreement (%)<sup>b</sup> | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| ATU included | 100.0 | 100.0 | 100.0 | 99.2 | 99.2 | 99.2 | 100.0 | 99.2 | 99.2 | 100.0 | 100.0 | 100.0 |
| ATU not included | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.1 | 99.2 | 99.2 | 100.0 | 100.0 | 100.0 |
| Discordant results (n) | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Major error | 1 | 1 | 1 | 1 | 1 |
| Very major error | 1 | 1 |
| Discordant results (%) | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Major error | 0.0 | 0.0 | 0.0 | 0.8 | 0.8 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Very major error | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

<sup>a</sup>BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

<sup>b</sup>Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.
TABLE 11 *Enterococcus faecalis* spiked BC results (n = 66 isolates)\(^a\)

| *E. faecalis* spiked BC parameters | Ampicillin | Imipenem\(^b\) | Vancomycin | Gentamicin | Linezolid |
|-----------------------------------|-----------|----------------|------------|------------|----------|
|                                    | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Isolates by category (n)           |     |     |     |     |     |     |     |     |     |     |     |     |
| Resistant                          | 5   | 6   | 6   | 21\(^c\) | 23\(^c\) | 21\(^c\) |     |     |     |     |     |     |
| ATU                                | 2   | 57  | 60  | 60  | 32  | 28  | 28  | 22  | 10  | 11  |     |     |
| Susceptible                        | 60  | 66  | 66  | 62  | 66  | 66  |     |     |     |     |     |     |
| Inhibition zone not readable       | 4   | 0   | 0   | 4   | 0   | 0   | 4   | 0   | 0   | 4   | 0   | 0   |
|                                    |     |     |     |     |     |     |     |     |     |     |     |     |
| Isolates by category (%)           |     |     |     |     |     |     |     |     |     |     |     |     |
| Resistant                          | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.1 | 9.1 | 9.1 | 33.9 | 34.8 | 31.8 |
| ATU                                | 3.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 91.9 | 90.9 | 90.9 | 51.6 | 42.4 | 42.4 |
| Susceptible                        | 96.8 | 100.0 | 100.0 | 100 | 100 | 100 | 0.0 | 0.0 | 0.0 | 14.5 | 22.7 | 25.8 |
| Inhibition zone not readable       | 6.1 | 0.0 | 0.0 | 6.1 | 0.0 | 0.0 | 6.1 | 0.0 | 0.0 | 6.1 | 0.0 | 0.0 |
|                                    |     |     |     |     |     |     |     |     |     |     |     |     |
| Categorical agreement (%)\(^d\)    |     |     |     |     |     |     |     |     |     |     |     |     |
| ATU included                       | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 95.0 | 95.0 | 95.0 | 100.0 | 100.0 | 100.0 |
| ATU not included                   | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 94.6 | 95.0 | 95.0 | 100.0 | 100.0 | 100.0 |
| Discordant results (n)             |     |     |     |     |     |     |     |     |     |     |     |     |
| Major error                        | 3   | 3   | 3   |     |     |     |     |     |     |     |     |     |
| Very major error                   |     |     |     |     |     |     |     |     |     |     |     |     |
| Discordant results (%)             |     |     |     |     |     |     |     |     |     |     |     |     |
| Major error                        | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 5.0 | 5.0 | 4.5 |
| Very major error                   | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

\(^a\)BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

\(^b\)Isolates with zone diameters greater than the ATU interval were reported as “Susceptible, increased exposure” (I).

\(^c\)High-level aminoglycoside resistance.

\(^d\)Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.

EUCAST Breakpoint Tables version 3.0. Therefore, special attention should be paid to isolates within the vancomycin-ATU.

The decision to include all positive BCs from the same patients enabled us to highlight the high repeatability and reproducibility of the RAST. No discordant results at the categorical level were observed among BCs from the same patient.

TABLE 12 *Enterococcus faecium* spiked BC results (n = 48 isolates)\(^a\)

| *E. faecium* spiked BC parameters | Ampicillin | Imipenem\(^b\) | Vancomycin | Gentamicin | Linezolid |
|-----------------------------------|-----------|----------------|------------|------------|----------|
|                                    | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h | 4 h | 6 h | 8 h |
| Isolates by category (n)           |     |     |     |     |     |     |     |     |     |     |     |     |
| Resistant                          | 45  | 45  | 45  | 47  | 46  | 46  | 22  | 23  | 23  | 0\(^e\) | 20\(^e\) | 20\(^e\) |
| ATU                                | 4   | 1   | 2   | 2   | 26  | 25  | 25  | 6   | 3   | 18  | 9   |     |
| Susceptible                        | 3   | 3   | 3   |     |     |     |     |     |     |     |     |     |
| Inhibition zone not readable       | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |     |
|                                    |     |     |     |     |     |     |     |     |     |     |     |     |
| Isolates by category (%)           |     |     |     |     |     |     |     |     |     |     |     |     |
| Resistant                          | 93.8 | 93.8 | 93.8 | 97.9 | 95.8 | 95.8 | 45.8 | 47.9 | 47.9 | 41.7 | 41.7 | 41.7 |
| ATU                                | 0.0 | 0.0 | 0.0 | 2.1 | 4.2 | 4.2 | 54.2 | 52.1 | 52.1 | 12.5 | 0.0 | 6.3 |
| Susceptible                        | 6.3 | 6.3 | 6.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 45.8 | 58.3 | 52.1 |
| Inhibition zone not readable       | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
|                                    |     |     |     |     |     |     |     |     |     |     |     |     |
| Categorical agreement (%)\(^b\)    |     |     |     |     |     |     |     |     |     |     |     |     |
| ATU included                       | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| ATU not included                   | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Discordant results (n)             |     |     |     |     |     |     |     |     |     |     |     |     |
| Major error                        |     |     |     |     |     |     |     |     |     |     |     |     |
| Very major error                   |     |     |     |     |     |     |     |     |     |     |     |     |

\(^a\)BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST= R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

\(^b\)Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.

\(^c\)High-level aminoglycoside resistance.
The fully automated RAST allows inoculated media to be transferred to the incubator immediately after deposition of the antibiotic disks. The medium plates are incubated under optimal growth conditions, served by a stable temperature and adequate atmosphere. The digital images taken at predefined time points can be inspected using magnification. All these elements explain the improved percentages of readable inhibition zones at the different time points. Another positive impact was the lower percentage of ATU compared to that in data published previously using the manual process (12, 15).

The manual processing of the EUCAST RAST and especially the need to read the inhibition zone diameters at strictly defined time points translate into a very labor-intensive method. A previous report highlighted these challenges (12). In contrast, total laboratory automation (TLA) perfectly addresses these constraints by automatically acquiring images at predefined time points. Moreover, the expert system facilitates the integration of different rules, in line with the observations made in this study, to optimize and improve the results released to physicians. For instance, it automatically defines which antibiotic results can be released at 4 h and which can be released at later, well-validated incubation times. The advantages of full automation facilitate the implementation of the RAST directly from positive BCs in routine. In our laboratory, positive BCs are processed by the WASPLab. To further improve the TAT of RAST, we defined a panel of antibiotics covering all species analyzed in this study. Thereby, RAST can be performed without waiting for microscopy results and Gram staining; non-pertinent antibiotics can then be excluded from reporting during the reading phase.

The system used in our laboratory for the fully automated RAST AST is composed of one WASP and a single “air atmosphere” incubator. These incubation constraints (i.e., the CO₂ requirement) prevented us from using RAST to assess *Streptococcus pneumoniae* in this study.

Conclusions. In our previous report, we validated the full automation of standardized antimicrobial disk diffusion susceptibility testing (11). Nowadays, 97% of our AST panels are performed using this system in routine (17). Based on this positive experience, we extended the use of the fully automated solution to the EUCAST rapid AST method directly from positive blood cultures. The spiked BCs and the clinical study established that the performance

| TABLE 13 *Enterococcus faecalis* clinical trial results (n = 50 independent positive BCs from 13 patients)ab |
|-------------------------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                                                    | Ampicillin                  | Imipenem                     | Vancomycin                   | Gentamicin                   | Linezolid                    |
| *E. faecalis* clinical trial parameters             | 4 h 6 h 8 h                 | 4 h 6 h 8 h                  | 4 h 6 h 8 h                  | 4 h 6 h 8 h                  | 4 h 6 h 8 h                  | 4 h 6 h 8 h                  |
| Isolates by category (n)                           |                              |                              |                              |                              |                              |                              |
| Resistant                                          |                               |                              |                              |                              |                              |                              |
| ATU                                                | 8 4 3                        | 46 50 50                     | 12 20 19                     | 9 5 7                       | 25c 24c 21c                  |
| Susceptible                                        | 38 46 47                     | 47 50 50                     | 9 6 10                       | 22 29 27                    | 4 0 0                        |
| Inhibition zone not readable                       | 4 0 0                        | 4 0 0                        | 4 0 0                        | 4 0 0                       | 3 0 0                        |
| Isolates by category (%)                           |                               |                              |                              |                              |                              |                              |
| Resistant                                          | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  |
| ATU                                                | 17.4 8.0 6.0                 | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  |
| Susceptible                                        | 82.6 92.0 94.0               | 100.0 100.0 100.0            | 100.0 100.0 100.0            | 100.0 100.0 100.0            | 100.0 100.0 100.0            |
| Inhibition zone not readable                       | 8.0 0.0 0.0                  | 8.0 0.0 0.0                  | 8.0 0.0 0.0                  | 8.0 0.0 0.0                  | 8.0 0.0 0.0                  |
| Categorical agreement (%)d                         |                               |                              |                              |                              |                              |                              |
| ATU included                                       | 100.0 100.0 100.0            | 100.0 100.0 100.0            | 100.0 100.0 100.0            | 95.7 96.0 96.0              | 100.0 100.0 100.0            |
| ATU not included                                   | 100.0 100.0 100.0            | 100.0 100.0 100.0            | 100.0 100.0 100.0            | 94.1 93.3 93.5              | 100.0 100.0 100.0            |
| Discordant results (n)                             |                               |                              |                              |                              |                              |                              |
| Major error                                        | 1 1 1                        |                              |                              |                              |                              |                              |
| Very major error                                   | 1 1 1                        |                              |                              |                              |                              |                              |
| Discordant results (%)                             |                               |                              |                              |                              |                              |                              |
| Major error                                        | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  |
| Very major error                                   | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  | 0.0 0.0 0.0                  |

*a* BC, blood culture; ATU, area of technical uncertainty. Very major error, RAST = S and reference AST = R; major error, RAST = R and reference AST = S; minor error, RAST = S or R and reference AST = I.

*b* Isolates with zone diameters greater than the ATU interval were reported as “Susceptible, increased exposure” (I).

*c* High-level aminoglycoside resistance.

*d* Categorical agreement for RAST at 4, 6 and 8 h of incubation compared to EUCAST standardized disk diffusion testing.

The fully automated RAST allows inoculated media to be transferred to the incubator immediately after deposition of the antibiotic disks. The medium plates are incubated under optimal growth conditions, served by a stable temperature and adequate atmosphere. The digital images taken at predefined time points can be inspected using magnification. All these elements explain the improved percentages of readable inhibition zones at the different time points. Another positive impact was the lower percentage of ATU compared to that in data published previously using the manual process (12, 15).
of this automation was consistently robust, even for the detection of ESBL, carbapenemase-producing bacteria, and MRSA. In addition, the automation enhances the percentage of readable inhibition zones and reduces the percentage of isolates categorized in the ATU. The fully automated RAST, coupled to the expert system, will substantially improve workflow by reducing hands-on times and the constraints linked to manual workup.

**Ethical approval.** In accordance with local ethical committees, routine clinical laboratories of our institution may use biological sample leftovers for method development after irreversible anonymization of data. The official name of the ethics committee is the “Commission cantonale d’éthique de la recherche” (CCER; https://www.hug-ge.ch/ethique).

**SUPPLEMENTAL MATERIAL**
Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 1.6 MB.

**ACKNOWLEDGMENT**
The authors declare no conflict of interest.
This study was supported by internal funding.

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