# Blood culture processing using a total lab automation (TLA) – reduced time to report using rapid antimicrobial susceptibility testing (RAST)

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## Background

A shortened time to report is crucial for patients with symptoms of bacteraemia, particularly in times of increasing infections with multi-resistant pathogens. Rapid molecular tests like PCR only deliver results for MRSA and VRE, while the rapid detection of multi-resistant gram-negative pathogens remains unsatisfactory. The objective of the current study was to reduce time to report using rapid antimicrobial susceptibility testing (RAST) on blood cultures processed in a total lab automation (TLA, BD Kiestra™).

## Materials and methods

Positive blood culture bottles (BACTEC™ Aerobic, BACTEC™ Anaerobic) were subcultured onto Columbia 5% SB agar, chocolate agar, MacConkey agar and Schaedler agar (if anaerobic). Additionally each bottle was subcultured onto a Mueller-Hinton agar (4 drops ≈ 150µL) and 6 antibiotic discs were added covering gram-negative and gram-positive therapy (cefoxitin, ampicillin, vancomycin, piperacillin/tazobactam, meropenem and ciprofloxacin). Subculture was done in the semi-automatic part of a TLA. Streaking was done using the rolling bead technology. Subcultures were imaged after 6h and 23h. RAST plates were only imaged after 6h (Figure 1). Zone sizes were measured using a tool in the ReadA Browser software of the TLA. Interpretation of zones was done using data from EUCAST conference papers (P0165, ECCMID 2017; O0746 ECCMID 2018) and the official clinical breakpoints (2018). Bacteria were identified using MALDI-TOF MS. MIC values were determined using VITEK2 panels for staphylococci, enterococci and gram-negative bacteria.

## Results

The Department for Infectious Diseases at the University Hospital Heidelberg received approximately 2600 blood culture bottles per month with a positivity rate of ≈20%. **Minimum time to RAST was 7h (median: 20h) and minimum time to MIC (Vitek) was 18h (median: 37h)**, both times depending on the exact time point of positivity (morning, afternoon, night).
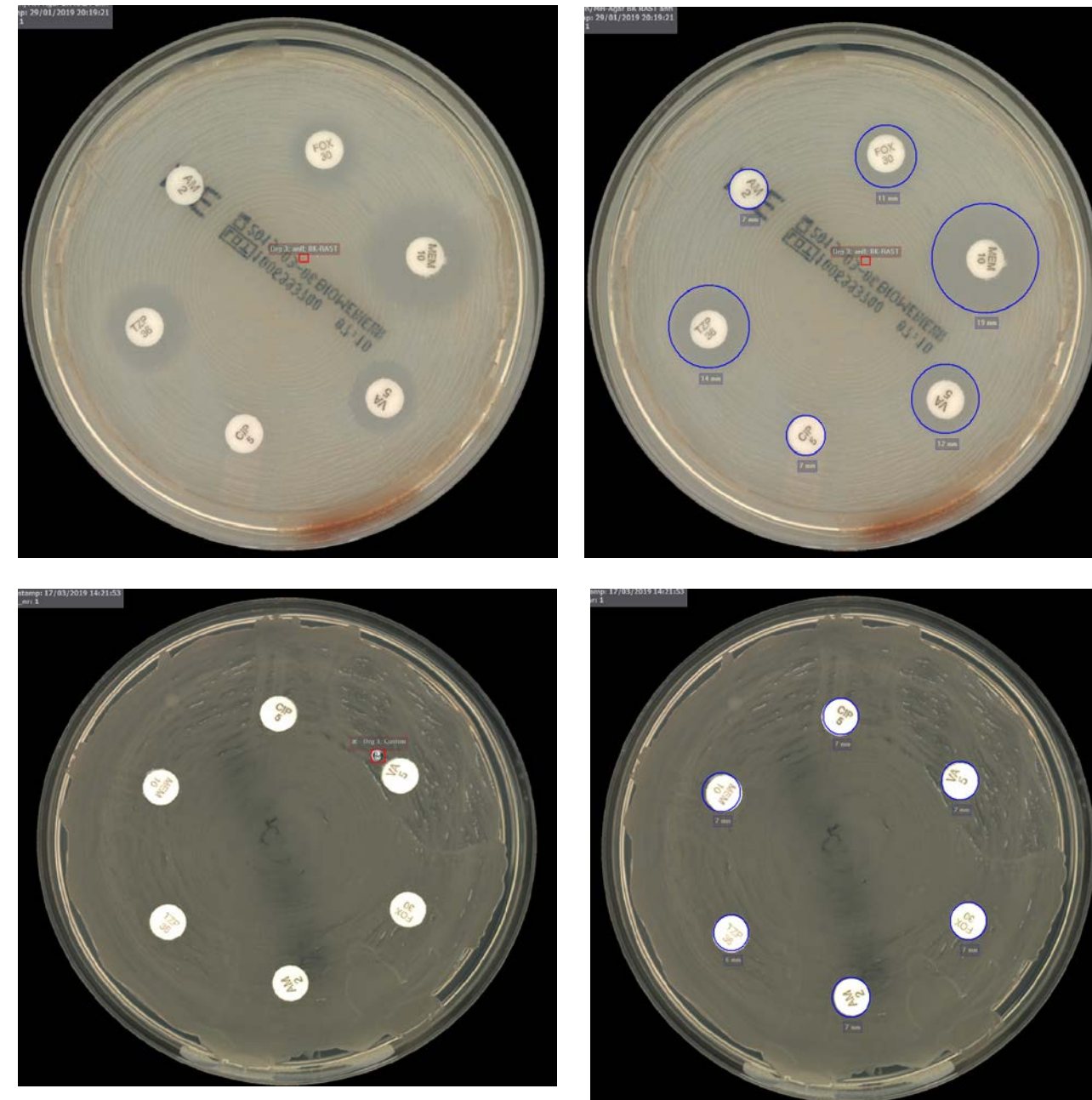
**Reduction of median time to report: 17h**



**Figure 1:** RAST images of MRSA (above) and multi-resistant *K. pneumoniae* (below) with and without measured zone sizes after 6h of incubation.

## Results

Results of agar diffusion were compared to MIC determination for *Escherichia coli* (n=100), *Staphylococcus aureus* (n=50), *Enterococcus* spp. (n=50), *Klebsiella pneumoniae* (n=25) and *Pseudomonas aeruginosa* (n=25), respectively. Accordance (S/R) between VITEK2 and RAST agar diffusion results was observed in ≈97% of cases (Table 1). Especially for Piperacillin/Tazobactam the number of ATU results was significant (47%, 32%, 40%).

## Conclusions

Switch from the classic to the automated workflow reduced the median time to report by 17h. Using the published zone sizes yielded accurate results compared to MIC determination. The number of ATU results implies that further investigation of the appropriateness seems recommendable.

| | | Old RAST | | | New RAST | | | Old RAST | | New RAST | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | ATU | R | S | ATU | R | Very major error | Major error | Very major error | Major error |
| S. aureus (n=50) | Cefoxitin | 94% (47/50) | - | 6% (3/50) | 94% (47/50) | - | 6% (3/50) | - | - | - | - |
| *Enterococcus* spp. (n=50) | Ampicillin | 46% (22/50) | - | 54% (30/50) | 42% (21/50) | - | 58% (29/50) | 2% (1/50) | - | 2% (1/50) | - |
| | Vancomycin | 78% (39/50) | - | 22% (11/50) | 74% (37/50) | - | 26% (13/50) | 2% (1/50) | - | - | 2% (1/50) |
| *E. coli* (n=100) | Piperacillin/ Tazobactam | 100% (100/100) | - | - | 50% (50/100) | 47% (47/100) | 3% (3/100) | - | - | - | 2% (2/100) |
| | Ciprofloxacin | 66% (66/100) | 9% (9/100) | 25% (25/100) | 62% (62/100) | 12% (12/100) | 26 % (26/100) | - | - | - | - |
| | Meropenem | 100% (100/100) | - | - | 100% (100/100) | - | - | - | - | - | - |
| *K. pneumoniae* (n=25) | Piperacillin/ Tazobactam | 88% (22/25) | - | 12% (3/25) | 44% (11/25) | 32% (8/25) | 24% (6/25) | 32% (8/25) | - | - | 8% (2/25) |
| | Ciprofloxacin | 68% (17/25) | 8% (2/25) | 24% (6/25) | 68% (17/25) | 8% (2/25) | 24% (6/25) | 8% (2/25) | - | 8% (2/25) | - |
| | Meropenem | 100% (25/25) | - | - | 100% (25/25) | - | - | - | - | - | - |
| *P. aeruginosa* (n=25) | Piperacillin/ Tazobactam | 40% (10/25) | 48% (12/25) | 12% (3/25) | 40% (10/25) | 40% (10/25) | 20% (5/25) | - | - | 4% (1/25) | 4% (1/25) |
| | Ciprofloxacin | 84% (21/25) | 4% (1/25) | 12% (3/25) | 60% (15/25) | 28% (7/25) | 12% (3/25) | 32% (8/25) | - | 12% (3/25) | - |
| | Meropenem | 64% (16/25) | 16% (4/25) | 20% (5/25) | 44% (11/25) | 32% (8/25) | 24% (6/25) | - | - | - | - |

**Table 1:** Comparison of old and new RAST in subcultured blood cultures and its very major and major errors.