

**Methodology - EUCAST rapid antimicrobial susceptibility testing (RAST)  
directly from positive blood culture bottles.**

**Version 6.0**

**March 2025**

## Changes from previous version (v. 5.0)

Section	Change
Introduction	Information on <i>Salmonella enterica</i> added.
Table 1	Information on <i>Salmonella enterica</i> added.
Table 2	Information on <i>Salmonella enterica</i> added.

The EUCAST RAST method is based on the EUCAST standard disk diffusion methodology, but with modified inoculum, incubation time, modified reading instructions and specific RAST breakpoints.

The purpose of the EUCAST RAST method is to allow rapid susceptibility test results directly from positive blood cultures. The RAST method provides specific breakpoints for readings at 4, 6 and/or 8 hours incubation. In addition, RAST breakpoints for 16-20 hours incubation have been developed. Results should only be read after 16-20 hours when it is not possible to read results after 4, 6 and/or 8 hours incubation, for example due to limited opening hours.

The method have been validated for the following species: *Escherichia coli*, *Klebsiella pneumoniae* (including *Klebsiella variicola* and *Klebsiella quasipneumoniae*), *Salmonella enterica*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium* and *Streptococcus pneumoniae*.

### Preparation of blood culture bottles

The EUCAST RAST method has been validated using blood culture bottles for BACTEC (Becton Dickinson), BacT/ALERT (bioMérieux) and VersaTREK (Thermo Fisher). The RAST method can be performed 0 – 18 hours after blood culture bottles have signalled positive. Do not remove positive bottles from the blood culture instrument until you are ready to proceed with the RAST. However, to allow for transport of positive bottles from one site to another, we have evaluated the impact of keeping bottles at room temperature after having removed them from the instrument. RAST results were not affected by a “delay” of up to 3 hours. The RAST method should not be performed on blood cultures with mixed species.

### Inoculation of agar plates directly from blood culture bottles

Take 125±25 µl of undiluted blood culture broth from the positive blood culture bottle to each 90-mm circular MH/MH-F agar plate. Spread the broth gently over the agar surface by swabbing in three directions or using an automatic plate rotator and apply disks as for standard AST. Use a maximum of 4 - 6 disks per plate to avoid interference between agents. Inoculate plates, apply antibiotic disks and incubate plates without any delay.

### Incubation and reading of plates

Incubate plates as described in Table 1. For 4, 6 and 8 hours: read inhibition zones at ± 5 minutes of the stated reading time (4, 6 and/or 8 hours). If needed, re-incubate the plates within 10 minutes to enable reading at a later time (6 and/or 8 hours). If it is necessary to incubate plates for longer than 8 hours, read inhibition zones within 16-20 hours. Do not incubate or read plates beyond 20 hours.

**Table 1.** Incubation conditions for antimicrobial susceptibility test plates.

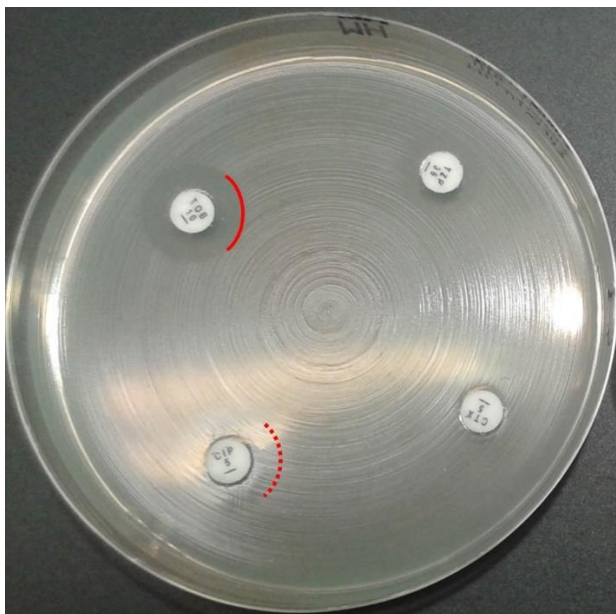
Organism	Incubation time	Medium	Incubation
<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Salmonella enterica</i> <i>Acinetobacter baumannii</i> <i>Staphylococcus aureus</i> <i>Enterococcus faecalis</i> <i>Enterococcus faecium</i>	4, 6 and 8 hours 16-20 hours	MH	35±1°C in air
<i>Pseudomonas aeruginosa</i>	6 and 8 hours 16-20 hours	MH	35±1°C in air

<i>Streptococcus pneumoniae</i>	4, 6 and 8 hours 16-20 hours	MH-F	35±1°C in 4-6% CO <sub>2</sub> in air
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### Examination of plates after incubation

#### 4, 6 and 8 hours incubation

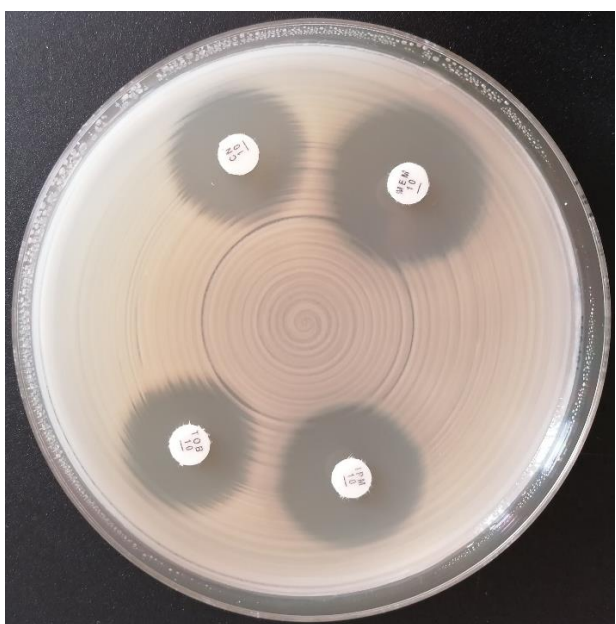
At 4, 6 and 8 hours, the growth on the Muller-Hinton agar plate will often appear less distinct than with the EUCAST standard disk diffusion method. **Inhibition zones should only be read when the growth is confluent and zone edges are clearly visible, see example in Figure 1.**



**Figure 1.** *E. coli* after 4 hours incubation. Zones with a clearly visible zone edge should be read (solid line) and zones with no clear zone should not be read (dotted line).

#### 16-20 hours incubation

At 16-20 hours incubation of RAST, the growth on the Muller-Hinton agar plate will often appear heavier compared with EUCAST standard disk diffusion method, **see example in Figure 2.**



**Figure 2.** *E. coli* after 16-20 hours incubation.

## Measurement of zone diameters

### General reading instructions

- Read MH plates against a dark background and MH-F plates against a light background. Hold the plate about 30 cm from the eye at a 45-degree angle from the work bench. Tilt the plate towards you to identify sharp zone edges.
- Measure inhibition zone diameters manually to the nearest millimetre. The RAST method has not been validated for automated zone readers.
- Thin growth within an inhibition zone with a clear zone edge should be ignored. This occasionally occurs at early reading for *E. coli* and *K. pneumoniae* and most often for  $\beta$ -lactam antibiotics.

### Specific reading instructions at 4, 6 and 8 hours incubation

- Read both MH and MH-F plates **from the front of the plate with the lid removed** and with reflected light.
- For *A. baumannii* with trimethoprim-sulfamethoxazole, read the outer zone edge and ignore growth within the zone.
- Sometimes there is no evident inhibition zone after 4 hours but a zone diameter can easily be measured after 6 hours (Table 2). It is not always possible to read inhibition zones for all tested antibiotic agents.

### Specific reading instructions at 16-20 hours incubation

- 16-20 hour read **MH plates from the back of the plate** with a reflected light and **MH-F plates from the front of the plate with the lid removed** and with reflected light.
- For *P. aeruginosa* with piperacillin-tazobactam, imipenem, imipenem-relebactam, meropenem and meropenem-vaborbactam, ignore isolated colonies inside the inhibition zone and read the outer zone edge, see example in Figure 3.



**Figure 3.** Ignore isolated colonies within the inhibition zone and read the outer zone edge.

**Table 2. The proportion of zone diameters (%) which are possible to read\* after 4 – 20 h of incubation.**

Organism	4 hours	6 hours	8 hours	16-20 hours
<i>Escherichia coli</i>	90	99	99	100
<i>Klebsiella pneumoniae</i>	96	98	98	100
<i>Salmonella enterica</i>	93	100	100	100
<i>Pseudomonas aeruginosa</i> **	-	88	97	100
<i>Acinetobacter baumannii</i>	99	100	100	100
<i>Staphylococcus aureus</i>	55***	91	95	100
<i>Enterococcus faecalis</i>	93	99	100	100
<i>Enterococcus faecium</i>	44	93	99	100
<i>Streptococcus pneumoniae</i>	68	83	95	100

\*The table displays “possible to read”, not “possible to interpret”, since some of the zone diameters will be in the ATU.

\*\*For some *P. aeruginosa* isolates there is weak growth at RAST, the isolates usually have weak growth at standard disk diffusion 16-20 hours incubation as well.

\*\*\*Cefoxitin and aminoglycosides are easy to read while norfloxacin and clindamycin are more difficult.

### Interpretation of results

- Interpret measured inhibition zone diameters according to the latest version of the RAST breakpoint tables.
- Sometimes it is not possible to report a susceptibility category for all tested antimicrobial agents, either because there is no growth, you cannot read the zone in a reliable way or because the zone diameter is in the ATU. In these cases, leave the report blank for the relevant agent. We suggest that laboratories include a comment in reports on positive blood cultures which explains why some results may be left blank at times. The comment could read: "Antimicrobial susceptibility testing directly from positive blood culture bottles, where results can often be offered after 4, 6 and/or 8 hours, requires that only reliable results are reported. A susceptibility report lacking results after short incubation, may be supplemented with more results at a later stage."

### Area of Technical Uncertainty (ATU)

The ATU is a range of inhibition zone diameters. There are ATUs for all organism-antimicrobial agent combinations with the EUCAST RAST method. The ATU represents an area where the separation between susceptibility categories is poor. Interpretative errors increase dramatically in this area and interpretation is not possible. Results above or below the ATU can reliably be reported.

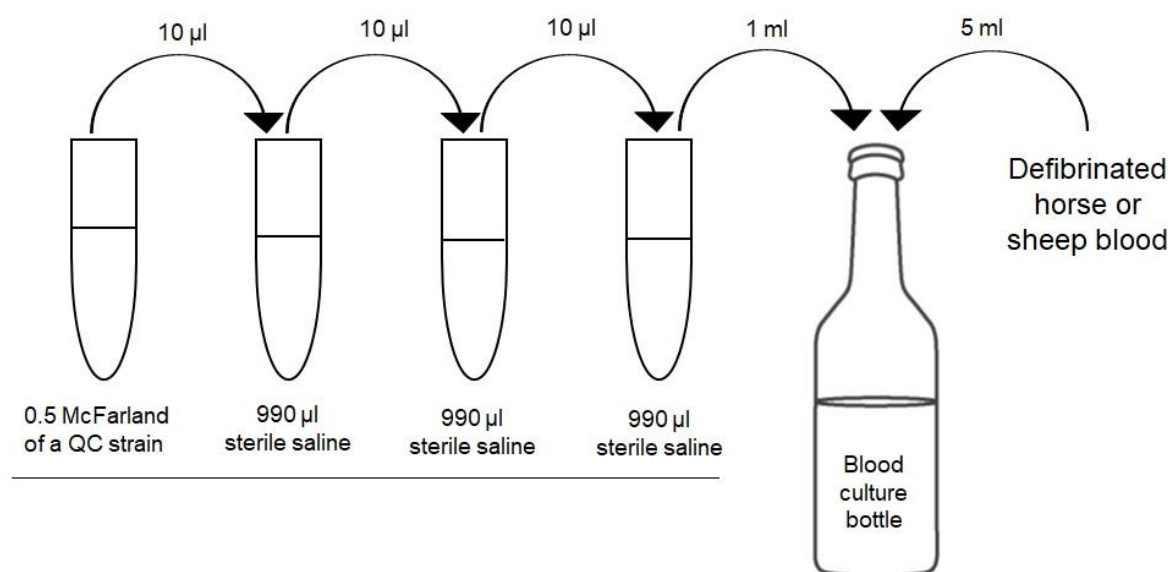
When a result is inside the ATU it cannot be interpreted. Do not hesitate to leave the report blank for the agent. At 4 hours, re-incubate plates within 10 minutes and read again at 6 hours and if needed at 8 hours and when necessary at 16-20 hours. If a complete result cannot be given after 8 or 16-20 hours incubation, perform AST with EUCAST standard disk diffusion method.

### Quality control recommendations

For EUCAST standard disk diffusion, EUCAST recommends that internal QC is performed daily to validate procedure and AST materials. EUCAST has also developed criteria for 4, 6, 8 and 16-20 hours for five QC strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619). The criteria are available in the RAST QC tables. The RAST QC procedure is performed mainly to calibrate and validate the implementation of the new procedure. All reading times utilized in the laboratory should be validated using the QC strains. Once the procedure is established and until new staff or new materials (a change of blood culture system or media or disks) are introduced, RAST QC is not needed. Regular internal QC with standard methodology should still be performed according to EUCAST recommendations to control materials and equipment used.

The QC strains are tested by inoculating blood culture bottles with 1 mL of a 100-200 CFU/mL suspension\* of the QC strain and with the addition of approximately 5 mL defibrinated horse or sheep blood. The inoculated bottles are incubated in the blood culture instrument and processed according to the RAST methodology following a positive signal.

\*100-200 CFU/mL = Suspension adjusted to 0.5 McFarland is diluted 1:1 000 000, see example in the graph below.



- Make a 0.5 McFarland dilution of a QC strain.
- Dilute according to dilution series above and add defibrinated horse or sheep blood to the blood culture bottle.
- Incubate bottle in the blood culture instrument.
- Process the bottles according to the described RAST methodology when the instrument signals positive.
- Use the RAST QC criteria available in the RAST QC document to assess the results.

## **Important considerations when using the EUCAST RAST method**

- Read inhibition zones only when the growth is confluent and zone edges are clearly visible.
- Read zones only at the designated reading times, i.e. 4, 6 and 8 hours, and when this is not possible (for example due to limited opening hours) at 16-20 hours.
- Use the EUCAST RAST breakpoint table, not the regular breakpoint table, to interpret the results into susceptibility categories.