Area of Technical Uncertainty (ATU) in antimicrobial susceptibility testing (1 June, 2020)

The Area of Technical Uncertainty (ATU) is a term coined by EUCAST to warn laboratories of uncertain interpretation of antimicrobial susceptibility testing (AST) results. These may be by MIC determination or disk diffusion using EUCAST methodology and breakpoints.

The ATU is defined by one (or on occasion more) MIC-value or by one (or a range of) inhibition zone diameter. It is not a susceptibility testing category like S, I and R and it does not interfere with the interpretation of results.

The ATU is a **warning** to laboratory staff that the value is in an area where there are interpretative difficulties. The reason is that a breakpoint is in a place where reproducible interpretation cannot be achieved. The ATU is not related to uncertainties in the testing procedures although the natural unavoidable variation in testing will influence the actions that may need to be taken. The ATU assumes that the test (MIC, inhibition zone diameter) is correctly performed and that the value obtained is correct in itself.

There is no mandatory action. The warning may be ignored or acted upon. On occasion an alternative test may solve the issue but otherwise a decision has to be taken to report as tested with or without a warning or to refrain from reporting a susceptibility category.

(1) If the decision is to act on the warning there are always several alternatives (see below) and the appropriateness of each will vary with the sample in which the organism was identified (UTI vs. septicemia), and the number of available antimicrobial alternatives. The individual laboratory may decide that results in the ATU should prompt laboratory technicians to consult with medical staff.

- **repeat the test** – this is only if there is reason to suspect a technical error.
- **perform an alternative test** (perform an MIC, a PCR, a test to determine the resistance mechanism) – this is relevant when the alternative test is conclusive (PCR to detect a *mecA* or *mecC* gene in Staphylococci, a *vanA* or *vanB* gene in enterococci).
- **report results in the ATU as "uncertain" with a comment**:
    - accept and include the interpretation but add a warning to the report
    - leave interpretation blank and add an explanation to the report
- **report results in the ATU as "R".** If there are several good alternatives in the AST report this may be the easiest and safest option.
- take the opportunity to **discuss the results with the clinical colleague**.

(2) If the decision is to ignore the warning, then interpret in accordance with the result obtained (MIC or zone). The ATU never interferes with the interpretation.

ATU warnings are listed in EUCAST Breakpoint Tables. They may change over time as breakpoints are updated.

Item in table	Suggested action
1.	The difficulties with *Enterobacterales* and amoxicillin-clavulanic acid are related to the systemic breakpoint (not with the UTI breakpoint). This is illustrated in Figure 1 a and b. The warning applies only to disk diffusion using systemic breakpoints. **Alternative actions:** Report blank with comment ("Unreliable susceptibility test result"), downgrade the susceptibility category (S to R), perform an alternative test. If one test result can be confirmed with another (disk diffusion and MIC-testing) this strengthens the interpretation.
2.	In *Enterobacterales,* a piperacillin-tazobactam result in the I-category is unreliable. In disk diffusion the overlap between 8, 16 and 32 and even 64 mg/L is considerable (Figure 2). **Alternative actions:** Report blank with comment ("Unreliable susceptibility test result"), downgrade the susceptibility category (S to I or I to R), perform an alternative test. If one test result can be confirmed with another (disk diffusion and MIC-testing) this strengthens the interpretation.
3.	For *Enterobacterales* and ceftaroline and (Figure 3) the overlap is considerable although the relationship between MIC and disk diffusion results is absolutely logical. The ATU is mainly to prevent false susceptibility. Even with the ATU false resistance will occur. **Alternative actions:** Report blank with comment ("Unreliable susceptibility test result"), downgrade the susceptibility category (S to R), perform an alternative test.
4.	For *Enterobacterales* and ciprofloxacin (Figure 4) the ATU is mainly to prevent false susceptibility. **Alternative actions:** Report blank with comment ("Unreliable susceptibility test result"), downgrade the susceptibility category (S to I or I to R), perform an alternative test. If one test result can be confirmed with another (disk diffusion and MIC-testing) this strengthens the interpretation.
5.	For *Pseudomonas* and piperacillin-tazobactam the ATU for disk testing is to prevent false susceptibility and to prevent organisms with an MIC of >16 mg/L from being reported as susceptible. **Alternative actions:** Report blank with comment ("Unreliable susceptibility test result"), downgrade the susceptibility category (S to R), perform an alternative test. If one test result can be confirmed with another (disk diffusion and MIC-testing) this strengthens the interpretation.
6.	For *Pseudomonas* and ceftazidime-avibactam the ATU for disk testing is to prevent false susceptibility and to prevent organisms with an MIC of >8 mg/L from being reported as susceptible. **Alternative actions:** Report blank with comment ("Unreliable susceptibility test result"), downgrade the susceptibility category (S to R), perform an alternative test. If one test result can be confirmed with another (disk diffusion and MIC-testing) this strengthens the interpretation.
7.	For *Pseudomonas* and colistin, the clinical colistin breakpoint (S≤2 mg/L) cuts into the wild type distribution of the species (ECOFF 4 mg/L). A value higher than 4 mg/L indicates the presence of a colistin resistance mechanism.
8.	The zone diameter ATU for the cefoxitin screen test in *S. epidermidis* (to identify methicillin resistance) is needed on Mueller Hinton media from some manufacturers. If in the ATU, perform a PCR to confirm the interpretation or report resistant..

9-10	For ceftaroline and ceftobiprole in *S. aureus* (Figure 5), the MIC and zone diameter breakpoints split the MRSA-population. Neither of the agents were approved for MRSA in general terms, only for *S. aureus* with MICs below a defined breakpoint. So the splitting of the MRSA populations was necessary. With the variability in both MIC and zone diameter testing, it is very difficult to guarantee a reliable test result. If one test result can be confirmed with another (disk diffusion and MIC-testing) this strengthens the interpretation.
11.	For S. aureus and amikacin it is difficult to distinguish between amikacin MICs of 8 and 16 mg/L, irrespective of which test is used. *S.aureus* which are wild type for kanamycin are never resistant to amikacin.
12 - 16	Susceptibility testing of *Haemophilus influenza* with mutations in the PBP3 is difficult. Not all mutations convey clinically significant resistance and also the loss of activity varies with the beta-lactam agent. To achieve reproducible categorization is difficult with both MIC and disk diffusion testing and the correlation between MIC and zone diameter formation is problematic. The benzylpenicillin 1U disk (see PCG1U in the flowchart in breakpoint table) will detect all beta-lactam resistance. • A negative screen allows the laboratory to report all relevant beta-lactam agents as susceptible. Further testing is not helpful. • A positive test indicates the presence of a mutation in PBP3 or beta-lactamase production but cannot distinguish between the two. Follow the flow chart. In *H. influenza* with PBP3 mutations and zone diameter values in the ATU, always either report “resistant”, leave blank with a comment or determine the MIC to categorise the isolate S, I and R to the agent.

Current ATUs

Enterobacterales

Amoxicillin clavulanic acid (systemic)
Piperacillin-tazobactam
Ceftaroline
Ciprofloxacin

Staphylococcus spp.

Cefoxitin (*S. epidermidis*)
Ceftaroline (*S. aureus*, pneumonia and non-pneumonia)
Ceftobiprole (*S. aureus*)
Amikacin (*S. aureus*)

Pseudomonas spp.

Piperacillin
Piperacillin-tazobactam
Ceftazidime-avibactam (*P. aeruginosa*)
Colistin (MIC only)

Haemophilus influenzae

Piperacillin-tazobactam (PBP3 mutations)
Cefepime (PBP3 mutations)
Cefotaxime (PBP3 mutations)
Cefpodoxime (PBP3 mutations)
Ceftriaxone (PBP3 mutations)
Cefuroxime (iv and oral, PBP3 mutations)
Imipenem (PBP3 mutations)

See graphs which illustrate ATUs and their use.