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1. EUCAST disk diffusion test - Medium

1. Which manufacturer of Mueller-Hinton agar does EUCAST recommend?

EUCAST does not recommend a particular manufacturer of Mueller-Hinton agar. Irrespective of the manufacturer used, each user should ensure that batches of media meet the internal quality control ranges published by EUCAST. These ranges have been checked with media from several manufacturers. For users of pre-poured commercial plates, it should be noted that the plate manufacturer not necessarily is the same as the Mueller-Hinton powder manufacturer.

A more extensive evaluation of MH agar from several manufacturers (21 brands from 17 manufacturers) performed by the EUCAST Development Laboratory is available in Clin Microbiol Infect. 2020 Oct;26(10):1412.e1-1412.e5. (also available at <https://www.eucast.org/about-eucast/publications-and-presentations/>).

2. What is the difference between Mueller-Hinton agar and Mueller-Hinton II agar?

The original specification of Mueller-Hinton agar did not define cation content, which is known to affect the activity of several agents, particularly aminoglycosides. Furthermore, the content of thymidine, which affects trimethoprim and trimethoprim-sulfamethoxazole activity, was undefined. Mueller-Hinton II agar is manufactured to contain a low concentration of thymidine and controlled concentrations of calcium and magnesium ions. Today, all Mueller-Hinton agars for susceptibility testing should be produced to meet the ISO technical specification 16782. Therefore, all Mueller-Hinton agars that yield inhibition zones within the acceptable ranges for EUCAST internal quality control strains can be used and EUCAST does not distinguish between MH and MH II.

An evaluation of MH agar from several manufacturers (21 brands from 17 manufacturers) performed by the EUCAST Development Laboratory is available in Clin Microbiol Infect. 2020 Oct;26(10):1412.e1-1412.e5. (also available at <https://www.eucast.org/about-eucast/publications-and-presentations/>).

3. Do we need to quality control each new batch of Mueller-Hinton agar?

Growth and inhibition zone diameters for antimicrobial agents used in routine practice should be checked on each new batch of Mueller-Hinton agar. Use strains recommended by EUCAST for internal quality control. Inhibition zone diameters outside control limits for gentamicin or tobramycin with *P. aeruginosa* ATCC 27853 may indicate high or low levels of cations and zone diameters below control limits for trimethoprim and/or trimethoprim-sulfamethoxazole with *E. faecalis* ATCC 29212 may indicate unacceptably high thymidine levels.

4. Can we use sheep blood instead of horse blood for the MH-F medium?

MH-F is the standard EUCAST medium for disk diffusion of fastidious organisms unless otherwise specifically stated. MH agar with 5% defibrinated sheep blood as the only supplement (MH-S) has been evaluated as an alternative medium for disk diffusion of fastidious organisms. For most species and agents, MH-S can be used instead of MH-F. The MH-S agar plates must contain 5% mechanically defibrinated sheep blood and have the same agar depth as MH-F (4.0 mm with a random variation of ± 0.5 mm). Specific reading instructions are needed for streptococci and trimethoprim-sulfamethoxazole.

However, EUCAST will not validate MH-S to the same extent as MH-F, and when using MH-S instead of MH-F, it is the responsibility of the user to validate the quality and usability of the medium, for all disks and species included in disk diffusion testing at the laboratory. This is primarily done by use of QC strains but also by comparing local distributions with those available from EUCAST. See <https://www.eucast.org/bacteria/methodology-and-instructions/media-preparation/> for more information.

5. Which β -NAD should we use?

We have evaluated β -NAD batches from several manufacturers and we recommend the use of β -NAD with a purity of $\geq 98\%$.

6. Can MH-F be used as medium for gradient tests?

For commercial products, such as gradient tests, testing should be performed as recommended by the manufacturer. See the manufacturers' instructions for information on which products are validated for MH-F.

7. It is stated in the EUCAST disk diffusion manual that the agar depth should be 4.0 ± 0.5 mm. Does this mean that it is acceptable to use plates with an agar depth of 3.5-3.7 mm?

No, the target value should be 4.0 mm, regardless of if in-house prepared or pre-poured commercial plates are used. If repeat measurements show the depth to be reproducibly above or below 4.0 mm, adjust the volume even when the agar depth is within 3.5 - 4.5 mm. Systematic use of plates that are close to the limits, particularly the lower limit, is more likely to result in erroneous inhibition zones.

8. We have problems with haze within the inhibition zones and growth of colonies close to the zone edge, particularly on the MH-F media. Can we do something to improve this?

If moisture is seen on the agar surface or inside the lid (moist film or droplets), it can explain a haze and/or fuzzy zone edges and it may be necessary to dry plates prior to inoculation. This is most common for plates stored in plastic bags or sealed containers. Make sure the agar surface is dry before inoculation. If needed, dry plates either at 20-22°C overnight or at 35°C for 15 min without lids. Storing plates unpacked in the fridge may also reduce problems with excess moisture. Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.

9. We have problems with fuzzy zone edges and haze within zones when testing anaerobic bacteria on FAA-HB media). How can we improve this?

When performing disk diffusion for anaerobic bacteria on FAA-HB (Fastidious Anaerobe Agar with 5% mechanically defibrinated horse blood), it is important to dry the plates before inoculation, either at 20-22°C overnight or at 35°C for 15 min without lids. For plates packed in plastic bags or sealed containers, it might be necessary to dry plates first at 20-22°C overnight, followed by drying at 35°C for 15 min without lids.

Hold plates at a 45-degree angle to the work bench when reading zones. If zone edges are fuzzy or if there is light haze within the zones, read the obvious zone edge. Tilt the plate towards you to better define the obvious zone edge.

10. Can we use Brucella blood agar supplemented with hemin and vitamin K instead of Fastidious Anaerobe Agar with defibrinated horse blood (FAA-HB) for disk diffusion of anaerobic bacteria?

The EUCAST disk diffusion method for anaerobic bacteria is developed for FAA-HB and alternative media cannot be used. For information on which anaerobic species that can be tested with disk diffusion according to EUCAST, see the latest version of the EUCAST Breakpoint Tables, <https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/>.

2. EUCAST disk diffusion test – Disks

1. Are EUCAST disk contents all the same as those used by CLSI?

Most are the same but several are different. Required contents for the EUCAST method are defined in the EUCAST QC and breakpoint tables. Alternative disk contents cannot be used when using EUCAST criteria. Each laboratory performing disk diffusion with EUCAST zone diameter breakpoints must perform quality control to check that the disks used perform according to the criteria in the EUCAST QC tables. There are significant differences in quality in disks from different manufacturers, see EUCAST evaluation of disks from 9 manufacturers, (<https://www.eucast.org/about-eucast/publications-and-presentations/>).

3. EUCAST disk diffusion test - Inoculum preparation

1. Do we have to measure the McFarland value on all suspensions?

It is not possible to judge the turbidity with the naked eye without a turbidity standard for comparison. The density of the inoculum suspension is most reliably adjusted by use of a photometric device calibrated to McFarland values. The density of the suspensions can be compared visually with that of a McFarland 0.5 turbidity standard but this is less reliable than using a photometric device. Easy-to-use photometric devices are commercially available.

2. Can we pick colonies from selective media?

Selective media contain substances that inhibit or promote growth of some organisms. It is a general recommendation for antimicrobial susceptibility testing to avoid picking colonies from selective media.

3. Should we pick more than one colony to be sure that we do not miss hetero-resistance?

Picking multiple colonies is not essential and will not affect detection of hetero-resistance, but is advisable to reduce the chances of selecting an atypical variant (such as a colony that has lost a resistance plasmid). In most cases it is necessary to pick more than one colony in order to have sufficient material to make a suspension of McFarland 0.5 density.

4. Can we use water or buffer instead of saline for inoculum preparation?

The EUCAST disk diffusion method is calibrated using 0.85% saline for the inoculum preparation. Commercial solutions labelled 0.9% are included in this recommendation. Laboratories that wish to use water or buffer instead of saline must validate this deviation locally.

5. In the EUCAST disk diffusion manual it is stated that we have to adjust the inoculum to a density of a McFarland 0.5 turbidity standard. What is the range we can use?

No range is given by EUCAST as the inoculum should be 0.5 McFarland. However, in practice it would be very time-consuming for laboratories to adjust all inocula to exactly 0.5 and a small variation is unlikely to affect results significantly. Laboratories using simple photometers may not be able to read more accurately than 0.1 McFarland unit and 0.4-0.6 will be used, but if you can adjust more accurately, we suggest that you do so.

6. Can flooding be used to inoculate plates for antimicrobial susceptibility testing?

No. Historically, flooding was used as an alternative to swabbing as a method for inoculation of plates. In most countries it is now considered unacceptable on safety grounds because pipetting or decanting high concentrations of organisms in suspensions onto the surface of plates and subsequent removal carries a high risk of production of aerosols and splashing. Moreover, flooding tends to produce higher density of microorganisms over the agar surface when compared with swabbing. For these reasons EUCAST does not recommend the use of flooding. Inoculation with a swab can be used with any size and shape of plate if the correct technique (evenly swabbing in three directions across the entire surface of the plate) is used. Alternatively, with round plates, a plate rotator (turntable) can be used.

4. EUCAST disk diffusion test - Reading zones of inhibition**1. Do we have to measure all inhibition zones?**

It is advisable to always measure and record inhibition zones. This enables the laboratory to compare their wild type zone diameter distributions with the reference inhibition zone diameter distributions available on the EUCAST zone diameter distribution database. It also enables local resistance surveillance. If alternative methods are used, e.g. automated zone readers or reading templates with EUCAST breakpoints, it is the responsibility of the laboratory to validate and maintain this system.

2. Should inhibition zones on both MH and MH-F be read against a dark background?

MH plates should always be read from the back of the plate against a dark background illuminated with reflected light. For MH-F plates, remove the lid and read from the front, with reflected light and preferably against a light background. Unless otherwise stated, read both MH and MH-F plates at a distance of 30 cm from the eye. Closer inspection may be needed to enable differentiation between haemolysis and growth on MH-F, between sharp and fuzzy zone edges (*Staphylococcus aureus* and benzylpenicillin, enterococci and vancomycin) and/or colonies within a zone (for the detection of heterogeneous resistance). See EUCAST Disk Diffusion Manual and Reading Guide for instructions.

3. Are all bactericidal and bacteriostatic agents read according to the same recommendations?

Yes, unless otherwise stated read zone edges for all antimicrobial agents at the point of complete inhibition as judged by the naked eye with the plate held at a distance of 30 cm from the eye (exceptions are listed in the EUCAST disk diffusion manual and in the EUCAST Reading guide).

4. Why is there sometimes growth within zones of beta-lactams for *Haemophilus influenzae* ATCC 49766?

Inhibition zones of *Haemophilus influenzae* ATCC 49766 and beta-lactam agents should be free from growth and within EUCAST quality control limits. Colonies within inhibition zones might be a result of a too heavy inoculum and/or excessively prolonged incubation time.

5. Are isolated colonies within mecillinam inhibition zones significant?

Mecillinam disk diffusion tests do sometimes produce colonies inside the zone of inhibition. Interpretation of mecillinam tests for species of Enterobacterales with mecillinam breakpoints is based on the obvious zone diameter and isolated colonies within zones should be disregarded.

6. Why are there sometimes colonies within the inhibition zones of carbapenems and *Pseudomonas aeruginosa* ATCC 27853?

Isolated colonies within the inhibition zones of carbapenems and *Pseudomonas aeruginosa* ATCC 27853 can be due to either loss of activity of the carbapenems in the disks (carbapenems are particularly sensitive to deterioration during storage), plates being too heavily inoculated, which facilitates emergence of resistant mutants, or the changes in the QC strain during subculturing. Performing daily QC is the best way to detect possible loss of activity of the antimicrobial disks. QC strains should be taken from the freezer each week and subcultured for no more than six days to maintain their properties. *P. aeruginosa* ATCC 27853 has two colony types and both must be included when subculturing the strain.

7. Why has EUCAST removed the specific reading instruction for linezolid disks when testing *Staphylococcus aureus*, i.e. reading zones with transmitted light?

When the EUCAST zone diameter breakpoints for linezolid and *Staphylococcus* spp. were established, we had access to few resistant isolates and decided to recommend the same reading instructions as CLSI, i.e. reading zones with transmitted light (plate held up to light). Since then, we have tested challenge collections of both *Staphylococcus* spp. and *Enterococcus* spp. and correlations between linezolid MIC values and inhibition zones with the EUCAST 10 µg disk were good both when zones were read with transmitted and reflected light respectively. We therefore decided to remove the specific reading instruction to facilitate routine reading of linezolid inhibition zones.

5. EUCAST disk diffusion test - General methodology

1. Do we have to follow the “15-15-15-minutes rule”?

Yes. EUCAST recommends that bacterial suspensions optimally are used within 15 minutes, and always within 60 minutes, of making the suspension. It is important to place the antimicrobial disks on the agar within 15 minutes of inoculating the plates and that plates are incubated within 15 minutes of placing disks on the inoculated medium. Extending these times may yield incorrect (systematically larger or smaller) inhibition zones.

2. Does EUCAST recommend “direct susceptibility testing”?

EUCAST has published a guidance document on “direct susceptibility testing” (For more information, see <https://www.eucast.org/eucastguidancedocuments>).

EUCAST has developed a specific method for rapid disk diffusion testing directly from positive blood cultures for sepsis species and antimicrobial agents relevant for bloodstream infections, see https://www.eucast.org/rapid_ast_in_blood_cultures/.

3. How should *Neisseria gonorrhoeae* be tested for antimicrobial susceptibility?

EUCAST has determined breakpoints for *N. gonorrhoeae* but is currently not recommending a specific method or medium. In collaboration with international experts on *N. gonorrhoeae*, EUCAST is in the process of evaluating alternatives. Until recommendations are published by EUCAST you should follow existing national or international guidelines. If commercial products are used for MIC determination, the manufacturer’s instructions should be followed, including the specific media recommended by the manufacturer.

4. Why does EUCAST recommend incubation at 35 ± 1°C when CLSI recommends 35 ± 2°C?

National standards for incubation temperature for susceptibility testing have been rather variable, but all other than CLSI have been based around ± 1°C. The ISO standard for broth microdilution (20776-1) also recommends 35 ± 1°C.

Modern incubators are specified to control temperature to well within ±1°C. Extensive work in calibrating the EUCAST disk diffusion method has been based on monitored temperatures of 35 ± 1°C and there has been no problem achieving this.

5. When implementing the EUCAST disk diffusion method is there a 20-day trial period, similar to CLSI, after which internal quality control (QC) testing frequency can be reduced from daily to weekly testing?

EUCAST recommends a training period (approximately 2 months) prior to routine use in order to teach all staff how to prepare and read plates.

Internal QC, using recommended QC strains, should be performed daily, or at least four times per week. Mean values of repeated tests (≥ 10) should optimally be on the target QC values ± 1 mm.

For a period of at least one month after introduction of the method, we recommend that all inhibition zone diameters be recorded and inhibition zone histograms are compared with reference distributions available on the EUCAST zone diameter distribution website. The median of the wild-type distribution should be within ± 1 mm of the median of the wild-type reference distribution. For EUCAST reference distributions, see <https://www.eucast.org/bacteria/mic-and-zone-distributions-ecoffs/>.

6. When performing disk diffusion of *Cutibacterium acnes*, we often fail to get confluent growth after 16-20 h incubation. Can we prolong the incubation time with an additional 24 hours?

EUCAST breakpoints are developed based on 16-20 h incubation unless otherwise stated. For *C. acnes*, prolonged incubation can result in zone diameters 5-10 mm larger compared with 16-20 h incubation, and incubation should therefore not be prolonged. To achieve confluent growth of *C. acnes* after 16-20 h incubation, make sure that the bacterial culture is in a good state (e.g. by subculturing before testing), to use a wet cotton swab and streak plates carefully when applying the inoculum suspension to the FAA-HB agar plate and to limit the number of disks on the plate (e.g. by using 2-3 disks on a 90-mm agar plate).

6. Breakpoints – general

1. Will there be breakpoints and methods for *Actinomycetes* spp., *Bordetella* spp., *Nocardia* spp., rapidly growing mycobacteria and *Streptomyces* spp.?

Breakpoints for these are under consideration. For some of them MIC testing only will be recommended and for others disk diffusion testing criteria will be developed.

2. Does EUCAST have clinical breakpoints or expert rules for veterinary use?

EUCAST clinical breakpoints specifically for veterinary use are under development. Human clinical breakpoints may be inappropriate for veterinary isolates, which may be from a variety of animal species. Among different animal species antimicrobial pharmacodynamics may vary widely. In this situation epidemiological cut-off values (ECOFFs) are a logical alternative to human clinical breakpoints, and ECOFFs have been used in preference to clinical breakpoints in veterinary resistance surveillance studies. EUCAST expert rules have been devised for human clinical use and, for the reason mentioned above, some may be inappropriate for veterinary situations although some might apply equally to human and veterinary situations. For more information, see EUCAST Veterinary Committee on Antimicrobial Susceptibility Testing (VetCAST), http://www.eucast.org/ast_of_veterinary_pathogens/.

3. What are the EUCAST breakpoints for the “I category” (susceptible, increased exposure) as none are given in the EUCAST breakpoint tables?

MICs or zone diameters between the S and R breakpoints given in the EUCAST breakpoint tables are within the “I category”. For example, measuring zone diameters to the nearest mm for breakpoints given as $S \geq 17$ mm, $R < 14$ mm, zone diameters ≥ 17 mm are susceptible, < 14 mm resistant, and therefore 14-16 mm within the “I category”.

4. EUCAST does not give breakpoints for oxacillin, cephalosporins and carbapenems for many staphylococci so how is susceptibility determined?

Susceptibility to these agents is inferred from the ceftiofur susceptibility. Some β -lactam agents may have activity against methicillin-resistant isolates in which case specific breakpoints are listed in the breakpoint table.

5. Why do breakpoints for nitrofurantoin relate to *E. coli* and not to other Enterobacterales?

Nitrofurantoin is recommended for treatment of uncomplicated urinary tract infection only. Urinary tract infections with Enterobacterales other than *E. coli* are more likely to be complicated or affect the upper urinary tract and hence they are excluded from recommendations. Also, ECOFFs for most other species are much higher than the ECOFF for *E. coli* rendering them as an expected resistant phenotype.

6. Why are there no tetracycline breakpoints for Enterobacterales?

The EUCAST Steering Committee did not set tetracycline breakpoints for Enterobacterales because it is no longer considered a clinically useful agent for treatment of patients with infections caused by Enterobacterales. We are aware that the agent is still sometimes used for prophylaxis and for this purpose an epidemiological cut-off value (ECOFF) for most Enterobacterales of 8 mg/L can be used to distinguish organisms with and without resistance mechanisms.

7. EUCAST has now established breakpoints for Enterobacterales and temocillin, but why is the breakpoint not valid for all species of Enterobacterales?

Temocillin recently received breakpoints for some but not all Enterobacterales. The rationale is given in the consultation document:

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2019/EUCAST_General_Consultation_on_temocillin_breakpoints_20191011.pdf

8. What happened to the PK/PD (non-species related) breakpoints?

Pharmacokinetics and pharmacodynamics (PK/PD) are important, but not the only tools for setting and revising clinical breakpoints. PK/PD targets are often based on a limited number of species. The selection of clinical PK/PD targets is highly dependent on the targeted patient population. Critically ill patients or immunocompromised patients will normally require higher antimicrobial exposure, and thus the PK/PD targets will be higher. As clinical PK/PD targets are often lacking, preclinical PK/PD targets determined in in vitro and animal models are often used. These models are not always validated with clinical data. Moreover, the animal models are usually limited to the neutropenic mouse thigh and lung infection model and may not have a translational value for all type of infections. Different PK/PD targets can be determined depending on i) the species, ii) the level of effect (stasis, 1-3 log kill, prevention of emergence of resistance), and iii) the within-species strain variation of PK/PD-targets.

Moreover, simulated pharmacokinetics (healthy vs. patients, different patient populations with different degree of renal/hepatic insufficiencies, levels of plasma proteins and other important covariates) will play a major role in determining PK/PD cut-offs. Critically ill patients have much higher variation in PK than other groups of patients. Calculations are usually made based on free drug concentrations in the plasma or epithelial lining fluid, which are presumed to relate to the concentration at the site of infection. Individual variations in protein binding may also affect the pharmacodynamically important drug exposure. Finally, PK/PD cut-offs may be based on various levels of probability of target attainment like 99%, 95% or 90%. All these factors may result in different PK/PD cut-off values that span in several two-fold dilutions.

A common misunderstanding is that PK/PD cut-offs can be used when clinical breakpoints are lacking. This is not the intention. Instead EUCAST has developed guidance on “When there are no breakpoints” (see <https://www.eucast.org/eucastguidancedocuments>) and removed the PK/PD cut-offs from the breakpoint tables. This is to underline that these values should never be used when clinical breakpoints are lacking.

9. For cefuroxime, the breakpoints for *Escherichia coli*, *Klebsiella* spp. (except *K. aerogenes*), *Raoultella* spp. and *Proteus mirabilis* relate only to high dosage (1.5 g x 3). What is the rationale for this?

With cefuroxime, PK/PD breakpoints are S \leq 4 mg/L and R $>$ 8 mg/L, the S breakpoint being based on a lower dose (0.75 g x 3) and the R breakpoint on a higher dose (1.5 g x 3). However, 4 mg/L falls in the middle of the wild type MIC distribution for *E. coli* and indicates that with a standard dosing regimen, patients would often be receiving marginal or inadequate treatment. The S breakpoint was moved to 8 mg/L to avoid splitting the wild type (which would result in poor reproducibility of susceptibility test results) and the high dosing regimen was specified to compensate for the raised breakpoint. The data on MIC distributions can be seen on the EUCAST MIC distribution website.

10. Why are breakpoints for trimethoprim restricted to certain species?

Trimethoprim breakpoints were reviewed in 2025. The previous breakpoints were poorly substantiated by either clinical data or PK/PD data and the new breakpoints are based on ECOFFs for *E. coli* and *Klebsiella* spp. For other species, there is insufficient clinical evidence of efficacy and/or lack of ECOFFs. For *Proteus* spp., the ECOFF can be used to exclude acquired resistance mechanisms (presence of resistance indicated by MICs $>$ 8 mg/L or trimethoprim 5 μ g disk zone diameter $<$ 14 mm).

11. Are you planning to give breakpoints for topical therapy with agents such as chloramphenicol, polymyxin B, tetracycline, neomycin and tobramycin?

Despite protracted discussions and two wide consultations on breakpoints for agents used topically, EUCAST has not set yet breakpoints for most topical agents. There are significant issues because for most topical agents it is not known what the free agent concentrations are at the site of infection, how long they are maintained or what variation there is in practice. For most agents there are no sound pharmacokinetic data and no data relating treatment to outcome other than anecdotal comment. Clinical breakpoints may not be appropriate and are not available for all agents used topically. The use of epidemiological cut-offs (ECOFFs) might underestimate the activity of some agents in topical preparations but would at least categorise isolates as wild type or non-wild type and would demonstrate reduced susceptibility, which may result in a higher probability of clinical failure. However, some felt strongly that use of ECOFFs, which are often close to

clinical breakpoints would be confusing. In the absence of clinical data on outcome related to MIC of infecting organisms EUCAST has been unable to reach a consensus that resolves the conflicting opinions on these two alternative proposals:

1. Use ECOFFs for all agents when used topically.
2. Use clinical breakpoints when available and ECOFFs when there are no clinical breakpoints.

A guidance document expanding the arguments for ECOFFs or clinical breakpoints has been released and gives both clinical breakpoints (when available) and ECOFFs for reference, <https://www.eucast.org/eucastguidancedocuments>.

12. Will EUCAST produce azithromycin breakpoints for *Salmonella* spp. and *Shigella* spp.?

Salmonella spp. and *Shigella* spp. with azithromycin is covered by the cut-off value of 16 mg/L. The corresponding zone diameter cut-off for the azithromycin 15 µg disk is 12 mm.

13. Which breakpoints should be used for non-fermenting Gram-negative rods other than *Pseudomonas* spp. and *Acinetobacter* spp.?

Breakpoints for *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* are available in the EUCAST Breakpoint Tables. Breakpoints for groups of organisms currently without specific breakpoints are being examined and, in the meantime, see the EUCAST Guidance Document "What to do when there are no breakpoints in the EUCAST Breakpoint Table" (<https://www.eucast.org/eucastguidancedocuments>).

Also, breakpoints for *Aeromonas* spp., glucose fermenting, an Enterobacterales genus, but oxidase positive, are listed separately in the EUCAST Breakpoint Tables.

14. Why has the nalidixic acid screen test for *Salmonella* isolates been removed from the clinical breakpoints and what should now be done?

There has been extensive discussion about screening for quinolone resistance in *Salmonella* spp., particularly low-level ciprofloxacin resistance. Nalidixic acid screening does not pick up all *qnr* positive isolates so it cannot be recommended alone as an indicator of ciprofloxacin susceptibility.

EUCAST recommends the use of the pefloxacin 5 µg disk for detection of ciprofloxacin resistance in *Salmonella* spp. (see EUCAST breakpoint table). This detects all currently known ciprofloxacin resistance mechanisms (*qnr*, QRDR and *aac'*) in *Salmonella* spp. and all isolates with MICs above the ECOFF (>0.06 mg/L). Hence, pefloxacin resistant isolates should be reported as ciprofloxacin resistant and pefloxacin susceptible isolates should be reported ciprofloxacin susceptible.

For more information, see Skov R. et al. 2015. Development of a pefloxacin disk diffusion method for detection of fluoroquinolone-resistant *Salmonella enterica*. J Clin Microbiol 53:3411–3417.

15. Can the ECOFF be used for ESBL detection and carbapenemase detection?

Yes, the ECOFF is the most sensitive phenotypic measurement to detect acquired resistance. However, it is not specific for ESBL and will detect several other mechanisms of 3rd generation cephalosporin resistance. Please refer to the EUCAST document on resistance mechanisms (https://www.eucast.org/resistance_mechanisms/).

16. Will EUCAST establish breakpoints for viridans group streptococci with agents used for urinary tract infections?

Viridans group streptococci in urine are most likely to be a result of contamination, and they very rarely cause urinary tract infection. Breakpoints for agents used for urinary tract infections are not likely to be produced by EUCAST. In identifying “viridans streptococci” from urine, it is important to ensure that they are not *Aerococcus* species, for which breakpoints are available.

17. What does "uncomplicated UTI only" mean for Enterobacterales and oral cephalosporins?

When setting breakpoints for oral cephalosporins and Enterobacterales EUCAST could not find clinical outcome evidence supporting use of these agents other than in uncomplicated UTI. These agents have low tissue levels and when PK/PD data are available it generally indicates that antimicrobial exposure is likely to be poor in systemic infections. See also the definition of “uncomplicated UTI” in the Notes sheet in EUCAST Breakpoint tables and the relevant guidance document, <https://www.eucast.org/eucastguidancedocuments>.

18. The nitrofurantoin breakpoints in the *Staphylococcus* spp. table refer to *S. saprophyticus* only. What would be your advice regarding the testing and interpretation of other *Staphylococcus* spp. from urines?

EUCAST advises against nitrofurantoin for staphylococci other than *S. saprophyticus*. Significant infections caused by *S. aureus* or coagulase-negative staphylococci other than *S. saprophyticus* are normally not just uncomplicated urinary tract infections and should not be treated with nitrofurantoin.

19. Why has the EUCAST clinical breakpoints for *Staphylococcus aureus* and mupirocin been removed?

The EUCAST breakpoint for *S. aureus* and mupirocin is available in the table on ECOFFs and breakpoints for topical agents (firstly included in the EUCAST Breakpoint Tables from version 7.0, 2017). More information on how to use these breakpoints is available on the EUCAST website (<https://www.eucast.org/eucastguidancedocuments>).

20. Some antimicrobial agents have comments on dosing regimens. Does the higher dosing regimen refer to the susceptible or the resistant breakpoint?

In EUCAST terms and terminology, the higher dose refers to the “Susceptible, increased exposure” category. See the dosing tab in the breakpoint table.

21. EUCAST notes that *E. faecium* resistant to penicillins can be considered resistant to all other β -lactam agents including carbapenems. Does this include amoxicillin-clavulanic acid?

Resistance to β -lactam agents in *E. faecium* is commonly mediated by modification or increased expression of PBPs. To our knowledge, β -lactamase-mediated resistance to penicillins has been described in *E. faecium* in very few publications. As most isolates of *E. faecium* are resistant to β -lactam agents because of the presence of alterations to PBPs, β -lactamase inhibitors would not restore susceptibility to ampicillin or amoxicillin. Isolates resistant due to β -lactamase only were apparently found in the Italian study as some appeared susceptible to ampicillin-sulbactam. Resistance mediated by β -lactamase has not been detected in major resistance surveillance studies in recent years and would

appear to be rare and geographically restricted. Also, there have been technical problems detecting resistance mediated by β -lactamase in enterococci; so the instruction that *E. faecium* resistant to penicillins can be considered resistant to amoxicillin-clavulanic acid is a cautious one. It may be necessary to revise this note if β -lactamase mediated resistance becomes more common.

22. For mupirocin: In the EUCAST breakpoint tables it says, "Breakpoints relate to nasal decolonization of *S. aureus*". For other *Staphylococcus* spp., is the intent to report an MIC only or to not report any result at all, especially since MIC distributions are shown for some coagulase-negative staphylococci?

Data on resistance mechanisms and clinical significance relate to *S. aureus* only, so report results for *S. aureus* only.

23. With EUCAST methods and breakpoints, several β -lactamase negative *Haemophilus influenzae* isolates are resistant to cefuroxime but susceptible to ampicillin. Can this be true?

EUCAST recommends use of the benzylpenicillin 1 unit disk to screen for β -lactam resistance in *H. influenzae*. The benzylpenicillin 1 unit disk is a sensitive marker for all types of β -lactam resistance, including both β -lactamases and different types of PBP mutations. If the benzylpenicillin zone is ≥ 12 mm, all β -lactams with clinical breakpoints can be reported susceptible (see the supplementary table in the EUCAST breakpoint table). Information on the benzylpenicillin screen is available in the EUCAST breakpoint table and on the EUCAST website: <https://www.eucast.org/bacteria/development-of-clinical-breakpoints-and-ecoffs/mic-zone-diameter-correlations/>.

The variety and multitude of PBP mutations in *H. influenzae* have increased over recent years. There are several different types of PBP mutations, some of which mainly affect penicillins (including ampicillin) and others mainly cephalosporins (and these usually have a particularly marked effect on cefuroxime). Cefuroxime is a sensitive marker for PBP mutations affecting cephalosporins. These mutations do not necessarily affect ampicillin or amoxicillin to the same degree.

24. Can breakpoints for *H. influenzae* be used for isolates of other species of *Haemophilus*?

EUCAST breakpoints have been defined for *H. influenzae* only, as clinical data relating to success or failure in treatment of infections caused by other *Haemophilus* species are scarce. MIC distributions for *H. parainfluenzae* are very similar to those for *H. influenzae*; so in the absence of specific breakpoints the *H. influenzae* breakpoints may be applied to this species. Disk diffusion criteria for *Haemophilus* other than *H. influenzae* have not been established.

25. Since we introduced EUCAST criteria in our lab, we always report cefuroxime axetil as "susceptible, increased exposure" for *H. influenzae*. Before, using the CLSI criteria, we usually reported *H. influenzae* isolates as susceptible to cefuroxime axetil. Can this agent be used with higher dosages? It is largely used in our region and our clinicians believe it to give satisfactory clinical results. What is the reason that isolates cannot be reported susceptible?

The activity of cefuroxime against *H. influenzae* is poor compared with the activity of many other beta-lactam agents and even with cefuroxime given intravenously it is doubtful whether effective concentrations are achieved in all patients. When EUCAST determined breakpoints for cefuroxime and cefuroxime axetil all aspects (MIC distributions, pharmacokinetics, pharmacodynamics, supporting clinical data and resistance mechanisms) were considered and there was no clinical evidence to support use of

cefuroxime axetil (or cefaclor) to treat respiratory infections or otitis media caused by *H. influenzae*. Clinicians may believe cefuroxime gives satisfactory clinical results because there is a high spontaneous cure rate in upper respiratory tract infection caused by *H. influenzae*, which makes it difficult to assess the effect of antimicrobial treatment.

Furthermore, with the increasing and now often high rates of chromosomally mediated (PBP3 mutations) beta-lactam resistance (beta-lactam resistance other than that caused by beta-lactamase) in *H. influenzae*, and the fact that this quite often affects cefuroxime (and cefuroxime axetil and cefaclor) more than other beta-lactams, empirical therapy with cefuroxime axetil should be avoided.

26. Why do breakpoints for nitrofurantoin relate to *Enterococcus faecalis* only and not to other *Enterococcus* spp, in particular *Enterococcus faecium*?

The MIC distributions of *E. faecalis* (ECOFF 32 mg/L) and *E. faecium* (ECOFF 256 mg/L) differ by at least three 2-folds dilutions. The breakpoint of $S \leq 64$ mg/L is appropriate for *E. faecalis* but would divide the *E. faecium* distribution in such a way that reproducible antimicrobial susceptibility categorisation would not be possible. This, in combination with the much lower intrinsic susceptibility of *E. faecium* and the lack of evidence of clinical efficacy, prompted EUCAST to exclude *E. faecium* from the breakpoint.

27. Why do benzylpenicillin breakpoints for staphylococci no longer apply to coagulase-negative staphylococci except *S. lugdunensis*?

The benzylpenicillin breakpoints for staphylococci were based on data for *Staphylococcus aureus* but were originally applied to all staphylococci as breakpoints for staphylococci normally apply to all *Staphylococcus* spp., with a few noted exceptions. New data have become available for species such as *S. lugdunensis* and the reliability of methods for detection of penicillinase has been shown to be poor, so the applicability of benzylpenicillin breakpoints to all species of *Staphylococcus* has been questioned.

Benzylpenicillin MIC breakpoints for *S. aureus* were principally based on separation of penicillinase-producers from penicillinase-negative isolates. The MIC breakpoint does not detect all penicillinase-producers and, to err on the side of caution, isolates shown to be penicillinase-producers should be reported resistant even when the MIC is below the breakpoint. Most methods for detection of penicillinase in staphylococci are unreliable, including widely used methods based on chromogenic cephalosporins. In *S. aureus*, the disk diffusion screening method based on sharp zone edges with penicillinase-producers has been shown to be reliable as long as care is taken when reading zones, but the method is not reliable for coagulase-negative staphylococci in general. With *S. lugdunensis* zone diameter breakpoints (as well as MIC breakpoints) will distinguish *blaZ* positive from *blaZ* negative isolates. *blaZ* genes encoding penicillinase in coagulase-negative staphylococci are variable and PCR methods may give different results depending on the primers used; so PCR methods cannot be taken as a reference method or the presence of *blaZ*. In addition, benzylpenicillin is unlikely to be an agent of choice for treating infections with coagulase-negative staphylococci except for *S. lugdunensis*, particularly as a large proportion of isolates is resistant.

The conclusion, based on currently available data, is that benzylpenicillin breakpoints are not applicable to coagulase-negative staphylococci other than *S. lugdunensis* and that there should be no requirement for such breakpoints.

28. We sometimes get susceptibility test results for *Haemophilus influenzae* that are susceptible for ampicillin but resistant for amoxicillin-clavulanic acid for beta-lactamase negative isolates with PBP3 mutations (positive in the benzylpenicillin disk screening test). How should we report these isolates?

Ampicillin-susceptible isolates should be reported susceptible for ampicillin, amoxicillin and amoxicillin-clavulanic acid. Aminopenicillin susceptibility tests on *H. influenzae* with altered PBPs are difficult and “Areas of Technical Uncertainties (ATUs) have been introduced to warn laboratories that results in some areas are uncertain. We recommend testing and reporting ampicillin for beta-lactamase negative isolates (from which amoxicillin and amoxicillin-clavulanic acid susceptibility can be inferred) and testing and reporting amoxicillin-clavulanic acid for beta-lactamase positive isolates.

29. Can moxifloxacin susceptibility of *Corynebacterium* spp. be inferred from the ciprofloxacin susceptibility?

Susceptibility of moxifloxacin can be inferred from the ciprofloxacin disk diffusion test result for *Corynebacterium* spp., but this will to some extent overcall moxifloxacin resistance.

30. What is the basis for EUCAST recommendations on reporting susceptibility of staphylococci and streptococci with dissociated resistance to clindamycin?

In staphylococci and streptococci, most resistance to macrolide, lincosamide, streptogramin type B (MLS_B) antibiotics is mediated by the *erm* genes and is induced by erythromycin, clarithromycin and azithromycin, but not by clindamycin (dissociated resistance or MLS_B inducible resistance). Hence inducible strains are resistant to erythromycin but not to clindamycin in antimicrobial susceptibility tests. Strains with MLS_B-constitutive resistance are resistant to both agents.

For many years there has been debate about whether staphylococci and streptococci with inducible clindamycin resistance (erythromycin-resistant, clindamycin-susceptible) should be reported resistant or susceptible as inducible strains segregate clindamycin resistant mutants, which may be selected during treatment, possibly leading to treatment failure.

Current opinion generally favours reporting staphylococci with dissociated resistance as resistant to clindamycin. In animal models of treatment with clindamycin there is regrowth of *S. aureus* strains with dissociated resistance to clindamycin. For *S. aureus* there are also references to clinical failures although it is not clear that clinical failures are common and it is probable that clindamycin may be used for less serious skin and soft tissue infections. It is therefore currently recommended that if dissociated resistance is detected the isolate should be reported resistant and consideration given to adding a comment to the report that clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as full resistance is unlikely to develop during such therapy.

The significance of inducible MLS_B resistance in streptococci is not so clear. In animal models of treatment with clindamycin there is regrowth of strains with dissociated resistance to clindamycin but to a lesser extent than seen with *S. aureus*. Clinical data for streptococci is rare although one recent report indicates that there may be treatment failures. EUCAST recommendations err on the side of caution and if dissociated resistance is detected the isolate should be reported resistant and consider adding a comment to the report that clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as constitutive resistance is unlikely to develop during such therapy. The significance of inducible resistance for combination therapy in severe infections with *S. pyogenes* is not known.

31. Why are there no daptomycin breakpoints for enterococci?

High-dose daptomycin has been thought to be effective in the treatment on enterococcal bloodstream infection and endocarditis, although published experience with the latter condition is limited. Although daptomycin is increasingly used for these conditions, especially when caused by vancomycin-resistant isolates, the EUCAST Steering Committee recognises that there are remaining uncertainties, particularly the inability of even the highest published doses (12 mg/kg/day) to achieve adequate exposure against all wild-type isolates of *E. faecalis* and *E. faecium*. The documented variation in susceptibility testing amplifies these uncertainties. Therefore, EUCAST has not proposed clinical breakpoints for daptomycin and *Enterococcus* species, but rather listed the breakpoint as “IE” = Insufficient Evidence with a referral to a Guidance document. In part, this decision is influenced by the dosing regimen that is required for bloodstream far exceeds that of the regimen licensed by EMA. More detailed guidance, including the use of high dosages, can be found at <https://www.eucast.org/eucastguidancedocuments>.

32. How can antimicrobial susceptibility tests be done on clinical isolates or agents for which there are no EUCAST breakpoints?

There are some bacterial groups and antimicrobial agents for which EUCAST has not yet determined breakpoints.

Breakpoints for new agents will be set as the agents go through the marketing approval application to the EMA and are released if the agent is granted approval. Breakpoints for some older agents may be set when a convincing need is established (e.g. nitroxoline and temocillin). There are also some less common organism groups (e.g. *Nocardia* spp.) for which breakpoints may eventually be determined.

There are some agents and organism groups where there may never be breakpoints. This mainly relates to older agents which have been replaced by more modern agents with clear advantages (greater activity, improved pharmacokinetics or reduced toxicity) over older agents in the same group. For example, this is the case for the aminoglycoside kanamycin, the quinolone sparfloxacin, the macrolide josamycin and the cephalosporin cefalothin. It is also less likely that breakpoints will be set for rarely isolated species such as *Erysipelothrix rhusopathiae*, *Campylobacter* spp. other than *C. jejuni* and *C. coli*, and groups for which there are difficulties in devising reproducible testing conditions such as *Acinetobacter* spp. for cephalosporins and *Stenotrophomonas maltophilia* for many agents.

In the absence of a breakpoint it will not be possible to proceed with assessment based on phenotypic testing unless a trustworthy and reproducible MIC value can be obtained for the isolate. If an MIC value can be obtained, guidance on interpretation is available in the guidance document “When there are no breakpoints”, see <https://www.eucast.org/bacteria/guidance-documents/>.

When no breakpoints are available, it is useful to ascertain whether the MIC for the isolate is consistent with the wild type MIC distribution for the species. Access the EUCAST MIC distribution website (http://www.eucast.org/mic_distributions_and_ecoffs/) and enter either the name of the species or of the agent. If you find a distribution which matches the relevant species (or that of a species related to the species in question) and agent you will be able to decide whether or not the MIC belongs to the wild type or not. If the MIC is consistent with the wild type, comparison can be made with other species for which a clinical categorization of the wild type already exists (i.e. breakpoints have already been determined) in order to interpret, with caution, the MIC for the relevant isolate. For example, assume you aim to find out whether or not an isolate of *Arcanobacterium haemolyticum* is susceptible to erythromycin. The MIC is determined as 0.5 mg/L. When displaying erythromycin MIC distributions on the EUCAST MIC distribution website you will at present not find data on *Arcanobacterium haemolyticum*, but you will discover that

all Gram-positive bacteria considered susceptible to erythromycin exhibit wild type MIC distributions below 1 mg/L and mostly below 0.5 mg/L. Hence it is reasonable to assume that your isolate is likely to be susceptible to erythromycin.

33. Why does EUCAST not recommend a beta-lactamase test before reporting penicillins as susceptible for enterococci while CLSI insists on that?

The EUCAST breakpoints are determined to disclose the presence of a significant resistance mechanism and thus there is no need for a beta-lactamase test until a penicillin/ampicillin resistant isolate has been detected.

34. What is the difference between “Susceptible, increased exposure” and “Susceptible Dose Dependent” (SDD) as defined by CLSI for cefepime?

All breakpoints are dose (or rather exposure) dependent. Hence, “susceptible increased exposure” is a more appropriate term than SDD and since 2019, this category has replaced “intermediate” in EUCAST documents.

35. How should we test and interpret results for *Staphylococcus saccharolyticus*?

Staphylococcus saccharolyticus is an anaerobic coagulase-negative staphylococci and should be tested according to the methodology for anaerobic bacteria, i.e. MIC determination. If a commercial method is used, follow the manufacturer’s instructions. MICs should be interpreted according to the Guidance document “When there are no breakpoints”, see <https://www.eucast.org/bacteria/guidance-documents/>.

36. What is the meaning of the new “I” susceptibility testing interpretive category and how shall we handle it in the laboratory?

EUCAST has decided to change the definitions of susceptibility testing categories S, I and R as shown below. Results of several consultations on the new definitions are available on the EUCAST website under “Consultations”.

S - Susceptible, standard dosing regimen: A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.

I – Susceptible, increased exposure*: A microorganism is categorised as "Susceptible, Increased exposure*" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

R - Resistant: A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

*Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

The new definitions are clearly related to agent exposure of the organism which is in turn related to dose, dosing frequency (including changing from repeated administration to intravenous infusion), route of administration, and to the pharmacokinetics of the agent and sometimes to the type of infection (urinary tract infections vs. meningitis).

Dosing and modes of administration related to S, I and R of agents are available in the last TAB of the EUCAST breakpoint table. See the EUCAST website (<http://www.eucast.org/newsiandr/>).

37. What is the meaning of the Area of Technical Uncertainty (ATU) and how shall we handle it in the laboratory?

ATUs are warnings to laboratories when there is technical or interpretative uncertainty. The ATU does not interfere with interpretation to S, I or R. The warning can be ignored or dealt with. ATUs are established in areas with uncertain results and are not related to a specific susceptibility category. ATUs can be a zone diameter interval, an MIC value or both.

How to deal with results within ATU depends on the situation. The type of sample (blood culture vs. urine culture), the number of alternative agents available, the severity of the disease, whether or not a consultation with clinical colleagues is feasible, will influence the action taken. See EUCAST Breakpoint Tables, tab “Technical Uncertainty” and EUCAST guidance document on ATU (<https://www.eucast.org/eucastguidancedocuments>) for more guidance.

38. How shall we interpret tigecycline test results for Enterobacterales other than *Escherichia coli* and *Citrobacter koseri*?

For these species, the wild-type MICs are not covered by a standard dosing regimen. There is also no approved high-dose, but for those who want to target treatment of such species more guidance can be found in the EUCAST guidance document regarding tigecycline dosing: <https://www.eucast.org/eucastguidancedocuments>.

39. Are EUCAST breakpoints for *Staphylococcus aureus* valid also for other coagulase-positive staphylococci?

For coagulase-positive species other than *S. aureus* (*S. argenteus*, *S. schweizeri*, *S. intermedius*, *S. pseudintermedius* and *S. coagulans*) there is limited information on the performance of breakpoints for most agents. Where such information exists, specific breakpoints are provided. For *S. argenteus*, breakpoints for *S. aureus* can be used without caveats.

40. Why doesn't EUCAST recommend screening for BORSA (borderline oxacillin resistant *Staphylococcus aureus*)?

Occasionally oxacillin MIC values are high in *S. aureus* in absence of *mec*-gene mediated resistance. These isolates have been called BORSA (borderline oxacillin resistant *S. aureus*). EUCAST does not recommend systematic screening for BORSA since the clinical significance of these strains is still in doubt many years after they were first described. Oxacillin, as many other beta-lactam agents but less so ceftazidime, are partially unstable to staphylococcal beta-lactamases.

41. The breakpoints for Enterobacterales and piperacillin-tazobactam were changed in 2021. Why was the “I category” removed?

Analysis of PK/PD data and clinical data did not provide clear support for maintaining an I group. To be able to cover 16 mg/L, one would need to dose 4 g q6h plus use extended infusion time. This dosing practice is not well established and additionally there are uncertainties about clinical outcomes at this MIC level. Finally, there are methodological challenges with the area of technical uncertainty which makes a breakpoint at 16 mg/L particularly challenging to work with.

42. How shall we report cefiderocol for *Acinetobacter* spp. and *Stenotrophomonas maltophilia*?

For both of these species, cefiderocol can be reported according to the guidance in the EUCAST Breakpoint Tables, which includes cut-off values for MIC and disk diffusion.

43. What does breakpoints in brackets mean in the EUCAST Breakpoint Table?

Breakpoints in brackets distinguish between isolates without and with phenotypically detectable resistance mechanisms. They are based on ECOFFs but since they may serve more than one species, the value may represent a best fit. For these agents, clinical evidence as monotherapy is usually lacking but for a specific indication or in combination with another active agent or measure they may still be used. Isolates with resistance can be reported R (resistant). Reporting S or I should be avoided and if considered necessary, there should be a comment to explain the need for adjunctive measures as mentioned above. For more information, see EUCAST Guidance Document on “Breakpoints in brackets in breakpoint tables” at <https://www.eucast.org/bacteria/guidance-documents/>.

44. Why do breakpoints for fosfomycin oral relate to *E. coli* and not to other Enterobacterales?

A scrutiny of PK/PD data in urine and clinical data has demonstrated that the UTI dose is only sufficient to cover *E. coli*, whereas other Enterobacterales, such as *Klebsiella* spp., are considerably less susceptible with high ECOFFs.

45. With the new EUCAST breakpoints for anaerobic bacteria, several breakpoints are considerably lower than the previous, non-species specific breakpoints. Because of this some resistance frequencies went from low to high – for example meropenem resistance in *Bacteroides fragilis* (from 5 to 17%). Why is this?

In the very beginning all bacteria (Gram-positive and Gram-negative) had the same breakpoints for any agent. Also, it was never specified whether or not a breakpoint was related to a defined species, disease or dose. This was inexact.

Later we learnt that different species behave differently and needed different breakpoints. The same is true for anaerobe species.

In routine laboratories in the past, anaerobes were difficult to grow, difficult to identify, difficult to test and resistance development was not much of a problem. Now, with MALDI-TOF technology, we can easily identify different species and subspecies and discover that their MIC distributions differ. For aerobic bacteria each species is evaluated separately, and now the turn has come to anaerobes. We also look for evidence to support the treatment of wild type isolates with respective agent, and for the evidence and PK/PD to suggest that not only wild type isolates but also some non-wild type isolates can be treated “provided the MIC is not greater than X or Y mg/L”. This is exactly what we agreed was needed for aerobic species and we have no reason to deal with anaerobes differently.

We have identified a medium which can be obtained from several manufacturers, and which will grow most/many anaerobes so developing MIC distributions and zone diameter distributions to make sure we can correlate the one to the other seems appropriate and in line with what we have done for aerobic bacteria over the last almost 20 years.

Older higher breakpoints were influenced by the thinking in the late 1990ies and early 2000. Clinical breakpoints were set to cover all the different species; this of course left large gaps between wild type isolates (ECOFFs) of several species and the breakpoints,

and no one really knows whether the non-wild type isolates when they gradually appear will be treatable or not.

46. How shall we test and report anaerobic bacteria belonging to species which don't have breakpoints in EUCAST Breakpoint Tables?

There is guidance on this in the document "When there are no breakpoints in the EUCAST tables", see <https://www.eucast.org/eucastguidancedocuments>. Perform an MIC according to the manufacturer's instructions and interpret according to the recommendations in the guidance document. Disk diffusion cannot be used.

47. For *Streptococcus agalactiae* (GBS) infections, can phenoxymethylpenicillin, for which there are no EUCAST breakpoints, be used instead of an aminopenicillin or benzylpenicillin, for which EUCAST has breakpoints?

EUCAST has not been able to find published data to support the clinical use of phenoxymethylpenicillin and therefore has no recommendation for the use or non-use of the agent in *Streptococcus agalactiae* infections. Benzylpenicillin wild type isolates will be devoid of resistance mechanisms to phenoxymethylpenicillin.

48. How were the staphylococcal breakpoints for clindamycin selected? There is no rationale document available to explain this.

In general, PK/PD information on this agent is very limited. Two papers provide some useful information: LaPlante et al., AAC 2008; 52:2156-62, and Klepser et al., AAC 1997; 41:630-5. They suggest that clindamycin is effective in vivo against strains of *S. aureus* without inducible resistance to MLSb agents. They do not however provide any information of the magnitude of the $fAUC_{24}/MIC$ target, which is a major consideration in setting breakpoints. It is for this reason that there are no PK/PD breakpoints for clindamycin (or indeed any of the MLSb group of agents) listed in EUCAST Breakpoint Tables. Instead, they are listed as "IE" = insufficient evidence. In these circumstances, EUCAST falls back on other data, such as that found in the above-mentioned papers, and the ECOFF, which for *S. aureus* is 0.25 mg/L.

49. What is the rationale for the new comment recommending treatment alternatives to carbapenems in Enterobacterales with confirmed carbapenemases?

In general, several papers suggest that if carbapenemase-production is detected there is an uncertainty in clinical outcomes when using carbapenems. Moreover, there are papers suggesting that high exposure should be used when treating carbapenemase-producers, and that combination therapy may be preferable to monotherapy. Finally, contrary to the situation in 2018 when carbapenem breakpoints were last reviewed, there are now several agents available that are recommended in clinical guidelines for treatment of carbapenemase-producing microorganisms. For these reasons, it is justified to include a warning comment to be sent out with the antibiogram, which highlights the caveats of using carbapenems to treat infections caused by carbapenemase-producing microorganisms. A proposed comment to be used is the following – only to be used if the isolate is S or I (if R, carbapenems should not be considered for therapy). For the exact wording of the comment, see EUCAST Breakpoint Tables, at <https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/>.

50. Why are there no carbapenem breakpoints for *Aeromonas*?

At the time that breakpoints were set, there was much discussion around the role of carbapenems against *Aeromonas*, because most of the species harbour chromosomal 'carbapenemases', usually metallo-beta-lactamase enzymes. Also, there is no treatment guideline that supported to use of carbapenems for *Aeromonas* infections.

51. Why does EUCAST have breakpoints for *Bacteroides* spp, vs. ampicillin-sulbactam and amoxicillin-clavulanic acid but not for ampicillin and amoxicillin?

Aminopenicillins without beta-lactamase inhibitors are rarely active against *Bacteroides* spp. and EUCAST has refrained from setting breakpoints for ampicillin and amoxicillin without inhibitors.

52. Why has EUCAST removed the recommendation to infer susceptibility for dalbavancin from the vancomycin susceptibility for staphylococci?

Resistance to glycopeptides and lipoglycopeptides in staphylococci is rare, but there are reports on isolates being resistant to dalbavancin and susceptible to vancomycin for both *S. aureus* and coagulase-negative staphylococci. Also, glycopeptide and lipoglycopeptide MICs are method dependent and should be determined with broth microdilution according to ISO 20776-1. To get reliable MICs for dalbavancin, EUCAST therefore recommends to use broth microdilution and to test dalbavancin to report dalbavancin susceptibility.

53. Why has EUCAST removed the recommendation to infer susceptibility for piperacillin-tazobactam from the ampicillin susceptibility for enterococci?

Enterococci with wild-type MICs for ampicillin can have non-wild type MICs for piperacillin-tazobactam. This is true also for *E. faecalis*, which is the only *Enterococcus* species with breakpoints for piperacillin-tazobactam in EUCAST Breakpoint Tables. Therefore, EUCAST recommends to test piperacillin-tazobactam to report piperacillin-tazobactam susceptibility in *E. faecalis*.

7. Breakpoints – zone diameter**1. EUCAST does not give zone diameter breakpoints for macrolides other than erythromycin. How is susceptibility determined?**

Susceptibility to erythromycin is used to infer susceptibility to other macrolides.

2. What does "IP" mean in the breakpoint tables?

In the EUCAST tables, a few zone diameter breakpoints are replaced with "IP" (in preparation). This means that breakpoints are being developed and will be given in a later version of the breakpoint table.

3. Why do some antimicrobial agents have susceptible zone diameter breakpoints of ≥ 50 mm?

A zone diameter breakpoint of "S ≥ 50 mm" is an arbitrary "off scale" zone diameter breakpoint used to signify that EUCAST clinical breakpoints do not recognise any organisms in the category "susceptible, standard dosing regimen" within the species, i.e. wild type organisms are categorised as "susceptible, increased exposure".

4. Can the results from the pefloxacin screening test for *Salmonella* spp. be used to infer susceptibility to fluoroquinolones other than ciprofloxacin?

The pefloxacin screening test has been shown to detect fluoroquinolone resistance due to QRDR mutations and plasmid-mediated resistance as *qnr* and *aac6* in *Salmonella* spp., but ciprofloxacin is the only agent for which EUCAST has set specific breakpoints for *Salmonella* spp..

5. Can the pefloxacin screening test be used to screen for fluoroquinolone resistance in species other than *Salmonella* spp.?

The pefloxacin 5 µg breakpoint used to screen for clinical fluoroquinolone resistance in *Salmonella* spp., can also be used to detect fluoroquinolone resistance mechanisms in other Enterobacterales such as *E. coli*, *K. pneumoniae* and *Shigella* spp. See the latest version of the EUCAST Breakpoint Tables for information on for which Enterobacterales species screening with pefloxacin is recommended.

6. Can EUCAST zone diameter breakpoints for *Campylobacter jejuni* and *C. coli* be used for other *Campylobacter* species?

No. EUCAST zone diameter breakpoints for *Campylobacter* spp. are valid only for *Campylobacter jejuni* and *C. coli*. For other species, determine the MIC.

7. Why has EUCAST changed the cefoxitin screen for *Staphylococcus epidermidis* and coagulase-negative staphylococci?

The EUCAST recommendations to categorise coagulase-negative staphylococci (CNS) as susceptible or resistant to methicillin pre-suppose that CNS are identified to the species. For laboratories that do not identify CNS to the species, breakpoints of $S \geq 25$ mm, $R < 25$ mm can be used, with an ATU (Area of technical Uncertainty) of 22-24 mm. For isolates with results inside the ATU: identify species, perform PCR for *mecA/mecC* or report resistant.

8. Will EUCAST establish fosfomycin zone diameter breakpoints for Enterobacterales other than *Escherichia coli*?

Following review of fosfomycin breakpoints, EUCAST breakpoints apply only to *E. coli* (see https://www.eucast.org/fileadmin/eucast/pdf/public_consultations/2023/EUCAST_General_Consultation_on_Fosfomycin_IV_20230712.pdf and EUCAST guidance document on the use of iv fosfomycin, <https://www.eucast.org/bacteria/guidance-documents/>)

9. Will EUCAST establish RAST zone diameter breakpoints for Enterobacterales other than *Escherichia coli* and *Klebsiella pneumoniae*?

The RAST method was developed for the most important and commonly isolated blood stream infection pathogens and agents. Since the RAST method was first published (November 2018), we have added breakpoints for *Acinetobacter baumannii*, *Salmonella enterica* and for additional agents. Breakpoints for additional agents and additional species are under consideration, but the RAST method will never cover all species and agents with breakpoints for standard methodology.

10. Why should we read benzylpenicillin 1 unit zone diameters for *Streptococcus pneumoniae* only for isolates with oxacillin 1 µg zone diameters <20 mm?

The oxacillin 1 µg disk reliably differentiates between *S. pneumoniae* without and with beta-lactam resistance mechanisms. For benzylpenicillin, the correlation between MICs and zone diameters is generally good, but there is overlap between isolates being susceptible and “susceptible, increased exposure” with benzylpenicillin disk diffusion. Therefore, EUCAST recommends to use the oxacillin 1 µg disk to predict susceptibility and the benzylpenicillin 1 unit disk to differentiate between “susceptible, increased exposure” and resistant in isolates with oxacillin 1 µg zone diameters <20 mm.

8. Quality control

1. Where can I get EUCAST quality control strains?

Control strains can be obtained from national culture collections (ATCC, NCTC, CIP, CCUG etc.). They are also sold in various convenient formats by companies supplying materials for antimicrobial susceptibility testing.

2. How often should quality control strains be tested?

Internal quality control (QC), using the EUCAST recommended strains, should be performed daily, or at least four times per week. No more than 1 in 20 consecutive tests should be outside control limits.

The frequent routine QC is needed to control both the materials and equipment used (media, disks, incubators etc) and the procedure (inoculum preparation, inoculation of plates, incubation and reading of zones). If QC is performed less regularly, problems related with media, disks or incubators will not be detected until after the results for many clinical isolates have been reported.

3. Can I use EUCAST quality control strains for quality control of automated systems?

Effective QC requires strains with MICs within the dilution ranges used in the automated system. Suitable strains should be provided by the manufacturers.

4. Where can I find reference susceptibility distributions for comparison with the distributions from our laboratory?

Reference distributions for both MICs and zone diameters with data from several sources are available from the EUCAST website, see <https://www.eucast.org/bacteria/mic-and-zone-distributions-ecoffs/>.

5. Many automated systems recommend the use of QC organisms for which the expected MIC range is not within the range on the AST panel. The ISO recommendations suggest that MICs for at least one QC organism should be within the panel MIC range. It is very difficult to accept QC results which have < or > because the QC organism MIC is not within the scale of the MIC range on the panel.

We agree. MIC test ranges in any method, including those in automated systems, should include the MIC range specified for the control strain, otherwise the control is ineffective. If MIC ranges are restricted, as in most automated systems, alternative QC organisms with MICs within the test range should really be used. In practice this is a problem as it requires multiple QC organisms to cover different agents. The current situation is that an

off-range control is a qualitative control with undefined sensitivity for detection of errors, and hence is a very poor control.

6. Why are there sometimes discrepancies between the MIC ranges for quality control recommended by EUCAST and CLSI?

In principle, there should be no differences between EUCAST and CLSI QC ranges for MICs. Both are based on the ISO standard 20776-1, and now EUCAST and CLSI collaborate to update QC ranges when needed. Publication of updates is not coordinated which may explain discrepancies. However, when test conditions differ, QC ranges for MICs and zone diameters may differ. For disk diffusion, main differences are related with antibiotic disk contents (potencies) and in some cases with media (e.g. for fastidious organisms).

7. How should we control penicillin beta-lactam-beta-lactamase inhibitor combination disks?

A beta-lactamase producing strain is needed to control the inhibitor component of beta-lactam-beta-lactamase inhibitor combination disks. The active component is controlled by a standard susceptible strain. For current recommendations, see EUCAST QC Tables, <https://www.eucast.org/bacteria/methodology-and-instructions/disk-diffusion-and-quality-control/>.

8. When should we perform the EUCAST QC procedure for Rapid AST from positive blood cultures (RAST)?

The EUCAST RAST QC procedure should be performed when implementing the RAST method in the laboratory, when training new staff and with any change in the system (e.g. blood culture system, disk or media manufacturer). QC with EUCAST standard disk diffusion methodology is performed to control the materials and equipment used and the procedures for disk diffusion in the laboratory. See http://www.eucast.org/rapid_ast_in_blood_cultures/ for more information.

9. How shall we control antimicrobial agents for which there are no EUCAST QC criteria for a strain of the same (or a similar) species as the organism to be tested, e.g. azithromycin for Enterobacterales or piperacillin-tazobactam for enterococci?

There is guidance on principal QC strains in the EUCAST QC Tables, see <https://www.eucast.org/bacteria/methodology-and-instructions/disk-diffusion-and-quality-control/>. If specific information is missing in that table, EUCAST recommends to test a QC strain using the same medium as the organism to be tested, e.g. using *S. aureus* ATCC 29213 for QC of azithromycin MIC and the combination of *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 for QC of piperacillin-tazobactam zone diameters.

9. Other questions

1. EUCAST breakpoints indicate a fixed concentration of beta-lactamase inhibitor for all beta-lactam beta-lactamase inhibitor combinations. Is this valid for MICs only and what is the reason for this?

The fixed concentration of inhibitor applies to MICs only. Clearly there is no way it can apply to disks if both agents are incorporated into the disks.

Historically, there has been a discrepancy with beta-lactamase inhibitor combinations regarding whether a fixed concentration of inhibitor or ratio of inhibitor to active agent is

tested. For amoxicillin-clavulanic acid and ampicillin-sulbactam a ratio has generally been used, whereas for piperacillin-tazobactam and ticarcillin-clavulanic acid a fixed concentration of inhibitor has been used. There is no logical reason for this difference and it is now widely accepted that a fixed concentration of inhibitor is appropriate and this approach is applied to all new inhibitor combinations. While some groups have retained the ratio for amoxicillin-clavulanic acid and ampicillin-sulbactam for continuity with historical data, EUCAST felt strongly that the error should not be perpetuated and testing should be changed to a fixed concentration. The objective is to determine whether the MIC of the active agent is changed by the presence of the inhibitor. The ratio of amoxicillin:clavulanic acid differs in different pharmaceutical preparations and there is not a fixed 2:1 ratio in the patient at the site of infection. Using a ratio means that as the MIC of the active agent increases, the concentration of inhibitor increases beyond any clinically achievable concentration.

This is also valid for MIC gradient tests and only MIC gradient tests with a fixed inhibitor concentration can be used for EUCAST MIC determination.

For more information, see EUCAST guidance document "Setting breakpoints for agent-inhibitor combinations" at <https://www.eucast.org/bacteria/guidance-documents/>.

2. Will EUCAST recommend standardised phenotypic/genotypic methods for confirming carbapenemase-producing strains?

See "EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance" on the EUCAST website (http://www.eucast.org/resistance_mechanisms/).

3. How should the laboratory respond to frequent updates from EUCAST?

EUCAST has, from 2012, published one update of the breakpoint tables per year. A preliminary version of the tables for comments will be published at the beginning of December and a final version will be published on the 1st of January each year. All changes from the previous table will be highlighted in pale yellow. All EUCAST News can be followed, and subscribed to (at no cost), via the News feed on the EUCAST website.

4. What does the abbreviation ND on the EUCAST MIC and zone diameter distribution website mean?

ND means that the ECOFF value is "Not Defined". This may be because there are too few isolates in the distribution or the data are not considered sufficiently reproducible or clear enough to set an ECOFF. Additional distributions are continually being added to the database and distributions are reviewed in the light of new data. Following such review ECOFFs may be defined in place of the ND designation.

See also EUCAST SOP 10, <https://www.eucast.org/eucastsops>.

5. According to the EUCAST breakpoint tables, MICs of beta-lactam beta-lactamase inhibitor combinations are determined using a fixed concentration of the inhibitor. Are MIC gradient tests available with a fixed concentration of inhibitor?

Some manufacturers offer gradient tests with a fixed concentration of the inhibitor along the strip. As with disk diffusion, both the beta-lactam (parent) agent and the beta-lactamase inhibitor, will diffuse into the agar from the strip, forming a gradient with gradually decreasing concentrations of both components. Manufacturers have been

instructed to calibrate their products against reference methodology (broth microdilution according to ISO 20776-1).

6. Why has the "other streptococci" group been replaced by "viridans group streptococci" and how do we deal with non-haemolytic isolates?

In the EUCAST breakpoint tables the tab "Other streptococci" was changed to "Viridans group streptococci" as the latter is a more scientific description. In practice the organisms intended to be included have not changed. The viridans group is a large group of species (over 30), including the *S. salivarius*, *S. bovis*, *S. mitis*, *S. mutans* and *S. anginosus* groups, each of which includes multiple species. Several of the species included in the viridans group may be non-haemolytic. Others are predominantly alpha-haemolytic and indeed some in the anginosus group may be beta-haemolytic. Most clinically significant non-haemolytic streptococci will be viridans group. In the EUCAST Breakpoint Table (v. 6.0, 2016), information on species included in the viridans group streptococci table has been added.

7. Does EUCAST have any advisory role with regards to the development of automated AST systems for companies?

EUCAST has no advisory role in the development of commercial AST systems. However, EUCAST does comment on AST systems and we make it clear that it is the responsibility of commercial companies to ensure that their systems are compliant with EUCAST guidelines, including reference methods.

8. EUCAST is not consistent in the use of abbreviations of two-fold dilution concentrations. How should we interpret a microorganism with an MIC of 0.125 against a EUCAST breakpoint listed as $S \leq 0.12$ mg/L?

By international convention MIC dilution series are based on twofold dilutions up and down from 1 mg/L. At dilutions below 0.25 mg/L, this leads to concentrations with multiple decimal places. There is some variation in the abbreviations used for some of these dilutions, largely depending on how dilution series for MIC determination are prepared. EUCAST has decided to use the following abbreviations, which are the mathematically correct abbreviations. The agreed terminology will be implemented as EUCAST documents are updated.

Actual concentration (mg/L)	EUCAST terminology	Alternatives used elsewhere
0.125	0.125	0.12
0.0625	0.06	0.064
0.03125	0.03	0.032
0.015625	0.016	0.015
0.0078125	0.008	-
0.00390625	0.004	-
0.001953125	0.002	-

9. In the EUCAST breakpoint table it is suggested that erythromycin can be included in the susceptibility test of viridans group streptococci to detect the presence of inducible clindamycin resistance, despite the lack of erythromycin breakpoints. How is this possible?

The erythromycin 15 µg disk is included in the susceptibility test only to detect inducible clindamycin resistance as a flattening of the clindamycin inhibition zone adjacent to the erythromycin disk. Erythromycin susceptibility cannot be interpreted from the erythromycin zone diameter.

10. EUCAST recommends MH-F broth for broth microdilution testing of streptococci, but the ISO standard 20776-1 states that Mueller-Hinton broth with 2.5-5% lysed horse blood should be used. Why is there a difference?

EUCAST has decided to recommend the same broth for streptococci and *Haemophilus influenzae* (in analogy with the solid MH-F medium): the MH-F broth (Mueller-Hinton broth with 5% lysed horse blood (LHB) and 20 mg/L β-NAD for broth microdilution of fastidious organisms (<https://www.eucast.org/bacteria/methodology-and-instructions/media-preparation/>). The β-NAD is not needed and has no effect on the streptococci and the 5% LHB is within the ISO recommendation.

There is no indication that the addition of 20 mg/L beta-NAD in any way affects MICs for streptococci and pneumococci.

Varying the concentration of LHB (2.5 or 5%) might have a small effect, but this has not been evaluated further. We are aware of that a lower LHB concentration (2.5%) facilitates the reading of results for most automated or semi-automated systems, but the higher LHB concentration (5%) is advantageous to promote growth of fastidious organisms such as *H. influenzae*, and manual reading is not difficult with the MH-F broth.

In summary, the EUCAST recommendation is to use MH-F broth for all fastidious organisms. Differences in MICs for streptococci when using MH-F or CAMBH with 2.5% LHB has so far been insignificant.

11. How shall we test a *Staphylococcus aureus* that does not grow when using standard disk diffusion methodology (un-supplemented Mueller-Hinton (MH) agar in air)?

The rare *S. aureus* (or other bacteria with the same recommendation) that does not grow when using standard methodology can be tested on MH and MH-F (MH with 5% defibrinated horse blood and 20 mg/L β-NAD) agar in CO₂, but one must be aware there may be small differences in zone sizes for several agents. For example, incubation in CO₂ affects the pH. Aminoglycosides and macrolides are less active at a lower pH, whereas tetracycline and fusidic acid are more active at a lower pH. Zones for highly protein bound agents (fusidic acid) may be smaller on MH-F but zones for most agents are affected very little by blood. If results are borderline when using non-standard recommendations such as CO₂ or MH-F, be cautious about interpretation.

12. Why does EUCAST advise against the use of MIC gradient tests for colistin MIC testing?

There have been several reports of the inadequacy of MIC gradient tests to correctly predict colistin susceptibility and resistance. EUCAST has evaluated colistin MIC testing methods using a challenge collection of 75 Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp.). In addition to gradient tests from two manufacturers, commercial broth microdilution (BMD) methods and disk diffusion were evaluated. Based on the results in this study, EUCAST has issued a warning against the use of gradient tests for colistin MIC determination (<http://www.eucast.org/warnings/>).

Results in summary:

- The broth microdilution tests we have evaluated all seem to give correct results both for susceptible and non-susceptible isolates, although some of them may benefit from further improvements.
- Disk diffusion cannot be used for susceptibility testing of colistin. It does not discriminate between susceptible and resistant isolates.
- Currently available gradient tests underestimate colistin MIC values and hence resistance, and should be avoided, even when quality control results are within range
- Quality control of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and the colistin resistant *E. coli* NCTC 13846 (mcr-1 positive). For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only occasionally be 2 or 8 mg/L.

13. We know that the zone diameter can be different between different laboratories. Do you take this point when you establish quality control (QC) criteria, zone diameter breakpoints and import data in the EUCAST reference database?

When we establish EUCAST QC criteria and zone diameter breakpoints, we use data produced with disks and media from more than one manufacturer and data produced by different laboratories to make sure that the criteria are robust. The EUCAST procedures for establishing zone diameter breakpoints and QC criteria for new antimicrobial agents are available in SOP 9 (<https://www.eucast.org/eucastsops>).

All zone diameter reference distributions in the EUCAST database (http://www.eucast.org/mic_distributions_and_ecoffs/) are based on data produced with materials from several manufacturers and by several technicians, but for some organism-agent combinations, the data is produced by few laboratories. Data from additional laboratories are added when available.

14. Is the expert rules document (v 2.0) still valid after publication of the expected susceptible (v_1.1) and expected resistant phenotypes (v_1.1 and v_1.2) documents?

Expert rules were extensively revised and were published in February 2020 as version 3.2. They were broken down into different files for different microorganisms with the same logic as the breakpoint tables and can be found on EUCAST webpage at <https://www.eucast.org/bacteria/important-additional-information/expert-rules/>.

15. Why has EUCAST abandoned the terms “intrinsic resistance” and “unusual resistance phenotype” and uses “expected resistant phenotypes” and “expected susceptible phenotypes” instead?

For many years EUCAST and other committees have struggled with the term “intrinsic resistance”. However, there is no agreed definition of this term and since breakpoints are always “exposure dependent” it is hard to agree on a definition which will survive changes in dosing, modes of administration and a sudden willingness to accept a new and higher level of toxicity because of a lack of alternatives. One advantage of defining a species as an “expected resistant phenotype” or “expected susceptible phenotype” in relation to an agent or class of agents, is that susceptibility testing becomes unnecessary and it allows colleagues to report the isolate as resistant and susceptible, respectively, without having performed a test. Also, it informs us of important characteristics, shortcomings and assets, of agents.

EUCAST has decided to replace the term “intrinsic” with the terms “expected susceptible phenotype” and “expected resistant phenotype”. For a species to be included in the “expected resistant phenotype”, 90% or more should be considered resistant (*Klebsiella pneumoniae* vs. ampicillin is an example). For a species to be included in the “expected susceptible phenotype” the wild type should be considered susceptible (S or I) to the agent and a very high proportion (99%) of isolates should be devoid of acquired resistance to the agent (*Streptococcus pyogenes* vs. benzylpenicillin is one example). In both cases, susceptibility testing is best avoided. A result which goes against the expected phenotype should be viewed with suspicion. See the latest versions of expected resistance phenotypes and expected susceptible phenotypes at EUCAST website <https://www.eucast.org/bacteria/important-additional-information/expected-phenotypes/> and in this publication: Gatermann S et al. Expected phenotypes and expert rules are important complements to antimicrobial susceptibility testing. Clin Microbiol Infect. 2022 Jun;28(6):764-767. doi:10.1016/j.cmi.2022.03.007.

16. How can we test fosfomycin, daptomycin and dalbavancin/oritavancin/telavancin, for which EUCAST recommends supplements to the Mueller-Hinton media?

Both EUCAST and CLSI recommend supplementation of the Mueller-Hinton media with glucose-6-phosphate for fosfomycin, Ca²⁺ for daptomycin and polysorbate-80 for dalbavancin/oritavancin/telavancin.

For commercial products (e.g. disks, gradient tests or freeze-dried broth microdilution panels), the supplement should be included in the test product. For example, fosfomycin disks and gradient tests should be supplemented with glucose-6-phosphate, and the antimicrobial susceptibility testing is performed on regular Mueller-Hinton agar. When preparing fresh or frozen broth microdilution panels, the supplements must be part of the broth containing the antimicrobial solution according to the instructions in ISO 20776-1.

17. How does EUCAST recommend to calculate categorical agreement with the “new” I category “susceptible, increased exposure”?

Following the change of the definition of the I group, the EUCAST two susceptible categories (S and I) obviate the traditional placements of minor discrepancy, major discrepancy, and very major discrepancy. For this reason, new interpretations of categorical discrepancies have been developed:

New EUCAST interpretations of categorical agreement

'True' susceptibility category	Tested and reported susceptibility category	Characterization of discrepancy	Rationale	Potential consequences
S	S	No discrepancy	Correct report	None
I	I	No discrepancy	Correct report	None
R	R	No discrepancy	Correct report	None
S	I	mD	Both are susceptible categories; the consequence is an unnecessary increased exposure	Increased risk of adverse reactions; Increased cost of agent and/or administration
S	R	MD	False resistance removes a therapeutic option	Therapeutic alternative(s) may be more expensive and/or more toxic
I	S	MD	Both are susceptible categories; the consequence is suboptimal antimicrobial exposure	Increased risk of therapeutic failure and resistance selection
I	R	MD	False resistance removes a therapeutic option	Therapeutic alternative(s) may be more expensive and/or more toxic
R	S	VMD	False susceptibility may have serious consequences	Risk of continuing or switching to ineffective therapy with increased risk of therapeutic failure
R	I	VMD	False susceptibility may have serious consequences	Risk of continuing or switching to ineffective therapy with increased risk of therapeutic failure

EUCAST, European Committee on Antimicrobial Susceptibility Testing; I, susceptible, increased exposure; MD, major discrepancy; mD, minor discrepancy; R, resistant; S, susceptible, standard dosing regimen; VMD, very major discrepancy.

For more information, see Turnidge et al, CMI, 2025, “How to: the application and analysis of categorical agreement in antimicrobial susceptibility testing using European Committee on Antimicrobial Susceptibility Testing breakpoints”.