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22. What does the "uncomplicated UTI only" mean for Enterobacteriaceae and cephalosporins?

23. 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?

24. Why has the EUCAST breakpoints for Staphylococcus aureus and mupirocin been removed?

25. Some antimicrobial agents have comments on dosages. Does the higher dose refer to the susceptible or the resistant breakpoint?

26. 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?

27. 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?

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

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

30. Since we introduced EUCAST criteria in our lab, we always report cefuroxime axetil as intermediate 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 it cannot be reported susceptible?

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

32. For S. pneumoniae, how should we report benzylpenicillin for meningitis in cases where the MIC is ≤0.06 mg/L but oxacillin zone diameter is <20mm?

33. Why do benzylpenicillin breakpoints staphylococci no longer apply to coagulase-negative staphylococci?

34. We sometimes get susceptibility test results for Haemophilus influenzae that are susceptible for ampicillin but resistant for amoxicillin-clavulanic acid. How should we report these isolates?
35. Can moxifloxacin susceptibility of *Corynebacterium* spp. be inferred from the ciprofloxacin susceptibility?

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

37. Why are there no daptomycin breakpoints for enterococci?

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

39. Why are breakpoints for ceftazidime-avibactam higher than breakpoints for ceftazidime alone?

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7. Breakpoints – zone diameter

1. Does EUCAST have zone diameter breakpoints equivalent to non-species-related breakpoints?

2. EUCAST does not give zone diameter breakpoints for macrolides other than erythromycin. How is susceptibility determined?

3. What does “IP” mean in the breakpoint tables?

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

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

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

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

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

9. Will EUCAST establish fosfomycin zone diameter breakpoints for Enterobacteriaceae other than *Escherichia coli*?

8. Quality control

1. Where can I get EUCAST quality control strains?

2. How often should quality control strains be tested?

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

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

5. Many automated systems recommend the use of QC organisms which do not measure the expected MIC range on-scale with that on the AST panel. The ISO recommendations suggest that at least one QC organism should be measured on the panel MIC range. It makes it very difficult to accept QC results of < or > because the QC organism MIC is not measure on the scale of the MIC range on the panel. How should we deal with this?
6. Why are there sometimes discrepancies between the CLSI and EUCAST MIC ranges for the same quality control strain?

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

9. Other questions

1. EUCAST breakpoints indicate a fixed concentration of beta-lactamase inhibitor for piperacillin-tazobactam, amoxicillin-clavulanic acid and ampicillin-sulbactam. Is this valid for MICs only and what is the reason for this?

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

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

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

5. According to the EUCAST breakpoint tables, MICs of amoxicillin-clavulanic acid must be tested with a fixed concentration of clavulanic acid (2 mg/L). Can gradient tests be done with a fixed concentration of clavulanic acid?

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

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

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≤0.12 mg/L?

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?

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?

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)?

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

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

14. Is the expert rules document (v2.0) still valid after publication of intrinsic resistant and resistant exceptional phenotype tables (v3.1)?

15. How is an “exceptional resistance phenotype” defined and why have the “exceptional susceptible phenotypes” been removed in the new expert rules document (v3.1)?
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. We have tested batches of Mueller-Hinton agar from four manufacturers (BBL, Oxoid, bioMérieux and Bio-Rad) repeatedly and have evaluated other media occasionally. We have also tested batches of pre-poured commercial MH-F (Mueller-Hinton Fastidious organisms; which is Mueller-Hinton agar with 5% mechanically defibrinated horse blood and 20 mg/L β-NAD) from the manufacturers mentioned above. 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.

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.

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?

No. All breakpoints are standardised and calibrated for Mueller-Hinton agar with 5% mechanically defibrinated horse blood and 20 mg/L β-NAD and are not valid if another medium is used. Haemophilus strains do not grow on Mueller-Hinton agar with 5% sheep blood and 20 mg/L β-NAD.

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 ≥ 98%.
6. Can MH-F be used as medium for gradient tests?

See the manufacturers’ instructions for information on which products are validated for MH-F.

Growth of anaerobes and Neisseria gonorrhoeae is frequently insufficient on MH-F and we recommend that testing should be performed using other media according to the gradient test manufacturer’s instructions.

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. Plates can be dried 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.

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 quality control and breakpoint tables. Alternative disk contents cannot be used when using EUCAST criteria.

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?
No. The EUCAST disk diffusion method is based on use of 0.85% saline for inoculum preparation.

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 measure and record inhibition zones when first changing to the EUCAST disk diffusion method. 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. Following that, templates calibrated to EUCAST breakpoints may be used as an alternative to measuring zones. Zones for control tests should always be measured and recorded.

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 (and gradient tests) do sometimes produce colonies inside the zone of inhibition. Interpretation of mecillinam tests for E. coli (but not for other Enterobacteriaceae) is based on the obvious zone diameter and individual 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) or plates being too heavily inoculated, which
facilitates emergence of resistant mutants. Performing daily QC is the best way
to detect possible loss of activity of the antimicrobial disks.

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 http://www.eucast.org/ast_of_bacteria/guidance_documents/). A method for direct
disk diffusion testing from positive blood cultures is under development.

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.

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 MIC method specifies that temperature should be within the range
34-37°C, a compromise to accommodate the option of setting incubators at 35 or
36°C and a ± 1°C variation.

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.

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 does not have clinical breakpoints or expert rules specifically for veterinary use. Human clinical breakpoints may be inappropriate for veterinary isolates, which may be from a variety of animals. 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 Intermediate category 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 Intermediate. For example, measuring zone diameters to the nearest mm for breakpoints given as S ≥17 mm, R<14 mm, zone diameters ≥17 mm are susceptible, <14 mm resistant, and therefore 14-16 mm intermediate.
4 EUCAST does not give breakpoints for oxacillin, cephalosporins and carbapenems for staphylococci so how is susceptibility determined?

Susceptibility to these agents is inferred from the cefoxitin 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 Enterobacteriaceae?

Nitrofurantoin is recommended for treatment of uncomplicated urinary tract infection only. Urinary tract infections with Enterobacteriaceae other than E. coli are more likely to be complicated or affect the upper urinary tract and hence they are excluded from recommendations.

6 Why are there no tetracycline breakpoints for Enterobacteriaceae?

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

7 Is trimethoprim-sulfamethoxazole the only available agent for Stenotrophomonas maltophilia?

For Stenotrophomonas maltophilia the only antimicrobial agent with clinical correlation between MICs and clinical outcome is trimethoprim-sulfamethoxazole (cotrimoxazole). It may be that in the future breakpoints are set for more agents, but current literature does not clearly indicate another agent for which it is reasonable to determine breakpoints. See guidance note on “Stenotrophomonas maltophilia”, http://www.eucast.org/ast_of_bacteria/guidance_documents/.

8 Will EUCAST establish breakpoints for Enterobacteriaceae and temocillin?

Temocillin has been discussed by the EUCAST Steering Committee on several occasions in the past and very recently and there are issues that have delayed the breakpoint setting process.

MIC distributions indicate that the ECOFFs for most Enterobacteriaceae are 16 mg/L or less. The MIC distribution for ESBL-producers overlaps the wild-type distribution. Many ESBL-producers are inhibited by 8-16 mg/L temocillin, most by 32 mg/L but for some MICs are higher. PK-PD data indicate that the lower dose licensed in the UK (1 g twice daily) is inadequate to treat systemic infections and the higher dose (2 g twice daily) is needed even to cover isolates with MIC 8 mg/L, which cuts into the wild type and does not cover many ESBL-producers. There are some issues with the PK-PD studies and Eumedica are supporting additional work at present. The BSAC set a systemic susceptible breakpoint of 8 mg/L based on the above data and noted that the high dose (2 g twice daily) should always be used. Clinical data on the treatment of blood stream infections are very limited but Balakrishnan et al has published (J Antimicrob Chemother 2011; 66):2628-31.) positive outcomes for treatment of most infections caused by isolates with MICs 8-16 mg/L, although numbers were very small.
Eumedica are promoting a higher dose of 2g three times daily and is applying for marketing approval for this dose, initially in Belgium, Luxemburg and France and then in the UK, although we have no dates for this. PK-PD studies with the higher dose might be expected to cover isolates with MIC 16 mg/L.

EUCAST has decided to wait for the additional PK-PD data (expected later this year) and some indication of the progress with regulatory authorities on the 2g three times daily dose before setting breakpoints. In the meantime, there are national breakpoints from the UK (BSAC), France and Belgium.

9 Are the PK/PD breakpoints (formerly called “non-species-related MIC breakpoints”) in the breakpoint tables of any use in the routine clinical laboratory?

The PK/PD breakpoints are based on pharmacokinetic and pharmacodynamics data only. They are used as the basis for determination of clinical breakpoints but clinical breakpoints may be modified from PK/PD breakpoints in the light of microbiological or clinical data. They may be used in the routine clinical laboratory with odd microorganisms for which there are no breakpoints, meaning that if there is a species or group of organisms which is not included or is not mentioned elsewhere in the breakpoint tables you can determine the MIC and then interpret the MIC on the basis of the PK/PD breakpoints. This gives some idea about the usefulness of the agent in question. If possible, the MIC should also be compared with the MIC distribution for the species (available from the EUCAST MIC distribution website). Such comparison will indicate whether or not the isolate is likely to express any phenotypically detectable resistance mechanism.

More information is available in the Guidance Document “What to do when there are no breakpoints in the EUCAST Breakpoint Table” (http://www.eucast.org/guidance_documents/).

10 For cefuroxime, the breakpoints for *E. coli*, *Klebsiella* spp. and *P. 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.

11 Why shouldn't I use cefuroxime in the higher dosage for other Enterobacteriaceae besides *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis* when they appear susceptible in susceptibility tests?

When setting harmonised breakpoints, cefuroxime was one of the most contentious and some countries would not accept that any Enterobacteriaceae should be reported susceptible to cefuroxime because its activity is so marginal. The compromise accepted by most was to restrict use to the most susceptible (and most common) species and to base reports on high dose therapy only.

In the current version of the breakpoints (see EUCAST website for latest version)
Klebsiella spp. and Proteus mirabilis are included with Escherichia coli in the cefuroxime note. During the breakpoint setting process the efficacy of cefuroxime for anything other than clearly uncomplicated infections was questioned. It was decided that an "uncomplicated" infection would be caused primarily by these species and that for other, less commonly isolated species the documentation on clinical efficacy was poor or non-existent.

12 Why is the breakpoint for trimethoprim given for all Enterobacteriaceae while nitrofurantoin is only for Escherichia coli? Both are for uncomplicated urinary tract infections only.

It is true that both nitrofurantoin and trimethoprim are for uncomplicated UTI only and that Enterobacteriaceae other than E. coli are more likely to be associated with complicated UTI. However, the activity of trimethoprim against Enterobacteriaceae is relatively uniform and the breakpoint creates no problems with reproducibility of antimicrobial susceptibility test results. E. coli is specified as the only target for nitrofurantoin because the activity of nitrofurantoin against Klebsiella pneumoniae is problematic as MICs often straddle the E. coli breakpoint, thereby making interpretation unreliable. Furthermore, the activity of nitrofurantoin against Proteus spp. and Providencia spp. is poor.

13 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, http://www.eucast.org/ast_of_bacteria/guidance_documents/.
14 Will EUCAST produce azithromycin breakpoints for *Salmonella* and *Shigella*?  
*Salmonella* spp. with azithromycin is covered by the epidemiological cut-off values (ECOFFs) of 16 mg/L. The corresponding zone diameter ECOFF is 12 mm. For *Shigella* spp. the ECOFF could also be applied but there are currently insufficient data to set the cut-off value.

15 Which breakpoints should be used for non-fermenting Gram-negative rods other than *Pseudomonas* spp. and *Acinetobacter* spp.?  
Breakpoints for groups of organisms currently without specific breakpoints are being examined and in the meantime, for practical purposes, application of the PK/PD breakpoints is recommended. For further information, see the EUCAST Guidance Document “What to do when there are no breakpoints in the EUCAST Breakpoint Table” ([http://www.eucast.org/guidance_documents/](http://www.eucast.org/guidance_documents/)).

16 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 mutants 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.

If nalidixic acid is used, any resistant isolates can be categorised as resistant to ciprofloxacin. Susceptible isolates need to be tested further by ciprofloxacin MIC determination.


17 EUCAST states in the breakpoint tables that the cephalosporin breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including ESBL and plasmid mediated AmpC). Does this mean that there is no need for additional testing for these mechanisms and to report susceptibility as found?  
EUCAST, like CLSI, recommends that susceptibility is reported "as found" in relation to Enterobacteriaceae and beta-lactams. Hence there is no need to detect resistance mechanisms for clinical reporting. However, there may be good arguments for detecting and characterising resistance mechanisms for public health or infection control purposes. See EUCAST guidance on detection of resistance mechanisms [http://www.eucast.org/resistance_mechanisms/](http://www.eucast.org/resistance_mechanisms/).
18 Can the ECOFF be used for ESBL detection and carbapenemase detection?  ▲

Yes, the ECOFF is the most sensitive phenotypic measurement. For ESBL screening, use cefotaxime AND ceftazidime ECOFFs and for carbapenemase screening use the meropenem ECOFF. See the “EUCAST guidelines for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance” (http://www.eucast.org/resistance_mechanisms/).

19 Benzylpenicillin breakpoints for Streptococcus pneumoniae are dosing regimen specific; how do we report these? Do all notes associated with the breakpoints need to be reported?  ▲

The National Antimicrobial Susceptibility Testing Committee (NAC) should decide which of the listed dosages is most often used in the country for treating pneumonia and recommend that laboratories use the breakpoints valid for this dosing regimen.

20 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.

21 If a pneumococcal strain is susceptible to penicillin, it can be reported susceptible to all beta-lactams, but if the strain is intermediate or resistant to penicillin what can I say about amoxicillin and amoxicillin-clavulanic acid?  ▲

If an isolate is intermediate or resistant to benzylpenicillin, or resistant in the oxacillin screen test it should be tested for susceptibility to ampicillin. Susceptibility to amoxicillin and amoxicillin-clavulanic can be inferred from the results of the ampicillin susceptibility test (no isolates producing beta-lactamase have ever been reported). For isolates categorised as intermediate to ampicillin oral treatment with ampicillin, amoxicillin or amoxicillin-clavulanic acid should be avoided.

22 What does "uncomplicated UTI only" mean for Enterobacteriaceae and oral cephalosporins?  ▲

When setting breakpoints for oral cephalosporins and Enterobacteriaceae 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 response is likely to be poor in systemic infections. Despite this there may be situations in which systemic treatment is successful and if EUCAST is provided with clinical outcome evidence supporting use to treat infections other than uncomplicated UTI we shall review the breakpoints. See also guidance document, http://www.eucast.org/ast_of_bacteria/guidance_documents/.
23 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.

24 Why has the EUCAST 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 (http://www.eucast.org/guidance_documents/).

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

The comment on the higher dose may refer to either of the breakpoints. When there is an intermediate category for systemic infections, the higher dose refers to this category. This is briefly explained in the rationale documents (http://www.eucast.org/documents/rd/) on the EUCAST website. There is a tab in breakpoint tables “Dosages” with dosing regimens that were used to establish EUCAST breakpoints.

26 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 only two publications, one from the USA in 1992 (Coudron et al 1992; AAC 36: 1125-6) and one from Italy in 2012 (Sarti et al 2012; 50: 169-72). 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.
27  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.

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28  With EUCAST methods and breakpoints, several β-lactamase negative *Haemophilus influenzae* isolates are resistant to ceffuroxime 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: [http://www.eucast.org/ast_of_bacteria/calibration_and_validation/](http://www.eucast.org/ast_of_bacteria/calibration_and_validation/).

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 ceffuroxime). Cefuroxime is a sensitive marker for PBP mutations affecting cephalosporins. These mutations do not necessarily affect ampicillin or amoxicillin to the same degree.

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29  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.

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30  Since we introduced EUCAST criteria in our lab, we always report ceffuroxime axetil as intermediate for *H. influenzae*. Before, using the CLSI criteria, we usually reported *H. influenzae* isolates as susceptible to ceffuroxime 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 ceffuroxime against *H. influenzae* is not good compared with the activity of many other agents and even with ceffuroxime given intravenously it is doubtful whether effective concentrations are achieved in all patients. When EUCAST determined breakpoints for ceffuroxime and ceffuroxime 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 ceffuroxime axetil (or cefaclor) to treat pulmonary infections or
otitis media caused by *H. influenzae*. Clinicians may believe cefuroxime gives satisfactory clinical results because there is a relatively 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 rates of chromosomally mediated 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 probably be avoided. Exacerbations in patients with COPD are often caused by a general invasion of upper respiratory flora (*H. influenzae, H. parainfluenzae, M. catarrhalis, S. pneumoniae* and others) and it is not easy to ascertain which of these should be the target of treatment. Possibly most patients benefit, at least in the short term, from any antimicrobial agent which can reduce bacterial counts, and it may be that this is the effect that your clinicians are registering.

### 31 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* (median MIC 8) and *E. faecium* (median MIC 64 or higher) differ by at least three 2-folds dilutions. The breakpoint of S≤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.

### 32 For *S. pneumoniae*, how should we report benzylpenicillin for meningitis in cases where the MIC is ≤0.06 mg/L but oxacillin zone diameter is <20mm?

The first step would be to repeat both tests. If you do not have time to repeat both oxacillin disk diffusion testing and the MIC determination (in meningitis you would not), we suggest the following: If the quality of your MIC determination is good (broth microdilution according to ISO standard) and well calibrated/quality controlled, trust the MIC and report “S”. If you are not certain about your MIC test, go with the worst-case scenario and report “R”, especially if the oxacillin zone is not borderline. The oxacillin disk diffusion test is generally a very reliable screen for β-lactam resistance in *S. pneumoniae*.

### 33 Why do benzylpenicillin breakpoints for staphylococci no longer apply to coagulase-negative staphylococci?

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.

34 We sometimes get susceptibility test results for *Haemophilus influenzae* that are susceptible for ampicillin but resistant for amoxicillin-clavulanic acid. 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 the zone diameter breakpoints are under revision. In the meantime, 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.

See also response to Breakpoints – general, item 28.

35 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.

36 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 in 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 \textit{S. aureus} strains with dissociated resistance to clindamycin. For \textit{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\textsubscript{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 \textit{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 \textit{S. pyogenes} is not known.

37 \textbf{Why are there no daptomycin breakpoints for enterococci?}

Pharmacokinetic-pharmacodynamic studies and limited clinical data indicate a low probability of successful treatment of serious infections caused by wild type enterococcal isolates when the licensed dosages of daptomycin (4 mg/kg/day) are used. Hence breakpoints are not appropriate. However, if daptomycin MICs are confirmed to be \( \leq 4 \) mg/L infections may be successfully treated with high doses of daptomycin (at least 8 mg/kg/day). (For more information, see [http://www.eucast.org/ast_of_bacteria/guidance_documents/](http://www.eucast.org/ast_of_bacteria/guidance_documents/)).

38 \textbf{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. \textit{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
**Erysopelothrix 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 may be available from the EUCAST rationale document or the EUCAST breakpoint table (www.eucast.org), where there may be PK-PD-based breakpoints that can be applied.

When no PK-PD breakpoints are available (because PK-PD data were not available for the agent when it as originally assessed) 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://mic.eucast.org/Eucast2) 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.

This is further explained in the EUCAST Guidance Document “What to do when there are no breakpoints in the EUCAST Breakpoint Table” (http://www.eucast.org/guidance_documents/).

See also response to Breakpoints – general, item 9.

**39 Why are breakpoints for ceftazidime-avibactam higher than breakpoints for ceftazidime alone?**

All breakpoints are related to the dosing regimen of the agent. The dosing regimen is significantly different between the two agents: ceftazidime alone has a standard regimen of 1 g x 3 per day (high dose 2 g x 3) infused over 0.5 h, while the standard regimen for ceftazidime-avibactam is 2 g + 0.5 g x 3 per day, infused over 2 h (extended infusion). Doubling the dose and increasing infusion time will translate to coverage of two additional MIC dilutions.

**40 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.
41 What is the difference between intermediate and “Susceptible Dose Dependent” (SDD) as defined by CLSI for cefepime?

One difference is that CLSI has defined SDD for cefepime only, whereas the principle in EUCAST has always been to consider Intermediate equivalent to “susceptible with the right dosing regimen”. From a semantic point of view, all susceptibility is dose dependent. Hence, “susceptible high-dose” would have been a more appropriate term than SDD. This term has been considered by EUCAST, but the decision was to use the intermediate category to capture this situation and not to modify a term that has been used from many years.

7. Breakpoints – zone diameter

1 Does EUCAST have zone diameter breakpoints equivalent to PK/PD breakpoints?

The breakpoints in the PK/PD table are MIC breakpoints only. There are no equivalent zone diameter breakpoints.

2 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.

3 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.

4 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 susceptible organisms within the species, i.e. wild type organisms are categorised as intermediate.

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

The pefloxacin screening test has so far been validated only for *Salmonella* spp. The clinical breakpoints for other Enterobacteriaceae and fluoroquinolones have been reviewed and revised in 2017 (EUCAST Breakpoint Table v 7.1). For other Enterobacteriaceae, the respective agent should be tested and reported.

7 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.

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

In the EUCAST Breakpoint Tables version 7.0, new zone diameter criteria for cefoxitin 30 µg were published for categorising coagulase-negative staphylococci (CNS) as susceptible or resistant to methicillin and most other beta-lactams. These recommendations pre-suppose that CNS are identified to the species. This resulted in some uncertainty and questions of interpretation. The Steering Committee (SC) reviewed the data and elected to revise the zone diameter criteria for *S. epidermidis*, as well as the wording of accompanying Note B. The revised version was published in the EUCAST Breakpoint Tables version 7.1.

For laboratories that do not identify CNS to the species, the SC decided to align the zone diameter criteria for all CNS with that of *S. epidermidis* (S≥ 25 mm, R< 25 mm). This is a compromise which correctly categorise *S. epidermidis* but which leave a small percentage of meca-containing *S. epidermidis* categorised as susceptible. These isolates have been further investigated and mostly contain non-expressed meca genes", a phenomenon previously shown to exist in *S. aureus*. For laboratories that identify CNS to the species, S≥ 22 mm, R< 22 mm should be used for all CNS except *S. epidermidis*.

9 Will EUCAST establish fosfomycin zone diameter breakpoints for Enterobacteriaceae other than *Escherichia coli*?

Work is on-going to investigate whether it is possible to develop a reproducible disk diffusion method and establish fosfomycin zone diameter breakpoints for *Klebsiella pneumoniae* and *Staphylococcus aureus*.

8. Quality control

1 Where can I get EUCAST quality control strains?

Control strains can be obtained from national culture collections (ATCC, NCTC, CIP, 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 (www.eucast.org) under “MIC distributions & ECOFFs” or “Zone diameter 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 with the point you make about QC. 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 principal, 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 disc charge and in some cases with media.
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.

9. Other questions

1 EUCAST breakpoints indicate a fixed concentration of beta-lactamase inhibitor for piperacillin-tazobactam, amoxicillin-clavulanic acid and ampicillin-sulbactam. 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 gradient tests and only gradient tests with a fixed inhibitor concentration can be used for EUCAST MIC determination. See also Other questions, item 5.

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

In 2013, the “EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance” was made available on the EUCAST website (http://www.eucast.org/resistance_mechanisms/). This document is currently under revision and will be submitted for publication soon.
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 (RSS) flow 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 10.0, http://www.eucast.org/documents/sops/.

5 According to the EUCAST breakpoint tables, MICs of amoxicillin-clavulanic acid must be tested with a fixed concentration of clavulanic acid (2 mg/L). Can gradient tests be done with a fixed concentration of clavulanic acid?

There is no reason why gradient MIC tests on amoxicillin cannot be done with a fixed concentration of clavulanic acid, in the same way that piperacillin-tazobactam is tested with a fixed concentration of tazobactam. At present, amoxicillin-clavulanic acid gradient test strips with a fixed concentration of clavulanic acid are not available from all manufacturers. Gradient test strips with a 2:1 ratio of amoxicillin:clavulanic acid cannot be used in place of a fixed concentration as MICs may be lower with the 2:1 ratio, particularly with more resistant isolates. Gradient tests with a fixed concentration of inhibitor for amoxicillin-clavulanic acid and ampicillin-sulbactam are available from at least one manufacturer.

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≤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.

<table>
<thead>
<tr>
<th>Actual Concentration (mg/L)</th>
<th>EUCAST terminology</th>
<th>Alternatives used elsewhere</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>0.125</td>
<td>0.12</td>
</tr>
<tr>
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<td>0.06</td>
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<td>-</td>
</tr>
<tr>
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<td>0.004</td>
<td>-</td>
</tr>
<tr>
<td>0.001953125</td>
<td>0.002</td>
<td>-</td>
</tr>
</tbody>
</table>

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.
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 gradient tests for colistin MIC testing?

There have been several reports of the inadequacy of 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 labs. 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 9.0 ([http://www.eucast.org/documents/sops/](http://www.eucast.org/documents/sops/)).

All zone diameter reference distributions in the EUCAST database ([http://www.eucast.org/mic_distributions_and_ecoffs/](http://www.eucast.org/mic_distributions_and_ecoffs/)) are based on data produced with materials from several manufacturers and by several technichians, 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 (v2.0) still valid after publication of intrinsic resistant and resistant exceptional phenotype tables (v3.1)?

The tables listing exceptional phenotypes were recently updated. The other parts of the expert rules document (v 2.0) are currently under revision. The new version is expected during 2018.

15 How is an “exceptional resistance phenotype” defined and why have the “exceptional susceptible phenotypes” been removed in the new expert rules document (v3.1)?

EUCAST is aware of the fact that what is considered an “exceptional phenotype” at one point in time may not be exceptional at a later point and that what is considered exceptional in one geographical area may not be exceptional in another. The need for repeated revision of the document is therefore self-evident.