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**Breakpoints – general**


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

3. Why are there two different breakpoints for testing *Enterobacteriaceae* for ampicillin?

4. What are the EUCAST breakpoints for the intermediate category as none are given in the EUCAST breakpoint tables?

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

6. Why do breakpoints for nitrofurantoin relate to *E. coli* and not to other *Enterobacteriaceae*?

7. Why are there no tetracycline breakpoints for *Enterobacteriaceae*?

8. For *Stenotrophomonas maltophilia* is trimethoprim-sulfamethoxazole the only available agent?

9. Why are there ciprofloxacin and ofloxacin breakpoints for *S. pneumoniae*?

10. Are non-species-related MIC breakpoints (PK/PD) in the breakpoint tables usable in the routine clinical laboratory?

11. For cefuroxime, the breakpoint relates only to high dosage (1.5 g x 3). What is the rationale for this?

12. 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?

13. 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.

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

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

16. Which breakpoints should we use for non-fermenting Gram-negative rods other than *Pseudomonas* spp. and *Acinetobacter* spp.?

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

18. Why are there no levofloxacin breakpoints for *Enterococcus* spp. when it is used for treatment of uncomplicated urinary tract infections?
19. 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?

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

21. Is it really not possible to report the susceptibility of *S. agalactiae* to trimethoprim for isolates from urinary tract infection? According to EUCAST we should report as them as resistant (- in the clinical breakpoint table).

22. Benzylpenicillin breakpoints for *Streptococcus pneumoniae* are dosage specific, how do we report these? Do all notes associated with the breakpoints need to be reported?

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

24. 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 can I say about amoxicillin and amoxicillin-clavulanic acid?

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

26. 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?

27. What about breakpoints for *Aeromonas hydrophila*? Should I use breakpoints for Enterobacteriaceae or non-species related MIC breakpoints?

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

29. EUCAST notes that *E. faecium* resistant to penicillins can be considered resistant to all other beta-lactam agents including carbapenems. Does this include amoxicillin-clavulanate?

30. 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 staphylocooci?

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

32. There is no EUCAST recommendation on how to screen for high-level aminoglycoside resistance in viridans group streptococci. Can we use the criteria for enterococci?

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

34. 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 drug 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?

**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?

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?

Other questions

1. The new EUCAST standard indicates a fixed concentration of beta-lactamase inhibitor for piperacillin-tazobactam, amoxicillin-clavulanate 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?
EUCAST Frequently Asked Questions

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) and will evaluate another (MAST) during the first part of 2010. We have also tested batches of MH-F (Mueller-Hinton Fastidious organisms; Mueller-Hinton agar with 5% horse blood and 20 mg/L β-NAD) from the manufacturers mentioned above. Whatever the source of medium, inhibition zones of EUCAST recommended strains for internal quality control must be within the ranges published by EUCAST. /2010-05-03

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 (affecting tests on several agents, particularly aminoglycosides) and limits of thymidine and thymine content (affecting tests on trimethoprim and trimethoprim-sulfamethoxazole). Mueller-Hinton II agar is manufactured to contain low levels of thymidine and thymine and controlled levels of calcium and magnesium ions. Today, all Mueller-Hinton agars for susceptibility testing should be produced to meet the current CLSI performance standard. Therefore, all Mueller-Hinton agars that yield inhibition zones within the acceptable range for EUCAST recommended strains for internal quality control can be used. /2010-05-03

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

Growth and inhibition zone diameters for antibiotics used in routine work have to be checked on each new batch of Mueller-Hinton agar. Use EUCAST recommended strains for internal quality control. Inhibition zone sizes 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 thymine/thymidine levels. /2010-05-03

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% 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. /2010-05-03

5 Which β-NAD should we use? ▲

We have evaluated β-NAD batches with a purity of ≥ 95% from seven manufacturers. All yielded similar growth and inhibition zone sizes, but we recommend the use of a β-NAD with a purity of ≥ 98%. /2010-05-03
6 Can MH-F be used as medium for gradient tests?

We have evaluated MH-F as medium for Etest and M.I.C.Evaluator and did not obtain any systematic differences compared with recommended media. Trimethoprim-sulfamethoxazole may be problematic on blood-containing media.

Manufacturers of gradient tests have also been validating their products for testing on MH-F. For information on which products are validated for MH-F, see the EUCAST file “Compliance of manufacturers of susceptibility testing devices and materials”.

Growth of anaerobes and *Neisseria gonorrhoeae* is frequently insufficient on MH-F and we recommend that testing should be performed according to the current manufacturer’s instructions. /Revised 2013-04-24

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**EUCAST disk diffusion test - Disks**

1 Are EUCAST disk contents all the same as CLSI?

Most are the same but several are different and required contents for the EUCAST method are defined in the EUCAST method description and breakpoint tables. Alternative disk contents cannot be used. /2010-05-03

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**EUCAST disk diffusion test - Inoculum preparation**

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

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 could be compared visually with that of a McFarland 0.5 turbidity standard but this is less reliable than using a photometric device. It is not possible to judge the turbidity with the naked eye without a turbidity standard for comparison. /2010-05-03

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. /2010-05-03

3 Should we pick more than one colony to be sure that we do not miss heteroresistance?

Picking multiple colonies is not essential and will not affect detection of heteroresistance, but may avoid selection of 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. /2010-05-03
4 Can we use water or buffer instead of saline for inoculum preparation?
No. The EUCAST disk diffusion method is based on saline for inoculum preparation. /2010-09-17

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 inocula 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. If you can adjust more accurately, we suggest 0.45-0.55. /2011-02-28

6 Can flooding be used to inoculate plates for antimicrobial susceptibility testing?
Historically, flooding has been used in some countries as an alternative to swabbing as a method for inoculation of plates. However, 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. For this reason 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 (even swabbing in three directions across the entire surface of the plate) is used. Alternatively, with round plates, a plate rotator (turntable) can be used. /2013-04-24

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 analyse the results against EUCAST inhibition zone distributions on www.eucast.org. Templates calibrated to EUCAST breakpoints may be used as an alternative to measuring zones. Zones for control tests should always be measured and recorded. /2010-05-03

2 Should inhibition zones on both MH and MH-F be read against a black background?
MH plates are always read from the back of the plate against a black background illuminated with reflected light. The plate is preferably held about 30 cm from the eye. MH-F plates are read from the front without the lid, with reflected light and preferably against a light background. MH-F plates can usually be read with plates held about 30 cm from the eye, but might need closer investigation to enable differentiation between haemolysis and growth. /2010-05-03
3 Are all bactericidal and bacteriostatic antibiotics read according to the same recommendations?

Yes, read zone edges for all antibiotics at the point of complete inhibition as judged by the naked eye. Hold the plate at a distance of 30 cm from the eye and do not look for films and tiny colonies that are not visible to the naked eye. /2010-05-03

4 Why is there sometimes growth within zones of beta-lactams for Haemophilus influenzae NCTC 8468?

Inhibition zones of Haemophilus influenzae NCTC 8468 and beta-lactam antibiotics 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. /2010-05-03

5 Are separate 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 is based on the obvious zone diameter and individual colonies within zones should be disregarded. /2011-02-28

EUCAST disk diffusion test - General methodology

1 Do we have to follow the “15-15-15-minutes rule”?  
EUCAST recommends that bacterial suspensions are used within 15 minutes of making the suspension. It is important to add the antibiotic disks within 15 minutes of streaking the plates and that plates are incubated within another 15 minutes. Extending these times may yield incorrect inhibition zones. /2010-05-03

2 Does EUCAST recommend “direct susceptibility testing”?  
No. EUCAST does not currently recommend “direct testing” because of the problems of controlling inoculums, mixed cultures and the potential transfer of substances interfering with susceptibility testing. /2010-09-17

3 How should Neisseria gonorrhoeae be tested for antimicrobial susceptibility?

EUCAST has determined breakpoints for gonococci but is currently not recommending a specific method or medium. In collaboration with international experts on Neisseria gonorrhoeae, EUCAST is in the process of evaluating alternatives. Until recommendations can be published by EUCAST you should follow existing national or international guidelines. /2011-02-28

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 a ±1°C variation plus the option of setting incubators at 35 or 36°C.

Modern incubators are specified to control temperature 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. /2011-02-28

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 to teach all staff how to prepare and read plates. Daily quality control is required after introduction of the method. Also for a period of at least one month after introduction of the method, we recommend that all inhibition zones should be recorded and inhibition zone histograms compared with reference histograms at www.eucast.org. When the methodology works satisfactorily, you might switch to weekly QC. /2011-02-28

Breakpoints - general


Breakpoints for many of these are under consideration during 2010 – 2013. For some of them MIC testing only will be recommended and for others disk diffusion testing criteria will be developed.

MIC and zone diameter breakpoints have now been published for Campylobacter jejuni, Campylobacter coli, Listeria monocytogenes and Pasteurella multocida. /Revised 2013-04-24

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 animals antimicrobial pharmacodynamics may vary widely. In this situation epidemiological cut-offs (ECOFFs) are a logical alternative to human clinical breakpoints, and ECOFFs have been used in preference to clinical breakpoints in some 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 many would apply equally to human and veterinary situations. /2010-09-17
3 Why are there two different breakpoints for testing Enterobacteriaceae for ampicillin?

This has caused considerable confusion and the presentation has been changed in the 2012 breakpoint tables to reflect the approach taken in most countries.

There are different reporting practices in different countries. This means that organisms without ampicillin resistance mechanisms (wild type organisms) may be categorised as either susceptible or intermediate to aminopenicillins.

In most countries, where it has been common practice to categorise wild type Enterobacteriaceae as susceptible, breakpoints are S≤8 mg/L, R>8 mg/L, corresponding to S ≥14 mm, R <14 mm. This is the format presented in the 2012 breakpoint tables. In a few countries, e.g. Germany and Norway, it has been common practice to categorise the wild type as intermediate S≤0.5 mg/L, R>8 mg/L, corresponding to S ≥50 mm, R <14 mm. This is presented as an alternative in notes in the 2012 tables.

A disk diffusion test breakpoint of "S ≥ 50 mm" is an arbitrary "off scale" zone diameter breakpoint corresponding to MIC breakpoint situations where wild type isolates are categorised as intermediate (i.e. no fully susceptible isolates exist).

/2010-09-17, revised 2012-02-10

4 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 tables are intermediate, as indicated in note 5 on page 1. For example, measuring zones to the nearest mm, for gentamicin with Enterobacteriaceae zone diameters ≥ 17 mm are susceptible, <14 mm resistant, and therefore 14-16 mm intermediate. /2010-09-17

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

Susceptibility to methicillin, oxacillin, and all other penicillinase-resistant beta-lactam agents is inferred from the cefoxitin susceptibility. Therefore S. aureus with a zone of <22 mm around a cefoxitin 30-µg disk are methicillin resistant.

You cannot report susceptibility of staphylococci to cephalosporins and carbapenems without testing susceptibility to cefoxitin. /2010-09-17

6 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. /2010-09-17
7 Why are there no tetracycline breakpoints for Enterobacteriaceae?

The EUCAST Steering Committee has declined to set tetracycline breakpoints for Enterobacteriaceae because it is no longer considered a reasonable drug 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. /2010-11-11

8 For Stenotrophomonas maltophilia is trimethoprim-sulfamethoxazole the only available agent?

For Stenotrophomonas maltophilia the only drug with clinical correlation between MICs and clinical outcome is trimethoprim-sulfamethoxazole (cotrimoxazole). It may be that in the future one or two more drugs may receive breakpoints, but current literature does not clearly indicate another drug for which it is reasonable to determine breakpoints. See guidance note “Stenotrophomonas maltophilia” /2010-11-11, revised 2012-02-10

9 Why are there ciprofloxacin and ofloxacin breakpoints for S. pneumoniae?

Breakpoints for susceptibility testing should not divide wild type distributions and levofloxacin is a more appropriate indication for respiratory infections. Ciprofloxacin and ofloxacin are both poor therapy for pneumococcal respiratory infections but could be used with a maximum dose. You are right that breakpoints should not divide wild type distributions. These two statements together explain why EUCAST decided to place wild type distributions for both agents in the intermediate category. With EUCAST breakpoints no wild type isolates will be categorised as susceptible to ciprofloxacin and ofloxacin. Wild type isolates will be categorised as intermediate and non-wild type isolates as resistant. See ciprofloxacin and ofloxacin MIC distributions. Levofloxacin is more active and moxifloxacin better still against pneumococci, but ciprofloxacin and ofloxacin (being first on the market) were approved for pneumococcal infections and for that reason we needed to set breakpoints. /2010-11-11

10 Are non-species-related MIC breakpoints (Pk/Pd) in the breakpoint tables usable in the routine clinical laboratory?

The non-species related breakpoints are based on pharmacokinetic and pharmacodynamics data only. They are used as the basis for determination of clinical breakpoints but in determination of clinical breakpoints the Pk/Pd breakpoint may be modified 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 in any other part of the breakpoint tables you can determine the MIC and then interpret the MIC on the basis of the "non-species related breakpoints". This gives some idea about the usefulness of the drug in question. If possible, one should also compare the MIC with the MIC distribution for the species in the EUCAST MIC distribution available on the EUCAST website. Thereby, you can also obtain guidance as to whether or not the isolate is likely to express any resistance mechanism. /2010-11-11
11 For cefuroxime, the breakpoint relates only to high dosage (1.5 g x 3). What is the rationale for this?

With cefuroxime, Pk/Pd breakpoints are \( S \leq 4 \, \text{mg/L} \) and \( R > 8 \, \text{mg/L} \), the S breakpoint being based on a lower dose and the R breakpoint on a higher dose. 4 mg/L falls in the middle of the wild type MIC distribution for \( E. \, \text{coli} \) and indicates that with a standard dose 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 not permit reproducible susceptibility test results) and the high dosage was specified to compensate for the raised breakpoint. The data on MIC distributions can be seen on the EUCAST MIC distribution website. /2010-11-11

12 Why shouldn’t I use cefuroxime in the higher dosage for other Enterobacteriaceae besides \( E. \, \text{coli}, \, K. \, \text{spp.} \) and \( P. \, \text{mirabilis} \) 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) \( K. \, \text{spp.} \) and \( P. \, \text{mirabilis} \) are included with \( E. \, \text{coli} \) in the cefuroxime note. During the decision process in the EUCAST Steering Committee, 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. /2010-11-11

13 Why is the breakpoint for trimethoprim given for all Enterobacteriaceae while nitrofurantoin is only for \( E. \, \text{coli} \)? 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 \( E. \, \text{coli} \) 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. \( E. \, \text{coli} \) is specified as the only target for nitrofurantoin because the activity of nitrofurantoin against \( K. \, \text{pneumoniae} \) is problematic as MICs often straddle the breakpoint and because the activity against \( P. \, \text{spp.} \) and \( P. \, \text{sp.} \) is poor. /2010-11-11

14 Are you planning to give breakpoints for topical therapy with agents such as chloramphenicol, polymyxinB, tetracycline, neomycin and tobramycin?

EUCAST is currently looking at several topical agents. The approach for these is to use systemic clinical breakpoints if available, and if not to use epidemiological cutoffs (ECOFFs). This approach has been endorsed by the EMA. Hence there are already breakpoints for staphylococci with tobramycin, tetracycline and chloramphenicol. /2010-11-11
15 **Will EUCAST produce azithromycin breakpoints for *Salmonella* and *Shigella***?  
*Salmonella* spp. and *Shigella* spp. with azithromycin will be covered by epidemiological cut-off values, set at 16 mg/L for *Salmonella* spp. For *Shigella* spp. there are currently insufficient data to set the cut-off value. /2010-11-11

16 **Which breakpoints should we could use 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 non-species related breakpoints is recommended. /2011-02-28

17 **Why has the nalidixic acid screen test for *Salmonella* isolates been removed from the last update of the clinical breakpoints and what should now be done?**  
There has been extensive discussion about screening for quinolone resistance in *Salmonella* spp. Nalidixic acid screening does not pick up *qnr* mutants so it cannot be recommended alone as an indicator of ciprofloxacin susceptibility. If nalidixic acid is used, any susceptible isolates should then be tested with ciprofloxacin (or both tested at the same time, nalidixic acid being a good screen for other ciprofloxacin resistance mechanisms). At this time the EUCAST committee felt it important to highlight the limitation of nalidixic acid screening by removing it completely. We recommend that the ciprofloxacin MIC is determined and that all *Salmonella* isolates with MIC >0.06 mg/L should be reported resistant. /2011-02-28

18 **Why are there no levofloxacin breakpoints for *Enterococcus* spp. when it is used for treatment of uncomplicated urinary tract infections?**  
EUCAST decided against setting breakpoints for any of the fluoroquinolones for enterococci. The clinical evidence was scarce, enterococci were often accompanied by other pathogens so significance is unclear, and resistance develops rapidly and is very common in enterococci.

Use of the norfloxacin 10 μg disk will reliably distinguish *E. faecalis* with (norfloxacin MIC > 8 mg/L) and without (norfloxacin MIC ≤ 8 mg/L) fluoroquinolone resistance. Data are not available for *E. faecium*, but fluoroquinolone resistance in *E. faecium* is very common and perhaps it is best to avoid fluoroquinolones. /2011-02-28

19 **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, is now recommending 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, not all producers of ESBLs, plasmid AmpC and carbapenemases will appear resistant to relevant beta-lactams and there may be good arguments for detecting resistance mechanisms for epidemiological or infection control purposes.
Cefoxitin susceptibility is a sensitive but not a specific indicator of AmpC production. Any isolates resistant to cefoxitin should be confirmed as AmpC producers by a more specific test. /2011-02-28

20 Can the ECOFF be used for ESBL detection and carbapenemase detection?

Yes, the ECOFF is the most sensitive phenotypic measurement. The recommendation is to use the clinical breakpoint to categorise clinically and report as S, I or R (do not delay reporting susceptibility – perform ESBL characterisation as the next step if needed) and the ECOFF to screen for those isolates that you want to test for ESBL or carbapenemase production. For ESBL screening, use cefotaxime AND ceftazidime ECOFFs and for carbapenemase screening use the meropenem ECOFF. /2011-02-28

21 Is it really not possible to report the susceptibility of S. agalactiae to trimethoprim for isolates from urinary tract infection? According to EUCAST we should report as them as resistant (- in the clinical breakpoint table).

Previously, EUCAST could find no clinical data to support treating uncomplicated S. agalactiae urinary tract infections with trimethoprim alone (rather than with trimethoprim-sulfamethoxazole). MIC distributions were scarce and there was thought to be no other evidence to support a breakpoint. It may be that “IE” (insufficient evidence) would have been more appropriate than “-“. We agreed to try to find more MIC distributions and look into this further. In November 2012 this was reviewed and in the light of additional data breakpoints were added to the 2012 breakpoint tables. /2011-02-28, revised 2012-02-10

22 Benzylpenicillin breakpoints for Streptococcus pneumoniae are dosage 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 the laboratories to use the breakpoints valid for this dosage. /2011-02-28

23 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 contamination, and they very rarely cause uncomplicated urinary tract infection. Breakpoints for agents used for uncomplicated urinary tract infections are not likely to be produced by EUCAST (and are not available from CLSI). /2012-02-10

24 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-clavulanate can be inferred from the results of the
ampicillin susceptibility test (no isolates producing beta-lactamase have ever been reported). /2012-02-10

25 What does the "uncomplicated UTI only" mean for Enterobacteriaceae and 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 “Why do EUCAST have no systemic breakpoints for Enterobacteriaceae with oral cephalosporins?” /2012-02-10

26 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. /2012-02-10

27 What about breakpoints for Aeromonas hydrophilia? Should I use breakpoints for Enterobacteriaceae or non-species related MIC breakpoints?

There is not much information available on susceptibility testing of Aeromonas hydrophilia and EUCAST has very limited MIC data for the organism. It falls somewhere between Enterobacteriaceae and Pseudomonas spp. and we would suggest that for the moment you use non-species-related breakpoints. If there are agents you need but for which there are no non-species-related breakpoints we suggest you use the Enterobacteriaceae breakpoints. We are currently addressing breakpoints for less commonly isolated organisms so we would expect more specific guidance in due course. /2012-02-10

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

The comment on the higher dose may refer to either of the breakpoints. This is briefly explained in the Rationale Documents on the EUCAST website. /2012-10-02

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

Resistance to β-lactam agents in E. faecium is commonly mediated by modification 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; but 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-clavulanate is a cautious one. It may be necessary to revise this note if β-lactamase mediated resistance becomes more common. /2013-04-24

30 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. /2013-04-24

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

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.

EUCAST recommend 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 v 3.1). Information on the benzylpenicillin screen is available on the EUCAST website. /2013-04-24

32 There is no EUCAST recommendation on how to screen for high-level aminoglycoside resistance in viridans group streptococci. Can we use the criteria for enterococci?

Neither EUCAST nor CLSI include guidance on detection of HLGR in viridans group streptococci. However, the definition of HLGR used for enterococci has been widely used for viridans streptococci in publications and it is unlikely that synergy will be seen with isolates with gentamicin MICs >128mg/L. /2013-04-24

33 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. /2013-04-24

34 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 drug 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?

The activity of cefuroxime against *H. influenzae* is not good compared with the activity of many other agents and even with cefuroxime given intravenously it is doubtful whether effective concentrations are always achieved in 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 it was decided that there was no clinical evidence to support use of cefuroxime axetil (or cefaclor ) to treat pulmonary infections or otitis media caused by *H. influenzae*.

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. /2013-04-24

**Breakpoints – zone diameter**

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

The breakpoints in the non-species-related table are MIC breakpoints only. There are no equivalent zone diameter breakpoints. /2010-09-17

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. /2010-09-17
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 will be given in a later version of the breakpoint table. /2010-11-11

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. /2012-02-10

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. /2010-09-17

2 How often should quality control strains be tested?
Control strains should be tested daily until performance is shown to be satisfactory (no more than 1 in 20 tests outside control limits), at which stage testing frequency may be reduced to once a week. /2010-09-17

3 Can I use EUCAST quality control strains to quality control automated systems?
Automated systems should be quality controlled for the dilution range used in the system. Suitable strains should be provided by the manufacturers. /2011-02-28

4 Where can I find reference susceptibility distributions for comparison with the distributions from our laboratory?
Reference distributions for both MIC and zone diameters with data from several sources are available from the EUCAST website (www.eucast.org) under “MIC distributions” or “Zone diameter distributions”. 2012-02-10

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. This is noted in the chapter on QA in the book “Quality Assurance: Principles and Practice in the Microbiology Laboratory” (Ed Snell et al, pp. 91-104, PHLS, London, 1999). The point has also been made in several EUCAST lectures and will be included in a EUCAST Quality Assurance document (currently under preparation). If MIC ranges are restricted, as in some commercial AST systems, alternative QC organisms with MICs within the test range should really be defined. 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. /2013-04-24

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

For the first version of the EUCAST QC tables, we used the MIC ranges published in the ISO standard, which refers to CLSI for control data. Some of these ranges have been updated by CLSI (a EUCAST representative is now on the CLSI QC group) and will be updated in the EUCAST tables accordingly. At present, we recommend the CLSI ranges for MICs and there are no additional EUCAST MIC ranges. However, we are developing control MIC ranges for *H. influenzae* NCTC 8468, a strain that has no CLSI criteria. We shall update the QC ranges according to CLSI updates (both for MIC and for disk diffusion where we use the same methodology, i.e. strain, media and disk content). The EUCAST QC tables are revised at least yearly. /2013-04-24

Other questions

1 The new EUCAST standard indicates a fixed concentration of beta-lactamase inhibitor for piperacillin-tazobactam, amoxicillin-clavulanate 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.

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-clavulanate and ampicillin-sulbactam a ratio has generally been used, whereas for piperacillin-tazobactam and ticarcillin-clavulanate a fixed concentration of inhibitor has been used. There is no logical reason for this discrepancy and EUCAST felt strongly that the fixed concentration is appropriate. The objective is to determine whether the MIC of the active agent is changed by the presence of the inhibitor. The ratio of amoxicillin-clavulanate differs in different pharmaceutical preparations and there 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 far beyond any clinically achievable concentration. There is still some ongoing discussion regarding what the fixed concentration of clavulanate should be - there are arguments for 4 mg/L rather than 2 mg/L (although 2 mg/L is used in ticarcillin-clavulanate), but the argument for a fixed concentration is accepted. /2011-02-28
2. **Will EUCAST recommend standardised phenotypic/genotypic methods for confirming carbapenemase-producing strains?**

   Not at this point. The literature is full of suggested methods. Different methods may be appropriate depending on prevalence, cost limitations, requirements for rapid testing, etc. /2011-02-28

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

   EUCAST will, from 2012, publish one update (in the period November 15th to December 15th) per year. This is to allow laboratories time to implement new and revised breakpoints on 1st of January each year. /2011-02-28

4. **What does the abbreviation ND on the EUCAST MIC and zone diameter 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. /2012-02-10

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. /2013-04-24

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 are 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. /2013-04-24
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. /2013-04-24