Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Comments and Subcommittee Responses

M02 (Disk Diffusion)

M2-A5: *Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition; Approved Standard*

General

1. What are the standards for testing topical antibiotics, specifically for eye and external ear infections? Is it recommended that they should not be tested and that topical antibiotics should be used empirically? Are there any good references for correlation of in vitro testing of topical antibiotics with clinical results?

- **The interpretive criteria outlined in this document are intended for systemically administered antimicrobial agents. To date, the subcommittee has not had the opportunity to review relevant studies that correlate in vitro susceptibility testing results with clinical response data for topical agents. Until specific guidelines can be developed, clinically significant isolates from eye infections for which interpretive criteria exist may be tested and reported in an effort to develop clinical correlations.**

2. We are experiencing a problem with *Escherichia coli* isolates that are resistant to ampicillin and intermediate to augmentin. I would expect the clavulanic acid to either work or not work and therefore result in augmentin being either sensitive or resistant. Is there another mechanism of ampicillin resistance in *E. coli* other than by β-lactamase, or have I got a more serious problem?

- **It is possible for enteric gram-negative rods, such as *E. coli* to be resistant to β-lactamase inhibitor combinations due to hyperproduction of β-lactamase, or due to porin changes. Either of these mechanisms could explain your observations.**

3. The rejection in M2-A5 of direct susceptibility testing (i.e., on urine) when there is evidence that it gives good correlation with other methods, and when it has been found to be clinically useful for many years, is inappropriate. While Kirby–Bauer testing may be of value in selecting antibiotics to be used in bone, soft tissue, and blood borne infections, it has been a major detriment to those of us who treat urinary tract infections. With the direct pour plate sensitivity testing and direct sensitivity disk plating, I am able to read sensitivity test data usually within four to six hours. On several occasions, this procedure may have saved a patient's life by allowing me to change to the appropriate antibiotic within four to six hours instead of having to treat the patient inappropriately for two full days while the laboratory performs the required standard 48 hour Kirby–Bauer test.

- **The second paragraph in Section 2 of this document addresses the potential use of direct susceptibility tests performed in clinical emergencies when the gram stain suggests that the infection is due to a single species. If such testing is performed, a follow-up standardized test should be performed once isolated colonies become available from the initial culture.**

Section 5.5 and 6.3.2

4. M2-A5 states clearly (see Sections 5.3 and 6.3.2) that when disk testing pneumococci, incubation should be in the presence of CO_2. M7-A2, however, states in Section 3.5 *(now Section 5.5, M7-A4)* that, when determining MICs, CO_2 should be avoided except for gonococci, meningococci and *Haemophilus influenzae.* As pneumococci are not mentioned, one presumes that CO_2 should be avoided for this species. Because the whole point of disk testing is to correlate with MIC results, it seems logical to use identical test conditions for the two techniques.

- **Incubation in a CO_2 atmosphere is not necessary for the broth microdilution test presently recommended for testing of pneumococci. However, CO_2 is necessary to obtain reliable growth with an agar system, such as the disk diffusion test or agar dilution. The agar dilution method is not specifically recommended for testing of pneumococci at this time, because sufficient recent data derived by that method have not been available for review by the subcommittee.**

Section 6.1

5. I have a concern with the *Haemophilus* Test Media (HTM) not being able to support reproducible antibiotic testing with certain of the cephalosporins and *Haemophilus influenzae* ATCC® 49247.

- **The subcommittee has designated a second *H. influenzae* control strain for use with several cephalosporins. Appropriate zone diameter ranges for those agents with *Haemophilus influenzae* ATCC® 49766 are indicated in Table 3A.**

6. Anywhere from 3 to 15% of haemophili do not grow on HTM. The problem was that HTM was not tested broadly in clinical practice before being sanctioned as a standard medium. We are unaware of whether the medium has been improved since then. The question of what to do with strains that do not grow on HTM is not addressed in the M2 standard.

- **Members of the subcommittee have also experienced growth failures with certain commercially prepared lots of HTM. Use of improperly prepared or stored HTM can give rise to excessive growth failures of *Haemophilus influenzae.* The *Haemophilus influenzae* ATCC® 10211 control strain may be used to troubleshoot growth problems of HTM lots by media manufacturers or clinical laboratories. Other contributing factors to poor growth or growth failures include preparing the test inoculum from culture plates that are more than 24 hours old, preparing the inoculum suspension in water rather than broth or saline, and failure to incubate the plates in a CO_2 atmosphere.**

Section 7.0

7. Do all enterococcus species need to have β-lactamase testing done or only those organisms found in blood and spinal fluid? Literature alludes to the need for β-lactamase testing of *Enterococcus* spp.; however, I cannot find any criteria for specific situations.

- **Because β-lactamase-producing enterococci continue to be extremely rare, laboratories may choose to perform direct β-lactamase tests only selectively, e.g., isolates from blood, CSF, or other normally sterile sites.**

8. Can we use complementary tests to detect ESBL-producing, gram-negative bacilli? If test results show ESBL, can we report the isolates resistant to all β-lactams except cephamycins, carbapenems, and moxalactam?

- **This edition of the document provides some general recommendations on the detection and reporting of ESBL-producing gram-negative bacilli. Until further information becomes available, laboratories may wish to report ESBL-producing isolates as resistant to all penicillins, cephalosporins, and aztreonam.**

9. Is there any need to screen gram-negative bacteria for inducible β-lactamases? Have inducible β-lactamases any importance in the antibiotic choice in therapy?

- **Because essentially all isolates of several genera and species of gram-negative bacilli [e.g., *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii, Providencia* spp., *Proteus* spp. (except *Proteus mirabilis)*, *Serratia marcescens, Pseudomonas aeruginosa*] possess the genes (in a normally repressed state) for Group I β-lactamase production, there is no useful information derived from demonstrating in vitro induction of the enzyme. Instead, laboratories should focus on repeat testing (every three to four days) of isolates repeatedly recovered from infected patients to detect selection of clones during therapy that constitutively produce Group I β-lactamase.**

Section 8.0

10. With regular frequency, we experience the phenomenon of colonies that grow within zones of inhibition with Kirby–Bauer testing. We have designated these colonies as "zoners" or "encroachers." It is our practice to repeat both identifications and susceptibilities when this happens. It is important to add that the *entire* panel of antibiotics is tested rather than just those with the zoners. The result of such testing is usually a different—a more resistant— antibiotic profile. Rather than making a "lab monster," it is our contention that such testing may expose small numbers of resistant organisms present in a mixed population. It seems likely that the clinician would prefer to aim therapy at these resistant organisms. There seems to be no information to guide us in dealing with this problem. Such testing is time-consuming, and, if not useful in providing the best clinical information, should be discontinued. Until more information is available, we intend to pursue this type of testing procedure. We would appreciate some recommendations.

- **Although the genera or species involved were not specified, this phenomenon could be the appearance of spontaneous mutants for Group I β-lactamase production among certain gram-negative bacilli. If the possibility of a mixed culture can be excluded with assurance, the presence of colonies within a zone of inhibition of a β-lactam antibiotic should lead the laboratory to report resistance to that agent.**

Section 9.0

11. M2-A5 does not address the issue of bias in its recommended quality control procedures. In fact, corrective action is recommended only when a result is outside the acceptable control limits. Not a single zone size diameter in any of the submissions to our laboratory proficiency testing program met this criteria for action. In the face of persistent analytical bias, it is likely that an isolate with a borderline susceptibility to the agent in question would be misclassified with potential adverse effects on the patient.

- **It is anticipated that at least 95% of repeated quality control values will be encompassed within the zone diameter limits described in Tables 3, 3A, 3B, and 3C. At times, uncontrolled technical factors, including reader bias, may result in values outside the control limits, thus prompting corrective action. Minor degrees of technical variation or bias should result in values encompassed within the control ranges.**

12. Is it still necessary to calculate the maximum allowable range for precision when performing weekly quality control for antimicrobial disk susceptibility tests? Our most recent NCCLS document M2-A5 has no mention of this procedure.

- **The calculation of maximum allowable ranges for disk diffusion quality control tests (formerly Table 4) was deleted from the document because, in most laboratories, it no longer provides useful information.**

13. When monitoring new lot numbers of Mueller-Hinton agar, is it necessary to run a sterility check? This procedure involves incubating one empty plate or tube to check for sterility.

- **Section 4.1.1 specifies that a sample of Mueller-Hinton agar plates should be incubated to assure sterility of the medium.**

Appendices/Tables

14. Our laboratory has been using the NCCLS protocol for antimicrobial disk susceptibility testing for several years now and a problem has emerged. When testing *Pseudomonas aeruginosa* ATCC® 27853 against norfloxacin, 10 µg, the resulting zone sizes are consistently too large (32 mm to 35 mm). I do not believe the disks are faulty because the combination of *Staphylococcus aureus* ATCC® 25923 and norfloxacin gives correct zone sizes. Media depth and pH are both correct, and the same results are obtained with two manufacturers' Mueller Hinton. I have replaced my organisms many times. Have you encountered this problem before and do you have any suggestions?

- **This problem has not been discussed by the subcommittee. However, a working group of the subcommittee is presently evaluating the approved quality control ranges with the goal being the correction of any ranges that consistently lead to discrepant values.**

15. I was excited to find susceptibility guidelines for *Streptococcus pneumoniae*. I purchased the recommended *Streptococcus pneumoniae* ATCC® 49619 strain and followed the recommendation for inoculating and incubating listed in M2-A5, Section 6.3. I compared my results to Table 3C and, to my dismay, found that I am getting zone sizes at the upper end of the recommended ranges. I, of course, repeated the test for 30 consecutive days. I still obtained the same results. I then purchased another *Streptococcus pneumoniae* ATCC® 49619 and sent the organism I was working on to a reference lab. Both strains yield the same results. The reference lab confirmed my findings. I don't test for all antibiotics listed in the table, but I hope that your committee can steer me in the right direction.

- **One inherent problem in testing *S. pneumoniae* is the fact that there are fewer viable cells at the 0.5 McFarland density than there are with most other organisms. Thus, it is critical to prepare the 0.5 McFarland suspension accurately and to inoculate test plates within 15 minutes of preparing the suspension. The McFarland suspension should be prepared in broth or 0.9% saline but not in sterile water. It may also be helpful to use relatively young cultures (16 to 18 hours) to prepare the suspension, rather than plates that have been incubated for 24 hours or longer. Last, it is essential to use a 5% CO_2 atmosphere for**

incubation of pneumococcal tests. Use of ambient air incubation will result in some growth failures and yield excessively large zones with several drugs.

16. The M100-S5, M2-A5 supplemental tables list several antibiotics for routine antimicrobial testing of *Streptococcus pneumoniae*. The footnote "g" can be interpreted a couple different ways. Is it acceptable to test all the drugs listed (penicillin, oxacillin, erythromycin, trimethoprim/sulfamethoxazole, vancomycin, tetracycline, and chloramphenicol) by the Kirby–Bauer technique for isolates from blood or spinal fluid, or are MICs required? Or is the previous list of drugs to be set only when the oxacillin screen is found to be resistant?

- **The subcommittee recommends that penicillin and extended-spectrum cephalosporin (cefotaxime or ceftriaxone) MICs be determined routinely on all pneumococcal isolates from cerebrospinal fluid or blood. This should be accomplished as soon as sufficient growth is available for testing, not following initial screening with an oxacillin disk. Non-β-lactam antibiotics (e.g., vancomycin and chloramphenicol) may be tested accurately by the disk diffusion method. Agents that do not have an FDA-approved indication for treatment of meningitis (e.g., erythromycin, tetracycline, trimethoprim/sulfamethoxazole) should not be reported routinely on cerebrospinal fluid isolates.**

17. In our laboratory we use the Kirby–Bauer method and follow NCCLS guidelines. In carrying out our weekly quality control susceptibility testing using a manufacturer's Mueller-Hinton agar, we have observed that our control strain *Staphylococcus aureus* ATCC® 25923 to clindamycin, 2 µg, does not fall within the stated range (24–30 mm). The range that we have achieved with this combination on 30 consecutive days of testing is 22–27 mm, with ten results being low. We then tested a fresh lot of disks, as well as an alternative brand of discs for five days each, with no change in results. An alternative brand of Mueller Hinton agar (home-made) did not rectify the problem.

- **This problem has not been discussed by the subcommittee. However, a working group of the subcommittee is presently evaluating the approved quality control ranges with the goal being the correction of any ranges that consistently lead to discrepant values.**

18. To my understanding, according to the M2-A5 standard, we are to perform the susceptibility test for *Streptococcus pyogenes* using plain Mueller-Hinton plates, along with the zone diameter interpretive standards from the M2 tables. As quoted in the M2-A5, "Susceptibility tests are seldom necessary when the infection is due to a microorganism recognized from penicillin-allergic patients; erythromycin or another macrolide may be tested to detect strains resistant to those agents." However, with the apparent increase in the severe invasive Group A streptococcal disease, we are called upon by our laboratory proficiency testing program, to be able to pick up resistant strains when performing susceptibility testing. In doing so, I have observed that the growth of *S. pyogenes* on plain Mueller-Hinton medium did not easily enable me to interpret the zone sizes as well as on Mueller-Hinton with 5% sheep blood. Does the guideline allow the use of Mueller-Hinton with blood as for *Streptococcus pneumoniae*?

- **The recommended medium for disk diffusion susceptibility testing of *all* streptococci is Mueller-Hinton sheep blood agar, with subsequent incubation in a 5% CO_2 atmosphere.**

19. I recommend including the enterococcus footnote on *all* sources. The sixth edition of *Clinical Microbiology* indicates that the caveat be included only in systemic, but ID physicians may wish it on all.

- **It is important to emphasize to physicians that bactericidal therapy of a serious enterococcal infection, such as endocarditis, can only be achieved with combined drug therapy. However, there is no evidence that combined therapy is required for less serious infections, e.g., urinary tract or wound infections.**

20. If gram-positive cocci were found to be gentamicin resistant, can we report this isolate resistant to all aminoglycosides?

- **Screening for high-level gentamicin and streptomycin resistance should be performed on enterococcal isolates from blood or CSF. High-level gentamicin resistance in enterococci also correlates with resistance to amikacin, kanamycin, netilmicin, and tobramycin.**

21. In the area in which I live, we have a high percentage of β-lactamase-positive *Haemophilus influenzae* rather than the β-lactamase-negative, ampicillin resistant strains. The 1994 *Guide to Antimicrobic Therapy* states that 25 to 30% of *H. influenzae* in this country are resistant to ampicillin and does not include it in the list of recommended therapy. The physicians in my area use this publication as a guide for treatment. They also want to know if the organisms I isolate are sensitive to amoxicillin/clavulanic acid and ampicillin/sulbactam. I am concerned about the discontinuation of sensitivity testing on ampicillin/clavulanic acid and ampicillin/sulbactam (two of the more widely used antibiotics for this organism) without providing any kind of rational explanation as to why.

- **The interpretive criteria for ampicillin/sulbactam and amoxicillin/clavulanic acid when testing *Haemophilus* spp. have been restored in Table 2A. However, a direct β-lactamase test can readily detect ampicillin-resistant strains that are predictably susceptible to β-lactamase inhibitor combinations, with the exception of the rare β-lactamase negative, ampicillin-resistant (BLNAR) strains. The testing of ampicillin serves better to detect the rare BLNAR strains than testing either of the β-lactamase inhibitor combinations by the disk method.**

22. Table 2, footnote "p" states, "Blood containing media (except for lysed horse blood) are generally not suitable for testing sulfonamides or trimethoprim." Please explain why Table 1A and Table 2C indicate testing of trimethoprim/sulfamethoxazole on blood Mueller-Hinton agar for *Streptococcus pneumoniae* is acceptable.

- **The use of Mueller-Hinton agar supplemented with sheep blood produces sharp, well-demarcated zones with trimethoprim/sulfamethoxazole tests of streptococci. Sheep blood should not be added to Mueller-Hinton agar for testing of other organisms with trimethoprim or sulfonamides because of medium antagonism that can result with some other genera.**

23. Please clarify disk diffusion testing on viridans streptococci. Table 2C has zone interpretations for organisms incubated in CO_2. Should *all* streptococci be incubated in CO_2 for susceptibility testing or just *Streptococcus pneumoniae* as noted in M2-A5? If viridans streptococci and β-hemolytic streptococci are to be incubated in O_2, what zone interpretations should be used? In addition, should the antibiotic gradient method for penicillin MIC on viridans streptococci be incubated in O_2 or CO_2?

- **The recommended medium for disk diffusion susceptibility testing of all streptococci is Mueller-Hinton sheep blood agar with incubation in a 5% CO_2 atmosphere. The laboratory should consult the manufacturer for the appropriate testing conditions for specific commercial test systems.**

Summary of Comments and Subcommittee Responses

M2-A7: *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Seventh Edition*

General

1. I am looking for guidelines regarding the optimal frequency for repeat susceptibility testing on bacterial isolates from the same patient and source. The M100-S9 guidelines footnote two organism/antimicrobial combinations (*Staphylococcus* spp. vs. quinolones and *Enterobacter, Citrobacter*, and *Serratia* spp. vs. cephems) as possibly requiring repeat susceptibility testing within three to four days after initiation of therapy, but does not give any other general recommendations for repeat susceptibility testing. Is there any other information available on this issue?

- **As stated in Section 11.3, "Some antimicrobial agents are associated with the emergence of resistance during prolonged therapy. Therefore isolates that are initially susceptible may become resistant after initiation of therapy. This occurs within three to four days, most frequently with *Enterobacter, Citrobacter*, and *Serratia* spp. with third-generation cephalosporins; in *P. aeruginosa* with all antimicrobial agents; and in staphylococci with quinolones." We are reluctant to delineate the number of days between repeat testing except when we know that failure to retest might lead to a serious medical error. In certain circumstances, repeat testing might be warranted earlier than three to four days based on the specific situation and the severity of the patient's condition. Laboratory guidelines on when to perform repeat susceptibility testing should be determined after consultation with the medical staff. When generating antibiograms, results from repeat testing should be excluded as recommended in the NCCLS guideline M39—*Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data.***

2. I was looking at the January 2000 disk diffusion standard (M2-A7) and noticed that the statements regarding the use of commercial systems to prepare the inoculum (e.g., Prompt®) and regarding the use of templates to read the zones sizes were deleted. Can you enlighten me on what these deletions mean and why they were deleted? Specifically I would like to know if these practices are no longer recommended (versus NCCLS remaining silent on the issue) and if so why. We have used Prompt and templates since the mid-1980s and have never had a problem with QC or anything else.

- **The subcommittee agreed that since commercial inoculum preparation systems had not been evaluated in a study reviewed by the subcommittee, we could not recommend their use. However, a laboratory could conduct an in-house evaluation of the method to determine its equivalence to the reference method for preparing inoculum. In terms of the use of templates, we wanted to encourage the reading and recording of actual zone diameters, which the use of templates precludes. In addition, changes in interpretive criteria made in M100 may not be updated in templates on an ongoing basis.**

Section 4.1.4 and Table 3

3. Our laboratory received a Phase II deficiency during a CAP inspection due to our discontinuation of testing *E. faecalis* 29212 against trimethoprim-sulfamethoxazole for low levels of thymidine as referenced in Note 1 of Table 3 in M100-S12 (disk diffusion). Our interpretation of Note 1 is that this testing was no longer necessary, but is always an option. Is our interpretation of this correct and discontinuing the testing of *E. faecalis* 29212 appropriate?

- **Current lots of Mueller-Hinton agar are essentially free of thymidine and thymine. The protocol for M6-A—*Protocols for Evaluating Dehydrated Mueller-Hinton Agar* that is used by manufacturers of Mueller-Hinton agar requires that media that are acceptable (and consequently labeled as having been tested by the M6 protocol) should have been checked for this component. Therefore, the need to test current lots is not as critical as it once was. New wording in M2 (Section 4.1.4) and M2, Table 3 states that testing of *E. faecalis* ATCC 29212 or *E. faecalis* ATCC 33186 against trimethoprim-sulfamethoxazole is optional but should be considered if quality control problems occur with the other organisms when tested against the sulfonamides or trimethoprim.**

Section 5.2

4. We are currently implementing your standards for disc susceptibility testing of bacteria (M2-A6), as many laboratories in the UK are, but are finding a problem with inoculation of plates (Section 5.2). Our previous experience used a rotary plater, which we feel gives better zone definition. Does this standard prohibit the use of rotary plating devices? And, if so, what is the reasoning behind this?

- **As for any variation from the standard method (see comment 2), to determine the equivalence of using a rotary plater to the routine three-plane inoculation of a disk diffusion plate, a laboratory could perform studies to show that the inoculation method is equivalent and that quality control ranges are within expected limits when using the plater.**

Section 6.5.1

5. In M7-A5, Section 9.2.1 (M2-A7, Section 6.5.1), NCCLS recommends MIC testing of *E. faecium* (recovered from blood or CSF) to ampicillin and penicillin. This is to test for potential susceptibility of synergy with aminoglycosides. I cannot find this in M100-S11. Is this still a recommendation?

- **In M2-A8, we continue to suggest that, for penicillin- or ampicillin-resistant strains of *E. faecium*, it might be appropriate to determine the actual MICs, since strains with penicillin MICs ≤64 μg/mL or ampicillin MICs ≤32 μg/mL may respond to treatment with a β-lactam and an aminoglycoside in the absence of high-level aminoglycoside resistance. However, because this is an optional step that might be worthwhile doing only in rare circumstances when requested by an infectious diseases physician managing the therapy of a patient with a serious infection, we feel that it is adequate to only mention it in the text and not the tables. The test can be performed using a broth-dilution or agar-based MIC method.**

Tables

6. It is our understanding that current standards exist for *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*, and *Acinetobacter* spp., but we are not aware, however, of any standards being available for any of the other members of this group.

   We have considered several options for susceptibility testing of these unusual organisms as follows:

   1. State that no standards exist and that susceptibility tests cannot be performed.

   2. Perform a broth dilution MIC and report the results without an S, I, R interpretation, with an appended comment that current validated testing reference is not available, and that susceptibility data does not necessarily correlate with clinical activity.

   3. Send the isolate to a reference laboratory.

- **Methods for disk diffusion testing of nonfastidious, glucose-nonfermenting, gram-negative bacilli exist only for *P. aeruginosa* and *Acinetobacter* spp. In addition, a general comment has been added to Table 2B that testing *P. aeruginosa* isolates from patients with cystic fibrosis can be performed, but that incubation for up to 24 hours may be necessary. The subcommittee is currently working on recommendations for testing both *S. maltophilia* and *B. cepacia* by disk diffusion. In the meantime, these organisms (and others in that group) can be tested by an MIC method and the results interpreted using the breakpoints given in M7 Table 2B for other non-Enterobacteriaceae (see general comment 1 in that table).**

Summary of Comments and Subcommittee Responses

M2-A8: *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eighth Edition*

General

1. Could you please clarify a point for me about cefuroxime testing? Your standard gives different zone size criteria for the parenteral and oral forms of the drug and specifies that different discs should be used. Is the implication that you could get an isolate testing sensitive with the one disk and resistant with the other? If this is true, could you use the parenteral (cefuroxime sodium) disk to predict sensitivity for both forms, accepting that you may miscall some strains resistant that would respond to oral, or is it the other way around? The standard gives no details about these particular recommendations.

- **Although there are two formulations for cefuroxime, one for parenteral and one for oral administration, there is only one disk for laboratory testing. Different interpretive criteria were developed based on the different pharmacodynamic/pharmacokinetic data and clinical indications for the two formulations. Tables 2A through 2J should be used to guide interpretation for individual organisms.**

2. Regarding the intention of the "Warning" on page 23 of M100-S14 regarding not routinely reporting clindamycin, macrolides, etc. for bacteria isolated from the "CSF": Should this be interpreted in its narrowest sense or does CLSI consider all central nervous system (CNS) sites similarly to CSF? Specifically, would it be incorrect to report clindamycin, etc. for a bacterial brain abscess aspirate? Infectious disease textbooks consider clindamycin as an acceptable alternative to treat brain abscesses.

- **The WARNING box in M100 refers only to bacterial meningitis. Clindamycin is mentioned among alternative therapies useful for brain abscess (*Principles and Practice of Infectious Diseases*, Mandell GL, Bennett JE, Dolin R, eds, 5th edition, Churchill Livingstone, Inc., Philadelphia, 2000), so it would <u>not</u> be incorrect to report clindamycin susceptibility test results on an aspirate from a brain abscess. There are insufficient data to provide comprehensive guidance on which test results to report routinely for pathogens isolated from brain abscesses.**

Table 2A

3. Could you clarify the comment in M100 regarding the "Warning" for *Salmonella* and *Shigella* in Table 2A, comment (4) on page 35? The comment states that first- and second-generation cephalosporins should not be reported as susceptible. Does that comment include the cephamycins also?

- **Yes, the statement has been clarified.**

Table 2C

4. There appears to be a discrepancy between the disk diffusion and MIC sections of the current M100 document as related to oxacillin:
   For disk diffusion testing of non-*S. epidermidis* coagulase negative staphylococci, disk diffusion "R" isolates that are *mec*A/PBP2a negative should be reported as "S" (pg. 42).

However, for MIC testing of these same non-*S. epidermidis* coagulase negative staphylococci, isolates that are *mec*A/PBP 2a negative should be reported as "S" if oxacillin MICs are between 0.5 to 2 mcg/ml, but as "R" if MICs are > 4 mcg/ml (pg. 105).

If moderately to highly oxacillin-resistant *mec*A/PBP2a negative isolates should be reported as "R" when performing the MIC test, shouldn't this also be the case when doing disk diffusion? Put another way, shouldn't the disk diffusion criterion also have a zone size below which a report of "R" be made, regardless of *mec*A/PBP 2a findings?

- **The reason for reporting strains exhibiting MICs ≥4 µg/mL as oxacillin resistant despite *mec*A/PBP2a status is based on pharmacokinetic/pharmacodynamic data and the possibility that other resistance mechanisms not yet discovered may be responsible for the increased MICs. Unfortunately, there is no zone diameter or range of zone diameters that correlates exactly with MICs ≥4 µg/mL. The data from the CLSI study used to establish the revised interpretive criteria (Tenover, et al. *J Clin Microbiol* 37: 4051-4058) show that 108 of 110 (98.2%) isolates with zone diameters of 6 mm (i.e., no zone) were *mec*A positive. Of 42 strains with zone diameters in the 7 to 17 mm range, 15 (35.7%) were *mec*A positive and 26 (64.3%) were *mec*A negative. Therefore, based on these data, it would be possible to do the following: if there is no zone to oxacillin, report as resistant; if there is any zone ≥7 mm, then perform a *mec*A test or a cefoxitin disk test for a definitive answer. However, recent studies show that using the cefoxitin disk in place of the oxacillin disk gives better correlation with *mec*A status for coagulase-negative staphylococci, and the cefoxitin zone is much easier to read.**

Table 2E

5. I am aware that the antibiotic tested is the one to be reported; however, I need clarification on how to address the reporting of doxycycline with the fastidious organisms when the tetracycline interpretation is resistant or intermediate. In Tables 2A, 2B, 2C, and 2D of M100-S15, all tetracycline comments end by stating, "...However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline..." But in Tables 2E (*Haemophilus*) and 2G (*S. pneumo*), this statement is not part of the comment. Am I to assume this statement does not hold true for the fastidious organisms, or can I deduce that it does? Literature leads me to believe that it does. Should tetracycline R or I be equated with doxycycline resistance? Will CLSI be developing zone sizes for doxycycline in the future?

   Some physicians are hesitant to use doxycycline because of presumed inactivity. Clinicians assume isolates reported resistant to tetracycline are also doxycycline resistant. Our pharmacologist notes this assumption does not agree with the literature and that extrapolating our tetracycline susceptibility data to doxycycline has no direct application. It doesn't relate to therapy or help in clinical studies. Citing literature, she says doxycycline is consistently active against all common typical and atypical bacterial causes of pneumonia. She is concerned this assumption of tetracycline resistance (which is common for *S. pneumo*) implying doxycycline resistance as well is sending those not familiar with infectious disease in the wrong direction (i.e., towards the use of more expensive alternatives with more harmful side effects).

- **Tetracycline susceptible isolates of various species are susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline. Currently, there are no interpretive criteria for doxycycline and minocycline against pneumococci and *H. influenzae*. Until additional studies are undertaken or reviewed by CLSI, it is premature to use tetracycline to predict doxycycline or minocycline resistance.**

Table 2G

6. Reading the CLSI documents of 2005, I wonder why the D-test that looks for inducible clindamycin resistance is not described for *Streptococcus pneumoniae*. Can you give me the reason behind this?

- **Isolates of *Streptococcus pneumoniae* can have *erm*-mediated resistance to erythromycin. However, the vast majority of these isolates are also resistant to clindamycin (i.e., the constitutive resistance phenotype). Rare isolates of pneumococci may have inducible resistance; however the clinical significance of this has not been established. Therefore, routine testing for inducible clindamycin resistance is not recommended for this species.**

Summary of Comments and Subcommittee Responses

M2-A9: *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition*

General

1. I am preparing to test minimal inhibitory concentration (MIC) values and had a question about the dilutions. Someone mentioned to me that it is recommended to make only four dilutions from each antibiotic and then make a new standard at a lower concentration. I cannot find reference to that in my reading of M07. Would it be possible to do serial dilutions of the antibiotics rather than the method outlined in Table 6 of M100? I am concerned about being told that serial dilutions are only good for four dilutions, because this is a standard practice I have always used to quantify CFU/mL; and if it is not accurate with these antibiotic standards, then who is to say it is accurate for quantifying CFU/mL? And if it is accurate for quantifying CFU/mL, then why is it not accurate for quantifying MIC values for the antibiotics? Sorry for being confused. I have been handed a protocol already in place that seems to have a lot of unnecessary dilutions and testing being done to determine the MIC and I am trying to scale it back.

- **In the experience of many of the subcommittee members who have been preparing reference dilution panels or plates for many years, they have never done what you describe, ie, prepare intermediate stock solutions when diluting more than four tubes. M07-A8 states in Section 10.4.1, "For the intermediate (10x) antimicrobial solutions, dilute the concentrated antimicrobial stock solution (see Section 7.3) as described in Table 7 (previous Table 6) of M100 or by making serial twofold dilutions."**

2. In CLSI document M02, the disk diffusion zone diameters are given with equivalent MIC breakpoints. In the overwhelming majority, they correspond to the MIC breakpoints printed in M07. However, some do not (eg, gentamicin and amikacin with *Enterobacteriaceae*). Why? Also, some of the MIC equivalent breakpoints are not in doubling dilutions (eg, in Table 2A, the Susceptible equivalent breakpoints are ≤ 12 μg/mL for netilmicin and ≤ 6 μg/mL for kanamycin). Why?

- **MIC equivalents listed in M02 represent the MIC breakpoints used when the zone size diameters were first determined. Since the M02 document was published before the M07 document, occasional discrepancies have existed and these mainly occur with the aminoglycosides. However, in M100-S19, the MIC and zone diameter interpretive criteria in all the Table 2s were combined in the same Table for each of the organism groups and the equivalent MIC breakpoints (or MIC correlates) for disk diffusion no longer appear in the Tables. A table listing the older MIC equivalents for zone diameters where discrepancies occurred between M02 and M07 is available in the Minutes of the AST Subcommittee meeting of 11-13 June 2008 as Attachment 3.**

3. Our pulmonologist has requested that we test *Staphylococcus* spp. and *Enterobacteriaceae* against moxifloxacin. The PharmD gave me a moxifloxacin product insert that gives different interpretive criteria (≥ 19 = susceptible) than those listed in M100 (≥ 24 mm = susceptible). Their product insert gives the same interpretive criteria for *Enterobacteriaceae,* and the CLSI document does not list ANY moxifloxacin interpretations for *Enterobacteriaceae.*

   I realize that we may use FDA or CLSI interpretive criteria, but the difference here is so great—19 mm would be RESISTANT per CLSI—that I don't feel comfortable reporting any results until I get a satisfactory explanation.

- **Although there are several reasons why the CLSI and FDA moxifloxacin breakpoints for staphylococci differ, the most important point for the laboratorian to understand is that CLSI breakpoints can be used for all staphylococci including MRSA, whereas the FDA breakpoints apply only to methicillin-susceptible staphylococci (per the FDA label for clinical use of the drug), so the laboratory should not report the drug on MRSA if using the FDA breakpoints. CLSI breakpoints for testing moxifloxacin with *Enterobacteriaceae* have not been determined, but FDA breakpoints are available for use. It is important to note that moxifloxacin is not approved for treatment of urinary tract infections due to low urinary concentrations and, thus, should not be tested on urinary isolates. The decision regarding which drugs to report for certain organism groups and which breakpoints to use should be made by the laboratory following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy & Therapeutics and Infection Control committees of the medical staff. Clinical laboratories may implement newly approved or revised disk CLSI breakpoints as soon as they are published in M100. If a susceptibility testing device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility to an agent using the CLSI MIC breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.**

4. What is the recommended frequency for quality control of various agar screening tests (eg, chromogenic media, vancomycin agar screen)?

- **Media containing antimicrobials used for primary isolation are not part of the scope of the susceptibility testing documents M02 and M07 (see CLSI document M22).**

  Single drug susceptibility tests/screens should be treated like other susceptibility tests multiple concentrations or multiple drugs) until such time that recommendations and appropriate supportive data are available to streamline

Tables 1 and 2B-4

5. In a recent College of American Pathologists (CAP) survey, participants were told that for *S. maltophilia,* they should have only reported results and interpretive breakpoints for the antimicrobial agents listed in Table 1. The question concerns minocycline, which is listed in the *S. maltophilia* column. Most laboratories can test tetracycline, but not minocycline. In footnote b, Table 1 in M100-S15, it states that tetracycline can be used to predict susceptibility (not Intermediate or Resistant) to minocycline. Is the same statement true for *S. maltophilia*? There are no tetracycline breakpoints listed in the draft of Table 2B-4, *S. maltophilia* (M100-S16). If minocycline is not available on the antimicrobial susceptibility testing medical device system the laboratory is using, and the *S. maltophilia* isolate is susceptible to tetracycline, should the laboratories report the tetracycline result or not?

- **It is true that isolates of *S. maltophilia* that are susceptible to tetracycline are also susceptible to minocycline and doxycycline. However, >90% of *S. maltophilia* strains (personal communication, R. Jones, Sentry Antimicrobial Surveillance Program) are resistant to tetracycline but susceptible to minocycline and doxycycline, so testing tetracycline as a surrogate in place of the other tetracyclines is not recommended, because the vast majority of strains would be called resistant. When testing was done to determine criteria for testing *S. maltophilia* and *Burkholderia cepacia,* the CLSI working group chose to include only agents that were active, that were recommended by experts as therapies of these infections, and for which the recommended breakpoints were proven to be reproducible.**

Tables 2A and 2B-5

6. *Enterobacteriaceae* and non-*Enterobacteriaceae,* which are resistant to tobramycin and amikacin, but susceptible to gentamicin, most likely produce a 6′-acetyltransferase. In this case, only one of the three gentamicin subcomponents, C_1, remains active. Since the fraction of C_1 varies between gentamicin formulations and C_1 appears to have different pharmacokinetics than gentamicin as a whole (*Antimicrob Agents Chemother*. 1975;7:328-332), are the gentamicin interpretive breakpoints accurate in these cases? Would it be reasonable to report gentamicin susceptibility as intermediate or provide a comment that gentamicin activity is uncertain?

- **The commenter raises an interesting question. The subcommittee has no data that support changing the susceptible category to intermediate or resistant. However, when an isolate that is gentamicin susceptible and amikacin and tobramycin resistant is encountered, and selective reporting is used by the laboratory, the susceptibility to gentamicin and the resistance to tobramycin and amikacin should all be reported.**

Table 2B-2

7. I am a microbiology supervisor with a question regarding interpretations for *Acinetobacter* to tigecycline. I have an infectious disease doctor complaining that this drug has been out for over a year, and still no interpretations and guidelines regarding this drug have been published. I have the 2008 standards and see this is true. Any time frame or information that you may have so that I could pass some pertinent information on to this doctor would be appreciated.

- **Interpretive criteria for tigecycline are not included in the CLSI documents for any genera, because the drug manufacturer has not presented the necessary data for review by the subcommittee for subsequent publication of breakpoints in M100. In the meantime, one ordinarily could refer to the drug package insert for the US Food and Drug Administration (FDA) breakpoints; however, breakpoints for *Acinetobacter* are not included in the FDA list at this time because there is no clinical indication for tigecycline against *Acinetobacter*.**

8. CLSI document M100-S17 has MIC susceptibility ranges for colistin and polymyxin B against *Acinetobacter* sp., but there are no standards listed for disk diffusion on this isolate. Our Infectious Disease staff sometimes requests that colistin and polymyxin B be tested against multidrug-resistant (MDR) *Acinetobacter* isolates; and since these drugs are not available on our commercial conventional microdilution panels, I order these antimicrobials as (MIC) antibiotic gradient strips from a commercial source. The company, however, requires that a disclaimer be signed stating that we will use colistin and polymyxin B for INVESTIGATIONAL USE ONLY; a disclaimer is only good for six months and a new disclaimer must be signed for each new order. Should colistin and polymyxin B not be used for clinical purposes and are indeed for investigational use only?

- **There are no disk diffusion criteria for *Acinetobacter* in M100 because the disk test does not correlate with MIC tests and is therefore unreliable. Questions about the commercial gradient strip test should be addressed to the manufacturer. The use of colistin or polymyxin B for clinical treatment is a medical decision.**

Table 2G

9. I have a question about reporting cefepime (meningitis) and/or cefepime (nonmeningitis). In M100-S18 Table 2G M07-MIC, cefepime (nonmeningitis) has a comment (11), "Only report interpretations for nonmeningitis and include the nonmeningitis notation on the report." There is not a US FDA-approved indication for the use of cefepime for meningitis. Just below the cefepime

(nonmeningitis) entry, cefepime (meningitis) is listed with interpretative values. When would it be appropriate to use this?

- **The CLSI documents are also for use outside the United States where cefepime might be used for treatment of meningitis, which is the reason those criteria are included in Table 2G. You should discuss with your Medical Director how to handle reporting of cefepime, but one solution in the United States might be to report only cefepime (nonmeningitis) with a note that cefepime is not US FDA-approved for treatment of meningitis.**

M7-A4, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Fourth Edition*

1. In some of the NCCLS documents such as M7 and M27 there are tables that tell people how to dissolve various antimicrobials. Some of the materials that are being used as solvents, methanol, DMSO, ethanol can be dangerous. DMSO for example is considered as an allergen, an irritant and if heated emits toxic vapors. More importantly it is a compound that is absorbed through the skin carrying with it anything dissolved in it. These documents have the "Universal Precautions" statement but no chemical safety statement. I suggest that a chemical safety statement be put into these documents. Preferably this statement should be put in the section where the recommendation to use the material is made.

- **We have added a footnote designation to those compounds that are potentially toxic, suggesting that the user consult the appropriate Material Safety Data Sheets that are available from the manufacturer for the compounds before using any of them. The standard precautions statement has been updated to reflect current practices.**

2. First- and second-generation cephalosporins are no longer included in NCCLS standard M7 Table 1A (M100-S6 or M100-S7) "Suggested Groupings of U.S. FDA-approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Fastidious Organisms by Clinical Microbiology Laboratories," for *Streptococcus* species, nor are there interpretative criteria in Table 2C (M2-A6 or M7-A4) for these organisms for these antibiotics, with the exception of MIC interpretations for cefuroxime for *S. pneumoniae* in M7-A4.

- **There was not sufficient pharmacokinetic, pharmacodynamic, or clinical data available to establish appropriate MIC breakpoints for all the parenteral and oral cephalosporins represented by testing cephalothin. However, it was agreed that penicillin-susceptible pneumococci and streptococci should be considered as also susceptible to the first- and second-generation cephalosporins. This point is now included in Table 2H.**

3. Many major references still list first- and second-generation cephalosporins as being active for *Streptococcus* sp. and less costly (e.g., especially the first-generation cephalosporin, cefazolin). The lack of interpretive standards implies that these drugs should not be used for these organisms. Can you provide me with the rationale behind the committee's decision to exclude these agents from the interpretive tables? Are there unpublished interpretative data for cefazolin and *Streptococcus* species that would provide guidelines for reporting interpretations for these organisms?

- **Please refer to the response to question 2 above.**

4. This letter is a formal inquiry to the NCCLS committee to address the quality control procedure for the Mueller-Hinton Agar with 4% NaCl and 6 mcg/mL Oxacillin. The current quality control organism is *Staphylococcus aureus* (ATCC® 43300) and is required to show visible growth in 24 hours using the recommended inoculation procedure in NCCLS document M7-A3. The previous organism recommended by ASM was *Staphylococcus aureus* (ATCC® 33591) using their inoculation procedure. We have been observing a

significant difference in the amount of growth observed using both organisms for quality control testing. *S. aureus* (ATCC® 33591) grows significantly better than *S. aureus* (ATCC® 43300) on the medium at 24 hours. In some cases the growth of *S. aureus* (ATCC® 43300) is barely visible at 24 hours or does not grow visibly until 48 hours. The following are some of my concerns:

1. How important is the inoculum size?
2. Is there a quantitative inoculum procedure for this medium type?
3. How important is the incubation temperature?
4. How stable is the resistance performance for *S. aureus* (ATCC® 43300)?
5. What is the MIC for S. aureus (ATCC® 43300) versus *S. aureus* (ATCC® 33591)?
6. Why is S. aureus (ATCC® 43300) recommended by NCCLS and not *S. aureus* (ATCC® 33591)?
7. What validation studies were done using the *S. aureus* (ATCC® 43300) and are there any published articles concerning this organism?

- **When the original studies were done to attempt to improve the reference methods for detection of oxacillin resistance in staphylococci, ATCC® 43300 (oxacillin MIC, 32 :g/mL) was selected as a control strain for the oxacillin agar screen test, because it is a "difficult-to-detect" heteroresistant, *mecA* positive MRSA. *S. aureus* ATCC® 33591 (oxacillin MIC, >64 µg/mL) appears to be homogeneously resistant. If the conditions (i.e., insufficient NaCl or inoculum, temperature, or the lot of medium) are inadequate, ATCC® 43300 is more likely to grow poorly and may appear susceptible when it is not. Inoculum and incubation temperatures are important for testing all staphylococci. Although no validation studies for ATCC® 43300 were ever published, the subcommittee believes that of these two strains, ATCC® 43300 represents the more sensitive quality control strain.**

5. Our laboratory has been using the NCCLS protocol for antimicrobial disk susceptibility testing for several years now. I would appreciate if you can clarify disk diffusion, agar dilution, and E-test testing on *Stenotrophonomas maltophilia*. Using the Kirby-Bauer, what zone interpretations should be used with piperacillin, piperacillin/tazobactam, and ticarcillin/clavulanic acid (*P. aeruginosa* or other gram-negative organisms)? I am concerned about the false susceptibility testing on aminoglycosides and TMP-SMX using the Kirby-Bauer. Is the E-test more accurate than agar dilution?

- **Current disk diffusion criteria are applicable to only *P. aeruginosa* and *Acinetobacter* spp. However, it should be possible to test *S. maltophilia* using one of the MIC methods in NCCLS document M7Σ*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* if the breakpoints for non-Enterobacteriaceae are used. Concerns about testing *Stenotrophomonas* and *Burkholderia* spp. are being addressed by a new working group whose goals are to determine which of the reference methods can be used for testing these organisms.**

  The NCCLS Antimicrobial Susceptibility Testing Subcommittee does not have the authority or obligation to comment on the performance or applicability of commercial susceptibility testing products or devices. The U.S. FDA has the responsibility of "clearing" susceptibility devices for use in clinical laboratories. The MIC control values developed and published by NCCLS are often applicable to tests performed using a commercial system. The laboratory should consult the approved product package insert of a particular system to determine recommended quality procedures for that product.

Section 4.2.8

6. It indicates that rifampin is a protein synthesis inhibitor. Rifampin is actually an RNA synthesis inhibitor.

- **This error has been corrected in M7-A5.**

Section 6.1 (5) (Now Section 6.6(1)

7. With regards to sulfonamide and trimethoprim susceptibility tests, it reads, "The end points should be easy to read (either as no growth or approximately 80% reduction as compared to the control)." I think this sentence is a bit confusing, and might be clearer if it says, "The end points should be easy to read (as 80% or greater reduction in growth as compared to the control)."

- **This statement has been clarified in M7-A5.**

Tables

8. On Table 1 of M100-S6/M7-A3, footnote j., the phrase "Penicillin-susceptible staphylococci are also susceptible to other penicillins, cephems, and carbapenems..." is confusing. Table 2 of M100-S6/M7-A3 lists "Cephalosporins and other Cephems." Please distinguish cephalosporins and cephems. Are cephalosporins included as cephems in footnote "j" of Table 1?

- **Cephem is the broader term that includes cephalosporins, cephamycins, carbacephems, and oxacephems. Please refer to the glossary of antibiotic terms that appears in this document for further clarification.**

9. In Table 1 of M7-A4, a footnote (a) is included for drugs intended for testing with the Enterobacteriaceae that specifies agents other than those given a U designation that might be considered for testing. Are the additional drugs in Groups A, B, and C that do not have a footnote (a) assignment not recommended for testing urinary Enterobacteriaceae isolates?

   In addition, there is no footnote (a) indication for any of the drugs (other that those in Group U) intended for testing other bacterial groups in the table, i.e., *P. aeruginosa* and other non-Enterobacteriaceae, *Staphylococcus* spp., and *Enterococcus* spp. Does this mean that no drugs other than those included in Group U are recommended for testing with these bacterial groups?

   Also, for some of the drugs indicated in Group U or footnote (a) for testing urinary tract isolates, FDA-approved indications for usage in urinary tract infections do not include some of the known urinary tract bacterial species. For example, the only drug from NCCLS-recommended Group U for reporting and testing *Acinetobacter* sp. that has an FDA-approved indication is tetracycline. What is the opinion of NCCLS regarding these discrepancies?

- **Because of the difficulty in maintaining the list of agents that might qualify for a footnote (a) designation, in addition to the related concerns that were pointed out in your letter, the subcommittee felt that the best solution would be to delete the footnote entirely for agents in**

Groups A, B, and C, leaving Group U for the agents that are limited to treatment of urinary infections only. Table 1 is not intended to be an exhaustive resource for therapy decisions, nor can it be.

10. It is my understanding that, as stated in document M7-A4, January 1997, Section 5.5, page 24, that agar dilution is NOT specifically recommended for pneumococci due to insufficient recent data to validate the method. This document does say that if you are using an agar system, including disk diffusion or agar dilution, it is necessary to always incubate in increased CO_2. I assume that this statement relates directly to testing pneumococci but not to other streptococci (e.g., viridans group strep). Correct?

    Table 2G, page 90, M100-S9 clearly indicates that the recommended method for pneumo continues to be broth microdilution. What is confusing, and about which I have received questions, is the new addition to Table 3A, page 99, M100-S9, that provides "Testing Conditions for Clinical Isolates and Performance of Quality Control." This table implies that agar dilution can, in fact, be used for testing pneumococci . . . Can you please clarify the intent/interpretation of this table for me? Does it really contradict Table 2G, as it seems to? Are there new data now available that validate the agar dilution method for pneumococci (I know this method is used extensively in Europe)? Am I correct in my interpretation that incubation of agar dilution MIC plates, when testing viridans group streptococci, should NOT be in CO_2 unless the strains are known to be CO_2-dependent?

- **Prior to M100-S9, there were separate QC tables (Tables 3A, 3B, and 3C) for the fastidious organisms. In M100-S9, it was decided to put all the fastidious QC strains into one table (Table 3A) and include the testing conditions for the organisms in a smaller table at the end of Table 3A. When that table was created, the agar dilution test for *S. pneumoniae* was incorrectly included. As stated on p. 24 of M7-A4, "the agar dilution method is not specifically recommended for testing of pneumococci at this time, because sufficient recent data derived by that method have not been available for review by the subcommittee." Unfortunately, that is still the case. We also have no data to support or deny the use of CO_2 when performing agar dilution with either pneumococci or other streptococci.**

11. As a member of our hospital infection control committee, I have been asked to review the microbiology laboratory reporting process. Upon reviewing the MIC reports and antimicrobial suppressions by our laboratory, I have several questions pertaining to the MIC interpretive criteria for *Pseudomonas aeruginosa* and other non-Enterobacteriaceae. My question is regarding the aminoglycoside interpretive standard for susceptible organisms. In the January 1998 M100-S8, page 55, it states that the microorganism is susceptible to amikacin with an MIC ≤16 :g/mL. As a clinical pharmacist, I am aware the aminoglycosides demonstrate concentration-dependent killing and possess a postantibiotic effect. It is well established that aminoglycoside toxicity is related to tissue accumulation. Having been taught that trough concentrations are used to monitor for accumulation, it is recommended to keep the trough concentrations of gentamicin/tobramycin ≤2 :g/mL and amikacin ≤10 :g/mL. My question is, "How do you treat a potentially susceptible organism without risking toxicity from a higher than desired trough?"

- **NCCLS uses microbiologic, pharmacokinetic, and clinical data to establish breakpoints. Amikacin interpretive criteria were established assuming the recommended dose of 7.5 mg/Kg every 12 hours, which results in mean peak serum levels of 38 μg/mL (IV administration) or 21 μg/mL (IM administration). These peak serum levels for amikacin (an important parameter for aminoglycoside efficacy) are above the susceptible breakpoint of ≤16 μg/mL.**

12. For antipseudomonal penicillins/β-lactamase combinations, the MIC breakpoints for Enterobacteriaceae include an intermediate classification. For *Pseudomonas,* which is associated with higher morbidity and mortality (up to 50% in ventilated patients with Pseudomonas pulmonary infections), there is not an intermediate classification, and the organism is considered susceptible over this intermediate range of MICs for the Enterobacteriaceae. I have seen failures in treatment with piperacillin/tazobactam due to the reported "susceptibility" of the organism. Physicians who have had success treating infections with low MICs don't realize the difference in MIC and see "susceptible as susceptible." Even when the drugs are dosed maximally the serum concentration falls below the MIC in only a few hours (the pharmacodynamic 'monitor' for efficacy). I don't understand the reason for this reporting. The fact that the footnote says that an aminoglycoside should be used in combination with maximum dose β-lactam agent is NOT a comment that the MD sees, and they continue to treat with max or sub-max doses and most often WITHOUT an aminoglycoside. I believe that although your reporting may be technically correct and an educated ID practitioner is aware of this information, the general physician is not. I believe that perhaps standards should be changed so that the reporting does not harm the patient. The current reporting in which micro labs do not report out your footnote, but just call a Pseudomonas isolate with an MIC of 64 susceptible, has caused adverse outcomes of which I am personally aware.

- **The susceptibility of the microorganism, site and type of infection, and overall condition of the patient are all important factors that should be considered for therapy. For documented pseudomonal infections, antimicrobials often are given at a higher dosage and with more frequency, since infections with these organisms are generally more difficult to treat. Some pseudomonal infections may be treated with monotherapy, but serious life-threatening *Pseudomonas aeruginosa* infections and those in granulocytopenic patients should be treated with maximum doses and in combination with an aminoglycoside. This is generally considered standard therapy and is included in package labels of β-lactams with this indication and in the NCCLS document. Laboratories can include therapy comments (such as the use of higher dosage in combination with an aminoglycoside) on their lab reports if they wish to do so.**

Summary of Comments and Subcommittee Responses

M7-A5, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Fifth Edition*

General

1. We are presently testing a panel of antimicrobial agents for *Moraxella catarrhalis* for patients in our area. Our question is:

   We would like to know if interpretive standards could be developed for *Moraxella catarrhalis*. In the future we will be sending out just the MIC value in the patient report but have no interpretive standards (S, I, or R) alongside of them. Is there any method that we can utilize to interpret these results? Also we will be sending them out with a comment referring to the fact it is a non-standardized method. Please comment and provide us with any information we can utilize.

- **The current recommendation in Section 10 is for the use of a β-lactamase test to detect resistance to penicillin, ampicillin, and amoxicillin in *M. catarrhalis*. The need for a specific test method and interpretive criteria for these and other agents for this organism is an issue that the subcommittee hopes to address in the future.**

Section 3.1

2. We follow NCCLS guidelines when preparing our in-house frozen MIC panels, and this has been relatively easy for us as the potency of the drug supplied by a pharma company is always clearly indicated on the outside of the vial. We are about to prepare a series of MIC plates in which the sponsor has supplied the powder but the potency has not been indicated. The certificate of analysis refers to the "assay value" (which is a percentage). The pharma company has advised us to adjust our calculations for water content (~ 8%), but this is something that is not discussed in the NCCLS M7 document. Is this something we should be doing?

- **Text has now been added to the document (see Section 3.2) that clarifies this issue.**

Sections 6.1 and 6.3

3. We are currently involved in *in vitro* testing of novel antibacterial agents against *Streptococcus* spp. using the NCCLS-recommended procedures in M7-A4. We have had very good correlation among our microbroth dilution, agar dilution, and Kirby Bauer susceptibility test methods. I have two questions for the committee:

a). I would appreciate knowing the historic reasoning for the committee's choice of LHB (lysed horse blood) as the media supplement for microbroth dilution, because sheep blood is the recommended media supplementation for agar dilution or Kirby Bauer testing.

- **Lysed horse blood was chosen for the broth microdilution method because it provided very good growth of pneumococcal clinical isolates, and because 1) it had been used for a substantial length of time by several laboratories with expertise in testing pneumococci; 2) it provided a clear medium; and 3) it did not contain the inhibitors of trimethoprim or sulfonamides that other bloods did. After this decision was made, it**

was discovered that sheep blood did not appear to inhibit the action of trimethoprim-sulfamethoxazole on pneumococci as it did against other organisms and that there was good correlation of MIC and zone diameters when sheep blood was used for disk diffusion testing of pneumococci.

b) Currently the only recommended method for the preparation of inoculum for *Streptococcus pneumoniae* is to prepare a 0.5 McFarland standard from an overnight sheep blood agar plate. In our hands, this method consistently yields a slightly lower inoculum than expected (approximately 10^7 CFU/mL). Would the broth culture method recommended for other organisms be a viable alternative to achieve the proper inoculum?

- **We realize that with some strains of pneumococci a 0.5 McFarland suspension does not achieve the target count of 1.5 x 10^8 CFU/mL. However, with both pneumococci and *Haemophilus* spp., the age of the plate from which the inoculum is prepared can affect the final counts (see Section 7.3.1). Inoculum suspensions for pneumococci should be prepared from a plate that is no more than 18 to 20 hours old. We prefer the direct inoculum preparation method for pneumococci, because pneumococci and other fastidious organisms often grow unpredictably in a broth medium.**

Section 7.3.1

4. I found a possible error in Section 7.3.1 (4) - under Broth Dilution Testing. After repeated calculations, we believe the statement, "After mixing, a 0.1-mL aliquot is spread ... the presence of approximately 50 colonies would indicate an inoculum density of 5 x 10^5 CFU/mL" is incorrect. Instead, a 0.001-mL (or 1-µL) aliquot should be spread in order to obtain ~50 colonies.

- **The subcommittee has used the following calculation: If there is 5 x 10^5 CFU/mL in the well of the MIC plate and one makes a 1:1000 dilution of it (by adding 0.01 mL to 10 mL), one then should have 5 x 10^2 CFU/mL (or 500 CFU/mL). If one then plates 0.1 mL of that onto a BAP, one should have ~50 colonies.**

Sections 9.2.1 and 9.3

5. I appreciate the more user-friendly format these particular documents have adopted in the recent past. Please pass on our compliments to the subcommittee for the fine work they've done in improving the M2 and M7 documents.

   Our only concern about this topic is that industry is not keeping with NCCLS recommendations by providing readily available (and especially automated) products to address emerging issues in the area of antimicrobial susceptibility testing. Examples of recent changes for which there are no readily available and easily implemented solutions include ESBLs (M7-A5, Section 9.3) and penicillin/ampicillin-resistant enterococci (M7-A5, Section 9.2.1). We are grateful that NCCLS so carefully studies and defines these issues, but we are also frustrated by the increasing amount of offline, manual testing required to comply with these recommendations.

   There are many factors contributing to this situation, including the time required to get FDA

approval for new products and methods. This is not really an NCCLS problem, but a reality in a rapidly changing brand of laboratory medicine.

- **We provide recommendations in our documents that we have determined to be optimum; however, it is clear that, as new mechanisms of resistance arise, current recommendations may not be optimum, and supplemental testing recommendations are needed. Whenever possible, we will continue to refine the basic methods in order to minimize the extra testing.**

6. In M7-A5, Section 9.2.1 (M2-A7, Section 6.5.1), NCCLS recommends MIC testing of *E. faecium* (recovered from blood or CSF) to ampicillin and penicillin. This is to test for potential susceptibility of synergy with aminoglycosides. I cannot find this in M100-S11. Is this still a recommendation?

- **In M7-A6, we continue to suggest that, for penicillin- or ampicillin-resistant strains of *E. faecium*, it might be appropriate to determine the actual MICs, since strains with penicillin MICs ≤64 μg/mL or ampicillin MICs ≤32 μg/mL may respond to treatment with a β-lactam and an aminoglycoside in the absence of high-level aminoglycoside resistance. However, because this is an optional step that might be worthwhile doing only in rare circumstances when requested by an infectious diseases physician managing the therapy of a patient with a serious infection, we feel that it is adequate to only mention it in the text and not the tables. The test can be performed using a broth dilution- or agar-based MIC method.**

Section 12.7.2.2

7. In attempting to reduce costs to the laboratory without compromising patient care, I have been reviewing the quality control requirements for susceptibility testing. We are currently using the VITEK system. I have asked the following question of the quality control department at Bio-Merieux: Do you interpret the saline diluent as a reagent component of their system? We currently purchase prepared saline from B-D for use with the VITEK system and are often sent multiple lot numbers. I was told that they did not have the recommendation, but I should check the NCCLS documentation. Depending on how one interprets NCCLS document M7-A5, page 20 (Section 12.7.2.2): Perform quality control testing once per week and whenever any reagent component of the test is changed, a laboratory could be doing the quality control two or three times per week. It is the desire of this laboratory to perform tests appropriately and correctly, but without extra work or expense. When we initially began to use the VITEK, we viewed the saline as a reagent component, but I would like this to be clarified by those that research and write the guidelines for testing. I would appreciate clarification on this issue.

- **We consider saline and/or water that is used to prepare an inoculum to be a reagent component that does not require lot-to-lot testing. However, the risk with either water or saline is contamination, especially when repeating dispensers are used to prepare aliquots for inoculum preparation. For MIC testing, it is advisable to do a purity check using a nonselective medium, and then contamination could usually be ruled out each time a test is performed.**

Section 12.7.2.2

8. Once an institution has established that QC can be done weekly by the 30-consecutive-day testing protocols, I think that it is excessive for the institution to have to do 30-day consecutive testing with multiple strains again when a new drug or panel is brought on line and the method of testing remains the same. For example, we use the Vitek instrument for MIC testing. We have performed 30-day QC testing for three different MIC panel types with multiple QC strains as recommended and had no QC failures. Under current guideline recommendations, if we want to select or update a panel, we must run the 30-day QC all over again with multiple strains.

   I recommend that when the method remains the same and a panel change occurs, a five- or seven-day consecutive QC test regimen be performed instead of 30 days. My reasoning is: 1) the manufacturer has already done extensive QC on the panels prior to releasing them; 2) the testing institution has already done R & D and 30-day QC for the method and thus have gained the necessary experience; 3) laboratories in general are being constantly squeezed to cut costs, of which excessive QC is a large component; and 4) panels change frequently due to new drug availability and changing recommendations.

   This seems reasonable to me, because in my 20 years of MIC testing, I have never had a 30-day QC failure with the variety of methods I have used. It is prudent to do the 30-day QC testing any time one changes methods, but once established, I think it is excessive to constantly do 30-day testing when five or seven consecutive days of testing would confirm what the manufacturer has already proven.

- **Although the original quality control recommendations were based more on statistical assumptions rather than hard data, we are reluctant to change them without valid studies or other support to show that less frequent testing would demonstrate adequate performance. Based on the current criteria for acceptable daily control performance (Section 12.7) an option was added to allow for a 20-day testing protocol to convert from daily to weekly quality control. This option provides a streamlined protocol with acceptable confidence limits. The subcommittee will evaluate alternate approaches in the future to further streamline quality control. In the meantime, we believe that the addition of a new drug (should a panel be changed or updated) is a circumstance that continues to warrant 20- or 30-day testing.**

Section 12.9.1

9. The part of this recommendation that I feel is excessive relates to the five consecutive days of QC testing recommended should a drug fail in QC with no obvious reason.

   I recommend that the panel/drug be repeated immediately and if it is OK, then no further action is necessary. My reasoning is: 1) if one is allowed three errors during the 30-day consecutive QC, then it seems inconsistent to assume one will not have an occasional random error with weekly testing; 2) in my experience with the occasional one-drug-out-of-control occurrence, the repeat has always been acceptable; and 3) cutting costs by eliminating waste is becoming crucial to laboratories.

In my proposal to do a single repeat test instead of the five-consecutive-day testing, should the repeat test not fall within the acceptable range, it would be followed up by the five-day testing protocols as outlined in the document.

- **As we stated in the answer to comment 7, we are reluctant to change recommendations without sufficient data to support a change.**

Tables

10. The oral formulation of cefuroxime gives lower serum and tissue concentrations than the parenteral form. So for gram-negatives, the breakpoints for the oral formulation are lower than for the parenteral form. This is correct. I do not understand and I think there is an error in M100-S10 (M7) Table 2G, where the breakpoint for the oral cefuroxime is higher for pneumococci (1 and 4 μg) than the parenteral one (0.5 and 2 μg). Could someone please check this item? If an error is made, it has broad consequences, since the oral formulation is usually understood and the risk for selection of resistance is increased.

- **The cefuroxime sodium (parenteral) susceptibility breakpoint for *Streptococcus pneumoniae* is lower than the value for cefuroxime axetil (oral) because cefuroxime is FDA-approved for the treatment of pneumococcal meningitis. The lower drug concentrations achieved in cerebrospinal fluid (CSF) compared to serum necessitates the lower breakpoint.**

11. I had a question regarding the current gentamicin breakpoints for *Pseudomonas*. Currently, a gentamicin MIC of 4 is read as sensitive and 8 as intermediate. However, given the way that gentamicin is dosed, if a patient is being dosed traditionally (i.e., 1-1.5mg/kg q 8-12h), the concentrations achieved are almost never above 6 to 9 μg/mL and more realistically, are between 5 to 7 μg/mL. These serum concentrations are then only 1-2x the MIC of the organism. With "once daily dosing" (7mg/kg) gentamicin peaks are usually between 13 to 20 μg/mL; better, but still not ideal for an organism with an MIC of 4. Certainly not all patients are even candidates for "once daily dosing" of gentamicin (i.e., elderly, low creatinine clearances, hypermetabolic, etc.), and traditional dosing in these patients is necessary.

    As you know, the goal behind once daily dosing of aminoglycosides is to maximize the pharmacokinetics and pharmacodynamics of these drugs by maximizing the peak to MIC ratio and therefore achieve better, more rapid killing with potentially less toxicity. It is generally regarded that this is best achieved when the peak is 8-10x the MIC value. For an organism with an MIC of 4 this cannot even remotely be achieved safely.

    My concern, therefore, is that these MIC breakpoints (4 and 8) do not reflect the practical issues surrounding gentamicin (and tobramycin) dosing in clinical practice. I am interested in your comments regarding this issue and whether or not this has ever been addressed or if alternative breakpoints have been proposed.

- **Pharmacodynamics is not the only criteria used by NCCLS for developing interpretive criteria. A lower breakpoint for gentamicin would split the population distribution of MICs with *Pseudomonas aeruginosa*. Small, uncontrolled, technical factors could then determine whether or not an organism was considered susceptible. While the current**

breakpoints are adequate for monotherapy of urinary tract infections, we recommend combination therapy at maximal doses for therapy of serious *Pseudomonas* infections (see Table 2B, comment 4 of M100).

12. I am looking for guidelines regarding the optimal frequency for repeat susceptibility testing on bacterial isolates from the same patient and source. The M100-S9 guidelines footnote two organism/antimicrobial combinations (*Staphylococcus* spp. vs. quinolones and *Enterobacter, Citrobacter*, and *Serratia* spp. vs. cephems) as possibly requiring repeat susceptibility testing with three to four days after initiation of therapy, but does not give any other general recommendations for repeat susceptibility testing. Is there any other information available on this issue?

- **As stated in Section 13.3, "Some antimicrobial agents are associated with the emergence of resistance during prolonged therapy. Therefore isolates that are initially susceptible may become resistant after initiation of therapy. This occurs within three to four days, most frequently with *Enterobacter, Citrobacter*, and *Serratia* spp. with third-generation cephalosporins; in *P. aeruginosa* with all antimicrobial agents; and in staphylococci with quinolones." We are reluctant to delineate the number of days between repeat testing except when we know that failure to retest might lead to a serious medical error. In certain circumstances, repeat testing might be warranted earlier than three to four days based on the specific situation and the severity of the patient's condition. Laboratory guidelines on when to perform repeat susceptibility testing should be determined after consultation with the medical staff. When generating antibiograms, results from repeat testing should be excluded as recommended in the NCCLS document M39—*Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data.***

13. I am writing this regarding the MIC cut-off for ciprofloxacin for *S. typhi*. As per the existing NCCLS recommendations ≤1 μg/mL is sensitive and ≥4 μg/mL is resistant. We are currently following this stipulation in reporting ciprofloxacin MIC. We have been isolating strains of *S. typhi* with MIC up to 0.5 μg/mL from patients who are clinically nonresponsive to ciprofloxacin treatment, and the clinicians tell us that fever does not defervesce for up to seven days following treatment. This has been the experience in various centers in India, and the strains from some of these centers sent to us also give an MIC ≤1 μg/mL. The corresponding zone size in disc diffusion test is 21 to 22 mm. We have been monitoring this upward climb in MIC in our center.

    Should we continue with the MIC cut-off as it is?

- **When these breakpoints were first developed, fluoroquinolone resistance had not been documented. We also are aware of several reports in the literature of clinical failures when the current breakpoints are used to detect fluoroquinolone resistance in *Salmonella*. The subcommittee intends to review existing data on this issue soon. Because we have insufficient data that would allow us to change the current breakpoints at this time, we have added the following suggestion to Table 2A (comment 13) that states that "Fluoroquinolone-susceptible strains of *Salmonella* that test resistant to nalidixic acid may be associated with clinical failure or delayed response in**

fluoroquinolone-treated patients with extraintestinal salmonellosis. Testing of extraintestinal *Salmonella* isolates for nalidixic acid resistance may be considered."

14. What is the rationale for having differing breakpoints for levofloxacin, gatifloxacin, and moxifloxacin as it pertains to *Streptococcus pneumoniae*? Moxifloxacin and gatifloxacin typically have lower MICs than levofloxacin, as demonstrated in the SENTRY data (*Clinical Infectious Diseases* 2001;32(Suppl 2):S81-93). NCCLS does not currently set breakpoints for a ciprofloxacin against *Streptococcus pneumoniae*. I believe that the current breakpoints lead the practicing physician to conclude that all fluoroquinolones are essentially equivalent against *Streptococcus pneumoniae*.

- **The breakpoints for each of the three quinolones cited were established based upon a review of the *in vitro* activity of each agent, including its activity against pneumococci with well- characterized resistance due to mutations in the genes that affect the gyrase and topoisomerase targets of this class. In addition, the pharmacokinetics and pharmacodynamics of each agent were assessed independently, and included calculations of the AUC/MIC for free drug concentrations of each agent. Lastly, clinical trials data describing clinical and bacteriological response rates for each agent were reviewed. Thus, each of the three drugs was assessed individually on the basis of specific data for that agent, not as a comparative trial of the three compounds. NCCLS has not established breakpoints for ciprofloxacin, in part because it is not advocated for therapy of pneumococcal infections by experts in treating such infections, nor is it listed among the recommended drugs in any of the published treatment guidelines for community-acquired pneumonia.**

15. We are currently struggling with some issues regarding reference antibiotic susceptibility testing (AST) for *Streptococcus* species, including *S. pneumoniae*. NCCLS document M7-A5, Section 8 recommends that AST testing be performed using lysed horse blood-supplemented (LHB), cation-adjusted Mueller-Hinton broth. However, the addition of LHB may occur either at the time of preparing AST panels before freezing or at the time of panel inoculation (microbroth dilution method). We would like to ask the following questions: 1) Is there any concern or impact to the results of AST testing, regarding the time of adding LHB to the AST panels? 2) Do both methods for adding LHB to the broth meet the current NCCLS guidelines defining the reference panels for use in *Streptococcus* AST testing?

- **Text has now been added to the document (see Section 7.1) that clarifies this issue.**

16. It is our understanding that current standards exist for *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*, and *Acinetobacter* spp., but we are not aware, however, of any standards being available for any of the other members of this group.

    We have considered several options for susceptibility testing of these unusual organisms as follows:

    i. State that no standards exist and that susceptibility tests cannot be performed.

    ii. Perform a broth dilution MIC and report the results without an S, I, R interpretation, with an appended comment that current validated testing reference is not available, and that

susceptibility data does not necessarily correlate with clinical activity.

iii. Send the isolate to a reference laboratory.

- **Methods for disk diffusion testing of nonfastidious, glucose-nonfermenting, gram-negative bacilli exist only for *P. aeruginosa* and *Acinetobacter* spp. In addition, a general comment has been added to Table 2B that testing *P. aeruginosa* isolates from patients with cystic fibrosis can be performed, but that incubation for up to 24 hours may be necessary. The subcommittee is currently working on recommendations for testing both *S. maltophilia* and *B. cepacia* by MIC and disk diffusion. In the meantime, these organisms (and others in that group) can be tested by an MIC method and the results interpreted using the breakpoints given in M7, Table 2B, for other non-Enterobacteriaceae (see general comment 1 in that table).**

17. We are truly concerned these days about the MIC range of ofloxacin for *Salmonella typhi*. According to NCCLS guidelines under Enterobacteriaceae it should be ≤2 μg/mL to be considered as susceptible. But there is evidence that even if it is 0.25 or 0.5 μg/mL, it does not remain a drug of choice, as patients do not respond. What do you suggest regarding this? Also, is it appropriate to check nalidixic acid susceptibility of blood isolates of *Salmonella typhi* to use as a crosscheck of ofloxacin susceptibility? What if we get a nalidixic acid-resistant and ofloxacin-susceptible isolate?

- **The subcommittee hopes to further address this issue in the future. Please refer to the response to comment 13.**

18. I have some questions about information presented in M100-S11. In Table 2G of M2-A7 (Zone Diameter Interpretive Standards and Equivalent MIC Breakpoints for *Streptococcus pneumoniae*), the recommended incubation is 35 °C in 5% CO_2 for 20-24 hrs. In Table 2G of M7-A5 (MIC Interpretive Standards for *S. pneumoniae*), the recommended incubation is 35 °C in ambient air for 20 to 24 hours. I use E-test strips for the MIC determinations of *S. pneumoniae* and often drop antibiotic disks on the same plate. Is this an acceptable practice, since the recommended atmospheres for MIC and disk diffusion are different? I really do not like the idea of having to set up two separate plates for each QC and patient isolate when I need to do both disk diffusion and MIC testing. In all the years I have performed E-test MIC determinations on *S. pneumoniae*, I have always incubated the QC and the patient strains in 5% CO_2. In fact, AB Biodisk (the manufacturer of the E-test strips) recommends incubation of *S. pneumoniae* isolates in 5% CO_2. I have not had any problems with the QC results falling outside acceptable ranges. Do the manufacturer recommendations override the NCCLS recommendations? Could you provide me with some background on the differences in the incubation recommendations?

- **The interpretive criteria in the NCCLS tables for *S. pneumoniae* were developed using CO_2 incubation for disk diffusion and ambient air incubation for broth microdilution tests. Correlation of MICs and zone diameters were made based on that testing. Correlation of agar dilution with disk diffusion was not done for *S. pneumoniae* when these interpretive criteria were established. Testing in CO_2 can change the results for certain drugs, e.g., fluoroquinolones, macrolides, and tetracyclines. The issue of how this might affect testing done by methods other than the NCCLS reference methods should be addressed by the manufacturers of those methods. Clearance by the FDA for commercial systems indicates that the agency concludes that commercial devices provide susceptibility results that are substantially equivalent to results generated using the NCCLS reference methods for the organisms and antimicrobial agents described in the FDA-approved pharmaceutical antimicrobial agent package insert.**

19. I note you have recommendations for MIC testing of *Helicobacter pylori* by agar dilution only. Are you intending to produce guidance on disc sensitivity test or epsilometer tests?

    The UK PHLS Helicobacter Working Group wishes to develop some guidance on disc testing, and we are currently reviewing all the literature. We would wish to mention any recommendations you may have. If you would be interested in our review we would be happy to supply it.

- **The agar dilution test for *H. pylori* was developed as a reference method that could be used in clinical trials, so that the results of those trials could be more accurately evaluated during the approval of new drugs by the FDA. There are no plans to develop a disk test for *Helicobacter*, which, in any case, may prove difficult to do because of the fastidious nature of the organism. Since the E-test is a commercial system, we cannot make recommendations for testing by that method.**

20. Recent changes in the susceptibility breakpoint categories for ceftriaxone, cefotaxime, and cefepime in the M100-S12 document, Table 2G M7-MIC are noteworthy. However, several important questions remain. I would like some feedback and comments for the following questions:

(a) Why have the penicillin G breakpoints remained unchanged?

- **The penicillin MIC breakpoints for *S. pneumoniae* were established many years ago, primarily to guide the treatment of pneumococcal meningitis, where penicillin MICs above 0.1 μg/mL were predictive of clinical failure. A penicillin-susceptible result, whether by MIC testing or through the use of the oxacillin disk-screen test, indicates that a pneumococcal strain remains susceptible essentially to all beta-lactam agents. Thus, the predictive value of a penicillin- susceptible result using the current breakpoint has considerable utility, particularly in laboratories where MIC testing is not possible. Over the course of several meetings, NCCLS members expressed concern that a second set of penicillin MIC breakpoints might lead some to believe that a susceptible result of ≤1 μg/mL would still indicate broad susceptibility to all beta-lactams, which would be incorrect. Furthermore, a detailed review of PK/PD data and Monte Carlo simulations suggested that a susceptible breakpoint of ≤1 μg/mL could not be supported even though limited clinical data suggested that patients with pneumococcal pneumonia caused by such strains responded favorably. The loss of the predictive value of a penicillin-susceptible result, the confusion over when to apply a second set of breakpoints, and the PK/PD data convinced the subcommittee to keep one set of breakpoints and footnote 5 as the best guidance for physicians.**

(b) What is the rationale for allowing amoxicillin and amoxicillin-clavulanate the luxury of having breakpoints, which are one dilution higher than parenteral agents?

- **Please refer to the response to comment 10.**

(c) How does the committee recommend the reporting of penicillin-resistant *S. pneumoniae*? Should reporting cefotaxime and ceftriaxone replace penicillin in reporting resistance?

- **No. The results of testing for each compound should be reported as outlined in Table 2G.**

(d) Can the committee explain why levofloxacin enjoys breakpoints, which are one dilution higher than gatifloxacin and moxifloxacin? The current breakpoints lead clinicians to believe that the potency of the newer fluoroquinolones are equivalent. I would greatly appreciate any insight.

- **Please refer to the response to comment 14.**

21. With the recent changes of interpretive standards for *Streptococcus pneumoniae* (Table 2G), the third-generation cephalosporins have different MICs depending on whether samples are from CSF or non-CSF sites. In the case of *S. pneumoniae* there are some potential problems for laboratories that they should be alerted to. We had a five-year-old patient with a crushed face; two days later he developed altered mental status. With antimicrobial agents started before a spinal tap could be done, blood cultures grew *S. pneumoniae*. Five hours later when the spinal tap was done, it was clear the patient did have meningitis but cultures and gram stains were negative. His MIC was 1 to cefotaxime by E-test.

    My concern is that the laboratory in the future will report this as "sensitive" and that the clinician will not be aware of the specific E-test MIC numbers and their significance and will treat this patient with cefotaxime as the sole drug for his meningitis, based on sensitivity to a peripheral blood isolate. In this case and probably many cases, patients receive therapy before their CSF is obtained. In this case, the culture was negative even though the patient clearly had meningitis. If he had grown the organism from spinal fluid, it would have been considered intermediately resistant and the patient would have been treated with therapy including cefotaxime and vancomycin.

    Hospitals across the country where infectious disease specialists may not be available need some type of alert on their reporting forms to draw attention to the clinician. We are implementing such an alert in our own hospital and feel that the communication system through NCCLS would be the best way to spread this information.

- **During the period that the subcommittee discussed the advisability of creating new breakpoints for patients who do not have meningitis, it was recognized that the microbiology laboratory staff often are not aware of whether a patient does or does not have meningitis unless there is a positive CSF stain or culture. Therefore, the new NCCLS document advises laboratories to report only the meningitis interpretations on CSF isolates, but to report both interpretations on all other specimens, so that clinicians can make the appropriate determinations for their individual patients. Comments 7 through 10 of Table 2G provide additional guidance for using these new breakpoints.**

  We recognize that putting the new breakpoints into practice will pose some challenges in the accurate communication of individual susceptibility results. We will closely monitor any such concerns during the next year. It is our aim to facilitate patient care by offering clinically relevant interpretive criteria for the injectable β-lactams to avoid the perception that potentially more toxic or more expensive agents are frequently necessary for non-CNS infections such as pneumonia.

Summary of Comments and Subcommittee Responses

M7-A6, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Sixth Edition*

General

1. What is the intention of the "Warning" (page 23 of the M100-S14 Vol. 24, No. 1 standards for antimicrobial susceptibility testing) regarding not routinely reporting clindamycin, macrolides, etc. for bacteria isolated from the "CSF"? Should this be interpreted in its narrowest sense, or does CLSI consider all central nervous system (CNS) sites similarly to CSF? Specifically, would it be incorrect to report clindamycin, etc. for a bacterial brain abscess aspirate? Infectious disease textbooks consider clindamycin as an acceptable alternative to treat brain abscesses.

- **The WARNING box in M100 refers only to bacterial meningitis. Clindamycin is mentioned among alternative therapies useful for brain abscess (*Principles and Practice of Infectious Diseases*, G. L. Mandell, J. E. Bennett, and R. Dolin (eds.), 5th edition, Philadelphia, Churchill Livingstone, Inc., 2000), so it would not be incorrect to report clindamycin susceptibility test results on an aspirate from a brain abscess. There are insufficient data to provide comprehensive guidance on which test results to report routinely for pathogens isolated from brain abscesses.**

Table 2A

2. Could you clarify the comment in M100 regarding the Warning for *Salmonella* and *Shigella* in Table 2A, comment (5) on page 35? The comment states that first- and second-generation cephalosporins should not be reported as susceptible. Does that comment include the cephamycins also?

- **Yes, the statement has been clarified.**

Table 2B

3. On page 95, footnote j states that "Other non-Enterobacteriaceae include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli except for *Acinetobacter* spp., *Burkholderia cepacia,* and *Stenotrophomonas maltophilia."* On page 108 under general comment (1), it states "Non-Enterobacteriaceae include *Acinetobacter* spp., *S. maltophilia, Pseudomonas* spp., and other…" Is this not contradicting the statement on page 95?

- **In order to avoid this confusion in the future, the wording in Table 1, footnote j has been revised.**

Table 2C

4. There appears to be a discrepancy between the disk diffusion and MIC sections of the current M100 document as related to oxacillin:

   For disk diffusion testing of non-*S. epidermidis* coagulase negative staphylococci, disk diffusion "R" isolates that are *mec*A/PBP2a negative should be reported as "S." (Pg. 42)

   However, for MIC testing of these same non-*S. epidermidis* coagulase negative staphylococci, isolates that are *mec*A/PBP 2a negative should be reported as "S" if oxacillin

MICs are between 0.5 to 2 mcg/mL, but as "R" if MICs are > 4 mcg/mL. (Pg. 105)

If moderately to highly oxacillin resistant *mec*A/PBP2a negative isolates should be reported as "R" when performing the MIC test, shouldn't this also be the case when doing disk diffusion? Put another way, shouldn't the disk diffusion criteria also have a zone size below which a report of "R" be made regardless of *mec*A/PBP 2a findings?

- **The reason for reporting strains exhibiting MICs ≥4 µg/mL as oxacillin resistant despite *mec*A/PBP2a status is based on pharmacokinetic/pharmacodynamic data and the possibility that other resistance mechanisms not yet discovered may be responsible for the increased MICs. Unfortunately, there is no zone diameter or range of zone diameters that correlates exactly with MICs ≥4 µg/mL. The data from the CLSI study used to establish the revised interpretive criteria (Tenover et al, *J Clin Microbiol.* 37: 4051-4058) show that 108 of 110 (98.2%) isolates with zone diameters of 6 mm (i.e., no zone) were *mec*A- positive. Of 42 strains with zone diameters in the 7 to 17 mm range, 15 (35.7%) were *mec*A-positive and 26 (64.3%) were *mec*A negative. Therefore, based on these data, it would be possible to do the following: if there is no zone to oxacillin, report as resistant; if there is any zone ≥7 mm, then perform a *mec*A test or a cefoxitin disk test for a definitive answer. However, recent studies show that using the cefoxitin disk in place of the oxacillin disk gives better correlation with *mec*A status for coagulase-negative staphylococci, and the cefoxitin zone is much easier to read.**

5. I am currently revising my SOP for β-lactamase testing of staphylococci, and I am training my staff to follow the CLSI/NCCLS guidelines stated in Table 2C of document M100-S14. Today one of my techs has a patient with a penicillin MIC of <0.03 µg/mL. Per CLSI/NCCLS, we should simply report the penicillin as susceptible. My tech did both a direct and an induced β-lactamase, and they were both positive. We are having a difficult time understanding why CLSI/NCCLS wants us to call the penicillin susceptible when we are getting a positive β-lactamase result.

   Following CLSI/NCCLS and reporting the penicillin susceptible on this patient is making me uncomfortable, because I know that it is β-lactamase positive. My tech is asking me to explain why CLSI/NCCLS is telling us not to do the β-lactamase testing when the MIC is <0.03 µg/mL, and I am hoping that you can help me provide her an answer. After this patient, if we follow the CLSI/NCCLS guidelines, and stop doing the β-lactamase testing on patients with an MIC of <0.03, we will always feel uncomfortable knowing that it could be β-lactamase positive. Any information you can provide that will help us understand this will be greatly appreciated.

- **The CLSI/NCCLS recommendation is not what is stated above. Comment 6 in Table 2C of M7-M100-S14 (now comment 8 in M7-M100-S15) states: "A penicillin MIC of ≤ 0.03 µg/mL <u>usually</u> implies lack of β-lactamase production, and MICs of ≥ 0.25 µg/mL should be considered resistant; staphylococci with penicillin MICs between 0.06 to 0.12 µg/mL may or may not produce β-lactamase, and an induced β-lactamase test can clarify these MICs (see M7-A6, Section 10.2)." Using CLSI reference methods, it should be extremely rare to find strains of staphylococci that exhibit penicillin MICs ≤ 0.03 µg/mL and that produce β-lactamase; however, should a strain be determined to produce β-lactamase, it should be reported as penicillin resistant despite the penicillin**

MIC. Those laboratories using commercial systems should follow the manufacturer's recommendations for guidance in this situation.

Table 2E

6. I am aware that the antibiotic tested is the one to be reported; however, I need clarification on how to address the reporting of doxycycline with the fastidious organisms when the tetracycline interpretation is resistant or intermediate. In Tables 2A, 2B, 2C, and 2D of M100-S15, all tetracycline comments end by stating, "...However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline..." But in Tables 2E (*Haemophilus*) and 2G (*S. pneumoniae*), this statement is not part of the comment. Am I to assume this statement does not hold true for the fastidious organisms or can I deduce it does? Literature leads me to believe it does. Should tetracycline R or I be equated with doxycycline resistance? Will CLSI be developing zone sizes for doxycycline in the future?

   Some physicians are hesitant to use doxycycline because of presumed inactivity. Clinicians assume isolates reported resistant to tetracycline are also doxycycline resistant. Our pharmacologist notes this assumption does not agree with the literature and that extrapolating our tetracycline susceptibility data to doxycycline has no direct application. It doesn't relate to therapy or help in clinical studies. Citing literature, she says doxycycline is consistently active against all common typical and atypical bacterial causes of pneumonia. She is concerned this assumption of tetracycline resistance (which is common for *S. pneumoniae*) implying doxycycline resistance as well is sending those not familiar with infectious disease in the wrong direction (i.e., towards the use of more expensive alternatives with more harmful side effects).

- **Tetracycline-susceptible isolates of various species are susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline. Currently, there are no interpretive criteria for doxycycline and minocycline against pneumococci and *H. influenzae*. Until additional studies are undertaken or reviewed by CLSI, it is premature to use tetracycline to predict doxycycline or minocycline resistance.**

7. Reading the CLSI documents of 2005, I wonder why the D-test that looks for inducible clindamycin resistance is not described for *Streptococcus pneumoniae*. Can you give me the reason behind this?

- **Isolates of *Streptococcus pneumoniae* can have *erm*-mediated resistance to erythromycin. However, the vast majority of these isolates are also resistant to clindamycin (i.e., the constitutive resistance phenotype). Rare isolates of pneumococci may have inducible resistance; however, the clinical significance of this has not been established. Therefore, routine testing for inducible clindamycin resistance is not recommended for this species.**

Summary of Comments and Subcommittee Responses

M7-A7, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition*

General

1. I am preparing to test minimal inhibitory concentration (MIC) values and had a question about the dilutions. Someone mentioned to me that it is recommended to make only four dilutions from each antibiotic and then make a new standard at a lower concentration. I cannot find reference to that in my reading of M07. Would it be possible to do serial dilutions of the antibiotics rather than the method outlined in Table 6 of M100? I am concerned about being told that serial dilutions are only good for four dilutions, because this is a standard practice I have always used to quantify CFU/mL and if it is not accurate with these antibiotic standards, then who is to say it is accurate for quantifying CFU/mL? And if it is accurate for quantifying CFU/mL, then why is it not accurate for quantifying MIC values for the antibiotics? Sorry for being confused. I have been handed a protocol already in place that seems to have a lot of unnecessary dilutions and testing being done to determine the MIC and am trying to scale it back.

- **In the experience of many of the subcommittee members who have been preparing reference dilution panels or plates for many years, they have never done what you describe, ie, prepare intermediate stock solutions when diluting more than four tubes. M07-A8 states in Section 10.4.1, "For the intermediate (10x) antimicrobial solutions, dilute the concentrated antimicrobial stock solution (see Section 7.3) as described in M100 Table 7 (previous Table 6) or by making serial twofold dilutions."**

2. In CLSI document M02, the disk diffusion zone diameters are given with equivalent MIC breakpoints. In the overwhelming majority, they correspond to the MIC breakpoints printed in M07. However, some do not (eg, gentamicin and amikacin with *Enterobacteriaceae*). Do you know why? Also, some of the MIC equivalent breakpoints are not in doubling dilutions (eg, in Table 2A, the Susceptible equivalent breakpoints are ≤ 12 µg/mL for netilmicin and ≤ 6 µg/mL for kanamycin). Why?

- **MIC equivalents listed in M02 represent the MIC breakpoints used when the zone size diameters were first determined. Since the M02 document was published before the M07 document, occasional discrepancies have existed and these mainly occur with the aminoglycosides. However, in M100-S19, the MIC and zone diameter interpretive criteria in all the Table 2s were combined in the same Table for each of the organism groups and the equivalent MIC breakpoints (or MIC correlates) for disk diffusion no longer appear in the Tables. A table listing the older MIC equivalents for zone diameters where discrepancies occurred between M02 and M07 is available in the minutes of the AST Subcommittee meeting of 11-13 June 2008 as Attachment 3.**

3. Our pulmonologist has requested that we test *Staphylococcus* spp. and *Enterobacteriaceae* against moxifloxacin. The PharmD gave me a moxifloxacin product insert that gives different interpretive criteria (≥ 19 = susceptible) than those listed in M100 (≥ 24 mm = susceptible). Their product insert gives the same interpretive criteria for *Enterobacteriaceae,* and the CLSI document does not list ANY moxifloxacin interpretations for *Enterobacteriaceae.*

   I realize that we may use FDA or CLSI interpretive criteria, but the difference here is so great—19 mm would be RESISTANT per CLSI—that I don't feel comfortable reporting any results until I get a satisfactory explanation.

- **Although there are several reasons why the CLSI and FDA moxifloxacin breakpoints for staphylococci differ, the most important point for the laboratorian to understand is that CLSI breakpoints can be used for all staphylococci including MRSA, whereas the FDA breakpoints apply only to methicillin-susceptible staphylococci (per the FDA label for clinical use of the drug), so the laboratory should not report the drug on MRSA if using the FDA breakpoints. CLSI breakpoints for testing moxifloxacin with *Enterobacteriaceae* have not been determined, but FDA breakpoints are available for use. It is important to note that moxifloxacin is not approved for treatment of urinary tract infections due to low urinary concentrations and, thus, should not be tested on urinary isolates. The decision regarding which drugs to report for certain organism groups and which breakpoints to use should be made by the laboratory following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy & Therapeutics and Infection Control committees of the medical staff. Clinical laboratories may implement newly approved or revised disk CLSI breakpoints as soon as they are published in M100. If a susceptibility testing device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility to an agent using the CLSI MIC breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.**

4. What is the recommended frequency for quality control of various agar screening tests (eg, chromogenic media, vancomycin agar screen)?

- **Media containing antimicrobials used for primary isolation are not part of the scope of the susceptibility testing documents M02 and M07 (see CLSI document M22).**

  Single drug susceptibility tests/screens should be treated like other susceptibility tests (multiple concentrations or multiple drugs) until such time that recommendations and appropriate supportive data are available to streamline.

Tables 1 and 2B-4

5. In a recent College of American Pathologists (CAP) survey, participants were told that for *S. maltophilia,* they should have only reported results and interpretive breakpoints for the antimicrobial agents listed in Table 1. The question concerns minocycline, which is listed in the *S. maltophilia* column. Most laboratories can test tetracycline, but not minocycline. In footnote b in Table 1 (M100-S15), it states that tetracycline can be used to predict susceptibility (not Intermediate or Resistant) to minocycline. Is the same statement true for *S. maltophilia*? There are no tetracycline breakpoints listed in the draft of Table 2B-4, *S. maltophilia* (M100-S16). If minocycline is not available on the antimicrobial susceptibility testing medical device system the laboratory is using, and the *S. maltophilia* isolate is susceptible to tetracycline, should the laboratories report the tetracycline result or not?

- **It is true that isolates of *S. maltophilia* that are susceptible to tetracycline are also susceptible to minocycline and doxycycline. However, > 90% of *S. maltophilia* strains (personal communication, R. Jones, Sentry Antimicrobial Surveillance Program) are resistant to tetracycline but susceptible to minocycline and doxycycline, so testing tetracycline as a surrogate in place of the other tetracyclines is not recommended,**

because the vast majority of strains would be called resistant. When testing was done to determine criteria for testing *S. maltophilia* and *Burkholderia cepacia,* the CLSI working group chose to include only agents that were active, that were recommended by experts as therapies of these infections, and for which the recommended breakpoints were proven to be reproducible.

Table 2A and 2B-5

6. *Enterobacteriaceae* and non-*Enterobacteriaceae,* which are resistant to tobramycin and amikacin, but susceptible to gentamicin, most likely produce a 6′-acetyltransferase. In this case, only one of the three gentamicin subcomponents, C_1, remains active. Since the fraction of C_1 varies between gentamicin formulations and C_1 appears to have different pharmacokinetics than gentamicin as a whole (*Antimicrob Agents Chemother*. 1975;7:328-332), are the gentamicin interpretive breakpoints accurate in these cases? Would it be reasonable to report gentamicin susceptibility as intermediate or provide a comment that gentamicin activity is uncertain?

- **The commenter raises an interesting question. The subcommittee has no data that support changing the susceptible category to intermediate or resistant. However, when an isolate that is gentamicin susceptible and amikacin and tobramycin resistant is encountered and selective reporting is used by the laboratory, the susceptibility to gentamicin and the resistance to tobramycin and amikacin should all be reported.**

Table 2B-2

7. I am a microbiology supervisor with a question regarding interpretations for *Acinetobacter* to tigecycline. I have an infectious disease doctor complaining that this drug has been out for over a year, and still no interpretations and guidelines regarding this drug have been published. I have the 2008 standards and see this is true. Any time frame or information that you may have so that I could pass some pertinent information on to this doctor would be appreciated.

- **Interpretive criteria for tigecycline are not included in the CLSI documents for any genera, because the drug manufacturer has not presented the necessary data for review by the subcommittee for subsequent publication of breakpoints in M100. In the meantime, one ordinarily could refer to the drug package insert for the US Food and Drug Administration (FDA) breakpoints; however, breakpoints for *Acinetobacter* are not included in the FDA list at this time because there is no clinical indication for tigecycline against *Acinetobacter*.**

8. CLSI document M100-S17 has MIC susceptibility ranges for colistin and polymyxin B against *Acinetobacter* sp., but there are no standards listed for disk diffusion on this isolate. Our Infectious Disease staff sometimes requests that colistin and polymyxin B be tested against multidrug resistant (MDR) *Acinetobacter* isolates; and since these drugs are not available on our commercial conventional microdilution panels, I order these antimicrobials as (MIC) antibiotic gradient strips from a commercial source. The company, however, requires that a disclaimer be signed stating that we will use colistin and polymyxin B for INVESTIGATIONAL USE ONLY; a disclaimer is only good for six months and a new disclaimer must be signed for each new order. Should colistin and polymyxin B not be used for clinical purposes and are indeed for investigational use only?

- **There are no disk diffusion criteria for *Acinetobacter* in M100 because the disk test does not correlate with MIC tests and is therefore unreliable. Questions about the**

commercial gradient strip test should be addressed to the manufacturer. The use of colistin or polymyxin B for clinical treatment is a medical decision.

Table 2G

9. I have a question about reporting cefepime (meningitis) and/or cefepime (nonmeningitis). In M100-S18 Table 2G M07-MIC, cefepime (nonmeningitis) has a comment (11), "Only report interpretations for nonmeningitis and include the nonmeningitis notation on the report." There is not a US FDA-approved indication for the use of cefepime for meningitis. Just below the cefepime (nonmeningitis) entry, cefepime (meningitis) is listed with interpretative values. When would it be appropriate to use this?

- **The CLSI documents are also for use outside the United States where cefepime might be used for treatment of meningitis, which is the reason those criteria are included in Table 2G. You should discuss with your Medical Director how to handle reporting of cefepime, but one solution in the United States might be to report only cefepime (nonmeningitis) with a note that cefepime is not US FDA approved for treatment**