CLSI Teleconference Questions 2005

NLTN CLSI M100-S15 Teleconference (January 2005)
Q&A

#	Question	Answer
1	When should labs perform the BHI-V6 agar screen?	If you are using a system for testing vancomycin that has been unreliable in detection of VRSA (e.g., automated commercial systems), it is essential to add the BHI-V6 in order to detect this rare, but important emerging resistance.
2	Last year at ASM, one of our lecturers said that if you have growth of *S. aureus* on the BHI-V6, you should subculture the growth to a BAP, then the next day sub again to another BAP, and then subculture back to BHI-V6. If there is growth, it is a true VRSA, but if no growth then this is a *S. aureus* showing vancomycin heteroresistance. Is this something that we should be doing?	The method you described is not currently recommended by CLSI for clinical laboratories. The overall significance of vancomycin heteroresistant *S. aureus* is uncertain at this time.
3	Regarding the vancomycin screen agar, are we no longer required to include *S. aureus* for QC? Earlier CDC recommendations stated that *S. aureus* and the two *Enterococcus faecalis* organisms should be used for QC.	The current CDC recommendation for QC of the BHI-V6 (when screening staphylococci OR enterococci) is to use *E. faecalis* ATCC 29212 (vancomycin-S control) and *E. faecalis* ATCC 51299 (vancomycin-R control).
4	What are your thoughts on suppressing vancomycin for oxacillin-susceptible staphylococci? Although I realize it is more effective for the pharmacy to prohibit usage, we wanted to try to make an attempt to decrease the vancomycin usage from micro.	Decisions on what to report should be made in consultation with your medical staff. Institutions use various mechanisms to encourage "prudent prescribing". Regardless, it is recommended that laboratories use a reliable method for detection of VISA and VRSA.
5	Is Trypticase Soy Broth satisfactory for inoculating BHI-V6? I did not see MH Broth specified in "CLSI" document nor was it specified in BBL technical insert.	Although TSB was not evaluated during CDC studies, it is likely that it would perform satisfactorily. In M7-A6 (page 10), it states that "broth" or saline can be used for inoculum preparation using the direct colony suspension method for agar dilution testing.
6	We have started receiving CoNS here at the Public Health Lab for confirmation of susceptibility results for vancomycin. Should we be concerned about the CoNS isolates or should just *S. aureus*? Also, at what point do we send isolates to CDC? We haven't had any vancomycin MICs greater than 4 µg/ml.	All confirmed *S. aureus* with vancomycin MICs of 4µg/ml or greater should be sent to your local health department and CDC. At this time, there is no specific recommendation for CoNS. However, if CoNS with resistance to vancomycin (e.g., MIC ≥32 µg/ml) were encountered from a particularly significant infection due to CoNS, you may wish to contact your local health department and/or CDC to see if they want the isolate.
7	My lab is reconsidering our workflow for staphylococcus sensitivity testing. We will be switching to cefoxitin to detect *mec*A and report oxacillin results. Can we use cefoxitin to induce β-lactamase as well, or do we still need to induce with oxacillin?	Although there are no data in the literature to confirm that cefoxitin is a good inducer of staphylococcal β-lactamase, anecdotal evidence suggests that this is true and cefoxitin should work for inducing staphylococcal β-lactamase. CLSI is re-examining penicillin (and β-lactamase testing) of staphylococci and improved recommendations may occur soon.
8	Should we use cefoxitin as an inducer for CoNS for determining *mec*A-mediated oxacillin resistance using the PBP2a test?	Cefoxitin is a good inducer of PBP2a. However, if you use cefoxitin instead of oxacillin as an inducer of CoNS prior to performance of the PBP2a

		assay (using the FDA-cleared kit), you would have to validate this procedure in your laboratory if it differs from that listed in the package insert. Perhaps the manufacturer can help you.
9	Scenario: *S. aureus*, PBP2a negative, oxacillin-R by disk diffusion, not multiply resistant. We informed MD that this is not an MRSA but isolate seems to have some type of atypical oxacillin resistance**.** How should we proceed?	The isolate may be one of the rare *mec*A negative strains with borderline oxacillin resistance (MIC 4-8 µg/ml) or borderline oxacillin susceptibility (MIC 1-2 µg/ml). An oxacillin reference broth microdilution MIC of ≥4 µg/ml and a cefoxitin susceptible disk diffusion result would further confirm this suspicion. By disk diffusion, cefoxitin is comparable to oxacillin in detection of *mec*A-mediated resistance in *S. aureus*, but may not detect the rare borderline oxacillin-R isolates.
10	For the rare *Staphylococcus aureus* that are *mec*A negative, but have oxacillin MICs of 4.0 µg/mL, is it likely these are hyper β-lactamase producers? 1. Should they be reported as MRSA, *mec*A negative? 2. Would the organism be susceptible to oxacillin in vivo? 3. Should one consider using oxacillin or cefazolin in spite of the oxacillin results? 4. Would additional testing necessary to R/O hyper β-lactamase production or other mechanisms?	It is possible that the isolate has borderline oxacillin resistance due to excessive β-lactamase production or another mechanism. There are no practical clinical laboratory tests to determine the mechanism of resistance and borderline oxacillin resistance is not currently addressed in CLSI standards. There are some suggestions that β-lactams could be used for treating infections caused by *mec*A-negative *S. aureus* that have MICs slightly above 2 µg/ml. One strategy would be to report these as oxacillin "R" (with the MIC) and explain the situation (e.g., isolate does not have "typical" *mec*A-mediated oxacillin resistance, but oxacillin MIC is resistant and higher than that encountered in most *mec*A-negative strains) to the MD and possibly add a qualifying comment to the report.
11	Recently, we discussed the need for keeping OX on our disk diffusion panels and the issue of losing the ability to detect "non-traditional" forms of methicillin resistance. Overall it was felt from the work of Chambers and other investigators that BORSA (and/or MODSA) organisms were interesting but very infrequent and of probable little clinical significance. We have had a couple "presumptive" BORSA that are *mec*A negative, confirmed *S. aureus* by light-cycle assays, PBP2a negative and have OX zone sizes b/w 7-11mm (also sensitive to FOX). For discrepant OX and FOX results over the last 6 months, we found a frequency of 0.45%. (single isolates from distinct patients) Are you aware of any clinical data that suggests that BORSA/MODSA strains should be of any concern for either the clinical lab or the physician? I am aware of both an animal study that was able to show the effectiveness of OX against these phenotypes as well as the potential inability to transfer resistance from BORSA strains to MSSA strains. What are your thoughts concerning the possibility that the clinical data is lacking because we as laboratorians and physicians might not be aware of these strains (i.e. previously difficult to detect based solely on OX)?	See above. As you mentioned, there is little information on isolates with borderline oxacillin MICs and there are mixed opinions related to the clinical significance of these strains. It is unclear at this point if the cefoxitin disk diffusion test will detect any of these.
12	In the past, when we got a discrepancy between the Vitek oxacillin MIC and the oxacillin screen agar for *S. aureus*, we arbitrated with PBP2a latex test.	Oxacillin Etest needs to be done on MHA with 2% NaCl and is probably not the most convenient test to arbitrate discrepant oxacillin results from

	If PBP2a positive, we reported oxacillin resistant; if PBP2a negative, then oxacillin susceptible. Now with note 4 in 2005 CLSI Staph (M2) Table 2C, I question this protocol. Can I use oxacillin ETEST to confirm an oxacillin MIC of 4 µg/ml with a negative PBP2a?	Vitek and the oxacillin salt agar screen for *S. aureus*. Studies that supported the suggestion to report PBP2a-negative staphylococci with oxacillin MICs of ≥ 4 µg/ml as oxacillin resistant were determined using CLSI reference oxacillin MIC methods (e.g., broth dilution). It is not known how the isolates examined in these studies would perform with Vitek or other commercial systems. Because PBP2a negative, oxacillin resistant staphylococci (when tested by CLSI reference methods) are rare, the PBP2a or cefoxitin disk diffusion test would be sufficient in most cases when arbitration is necessary. The most definitive test would be an assay for *mec*A.
13	Please explain further the problem with overcalling oxacillin resistance in CoNS and the benefit of cefoxitin disk testing on these isolates. For CoNS causing endocarditis that are oxacillin-R by Vitek, should we do a cefoxitin disk test? Might we encounter isolates that are oxacillin-R and cefoxitin-S by disk diffusion testing?	Some non-epidermidis CoNS that have oxacillin resistant MICs of 0.5-2.0 µg/ml or zones in the resistant range do not contain *mec*A and would thus be reported as falsely resistant to oxacillin. However, these strains usually test "S" with the cefoxitin disk diffusion test. Therefore, the cefoxitin disk diffusion test is less likely to overcall oxacillin resistance in *mec*A-negative CoNS.
14	We have CoNS isolates from our nursery with MicroScan and Etest vancomycin MICs of ≥4 µg/ml. Most of these have been identified by MicroScan as *S. capitis subsp. ureolyticus*. We use BHI-V6 on all staphylococci and the above-mentioned isolates grew on BHI-V6. How should we report the CoNS with vancomycin MICs of 6 or 8 µg/ml? Currently, we only report the verified MIC (without interpretation) and add a comment "Reduced vancomycin susceptibility".	Most of the work with BHI-V6 has been done with *S. aureus*. Etest is reliable in detecting *S. aureus* with reduced vancomycin susceptibility (VISA and VRSA) and CoNS with reduced vancomycin susceptibility. There are some species of CoNS (e.g., *S. hemolyticus*) that are known to be less susceptible to vancomycin. I do not know if this is true for *S. capitis* and you may want to check the literature. Nevertheless, the CLSI tables can be used to interpret the vancomycin MIC from Etest and your additional comment seems reasonable. Because your CoNS isolates are from a single location and assuming the antibiograms and biotypes are identical, you may wish to inform your infection control team, particularly if the strains are likely to be associated with an infection.
15	As far as *S. lugdunensis*, how important is it to identify coagulase positive staphylococci that are penicillin-S to rule out *S. lugdunensis*. If isolate is coagulase neg, we are reporting a more conservative susceptibility anyway, which should be less danger to the patient. How far does this really need to go?	Some feel it is important to identify *S. lugdunensis,* particularly when isolated from sterile body sites since some infections with this species may be more difficult to manage than those due to other CoNS. If a patient had endocarditis with *S. lugdunensis*, a β-lactam, if it tests susceptible, would likely be preferred over vancomycin. If using CoNS breakpoints, many *mec*A-negative *S. lugdunensis* test oxacillin-R (and are then reported as R to all β-lactams). Many of us are rethinking workup of staphylococci to catch *S. lugdunensis*, particularly from significant sites, and you will probably see more suggestions for dealing with this in the near future.
16	For CoNS that are PBP2a negative but oxacillin-R (Vitek), we assume we should go with the PBP2a result. However, other β-lactams are edited to R by Vitek in this case. Would it be acceptable to include a comment something like "Oxacillin-S staphylococci are susceptible to other	Yes, in fact it is suggested in CLSI [page 111 comment (7)] that oxacillin and penicillin results be used to predict results of other anti-staphylococcal β-lactams for staphylococci. You are correct that oxacillin-S or PBP2a negative staphylococci would be susceptible to other antistaphylococcal β-

	antistaphylococcal β-lactams?	lactams.
17	I understand that use of cefoxitin disk will eliminate some false oxacillin-R for CoNS. We use Vitek for CoNS testing. If I wanted to do some spot checks, do you think we would get valid results by dropping a cefoxitin disk on our purity plate rather than perform a standard disk diffusion test?	Performance of the cefoxitin disk diffusion test for staphylococci has only been validated for use with the standard disk diffusion method. Since purity plates generally use an inoculum lower than a McFarland 0.5, it is unlikely that the specified cefoxitin breakpoints would work on purity plates. Consequently, you would have to determine and validate cefoxitin zone breakpoints that would work with this modified purity plate method.
18	We use the Vitek for susceptibility testing for all CoNS and *S. aureus* isolated from specimens other than MRSA screening cultures. We currently test staphylococci from MRSA screening cultures using an oxacillin Kirby Bauer test. You emphasized that cefoxitin was a surrogate and especially improved detection of oxacillin resistance in CoNS. It looked to me that the data for the two drugs are essentially the same for *S. aureus*. Am I correct?	Yes. Current data suggests that for *S. aureus*, results for disk diffusion testing with oxacillin are comparable to those for disk diffusion testing with cefoxitin for predicting *mec*A mediated oxacillin resistance.
19	I am still unclear about cefoxitin testing vs. oxacillin interpretation on staphylococcal isolates. My understanding is that the cefoxitin disk results guide the oxacillin interpretation i.e. if cefoxitin is S and oxacillin is R, the isolate would not be an MRSA.	Results from cefoxitin disk diffusion testing are used for reporting oxacillin. For CoNS, the cefoxitin disk diffusion test is considerably more specific than the oxacillin disk diffusion test such that oxacillin-R and cefoxitin-S isolates may be encountered. In these cases, it is likely that the oxacillin disk diffusion test result is falsely resistant since we know the oxacillin zone diameter interpretive criteria overcalls resistance in non-epidermidis CoNS. For *S. aureus*, it may be possible that a BORSA strain would give an oxacillin-R and cefoxitin-S disk diffusion result. Studies are ongoing to clarify these types of discrepancies. In the interim, the most definitive test would be an assay for *mec*A.
20	It seems that all labs are routinely doing the D zone test on all MRSA and now β streptococci. This may be easy for labs performing disk diffusion testing routinely, however requires extra effort for those of use using MicroScan or other automated systems. How can we do this most efficiently? Is routine D zone testing an "official" requirement? If those who receive our lab reports are not interested in results for clindamycin, do we still need to report clindamycin?	When deciding which routine and supplemental antimicrobial susceptibility tests to perform, each lab must determine if, how, and when results from these tests will be used. D zone testing is performed to determine if clindamycin would be appropriate for treating staphylococcal or β streptococcal infections due to erythromycin-R and clindamycin-S strains. If physicians are not interested in using clindamycin in your facility, there is no point in expending supplemental resources to do the D zone test. Interaction with your medical staff is essential to determine which tests are useful for your patients. Many labs perform D zone testing on request only. They suppress reporting of clindamycin-S on erythromycin-R and clindamycin-S staphylococci and β-hemolytic streptococci and include a comment such as "Contact laboratory if clindamycin results clinically indicated".

21	What are we to do with an isolate that is intermediate to erythromycin?	The distribution of erythromycin results for staphylococci and streptococci is usually bimodal, i.e., isolates are either very R or very S. There should not be many "I" results. Sometimes "I" can result when inoculum is too heavy and "trails". Nevertheless, when an "I" result is confirmed in an isolate that is clindamycin-S, it would be best to do the D zone test before reporting clindamycin as "S".
22	For the D zone test, it states in M100-S15 to place the disks 15 mm apart but in your teleconference you use 12 mm. Why the difference? Is a range of 12-15 acceptable?	In M100-S15, it states that for the D zone test on β-hemolytic streptococci, the disks are to be placed 12 mm apart, whereas for staphylococci it states 15 mm for the purity plate method with MIC tests and 15-26 mm for routine disk diffusion testing. Our experience with the purity plate method is that 12-13 mm is easier to interpret as compared to 15 mm. Some who manually place the disks on standard disk diffusion plates prefer distances closer to 15 mm than 26 mm. If using a disk dispenser, the inner ring tends to drop disks closer together. If disks are too far apart, the D zone reaction may be difficult to interpret and could be reported as falsely negative.
23	Is the use of TSA with 5% sheep blood agar an acceptable medium for D zone testing or should we be using Mueller Hinton agar?	As described in M100-S15, page 114 (26), "standard blood agar plate" used for purity check is acceptable for D zone testing with MIC methods. Most would agree that TSA with 5% sheep blood would be considered a "standard blood agar plate" and could be used for the D zone test.
24	Should the D zone test be performed on CoNS?	*S. aureus* as well as CoNS may have inducible clindamycin resistance which could be detected with the D zone test. The same testing and reporting rules apply to *S. aureus* and CoNS. However, it is very unusual for clindamycin to be used for CoNS infections. Therefore, a practical strategy would be to refrain from reporting clindamycin on erythromycin-R and clindamycin-S CoNS and including a comment to "Contact laboratory if clindamycin clinically indicated". At that time, the D zone test could be performed.
25	Is it ok to report the clindamycin result along with a comment stating it may have inducible resistance?	The decision on what to report must be made with your medical staff and the needs of those using your reports for patient management. Some infections caused by erythromycin-R and clindamycin-S staphylococci or β-hemolytic streptococci with inducible clindamycin resistance may not respond to clindamycin therapy. Physicians tend to look at "S" or "R" before they read a comment. You must take this into consideration when developing your reporting protocols.
26	For D zone test, we have not been doing zone QC of the clindamycin and erythromycin discs. We have been looking for the "D zone" with *S. aureus* ATCC 25923 and a "wild strain" which is D zone positive each time we have a patient request due to the infrequency of requests. Should we be performing the QC of the discs even if we do not report Kirby Bauer zone	It is important to perform QC of disks even if you do not report results for clindamycin and erythromycin from the disks. Do not perform the D zone test on *S. aureus* ATCC 25923, which is susceptible to both erythromycin and clindamycin. Please see additional information provided above.

	results on patients? We only use the clindamycin and erythromycin discs for the D test.	
27	We are currently offering the D zone test on request only. Our QC for the test states that the erythromycin and clindamycin are checked on a weekly basis in routine KB QC.	Like QC suggestions for most other antimicrobial susceptibility tests, QC can be done daily, weekly (once reliable daily testing is confirmed according to CLSI rules), or concurrent with testing patient isolates. If D zone test is only done once a month or so, it might be most efficient to QC the erythromycin and clindamycin disks at the time the patient isolate is tested.
28	Does CLSI or CAP or CLIA or you have any recommendation on frequency of QA on D zone test? Should QA strains be tested once per lot/shipment of the erythromycin and/or clindamycin disks?	No. Each laboratory must decide on the frequency of QA testing for the D zone test. As you know, QA strains *S. aureus* ATCC BAA -976 and BAA-977 is included for training, competency or test evaluation.
29	Please clarify D zone QA/QC	The following strategy could be used for the D zone test QA/QC for staphylococci or β streptococci: 1) Perform QC of erythromycin and clindamycin disks with *S. aureus* ATCC 25923 and unsupplemented MHA (ambient air) OR *S. pneumoniae* ATCC 49619 on BMHA and incubate in 5% CO2. Note: *S. aureus* ATCC 25923 or *S. pneumoniae* ATCC 49619 are erythromycin-S and clindamycin-S so D zone phenomena cannot be monitored with these strains. 2) Record zone diameters. Troubleshoot any result that does not fall within expected range as indicated in Table 3 in M100-S15. Reliability of the D zone test is dependent on the disks having appropriate content of drug. This is checked by testing ATCC QC strains that have defined QC ranges. 3) Once 20 or 30 days of daily QC testing has been completed, weekly QC testing is acceptable. (Note, if you have previously established weekly QC is acceptable for erythromycin and clindamycin disk diffusion testing in you laboratory, it is not essential to redo this for the D zone test). 4) Use *S. aureus* ATCC BAA-976 and BAA-977 for QA. Perform D zone test with these isolates using standard disk diffusion method (unsupplemented MHA). If D zone testing will be done on MIC purity plates in your lab, set up a standard MIC and purity plate for *S. aureus* ATCC BAA-976 and BAA-977 5) Have all staff members who will be doing D zone test participate in testing *S. aureus* ATCC BAA-976 and BAA-977. Use for competency

		assessment and document. Demonstrate how placing disks too far apart may lead to false negative results with BAA–977. 6) Once QA has been performed on *S. aureus* ATCC BAA-976 and BAA-977, each lab must decide how often it will be necessary to retest. At minimum, use to train staff who had not previously performed the D zone test or if any test parameter changes (e.g., new media or disk supplier, switch from disk diffusion to purity plate method of D zone testing, etc).
30	Regarding D zone test for Group B streptococci: we do very few Kirby Bauer tests. I understand *S. aureus* 25923 should be incubated at 33 – 35°C in ambient air for 16 – 18 hours on plain Mueller Hinton agar. However, the Group B streptococci patient isolates should be incubated at 33 – 37°C in CO2 for 20 – 24 hours on blood Mueller Hinton agar. I am not comfortable with treating the control and patient isolates differently. Have I misinterpreted the instructions?	Reliability of the D zone test is dependent on the disks having appropriate content of drug; This is checked by testing ATCC QC strains that have defined QC ranges. Furthermore, placement of disks at appropriate distances and determining if sufficient growth is present on the test plate are checked during QA activities. Studies have shown that providing the disks contain the appropriate content of drug and the test is performed as recommended, further QC testing is not needed for this qualitative assay.
31	I understand the difference in QA versus QC and the new D test organisms. We have been using the D zone test for some time now and have routinely shown a positive and negative test to those training in our department. It seems to me that since one of the points of QA is determining accuracy of test results, it would be an advantage to implement using these organisms as QC daily or weekly at least, now that they are available. Is this something that is coming in the future anyway and why is it not being recommended now? The way it is listed on the new chart as a minimal requirement, led me to think that it was being recommended for actual testing and not just for competency and training purposes. What are most people planning to do about this and what do you recommend?	Potential reasons why it is not essential to test QA strains daily or weekly include: • D zone test is a qualitative test where the endpoint is merely "flattening" or "no flattening" • Inoculum concentration need not be precisely standardized as long as there is a sufficient lawn of growth that is not excessively heavy • Distance between two disks can vary by several mm (as long as they do not exceed recommended limits) and not affect the results • Disk content is critical and this is best checked using standard disk diffusion QC methods It is likely that most will QC the disks daily, weekly, or concurrent with patient testing using *S. aureus* ATCC 25923 or *S. pneumoniae* ATCC 49619 by following routine QC procedures described in M2-A8. *S. aureus* ATCC BAA-976 and BAA-977 will be used for QA and frequency of testing will be defined in each lab.
32	Our lab does not perform disk diffusion testing routinely nor do we stock supplies for this test. For initial D zone QC testing, we used an in-house positive strain and *S. aureus* 29213 for the negative control. We tested for 20 days and used these strains for training and competency assessment. 1) Do we need to now use *S. aureus* strains ATCC BAA-976 and BAA-977? 2) Do we need to perform daily QC on *S. aureus* ATCC 25923 for 20 – 30 consecutive days and then weekly QC? 3) We use BAPs to do the D zone test. Do we need to do weekly QC on Mueller Hinton?	Each laboratory must decide the extent of testing required to validate the test. One suggestion would be to perform QA/QC as above and test 5 patient strains that are positive for D zone but more difficult to detect than BAA-977 and 5 strains that are D zone negative but erythromycin-R and clindamycin-S. Perform testing with disks at various distances to prove that disks placed too far apart will reveal false negative results. Another way to validate would be to "swap" 10 erythromycin-R and clindamycin-S isolates with another lab in your area. The D zone test is only applicable to staphylococci and β streptococci that are erythromycin-R and

		clindamycin-S.
33	Is the 12 mm distance for the E disk and CC disk only for β-strep? We have been using 15 mm when performing the D-test on staphylococci.	The 12 mm distance between erythromycin and clindamycin disks is recommended for D zone testing on β streptococci in M100-S15. Our lab has found that 12-13 mm distance is preferable for staphylococci when performing the D zone test on routine purity plates with MIC testing.
34	Regarding the D zone test for β streptococci, is it set up on MHA with 5% sheep blood and incubated in 5% CO2? That then brings up a question regarding QC for the MHA with 5% sheep blood. We do not currently use that plate in our lab & since it is not being officially used for susceptibility testing, what QC should be performed? Or would documented QA testing be acceptable in this case?	Yes, D zone testing for β hemolytic streptococci is performed on MHA supplemented with 5% sheep blood and incubated in 5% CO_2. CDC has recently demonstrated that purity plates from MIC testing of can also be used for D zone testing of β -hemolytic streptococci. CO_2 incubation is used here too. See above.
35	When testing Group B Streptococcus for inducible clindamycin resistance, is Mueller Hinton with 5% sheep blood the medium of choice?	D zone testing on β-hemolytic streptococci is done on BMHA using the standard disk diffusion method. Erythromycin and clindamycin disks are placed 12 mm apart and test is incubated in 5% CO_2. CDC has recently demonstrated that purity plates from MIC testing can also be used for D zone testing of β streptococci. Incubation is in 5% CO_2
36	Scenario: S. aureus, oxacillin MIC 8 μg/ml, and no growth on the oxacillin screen agar, oxacillin zone 15 mm (faint haze in zone). What should I do? Is it still acceptable to use oxacillin salt agar rather than changing to the cefoxitin disk or a PBP2A latex test? We like the oxacillin salt agar!	Some heteroresistant *mec*A positive *S. aureus* will show hazes around the oxacillin disk and could result in oxacillin resistant MICs of 8 μg/ml. As you know, any haze within an otherwise apparent zone around oxacillin should be considered resistant for staphylococci. Use of cefoxitin disks to predict oxacillin resistance usually produces clearer endpoints. It is still acceptable to use the oxacillin salt agar plate. However, very recent preliminary information from CDC comparing oxacillin salt agar with cefoxitin disk diffusion testing suggests the latter is more sensitive and specific. For some strains that give equivocal results with phenotypic tests, it may be useful to perform a *mec*A or PBP2a analysis.
37	When using a commercial AST system, should we routinely drop a cefoxitin disk or perform another backup test on all too accurately determine oxacillin resistance, or only when results are questionable?	If oxacillin resistance in staphylococci can be reliably detected with your primary AST method (which would include automated methods) in your laboratory with your staff, it is not essential to perform multiple tests and oxacillin results can be reported from your routine system. Use of cefoxitin disk (or another backup method) should be considered if there is any suspicion that your primary testing method may miss or overcall MRS. Remember, no system is perfect.
38	We recently had a physician call us interested in a new community acquired MRSA which produces leukocidase??? Have you heard of this strain and is anyone doing a test for look for this production of leukocidase?	Production of PVL or Panton Valentine Leukocidin has been described primarily in community-associated MRSA (CA-MRSA). More information on this can be found on CDCs CA-MRSA website at http://www.cdc.gov/ncidod/hip/aresist/mrsa_spotlight.htm or Los Angeles County Health Department website at http://www.eurosurveillance.org/em/v09n11/0911-222.asp

39	In the 2005 standards, the oxacillin-salt agar screen is mentioned in Table 2C (Disk Diffusion) Comment (10), page 46 but the method is only listed on page 115 for Table 2C (MIC) even though it is not mentioned in that table. I found this a bit confusing.	You are correct in that the oxacillin salt agar screen should be mentioned in Table 2C (MIC) page 115 (11). CDC is currently comparing the oxacillin salt agar screen plate with the cefoxitin disk diffusion test.
40	Does incubation range of 33-35°C for staphylococci refer to the oxacillin salt agar plate only or does this also include the automated susceptibility testing instruments?	The incubation range of 33-35°C is for the CLSI reference disk diffusion and dilution methods. This would include the oxacillin salt agar screen plate although this is not clarified in M100-S15. When using a commercial system, follow the manufacturer's recommendations.
41	For staphylococci, we want to replace our oxacillin agar screen plate with the cefoxitin disk. We validated this test with patient isolates. Is it necessary to do 20-30 consecutive days (and then weekly) QC for this intended use?	Yes, if you have not previously tested cefoxitin with *S. aureus* ATCC 25923 and determined that weekly QC is acceptable following 20-30 days of daily QC. Any time we add a new drug to a panel, we must follow the CLSI QC frequency recommendations to reduce daily testing to weekly.
42	Regarding the use of cefoxitin disk for detecting oxacillin resistance, is it necessary to do a validation study comparing oxacillin against the cefoxitin disk, or is it OK to just switch and do the 20-30 consecutive days of QC testing with cefoxitin?	It is my opinion that 50–100 bench isolates of staphylococci should be monitored using the old and new methods. For disk diffusion users, this means adding the cefoxitin disk to the routine MHA plate. For MIC users, this means performing a cefoxitin disk diffusion test following the standard disk diffusion method (using the same 0.5 McFarland inoculum used for MIC testing, if available) and comparing the oxacillin S or R result obtained from testing the cefoxitin disk to the oxacillin S or R MIC interpretation. Cefoxitin and oxacillin disk diffusion tests should be monitored with *S. aureus* ATCC 25923 and the QC strain for oxacillin MICs is *S. aureus* ATCC 29213. Only results for oxacillin and cefoxitin would have to be tallied.
43	Will Vitek software release soon add their new breakpoints for the fluoroquinolones? Do we need to be confirming the Vitek Levofloxacin MIC with another method until to software update comes?	There are FDA-CLSI issues with breakpoints for fluoroquinolones and staphylococci at this time and these affect diagnostic manufacturers (the verdict is still out!). If the isolate tests levofloxacin resistant (MIC ≥ 4 µg/ml), you can interpret and report. Options if the MIC is < 4 µg/ml: Do not report Do not report and add comment "contact laboratory if levofloxacin results clinically indicated" Use old (FDA) breakpoints until the FDA-CLSI controversy is resolved. Under all circumstances, the medical staff should be appraised of the situation and provide input into your decision.
44	For automated systems, how do you address the issue of waiting for software updates when you are aware of new standards? We obviously are tied to the version in place currently and can only address issues of back up or manual methods. How do inspectors feel about these issues?	Commercial manufacturers participate in CLSI meetings and are informed of changes as they are discussed. They try to accommodate changes quickly but this is not always possible, considering FDA-clearance requirements. Each lab must determine if it is essential to implement a new recommendation the day it is released or develop a plan to implement the change when it becomes practical to do so. The nature of the change will dictate the urgency and it is likely surveyors will view implementation in

		this context also, e.g., not cite a lab if they have a logical plan in progress for implementation and current testing/reporting protocols are unlikely to have a negative affect on patient care.
45	We recently got a Vitek2. How long do we need to do correlation studies and what exactly am I looking at doing in that regard. We are currently using a Vitek Jr.	There is no "standard" guideline for validation of a new antimicrobial susceptibility test system, although we agree this would be beneficial. It is suggested that isolates with known resistance mechanisms (often stock isolates) as well as fresh clinical isolates should be tested using the new system and if possible, in parallel with the CLSI disk diffusion or MIC reference methods. If use of parallel testing with a reference method is not possible, results obtained with the new system could be compared to those obtained with the old system. Discrepancies should be arbitrated with a CLSI reference method. There are suggestions for validation on the CDC CD-ROM on antimicrobial susceptibility testing in the "Automated Systems" section. This free CD-ROM can be obtained by visiting … **http://www.phppo.cdc.gov/dls/master/default.aspx**. There is also a Cumitech on Validation (#31 www.asmpress.org) that includes some guidance for verification and validation of antimicrobial susceptibility test systems.
46	It is nice to have the KB control ranges in the CLSI for colistin again. However, you indicated that we should not be testing colistin by KB as we may be missing some resistance. In addition, there are no disk diffusion breakpoints for colistin. Why does CLSI have control ranges for this drug, but no interpretive data for patient isolates?	The investigators who proposed the MIC breakpoints for polymyxin B (PMB) are continuing to work on defining reliable methods for testing PMB and colistin. It is known that disk diffusion testing with PMB and colistin is not reliable for some organisms (e.g., Acinetobacter) and may not be reliable for any organisms for which testing would be useful (e.g. non-Enterobacteriaceae). This is primarily because both agents diffuse poorly in agar.
47	We have Etest strips for colistin. Can we use the MIC breakpoints that are in the 2005 tables for polymyxin B and apply them to colistin since the reverse is acceptable?	The investigators who proposed the MIC breakpoints for polymyxin B (PMB) will likely present colistin MIC breakpoint data to CLSI in the near future. It is possible that there will be a difference in colistin and PMB breakpoints, although some have applied a susceptible breakpoint of ≤4 µg/ml to colistin too (see references provided with M100-S15 teleconference). At this time, it might be best to use and interpret results from PMB. Isolates that are PMB-S can be considered colistin-S and isolates that are PMB-R can be considered colistin-R.
48	Do you do a β-lactamase on Enterococcus if it is penicillin sensitive, like we do with staphylococci?	The CLSI suggestion for β-lactamase testing on enterococci is to test sterile body site isolates. β-lactamase-producing enterococci give susceptible zones and MICs for ampicillin and penicillin. However, because β-lactamase producing enterococci have not been reported in over a decade, many labs have discontinued β-lactamase testing on any enterococci.
49	On the Vitek, ESBL is screened and confirmed on certain cards for *E coli*	M100-S15 states in Table 3C that "acceptable MIC QC limits for FDA-

	and *Klebsiella pneumoniae*. QC calls for *E coli* ATCC 25922. Should we add *Klebsiella pneumonia* ATCC 700603 even though not stated currently in our QC program dictated by Vitek?	cleared antimicrobial susceptibility tests may differ slightly from acceptable CLSI QC limits. Users of each device should utilize manufacturer's procedures and QC limits as indicated in the instructions for use." Please contact bioMerieux to obtain their advice on this topic.
50	Is the standard disk diffusion test using ceftazidime and ceftazidime-clavulanic acid and cefotaxime and cefotaxime-clavulanic acid considered a confirmatory test for ESBL producing *E. coli* and *Klebsiella* spp.? Once confirmed with this test, what comment would you recommend on the patient's report?	Yes, at this time, disk diffusion testing with cefotaxime and ceftazidime alone and with clavulanic acid is considered a confirmatory test for ESBL production in *E. coli* and *Klebsiella* spp. and now also *Proteus mirabilis*. Once an ESBL producer is confirmed, a susceptible result for any cephalosporin, penicillin, or aztreonam that would be routinely reported must be edited to resistant. There is no standard report comment recommended, however, some laboratories may highlight identification of an ESBL-producing strain by noting something like "This *Klebsiella pneumoniae* produces extended-spectrum β-lactamases (ESBL)"
51	It appears that ceftriaxone cannot be used to screen for ESBL production in *Proteus mirabilis*. We routinely use ceftriaxone for all of our ESBL testing. What should we do?	When initial studies were done to identify screening tests for ESBL-producing *P. mirabilis*, it was noted that ceftriaxone did not perform as well as cefotaxime, ceftazidime and cefpodoxime in identifying ESBL-producing strains. However, if elevated MICs to ceftriaxone (e.g., > 1 µg/ml) are noted, the isolate should be considered suspicious for ESBL production. Rather than change panels, to optimize detection of ESBL-producing *P. mirabilis* from sterile body sites, you may want to additionally perform the cefpodoxime screening test. Currently, because the incidence of ESBL-producing *P. mirabilis* is low in the US, the recommendation for ESBL testing of *P. mirabilis* is to routinely screen isolates selectively, i.e., those from sterile body sites.
52	What is the slight modification for ESBL testing in *Proteus mirabilis*?	Slight modification for ESBL testing of *P. mirabilis* involves using ceftazidime, cefotaxime, and cefpodoxime as screening agents (not aztreonam or ceftriaxone). Also, for the MIC screen test, the breakpoint for cefpodoxime is ≥ 8 µg/ml (not ≥ 2 µg/ml as for *E. coli* and *Klebsiella* spp.)
53	We are currently performing ceftazidime and cefotaxime with clavulanic disk diffusion QC with *K. pneumoniae* ATCC 700603 on a weekly basis. We seldom run patients because our Vitek reports ESBLs and only when Vitek states a possible ESBL, do we test a patient with the ESBL disk diffusion confirmatory test. Do you feel that is sufficient? We do not perform ESBL testing very often. Can we QC our disks each time we do the test or must we do QC weekly?	As with QC for most other antimicrobial susceptibility tests, QC can be done daily, weekly (once reliable daily testing is confirmed according to CLSI rules), or concurrent with testing patient isolates. If the ESBL confirmatory test is done infrequently, it might be most efficient to perform QC testing with *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 the at the time the patient isolate is tested.
54	Would using the MicroStrep Plus 1 panel from MicroScan be acceptable for *Neisseria meningitidis* testing?	At this time, there is no FDA cleared-commercial product for antimicrobial susceptibility testing of *Neisseria meningitidis*. FDA clearance cannot be

	Has Etest been approved for testing *N. meningitidis*?	granted until the manufacturer proves their product performs comparably to the CLSI "reference method". Prior to M100-S15, there was no "reference method" for *N. meningitidis.* However, now it is conceivable that commercial manufacturers will seek clearance for their products for testing this species. Since MicroStrep performed comparably to the CLSI reference method (and is FDA cleared) for testing streptococci and since the same reference method (CAMHB-LHB) but with CO_2 incubation is approved for *N. meningitidis*, MicroStrep may perform comparably to CLSI references methods for *N. meningitidis*. At this time, if you were to use MicroStrep for *N. meningitidis*, you would have to consider this use off label as you would for any other commercial product that is not cleared for the purpose in question. Likewise, Etest is not yet cleared for *N. meningitidis* but AB Biodisk may seek clearance for this species in the future. Contact the manufacturers of the respective products for further information.
55	For *N. meningitidis* testing, you mentioned doing β-lactamase testing as an option. We use the cefinase disc for this and the product insert does not list this organism as appropriate for testing. What other β-lactamase test would work	β-lactamase testing is currently not recommended for *N. meningitidis*. There have been rare reports of β-lactamase positive *N. meningitis,* the most recent occurring nearly a decade ago.
56	My question is about Table 1 in M100-S15, specifically the "*Pseudomonas aeruginosa* and Other Non-Enterobacteriaceae" column. From your slides in the presentation (slide 30), I noticed that your interpretation of footnote "k" is different than the printed copy of the document. Is *Pseudomonas aeruginosa* not considered part of the *Pseudomonas* spp?	I apologize in that the footnote I was referring to should have been "j" not "k'". I believe my comments are identical for footnote "j" as those on page 95 in M100-S15. However, some of the drugs listed with a superscript "k" in the "*Pseudomonas aeruginosa* and Other non-Enterobacteriaceae" column in Table 1 (page 92) are appropriate for non-Enterobacteriaceae other than *P. aeruginosa*.
57	Just want to confirm Stenotrophomonas susceptibility. It is my understanding that KB for SXT could be done and reported as per the new CLSI document. Please advise.	Yes, trimethoprim-sulfamethoxazole can be reliably tested by disk diffusion against *S. maltophilia*.
58	Recently, we recently isolated Group B streptococcus that was resistant to erythromycin and clindamycin. Is this unusual?	No, there are some isolates of Group B streptococci that have *erm* gene that can show resistance to both erythromycin and clindamycin. In this case, resistance to clindamycin is constitutive.
59	Our patient population is pediatric oncology. What is the best way to handle Group B testing? Occasionally our patients are pregnant, but not being seen at our hospital for prenatal care. Currently, we only perform sensitivities on Group B streptococci from sterile body sites. Would this exempt us from the new recommendations because of our patient demographics? Or would we need to do a D test on a β streptococcus from a blood culture, for example	As you know, we perform susceptibility tests when results are needed to guide MDs in prescribing antimicrobial therapy. You need assistance from your medical staff to help determine when and what to test. During prenatal care, anovaginal cultures are obtained to determine if Mom is colonized with Group B streptococcus. If Group B streptococcus is recovered, the CDC recommendation is to give the Mom prophylactic

	whether or not the patient is pregnant?	therapy during vaginal delivery. If Mom has no penicillin allergy, a β-lactam is administered. If Mom is at high risk for anaphylaxis with penicillin, then erythromycin or clindamycin is administered and there is some resistance to erythromycin and clindamycin, so susceptibility testing would be warranted. In addition, clindamycin should not be reported as "S" for erythromycin-R and clindamycin-S strains without performance of D zone test, as some strains with this profile have inducible clindamycin resistance.
60	I was hoping to hear that it is no longer necessary for hospital labs to routinely perform susceptibility testing on *Streptococcus pneumoniae*. I have noticed in the last few years that physicians very rarely inquire about *S. pneumoniae* susceptibility testing, even on blood culture isolates. Perhaps physicians are treating these infections empirically or they are using Sanford's Guide to Antimicrobial Therapy as it is a faster reference for the physicians than our next day susceptibility results. However, I am unsure if hospital laboratories should stop performing susceptibility testing on *S. pneumoniae*. Please clarify, since I need to order very expensive E Test strips if we are to continue susceptibility testing.	As you know, we perform susceptibility tests when results are needed to guide MDs in prescribing antimicrobial therapy. You need assistance from your medical staff to help determine when and what to test. Many laboratories continue to perform antimicrobial susceptibility tests on *S. pneumoniae*. It is particularly important to know the susceptibility to cefotaxime and/or ceftriaxone for *S. pneumoniae* isolated from patients with meningitis. Sanford Guide contains general recommendations and is typically used prior to and/or in conjunction with antimicrobial susceptibility test results for many isolates causing a variety of infections.
61	Is it acceptable to combine a regular weekly QC on the Vitek with a new lot or shipment QC? We've done this in the past with documentation that the test results represented both the weekly QC and the new lot testing.	Yes, as long as you do QC at least weekly and QC is performed on any new lot or shipment before or concurrent with use for testing patient's isolates.
62	Occasionally, when performing weekly Vitek QC we will experience an out of range result. There is no noticeable problem that we believe would have caused the error (wrong bug, older bug, over inoculated, etc). Accordingly, we are supposed to run daily QC for 5 days before reverting back to weekly. Inevitably, the repeats are satisfactory. I have talked to a number of other labs that just repeat the test and if results are satisfactory on the next day, they revert to weekly testing. What are your suggestions?	The CLSI suggests the 5 days as you are doing. However, it is expected that occasional results will be out of control due to "chance". If the same lot numbers of materials are in use and no other testing parameters change, it is conceivable that this is a "random" problem that would not affect patient results. The problem is with your confidence in the results vs. regulations. A lab may wish to document that these types of problems have corrected in the past, are they are using a system that has performed acceptably in their laboratory for a considerable length of time (if true), and conclude the observations are "random". It is up to the surveyor of your laboratory as to whether or not you would be cited for not following a regulation. (CLIA regulations pretty much defer to CLSI recommendations).
63	Our lab discontinued all KB/disk testing so I had not purchased the M2-A8. Should labs keep an updated document on hand even if not performing any procedures by disk method? If we perform the Cefoxitin disk or D zone test for staphylococci, would these be considered a disk diffusion test and would we need the M2 CLSI book?	For both the D zone test and cefoxitin disk test, disks are controlled by following the standard disk diffusion QC procedure. It is important to make sure you are using the method described in M2-A8 and applying QC limits as listed in M100-S15. When testing patient isolates with the cefoxitin disk, the standard disk diffusion method must be used. Unless the purity plate method is used for

		D zone testing, the standard disk diffusion method is used for staphylococci and β-hemolytic streptococci.
64	NCCLS recommends periodic colony counts on inoculum suspensions to ensure a final inoculum concentration that closely approximates to 5 x 105 CFU/ml. Shouldn't there be a range given (example 5 - 7 x 105 CFU/ml) ; how do we interpret the acceptability of our result if, for example our colony count was 5.5 or 6.5 x 105 CFU/ml?	You are correct in that a range would be helpful. In our laboratory, we use 3-7 x 10^5 CFU/ml (in-house prepared MIC trays). It is important to note that counts may differ for the various QC strains. Counts for *E. coli* and *S. aureus* QC strains are fairly reliable in contrast to those for *P. aeruginosa* and *S. pneumoniae* which can show considerable variability. For many drug/bug results, it is unlikely that MICs will change significantly unless the counts fall outside of the 10^5 range. If you are using a commercial system, contact the manufacturer to obtain their advice on this topic.
65	I would like to ask you for guidance on where to find more information about special additives that might be required for broth dilution susceptibility for certain drug/bug combinations. You mentioned that daptomycin required the addition of 50 µg/ml calcium to CAMHB. Are you aware of a source or reference where I can find more information about this topic in general?	The information necessary for testing drugs for which breakpoints are listed in CLSI M100-S15 are found in that standard and in M7-A6. Since M7 is updated every 3 years, it is conceivable that information added to M100 (updated yearly) may not yet appear in M7. If additional information is needed for testing, contact the manufacturer of the drug.
66	We noticed that there is a disparity in the break point values of E test for viridians streptococci and *Streptococcus pneumoniae*, especially with cefepime and another drug or two. Your checklist recommends following manufacturer's package insert and the package insert and product rep recommends following NCCLS standards. The NCCLS standards are more stringent, and therefore we feel that is the best way to go for best patient care. What do you recommend and how are other people addressing this dilemma?	CLSI and FDA are aware of some disparities between FDA and CLSI breakpoints. This is currently under discussion between the two organizations. Your suggestion to go with the most conservative breakpoints (e.g., CLSI) seems reasonable.
	Compliance and CPT Coding .	Some have asked about billing for various susceptibility screening tests. There are numerous Q&A's related to CPT coding and billing on ASM's Askit http://www.asm.org/division/c/index.htm: look under the "Compliance" section in the Askit archives. Two of the questions submitted following the M100-S15 audioconference and the Askit answers are copied below. DISCLAIMER Askit is for general information purposes only and may not be relied upon by users for decisions or action in specific circumstances. Neither ASM nor the expert assures the accuracy or completeness of information provided. The advice and comments do not necessarily reflect the views of ASM. ASM and INDIVIDUAL experts expressly disclaim any and all liability for any direct, indirect, or special damages or loss of any nature incurred as a consequence of use of this site or any advice or comment by any expert. By using Askit, the user accepts the waiver of claims and all terms of use of ASM's site, including the disclaimers set forth in the "Legal Rights" link on ASM' home page.

67	I've read the questions and answers from 6/13/03 and 10/10/03 and had additional comment and question. 1) We do screen tests to back up other tests in the event that the screen shows a VERY useful and reportable result. This back up isn't because we, at our respective hospital, are having problems with our instrument. This is a mandated back up test that has to be done. CDC has mandated that users of automated susceptibility testing perform a backup test for Vancomycin that can reliably detect the VISA and VRSA strains. Since this is mandated and could provide reportable and useful information, wouldn't it make sense to bill for the vancomycin screen?	The issue of when it is appropriate to bill for an additional related procedure and when it is not is one of the more problematic compliance issues that the microbiology laboratory faces, particularly because there are many circumstances in which best practice guidelines direct that a second related test be performed. The example you provide of CDC recommended vancomycin agar testing to supplement both disk and MIC methods of testing for staphylococci is an excellent one. The compliance concerns arise because of statements made in the National Correct Coding Initiative narrative and the consequent Medicare claims processing edits (which are often also used by other third party payers). NCCI states, "If after a test is ordered and performed, additional related procedures are necessary to provide or confirm the result, these would be considered part of the ordered test". Examples given of inappropriate billing are confirmation of an automated hemogram by a manual differential, and repeat testing of a sample with an abnormal result to verify it. In addition, NCCI states that "multiple tests to identify the same analyte, marker, or infectious agent should not be reported separately". The example given is inappropriate billing of a direct and amplified probe for the same analyte. However, neither of these cases accurately represents the coding and billing dilemma that susceptibility testing for emerging resistance mechanisms imposes on the microbiology laboratory. In these cases, the laboratory may be scientifically (as well as in a regulatory or policy sense) required to perform not confirmatory testing but supplemental testing to assure that unusual resistance mechanisms are reliably detected. Such is the case with vancomycin resistance in *S. aureus* where CDC algorithms strongly recommend supplemental agar screening on all isolates as primary methods do not reliable detect such resistance. In this specific circumstance, a direct question posed to Dr. Niles Rosen, Carrier Medical Director for Administar Federal (the Contractor responsible for NCCI) regarding acceptability of billing for the vancomycin screen using CPT 87181-59 (modified to bypass the mutually exclusive edit) yielded the following response: "It is appropriate to bypass the edit in the situation described. It would NOT be appropriate to bypass the edit if the additional supplemental testing is confirmatory. However, if the additional supplemental test provides medically reasonable and necessary information, it can be billed utilizing NCCI associated modifiers". As always, one should take any decision to bill or not to bill through the institutional Compliance Committee and to continually monitor NCCI annual narratives and quarterly edits for changes. (from Askit *answered 02/08/2005*)

68	What CPT code should be used for D test?	The first part of this question is straightforward. The "D-test" uses disk diffusion methodology, so it is best coded as CPT 87184. However, the second part of this question which may not be obvious is also not straightforward, and that is whether the D-test can be coded for and billed as a supplemental susceptibility procedure. All susceptibility procedures are included in Medicare Correct Coding Initiative mutually exclusive edits which are used not only for Medicare claims processing, but also by many other third party payers. To bill more than one type of susceptibility procedure concurrently, it is necessary to add a modifier if the supplemental procedure provides medically reasonable and necessary information and it is not simply confirmatory. In the case of the D test, it would be "reasonable and necessary" to reflex to a D-test whenever an MIC result shows erythromycin to be resistant and clindamycin to be sensitive if your medical staff and/or clients have approved and/or been informed of such. (from Askit *answered 11/08/2004*)