CLIA Regulations for Antimicrobial Susceptibility Testing

Laboratories performing bacteriology antimicrobial susceptibility testing either by minimum inhibitory concentration (MIC) or disk diffusion methods, must meet the following CLIA regulation:

§493.1261 Standard: Bacteriology.
(b) For antimicrobial susceptibility tests, the laboratory must check each batch of media and each lot number and shipment of antimicrobial agent(s) before, or concurrent with, initial use, using approved control organisms.
(b)(1) Each day tests are performed, the laboratory must use the appropriate control organism(s) to check the procedure.
(b)(2) The laboratory’s zone sizes or minimum inhibitory concentration for control organisms must be within established limits before reporting patient results.

The Centers for Medicare & Medicaid Services (CMS) CLIA program has adopted the microbiology quality control guidelines established by the Clinical Laboratory Standards Institute (CLSI), which allows the laboratory to reduce the frequency of antimicrobial susceptibility testing whether by minimum inhibitory concentration (MIC) or disk diffusion methods from daily to weekly. CLSI frequently updates the tables through new editions of the standards and supplements. Users should have the most recent editions. The current standards may be obtained from CLSI, 950 West Valley Road, Suite 2500, Wayne, PA 19087; telephone: 610.688.0100; website: www.clsi.org.

The current CLSI documents for the performance of antimicrobial susceptibility testing by MIC methods and the quality control accuracy limits are

M7-A9, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically – Ninth Edition; Approved Standard (January 2012); and

The current CLSI documents for the performance of antimicrobial susceptibility testing by disk diffusion and the quality control accuracy limits are

M2-A11; Performance Standards for Antimicrobial Disk Susceptibility Tests – Eleventh Edition; Approved Standard (January 2012); and

As a reminder, if your laboratory holds a certificate of accreditation, be sure to check with your accrediting agency for additional requirements.
General Quality Control

General QC procedures when performing antimicrobial susceptibility testing include, but are not limited to, the following:

- Establish written procedures for all aspects of testing, including the corrective action to be taken when control results failed to meet the laboratory criteria for acceptability.
- Ensure proper standardization of inoculum (e.g. use a 0.5 McFarland standard or its optical equivalent, or follow the manufacturer’s instructions for a commercially available system). If using a McFarland standard, check the expiration date.
- Document lot numbers of reagents, disks, plates, media, etc. (including the condition of any media upon receipt).
- Perform equipment maintenance and function checks (including manufacturer’s requirements and recommendations, incubator and refrigerator temperatures, loop and pipette calibrations, etc.).
- Although it is not a CLIA regulation, it is a good laboratory practice to perform a purity check plate on the test inoculum suspension. It is important to verify that the antimicrobial susceptibility testing results were generated from a pure culture.

Minimum Inhibitory Concentration (MIC) QC

Each new batch of macrodilution tubes, microdilution trays or agar dilution plates must be checked as follows:

<table>
<thead>
<tr>
<th>Appropriate Control Strain</th>
<th>Each New Batch of Media</th>
<th>Each Day If Isolates Are:</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus ATCC 29213 or equivalent*</td>
<td>X</td>
<td>Staphylococcus spp</td>
</tr>
<tr>
<td>E. coli ATCC 25922 or equivalent*</td>
<td>X</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853 and E. coli ATCC 25922 or equivalent*</td>
<td>X</td>
<td>Non-Enterobacteriaceae to include Acinetobacter spp., Stenotrophomonas maltophilia, Pseudomonas spp. And other nonfastidious, glucose nonfermenting, gram-negative bacilli</td>
</tr>
<tr>
<td>E. faecalis ATCC 29212 or equivalent*</td>
<td>X</td>
<td>Enterococcus spp.</td>
</tr>
</tbody>
</table>

NOTE 1: To determine the suitability of the Mueller-Hinton broth for sulfonamide and trimethoprim tests, MICs may be performed with *E. faecalis* ATCC 29212. Routine quality control testing of commercially manufactured panels for thymine and thymidine is not needed. However, should problems with QC of sulfonamides and trimethoprim occur, an MIC test should be performed with *E. faecalis* ATCC 29212 with trimethoprim-sulfamethoxazole. If the MIC for trimethoprim-sulfamethoxazole is < 0.5/9.5 ug/ml, the medium may be considered adequate.

NOTE 2: If testing beta-lactam/beta-lactamase inhibitor antimicrobial agents (e.g., ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, or ticarcillin-clavulanic acid), the laboratory should test *E. coli* ATCC 35218.

NOTE 3: If performing extended spectrum beta-lactamase (ESBL) tests, the laboratory should test *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing strain).

NOTE 4: If performing oxacillin salt agar screen tests, the laboratory should test *S. aureus* ATCC 29213 and 43300.

NOTE 5: If performing vancomycin BHI screen tests, the laboratory must test *E. faecalis* 29212 and 51299.
An equivalent strain is one which demonstrates reactivity similar to an ATCC strain and for which limits have been established. Organisms which manufacturers recommend or require for use in their systems are acceptable strains of control organisms.

Each day the test is performed, the appropriate control strain(s) must be included to check the test system.

When QC testing is performed daily, for each antimicrobial agent/organism combination, 1 out of every 20 consecutive results may be out of the acceptable range. Any more than 1 out-of-control result in 20 consecutive tests requires corrective action.

Refer to the CLSI Standard, “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Ninth Edition (M7-A9)”, or the most current edition, to determine the control strain to be used when performing MIC tests on isolates of Campylobacter jejuni, Haemophilus spp., Neisseria gonorrhoeae, Streptococcus pneumoniae or other organisms as applicable.

Conversion from Daily to Weekly QC Testing
The laboratory may test each appropriate control strain(s) a minimum of once each test week if the following requirements are met:

- The laboratory must document that appropriate control strains are tested for a minimum of 20 or 30 consecutive test days.
- For each antimicrobial agent/organism combination, no more than one out of 20 or three out of 30 MICs values (i.e., MIC values obtained from one antimicrobial agent/organism combination for 20 or 30 consecutive test days) may be outside established acceptable limits for quality control strains.
- These limits may be established by the laboratory or the laboratory may use the acceptable range provided in the CLSI document, “Performance Standards for Antimicrobial Susceptibility Testing; Twenty Third Information Supplement (January 2013)”, or most current edition. The quality control limits are valid only if the methodology in the most recent CLSI standard is used.

Corrective Action
Whenever an MIC value is observed outside the established acceptable limits for quality control strains during weekly quality control testing, the following corrective actions are required:

- If there is an obvious reason for the out-of-control result (e.g., use of the wrong control strain, obvious contamination of the strain or the medium, inadvertent use of the wrong incubation conditions), document the reason and retest the strain on the day the error is observed. If the repeat result is within range, no further corrective action is necessary.
- If there is not an obvious reason for the out-of-control result, test the implicated antimicrobial agent/organism combination on the day the
error is observed and test for a total of five (5) consecutive test days. If all 5 MICs for the antimicrobial agent/organism combination are within the established acceptable range, no additional corrective action is necessary.

- For the last item mentioned above, if any of the 5 MICs is outside the established acceptable range, additional corrective action is necessary. Daily control of tests must be continued until final resolution of the problem is achieved. Once the problem is corrected, in order to return to weekly quality control testing, documentation of satisfactory performance for another 20 or 30 consecutive days is required.

E-test

NOTE: CLSI does not address performance issues or make recommendations about any commercial test system such as the E-test.

The E-test is a quantitative technique test that determines antimicrobial susceptibility by using a predefined antibiotic gradient to determine the MIC of an individual antibiotic when tested on agar media by overnight incubation. Laboratories using the E-test should rely on the manufacturer to establish the appropriate accuracy control limits and may go to weekly quality control testing after meeting the requirements mentioned in the exception for the procedure to be used for demonstrating satisfactory performance for conversion from daily to weekly quality control testing of the MIC test.

Antimicrobial Disk Diffusion Susceptibility Testing QC

(Bauer, Kirby, Sherris and Turk Method)

<table>
<thead>
<tr>
<th>Appropriate Control Strain</th>
<th>Each New Batch of Media and Disks</th>
<th>Each Day If Isolates Are:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> ATCC 25923 or equivalent*</td>
<td>X</td>
<td><em>Staphylococcus spp.</em></td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922 or equivalent*</td>
<td>X</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td><em>P. aeruginosa</em> ATCC 27853 and <em>E. coli</em> ATCC 25922 or equivalent*</td>
<td>X</td>
<td><em>Pseudomonas aeruginosa Acinetobacter spp.</em></td>
</tr>
</tbody>
</table>

Each new batch of medium and each new lot/shipment of antimicrobial disks must be checked as follows:

NOTE 1: Routine quality control testing of commercially prepared Mueller-Hinton agar for thymine and thymidine is not needed. However, if problems with quality control of sulfonamides and trimethoprim occur, the Mueller-Hinton agar should be checked with *E. faecalis* ATCC 29212 or alternatively, *E. faecalis* ATCC 33186 with trimethoprim-sulfamethoxazole disks. Satisfactory media will provide essentially clear distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

NOTE 2: If testing beta-lactam/beta-lactamase inhibitor antimicrobial agents (e.g., ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, or ticarcillin-clavulanic acid), the laboratory should test *E. coli* ATCC 35218 (beta-lactamase producing strain).

NOTE 3: If performing extended spectrum beta-lactamase (ESBL) tests, the laboratory should test *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing strain). Zone sizes must be recorded for each antimicrobial control and limits must be established.

*An equivalent strain is one which demonstrates reactivity similar to an ATCC strain and for which limits have been established. Organisms which manufacturers recommend or require for use in their systems are acceptable strains of control organisms.
Each day the test is performed, the appropriate control strain(s) must be included to check the test system.

When testing is performed daily, for each antimicrobial agent/organism combination, 1 out of every 20 consecutive results may be out of the acceptable range. Any more than 1 out-of-control result in 20 consecutive tests requires corrective action.

Refer to the CLSI Standard, “Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition (M2-A11)”, or most current edition, to determine the control strain to be used when performing antimicrobial disk susceptibility tests on isolates of Haemophilus spp., Neisseria gonorrhoeae, Streptococcus pneumoniae or other organisms as applicable.

Conversion from Daily to Weekly QC Testing
The laboratory may test each appropriate control strain a minimum of once each week, provided the following requirements are met:

- The laboratory must document that appropriate control strains were tested for 20 or 30 consecutive test days.
- For each antimicrobial agent/organism combination, no more than one out of 20 or three out of the 30 zone diameters (i.e., zone diameters obtained from one antimicrobial agent-organism combination for 20 or 30 consecutive test days) may be outside established acceptable limits for quality control strains.
- These limits may be established by the laboratory or the laboratory may use the acceptable range provided in the CLSI document, “Performance Standards for Antimicrobial Susceptibility Testing; Twenty Third Information Supplement (January 2013)”, or most current edition.

NOTE 1: CLSI frequently updates the tables through new editions of the standards and supplements. Users should have the most recent editions. The current standards may be obtained from CLSI, 950 West Valley Road, Suite 2500, Wayne, PA 19087; telephone: 610.688.0100; website: www.clsi.org.

NOTE 2: This procedure is to be used only for demonstrating satisfactory performance for conversion from daily to weekly quality control testing of the disk diffusion test.

Additional QC Testing
After a laboratory has implemented weekly quality control testing of the disk diffusion test:

- Quality control testing must be performed whenever any reagent component of the test is changed (e.g., a new lot of agar or a new lot of disks).
- Corrective action is required if any of the weekly quality control results are outside of the established acceptable range.
- If a new antimicrobial agent is added, it must be tested for 20 or 30 consecutive days and have satisfactory performance documented before it can be tested on a weekly schedule.
- If there is a major change on the method of reading test results, such as conversion from manual zone measurements to an automated zone reader, 20 or 30 days of testing is required.

Corrective Action
If a zone diameter is observed outside the established acceptable limits for quality control strains during weekly quality control testing, the following corrective action(s) are necessary:

- If there is an obvious reason for the out-of-control result (e.g., use of the wrong disk, use of the wrong control strain, obvious contamination of the strain, inadvertent use of the wrong incubation temperature or conditions), document the reason and retest the strain on the day the error is observed. If the repeat result is within range, no further corrective action is necessary.
- If there is not an obvious reason for the out-of-control result, test the implicated antimicrobial agent/organism combination on the day the error is observed and test for a total of 5 consecutive test days. If all 5 zone diameter measurements are outside the established acceptable range, no additional corrective action is necessary.
- For the last item mentioned above, if any of the 5 zone diameter measurements are outside the established acceptable range, additional corrective action is necessary. Daily control of tests
must be continued until final resolution of the problem is achieved. Once the problem is corrected, in order to return to weekly quality control testing, documentation of satisfactory performance for another 20 or 30 consecutive days is required.

**Direct Susceptibility Testing**

Direct susceptibility testing is a modification of the standardized disk diffusion susceptibility testing method. Therefore, the laboratory must establish the interpretive zone diameters for patient specimens, as well as establish the zone diameters for quality control organisms. Since direct susceptibility testing is not a recommended CLSI method, the laboratory may not go to weekly quality control, but must perform quality control daily using appropriate control organisms.

**References**