SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1.1 Purpose

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has published a guideline for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test results.

1.2 References

M100-S24, January 2014 (Performance Standards for Antimicrobial Susceptibility Testing)
M7-A9, January 2012 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

1.3 Definition of Terms

Reference Culture: A reference culture is a microorganism preparation that is acquired from a culture type collection.

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time.

1.4 Important Considerations

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC
- CLSI requires that QC be performed either on the same day or weekly (only after 30 day QC validation)
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides
- Periodically perform colony counts to check the inoculum preparation procedure
Ideally, test values should be in the middle of the acceptable range

Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems

1.5 Storage of Reference Strains

Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen. (Alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

1.6 Frequency of Testing

Weekly vs. daily testing

Weekly testing is possible if the lab can demonstrate satisfactory performance with daily testing as follows:

- Documentation showing reference strain results from 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more than 3 out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

Corrective Actions

If an MIC is outside the range in weekly testing, corrective action is required as follows:

- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

The problem is considered resolved only after the reference strain is tested for 5 consecutive days and each drug/organism result is within specification on each day.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.
Appendix A. Quality Control Protocol Flow Charts

Quality Control (QC) Protocol: Daily Testing

1. QC each test day (Section 16.7.1)
2. ≤3 out of 30 results out of range
   - QC each test day. (For conversion to weekly testing, see Section 16.7.2.)
   - Error identified and corrected: Retest
   - Results in range – continue QC each test day
3. >3 out of 30 results out of range
   - Corrective Action (Section 16.9)
   - No error identified: Immediate Corrective Action (Section 16.9.2.1)
   - Retest and monitor for five consecutive test days
   - All results in range: Continue QC each test day
   - Any results out of range: Additional Corrective Action (Section 16.9.2.2)
     - Investigate possible source of errors

Reference: CLSI M7-A9, page 46
Appendix A. (Continued)

QC Protocol: Weekly Testing

Demonstrate Satisfactory Performance (Section 16.7.2.1)

\( \leq 1 \) out of 20 or \( \leq 3 \) out of 30 results out of range

Implement weekly QC testing (Section 16.7.2.2)

Any result out of range

Corrective Action (Section 16.9)

Error identified and corrected
Retest

Results in range
Return to weekly QC testing

Results out of range

Immediate Corrective Action (Section 16.9.2.1)

Retest and monitor for five consecutive test days

All results in range
Return to weekly QC testing

Any results out of range

Additional Corrective Action (Section 16.9.2.2)

Investigate possible source of errors

Reference: CLSI M7-A9, page 47