

# SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

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## 1 PURPOSE AND REFERENCES

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

The following can be regarded as a summary of information that should be followed for subculturing and maintaining QC-strains when performing AST by broth dilution methods. For full information related to this subject, the following standards are relevant: M100 (Performance Standards for Antimicrobial Susceptibility Testing) and M7 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard).

## 2 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.

## 3 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 (after 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.

#### 4 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.

#### 5 FREQUENCY OF TESTING

##### Weekly vs. daily testing

Weekly testing is possible if the laboratory can demonstrate satisfactory performance with daily testing according to the descriptions in the CLSI guidelines.

- Documentation showing reference strain results from 20 or 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more one out of 20 or three 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

If five acceptable QC results are available, no additional days of QC-testing are needed.

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

Repeat the 30 days validation before resuming weekly testing.