Troubleshooting and Common Mistakes Made in AST

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Basingstoke
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Bangkok, 7-8 October 2014

"For clarity, these are solely my personal views/opinions and do not necessarily represent those of any organization to which I am affiliated."

CLSI Standard vs. CLSI Guideline

Standard
Clearly identifies specific and essential requirements for materials, methods, and practices to be used in an unmodified form

May, in addition, contain discretionary elements, which are clearly identified

Guideline
Describing criteria for a general operating practice, procedure, or material for voluntary use.

A guideline can be used as written or modified by the user to fit specific needs

The current CLSI document for testing antimicrobial susceptibilities of bacteria isolated from animals Vet01-A4 is an **approved standard** and cannot be used in a modified form

CLSI Standard – Vet01-A4

Clear and precise instructions on how to perform susceptibility tests are given. They include for example:

- the approved medium specifications (including pH, cation concentration, and nutritional supplements)
- inoculum density
- incubation time
- incubation temperature and test conditions

For disk diffusion testing, CLSI indicates the specific disk potency for testing, as well as the depth of the test medium

All such instructions are not optional, but are strict rules to be adhered to for good laboratory practice

Common Deviations from CLSI Standard

Statements such as “Susceptibility testing mainly followed the recommendations given in the CLSI document Vet01-A4 **are not acceptable**.

Unfortunately, the scientific literature is full of papers which claim to use CLSI methods, but which display methodological details incompatible with CLSI recommendations.

Common errors in the application of CLSI methods include:

- the use of an incorrect medium
- different antimicrobial potency for disk diffusion testing (*e.g.* utilizing a 15 µg or 30 µg gentamicin disk rather than the required 10 µg disk)
- results for drug combinations for which there are no CLSI clinical breakpoints (*e.g.* amoxicillin and *Enterobacteriaceae*).

Common Deviations from CLSI Standard

All standards for performance of AST contain interpretive criteria which refer specifically to that particular methodology. A specific method and its associated clinical breakpoints are inseparable. It is not scientifically valid to "mix and match" testing methods and clinical breakpoints issued by different organizations e.g. BSAC method and CLSI interpretive criteria

Another common error is the use of out-of-date clinical breakpoints, especially if findings from multiple years are compared:

- Researchers should use the most recent clinical breakpoints available at the time that statistical analyses are performed – not the criteria current at the time that susceptibility tests were performed.

For example, if analyzing trends in % resistance from 2000 through 2010, the 2010 breakpoints should be applied to MIC measurements from all years to determine the correct interpretation, as best understood in 2010.

	Errors and Deviations
Methodology	**- Mistakenly claim to follow CLSI methodology** **- Use of altered inoculum sizes, incubation times, incubation conditions** **- Use of media other than recommended in CLSI documents** **- Lack of cation supplementation of the MH medium** **- Use of growth supplements not recommended by CLSI** **- Use of CLSI methodology for bacteria for which the respective methodology has not been approved** **- Use of own, self-made, non-approved methodology**

	Errors and Deviations
QC Aspects	**- Lack of QC data** **- Replacement of approved QC strains by laboratory-specific strains** **- Use of a QC strain for broth microdilution which, however, has been approved for disk diffusion (and vice versa), e.g. S. aureus ATCC®25923 (disk diffusion) versus S. aureus ATCC®29213 (broth microdilution)** **- Use of QC ranges (approved for broth microdilution) of reference strains for E-test applications** **- Test concentrations do not cover the acceptable QC range of the reference strains** **- Testing of antimicrobial agents for which no acceptable QC ranges of the reference strains are available** **- Testing of antimicrobial agents within the same class as antimicrobials for which acceptable QC ranges are available and claiming the QC ranges are applicable across the class** **- Use of QC strains that are not related to the test strains (e.g. P. aeruginosa ATCC®27853 for staphylococci)**

	Errors and Deviations
Interpretive Criteria	**- Use of outdated breakpoints** **- Use of breakpoints not approved for the respective combination of bacterium/animal species/disease condition** **- Use of breakpoints from other publications** **- "Mix and match" of ECVs and clinical breakpoints** **- "Mix and match" of clinical breakpoints from CLSI documents and other antimicrobial susceptibility testing documents (e.g. BSAC, DIN, CASFM)** **- Use own, self-defined breakpoints** **- Use of wrong antimicrobial disk content** **- Conversion of zone diameters into MICs** **- Modification of CLSI breakpoints to support specific needs (e.g. lowering breakpoints of fluoroquinolones to avoid fluoroquinolone use to control strains that already show single step mutations)**

Questions?