Rationale for defining a reference method for the determination of minimum inhibitory concentrations (MIC) of anti-tuberculous agents for *Mycobacterium tuberculosis* complex

Since the 1960-ies, many different media and strategies have been used for MIC determination of anti-tuberculous agents for isolates of the *Mycobacterium tuberculosis* complex (MTBC; 1). These methods were used for assessing isolates as susceptible or resistant based on breakpoints traditionally referred to as critical concentrations (1).

The need for a reference method for MTBC became evident in the last decade when new anti-tuberculous agents, such as delamanid and bedaquiline, were developed and submitted to the European Medical Agency (EMA). For anti-mycobacterial agents, the lack of a reference method has resulted in insufficient data to set proper ECOFFs (2,3), confusion about which MIC method to use for PK/PD studies, difficulties with evaluating cross-resistance and challenges in which method to use while evaluating phenotype-genotype correlations.

EUCAST required the subcommittee of anti-mycobacterial susceptibility testing (AMST) to develop a non-commercial reference method for the MIC determination of agents active against MTBC. Detailed protocols for MIC determination for MTBC in liquid culture (broth microdilution (BMD) in Middlebrook 7H9) and on solid medium (agar dilution in Middlebrook 7H10) were developed and their reproducibility evaluated in a multi-centre study. Based on these results and other factors (e.g. cost, labour requirements, published MIC data, and experiences from other pathogens), Middlebrook 7H9 BMD is proposed as the EUCAST reference method for MTBC.

The EUCAST AMST subcommittee wishes to make the following clarifications regarding the proposed reference MIC method for MTBC:

- The technical protocol and the data summary from the AMST study will be available on the EUCAST website (http://www.eucast.org/documents/consultations/).
- The proposed reference method will be open for consultation for six weeks and then presented to the EUCAST steering committee for a formal decision.
- The final technical protocol will be published following necessary amendments as a result of the consultation along with an SOP which specifies the requirements for calibration of other methods to the reference method.
- When other methods are to be used in clinical trials and routine susceptibility testing for the MTBC, it is necessary to properly calibrate and validate these against the reference method.
- EUCAST will set clinical breakpoints according to EUCAST principles (i.e. ECOFFs, PK/PD and clinical outcome data; 3) for the reference method only.

For EUCAST AMST,

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References

