Written responses to open questions of the webinar 'Susceptibility testing in antibacterial drug R&D' by Dee Shortridge and Rafael Cantón, originally broadcast on 2 March 2023. See webinar recording here: https://revive.gardp.org/susceptibility-testing-in-antibacterial-drug-rd/

Question asked	Response from the speakers
How can we relate the dose of compounds used in mice PK to the dose to be given to human?	There is a reference here with links to presentations: Byrne JM, Waack U, Weinstein EA, Joshi A, Shurland SM, Iarikov D, Bulitta JB, Diep BA, Guina T, Hope WW, Lawrenz MB, Lepak AJ, Luna BM, Miesel L, Phipps AJ, Walsh TJ, Weiss W, Amini T, Farley JJ. FDA Public Workshop Summary: Advancing Animal Models for Antibacterial Drug Development. Antimicrob Agents Chemother. 2020 Dec 16;65(1):e01983-20. doi: 10.1128/AAC.01983-20. PMID: 33106262; PMCID: PMC7927847.
My question is why is CAMHB a good choice in all the testing for standard MBC?	MBC is determined by subculturing wells after the MIC test is read. CAMHB is the standardized medium for both tests.
How can we do MIC for plant extracts provided the choice is disk diffusion?	You can put 10 microliters of any solution on a paper disk and place it on agar that has been inoculated with the isolate of interest to see if you get a zone of inhibition around the disk. You can test various dilutions of the extract if the zone is large.
How can we know if the increased MIC value is due to the inability of the drug to permeate the membrane?	You can suspect this because you previously demonstrate that the drug is acting to the target in a biochemistry experiment. Also because with key chemical modifications you have activity (see slide of "eNTRy" strategy in Rafael Cantón's presentation)
Is there any universal limit for MIC value? How would we assess which MIC is preferable? Or does it depend on the type of strain?	There is no limit of MIC but we normally go up 1024 mg/L. With higher concentration you will start with solubility problems and also, you will use a high quantity of powder of the drug testing!! The preferable MIC in an initial assay is 1 mg/ml but you do not need to discharge a drug if the MIC of the compound is initially 8 mg/L. With chemical modifications you can decrease this value. Latter, and quite important, you might need to demonstrate no toxic effect.
@Dee Shortridge: Is it possible to obtain free resources which include with the CLSI standards that you commented during your presentations? Thanks.	Some of the CLSI documents are free online. Go to CLSI.org and register.

Remaining audience questions from the webinar 'Susceptibility testing in antibacterial drug R&D' by Dee Shortridge and Rafael Cantón, originally broadcast on 2 March 2023

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Why DMSO 1.0% used during MIC?	DMSO higher than 1% can inhibit the growth of some organisms, which will make it hard to determine the MIC of your compound. Use less than 1% final concentration in MIC.
Will this influence CLSI guidelines for MIC testing?	CLIS and EUCAST use the same method for MIC calculation This is the ISO-20776-1
Question for Dee Shortridge: Besides the basic parameters you mentioned that should be tested for new drugs. What else is relevant to be tested for automated AST devices developing their methods for these drugs?	Some device manufacturers may need your compound's preliminary breakpoints to develop the test. DMSO is a difficult solvent for manufacturing, water is preferred. A reproducible reference method is the most important.
How does the MIC relate or take into account the beneficial GI microbiome?	MIC determination is for a single microorganism. We do not perform MIC for a "bacterial community".
How long can isolates be stored at -20°C?	A few weeks at -20C, several years at -80C in TSB+20% glycerol.
Is MHB is more preferable or nutrient broth can also be used in MIC?	MHB is preferred because it is standardized. Nutrient broth is richer and non-standardized medium that may result in higher and variable MICs.
How can we begin to use MIC in clinical settings in regions where we largely lack the required experience?	You can find some tutorials (videos) in YouTube. I have not specifically check them but google "MIC determination tutorial" or "Determination of MIC" and you will find them. You have to be sure before implementation that they are following ISO-20776-1