Colistin Drop Test

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Strengths	• Colistin solution is stable for more than 1 year in the refrigerator • Test can be carried out on the same plate on which disks have been placed to carry-out an antibiogram • Is capable to detect colistin resistance mediated by traditional (chromosomic) as well as transferable plasmidic (*mcr*) mechanisms.
Limitations	• Cation-adjusted Mueller-Hinton Broth (CA-MHB) required • Validated for Difco®, Oxoid® and BD Phoenix™ AST broth (Cat. 246003) Mueller Hinton Broth • Validated for Oxoid® and BBL® paper disks
Organism group	*Enterobacterales, Pseudomonas aeruginosa* and *Acinetobacter* spp.
Medium	• Agar Mueller-Hinton plates (as used for the disk diffusion method) • CA-MHB
Antimicrobial concentration	Colistin sulfate 16 µg/ml
Source of antimicrobial	10-µg colistin paper disks *Alternatively, colistin sulphate can be used*
Inoculum	• Using a loop or a swab, pick 3-5 colonies from a fresh (<24 h) culture (selective or non-selective media can be used) and transfer to sterile saline or broth. • Adjust turbidity to equivalent of a 0.5 Mc Farland turbidity standard
Test procedure	*A) Preparation of the colistin sulfate 16 µg/ml solution:* • Add 8 colistin disks (10-µg) in a glass tube containing 5 ml of CA-MHB. • Incubated at room temperature for at least 30 minutes but not longer than 60 minutes to allow colistin to elute from the disks. • Remove aseptically the disks and discard. • The remaining broth is the working solution (colistin final concentration of 16 µg/ml). • Colistin solution can be stored for up to 1 year at 4 ºC. • It is suggested to monthly control the sterility of the solution by overnight incubation at 35 ° C of 100 µl

	and observation of turbidity. If this happens, discard the solution. Alternatively, it can be re-sterilized by filtration (0.2µm filter pore size required). *B) Drop Test procedure:* • Swab the surface plate with a 0.5 Mc Farland suspension of the bacterial isolate. • Preferably, leave the plate to rest with its lid on for approx. 15 min to dry the inoculum. • Using a pipette or a calibrated loop, add a 10 µl drop of colistin sulfate solution (16 µg/ml) over the plate surface already inoculated. • If necessary, reserve the plates for 15 minutes at room temperature to allow complete absorption of the drop, then invert the plate. • Incubate for 16 to 18 hours at 35 °C. • After incubation, examine the purity plate to ensure inoculum was pure. • Observe the presence or absence of an inhibition zone.
Results	• COLISTIN-SUSCEPTIBLE: Clear inhibition zone, of any diameter. • COLISTIN-RESISTANT: o Absence of inhibition zone or o Defined colonies or haze growth within the inhibition zone (indicative of hetero-resistant subpopulations) *See figure in Annex for clarification*
Additional testing and reporting	
QC recommendations	• Colistin susceptible: *Escherichia coli* ATCC 25922 • Colistin resistant: *E. coli mcr* producer • *The quality of each batch of Agar Mueller-Hinton plates and CA-MHB should be previously controlled according to the latest edition of CLSI[1,2] or EUCAST[3]*

Reference	[1] Clinical and Laboratory Standards Institute. M02: Performance Standards for Antimicrobial Disk Susceptibility Tests. [2] Clinical and Laboratory Standards Institute M07: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically [3] European Committee on Antimicrobial Susceptibility Testing. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method [4] Development and validation of simple tests (agar spot, colistin drop, 1ml-broth disk elution MIC and tablet pre-diffusion) as an alternative to improve accuracy in screening chromosomal and plasmid-mediated colistin resistance in GNB. F. Pasteran, D. Danze, C. Cabrera, C. Lucero, A. Menocal, E. Albornoz, I. Castillo, M. Rapoport, P. Ceriana, P. Gagetti, A. Corso. O0952. 28º ECCMID, 2018

Annex.

Figure. Example of colistin susceptible and colistin resistant bacteria.