

# Colistin Micro Broth Disk Elution Test

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| Strengths                    | <ul style="list-style-type: none"> <li>Is an adaptation of the CLSI colistin disk elution test.<sup>1</sup></li> <li>Is capable to detect colistin resistance mediated by traditional (chromosomic) as well as transferable plasmidic (<i>mcr</i>) mechanisms.</li> </ul>   |
| Limitations                  | <ul style="list-style-type: none"> <li>Cation-adjusted Mueller-Hinton Broth (CA-MHB) required</li> </ul>  |
| Organism group               | <i>Enterobacteriales</i> , <i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp.  |
| Medium                       | CA-MHB  |
| Antimicrobial concentrations | Colistin 1 - 4 µg/ml  |
| Source of antimicrobial      | 10-µg colistin paper disk   |
| Test procedure               | <p><b>A) Tubes Preparation (1 ml final volume)</b></p> <ul style="list-style-type: none"> <li>Label 4 glass tubes for each test strain as Control, 1, 2 and 4.</li> <li>Place 10 ml of CAMHB in each tube.</li> <li>Aseptically add 0, 1, 2 and 4 colistin disks to tubes labelled as Control, 1, 2 and 4 respectively obtaining a final concentration of 0, 1, 2 and 4 µg/ml.</li> </ul> <div style="text-align: center;"> <p>Control    1 µg/ml    2 µg/ml    4 µg/ml</p> </div> <ul style="list-style-type: none"> <li>Incubated at room temperature for at least 30 minutes but not longer than 60 minutes to allow colistin to elute from the disks.</li> <li>Remove aseptically the colistin disks and discard</li> <li>Homogenize tube 1.</li> </ul> |

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|                                  | <ul style="list-style-type: none"> <li>• Divide the entire contents into 10 glass tubes with screw caps (or similar to allow storage) dispensing 1ml in each tube and Label as “1”.</li> <li>• Proceed in the same way with tubes 2, 4 and control.</li> <li>• Store tubes at -20°C properly closed up to 6 months.</li> </ul> <p><b>B) Colistin Micro Broth Disk Elution procedure:</b></p> <ul style="list-style-type: none"> <li>• Remove a set of 1ml tubes (one of each: control, 1, 2 and 4) from the freezer and allow them to reach room temperature.</li> <li>• Using a pipette of a calibrated loop, add <b>5 µl</b> of the inoculum to each tube (1, 2, 4 and control) (final concentration: approximately <math>7.5 \times 10^5</math> CFU/ml).</li> </ul> <p><i>Note: If you don't have a 5 µ micropipette, you can prepare a two-fold dilution of standardized inoculum and add 10 µl to each tube using 10 µl calibrated loop</i></p> <ul style="list-style-type: none"> <li>• Mix each tube gently.</li> <li>• Incubate for 18-20 hours at <math>35 \pm 2</math> °C for <i>Enterobacteriales</i>, <i>Pseudomonas aeruginosa</i> and 24 hours for <i>Acinetobacter</i> spp.</li> <li>• After incubation, examine the growth tube which must demonstrate obvious turbidity for the test to be valid<br/>Note: some <i>P. aeruginosa</i> isolates may grow near the meniscus.<sup>1</sup></li> <li>• Read the MIC as the lowest colistin concentration that completely inhibits growth of the isolate.</li> </ul> |
| Results                          | <p>COLISTIN SUSCEPTIBLE: MIC <math>\leq</math> 2 µg/ml.<br/>COLSITIN RESISTANT: MIC <math>\geq</math> 4 µg/ml.</p>   |
| Additional testing and reporting | <p>If there are an inconsistent growth pattern (for example, no growth in tube 2, but growth in tubes 1 and 4) repeat the test. This could be due to:</p> <ul style="list-style-type: none"> <li>• Contaminated tube.</li> <li>• No inoculated tube.</li> <li>• Hetero-resistant isolates.</li> <li>• Inadequate colistin concentration in tubes.</li> </ul>   |
| QC recommendations               | <p>It is suggested to test a positive and negative control each time a batch of ten determinations is prepared:</p> <ul style="list-style-type: none"> <li>• <i>Escherichia coli</i> ATCC 25922 (MIC range <math>\leq</math>1 – 2 µg/ml)</li> <li>• <i>P. aeruginosa</i> ATCC 27853 (MIC range <math>\leq</math>1 – 4 µg/ml)</li> <li>• Colistin resistant: <i>E. coli mcr</i> producer</li> </ul> <p>Note: Disks content may vary between manufacturers. If different batches or brands of disks are used, they should be controlled with adequate quality control strains.</p>   |

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| Reference | <sup>1</sup> Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests.<br><sup>2</sup> 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. 28 <sup>o</sup> ECCMID, 2018 |
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**Figure. Examples of a colistin-resistant and colistin-susceptible strains**

