

## Colistin pre-diffusion test with paper disks or tablets

Version

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Strengths	<ul style="list-style-type: none"> <li>No special media necessary. It is an adaptation of the diffusion method</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>Test time is 48 hours (if the plates are not pre-diffused previously)</li> <li>Validated for Oxoid® and BBL® disks and NeoSensitab Rosco® tablets</li> </ul>
Organism group	<i>Enterobacterales</i> , <i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp.
Medium	Agar Mueller-Hinton plates (as used for the disk diffusion method)
Antimicrobial concentration	10-µg colistin paper disk or tablets
Source of antimicrobial	Paper disks or tablets
Inoculum	<ul style="list-style-type: none"> <li>Using a loop or a swab, pick 3-5 colonies from a fresh (&lt;24 h) culture (selective or non-selective media can be used) and transfer to sterile saline or broth.</li> <li>Adjust turbidity to equivalent of a 0.5 Mc Farland turbidity standard</li> </ul>
Test procedure	<ul style="list-style-type: none"> <li>Place one 10 µg colistin disk or tablet on a non-inoculated Mueller Hinton agar plate. Note: two paper disk or tablets can be placed in the same plate in order to test two different strains (see picture).</li> <li>Incubate the plate 2 h on a stove at 35-37°C.</li> <li>After incubation remove the disk or tablet by knocking the plate against the table.</li> <li>Maintain the plate at room temperature for further 18 – 22 h before use.</li> <li>After the incubation at room temperature, the plate can be used immediately or can be kept in the refrigerator (4°C) for one week.</li> <li>To inoculate the pre-diffused plate, swab the surface with a 0.5 Mc Farland suspension of the bacterial isolate.</li> </ul>

	<ul style="list-style-type: none"> <li>• Incubate the plate at 35-37°C for 18-20hs.</li> <li>• Examine the purity plate to ensure inoculum was pure</li> <li>• Measure and record the diameter of the inhibition zones.</li> </ul>
Results	See table in annex
Additional testing and reporting	An intermediate result needs to be evaluated by the same or another approved method
QC recommendations	<ul style="list-style-type: none"> <li>• Colistin susceptible: <i>Escherichia coli</i> ATCC 25922</li> <li>• Colistin resistant: <i>E. coli mcr</i> producer</li> <li>• <i>The quality of each batch of Agar Mueller-Hinton plates should be previously controlled according to the latest edition of CLSI<sup>1</sup> or EUCAST<sup>2</sup></i></li> </ul>

Reference	<p><sup>1</sup> Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests.</p> <p><sup>2</sup> European Committee on Antimicrobial Susceptibility Testing. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method</p> <p><sup>3</sup> <a href="http://www.rosco.dk/gfx/pdf/98018%20-%20Print%20Insert2016.pdf">www.rosco.dk/gfx/pdf/98018%20-%20Print%20Insert2016.pdf</a></p> <p><sup>4</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</p>
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Annex.

**TABLE Interpretative Breakpoints.**

Reservoir brand	Interpretative Categories	Breakpoints (in mm) for the indicated species		
		<i>Enterobacterales</i>	<i>P. aeruginosa</i>	<i>Acinetobacter spp.</i>
ROSCO <sup>3,4</sup>	Susceptible	≥16	≥16	≥20
	Intermediate	12 -15	12 -15	14 - 19
	Resistant	≤11	≤11	≤13
OXOID <sup>4</sup>	Susceptible	≥20	≥16	≥16
	Intermediate	18 – 19	12 - 15	12 - 15
	Resistant	≤17	≤11	≤11
BBL <sup>4</sup>	Susceptible	≥16	≥16	≥16
	Intermediate	12 - 15	12 -15	12 - 15
	Resistant	≤11	≤11	≤11

**Figure. Examples of a colistin-resistant and colistin-susceptible strains**

