

# Colistin Agar Spot Test

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Strengths	<ul style="list-style-type: none"> <li>• Ability to tests up to 12 isolates at once.</li> <li>• Commercially available in some countries.</li> <li>• Is capable to detect and differentiate colistin resistance mediated by traditional (chromosomic) from transferable plasmidic (<i>mcr</i>) mechanisms by using an adaptation of this method (EDTA-Agar Spot).</li> </ul>
Limitations	<ul style="list-style-type: none"> <li>• Colistin sulfate powder required.</li> <li>• Validated with Difco®, Oxoid® and Britania® Mueller Hinton.</li> </ul>
Organism group	<i>Enterobacterales</i> , <i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp.
Medium	Agar Mueller-Hinton
Antimicrobial concentration	Colistin sulfate 3 µg/ml
Source of antimicrobial	Colistin sulfate powder. WARNING: Methane-sulfonate colistin (used for <i>in vivo</i> therapy) must not be used for this method
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	<p>A) <i>Preparation of the colistin stock solution:</i></p> <ul style="list-style-type: none"> <li>• Prepare a stock solution of <b>60 µg/ml</b> sulphate salt colistin:</li> </ul> <p>Sulphate salt colistin potency is expressed in UI/mg. Remember that 1UI is equivalent to 0.0332 µg.</p> <p>Express the potency as µg/mg to include it in the formula:</p> $\text{Volume (ml)} = \frac{\text{weight (mg)} \times \text{Assay potency (}\mu\text{g/mg)}}{\text{Concentration (}\mu\text{g/ml)}}$ <p>Use the formula to calculate the exact volume of water to be added to obtain the desired stock solution.</p>

	<p><i>B) Preparation of the COLISTIN AGAR-SPOT plates</i></p> <ul style="list-style-type: none"> <li>• Add 1ml of the 60µg/ml colistin stock solution and 19ml of sterile molten Mueller-Hinton agar equilibrated to 50°C (1/20 dilution) to a 90x15mm Petri dish. <b>Final colistin concentration in the plate will be 3 µg/ml.</b></li> </ul> <p>Note: It's convenient to add first the 19 ml Mueller-Hinton agar in order to avoid colistin binding to plastic and then to add 1 ml of the colistin stock solution.</p> <ul style="list-style-type: none"> <li>• Cover the plate with the lid and mix gently to homogenize.</li> <li>• Let the agar solidify.</li> <li>• Store the plates at 2 to 8° until use for up to 3 months.</li> </ul> <p><i>C) Colistin Agar Spot procedure:</i></p> <ul style="list-style-type: none"> <li>• Dip a sterile cotton swab into the 0.5 McFarland suspension of the test strain.</li> <li>• Remove the excess liquid by pressing firmly the swab on the inside wall of the tube above the fluid level.</li> <li>• Spot an agar surface area approximately 20 mm in diameter.</li> </ul> <p><i>Note: The same 90x15mm plate allows up to 12 strains to be tested at the same time.</i></p> <ul style="list-style-type: none"> <li>• Incubate the plate for 18 to 20 h at 35 °C.</li> <li>• After incubation, examine the purity plate to ensure inoculum was pure.</li> <li>• Observe the presence or absence of any growth.</li> </ul>
Results	<ul style="list-style-type: none"> <li>• COLISTIN-SUSCEPTIBLE: no bacterial growth at all or 1 colony.</li> <li>• COLISTIN-RESISTANT: growth of &gt;1 colony.</li> </ul> <p><i>See figure in Annex for clarification</i></p>
Additional testing and reporting	<p>Growth of only 1 colony could be considered cross contamination, if more than one isolate was tested. It is suggested to retest the isolate by this or another approved method.</p>
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	<sup>1</sup> Clinical and Laboratory Standards Institute M07: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically <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 <sup>3</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 <sup>4</sup> Phenotypic Detection of Plasmid-Mediated Colistin Resistance in Enterobacteriaceae J Clin Microbiol. 2020 Feb 24;58(3):e01555-19. doi: 10.1128/JCM.01555-19. Edgar Gonzales Escalante, Katherine Yauri Condor, Jose A Di Conza, Gabriel O Gutkind.
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**Annex.**

**Figure. Examples of a colistin-resistant (b, c, d, e, f, g, h) and colistin-susceptible (a, i) strains by the in-house agar test with 3mg/L concentration of colistin.** Strains c, d and f displayed semi-confluent growth. The species and the colistin broth microdilution MICs are indicated for each strain.

