## Colistin Micro Broth Disk Elution Test

**Version**  
EN 1.0  
**Last update:** May 2020  
Pages: 3

### Strengths
- Is an adaptation of the CLSI colistin disk elution test.\(^1\)
- 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

### Organism group
*Enterobacteriales, Pseudomonas aeruginosa* and *Acinetobacter* spp.

### Medium
CA-MHB

### Antimicrobial concentrations
Colistin 1 - 4 µg/ml

### Source of antimicrobial
10-µg colistin paper disk

### Test procedure
**A) Tubes Preparation (1 ml final volume)**
- Label 4 glass tubes for each test strain as Control, 1, 2 and 4.
- Place 10 ml of CAMHB in each tube.
- 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.

- 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 colistin disks and discard
- Homogenize tube 1.
- 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”.
- Proceed in the same way with tubes 2, 4 and control.
- Store tubes at -20ºC properly closed up to 6 months.

**B) Colistin Micro Broth Disk Elution procedure:**

- Remove a set of 1ml tubes (one of each: control, 1, 2 and 4) from the freezer and allow them to reach room temperature.
- Using a pipette of a calibrated loop, add 5 µl of the inoculum to each tube (1, 2, 4 and control) (final concentration: approximately 7.5 x 10^5 CFU/ml).

  *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*

- Mix each tube gently.
- Incubate for 18-20 hours at 35 ± 2 ºC for *Enterobacterales, Pseudomonas aeruginosa* and 24 hours for *Acinetobacter* spp.
- After incubation, examine the growth tube which must demonstrate obvious turbidity for the test to be valid.

  *Note: some *P. aeruginosa* isolates may grow near the meniscus.

- Read the MIC as the lowest colistin concentration that completely inhibits growth of the isolate.

### Results

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<th>COLISTIN SUSCEPTIBLE: MIC &lt;= 2 µg/ml.</th>
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<td>COLISTIN RESISTANT: MIC &gt;= 4 µg/ml.</td>
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### Additional testing and reporting

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:

- Contaminated tube.
- No inoculated tube.
- Hetero-resistant isolates.
- Inadequate colistin concentration in tubes.

### QC recommendations

It is suggested to test a positive and negative control each time a batch of ten determinations is prepared:

- *Escherichia coli* ATCC 25922 (MIC range <=1 – 2 µg/ml)
- *P. aeruginosa* ATCC 27853 (MIC range <=1 – 4 µg/ml)
- Colistin resistant: *E. coli mcr* producer

*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.*
Reference


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

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