# Minimal Inhibitory Concentration (MIC) Assay for *Acinetobacter baumannii*

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**[Abstract]** Minimal inhibition concentration (MIC) is the lowest concentration of an antimicrobial agent that can inhibit the visible growth of a microorganism after overnight incubation. MIC determination is used as not only a diagnostic tool in treating bacterial infections for clinicians but also a research method in evaluating the efficacy of an antimicrobial. Multidrug resistance *Acinetobacter baumannii* (*A. baumannii*) has emerged in recent years. Accurate determination of resistance by MIC assay is important in coping with this superbug. Here we described a protocol for determining MIC for *A. baumannii* in hope of assisting researchers and physicians in confirming resistance of clinical isolates correctly.

## Materials and Reagents

1. *A. baumannii* (ATCC, catalog number: 17978)
2. *Escherichia coli* (*E. coli*) (ATCC, catalog number: 25922)
3. Mueller Hinton broth (Sigma-Aldrich, catalog number: 70192)
4. Tigecycline (Wyeth, catalog number. 0220620-09-7)
5. NaCl (MDBio, catalog number: 101-1647-14-5)
6. KCl (Sigma-Aldrich, catalog number: P1147)
7. $Na_2HPO_4$ (J.T.Baker®, catalog number: 3828-01)
8. $KH_2PO_4$ (J.T.Baker®, catalog number: 4921-07)
9. HCl (J.T.Baker®, catalog number: 9535-03)
10. Tryptone (Pronadisa, catalog number: 1612)
11. Yeast extract (Pronadisa, catalog number: 1702)
12. Cation-adjusted Mueller-Hinton broth (CAMHB) (see Recipes)
13. PBS (1 L) (see Recipes)
14. Lysogeny broth (LB) (see Recipes)

## Equipment

1. 50 ml polystyrene culture tubes (sterile)
2. Spectrophotometer to measure absorbance of cell culture ($OD_{600}$)
3. 37 °C shaking and static incubators
4. Multichannel pipette (volume ranges 10 µl-1,000 µl)
5. 1.5 ml Eppendorf tube
6. A centrifuge machine
7. 1 ml cuvette

## Procedure

A. Preparation of antibiotic stock solution and dilution range

1. Obtain antibiotic powder from the pharmaceutical company and make a note of the relevant information, including expiry date, potency, stability and solubility.
2. Prepare 1 ml 10 mg/ml tigecycline stock solution.
3. Choose a suitable range of antibiotic concentrations to be tested for *A. baumannii* if available. If the range is not available, maximal concentration 512 µg/ml and serial diluted concentrations with CAMHB solution are used. The lowest dilution concentration is depended on the possible minimal inhibition concentration. 0.125 µg/ml is the lowest possible dilution concentration.
4. To get different tested concentrations, solution of 10-time maximal concentration is prepared by dispensing the appropriate amount of stock solutions with micropipette and diluting with CAMHB solution.
   For example, to get 1 ml 5,120 µg/ml solution, dispense 0.512 ml stock solution and dilute with 0.488 ml CAMHB solution.

B. Preparation of inoculum

5. Dissolve a single colony of *A. baumannii*, which is picked from a LB streak plate, in 3 ml LB broth and incubate overnight at 37 °C, 220 rpm.
6. Check $OD_{600}$ (1 $OD_{600}$= $10^9$ CFU/ml) with a spectrophotometer.
7. Dilute the bacterial solution with LB broth to get 0.1 $OD_{600}$ suspension and incubate at 37 °C, 220 rpm till mid-log phase (~2 h).
8. Put 1 ml mid-log phase bacterial solution in 1.5 ml Eppendorf tube, centrifuge at 6,000 rpm for five min, and wash with 1 ml PBS solution. Repeat the washing procedure twice.
9. Dissolve the bacterial pellet with 1 ml CAMHB solution.
10. Get 100 µl the above bacterial solution and mix it with 900 µl PBS, then check $OD_{600}$ with a

spectrophotometer. The bacterial concentration can be deduced from the measured value x 10.

11. Adjust the bacterial concentration to 1 x $10^7$ CFU/ml with CAMHB solution (1 $OD_{600}$ ~$10^9$ CFU/ml).

C. Inoculation and incubation

12. Mix 50 µl adjusted *A. baumannii* bacterial solution (1x$10^7$ CFU/ml), 850 µl CAMHB and 100 µl solutions of 10-time serial tested antibiotic concentration. Use the *E. coli* ATCC25922 bacterial solution as a control.
13. Use 900 µl CAMHB and 100 µl solutions of 10-time serial tested antibiotic concentration for $OD_{600}$ measurement comparison as a negative control.
14. Incubate at 37 °C, 220 rpm for 20-24 h.

D. Reading and interpretation

15. Check $OD_{600}$ with a spectrophotometer.
16. Read the MIC endpoint as the lowest concentration of antibiotic at which there is no visible growth of bacteria (no solution turbidity on naked eyes), and the difference of measured and background $OD_{600}$ is less than 0.01.

## Recipes

1. CAMHB
   Dissolve 23 g Mueller Hinton broth in 0.9 L of distilled water
   Adjust pH to 7.2 using HCl
   Then fill up to 1,000 ml with distilled water
   Sterilized by autoclaving at 121 °C for 15 min
   And added
   2 ml 10 g/L $Ca^{2+}$ (8.36 g $MgCl_2 \cdot 2H_2O$ in 100 ml $ddH_2O$)
   1 ml 10 g/L $Mg^{2+}$ (3.68 g $CaCl_2 \cdot 6H_2O$ in 100 ml $ddH_2O$)
   Stored at 4 °C
2. PBS (1 L)
   8 g NaCl
   0.2 g KCl
   1.44 g $Na_2HPO_4$
   0.24 g $KH_2PO_4$
   Dissolve in 900 ml $ddH_2O$

 

Adjust pH to 7.2 using HCl

Sterilized by autoclaving at 121 °C for 15 min

3. LB

   10 g tryptone

   5 g yeast extract

   5 g NaCl

   Fill to 1 L with dd$H_2O$

   Sterilized by autoclaving at 121 °C for 15 min

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**References**

1. Lin, M. F., Lin, Y. Y., Yeh, H. W. and Lan, C. Y. (2014). Role of the BaeSR two-component system in the regulation of *Acinetobacter baumannii* adeAB genes and its correlation with tigecycline susceptibility. *BMC Microbiol* 14: 119.

 