Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

SC 2019 74 09

MIC determination by broth micro dilution using Sensititre™ plates from Thermo Scientific™

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1. Introduction

This protocol has been prepared for the purpose of presenting and describing the laboratory activities covered by the FWD-AMR-RefLabCap Training Course hosted at DTU Food, Denmark in May 2022.

Minimal Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that is required to inhibit growth of an organism. The MIC of a bacterium to an antimicrobial agent gives a quantitative estimate of the antimicrobial susceptibility.

MIC determination with the commercial Sensititre™ panel from Thermo Scientific™ is performed in 96 well microtiter plates with dehydrated antimicrobial agents in two-fold dilutions. The panel is inoculated with a standardized inocolum of the organism tested and subsequently incubated. After incubation, the MIC value is read as the lowest concentration that inhibits visible growth.

The method is sensitive to variations in broth composition, inoculum, incubation time, temperature and reading procedures. Therefore it is important to follow a well-standardized method and to perform QC regularly.

The method follows ISO 20776-1:2020.
The interpretation of the MIC values should be done from well-defined criteria.

References

ISO 20776-1:2020
EUCAST: MIC determination

Publications Office

2. Equipment and reagents

- 50 µl and 100 µl pipettes
- Thermo Scientific™ Sensititre™ McFarland 0,5 standard
- Thermo Scientific™ Sensititre™ nephelometer
- Thermo Scientific™ Sensititre™ dosing heads
- Thermo Scientific™ Sensititre™ autoinoculator
- Thermo Scientific™ Sensititre™ MIC-panels
- For Salmonella: Incubator 36-37 ºC aerobic enviroment 18 ±2 h
- For Campylobacter: Microaerophilic environment (10% CO_2, 5% O_2, 85%N_2) incubator 41°C 24 h
- Tube with 5 ml 0,85% sterile saline
- For Salmonella: Thermo Scientific™ Sensititre™ glass tubes with 11 ml CAMHB (MH broth w. TES)
- For Campylobacter: MH-F broth - 11 ml CAMHB (MH broth w. TES) + 5% lysed horse blood
- Blood agar plates

3. Procedure day 1

- Standardize the inoculum: From a pure overnight culture, pick material from at least 3-4 colonies. Suspend in a 5 ml in a saline tube of the same type as the one for the McFarland 0.5. Mix. Adjust to McFarland 0.5 using a nephelometer. Calibrate the nephelometer before use using the MacFarland 0.5 standard and gently turn all invert you test suspension a couple of times by turning the tube upside-down before measuring. If necessary, adjust turbidity of inoculum to match the standard by adding either more colony material or more saline to the inoculum.

- Dilute the McFarland 0.5 suspension as follows:

  Campylobacter: Transfer 100 µl of your saline suspension into your broth
  Salmonella: Transfer 50 µl of your saline suspension into your broth

- Vortex (after this step, the suspension should be used within 15 minutes)

- Inoculate the plate. Transfer 100 µl per well for Campylobacter and 50 µl per well for Salmonella (this volume is panel depended). Use either a Thermo Scientific™ Sensititre™ autoinoculator or a multichannel pipette

- Seal the plates with perforated seals for Campylobacter and non-perforated seals for Salmonella and incubate them

- For purity control, spread 10 µl from the inoculum suspension on a blood agar plate and incubate it together with the MIC-panel

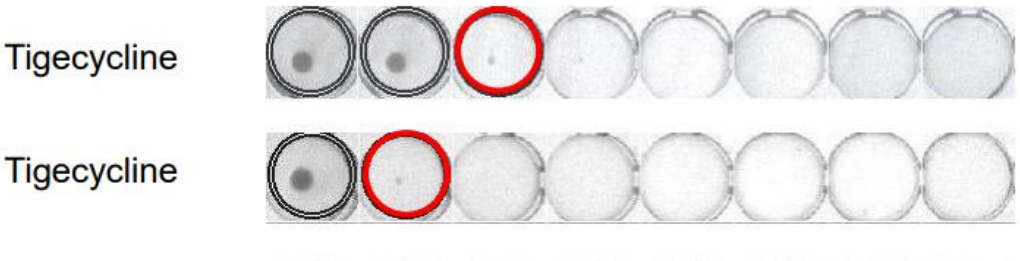
4. Procedure day 2

- Take you plates out of the incubator
- Check your purity control. If it is not pure, your results cannot be reported.
- Read the MIC's as the lowest concentration without visible growth. You can either read the panel using Thermo Fisher's Vizion or manually with a mirror.
- Start by reading your quality control strains and then proceed with your test strains.
- Check that there is growth in the positive control wells
- Be aware of special reading rules, e.g. for bacteriostatic antimicrobial agents, trimethoprim etc.
- The acceptable MIC ranges for the quality control strains as recommended by EUCAST are find here v_12.0_EUCAST_QC_tables_routine_and_extended_QC.pdf

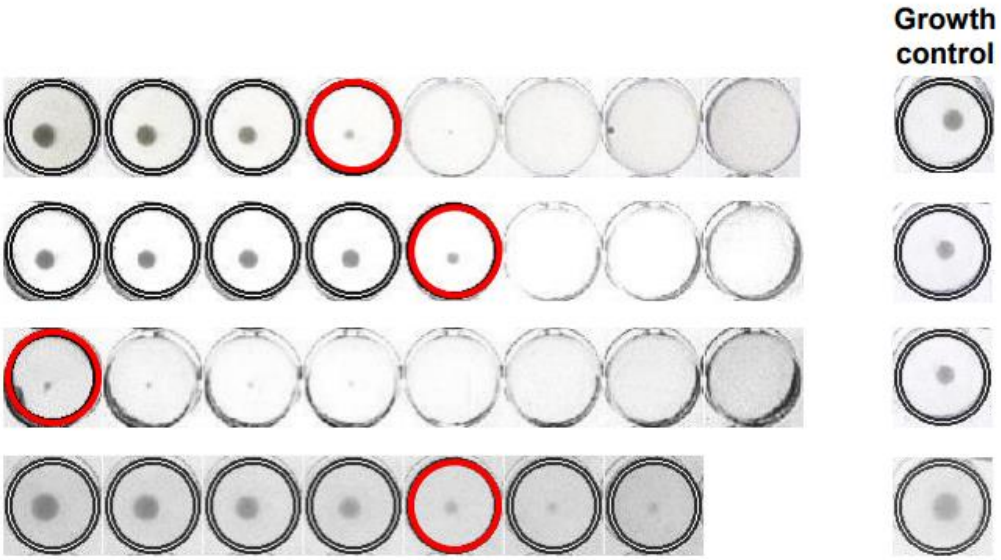
5. Reading examples

For Campylobacter that are tested in Mueller Hinton with blood, haemolysis of the blood may be observed, and is often accompanied by turbidity or a pellet. This should be regarded as growth when reading the endpoint. The black ring indicates where the MIC should be determined.

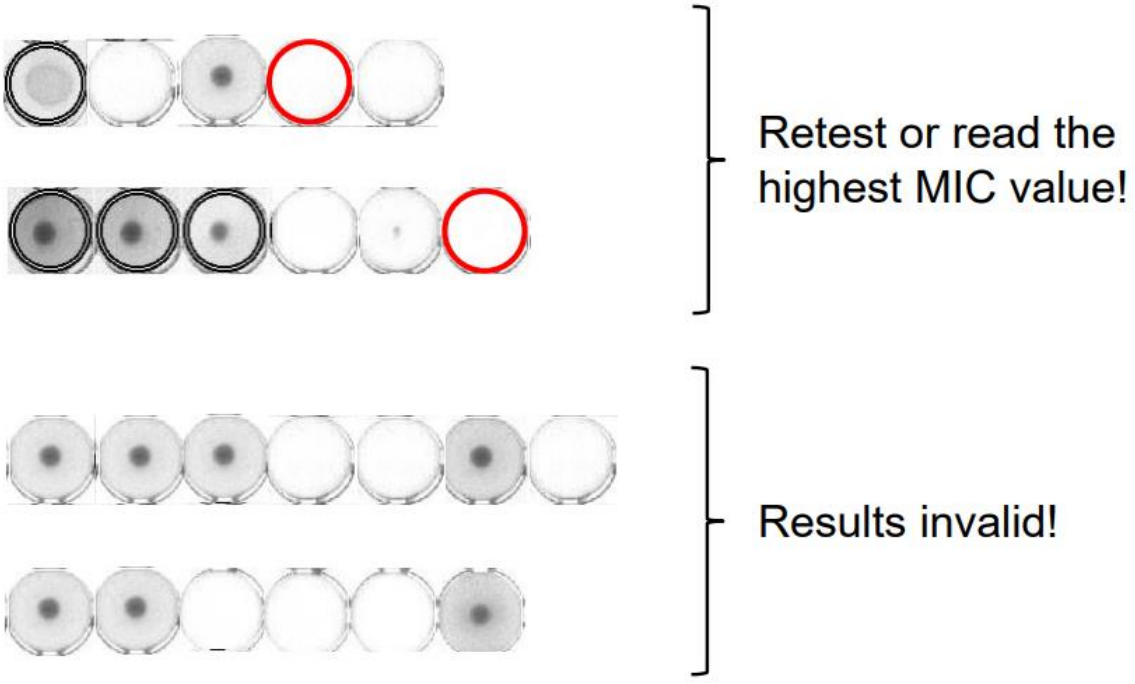
For Salmonella, you may observe that bacteriostatic antimicrobials present pinpoints as trailing endpoints. These should be disregarded. The red ring indicates where the MIC should be determined.



For trimethroprim and sulfamethoxazole, the MIC should be read as the lowest concentration that inhibits ≥80 % of the growth compared to the control well. The red ring indicates where the MIC should be determined.



Examples for skipped wells and how to process them. The red ring indicates where the MIC should be determined.



All pictures are from EUCAST reading guide. See full reading guide at [EUCAST BMD reading guide]

6. Quality control

To ensure that you obtain reliable results when performing antimicrobial susceptibility testing, quality control (QC) is crucial.

At the current training course, the quality control of the test results encompasses ATCC 25922 Eschericia coli for Salmonella testing and ATCC 33560 Campylobacter jejuni for Campylobacter testing.

Traceability is key when performing quality control. Ensure that you perform check of new batches of media etc. and ensure that your document and track your QC results allowing you to trace back if you need to troubleshoot, for example if you observe that your QC strain is one step out of range you can trace back to check if this might have started when you started to use a new batch of something.

If you do not perform a particular type of test routinely, ensure that you consider which QC-measures are relevant, for example including a QC strain in parallel to your test strains. If you obtain results from the QC strain that are within the acceptance range, you have an indication that your test strain results are reliable.

Annually, in January, EUCAST update their breakpoint tables and QC tables. This might include updated ranges or the addition of breakpoints and ranges for new antimicrobial agents. Keep updated on the newest version when interpretating your results by looking into the most recent QC table. The current QC table is V.12 and can be found here
EUCAST: Quality Control

7. Media preparation

For media preparation commercial media can be used if they follow EUCAST recommendations.

For EUCAST media preparation guide see EUCAST: Media preparation