Application Note

INTEGRA

Automated Minimum Inhibitory Concentration Testing

Introduction

Antibiotic resistance is a major threat to public health worldwide. One of the causes of antibiotic resistance is the overuse and misuse of antibiotics in healthcare and agriculture. One of the most important approaches used to define the effectiveness of an antimicrobial compound is to determine the minimum inhibitory concentration (MIC). Serial dilution of a compound is performed by mixing it with liquid growth medium, followed by the addition of a bacterium. Bacterial growth is then measured in the presence of different compound concentrations. The MIC is defined as the lowest concentration of an antimicrobial compound that inhibits the growth of the bacterium. Automating the transfer and mixing steps with the ASSIST PLUS pipetting robot enables serial dilutions to be performed faster and more reproducibly. Pipetting heights, speeds and mixing parameters are consistent across all dilutions, and the potential ergonomic hazards of manual pipetting are avoided.

Key benefits:

- Serial dilutions are laborious and error prone but, with the ASSIST PLUS, it's a walk in the park. The user does not lose track of the pipetting scheme, their hand does not tire through manual pipetting, and repetitive strain injuries do not develop. This is because the ASSIST PLUS, together with the VOYAGER adjustable tip spacing pipette, does the majority of the work. The user just has to define the key parameters for the serial dilution, such as the volumes and the mixing steps.
- Automating MIC determination on the ASSIST PLUS increases reproducibility by providing optimal and consistent pipetting heights, angles and immersion depths, as well as automating tip exchange.
- With the VOYAGER adjustable tip spacing pipette, compounds and bacterial inoculum stored in tubes can be transferred to the dilution plate eight times faster than with a single channel pipette.
- Changes in volumes, mixing cycles or labware can be effortlessly adjusted in the easy-to-use VIALAB software.
- The whole workflow can be fully automated in just one program, providing a complete walk-away solution that frees up highly valuable time.

Step-by-step procedure:

The ASSIST PLUS pipetting robot and an 8 channel VOYAGER 125 µl adjustable tip spacing electronic pipette with 125 µl Sterile, Filter GripTips can be used for the automated determination of MICs. In this microbroth dilution assay, eight different antibiotics were tested across a range of 10x twofold dilutions (doubling dilutions) with one bacterial strain in a single 96 well plate (**Figure 1**). Growth controls (growth media and bacterial inoculum) and sterility controls (growth media only) were also included. The pipetting program was generated using the VIALAB software.

Before starting the MIC test, the antimicrobial compound should be dissolved and diluted to the appropriate starting concentration. The bacterial inoculum should derive from morphologically similar colonies cultured overnight, and have a concentration of $5x10^5$ CFU (colony forming units)/ml.

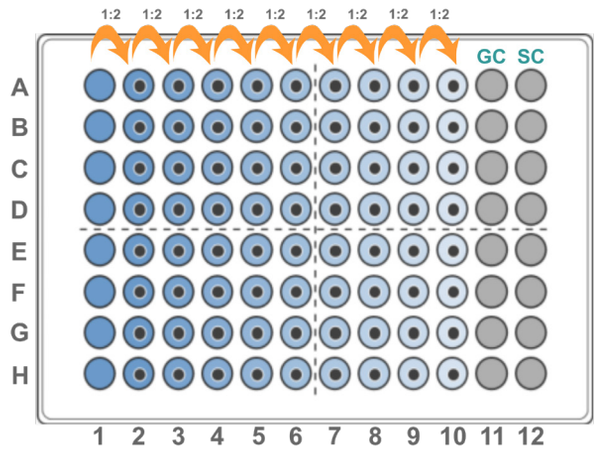

Figure 1: Schematic representation of the 10x twofold serial dilution (GC: growth control, SC: sterility control).

Figure 2: INTEGRA rack for 1.5/2 ml microcentrifuge tubes.

Program: Automated MIC assay (MIC_Assay)

Experimental set-up

Deck position A: 10 ml multichannel reagent reservoir with SureFlo™ anti-sealing array.
Deck position B: INTEGRA rack for 1.5/2 ml microcentrifuge tubes (**Figure 2**) with two sliders.
Deck position C: 96 well, flat bottom plate (Greiner Bio-One International).

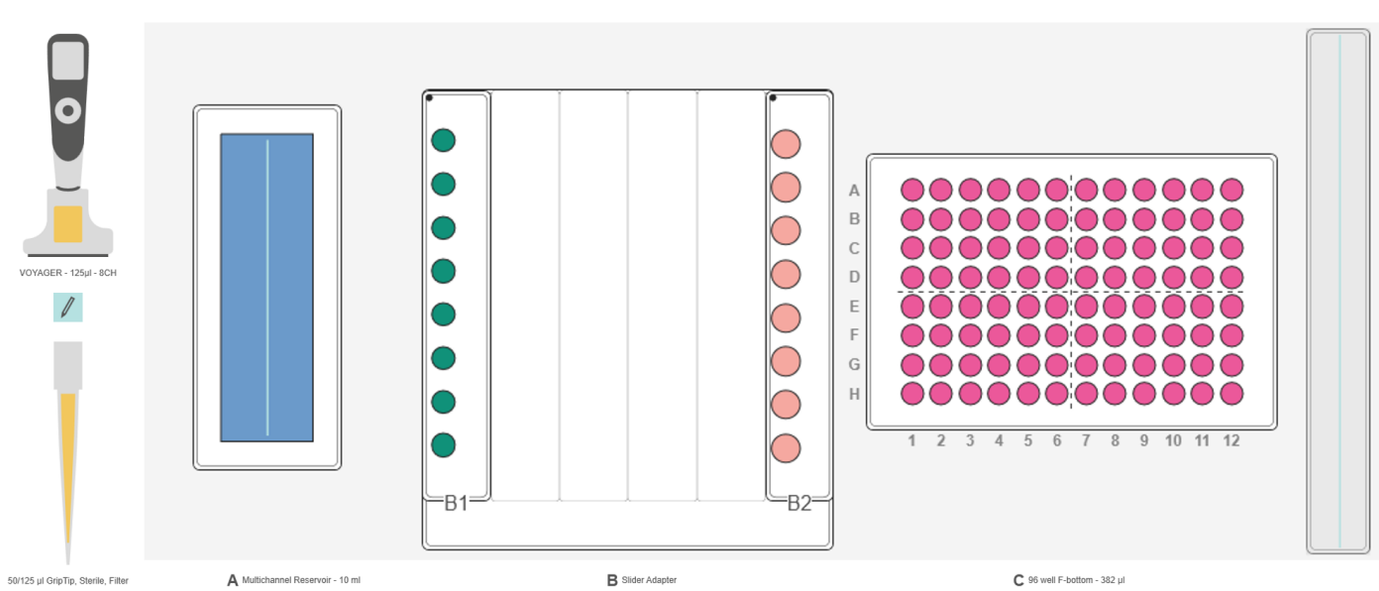

Figure 3: Deck set-up for determining MIC. **Position A**: 10 ml multichannel reagent reservoir filled with 8 ml fresh bacterial growth medium (blue). **Position B**: INTEGRA rack for 1.5/2 ml microcentrifuge tubes. One slider with 0.5 ml screw cap tubes (STARLAB) filled with eight different antimicrobial compounds to test (green), one with 1.5 ml reaction tubes (Greiner Bio-One International) filled with the bacterial inoculum (light pink) **Position C**: 96 well flat bottom plate (magenta).

1. Add the medium	**STEP:** Addition of bacterial growth medium to the sample, sterility, and growth control wells	**HOW TO:** Fill a 10 ml multichannel reservoir with 8 ml of fresh bacterial growth medium and load it on deck position A. Place the INTEGRA rack on deck position B. Fill the 0.5 ml screw cap tubes with eight different antimicrobial compounds to be tested against the bacterial strain (**Figure 3**, green). Fill the 1.5 ml reaction tubes with the bacterial inoculum (**Figure 3**, light pink). Place the 96 well flat bottom plate on deck position C (**Figure 3**, magenta). Select and run the VIALAB program 'MIC_Assay' on the VOYAGER pipette. The pipette transfers 50 µl bacterial growth medium to columns 2 to 11 of the microplate. The 5 µl post-dispense ensures precise pipetting, even if the growth medium tends to form bubbles during pipetting. In the next step, the pipette transfers 100 µl of bacterial growth medium to column 12 of the microplate. The wells in this column will be the sterility controls, containing only bacterial growth medium. This step uses a single set of sterile filter tips, as it only involves transfer of sterile medium to an empty, sterile plate. **Tip:** • The workflow parameters can be easily modified in VIALAB software. If the samples and reagents are in a different labware format, this can be set up quickly.
2. Serial dilution	**STEP:** Twofold serial dilution of the antimicrobial compound	**HOW TO:** The pipette transfers 100 µl of the antimicrobial compounds to the wells of column 1, then performs a twofold serial dilution (**Figure 1**), starting from column 1 (highest concentration) and finishing at column 10 (lowest concentration = 1:512). The pipette aspirates a 50 µl volume from the wells of one column, then dispenses it into the wells of the next column. Fully mixing the components is crucial in serial dilution, so the program includes an optimized mixing step with three mixing cycles before aspirating and after dispensing. The last aspirate stays in the tip, and is subsequently discarded to waste with the tip. This ensures that there is an equal volume in every well of the serial dilution. **Tips:** • The mixing volume (100 µl) in the serial dilution is set higher than the transfer volume (50 µl), resulting in a homogeneous mix without increasing the assay time significantly. • Productivity can be significantly increased by using the VOYAGER adjustable tip spacing pipette when transferring compounds from tubes to the MIC assay plate.
3. Add the bacterium	**STEP:** Addition of the bacterial inoculum	**HOW TO:** In this step, the ASSIST PLUS pipetting robot transfers 50 µl of bacterial inoculum to the wells of columns 1 to 11. When determining the MIC, it is very important that the concentration of the bacterial inoculum is the same in every well, to ensure consistent and reproducible results. An identical inoculum concentration is ensured through the implementation of mixing steps before every aspiration and after every dispense. **Tip:** • Using disposable sterile filter tips allows contamination-free processing.

4. Incubation and plate reading	**STEP:** Incubation for bacterial growth and results read-out	**HOW TO:** The ASSIST PLUS pipetting robot informs the user to incubate the 96 well plate, then prompts them to read the results with a microplate reader. The optical density is measured and corrected using the blank wells of the sterility control, and the bacterial growth is determined. For each compound, the lowest concentration without bacterial growth is defined as the MIC (**Figure 4**).

Figure 4: MIC testing results. Eight different antimicrobial compounds were tested (rows A-H) against one bacterial inoculum. Growth (column 11) and sterility (column 12) controls were also used.

Remarks

VIALAB software:	The VIALAB programs can be easily adapted to your specific labware and protocols, for instance, when partial plates are needed.
Partial plates:	Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

Conclusion

- Determination of the minimum inhibitory concentration is a very important and widely used approach in both antibiotic development and routine antimicrobial susceptibility testing by clinical microbiology laboratories.
- Performing MIC testing with the ASSIST PLUS pipetting robot and VOYAGER adjustable tip spacing pipette provides a fully automated solution, with the flexibility to adapt to different labware formats and user needs.
- Serial dilutions are error-prone processes, but the reproducibility of MIC testing can be increased significantly with the ASSIST PLUS.
- Contamination can be avoided by automated tip exchange and the use of sterile, filtered disposable tips.
- The potential ergonomic hazard of prolonged pipetting during serial dilution steps can be avoided by fully automating MIC testing.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	VOYAGER 8 channel 125 µl electronic pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4540	Rack for 1.5/2 ml microcentrifuge tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	6465	125 µl Sterile, Filter Grip Tips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	4370, 4371	10 ml Reagent Reservoir, Sterile, SureFlo anti-sealing array, polystyrene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
STARLAB	E1405-2130	0.5 ml Screw Cap Tube, Plain, Conical, Natural	https://www.starlabgroup.com/GB-en/product/0.5ml-screw-cap-tube-plain-conical-natural-e1405-2130.html
Greiner Bio-One International	616201	Reaction Tube, 1.5 ml, PP, Natural, Attached Cap	https://shop.gbo.com/en/switzerland/products/bioscience/reaction-tubes-analyser-cups/bs-reaction-tubes/616201
Greiner Bio-One International	655161	96 Well Microplate, PS, F-Bottom	https://shop.gbo.com/en/germany/products/bioscience/microplates/96-well-microplates/96-well-microplates-clear/655161

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