

High-throughput MIC hit detection and Synergy Screening

Warwick Antimicrobial Screening Facility are now offering automated, high-throughput MIC hit detection and synergy screening of compound libraries. Implementation of our Tecan Freedom EVO liquid handling platform enables us to provide a faster and more economical antimicrobial screening service whilst retaining reliability. The technology is specially designed to process medium to large sized libraries for academic and industrial clients at an affordable price, making screening more accessible than would be possible with manual handling.

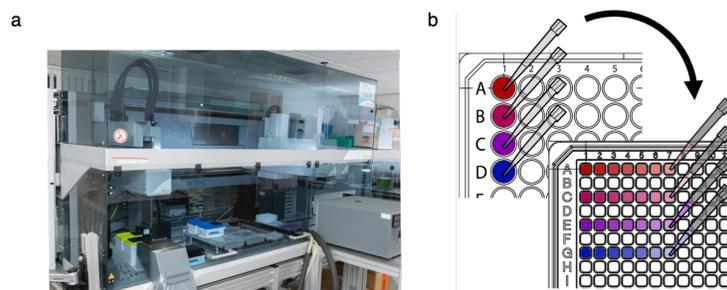


Figure 1: Liquid handling system a) The Tecan platform used to prepare and measure plates. b) Compounds, supplied by the client in 96 well plates, are transferred onto 384 well plates and then inoculated

Capabilities

MICs can be run with:

- 12 points
- 4 points
- Single well reads

Rapid results turnaround: next day reporting of 1000 compounds with 4 point reads.

Checkerboards can be run with:

- 11 x 7 grids
- 4 x 4 grids for increased throughput screening

The robot can be run 24 hours a day and takes 1 hour of human interaction to set up. The hourly charge for the robot is a third of the price of a technician. In addition, automation avoids potential human error typical with repetitive tasks.

In all cases, reduced volumes (80 in 384 plates allow 2.5x less compound usage.

Custom layouts, strains, and requirements can be easily accommodated, compounds can be supplied in water or DMSO. Dilution of powders can be handled at additional cost.

Positive and negative controls as well as 2 known antibiotic controls are included on every plate.

MIC results are determined using a custom matlab script.

Strains

The system is optimised to an *E. coli* screening strain with a *ToIC* knockout which reduces issues with permeation and increases hit rate, allowing the potential of the chemistry to be realised. Other ESKAPE organisms and β -lactamase hyper-producers are available on request.

Examples

High-throughput MICs

Manual and robotic 12 point MICs were carried out on 51 novel compounds in DMSO along with 5 known antibiotics (Amikacin, Ticarcillin, Minocyclin , Rifampin, Clarithromycin). In triplicate analysis, 77% of evaluations were within one dilution of the manual read. 23 of the compounds within the library were classified as hits (MIC of $<32 \mu\text{g/ml}$), robotMIC was able to correctly determine a hit 85% of the time. This is comparable to results achievable from manual screening.

Checkerboards

A Checkerboard between clavulanic acid and amoxicillin was prepared using the liquid handling robot. Figure 2 shows the synergistic interaction between clavulanic acid and amoxicillin against β -lactamase expressing *E. coli*, with amoxicillin able to inhibit growth once β -lactamases are inhibited.

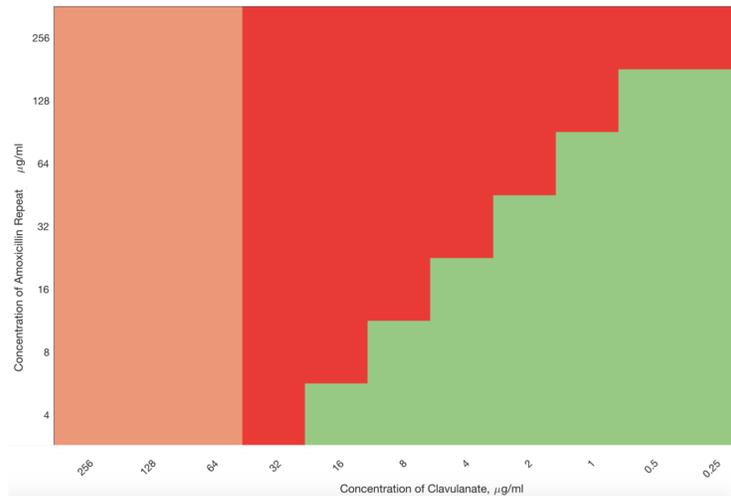


Figure 2: An 7x11 grid checkerboard between clavulanic acid (MIC of 64 $\mu\text{g/ml}$) and amoxicillin (MIC of $>256 \mu\text{g/ml}$) in the ESBL-expressing *E.coli* strain NCTC 13353. Pink indicates inhibition of growth due to the clavulanic acid, green indicates growth and red indicates inhibition of growth due to the amoxicillin in the presence of clavulanic acid. The amount of red illustrates that far more inhibition is possible once the drugs are combined.

Checkerboards allow for easy identification of synergy between two compounds and can be used to investigate mechanism of action. However due to the number of conditions tested, these assays are slow and labour intensive to carry out by hand. By automating the liquid handling, larger screens can be carried out, allowing greater exploration of the many possible combinations. By reducing the size from 7 x 11 to 4 x 4 (figure 3) , throughput can be increased further.

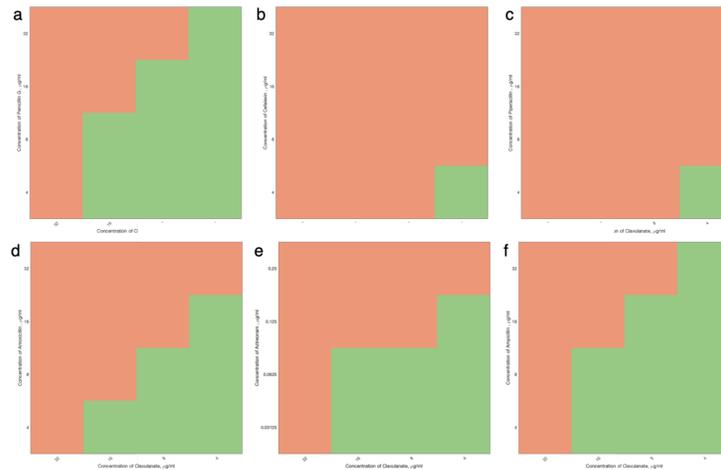


Figure 3: A 4x4 checkerboard allow increased throughput of synergy studies, typically a very slow process. 6 β -lactams: penicillin G, cephalexin, piperacillin, amoxicillin, aztreonam and ampicillin (a-f respectively) were tested in synergy with clavulanic acid against ESBL-expressing *E.coli* strain NCTC 13353. These results are triplicates, which were shown to be highly reproducible.

Follow-up Work Available

All hits identified by RobotMIC can be rapidly followed up with manual MICs and further organisms in the Warwick Antimicrobial Screening Facility. Our expert scientists are always on hand to help with data analysis and investigation, to make sure you get the as much from your data as possible.