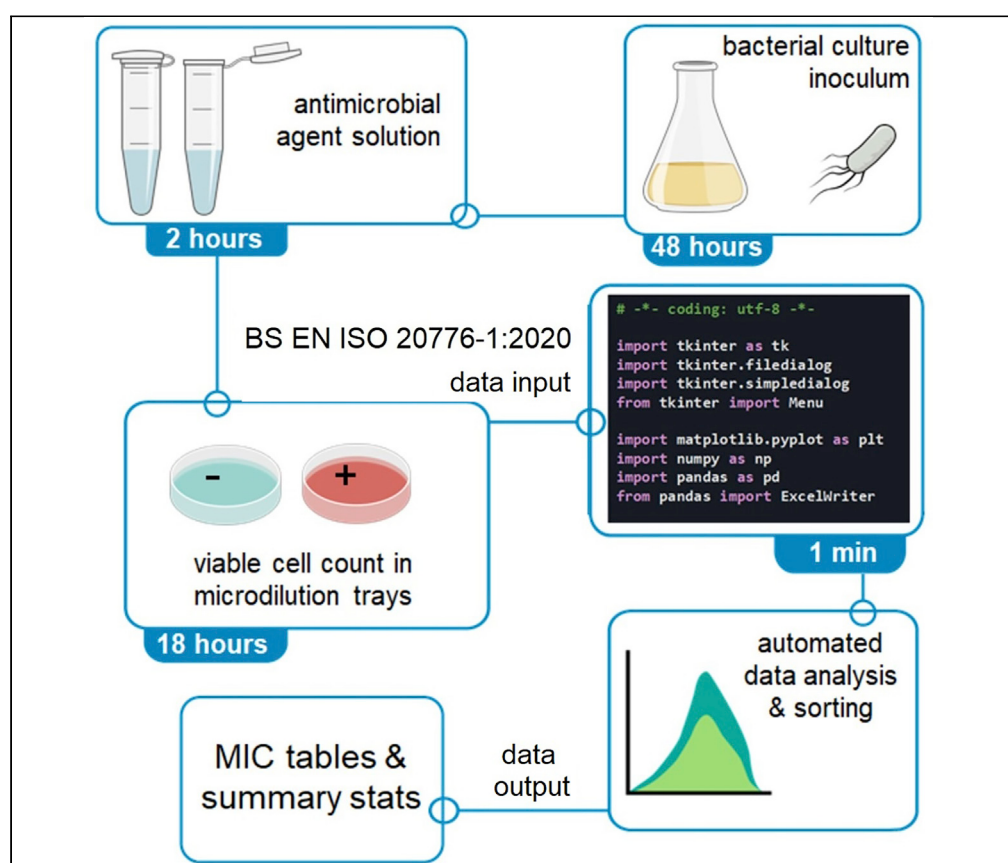


Protocol

DANAMIC: Data analyzer of minimum inhibitory concentrations – Protocol to analyze antimicrobial susceptibility data



Javier Garcia-Ruiz,
Maxim G. Ryadnov
max.ryadnov@npl.co.uk

Highlights

Open-source software to analyze antimicrobial susceptibility data

Detailed reference procedures for obtaining antimicrobial activity data

Automated conversion of the obtained data into minimum inhibitory concentrations

Automated data analysis compatible with high-throughput screening

This protocol describes an open-source software developed to analyze experimental data obtained using antimicrobial susceptibility assays. We first describe experimental procedures for testing the activity of antimicrobial agents *in vitro* based on reference standards (BS EN ISO 20776-1:2020). We then describe the software protocol to analyze and convert the data obtained using these procedures into minimum inhibitory concentrations. This approach enables automated data analysis for microdilution assays and can be adapted for high-throughput antimicrobial screening.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Protocol

DANAMIC: Data analyzer of minimum inhibitory concentrations – Protocol to analyze antimicrobial susceptibility data

Javier Garcia-Ruiz^{1,3} and Maxim G. Ryadnov^{1,2,4,*}¹National Physical Laboratory, Teddington, Middlesex TW11 0LW, UK²Department of Physics, King's College London, Strand Lane, London WC2R 2LS, UK³Technical contact⁴Lead contact*Correspondence: max.ryadnov@npl.co.uk
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SUMMARY

This protocol describes an open-source software developed to analyze experimental data obtained using antimicrobial susceptibility assays. We first describe experimental procedures for testing the activity of antimicrobial agents *in vitro* based on reference standards (BS EN ISO 20776-1:2020). We then describe the software protocol to analyze and convert the data obtained using these procedures into minimum inhibitory concentrations. This approach enables automated data analysis for microdilution assays and can be adapted for high-throughput antimicrobial screening.

BEFORE YOU BEGIN

The spread of antimicrobial resistance prompts the search for more effective antimicrobial agents (Laxminarayan et al., 2016). An increasing emphasis is on identifying novel molecular and supramolecular classes that have not had an antibiotic before (O'Neill, 2016). In contrast to more traditional approaches, which focus on finding individual molecules or modifying existing antibiotics, such a search paradigm renders antimicrobial discovery more systemic and is anticipated to underpin pre-clinical pipelines of antimicrobials making anti-infective medicine more sustainable (World Health Organization, 2021).

Regardless of which molecular class is being developed, antimicrobial susceptibility assays constitute an indispensable part of antimicrobial evaluations. Results of such tests are expressed as Minimum Inhibitory Concentrations (MICs), indicating the lowest concentrations preventing a visible bacteria growth. Because such assays use serial micro-dilutions, MICs are reported as dilutions at which bacteria stop growing. More precise MICs can be established within the identified dilution with an additional round of testing. However, one- or even two-dilution variations in MICs are also common (EUCAST, 2020). Therefore, MICs are often reported as a range of concentrations with an average or target concentration. The areas where MIC assays add most value are the speed of assessment for virtually any sample size and their adaptability to high-throughput screening. With the need to increase the throughput of antimicrobial analyses (Hammond et al., 2021), it is not surprising that attempts have been made to standardize the assays or at least provide guidance on harmonizing MIC procedures. Reference and performance standards are made available via International Organization for Standardization (ISO), e.g., ISO 20776-1:2020 and its earlier versions, and the Clinical and Laboratory Standards Institute (CLSI, 2018), e.g., M02, M07 and M11 standards.



These documents describe methodologies under the test conditions that are typical for *in vitro* susceptibility tests and are offered as reference methods for the validation of other susceptibility tests to ensure comparability and reproducibility of MIC measurements. The documents also consider other factors impacting on antimicrobial activity, i.e., pharmacokinetics and bacterial resistance mechanisms, but require experimental and component modifications when it comes to the clinical interpretations of the results. Some of these modifications are given and updated in separate annexes of the documents. Overall, the performance standards describe basic methods in the accepted practice of antimicrobial testing, which are sufficient for MIC determinations (Hammond et al., 2020). However, the analysis of the data obtained remains laborious, lacks efficiency and is prone to carry over reproducibility errors when challenged by statistically significant sample numbers.

With this in mind, we designed this protocol which facilitates the automated analysis of results obtained using methods described in the performance standards. The protocol is based on molar concentrations, at which no discernible growth of a given microorganism can be determined using spectrophotometric methods after incubation with a given antimicrobial agent over 24 h (Andrews, 2001).

Institutional permissions

This protocol requires biosafety level 2 containment facilities to obtain antimicrobial susceptibility data. Standard operating procedures, risk assessments, disinfectant reagents and methods should be approved by the Institutional Biosafety Committee, with all work performed in specialist biosafety cabinets.

Preparation of stock solutions

⌚ Timing: 60 min

1. Prepare a correct stock concentration of an antimicrobial agent, which is necessary for the measurements at serial dilutions. Concentrations should be prepared at 1 g/L or greater depending on the solubility of the agent tested. The stock solutions should be clear and transparent.
 - a. Weigh out the correct amount of an antimicrobial agent.
 - b. Use an analytical balance pre-calibrated with weight standards.
 - c. Pre-calibrate spectrophotometers used for concentration and MIC measurements with calibrations standards as per ISO 17025.
2. Factor the powder potency of an antimicrobial agent (commercial antibiotics are supplied with this value), to obtain the amount (in g) of substance or the volume (in L) of a diluent needed to make a standard solution:

$$m = \frac{V * c}{P} \quad (\text{Equation 1a})$$

$$V = \frac{m * P}{c} \quad (\text{Equation 1b})$$

m: mass of the antimicrobial agent in grams (g).

V: volume of diluent in liters (L).

c: concentration of the stock solution (mg/L).

P: potency of the antimicrobial agent (mg/g).

Note: This protocol uses molar concentrations (M) since mole is the base unit for the amount of substance under the International System of Units (SI), whereas the designated unit for MIC values in performance standards is mg/L.

3. [Equation 1](#) is modified to convert the units to a more practical format ([Andrews, 2001](#)) and further to molar concentrations:

$$c_1 = \frac{m * P}{V} \quad (\text{Equation 2})$$

$$c_1 \equiv c_2$$

$$M = \frac{c_2}{1000} * \frac{1}{MW} \quad (\text{Equation 3})$$

m: mass of the antimicrobial agent (mg).

V: volume of diluent (mL).

c₁: concentration of the stock solution (μg/mL).

c₂: concentration of the stock solution (mg/L).

P: potency of the antimicrobial agent (μg/mg).

MW: molecular weight of an antimicrobial agent (g/mol).

M: molar concentration (mol/L).

Note: [Equations 1–3](#) are recommended for commercial antimicrobials with known potencies. For experimental antimicrobials the following method is recommended:

4. Weigh out 2–7 mg of powder of an antimicrobial agent into an Eppendorf tube (1.5 mL) and dissolve the powder in 1 mL of filtered (0.22 μm) water with a resistivity of 18.2 MΩ.
5. Record the absorbance of the resulting solution using a pre-calibrated spectrophotometer at wavelengths for which molar extinction coefficients are known. For example, for ampicillin at 235 nm and ceftazidime at 260 nm ([Cantu and Palzkill, 1998](#)), for agents containing amino acids and peptide bonds at 214 nm and for agents containing aromatic groups at 280 nm ([Kuipers and Gruppen, 2007](#)). The range of A₂₈₀ should be below 10 absorbance units (AU), a 1:10 dilution of the sample must be used to adjust the concentration in case it is too high. If molar extinction coefficients are unknown an estimation of the concentration weight per volume should be used.
6. Determine the stock concentration using the Beer-Lambert law:

$$A * DF = \epsilon * c * l \quad (\text{Equation 4a})$$

$$c = \frac{A}{\epsilon * l} * DF \quad (\text{Equation 4b})$$

A: absorbance measured for an antimicrobial agent.

ε: molar extinction coefficient at a given wavelength (M⁻¹ cm⁻¹).

c: concentration of the stock solution (M, mol/L).

l: path length (cm).

DF: dilution factor.

Preparation of bacterial culture and pre-culture inoculum

⌚ Timing: 48 h

7. Source bacterial cell lines (e.g., from ATCC) and antibiotics.
8. Pre-culture the sourced bacteria in Mueller-Hinton (MH) broth:
 - a. combine 50 μ L of the bacterial inoculum with 5 mL of the MH broth in a 50 mL conical centrifuge tube (e.g., Falcon™ or Corning™).
 - b. incubate at $35 \pm 1^\circ\text{C}$ with orbital shaking at 170 rpm over 18–24 h.
9. Adjust the optical density (OD) of the obtained culture at 625 nm:
 - a. dilute the culture to reach the turbidity of the 0.5 McFarland Standard (OD_{625} 0.08–0.12) corresponding to an inoculum containing $1\text{--}2 \times 10^8$ colony-forming units per milliliter (CFU/mL).
10. Further dilute the culture to the final concentration of 10^6 CFU/mL, by adding an appropriate aliquot of the culture to 10 mL of the MH broth. Use the prepared inoculum for testing.

⚠ CRITICAL: Perform purity checks of bacteria culture avoiding cross-contamination and perform viable colony counts every time when testing.

Note: Use a control tube with the broth without bacteria to probe for potential contamination in the culture media which can impact optical density measurements.

Note: The broth microdilution method is for mesophilic aerobic bacteria, some of which may require lower temperatures, which should be adjusted accordingly. The testing of anaerobic bacteria by broth microdilution methods is also performed by adjusting conditions (Cordova and Ambretti, 2020).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
<i>E. coli</i> ATCC 25922	CECT	CECT 434
<i>S. aureus</i> ATCC 29213	CECT	CECT 794
<i>P. aeruginosa</i> ATCC 27853	CECT	CECT 108
<i>Enterococcus faecalis</i> ATCC 29212	CECT	CECT 795
<i>Streptococcus pneumoniae</i> ATCC 49619	CECT	CECT 8737
Chemicals, peptides, and recombinant proteins		
Chloramphenicol 98%	Acros Organics	Product Code. 10368030
Ampicillin Sodium Salt (Crystalline Powder)	Fisher Bioreagents	Product Code. 10419313
Vancomycin hydrochloride	Alfa Aesar	Stock No. J62790.06
Gentamicin sulfate	Acros Organics	Product Code. 15433221
Polymixin B Sulfate	Fisher Bioreagents	Product Code. 10207607
Kanamycin Sulfate (White Powder)	Fisher Bioreagents	Product Code. 10031553
Amoxicillin sodium salt, 87%	Alfa Aesar	Stock No. J66675.03

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
SoftMax Pro 7.0	Molecular Devices	https://support.moleculardevices.com/s/article/SoftMax-Pro-7-1-software-Download-page
Python v3.8.2	Python Software Foundation	https://www.python.org/
Pandas v1.0.5	McKinney (2010)	https://pandas.pydata.org/
Numpy 1.10.0	Harris et al., 2020	https://numpy.org/
Matplotlib 3.2.2	Hunter (2007)	https://matplotlib.org/
XlsxWriter 1.2.9	https://github.com/jmcnamara/XlsxWriter	https://xlsxwriter.readthedocs.io/
Other		
Mueller-Hinton broth (dehydrated)	Thermo Scientific	Product Code. 10681675
Mueller-Hinton (MH) agar (dehydrated)	Thermo Scientific	Product Code. 10452453
Spectramax i3x (Microplate reader)	Molecular Devices	https://www.moleculardevices.com/products/microplate-readers/multi-mode-readers/spectramax-i3x-readers
Filter-based multi-mode microplate reader	BMG LABTECH	FLUOstar Omega (UVvis)
Biowave CO8000 cell density meter	Scientific Laboratory Supplies	https://www.scientificlabs.co.uk/product/COL3430
Corning™ Clear Polystyrene 96-Well Microplates	Fisher Scientific	Product Code. 10216341
Thermo Scientific™ MaxQ™ 6000 Incubated/Refrigerated Stackable Shakers	Fisher Scientific	Product Code. 10449933
Combitips® advanced, Biopur®, 5.0 mL	Eppendorf UK Ltd	Catalog No. 0030089669
Combitips® advanced, Biopur®, 10 mL	Eppendorf UK Ltd	Catalog No. 0030089677
Standard 90 mm Petri Dishes	Thermo Scientific	Product Code 11901518
Multipette® E3x	Eppendorf UK Ltd	Catalog No. 4987000029
Falcon 50 mL Conical Centrifuge Tubes	Fisher Scientific	Product Code 10788561
Falcon™ 15 mL Conical Centrifuge Tubes	Fisher Scientific	Product Code 11507411
Sartorius™ Secura™ Standard Laboratory Balance	Fisher Scientific	Product Code 15653816
Eppendorf™ Polypropylene Protein LoBind Microcentrifuge Tube 1.5 mL	Fisher Scientific	Product Code 10708704
Prestige Medical™ Portable autoclave classic media	Fisher Scientific	Product Code 12755355
Disposable cuvettes, 1.5 mL	Fisher Scientific	Product Code 11602609

MATERIALS AND EQUIPMENT

Culture medium

Reagent	Final concentration	Amount
Mueller-Hinton (MH) broth	21 g/L in water	1 L
Mueller-Hinton (MH) agar	38 g/L in water	500 mL
Antimicrobial agent	100 µM in MH broth	7 mL
Milli-Q® water	N/A	1.5 L
Total	N/A	N/A

Use all reagents fresh. Store MHB and MHA at 4°C up to two and ten weeks, respectively.

STEP-BY-STEP METHOD DETAILS

Preparation of antimicrobial solutions

⌚ Timing: 60 min

Dilutions of antimicrobial agent solutions prepared according to ISO 20776-1:2020:

Starting concentration (µM)	V. stock (mL)	V. broth (mL)	Final concentration (µM)
X	Xi	7	100
100	1	1	50

(Continued on next page)

Continued

Starting concentration (μM)	V. stock (mL)	V. broth (mL)	Final concentration (μM)
100	1	3	25
100	1	7	12.5
12.5	1	1	6.25
12.5	1	3	3.125
12.5	1	7	1.5625
1.5625	1	1	0.78125
1.5625	1	3	0.390625
1.5625	1	7	0.1953125

X: the stock concentration.

Xi: the volume of the stock solution at concentration X.

Note: Use antimicrobial agent solutions on the same day, unless following instructions available on the stability of the solutions under specified storage conditions.

Note: Avoid repeated freeze-thaw cycles. These can accelerate the degradation of antimicrobial agents, e.g., β-lactams, or their aggregation.

Preparation and incubation of micro-dilution trays with antimicrobial agents

⌚ **Timing:** 18 h for the preparation of micro-dilution trays

1. Dispense 50 μL of the antimicrobial solutions in each well of micro-dilution trays in triplicates in a descending order.

Note: Use twice the required concentration of an antimicrobial agent to compensate for dilutions with 50-μL of the prepared bacterial inoculum.

Note: Use at least three wells with 50 μL each of the MHB and inoculum as a positive growth control and add extra 50 μL of the fresh MH broth to reach 100 μL and at least three wells with 100 μL of the MHB as a negative growth control.

2. Combine 50-μL of the prepared inoculum with 50 μL of an antimicrobial agent solution in each well of micro-dilution trays to reach the final inoculum of 5×10^5 CFU/mL (target range, 2×10^5 CFU/mL to 8×10^5 CFU/mL).
3. Place an adhesive seal around the microplates before incubation to prevent the desiccation of the wells. Incubate over 18 ± 2 h at $35 \pm 1^\circ\text{C}$ in ambient air.

Note: Do not exceed four micro-dilution trays in one stack to avoid uneven heating and place a single tight lid on the top of the stack.

⚠ **CRITICAL:** Use a different 96-well plate for each microorganism to avoid cross-contamination.

⚠ **CRITICAL:** Use controls for each strain tested.

Note: Store stock cultures of strains used frozen at $\leq -60^\circ\text{C}$. Make working cultures of the strains by sub-culturing the stock cultures on a non-selective nutritive agar medium.

Note: Use at least two relevant quality control strains every day when testing is carried out. Process these strains in the same way as routine cultures.

△ **CRITICAL:** Use the trays with an antimicrobial agent immediately. If not possible, store the trays before the time the agent starts degrading, as per documented quality control evidence, but no longer than three months (ISO, 20776-1:2020). In this case, seal the trays in plastic bags and place in a freezer ($\leq -60^{\circ}\text{C}$).

△ **CRITICAL:** MICs against control strains should be within the ranges given in quality control documents by EUCAST or CLSI. The MIC for the control strains should be within one two-fold dilution of the expected MIC (Andrews, 2001).

Viable cell count in micro-dilution trays

⌚ **Timing:** 18 h to probe viable cell count

4. In parallel with the preparations 1–3, probe the target viable cell count (5×10^5 CFU/mL) on the test suspension.
 - a. Take 10 μL of the culture from the positive growth control immediately after inoculation.
 - b. Dilute it (1:1,000) in 10 mL of MHB.
5. Spread 100 μL of the obtained suspension over an MHA plate.
6. Incubate over 12–18 h.
7. 20–80 colonies formed indicate an acceptable test suspension.

Note: All trays should be inoculated within 30 min in order to maintain the viable cell number.

△ **CRITICAL:** If the acceptable suspension of 20–80 colonies is not achieved, restart inoculum preparation.

Data recording

⌚ **Timing:** 20 min to record the data

8. Transfer microdilution trays into a micro-plate reader and take the readings after 18 h of incubation.

△ **CRITICAL:** Readings should only be taken if:

- a. there is visible bacterial growth in the positive control ($\text{OD}_{625} > 0.009$).
 - b. there is no visible growth in the negative control ($\text{OD}_{625} < 0.009$).
 - c. the inoculum has a viable cell count with no cross-contamination (see step 16).
9. Record the lowest concentration of the agent at which there is no visible growth ($\text{OD}_{625} < 0.009$).

Note: Absorbance values qualifying for a minimum inhibitory concentration are up to 0.009 AU (Table 1).

Interpretations of the results

⌚ **Timing:** 20 min to analyze the recorded data

10. To analyze the data use reference breakpoints (target and range of concentrations) for a specific bacterium and a specific antimicrobial agent.

Note: Refer to clinical breakpoints set by CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Tables 2 and 3).

Table 1. Example readings in absorbance units from a microdilution tray for conventional antibiotics

Bacteria	Sample	Concentration in μM									
		50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0
<i>E. coli</i> ATCC 25922	Amoxicillin	0.452	0.710	1.067	1.009	1.083	0.912	1.114	1.106	1.179	1.117
	Ampicillin	0.000	0.001	0.611	0.801	0.996	0.990	1.077	0.992	1.178	1.094
	Gentamycin	0.000	0.000	0.000	0.000	0.000	0.000	0.686	0.758	1.067	1.050
	Kanamycin	-0.001	-0.002	-0.001	-0.001	0.725	0.972	1.034	1.150	1.172	1.170
	Polymyxin B	0.000	0.000	0.000	0.000	0.001	0.158	1.035	1.034	1.185	1.112
<i>P. aeruginosa</i> ATCC 27853	Amoxicillin	0.700	0.752	0.858	0.786	0.752	0.733	0.688	0.658	0.714	0.656
	Ampicillin	0.638	0.861	0.880	0.848	0.714	0.795	0.750	0.718	0.757	0.618
	Gentamycin	0.000	0.002	0.002	0.002	0.004	0.006	0.004	0.719	0.758	0.620
	Kanamycin	0.682	0.675	0.692	0.670	0.679	0.676	0.665	0.647	0.678	0.704
	Polymyxin B	0.00	0.005	0.008	0.006	0.000	0.002	0.391	0.678	0.691	0.667
<i>S. aureus</i> ATCC 29213	Amoxicillin	0.130	0.043	0.889	0.257	0.951	0.971	0.938	0.904	0.897	1.007
	Ampicillin	0.000	0.000	0.000	0.000	0.673	0.778	1.069	1.073	1.054	0.975
	Gentamycin	0.001	0.001	0.001	0.001	0.002	0.001	0.327	0.950	1.135	1.176
	Kanamycin	0.001	0.001	0.027	0.002	0.546	0.986	1.103	1.075	1.154	0.969
	Polymyxin B	0.220	0.344	0.332	0.347	0.442	0.370	0.478	0.434	0.474	0.507

Note: Breakpoints are concentrations at which bacteria can be identified as susceptible or resistant to a given antibiotic. If the MIC is less than or equal to the susceptibility breakpoint the bacteria is susceptible to the antibiotic. If the MIC is greater than this value, the bacteria is intermediate or resistant to the antibiotic.

DANAMIC: Automation and data analysis

Data import

⌚ Timing: 5 min to prepare and import data into DANAMIC

DANAMIC is a bespoke Graphical User Interface (GUI) designed to automate data analysis from Excel data files exported by SoftMax Pro v7.0 from experimental data collected in micro-plate readers. The program is designed to work with Windows 10 and is compatible with any *NIX system provided that the libraries are installed correctly (Figure 1).

11. Prior to the automated analysis save experimental data in SoftMax Pro as follows:

Table 2. Exemplar reference breakpoints in μM based on the data from (ISO 20776-1:2006*; EUCAST)

Bacteria	Reference concentration EUCAST QC 2020-01-01 (μM)							
	Amoxicillin		Ampicillin		Gentamycin		Kanamycin	
	Target	Range	Target	Range	Target	Range	Target	Range
<i>E. coli</i> ATCC 25922	10.33	5.16–20.65	10.68	5.34–21.37	0.97	0.48–1.94	–	1.71–6.87 *
<i>P. aeruginosa</i> ATCC 27853	–	–	–	–	1.94	0.97–3.87	–	–
<i>S. aureus</i> ATCC 29213	–	0.64–2.58 *	–	1.34–5.34 *	0.48–0.97	0.24–1.94	–	1.71–6.87 *
<i>S. pneumonia</i> ATCC 49619	0.15	0.08–0.32	0.33	0.16–0.67	–	–	–	–
<i>E. faecalis</i> ATCC 29212	2.58	1.29–5.16	–	1.34–5.34 *	15.49	7.74–30.97	–	27.46–109.86 *
Bacteria	Polymyxin B		Chloramphenicol		Ciprofloxacin		Vancomycin	
	Target	Range	Target	Range	Target	Range	Target	Range
	Target	Range	Target	Range	Target	Range	Target	Range
<i>E. coli</i> ATCC 25922	–	0.19–1.54 *	12.38	6.19–24.76	0.02	0.01–0.05	–	–
<i>P. aeruginosa</i> ATCC 27853	–	0.19–1.54 *	–	–	0.75–1.51	0.38–3.02	–	–
<i>S. aureus</i> ATCC 29213	–	–	12.38–24.76	6.19–49.52	0.75	0.38–1.51	0.67	0.34–1.35
<i>S. pneumonia</i> ATCC 49619	–	–	12.38	6.19–24.76	–	–	0.17	0.08–0.34
<i>E. faecalis</i> ATCC 29212	–	–	–	6.19–49.52	1.51–3.02	0.75–6.04	1.35	0.67–2.69

Table 3. Exemplar reference breakpoints in mg/L based on the data from (ISO 20776-1:2006*; EUCAST)

Bacteria	Reference concentration EUCAST QC 2020-01-01 (mg/L)							
	Amoxicillin		Ampicillin		Gentamycin		Kanamycin	
	Target	Range	Target	Range	Target	Range	Target	Range
<i>E. coli</i> ATCC 25922	4	2–8	4	2–8	0.5	0.25–1	–	1–4 *
<i>P. aeruginosa</i> ATCC 27853	–	–	–	–	1	0.5–2	–	–
<i>S. aureus</i> ATCC 29213	–	0.25–1 *	–	0.5–2 *	0.25–0.5	0.125–1	–	1–4 *
<i>S. pneumonia</i> ATCC 49619	0.06	0.03–0.125	0.125	0.06–0.25	–	–	–	–
<i>E. faecalis</i> ATCC 29212	1	0.5–2	–	0.5–2 *	8	4–16	–	16–64 *
Bacteria	Polymyxin B		Chloramphenicol		Ciprofloxacin		Vancomycin	
	Target	Range	Target	Range	Target	Range	Target	Range
	Target	Range	Target	Range	Target	Range	Target	Range
<i>E. coli</i> ATCC 25922	–	0.25–2 *	4	2–8	0.008	0.004–0.016	–	–
<i>P. aeruginosa</i> ATCC 27853	–	0.25–2 *	–	–	0.25–0.5	0.125–1	–	–
<i>S. aureus</i> ATCC 29213	–	–	4–8	2–16	0.25	0.125–0.5	1	0.5–2
<i>S. pneumonia</i> ATCC 49619	–	–	4	2–8	–	–	0.25	0.125–0.5
<i>E. faecalis</i> ATCC 29212	–	–	–	4–16 *	0.5–1	0.25–2	2	1–4

- a. One bacterium strain per plate.
- b. Two antimicrobial agents per plate, with concentrations shown from left to right starting with the highest, with each concentration in triplicate, as shown in Figure 2.
12. Export the saved data in the correct format as follows:
 - a. Click in the top left microplate icon (📄) → Click “Export”
 - b. A dialog window as in Figure 3 will appear. Select the plates you want to export. Select the “Raw” option in Plata Data Options and “Plate (.txt or .xls)” in Output Format. Click “Ok”.
13. Open the .xls file in Excel and convert the exported data into *.csv file and run the script as described in the next section.

Data analysis

⌚ Timing: 3 min to run data analysis in DANAMIC

14. Download the software package from <https://github.com/JavierGarcia-Ruiz/DANAMIC>.
15. Open PowerShell and type:

```
PS [your_path] > cd <your_path_to_DANAMIC_directory>
PS [your_path] > python DANAMIC.py
```

Note: Substitute <your_path_to_DANAMIC_directory> with your own path in your computer, e.g., C:\Users\Desktop.

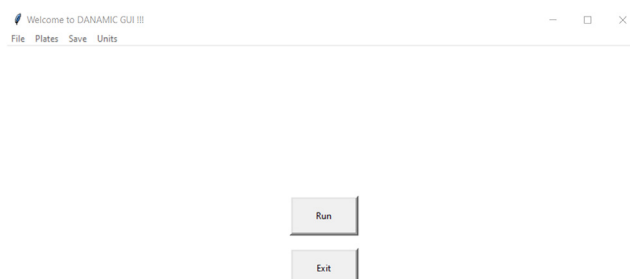


Figure 1. View of the main program window

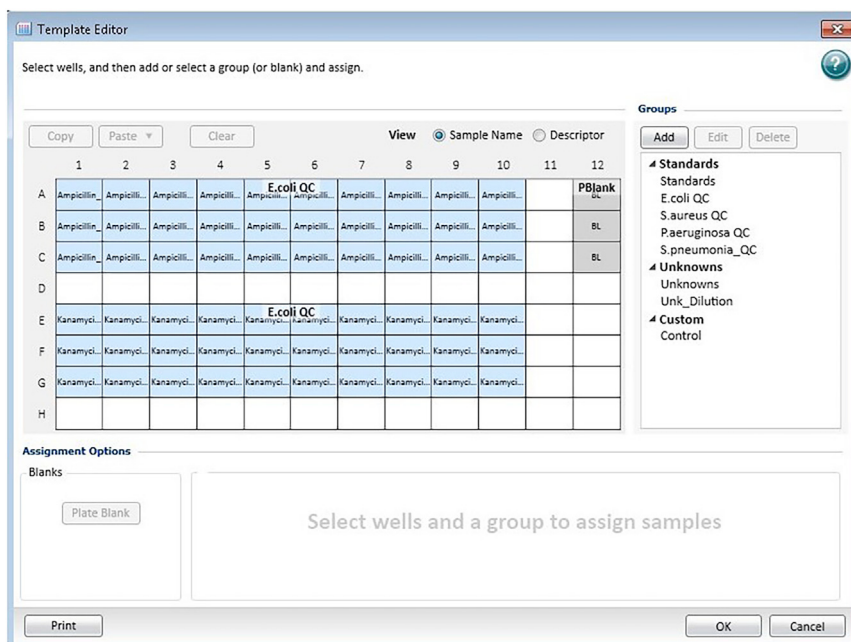


Figure 2. Exemplar design view of structuring an experiment in SoftMax Pro

16. DANAMIC will prompt a window where you need to fill in with 4 parameters:

File: it will open a window to search the .csv file you want to analyze.

Plates: it will open a window where you will need to type the number of plates your file contains.

Units: choose Concentration μM or Concentration (mg/L).

Save: it will open a window to save the data in the folder of your choice. You must type .xlsx at the end of the name. i.e., my_data_analyzed.xlsx.

Note: The 4 parameters can be filled in in any order.

17. Examples of the output can be found in <https://github.com/JavierGarcia-Ruiz/DANAMIC/tree/main/Output%20Examples>.

EXPECTED OUTCOMES

Following the analysis in DANAMIC, the data of antimicrobial susceptibility measured should be sorted into a tabulated format of MIC values at concentrations chosen (i.e., μM or mg/L). These values are organized against bacteria and antimicrobials used. In addition to this tabulated data, there is an extra table in the same section with statistics for the absorbance values collected.

Other sections include the processed data organized alphabetically by the name of the antimicrobial agent used, absorbance units versus bacteria strains used, absorbance units versus the antimicrobial agent used and histogram plots to visualize the data analyzed.

LIMITATIONS

The code is exemplified to work with SoftMax Pro 7.0 – software supplied by Molecular Devices for spectrophotometer SpectraMax i3X. The code can be adapted for another software provided that

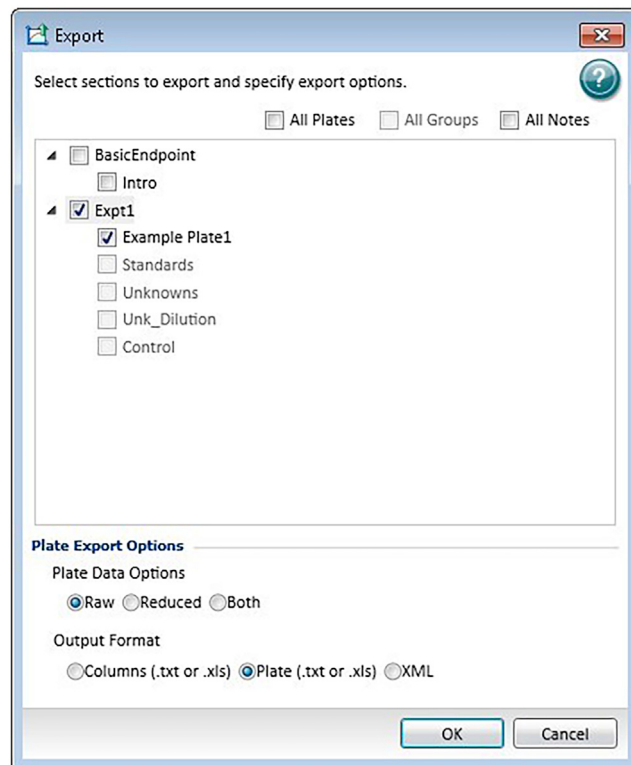


Figure 3. Exporting the saved data in the correct format

the data output format is similar (plate readout and summary table). Attention to detail and knowledge of Python3 will be necessary to perform custom adaptation and modifications. An example of .csv output can be found in: <https://github.com/JavierGarcia-Ruiz/DANAMIC/tree/main/Tests>.

TROUBLESHOOTING

There are several situations where the program may not be able to correctly interpret the input data.

Problem 1

A given .xls file has no extra line when saving file in SoftMax Pro. Soft Max Pro might save files without an extra, blank row between datasets of different plates. In this case, the program would fail to locate the data correctly (Figure 4) (See steps 10–13).

Potential solution

- Open the DANAMIC.py in your IDE or Text Editor of your preference.
- Change the variable.

```
# 2. Number of plates:
def number_of_plates_rows_drop(ncolumns, nrows=12):
    MIC_drop_rows = MIC_raw.drop(np.arange(0, nrows * ncolumns))
    return MIC_drop_rows
```

##BLOCKS=15													
Plate:	EC- Amp1 +	1.3	PlateForm	Endpoint	Absorbanc	Reduced	FALSE	1					
		1	2	3	4	5	6	7	8	9	10	11	12
		0.004933	0.419933	0.831933	1.053633	1.075233	1.090733	1.112333	1.084033	1.096833	1.102433	1.010533	0.000133
		0.106733	0.443933	0.936533	1.054233	1.144533	1.141033	1.107733	1.095533	1.127833	1.053833	1.020833	0.000433
		0.043633	0.545433	1.007533	1.091533	1.156233	1.187033	1.156833	0.991633	1.019233	0.883933	1.030733	-0.00057
		-0.00537	-0.00527	-0.00537	-0.00527	-0.00487	-0.00497	-0.00527	-0.00487	-0.00467	-0.00477	-0.00487	-0.00457
		-0.00097	-0.00057	-0.00087	-0.00077	-0.00077	0.195333	0.596033	0.386633	0.667733	1.018533	-0.00527	-0.00507
		-0.00207	-0.00257	-0.00207	-0.00137	-0.00237	-0.00157	0.593633	0.892933	0.937133	0.967033	-0.00537	-0.00507
		-0.00297	-0.00177	-0.00267	-0.00167	-0.00177	-0.00257	0.748833	0.901933	1.012833	0.637533	-0.00587	-0.00617
		-0.00717	-0.00577	-0.00637	-0.00657	-0.00627	-0.00627	-0.00647	-0.00677	-0.00727	-0.00587	-0.00597	-0.00577
~End													
##BLOCKS=15													
Plate:	EC- Amp1 +	1.3	PlateForm	Endpoint	Absorbanc	Reduced	FALSE	1					
		1	2	3	4	5	6	7	8	9	10	11	12
		0.004933	0.419933	0.831933	1.053633	1.075233	1.090733	1.112333	1.084033	1.096833	1.102433	1.010533	0.000133
		0.106733	0.443933	0.936533	1.054233	1.144533	1.141033	1.107733	1.095533	1.127833	1.053833	1.020833	0.000433
		0.043633	0.545433	1.007533	1.091533	1.156233	1.187033	1.156833	0.991633	1.019233	0.883933	1.030733	-0.00057
		-0.00537	-0.00527	-0.00537	-0.00527	-0.00487	-0.00497	-0.00527	-0.00487	-0.00467	-0.00477	-0.00487	-0.00457
		-0.00097	-0.00057	-0.00087	-0.00077	-0.00077	0.195333	0.596033	0.386633	0.667733	1.018533	-0.00527	-0.00507
		-0.00207	-0.00257	-0.00207	-0.00137	-0.00237	-0.00157	0.593633	0.892933	0.937133	0.967033	-0.00537	-0.00507
		-0.00297	-0.00177	-0.00267	-0.00167	-0.00177	-0.00257	0.748833	0.901933	1.012833	0.637533	-0.00587	-0.00617
		-0.00717	-0.00577	-0.00637	-0.00657	-0.00627	-0.00627	-0.00647	-0.00677	-0.00727	-0.00587	-0.00597	-0.00577
~End													

Figure 4. Data location error as a result of missing a blank row between datasets of different plates

The correct format (upper) incorporates a blank row (gray). The incorrect format (lower) missing the row introduces an error in data location and interpretation.

To:

```
# 2. Number of plates:

def number_of_plates_rows_drop(ncolumns, nrow=11):

    MIC_drop_rows = MIC_raw.drop(np.arange(0, nrow * ncolumns))

    return MIC_drop_rows
```

Note: Type the correct number of dataset plates. The number of plates typed must correspond to the exact number of datasets in the file.

Problem 2

Concentration units (μM or mg/L) were not specified correctly. Any unit outside this selection would need to be introduced manually in the code (See steps 14–16).

Potential solution

- Introduce other units in this section of the program:

```
def molar_unit():

    global unit

    unit = "Concentration  $\mu\text{M}$ "

    print(unit)

    label_molar = tk.Label(window, text="Concentration  $\mu\text{M}$ ", font=("Calibri", 10), bg="light sky blue", fg="black")

    label_molar.grid(column=0, row=3)

def milligram_unit():
```

```
global unit
unit = "Concentration (mg/L) "
print(unit)

label_milligram = tk.Label(window, text="Concentration (mg/L) ", font=("Calibri
    Bold", 10), bg="light sky blue", fg="black")
label_milligram.grid(column=0, row=3)

menu = Menu(window)
new_item1 = Menu(menu, tearoff=0)
new_item2 = Menu(menu, tearoff=0)
new_item3 = Menu(menu, tearoff=0)
new_item4 = Menu(menu, tearoff=0)
new_item5 = Menu(menu, tearoff=0)
new_item6 = Menu(menu, tearoff=0)

new_item1.add_command(label='Open', command=openfile)
new_item2.add_command(label='Number of plates', command=numberplates)
new_item3.add_command(label='Save', command=savefiles)

run_button = tk.Button(window, text="Run", command=window.destroy, height=2,
width=10, padx=5, pady=5, bd=4).place(x=380, y=200)

new_item4.add_command(command=run_button)
new_item5.add_command(label="Concentration  $\mu$ M", command=molar_unit)
new_item5.add_command(label="Concentration mg/L", command=milligram_unit)
```

Note: Knowledge in the use of the Tkinter library is required.

Problem 3

When at least one of the inputs has not been entered correctly in the program the following message is displayed: "Some input was incorrect. Please try again". In most cases, this would be due to that the plate number was entered incorrectly, the file was not .csv or the saved file was not saved as .xlsx, or the units were not correctly entered (See steps 11–17).

Potential solution

- always follow the structure specified in [Figure 2](#) in SoftMax Pro.
- export it in the correct form as shown in [Figure 3](#).

Note: DANAMIC does not identify what type of error it encounters but will always encourage you to try again.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Maxim G Ryadnov (max.ryadnov@npl.co.uk).

Materials availability

This study did not generate unique reagents.

Data and code availability

The code and example datasets are available at GitHub: <https://github.com/JavierGarcia-Ruiz/DANAMIC> and archived at Zenodo with a <https://doi.org/10.5281/zenodo.7086463>.

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AUTHOR CONTRIBUTIONS

J.G.R. and M.G.R. conceived, compiled, and wrote the protocol. J.G.R. performed the experiments and created the open-source code used to analyze the data. M.G.R. supervised the work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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